

3D Printed Medical-Grade Metal-based Microneedles as a Modular Platform for Minimally Invasive Therapeutic Delivery



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List of Abbreviations

2Dimensional	2D
3Dimensional	3D
Additive Manufacturing	AM
Analysis of Variance	ANOVA
Box–Behnken Design	BBD
Computer-aided design	CAD
Central Composite Design	CCD
Anti-calcitonin gene-related peptide	CGRP
Confidence interval	CI
Carboxymethyl cellulose	CMC
Diameter	d
Particle Diameter below 10%	D ₁₀
Particle Diameter below 50%	D ₅₀
Particle Diameter below 90%	D ₉₀
Deionised	DI
Direct metal laser sintering	DMLS
Deoxyribonucleic acid	DNA
Design of Experiment	DoE
Extra High Tension	EHT
2-hydroxy-2-methyl-propiophenone	HMP
Steady-state flux	J
Metal injection moulding	MIM
Number Average Molecular Weight	M _n
Microneedle	MN
Average Molecular Weight	M _w
Optical Coherence Tomography	OCT
Phosphate-buffered saline	PBS
Polycaprolactone	PCL
Polyethylene glycol	PEG
Poly(ethylene glycol) diacrylate	PEGDA
Poly(l-lactide-co-d,l-lactide)	PLA
Poly-Lactic Acid	PLA

Poly D,L-lactic-co-glycolic	PLGA
Polyvinyl alcohol	PVA
Polyvinylpyrrolidone	PVP
Surface roughness	Ra
Rhodamine B	RhoB
Ribonucleic acid	RNA
Round window membrane	RWM
Standard Deviation	SD
Scanning Electron Microscopy	SEM
Transdermal drug delivery	TDD
Ultraviolet	UV
White adipose tissue	WAT
Displacement	δ

List of Units

Millilitre	mL
Microlitre	μL
Grams	g
Microgram	μg
Millimetre	mm
Micrometre	μm
Nanometre	nm
Megapascal	MPa
Gigapascal	GPa
Kilonewton	kN
Newton	N
Millinewton	mN
Kilovolt	kV
Volt	V
Ampere	A
Watt	W
Hour	h
Minutes	min

Seconds	s
Per second	s ⁻¹
Units of active insulin	U
Degree Celsius	°C
Roughness Average	Ra
Revolutions Per Minute	RPM
Molarity	M
Kilodalton	kDa
Millimole per hour	mM/h
Centimetre per minutes	cm/min
Millimetre per minutes	mm/min
Microgram per centimetre square per minute	µg/cm ² /min
Degree celsius per minute	°C/min
Millimetre per second	mm/s
Pascal second	Pa*s
Microliter per second	µL/s
Milligram per decilitre	mg/dL
Nanogram per millilitre	ng/mL
Gram per centimetre cube	g/cm ³
Microgram per centimetre square	µg/cm ²
Percentage weight per weight	% w/w
Newton per needle	N/needle

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List of Publications and Oral/Poster Presentation

Publications

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2. Sargioti N., Shu W., Levingstone T.J., O'Cearbhaill E., McCarthy H., Dunne N. “Optimising Metallic Microneedles for Transdermal Drug Delivery: A Multi-Objective Parameter Optimisation Approach via Design of Experiments in Additive Manufacturing”, *Acta Biomaterialia*, Elsevier. 2023 (Under Review)

Oral Presentations

1. Sargioti N., Levingstone T.J., O'Cearbhaill E., McCarthy H., Dunne N. “*Additive Manufactured Metal-Based Microneedles as Minimally Invasive Drug Delivery System*”. 2021 International Student Conference in Metallic Materials - 'Metals for A Better Tomorrow' (Virtual, 12th – 13th July 2021)
2. Sargioti N., Subrata Mondal S., Levingstone T.J., O'Cearbhaill E., McCarthy H., Dunne N. “*3D Printed Microneedles as a Modular Platform for Minimally Invasive Therapeutic Delivery*”. 31st Conference of the European Society for Biomaterials 2021 (Virtual, 5th – 9th September 2021)
3. Sargioti N., Levingstone T.J., O'Cearbhaill E., McCarthy H., Dunne N. “*3D Printed Microneedles as a Modular Platform for Minimally Invasive Therapeutic Delivery*”. Research Day 2022 (Dublin, Ireland, 4th April 2022)
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7. Sargioti N., Levingstone T.J., O'Cearbhaill E., McCarthy H., Dunne N. "*Development 3D Printing Microneedle Arrays as Minimally Invasive Drug Delivery Systems*". 28th Annual Conference of the Section of Bioengineering of the Royal Academy of Medicine in Ireland (Enfield, Co Meath, Ireland, 27th – 28th January 2023)
8. Sargioti N., Levingstone T.J., O'Cearbhaill E., McCarthy H., Dunne N. "*Optimisation of Post-Processing and Polymer Coating Parameters for Metallic Microneedle Arrays as an Effective Drug Delivery System via 3D Printing Technology*". (Davos, Switzerland, 4th – 8th September 2023)

Poster Presentations

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3. 1. Sargioti N., Levingstone T.J., O'Cearbhaill E., McCarthy H., Dunne N. "*3D Printed Medical-Grade Metal-Based Microneedles as a Modular Platform for Minimally Invasive Therapeutic Delivery*". I-FORM Gathering 2020 (Virtual, 25th November 2020)
4. Sargioti N., Levingstone T.J., O'Cearbhaill E., McCarthy H., Dunne N. "*Development of Metallic Microneedles (MNs) as Minimally Invasive Drug Delivery Systems*". Research Day 2023 (Dublin, Ireland, 20th April 2023)

Awards

1. AMS CDT 2020 National Student Conference in Metallic Materials – **Best Poster Award in the section of Modelling & Characterisation** (Virtual, 16th July 2020)
2. I-FORM Gathering 2020 – **Best Poster with Greatest potential for impact on industry or society** (Virtual, 25th November 2020)
3. 2021 International Student Conference in Metallic Materials - 'Metals for A Better Tomorrow' - **Best Oral Presentation Award in the section of Additive Manufacturing** (Virtual, 12th – 13th July 2021)

Abstract

Nikoletta Sargioti

3D Printed Medical-Grade Metal-based Microneedles as a Modular Platform for Minimally Invasive Therapeutic Delivery

Microneedles (MNs) enhance transdermal drug delivery (TDD) by enabling better drug absorption and avoiding pain and needle-related risks, thereby improving treatment for chronic conditions and enhancing patient comfort. This study introduces optimised 3D printed stainless-steel MN arrays coated with biocompatible polymers. Conical-shaped MNs (36 MNs/patch) were fabricated using the direct metal laser melting process and optimised through a Design of Experiments approach. Optimal MNs, with height of $\sim 780\ \mu\text{m}$ and tip diameter $\sim 25\ \mu\text{m}$, with reduced roughness, were developed. These MNs demonstrated a skin penetration depth ranging between $400\text{--}700\ \mu\text{m}$ without potential for reaching the pain receptors. *Ex vivo* studies on porcine skin demonstrated the efficacy of the MNs since force of $\sim 4\ \text{N}$ is required for them to penetrate the *stratum corneum* with no tip fracture. MNs were dip-coated with poly(vinyl-alcohol) (PVA), polyvinylpyrrolidone (PVP), and carboxymethyl-cellulose (CMC) loaded with Rhodamine B (RhoB, $M_w=479.02\ \text{g/mol}$) as a model drug. A 5-layer coating with 10% PVA-RhoB, 20% PVP-RhoB or 8% CMC-RhoB resulted in a homogeneous coating on MNs without affecting the geometrical characteristics or the penetration depth, leading to a drug loading capacity of $11.0\pm 2.0\ \mu\text{g}$, $15.0\pm 1.5\ \mu\text{g}$ and $12.5\pm 3.0\ \mu\text{g}$ respectively. PVA/PVP/CMC-RhoB coated-MNs resulted in 60-80% drug delivery to porcine skin within 5 min of MN application. Additionally, the 2-h *in vitro* permeation study presented slower drug permeation for PVA-RhoB and CMC-RhoB, with a steady-state flux (J) ranging from $0.017\text{--}0.044\ (\mu\text{g}/\text{cm}^2)/\text{min}$, than PVP-RhoB ($J=0.23\ (\mu\text{g}/\text{cm}^2)/\text{min}$). Despite differing kinetics, all polymers achieved 80-95% drug permeation after 2-h. The coated metallic-MN technology offers a promising solution to the limitations of current TDD systems, providing a painless and controllable method for drug administration. Future work includes *in vivo* studies to verify efficacy, and potential applications in managing pain and chronic illnesses, aiming for a minimally invasive and patient-compliant delivery system.

Chapter 1: Introduction and Literature Review

Sections of this Chapter have previously been presented (with some modification) in a literature review published in MDPI: Bioengineering (2023): **Sargioti, Nikoletta, Tanya J. Levingstone, Eoin D. O’Cearbhaill, Helen O. McCarthy, and Nicholas J. Dunne. "Metallic Microneedles for Transdermal Drug Delivery: Applications, Fabrication Techniques and the Effect of Geometrical Characteristics" Bioengineering 10, no. 1: 24. <https://doi.org/10.3390/bioengineering10010024>.**

1.1. Overview

Various approaches are presently employed for drug delivery, including oral administration, conventional hypodermic needles, topical creams and transdermal patches [1]. Each route has its specific benefits and limitations that need to be considered in drug delivery strategies. Oral drug delivery is considered one of the most desired routes of administration when compared to other routes due to high patient compliance, cost-effectiveness, fewer sterility constraints, flexibility in dosage delivery and ease of production. However, it results in the poor bioavailability of drugs due to factors relating to dissolution, permeability, and solubility [2]. Conventional hypodermic needles can cause pain to the patient as they penetrate deep into the *dermis* where pain receptors are present. Their use is particularly challenging for needle phobic patients. The traditional use of subcutaneous injection for the delivery of macromolecules also has safety concerns for healthcare workers as needle stick injuries are a common occurrence. In some cases, subcutaneous injections are expensive as there may be a need for multiple or chronic administration by trained medical professionals [3]. The topical application and administration of drugs, using a topical cream, gel or ointment or a non-invasive transdermal patch, allows for the penetration of the drug into the skin without pain [4]. These topical methods have a limited ability to administer drugs with large particles (*e.g.*, nucleic acids, large drug molecules) as the *stratum corneum* layer of the skin acts as a natural barrier [5]. The ability of a drug to penetrate the skin is influenced by the skin physiology and permeability, and various other factors including the physiochemical properties of the drug (*i.e.*, size, molecular weight, concentration, partition coefficient and solubility) and formulation characteristics (*i.e.*, release rate, ingredients and the presence of permeation enhancer) [6]. Additionally, the administration of ionic drugs, drugs with high concentrations, or those exhibiting with very low/high partition coefficients can result in skin irritation, non-systematic circulation and poor permeability [7]. Overall, while these topical

methods have the advantage of being painless, they lack bioavailability and can lead to skin irritations, allergic reactions or non-controlled drug release [8].

Because of the disadvantages of existing techniques, there is an increasing imperative for innovative methods for the delivery of therapeutic agents. For this reason, many studies have focused on the investigation of microneedle (MN) arrays as transdermal drug delivery (TDD) systems. MN arrays are minimally-invasive devices that can penetrate the *stratum corneum*, one of the most important barriers for topically-applied drugs, thus creating a pathway for drug permeation to the dermal tissue below [9]. MN arrays can enhance skin permeability compared to non-invasive patches enabling a faster onset of action and good bioavailability. Using minimally invasive MN-based transdermal patches for TDD offers several advantages over traditional drug delivery methods. These advantages include: (i) easy and controlled drug delivery; (ii) the enhancement of therapeutic efficiency with fewer side effects; (iii) less pain than with traditional hypodermic needles; and (iv) the maintenance of a steady plasma level of the drug [10,11]. To date, MN arrays have been used in several biomedical applications including diabetes treatment [10], cancer diagnosis and therapy [11], infections, inflammation and chronic pain treatment and the treatment and control of obesity [12], and also for other applications including the sampling of blood and interstitial fluids [13]. However, current MN-based TDD systems have associated limitations including inconsistent penetration depth, skin irritation and inflammation and limited applicability for large drug molecules and loading capacity particularly for polymeric MNs, which results in limited drug delivery efficiency and the wastage of valuable medication [14–17]. Variability in penetration depth can result from differences in skin types, needle design, and application force, leading to insufficient and non-consistent depth to effectively deliver drugs. have been also reported post application. Large molecules are difficult to deliver through MNs because of their size and the need to maintain their stability and activity. The challenge of delivering large molecules through MNs

has been addressed by encapsulating these molecules in biodegradable nanoparticles, allowing for their stable and efficient transport through the skin.

Metallic MN offer the potential to overcome the challenges associated with polymeric MNs arrays. Metallic MNs, typically made from materials like stainless steel or titanium, offer greater mechanical strength than the polymeric. This characteristic allows for more consistent penetration depths without bending or breaking, which is crucial for reliable drug delivery. Furthermore, metallic MNs have been adapted to deliver large molecules by creating hollow or porous structures. Hollow MNs allow for the delivery of larger molecules directly through the needle pore, functioning almost like a mini-injection. This setup is particularly useful for high molecular weight drugs or particles, that are difficult to diffuse through solid MNs [12,18]. Although metallic MNs traditionally do not have a high drug loading capacity like some polymeric systems, the development of hollow or coated metallic MNs can address this limitation. Drugs have been either encapsulated in a coating on the needle surface or filled into the pore of hollow needles, significantly enhancing the amount of drug that can be delivered per application. However, several existing challenges limiting the translation of metallic MN arrays as a successful TDD system remain, including: (i) current methods for metallic MN array fabrication involve a multi-step process that is not cost-effective; (ii) the lack of clinical data relating to the cytotoxicity of the metals used for MN fabrication; (iii) limited drug loading; and (iv) challenges in maintaining mechanical properties and piercing capacity especially with high porosity structures [19]. For instance, the volume of voids within the structure of porous MNs can result in MN tip collapse, reducing the strength to only 2 N compressive force for titanium MNs when estimated using the uniaxial compressive stress. In addition to the decrease of mechanical properties, decrease of tip sharpness was also observed with increased fragility during the fabrication process [20,21]. Taking into account the porosity and the effect on the mechanical performance it was obtained that Selective laser

melting (SLM) printed lattices with a porosity 40-70% provided lower ultimate strength of approx. 200MPa compared to 900 MPa from solid samples [22].

Additive manufacturing techniques such as selective laser melting (SLM), electron beam melting (EBM), direct metal laser sintering (DMLS), etc. offer an innovative platform for design optimisation and the cost-effective manufacture of medical-grade metallic MN arrays [23]. For instance, SLM is a powder bed fusion process used to construct high-density metallic structures by selectively melting powder layers using a high-power laser [24]. This method is particularly suited for creating complex, high-strength metallic objects like MN arrays, allowing for precise control over needle geometry and density. Similar to SLM, EBM uses an electron beam instead of a laser to melt metal powder. It's especially effective for producing components from high-melting-point metals such as titanium, which is commonly used for medical devices due to its strength and biocompatibility. DMLS is another powder bed fusion technique that involves sintering rather than fully melting the metal powder. It's well-suited for creating detailed metallic structures and can use a variety of alloys, making it ideal for customized MN arrays.

This chapter describes the various types of MNs currently in development and their potential for use clinically in the treatment of diabetes, the diagnosis and treatment of cancer, management of chronic pain and treatment of obesity. The potential advantages of metallic MN are highlighted and the different methods that have been used for the development and manufacture of metallic MN arrays as potential TDD systems are discussed focusing on additive manufacturing. Additionally, a synopsis of existing and in development MNs for therapeutic drug delivery, and diagnosis and treatment applications is presented.

1.2. Anatomy of Skin

The skin is the largest organ in the human body and plays a vital role in protecting us from the external environment [25]. It serves as a physical barrier that prevents the entry of harmful substances, microbes, and ultraviolet (UV) radiation [26,27]. Additionally, the skin regulates body temperature, senses touch and pressure, and is also an important component of the immune system [28,29].

The skin has four main layers: the *stratum corneum*, *epidermis*, *dermis*, and subcutaneous tissue [30–33] (Figure 1.1). The *stratum corneum* is the outermost layer of the *epidermis* (approx. thickness of 10-40 μm), which consists of several layers of dead skin cells called corneocytes that are held together by a lipid matrix [34,35]. This layer serves as the primary barrier against various environmental stressors, including pathogens, toxins, and UV radiation. The *stratum corneum* also helps to regulate water loss from the body and maintain skin hydration. Due to its dense structure and low permeability, the *stratum corneum* can pose a significant challenge for the delivery of drugs and other therapeutic agents through the skin. Therefore, a better understanding of the composition of the *stratum corneum* and its properties is crucial for developing effective skin delivery strategies.

The *epidermis* (approx. 0.1 mm to 1 mm thickness) is the outermost layer, which is composed of several layers of cells, including keratinocytes, melanocytes, and Langerhans cells [25,36]. The keratinocytes are responsible for producing the protein keratin, which forms a protective barrier that prevents the loss of water and electrolytes from the body. The melanocytes produce the pigment melanin, which protects the skin from the harmful effects of UV radiation [37]. The Langerhans cells are part of the immune system and play a role in protecting the body from infection [32,33].

The *dermis* (approx. thickness of 2-3 mm) is the second layer of the skin and is composed of connective tissue, including collagen and elastin fibres, as well as blood vessels, nerves, and hair follicles [25,36]. The collagen and elastin fibres provide strength and elasticity to the skin, while the blood vessels supply nutrients and oxygen to the cells in the skin [38–40]. The nerves in the *dermis* sense touch, pressure, and temperature changes [41]. The subcutaneous tissue (approx. thickness of 8-12 mm), is the deepest layer of the skin and is composed of adipose tissue, blood vessels, and nerves [42–44]. It provides insulation to the body and serves as a cushion that protects the internal organs from injury.

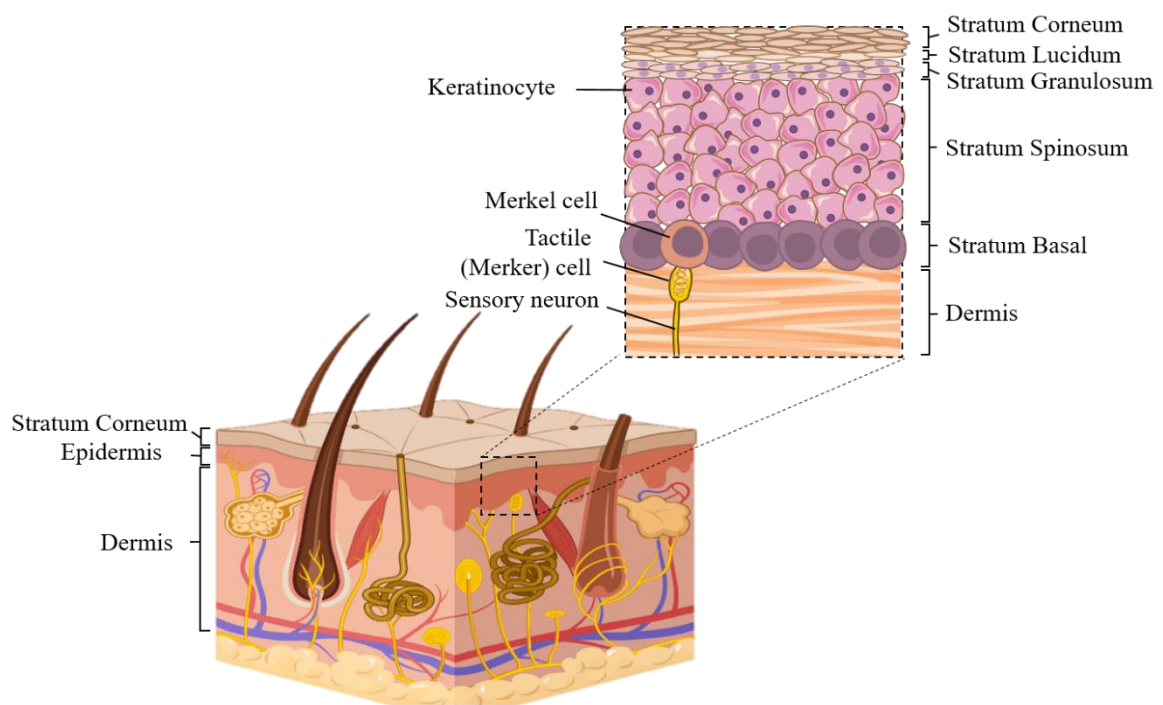


Figure 1.1: Anatomy of Skin – Schematic representation of the four main layers of the skin: *stratum corneum*, *epidermis*, *dermis*, and subcutaneous tissue. This illustration provides a comprehensive overview of the distinct layers comprising the human skin. Created with BioRender.com

1.2.1. Skin Barrier and Drug Penetration

The skin serves as the outermost organ of the body, functioning primarily as a protective barrier that retains internal moisture and shields against the infiltration of external substances. However, in addition to being a barrier against diffusion, the skin is also a living tissue having its own metabolism and contributing to the life of the overall body. In particular, skin provides an alternative route for drug administration, allowing sustained drug delivery to the blood circulatory system and providing greater comfort for the patient, while avoiding several side effects of oral and parenteral administration. Despite several advantages such as a large available surface area, relatively low enzymatic degradation and the extremely long application times made possible by transdermal delivery, the considerable barrier function of the *stratum corneum* makes transdermal delivery challenging. There are two possible routes of drug penetration across the intact skin, namely the transepidermal and transappendageal pathways, which have been diagrammatically presented in Figure 1.2. Transepidermal penetration can be termed intracellular or intercellular. The intracellular route through corneocytes, allows the transport of hydrophilic or polar solutes allowing diffusion of lipophilic or non-polar solutes through the continuous lipid matrix. The intercellular lipid matrix is a key component of the barrier function of the skin and is responsible for the low permeability of the *stratum corneum* to hydrophobic and lipophilic molecules. The transappendageal route involves the passage of molecules through sweat glands and across the hair follicles.

The dermal absorption describes the passage of compounds across the skin. The process is divided into four steps: (i) penetration of a substance into a particular skin layer, (ii) permeation into *epidermis*, (iii) diffusion through the viable *epidermis* and into the upper *dermis* and (iv) resorption of a substance into the vascular system. The understanding of the kinetics of skin permeation is necessary for development of successful TDD systems.

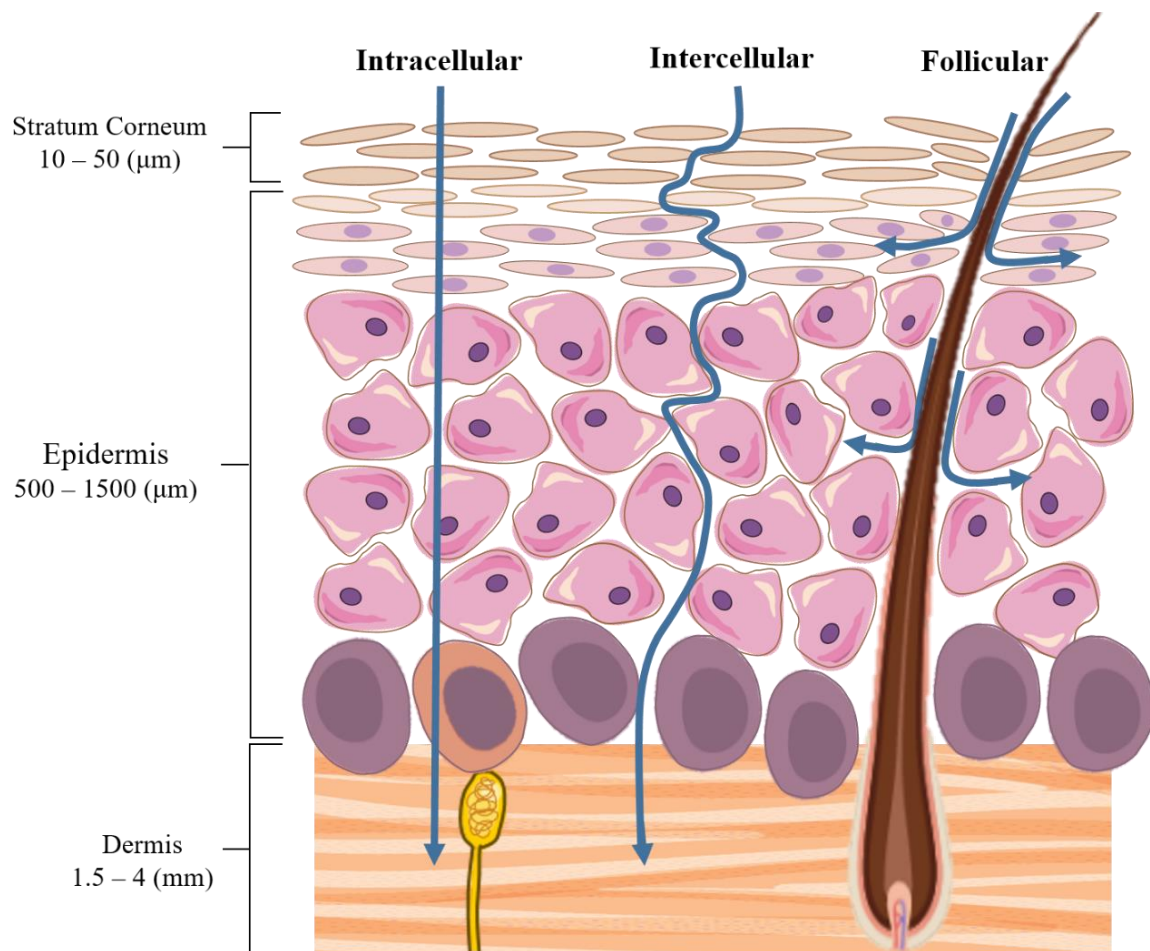


Figure 1.2: Drug Penetration – Schematic representation of the transepidermal and transappendageal pathways of drug penetration across the intact skin. The diagram illustrates the two distinct routes through which drugs can permeate the skin barrier. Created with BioRender.com

1.3. Overview of Current TDD Systems

TDD systems are a type of drug delivery system that allows medication to be absorbed through the skin and into the bloodstream [45,46]. These systems can be used for a variety of medications, including hormones, pain relievers, and nicotine replacement therapy. TDD systems offer several advantages over other drug delivery methods, including ease of use, consistent drug levels, and reduced side effects [47]. The system consists of a patch or other device that is applied to the skin and delivers the medication through the skin and into the bloodstream over a specified period [8,48].

TDD systems represent effective and convenient drug delivery methods that can improve medication adherence and patient outcomes. Better adherence directly correlates with more effective disease management and better overall health outcomes, making TDD systems a significant advancement in drug delivery technology. The main advantage of TDD systems is their ability to provide a controlled release of medication over an extended period [45,49]. This eliminates the need for frequent dosing and allows for consistent drug levels in the bloodstream. Additionally, TDD systems avoid the digestive system, reducing the risk of side effects that may occur when medications are taken orally [8]. TDD systems are particularly useful for medications that have a narrow therapeutic index, meaning that a small change in dose can have a significant impact on efficacy or safety [33]. Also, these systems provide a non-invasive method of drug administration, which is particularly advantageous for patients who are needle-phobic or have difficulties with oral dosages. This non-invasive nature significantly reduces the discomfort associated with drug administration, thereby promoting better adherence to treatment. TDD systems are not only easy to use but can also be self-administered, which is a significant advantage in terms of convenience and independence in managing health conditions. In cases where adverse effects occur or if drug delivery needs to be stopped, the patient can simply remove the transdermal patch or device, thus quickly

terminating the drug's delivery. This feature also helps in reducing the potential for systemic side effects and adverse reactions, as the drug delivery is more targeted and minimises exposure to non-target tissues.

Although TDD systems offer several advantages over other drug delivery methods, there are some limitations to their use [8]. For instance, limited number of drugs that can be delivered through the skin. The skin barrier can be a significant obstacle to drug delivery, and many drugs are not suitable for transdermal delivery due to their size, polarity, or other physicochemical properties. Furthermore, some drugs require a rapid onset of action, and transdermal delivery may not be able to provide the desired rate of absorption [47]. Another limitation of TDD systems is the variability in drug absorption between individuals. Factors such as skin thickness, hydration, and blood flow can affect the rate of drug absorption through the skin, leading to variability in drug levels among individuals [50,51]. In some cases, this variability can be significant enough to affect the efficacy and safety of the medication. Finally, TDD systems can also cause skin irritation or allergic reactions in some individuals [52]. This can be due to the adhesive used in the patch or the medication itself. Skin irritation can limit the duration of use or require additional treatments to manage the reaction. Overall, while TDD systems represent effective and convenient drug delivery methods, there are limitations to their use that must be considered when selecting a drug delivery method for a particular medication.

1.4. MNs for Drug Delivery

The use of minimally invasive MN-based transdermal patches offers several advantages over traditional drug delivery methods, including easy and controlled drug delivery with wider range of drugs than topical TDDs, enhanced therapeutic efficiency with fewer side effects, less pain than traditional hypodermic needles, and the maintenance of a steady plasma level of the drug [8,53]. Various biomedical applications, such as diabetes treatment [10], cancer

diagnosis and therapy [11], and the treatment and control of obesity [54], have been explored using MN arrays, and they have also been utilised for sampling blood and interstitial fluids [13]. However, current MN-based TDD systems still have limitations, such as incomplete insertion, particularly for polymeric MNs, which results in limited drug delivery efficiency and medication wastage [15]. Incomplete insertion can result primarily from the mechanical properties of the polymer MNs. These might not possess sufficient strength to fully penetrate the *stratum corneum*, especially if the needle design does not support optimal penetration. Additionally, the design and fabrication of the MNs, including their geometry and sharpness, greatly influence their ability to insert completely. This incomplete insertion leads to limited drug delivery efficiency since the full dose of the medication is not delivered, which can significantly impact the effectiveness of the treatment. Moreover, the variability in the depth of penetration can lead to inconsistent dosing, which may affect therapeutic outcomes and patient compliance. This issue also results in medication wastage, as drugs that remain on partially inserted needles are not utilised and thus wasted. This not only undermines the cost-effectiveness of the treatment but also increases overall healthcare costs due to the need for more treatments or higher doses to achieve therapeutic effects.

Metallic MNs offer several advantages of polymeric MNs. While high start-up costs are initially required for the fabrication of metallic MNs and post-processing following fabrication is normally necessary, they have shown considerable promise as efficient minimal invasive delivery systems [21]. In particular, recent studies have shown that the manufacture of metallic-based MNs can achieve optimal geometry for constant, efficient and controlled minimal invasive drug delivery system [21,52,55]. Due to their high mechanical strength, they can easily penetrate the *stratum corneum* without failing and can effectively create pathways across skin enhancing drug flux [55]. They have been shown to exhibit a high degree of dimensional accuracy and reproducibility, with a printing resolution typically better

than 0.5 mm, dependent on careful control of printing parameters, material properties, and post-processing techniques [56], while their unique physical and chemical properties afford them the ability to deliver small, lipophilic molecules through soft tissues such as skin to a range of organs [57,58]. Furthermore, metallic MNs have higher fracture toughness and yield strength values (greater than 200 MPa) than polymeric MNs. They have been shown to exhibit a high degree of dimensional accuracy and reproducibility, while their unique physical and chemical properties afford them the ability to deliver small, lipophilic molecules through soft tissues such as skin to a range of organs [18]. A number of manufacturing techniques have been employed including stereolithography, laser cutting and ablation, wet/dry etching, metal electroplating, injection moulding and laser sintering, leading to metallic solid or hollow MNs with complex geometries [59].

A range of metals including stainless steel, titanium, palladium, nickel, tantalum, platinum and palladium–cobalt alloys have been successfully used for biomedical applications [60,61]. For the manufacture of MN arrays, stainless steel and titanium are most used in research at the present time. Compared to stainless steel, titanium can lead to lower corrosion rates combined with higher mechanical properties resulting in the development of solid applications, while hollow applications can be also used as base of the coated MNs [61]. In addition to their good mechanical properties and biocompatibility, stainless steel and titanium allow penetration of the skin without fracture of the MN–based arrays, thus offering advantages compared to counterparts composed of other materials [62]. Nickel has issues relating to biocompatibility, leading to the risk of toxicity so requires careful use [45]. For instance, nickel sensitivity or development of dermatitis have been observed following contact with alloys containing approximately 14% nickel [63,64]. Meanwhile, even MNs containing a low percentage of nickel (8%) have been reported to cause allergic contact

dermatitis [65]. Palladium and platinum have not been adequately studied for use in the manufacture of MNs, due to their high price and high melting temperature [66].

Stainless steel is an iron-based alloy that contains a high percentage of chromium (11-30 wt. %) and varying amounts of nickel [60]. For this reason, the composition of stainless steel affects its biocompatibility. The optimal biocompatibility is provided by 316 L stainless steel, which is also widely used for endovascular devices and surgical applications. Nevertheless, this is not critical for MN arrays since their application does not require long-term contact time with the body. Their use is similar to traditional stainless steel (304 stainless steel) hypodermic needles, which are commonly used and considered as biocompatible [62]. Due to the high biocompatibility and mechanical properties (e.g. Young's modulus of 200 GPa) of stainless steel, it was the first metal used in the manufacture of MN arrays [67,68]. The simplest method for the fabrication of stainless steel MN arrays involves holding traditional hypodermic needles in a supporting material and changing the length of needles [62]. More advanced stainless steel MNs with a variety of designs and geometries can be produced using microfabrication technology [68].

1.4.1. Types of MNs

MN arrays can be classified into four types: solid, coated, hollow, and dissolvable, as shown in Figure 1.3 [57,69,70]. These arrays can also be further categorised based on their mode of drug delivery and the materials used for their production [71]. There are four main modes of drug delivery: (i) "poke-detach-diffuse" for solid MNs; (ii) "coat and poke" for coated MNs; (iii) "poke and flow" for hollow MNs; and (iv) "poke and release" using dissolvable MNs [72]. In the "poke-detach-diffuse" method, solid MNs are used to create microchannels through the *epidermis* into the *dermis* [54]. Once the MN array is removed, the drug formulation is applied to the skin surface through topical creams or transdermal patches, and the drug is delivered through the microchannels created [57]. Coated MN arrays involve

coating solid MNs with a specific drug formulation, and delivering the drug during the insertion of the needles into the skin, known as the "coat and poke" method [54]. The coating of MNs can be achieved by dipping or spraying the surface of the solid MNs with the solubilised drug [73]. Following the penetration of the MN into the skin, the drug is delivered by the dissolution of the coating, which allows for the diffusion of the drug, and the MNs are then removed [73]. Solid MNs can be manufactured from various materials, including metals (e.g., stainless steel or titanium), ceramics (e.g., silicon), and polymers (e.g., poly D, L-lactic-co-glycolic acid (PLGA) and polyethylene glycol (PEG)), using different fabrication methods [54,58,72]. The length and shape of the channels formed depend on the needle design and geometry. Martiano *et al.* used triangular stainless steel MNs (height = 1 mm, width = 0.2 mm) to deliver insulin through the "poke-detach-diffuse" method, demonstrating the potential for effective TDD of macromolecules using solid MNs [59]. Additionally, titanium solid MNs (330 μ m height) were used in the "coat and poke" method, coated with protein antigen for vaccine delivery, demonstrating rapid and reproducible intracutaneous administration of dry-coated antigen [59].

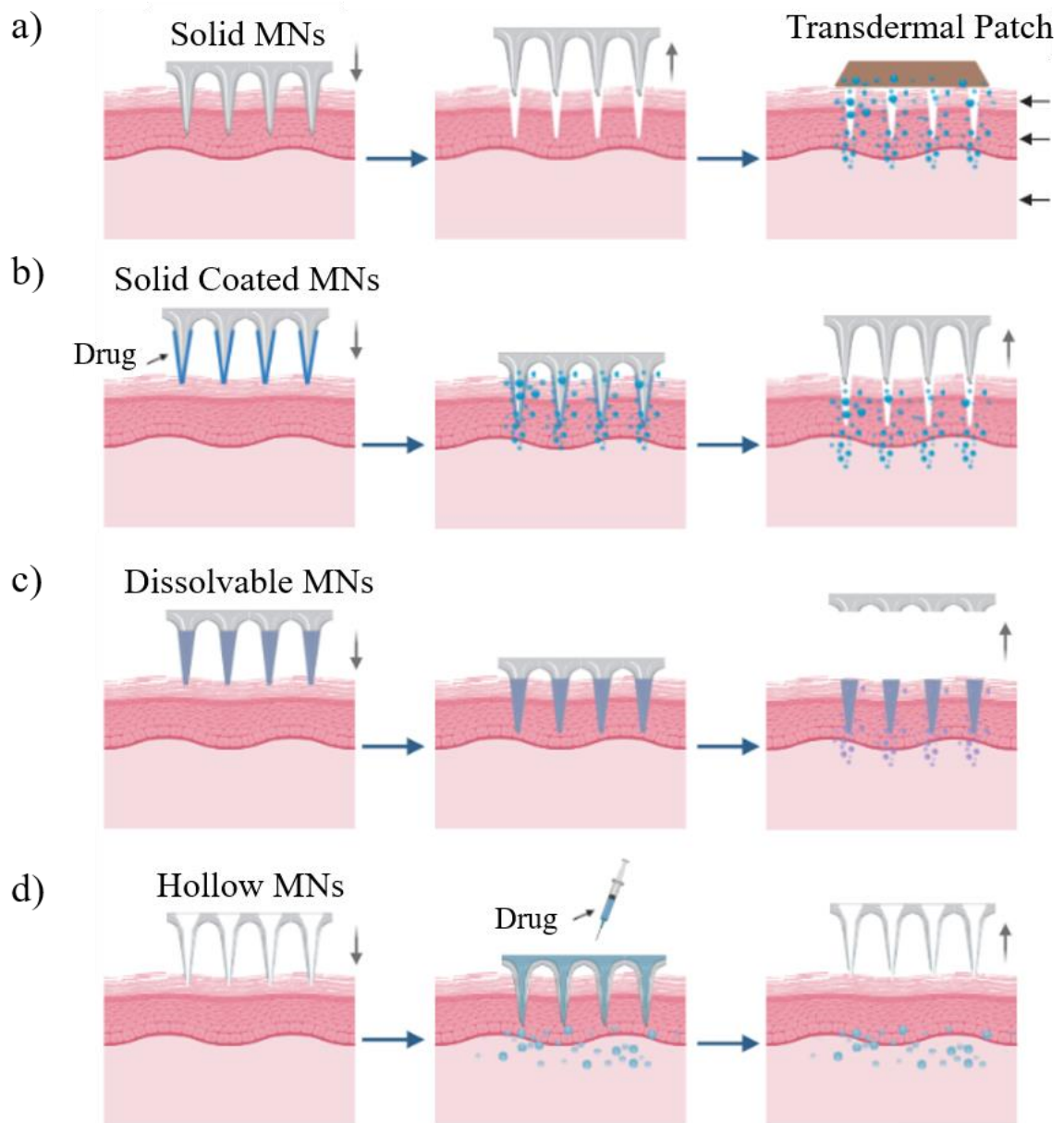


Figure 1.3: Type of MNs – Delivery procedures of solid MNs through the (a) ‘poke-detach-diffuse’ method, in which the solid MNs are used only for the creation of holes and the drug administration is provided by a transdermal patch and (b) ‘coat and poke’ method where the drug is coated on the needles and the drug administration is provided from the MNs. (c) “poke and release” method for dissolvable MNs which are inserted and dissolved in the skin and (d) “Poke and flow” method in which the drug is inserted through hollow MNs and delivered in the body. Created with BioRender.com

Recently, researchers have incorporated porosity into the structure of solid MNs, made from metals, polymers, or ceramics, to enhance their ability to deliver drugs and therapeutic agents [74]. These porous MNs have varying percentages of porosity, ranging from approximately 30% to 40%, with an average pore diameter of 1.3 μm to 2.22 μm within their structure, allowing them to absorb drugs within their pores and release them upon insertion into the skin [19,20,75]. However, while these MNs have shown potential for improved TDD, the presence of voids within the structure can cause the tip of the MN to collapse due to its porous nature [19]. Even if limited studies have been studied mechanical performance of MNs under compression test, it was obtained that the increase of porosity from 1.2% to 30% on MNs when tested under dynamic compressive loading conditions, decreases the yield strength of the material from 29.4~49.8 MPa to 14.6~26.0 MPa, and the compressive strength from 60.3~78.6 MPa to 30.2~40.5 MPa [76]. For instance, the porosity within a structure has been found to reduce the compressive force to 2 N for titanium MNs and 0.6N for polymeric MNs, limiting in this way the penetration ability. As a result the existence of porosity decreased mechanical properties, tip sharpness, and increased fragility during fabrication [21,75]. Hence, further optimisation of the selected particle size of the powder material and the pore diameter of the final part is necessary to produce porous MNs that meet clinical requirements [19].

Hollow MN arrays consist of an empty cavity within the MN and a bore at the tip, allowing for drug delivery through the ‘coat and flow’ method. This method permits the administration of micro-volumes of drugs directly into the skin with high accuracy in dosage [54,77]. Hollow MNs are typically manufactured from metals and ceramics using similar fabrication techniques as those used for solid MN arrays [71]. The last type of MN arrays use dissolvable MNs, which are biodegradable and are normally fabricated from water-soluble materials or degradable polymers [70]. The drug is embedded in the matrix of the MN array, which

possesses enough mechanical stability to enable insertion into the skin. The matrix then dissolves upon insertion, resulting in drug release via the ‘poke and release’ mechanism [71].

1.4.2. Fabrication Methods of MNs

Between 1990 and 1999, technological advancements in microelectronics led to the development of microfabrication tools suitable for producing MNs for medical applications [78]. Initially fabricated from silicon, different materials such as metals, polymers, ceramics, and glass have since been utilised in MN fabrication. Metallic MNs are designed with a focus on achieving optimal geometry, size, shape, and tip sharpness to increase mechanical strength and minimise the force required for penetration into the skin [67]. Fabrication methods include 3D printing techniques, such as Direct Metal Laser Sintering (DMLS), as well as laser cutting, laser ablation, etching, electroplating, hot embossing, and metal injection moulding (MIM). Table 1.1 provides a summary of these techniques. 3D printing techniques such as DMLS and stereolithography (SLA) are particularly advantageous as it allows for versatility and customisation in manufacturing, along with the ability to produce highly accurate and precise complex structures (with dimensional fidelity within $\pm 5\%$ of desired dimensions). It also enables rapid modification of key properties that influence MN performance. Despite their many benefits, MNs produced via 3D printing techniques like DMLS and SLA have drawbacks, including potentially high production costs, limitations in the materials that can be used which may affect biocompatibility, and challenges in achieving uniform drug loading and release profiles across batches.

1.4.2.1 Direct Metal Laser Sintering (DMLS) for 3D Printed MNs

Direct metal laser sintering (DMLS) is an additive manufacturing technique for the fabrication of metallic parts. DMLS is a power bed fusion technology that can combine this single fabrication step with the ability to produce three- dimensional parts [79]. It is one of the most widely used technique due to the accuracy in the geometry, low cost and the ability

to provide parts close to the shape and aspect ratio of the final product (near net shaped parts) [80]. This technique has already been used in medical applications, including for orthopaedic and dental applications [81,82]. The process uses a high power laser beam in order to melt together different layers of powder to a required layer thickness as specified in the computer-aided design data [80]. Krieger *et al.* published the first article on the fabrication of 316L stainless steel MN with the use of DMLS technology followed by electropolishing [83]. In this study, they developed 3D-printed dry MN electrodes, 5x5 MN arrays, for measurement of electrical muscle activity in humans. This study presented high quality surface electromyography signals, with reduced electrode–skin contact resistance about 63%, showing the potential of these MN electrodes to be used for biosignal recording. In terms of mechanical properties, Krieger *et al.* [77,83] used uniaxial compressive stress testing and Euler buckling analysis to evaluate the structural integrity of these MNs. For example, the printed MNs maintained mechanical robustness and did not exhibit tip fractures under the applied forces, which is critical for their reliable performance in skin penetration applications. The optimisation of the 3D printing process, including post-processing steps like electropolishing, enhanced the tip sharpness and overall smoothness of the needles, further improving their mechanical performance and user comfort during application.

1.4.2.2 Stereolithography (SLA) for 3D Printed MNs

Stereolithography (SLA) is the most rapid, low cost additive manufacturing technique that is able to fabricate specimens from different materials, layer-by-layer, from a computer-aided design (CAD) file. Stereolithography, as well as micro-stereolithography (μ SL) are capable of fabricating 3D micro-sized structures with complex geometries for various biomedical applications (e.g. MNs, cardiovascular stents, tissue scaffolds, etc.) [84]. Usually, the stereolithography apparatus includes a resin tank, a laser source for the solidification of the resin with scanning mirrors, that direct the laser beam, a movable build platform in which the

model is produced and an elevator to move the build platform. After solidification of the first layer, the platform lowers into the resin tank and the next layer of the part is then solidified on top. The same process is repeated until the production of the final designed 3D product [85]. SLA is capable of achieving the small dimensions (ranging between 0.08 mm to 1 mm) required for MN array fabrication. Economidou *et al.* successfully fabricated different MN geometries (i.e. conical, pyramid and flat spear shape) using SLA [86,87]. The optimisation of this 3D printing process led to the fabrication of MNs with a minimum applied force for skin penetration within the range of 2-5 N, with the highest force needed for flat spear-shaped and the lowest force for conical MN arrays.

In a recent study, two different shaped needle structures, i.e. pyramidal and flattened needles, were fabricated using wet etching for the determination of the mechanical characteristics [88]. The height and pitch of both needle types were 120–250 μm and 230–280 μm , respectively. The compression strength required for the penetration was approx. 40 mN for individual MNs and 10 N for a total array of 160 needles. Cahill *et al.* fabricated 316L stainless steel porous MNs with 36% of porosity using hot embossing of a patch of stainless steel-based feedstock. After fabrication, the MN were sintered at 1100 °C and electropolished [19]. Due to the porosity of 36% the MNs were able to wick-up $27 \pm 5 \mu\text{L}$ of fluid through capillary action. The drug delivery performance of the porous 316L stainless steel MNs ($22.4 \pm 4.9 \text{ mg/cm}^2$) appeared to be three times higher than the topical administration ($7.1 \pm 4.3 \text{ mg/cm}^2$). Furthermore, the metallic porous patches were able to penetrate porcine skin under a load of 19 N without leading to a failure despite the existence of porous. Porous stainless steel MNs using the hot embossing process were also developed by Ullhan *et al.* and were coated with a thin polymer layer achieving porcine skin penetrate without fracturing, delivering calcein dye to a depth of 750 μm [89]. Comparing the coated with non-coated MNs a significant improvement in mechanical properties was observed, with the failure force reaching the 25 N.

In addition to mechanical properties the porous polymer-coated stainless steel MNs were able to store higher amount of rhodamine B and lidocaine 5 to 25-fold higher drug delivery

1.4.2.3 MN Design Criteria

Several important design criteria require consideration when designing and fabricating metallic MNs. The material that is used for the MN fabrication plays a significant role in the fracture properties of the MNs and their ease of insertion into the skin. The geometric characteristics of individual MNs and MN arrays have a significant effect on their mechanical and penetration properties, in particular the height, diameter, thickness, aspect ratio and the shape of the MNs (summarised in Table 1.2) [90]. The needle height controls the depth to which a therapeutic agent may be delivered while the aspect ratio of the needle influences ease of insertion and mechanical integrity [77]. The tip diameter is also a key parameter influencing MN skin insertion. The penetration properties are also affected by the number of vertices of the individual MNs [91].

Moreover, the MN design can affect the delivery of therapeutic agents *in vivo* and the pain experienced by the patient during insertion. The increase of MN height can improve the drug loading, but this can be more painful during the insertion in the human body. Factors such as MN length, tip angle, thickness and width can also affect the insertion pain. Longer MNs can penetrate deeper into the skin, which might be necessary for effective delivery of some therapeutic agents into the dermal layers. However, increased length can also make the insertion process more painful or uncomfortable for the patient. The angle of the MN tip affects how easily the needle can penetrate the skin. A sharper angle can reduce the force required for skin penetration, potentially decreasing the pain experienced during insertion. Conversely, a blunter angle might require more force, increasing discomfort. Thicker needles might provide more durability and less risk of breaking upon insertion, but they could also increase the sensation of pain because they displace more skin and tissue. Often related to

thickness, width refers to the overall girth of the MN. Wider needles might enhance the delivery rate of the drug by creating larger microchannels in the skin, but they can also intensify the pain due to greater tissue displacement during insertion. Gill *et al.* [92] examined the effect of MN design on the pain levels experienced by human volunteers. Geometric characteristics, such as MN length, tip angle and thickness and width, were tested using different MN dimensions. The study showed that MNs with a length between 450 μm and 700 μm did not cause any pain for 100% volunteers tested and 90% of volunteers experienced pain when the MN length was increased to 1,450 μm . For MNs with a length of 480 μm - increasing the tip angle from 20° to 90° led to a painless penetration. Furthermore, there was no significant ($p\text{-value} > 0.05$) difference in pain levels reported when changing the MN width (from 160 μm to 465 μm) for solid MNs or the MN thickness (30 μm to 100 μm) for hollow MNs [93]. However, the array pattern presented influenced the final characteristics in terms their application. For instance, a 5-MN arrays reduced the pain levels by 10% compared with the pain experienced from hypodermic needle insertion while 50-MN arrays show 10 times higher levels of pain (which corresponded to 25% of the pain associated with hypodermic needle insertion) [92].

The base diameter of the MN is considered as another factor that can affect both mechanical and penetration properties. The increase of the base diameter leads to higher penetration forces, which can cause the failure of the needle during insertion into the skin. According to a study that investigated the effect of the base diameter on the efficiency of the drug delivery, TDD improvement was observed when the MN base diameter was increased from 40 μm to 125 μm [94]. Consequently, increasing the base diameter led to increased mechanical stability. Conversely, keeping the base diameter constant and increasing the MN height makes them more likely to fail due to buckling, leading to a more ductile behaviour. This buckling failure can be further explained through the Euler's critical load, which indicates that the

buckling load for a column is inversely proportional to the square of its length, making taller MNs inherently less stable under compression. Even though a larger base diameter leads to a MN array exhibiting a higher surface for drug coating, many studies have shown that the skin permeability is not affected significantly by changing the MN geometries [95], [96]. Moreover, the MN thickness is related to mechanical strength, in the same way as the insertion force, since the margin of safety (*i.e.* ratio between MN failure due to buckling and skin penetration force) reaches the highest values with large MN thickness. Similarly, with the base diameter, the thickness does not affect the drug permeability [92].

Also, the needle-to-needle distance, number of MNs within the array and array pattern influence the penetration force [97]. Furthermore, the penetration force is also affected by the total number of MNs combined with the parameters such as needle-to-needle distance, with the increase of the needle-to-needle distance leading to lower forces for skin penetration [95], [98]. The needle-to-needle distance is closely connected with the array pattern. The array pattern refers to the arrangement of the MNs within the patch, e.g., triangular, square and hexagonal MN array patterns [96]. The MN array pattern is one of the main factors that affects skin permeability. Studies have shown that the hexagonal pattern demonstrates the greatest skin permeability when compared to the square or triangular pattern arrangements. While a square pattern has a greater interface area that results in higher diffusion of the drug into the skin [99].

The recent development of porous MNs has shown potential for enhanced TDD [74]. Further optimisation of these MNs is required to achieve a high volume of pores while maintaining the mechanical integrity of the MN. Until recently, porous MNs have attracted less attention due to their complex fabrication processes used for their manufacture. However, recent advances in additive manufacturing offer great potential for manufacture of porous metallic

MNs, whereby the printing parameters can be more easily adjusted to provide the desired porous properties to the final part [100].

Table 1.1: Overview of techniques used for MNs fabrication.

Fabrication Technique	Types of MNs	Material used	Key geometric features	Advantages	Limitations	References
Laser cutting	Solid, hollow	Stainless steel	Height: 700 μm , width: 200 μm Outer and inner diameter of 50 μm and 20 μm Height >10 μm	Mass productivity, Low cost	Post-processing (<i>i.e.</i> electropolishing) is required (poor surface finishing) Required thin metallic sheet,	[101–103]
Laser ablation	Solid	Stainless steel Tantalum	Tip diameter: 0.3-0.5 μm Aspect ratio: 1-4.5 Thickness: 2.5-10 μm	Fast	Might cause cracks in the final structure Chemical contamination	[104,105]
Etching	Solid Hollow	Titanium Nickel	Height: 120-250 μm Pitch: 230-280 μm	Simple process, Controllable etching rate	High cost	[69,106,107]
Electroplating	Solid Hollow	Palladium Copper	Height < 500 μm Base diameter: 100-250 μm	Controlled thickness and the deposition rate	High cost	[108,109]
Hot embossing	Solid	Stainless steel Titanium	Porosity: d_{90} :1.56 - 2.93 μm Height >300 μm Tip: 30-90 μm	Mass production, Cost effective, Complex parts	Multi-step process	[19,54]
Metal injection moulding (MIM)	Solid	Stainless steel Titanium	Porosity: d_{50} : 1.3 μm Height: 460 $\pm$ 40 μm Tip diameter: 20 $\pm$ 4 μm	Mass production	Multi-step process	[20]
DMLS	Solid	Stainless steel Titanium	Height: 250-700 μm Tip radius <50 μm Resolution > 0.5 mm	Single fabrication step, Near net shaped parts, Mass production	High cost, Post-processing is required	[21,79,80,83]

Table 1.2: Overview of the geometrical characteristic affect the MN arrays performance.

Geometrical Characteristics	Mechanical Strength	Skin Insertion	Skin Permeability	Pain Levels	Drug Delivery	References
Needle length	The increase of needle length ($> 1000 \mu\text{m}$) can reduce the capacity of MNs to resist buckling.	Increasing the needle length increases the risk of bleeding during the insertion. Increasing the needle height ($300\text{-}900 \mu\text{m}$) lead to increase of penetration depth.	Increased of needle length enhances the skin permeability.	Increasing the MN length can increase the pain levels during penetration by reaching the pain receptors.	Increase of MN length can improve the drug release.	[92,93]
Needle tip diameter/angle	Greater MN tip diameter increase the margin of safety (<i>i.e.</i> ratio between fracture force and insertion force). Greater needle tip diameter increases the mechanical strength.	The small tip diameter improves the skin penetration leading to easier skin insertion	N/A	Sharper tips lead to decrease of pain.	N/A	[69,92,95,110]
Needle base Diameter	Increasing the base diameter (approx. $>20 \text{ mm}$) led to increased mechanical stability.	Increasing the base diameter led to the increase of the penetration depth due to enhanced force distribution, reducing the risk of failure and enabling deeper penetration.	Increasing the base diameter led to effective skin permeability.	Increasing the base diameter can cause increase of pain.	Increasing the base diameter ($40 \mu\text{m}$ to $125 \mu\text{m}$) lead to improvement of TDD by increasing the drug coating.	[92,95,111]

Needle thickness	Greater MN thickness led to greater margin of safety. Fracture force increases with the increase of thickness	Increasing the thickness led to limited and more difficult skin insertion.	Increasing the thickness enhanced the effectiveness skin permeability.	Increasing the needle thickness for both solid and hollow increased the pain levels.	N/A	[69,92,95]
Aspect ratio (height: base)	High aspect ratio led to buckling of the needles. Increasing the aspect ratio led to decrease of mechanical strength. Stiffness increased with the decrease of aspect ratio.	Increase of aspect ratio increased the penetration depth. Low aspect ratio limited the skin penetration.	Lower aspect ratio limits the skin permeability,	N/A	Increased aspect ratio led to increase of drug loading and release, due to higher surface area.	[95,110]
Array pattern	Increasing the needle-to-needle distance reduce the force during the penetration. Increasing the needle vertices (3 to 6 vertices) the needles can withstand higher compressive loads.	Increasing the needle-to-needle distance (30-600 μm) lead to increase of the penetration depth.	Increasing the needle vertices to 6 increases the skin permeability.	Increasing the number of MNs in the array lead to increase of pain.	N/A	[92,93,95,112]

1.4.3. Applications of MNs

Each of the different types of MNs previously described (*i.e.*, solid, hollow, dissolving and coated) are fabricated from a range of different materials and have been used in drug delivery applications (e.g., insulin, growth hormones), vaccines, genes, ribonucleic acid (RNA) and proteins [12]. In biomedical applications, MNs have been used in diabetes treatment, cancer therapy and diagnosis, the treatment of chronic pain, the treatment and management of obesity and for other applications [73].

1.4.3.1 MNs in Diabetes Management

The demand for therapies such as insulin is increasing among the adult population (9% of diabetic patients) according to the World Health Organisation, with diabetes projected to be the 7th leading cause of death by 2030, with an estimated 439 million cases [113]. Diabetes mellitus is a group of metabolic diseases characterised by hyper or hypoglycaemia (approximately 250 million diabetic people) due to disorders in insulin secretion, action or both [114]. Currently, the treatment of diabetes involves the use of insulin delivery pumps or pens, insulin therapy, syringes, jet injectors and hypodermic needles to provide transdermal delivery of a bolus delivery of insulin [115]. These methods of insulin delivery pose challenges in terms of insulin administration and can lead to poor clinical outcomes [116]. Manual techniques involve human error and patient noncompliance, which can prevent optimum and accurate insulin administration from being achieved. Furthermore, injections during self-administration lead to pain [117]. A further challenge relating to insulin administration relates to the size of insulin which is a large drug molecule (*i.e.*, molecular weight of 5,808 Daltons and contains 51 amino acids) and it is difficult to deliver using TDD systems. In addition, insulin has a negative charge at pH 7.4 (*i.e.*, anionic cargo) and as a result insulin release into the systemic circulation is slow and has poor bioavailability and

cellular uptake. Limitations relating to insulin release can lead to poor glucose control in diabetic patients causing high risk complications [10].

Therefore, due to the low accuracy and high risks of traditional insulin administration methods, the development of new efficient and precise insulin delivery systems is required. The development of “smart” systems capable of combining both diagnosis and therapy of Type 1 and Type 2 diabetes in response to body signals has provided a distinct advantage for patients suffering from the disease [117]. MNs offered the potential to deliver insulin and at the same time measure blood glucose levels, as such these “smart” systems have been an attractive solution for insulin delivery. Specifically, the MN-based arrays are capable of carrying and delivering insulin molecules, significantly reducing tissue trauma and pain [118].

Several studies have shown the potential of MNs to overcome the insulin administration challenges that occur using traditional methods [117]. In one such system, a MN array loaded with the diabetes therapy, exendin-4, was fabricated from hyaluronic acid using micromoulding. This system allows penetration of the skin without causing any skin damage, achieving accurate and rapid delivery [118]. Yu *et al.* [117] developed a glucose-responsive MN array containing glucose-responsive vesicles (*i.e.*, self-assembled from hypoxia-sensitive hyaluronic acid) that acts as a closed-loop system to mimic the function of pancreatic cells by combining the diagnosis and therapy of diabetes. This system provides a potentially painless way to prevent hyperglycaemia or hypoglycaemia and improve the health and life of Type 1 diabetic patients [117]. In a further study, a portable electrochemical device for Type 2 diabetes therapy with the capability to translate real-time data and predict glucose levels was developed. Bioresorbable polymer-based MNs, coated with the required drug, release the drug into the bloodstream when the programmed threshold temperature is exceeded. A reduction in blood glucose levels in diabetic mice was demonstrated using this device [119]. A dual mineralised MN array patch, consisting of exendin-4 and glucose oxidase, enhanced

skin penetration to 100% due to the strong crosslinking of alginate-based MN arrays [120]. The study showed that painless and non-invasive TDD could be achieved making it an attractive candidate for long term, safe and on-demand therapy. However, an incomplete insertion of the MN array into the skin using this system was observed. Finally, dissolvable starch and gelatin MN arrays have also been developed for the release of the encapsulated insulin, leading to complete dissolution within 5 min of insertion into the skin. This research confirmed the stability of the encapsulated insulin and great potential for its TDD in a relatively painless, rapid and convenient manner [121].

However, despite their benefits, the use of these MN arrays presents challenges, as these substances are quite soft with low modulus and strength, leading to failure upon insertion, limiting ability of the MN arrays to fully penetrate the skin without plastic deformation on the tip. Therefore, research efforts have focused on enhancing the penetration properties and the tip sharpness or failure forces of the MNs to improve insulin delivery. For instance, Invernale *et al.* fabricated platinum-coated 316 L stainless steel 2D MN arrays with a length of 680 μm and width of 250 μm for continuous glucose monitoring [122]. The MNs penetrated the skin without fracture of the needle and achieved continuous monitoring and the reduction of glucose levels for up to 7 days. Although cytotoxicity and biocompatibility are among the greatest issues facing such MN-based sensors, the proposed technology demonstrated no significant negative impact on cell viability. Finite element models have also been applied to further investigate the performance of the metallic 2D MN arrays. In a further study, a titanium hollow patch, consisting of needles with a length of 500 μm and a tip diameter of 75 μm , achieved pain-free administration and continuous drug delivery in a diabetic rat model [123]. The MNs showed sufficient strength to pierce the skin without breaking, improving the glucose levels by 50% within 4 h with a total of 0.43 ng/mL of insulin being released. An *in vivo* study, conducted by Martanto *et al.* in a diabetic rat model, investigated TDD of insulin

using laser-cut solid stainless steel MNs [124]. The MNs demonstrated full penetration into the rat skin with high skin permeability to insulin. The decrease in blood glucose achieved was similar to that from subcutaneous insulin injection (e.g., 0.05-0.5 U) with 0.5-7.4 ng/mL insulin concentration. High insulin concentration with shorter insertion time was obtained within increased transdermal insulin delivery and lower blood glucose levels by as much as 80%. Porous titanium MNs have also shown promise for insulin delivery [125]. The force-displacement graph during the penetration in the pigskin (with a thickness of 1-2 mm) indicated that full penetration was achieved with a force of only ~3 N required without the fracture of the tip. Successful and efficient insulin delivery was achieved in a diabetic rat model within 2 h, decreasing the blood glucose levels to normal values.

The MNs described here have shown an ability to provide continuous drug delivery without causing any pain compared to conventional hypodermic needles. Polymeric-based MNs have difficulty penetrating the *stratum corneum* and they have a high risk of needle breakage [121]. Mineralised MNs, formed by the chemical crosslinking of the polymer, have limitations relating to the toxic traces from the crosslinking process, which can potentially lead to skin irritation or infections [126]. However, the limitations related to the penetration of the *stratum corneum* barrier and the amount of drug encapsulation that can be achieved has shown an improvement with the use of metallic MNs [127]. Overall, despite the extensive research in the field of MN technology for the treatment of diabetes, further research is required to enhance their functionality and the control of release behaviour.

1.4.3.2 MNs for Obesity Treatment

According to the World Health Organisation in 2016, more than 1.9 billion adults aged 18 years and older were overweight [128]. Of these, over 650 million adults were obese. The number of incidents increased in 2019, with an estimated 38.2 million children under the age of 5 years being overweight or obese [128]. Obesity is highly connected with several diseases,

namely leading to a high risk of diabetes and cancer, which are responsible for 20% of deaths in adults [129]. Different factors influence the development of obesity, such as environmental and genetic factors, as well as an imbalance between calorie consumption and energy expenditure [130].

Current approaches towards long-term weight management for patients suffering from obesity include diet, physical exercise, pharmacological therapy and surgical approaches. However, these current treatments are associated with low effectiveness or undesired systemic side effects. Many studies have been focused on the use of surgical treatment approaches, which involve invasive procedures and have high risks. For instance, the modification of the anatomy of the gastrointestinal tract can lead to a decrease in food absorption and consumption [131]. Inflammation, infection, serious morbidity or even death can also result from using surgical approaches. Other methods involve the use of pharmacological agents, which have the benefit of not requiring invasive procedures. However, currently, these pharmaceutical approaches include limitations, leading to inefficient results, undesired side effects on human organs such as gastro-intestine, liver and kidney and limited applications [132].

There is a clear need for new approaches that can effectively treat obesity while overcoming the significant risks arising from the traditional treatment approaches. MN-based arrays have potential as minimally invasive devices that could provide an effective approach for the treatment and control of obesity. The MN-based arrays could provide a localised, convenient, and painless administration method for pharmacological agents for the treatment of obesity. This approach would also allow the side effects relating to current surgical procedures to be avoided. The MNs penetrate only the upper layer of the skin, minimising trauma, lowering infection risks, and eliminating the need for recovery time—contrasting with the risks of surgical procedures that require incisions and anaesthesia, which can be

particularly risky for overweight or obese patients. The simplicity and non-invasiveness of MN-based arrays not only facilitate easier and broader accessibility, potentially even from home, but also improve patient compliance by avoiding the daunting nature of surgery, thus reducing psychological barriers and anxiety associated with more invasive obesity treatments. A recent study reported the development of hyaluronic acid-based dissolving MN arrays for the TDD of caffeine for the treatment of obesity [133]. The results indicated the significant improvement of lipolysis resulting in a considerable weight loss leading to the $12.8 \pm 0.75\%$ reduction of obesity using the caffeine loaded dissolving MNs three times per week in obese C57BL/6J mice [133]. A further study reported the use of dissolvable MN-based arrays encapsulating rosiglitazone nanoparticles for the delivery of browning reagents, for the long term management of weight [134]. The browning reagents are capable of turning energy from food into heat in the body in a safe and effective manner for the inhibition of adipocyte hypertrophy improving the metabolism action. The delivery of browning agents reduced the inguinal fat pad of the obese mice during a four-week treatment achieving approx. 100% normalisation of the body weight and glucose levels to ~ 110 mg/dL from 140 mg/dL [134]. The delivery of these agents *via* MN arrays is just one of the methods that could be used however there are other ways such as oral administration and injections but come with their own sets of challenges and benefits like drug degradation and poor absorption, leading to possible systemic side effects, be painful and require medical supervision. In addition to obesity treatment, this TDD approach led to improved insulin sensitivity having a positive effect not only in the fight against obesity but also against diabetes. Hyaluronic acid-based dissolvable MN patches (10×10 arrays) with a height of 600 μm have been used for the TDD of the anti-obesity compounds (*i.e.*, $\beta 3$ -adrenoceptor agonist and the thyroid hormone, triiodothyronine) to white adipose tissue (WAT) to increase energy expenditure and transform the calorie-storing white fat into calorie-burning brown fat [135]. The optimal MNs required

a lower effective dosage compared to systemic administration with only 5 mg at day 1, resulting in a reduction of the side effects associated with the over-activation of β 3-adrenoceptors, such as increased heart rate and blood pressure. These dissolving MNs effectively promoted WAT browning and suppressed body fat and weight gain in a diet-induced obese mouse model, without the need for daily administration since a slow release of β 3-adrenoceptor agonist can be achieved [135].

In a further study, An *et al.* developed dissolvable MNs composed of the natural polymer gelatin for the treatment of obesity [136]. The gelatin-based MNs showed a reduction of local subcutaneous fat by up to 60% compared to non-treated control samples, demonstrating the direct effects of gelatin on the fat accumulation at the applied body part [136]. This was considered as an effective and simple method to target the local reduction of subcutaneous adipose tissue.

1.4.3.3 MNs in Cancer Diagnosis and Treatment

Cancer is considered a major public health problem. According to GLOBOCAN data, 18 million of the world population were diagnosed with cancer prior to 2018, with 0.5 million new cases in 2018 [137]. While cancer vaccination has been demonstrating promising anticancer results, it is predicted that by 2040 cancer incidence will have increased by 11.4 million new cases worldwide in total [73]. Traditional cancer treatment includes surgery, chemotherapy and radiotherapy approaches, which often lead to side effects (e.g. drowsiness, exhaustion, vomiting, etc.), acute toxicity or tumour recurrence [11]. An appealing solution would be the use of a controllable and easily applicable minimal invasive process (*i.e.*, MN-based arrays) for immunotherapy and gene-based therapy for both cancer vaccination and treatment [11].

The treatment of cancer using MN arrays has garnered significant attention by the scientific community, resulting in many studies being conducted on the application of MN patches containing immunostimulatory adjuvants and/or antigens as anticancer therapeutic approaches capable of achieving the clinical requirements. For instance, drug-loaded dissolving MN patches comprised of hyaluronic acid have been developed for the treatment of multiple tumours and the delivery of long-term treatments [138]. These MNs were shown to be light-activated without affecting the structure of the MN array, or their ability to penetrate the skin, able to achieve highly controllable on-demand delivery and to dissolve after the release of cargoes within the tumour site. These dissolvable MN arrays present a promising platform for the treatment of superficial skin tumours providing safety, effectiveness, good handling properties, low-toxicity and a minimally invasive approach [139]. Ye *et al.* reported the use of dissolvable hyaluronic acid-based MN arrays for the delivery of immunotherapy for the treatment of melanoma [140]. Maaden *et al.* used fused silica hollow MNs for the delivery of a therapeutic cancer vaccine, achieving stronger functional cytotoxic and T-helper responses in mice, while requiring lower volumes compared to traditional intradermal immunisation [141].

Chablani *et al.* developed metallic MNs, 1,100 μm in length, for the treatment of breast cancer [142]. These metallic patches demonstrated the successful delivery of a particulate breast cancer vaccine through 50 μm holes created in the skin. *In vivo* assessment using a mouse model showed five times more tumour suppression in vaccinated animals, confirming successful immune response activation and protection. The same metallic MN array were used for the transdermal delivery of an ovarian cancer vaccine by Tawde *et al.*, achieving high tumour suppression [143]. The use of coated metallic MNs for the improved delivery of a skin cancer drug has also been reported [55]. These MN arrays significantly suppressed tumour growth with a delivery efficiency of 90% within 5 min. Gill *et al.* fabricated and

investigated coated stainless steel MNs for the delivery of different proteins (e.g., calcein, vitamin B, etc.) and deoxyribonucleic acid (DNA). The coating was absorbed within 20 s, achieving a controllable and rapid delivery into the skin [144].

MN arrays are highly advantageous for delivering therapeutic agents due to their ability to target the immune-rich dermal layer for enhanced response, provide a minimally invasive and less painful alternative to traditional injections, improve bioavailability by bypassing gastrointestinal and liver metabolism, simplify administration for greater accessibility and patient compliance, and stabilise sensitive agents without the need for cold storage, thus facilitating distribution. Overall, due to these attributes, the use of MN array for immunotherapy, gene-based therapy and vaccine administration can be considered as a highly attractive approach, which requires further investigation.

1.4.3.4 MNs in Chronic Pain Management

Pain, acute and chronic, is the main reason for daily suffering for approximately 1.5 billion people, regardless of age [11]. Sources of pain are commonly the result of trauma, infections, inflammation, tumours, metabolic disease or endocrine diseases [143]. Acute pain is usually associated with an injury and has a short duration, whereas pain that persists for a longer duration, e.g., greater than three months is considered chronic. The conventional method for the delivery of analgesics for the temporary relief of acute and chronic pain is through oral administration or/and injection. The use of MN patches provides a promising technology for pain management offering additional benefits over the conventional methods, including a reduced risk of systemic side effects. According to Xie *et al.* [145] the fabrication of dissolvable MNs made of sodium carboxymethyl cellulose (molecular weight ~90,000) has been shown to be safe and effective for the local delivery of anti-calcitonin gene-related peptide (CGRP) that produces selective anti-hypersensitivity through antagonism of peripheral CGRP receptors for the treatment of neuropathic pain. In these studies, the use of

the MNs resulted in significant pain relief without skin irritations in contrast with traditional treatments, while avoiding the side effects associated with systemic exposure [146]. Lidocaine works as an analgesic for both chronic and acute pain, which is administrable via injection or non-invasive TDD patch. The topical delivery of lidocaine using non-invasive TDD patches is less efficient than traditional injections due to the low drug permeation (*i.e.*, the undesired delay of drug release) and the variability of drug absorption among individuals. Hence, to address the clinical problem, a solid drug-loaded integrated transdermal polymer-based MN patch was developed. Briefly, poly(ethylene glycol) diacrylate, (PEGDA ($M_n = 258$)), 2-hydroxy-2-methyl-propiophenone (HMP) were mixed and exposed to a high-power UV light source leading to a significantly higher amount of drug encapsulation and a faster onset of drug permeation compared to commercial patches. These MNs demonstrated an appropriate release and delivery of the active ingredient (lidocaine) for the relief of acute and chronic pain [147]. The development and use of a photo-triggered dissolvable polycaprolactone (PCL)/poly(l-lactide-co-d,l-lactide) (PLA) MN array for the on-demand lidocaine delivery has also been reported [148]. Specifically, more convenient, comfortable and effective pain control than standard injection was achieved with the use of this system, with rapid absorption into the blood circulation and high bioavailability (95%) reported.

1.4.3.5 Other Applications

MNs have also been extensively studied for several other biomedical applications, including blood and interstitial fluid sampling and for biosensing applications. MNs can be used for blood sampling since the blood capillaries are presented under the *dermis* at a depth of 400 μm . However, the tips of the nerves are in a similar depth and thus some of the MNs in the array might graze the uppermost nerve cells [10]. A study exploring the effect of MN length on pain using a 26-gauge hypodermic needle reported that shorter MNs measuring

480 μm were associated with lower pain scores at 5% (refer to the percentage of participants reporting pain at certain levels), while longer needles up to 1450 μm led to higher pain scores up to 40%. [92] and thus minimising the MN length is important to result in a minimum of pain. The use of MNs for blood sampling resulted in a reduction of pain by up to eight times when compared to conventional hypodermic needles [149]. In order to extract blood, MNs require mechanical and penetration properties that can achieve skin penetration without buckling. For example, an average force value for human skin puncture is 6.0 N, it is 2.0 N for subcutaneous fat tissue and 4.4 N for muscles and therefore metallic MNs are usually used [150]. These MNs require a large inner diameter (40 μm -125 μm) to allow the largest blood cells to pass while at the same time preventing the risk of failure due to buckling [95]. Many factors can affect the process of the blood extraction, such as the fluid density, the MN diameter and the material. The use of a microfluidic pumping device can improve the process as well as the capillary action, a natural action, which is enough for the blood extraction process [10]. Kawanaka *et al.* developed titanium MNs (inner diameter 100 μm) with a vacuum chamber for blood extraction [151]. The results show a good volumetric efficiency up to 90%. Another study presented stainless steel MNs (outer diameter 210-250 μm) as a blood/interstitial fluid extraction system for pharmacokinetic studies [152]. The *in vivo* studies on mice presented the ability of the MNs (inner diameter 115 μm) to successfully collect the blood through capillary action within a 1 μL steel reservoir.

Similarly, for the sampling of interstitial fluids, the MNs have to penetrate the *stratum corneum* and reach the *dermis* layer where the blood vessels are [153]. The sampling can be achieved through capillary action combined with a pumping device, however this method is not cost-effective due to the price of the device [10]. While, for the MNs, the capillary forces enhance the hydrophilicity and help with the extraction of the fluids leading to faster and easier blood extraction without the use of any device that makes it cost effective [152]. Mishra

et al. tested MNs with 500 μm height and 40 μm inner diameter for the optimisation of the hollow MNs design [154]. The flow rate was tested resulting in 0.93 $\mu\text{L/s}$ through the MN lumen [154]. In contrast, Kim *et al.* developed hollow metallic 10×10 MN arrays with a height of 400 μm and smaller inner diameter of 20 μm for the extraction of interstitial fluids [155]. These type of MN arrays delivered the fluids in a controlled manner with a fluid flow rate of 0.69 $\mu\text{L/s}$. For interstitial fluid extraction, the inner diameter of the MNs plays a significant role, whereby decreasing the diameter led to a decrease of the flow rate through the MN.

In addition, MNs have been used as biosensors providing the ability for minimally invasive measurements of biological or chemical reactions and monitoring. Metallic-based solid, hollow and solid porous MNs made by stainless steel, platinum, carbon and gold have been fabricated as biosensors [156]. Biosensors are assembled using hollow MN by placing wires of platinum, silver or carbons inside the channel of hollow needles. On the other hand, solid MNs have been moulded using the necessary biological recognition elements [156]. Cahill *et al.* fabricated porous 316 L stainless steel MNs designed for the storage, delivery and absorption of fluids inside the porous network for biosensing [157]. These MN arrays were fabricated using hot embossing of the patch followed by sintering at 1100 $^{\circ}\text{C}$ and electropolishing at 2 A and 10 V for 1 min. They achieved porosity up to 36% with average pore diameter of 2.22 μm and 27 ± 5 μL of fluid was able to wick up through capillary action.

Another challenging application is the accessibility of the inner ear for the delivery of drugs due to the fluids that exist in the cavity within the temporal bone. The only way to reach the inner ear without the breakage of bone is the round window membrane (RWM) in the middle ear. Studies have explored the use of polymeric MNs, with a tip diameter of 500 nm, for the delivery of drugs for this challenging application [158]. Due to the risk of the tip/needle failure because of the lack of mechanical strength (~ 0.2 N/needle, typical failure force under axial

load [159]) of the polymeric materials, further research has led to the development of gold-coated copper-based MNs for the penetration of the RWM [160]. In this study, MNs with a height of 430 μm and tip radius of 1.5 μm were fabricated using an electrodeposition process. The ultra-sharp MNs were tested in guinea pigs and achieved a mean perforation force (*i.e.*, maximum force that is exerted on the needles during the indentation) of 3.8 ± 0.3 mN. While a minimal degree of trauma was observed since the shaft diameter was 100 μm without introducing uncontrolled ripping of the RWM and at the same time, the MNs remained undamaged.

1.5. Summary

Considering the need and the disadvantages of the existing TDD systems for the treatment and diagnosis of different diseases and incidence, a micro-sized device presents significant potential as a painless minimal invasive device. Several TDD systems have been developed (*e.g.*, conventional hypodermic needles, oral administration, topical creams and non-invasive transdermal patches) to date, to overcome these drawbacks. However, these systems still lack the ability to overcome the main challenge, which is painless penetration without the fracture of the device and at the same time, controlled and continuous or rapid drug release. MN arrays have the potential to offer advantageous properties including good bioavailability, enhanced skin permeability [53] without skin irritation or allergic reactions, while at the same time combining the requirement for painless application and controlled drug delivery.

To date, a range of such devices have been investigated for use in the treatment of several diseases (*e.g.*, treatment of diabetes, cancer diagnosis and therapy, chronic pain treatment and control/treatment of obesity). However, these studies have been mainly focused on polymeric and dissolvable needles. For instance, studies reported the use of polymeric MNs for the diagnosis and therapy of Type 1 and Type 2 diabetes [161,162] and for the treatment of multiple tumours as anticancer therapeutic approaches [53]. Although, these polymeric MNs

offer a significant advantage as they can function as painless TDD systems, they have shown difficulties in penetrating the skin, insufficient biocompatibility and poor drug loading capacity. Currently, the scientific knowledge and understanding of the fabrication method required and the design rules associated with mechanical properties and geometrical characteristics is limited and considerable research efforts are being invested.

Initially, investigation demonstrated that the development of polymer-based MNs obtain poor mechanical properties with forces that lead to tip fracture before skin penetration. Metallic MNs show potential to overcome these challenges and are able to deliver relatively larger amounts of drugs (e.g., via drug coating or the drug loading of porous MNs) with higher mechanical integrity and piercing strength. In particular, recent studies have shown that the manufacture of solid metallic-based MNs can achieve optimal geometric characteristics for constant and controlled drug delivery [122]. However, since MNs can be manufactured in several shapes and types, MNs with optimal geometric characteristics can combine increased mechanical strength and reduced penetration forces to penetrate the *stratum corneum*, one of the main barriers of the skin. Nevertheless, there are challenges remaining in this area so, further investigations into the ideal properties, such as porosity and density, of the solid metallic-based MN arrays are required. For instance, there is a need for new research in order to achieve optimal mechanical properties as well as drug loading ability and biological performance, and ultimately improved disease treatment and management.

Research development of the metallic MN arrays is focused on exploring its potential as a drug delivery system for the controlled and localised delivery of cells, growth factors and small molecules focusing on the synchronisation of the load and the release of these elements. Another area of research focus relates to the investigation and optimisation of the additive manufacturing process to achieve improved penetration and mechanical properties. Considerable research remains on improving the suboptimal mechanical, physical properties

(e.g., compression strength, bulk modulus and ultimate strain before failure) and drug release rate and/or amount. Therefore, future studies should focus on the understanding of the fundamental principles of AM, such as the enhancement of laser beam or nozzle features, leading to the ultimate resolution of printed MNs [94]. This new knowledge will be very important to ensure the ease of handling, safety and effectiveness of the product(s) developed leading to the successful fabrication of MN arrays and understanding the structure-property-performance relationship.

1.6. Project Aim and Objectives

The current drug delivery systems in use are both painful and limited when it comes to administering large molecules. This PhD research is dedicated to addressing these drawbacks by focusing on the design, fabrication, and performance assessment of 3D printed metal-based MN arrays. The goal is to develop a painless device that enables the transdermal delivery of therapeutic drugs while minimising side effects. By utilising additive manufacturing techniques, micro-sized stainless-steel needle arrays will be created, allowing for effective delivery of drug molecules for diagnosing and treating damaged or diseased tissue. The novelty of this work lies in its systematic optimisation of both the 3D printing and post-processing parameters, using a Design of Experiments (DoE) approach, to achieve MNs with optimal geometrical and mechanical properties suitable for clinical application. Furthermore, the integration of advanced coating techniques has resulted in MNs that offer controlled drug release profiles, significantly improving therapeutic outcomes. This research not only addresses the limitations of existing TDD systems but also aims to the development of MN arrays with unique physical and chemical properties, which make them capable of delivering lipophilic molecules through the skin to various organs, ultimately enhancing the quality of life for patients.

Objectives to achieve the aim of the PhD are:

Objective 1: Analysis and optimisation of 3D printing parameters (laser power, speed and trace width) for the development of optimal solid metal-based MN arrays that are structurally robust and capable of efficient drug delivery to establish the optimal conditions for producing MN arrays with precise geometrical and mechanical properties suitable for painless transdermal drug administration.

Objective 2: Analysis and optimisation of electropolishing parameters (current and time) to enhance the surface quality, drug loading/delivery and penetration performance of solid metal-based MN arrays, thereby reducing skin irritation and improving drug delivery efficiency.

Objective 3: Analytical characterisation of the metal-based MN arrays to assess their functional properties, specifically focusing on drug elution profiles and penetration capabilities to quantify the efficiency of drug release and the depth of penetration into dermal tissue, ensuring optimal design for targeted and effective drug delivery.

Objective 4: Development and assessment of a novel coating method for metal-based MN array to improve drug loading and controlled release profiles and establish an effective technique that ensures uniform coating, enhances drug encapsulation, and facilitates precise drug release rates, improving therapeutic efficacy and patient outcomes.

Chapter 2: Design and Optimisation of the 3D Printing Parameters for the Development of Metal-based Microneedles as Drug Delivery System

2.1. Introduction

Metallic microneedles (MNs) offer a promising alternative to polymeric MNs due to their high mechanical strength, biocompatibility, and the ability to enhance drug flux effectively by penetrating the *stratum corneum* without failure [21,163]. The high drug flux and tensile strength of metals, with steel alloys demonstrating 0.5-10 KJ/m² and 1,000-3,000 MPa, and nickel showing around 100 KJ/m² and 20-500 MPa, make metallic MN arrays highly advantageous. These arrays are produced using additive manufacturing (AM) techniques with medical-grade metals, which not only allows for the optimization of MN array design but also makes their production cost-effective, as evidenced in several studies [13,23,152,155,164]. However, fabricating metallic MN arrays requires consideration of critical design criteria such as mechanical strength, needle height, aspect ratio, and tip diameter, which can be controlled by adjusting printing parameters [165]. These design elements directly impact the performance of MNs, enhancing their skin penetration efficiency, allowing for customizable drug release profiles, increasing drug loading capacities, and facilitating patient-specific treatments. This controlled customization not only improves the therapeutic efficacy but also enhances patient comfort, demonstrating the significant advantages of 3D-printed microneedle arrays in transdermal drug delivery. Therefore, it is necessary to efficiently explore a wide range of printing level combinations and achieve the optimal parameters that ensure precise and reliable MN fabrication.

The most vital factors that affect the mechanical integrity are the height, diameter, thickness, aspect ratio and the shape of the MNs [90]. The needle height controls the depth to which a drug/vaccine may be delivered while the aspect ratio together with tip diameter of the needle influences ease of insertion and mechanical integrity [91]. Moreover, the MN design can affect the delivery of therapeutic agents *in vivo* and the pain experienced by the patient during insertion. The increase of MN height can improve the drug loading, but this can be more

painful during the insertion in the human body [92]. The tip diameter is also a key parameter influencing MN skin insertion. The effect of MN design on the pain levels experienced by human volunteers has been examined by Gill *et al.* [92]. This study showed that MNs with a length between 450 μm and 700 μm did not cause any pain for 100% of volunteers tested and 90% of volunteers experienced pain when the MN length was increased to 1450 μm . For MNs with a length of 480 μm , changing the tip angle from 20° to 90° led to a painless penetration. Furthermore, there was no significant ($p\text{-value} > 0.05$) difference in pain levels reported when changing the MN width of solid MNs (from 160 μm to 465 μm) or the MN wall thickness of hollow (30 μm to 100 μm) [93]. The needle-to-needle distance, number of MNs within the array and array pattern also influence the force required during penetration into the skin [97]. For instance, studies have shown that a square array pattern due to high interface area results in higher diffusion of the drug into the skin. However, the various clinical requirements required for using metallic MN arrays for transdermal drug delivery (TDD) have not yet been achieved and therefore further experimentation is needed [99].

Direct metal laser sintering (DMLS) is an AM technique based on powder bed fusion technology that can combine a single fabrication step with the ability to produce 3D printed parts [79]. It is one of the most widely used techniques due to the accuracy in the geometry, low cost and the ability to provide parts close to the shape and aspect ratio of the final product (near net shaped parts) [80]. This technique has already been used in medical applications, particularly for orthopaedic and dental applications [81,82]. However, the process/property relationships for 3D printed metallic MNs are not well understood, and further optimisation of the process is required to achieve 3D printed metallic MNs that meet necessary clinical requirements. Furthermore, the ability to control the printing resolution can improve the accuracy of the final features.

To provide optimal design control, a Design of Experiments (DoE) (Design Expert V5 Software) was used to optimise the process parameters systematically and efficiently, generating statistically optimal experimental designs with minimal trials.

2.2. Chapter Aim

The chapter aims to understand the fabrication-structure-property relationship of the solid metal-based 3D printing MN arrays. This in-depth analysis is essential to verify its suitability for clinical application as efficient TDD systems. The initial focus was to analyse and optimise the 3D printing parameters offering customisation and cost-efficiency using low-cost raw printing materials while achieving improved accuracy of the MN geometry and surface/structural morphology. The specific objectives of this chapter were the:

- Analytical assessment of the 316 L stainless steel powder that was used for the 3D printing of the metallic MN arrays.
- Determination of the effects of key process parameters (e.g., the laser power, laser speed and trace width) on the geometrical properties, surface morphology and mechanical properties of 3D printed metallic MN arrays using the DoE approach.
- MN geometry and surface/structural morphology optimisation using DoE and validation of DoE studies comparing the optimal predicted values with the actual experimental properties through t-test analysis.

2.3. Material and Methods

2.3.1. DoE Parametric Study

DoE (Design Expert V5 software, Stat-Ease Inc. USA) approach was used to investigate the influence of 3D printing parameters on the geometrical properties, surface morphology and mechanical properties of the MN arrays. A three-level factorial design named Box–Behnken Design (BBD) was used to determine the factor-response relationships, identify the most

significant factors and the optimal levels required to achieve MN arrays with the desired properties. The critical factors for the 3D printing process were laser power, laser speed and trace width (Table 2.1), resulting in 17 experimental runs (Appendix 2.1) consisting of 12 unique design points and five centre points, to assess the repeatability and stability of the process under constant conditions. By conducting multiple experiments at these center points, where all variables are held at their mid-range values, the consistency of the results can be evaluated, ensuring high reliability and precision in the measurement of the printing process's performance. The number of runs represents less than one-half of all possible combinations for three factors. The responses investigated in the study were the tip diameter, needle height, base diameter, surface roughness, porosity and compressive strength. The statistical significance of the terms in each regression equation generated from the DoE for each response were examined using the sequential lack-of-fit and ANOVA, with a p-value < 0.05 deemed statistically significant.

Table 2.1: Factors and their levels used in the BBD for the analysis and optimisation of the printing parameters of MN arrays.

DoE Study	Factors	Levels
3D Printing Process	Laser Power (W)	30, 50, 70
	Laser Speed (mm/sec)	500, 600, 700
	Trace Width (mm)	0.09, 0.11, 0.12

There are two types of optimisations within the DoE: (i) numerical optimisation and (ii) graphical optimisation. For these methods, each response is assigned a level of importance

ranging from the minimal significance (value of 1 or *) to the highest significance (value of 5 or *****) as shown in Table 2.2. In the optimisation process of the MN device, particular emphasis was placed on the tip diameter, base diameter, and length of the needles. These properties were selected based on their critical roles in achieving the dual objectives of ensuring dimensional accuracy and facilitating a painless application. The tip diameter is crucial because it directly affects the skin's penetration ease, and the sensation experienced by the user. A smaller tip diameter is generally associated with less pain upon insertion, which is vital for patient compliance, especially in settings where repeated applications are required. Needle length is critical for ensuring that the MNs penetrate to an optimal depth—deep enough to bypass the stratum corneum (the outermost layer of skin) for effective drug delivery but not so deep as to reach nerve endings or blood vessels, which could cause pain or bleeding. The base diameter influences the structural integrity of the needle. A larger base can provide better support for the needle, reducing the risk of breakage during insertion and withdrawal. However, it must be optimized to not overly increase the footprint of the needle array, which could impact patient comfort. The approach is being applied to fabricate a pain-free MN device while ensuring that its dimensional characteristics meet specific clinical requirements. The numerical optimisation algorithm searches for one or more points within the factor ranges that meet the desired goals. During optimisation, the software identifies the optimal factor levels that satisfy the clinical requirements set for each response. Following optimisation, MN arrays were printed and a percentage error between the predicted and actual results was determined.

Table 2.2: Requirements including acceptable levels for the DoE numerical optimisation as well as the order of importance.

Responses	Requirements	Importance
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Tip diameter [11]	minimise	*****
Needle height [10,11]	maximise	*****
Base diameter [10,11]	~250 μm	***
Surface Roughness [19,20]	minimise	**
Porosity [21]	minimise	**
Compressive strength [22]	maximise	***

2.3.2. Fabrication of MN Arrays: Design and Printing

The aspect ratio of 4:1 for the MNs was selected to balance several critical factors, including printability, penetration ability, and the likelihood of failure during application. Higher aspect ratios, while potentially allowing for deeper penetration, have been found to compromise the printability and structural accuracy during the 3D printing process. Furthermore, MNs with very high aspect ratios are more susceptible to bending or breaking upon insertion into the skin. The chosen aspect ratio of 4:1 ensures a robust structure that can withstand the forces encountered during skin penetration without compromising the ease and reliability of the manufacturing process. [77]. The computer-aided design (CAD) software SolidWorks (DS SolidWorks Corp., USA) was used to create conical solid MNs with a base cross-sectional diameter of 250 μm and a length of 1,000 μm . The MNs were arranged in 6 x 6 arrays on a solid square patch of 10 $\times$ 10 $\times$ 1 mm, leading to 36 MNs per patch (Figure 2.1a). The MN arrays were fabricated using 316L stainless steel (Concept Laser GmbH, GE Additive, Bressanvido, Italy) in powder form. The powder was placed on a 50 x 50 x 80 mm build plate in a high-resolution DMLS (Concept Laser GmbH, Lichtenfels, Germany) system (Figure 2.1b). The system uses a laser beam, with laser power and speed following the runs provided by the 3D printing DoE study, to melt the different layers of powder to a layer thickness of 0.025 mm and spot size of 40 μm . Furthermore, the trace width (width of a single layer of the

laser beam as it melts and fuses the metal powder), laser power and laser speed were adjusted according to DoE runs within the range of 0.09-0.12 mm, 30-70 W and 500-700 mm/sec respectively. These levels were defined based on the maximum and minimum capacities of the 3D printer. Using the entire range provided by the printer is beneficial as it allows for a comprehensive exploration of the parameter space, enabling the identification of optimal settings when DMLS printing is used for the development of MNs as effective and reliable drug delivery systems. Also, this ensures that the printing process is thoroughly optimised, leading to the best possible quality and performance of the MNs.

Also support structure with an angle (the angle at which support structures are built to uphold parts of a print) of 60° was used. Even if there are no overhanging features the use of support structure is beneficial for distributing heat more uniformly across the structure, reducing the likelihood of warping due to thermal stresses. The angle of 60° is chosen because it provides a good balance between accessibility for removal post-printing and structural stability during the print process. During the printing a combined laser scan pattern of perimeter (contour) and infill were used for optimal results. The perimeter scan is used to define the precise external boundaries of the MNs, ensuring high precision and a smooth surface finish. Following the perimeter scan, the infill scan employing a hatch pattern was applied to fill the interior volume of the MNs. This step ensured structural integrity and robustness, which are vital for the mechanical strength of the MNs. This combined approach improved heat distribution during the printing process, minimising residual stresses and preventing cracking.

2.3.3. Physical Properties of 316L Stainless Steel Powder

The particle size distribution of the 316 L stainless steel was analysed with a Malvern Mastersizer 3000 Particle Analyzer (Malvern Panalytical, UK) following the guidelines outlined in ISO 13320. The measurements included the values of D₁₀, D₅₀, and D₉₀, which represent the cumulative distribution at 10%, 50%, and 90% of the total particle size range.

The particle size analyser was set up for spherical stainless-steel particles with a particle refractive index between 2.76 and 3.79 and the water was selected as dispersant. The results were reported in micrometres (μm) with six samples analysed for each measurement. Scanning electron microscopy (SEM) (Zeiss EVOL S15 SEM, Germany) was used to qualitative analyse the powder particles using an extra high tension (EHT) voltage of 15 kV. ImageJ (ImageJ software V1.8, National Institutes of Health, Maryland, USA) was used for image analysis to obtain the average particle size and to assess the morphology and circularity of the powder particles. According to the definition of a circularity of 1 denotes to a spherical shape while a circularity of 0 corresponds to a straight line. The magnification of the images used for the analysis was x50 and six images were analysed.

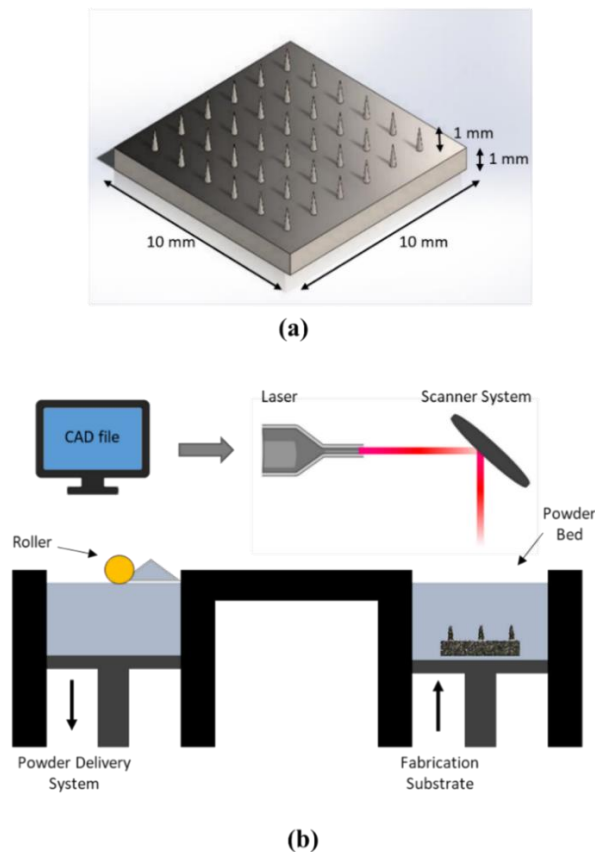


Figure 2.1: CAD Design and Schematic of the DMLS Process – a) Design of 6 x 6 arrays of solid MNs on a $10 \times 10 \times 1$ mm square patch and b) a layer-by-layer sintering process using the CAD file, while a laser beam directly melting the metal powder layer [166], resulting in a total of 36 MNs per patch.

2.3.4. Analytical Assessment of MNs for Printing DoE study

2.3.4.1 Geometry of MNs: Height – Tip Diameter – Base Diameter

MN array patches were examined after printing using a Keyence VHX-700 (KEYENCE Corporation of America, Itasca, IL, USA) digital microscope fitted with a high-performance zoom lens (VH-Z20R/Z20T). Imaging was completed at a magnification of x20. The dimensions of the needle height, tip, base and mid-height diameter was determined from the side view of the 3D images. For each MN patch a representative sample 24 needles out of 36 were measured from the two outer and the two middle rows of each MN patch, providing sufficient data to statistically infer the properties of the entire array. Furthermore, choosing needles from both the outer and middle rows, the variability across the patch can be assessed. This sampling method allows to gain meaningful insights without measuring every single needle and helps to verify that the needles' characteristics are consistent from edge to center, addressing any concerns about variability in the printing process. The 3D images were analysed, and the dimensions were measured using ImageJ software.

2.3.4.2 Surface Roughness

To determine the surface roughness (Ra) and the effect of printing parameters, a non-contact optical surface profilometer (Bruker Contour GT 3D, Bruker, Germany) was employed in each sample at six needle areas that were randomly selected, and average value recorded. The measurements conducted at x20 magnification. This method allowed for the quantification of the topographical features of the sample surface on the sides of the needles without causing any physical contact, thus avoiding any potential alterations to the surface characteristics during the measurement process. In addition, qualitative analysis was conducted on the surface of the MNs through SEM images post-printing as per DoE study. Each SEM image was analysed, and the effect of the different printing factors was observed.

2.3.4.3 Porosity

The experimental procedure for determining the density of the samples ($n=3$) post-printing and, thereby, the porosity was completed in accordance with the method outlined by Terris *et al.* [167]. The absolute density was determined using the helium pycnometer on the AccuPyc 1330 device (Micromeritics Instrument Corporation, GA, United States). Prior to the helium pycnometry measurements, the samples were subjected to oven-drying at 110°C for 30 min and subsequently air-cooled. The measurement process involved placing the sample into the measurement chamber, at atmospheric pressure. Helium gas was then introduced into the tank and then was expanded in a separate vessel, known as the expansion vessel, which was at atmospheric pressure. To ensure accurate measurements, several purge cycles were conducted prior to the actual measurements to remove any air present in both the tank and the open pores of the sample. Six consecutive measurements were performed with the tank kept closed and sealed.

Qualitative analysis of the porosity was also obtained from SEM micrographs of the samples by cutting, resin mounting, and polishing them to analyse the effect of the printing parameters on the microstructure of the MN arrays. Initially, the samples were mounted in resin to stabilise them during the polishing process. For consistency and repeatability in the images taken every sample was polished initially using a coarse polishing disc (P240) to remove any large surface irregularities and achieve a flat surface. Following by finer abrasive discs, moving to higher grit sizes using P600 with continues water stream. The fine polishing was carried out using a series of increasingly fine diamond suspensions to achieve a high-quality finish suitable for microscopic examination. The process began with a 9-micron diamond suspension, progressing to intermediate stages of 6-micron and 1-micron suspensions, and concluded with a final polish using an ultra-fine 0.05-micron diamond suspension, each polishing grade was applied for 3 min at 300 RPM. This sequence was performed using

specialized polishing cloths tailored for each stage to ensure a gradual and uniform reduction in surface roughness. The micrographs were captured on two planes, *i.e.*, perpendicular and parallel to the manufacturing direction using SEM. The images were then analysed using ImageJ software to determine the sample porosity. This method is helpful because it allows the pores within the sample to be located and their size and shape to be evaluated. It should be noted that the micrographic method is limited to the observed regions and does not consider the entire sample. However, both methods are included as responses in the DoE study.

2.3.4.4 Mechanical Properties

Accurate mechanical characterisation of MNs is a key aspect in the development of successful MN arrays. To ensure their effectiveness, MNs need standardised inherent strength that prevents array failure caused by stresses during insertion due to the non-uniformity of the skin surface. Moreover, stresses arise from the unavoidable movements during insertion as well as the forces exerted during removal. These stresses can lead to various modes of failure, such as MN bending, buckling, and base-plate fracturing.

The mechanical properties of the 3D printed MN arrays were assessed under compressive loading using the Zwick Testing System (Zwick Roell, UK). Before the test, the printing support located at the base of the MN arrays was carefully removed by grinding. Prior to testing, each MN array was securely attached to the lower compression test plate using double-sided tape affixed to the base of each patch. A compressive load was then applied at a displacement rate of 5 mm/min using a 5 kN load cell. Three samples were tested for each group. The stress-strain curve was plotted to determine the mean compressive strength, along with the corresponding standard deviation. To determine the compressive strength for each MN on the patch, the approach proposed by Du et al. [168] was selected due to its appropriateness for the conical shape of the MNs. This method is particularly well-suited

because it addresses the specific challenge of quantifying strength in structures that are prone to buckling at the tip, which is a common failure mode for conical MNs. This approach involved calculating the effective compressive strength of an individual MN by dividing the applied compression force by the MN fractured area, as expressed by Equation 2.1. Where r_δ is needle radius at the compression displacement δ and can be calculated by Equation 2.2.

Equation 2.1: Effective compressive strength of an individual MN.

$$\sigma = F / \pi r_\delta^2$$

Equation 2.2: Needle radius at the compression displacement δ .

$$r_\delta = \frac{1}{2} \left(-r_1 + \sqrt{\frac{3V}{(H - \delta)} - 3r_1^2} \right)$$

$$\text{Where } V = \frac{4}{3r_1^2} (H + H') - \frac{4}{3r_2^2 H'}$$

Where, r_1 : measured radius at the base of the MN, V : volume of the MN (based on the dimensions as defined by measuring them with Image J), H : measured height of MN before compression and, H' : length of MN tip (around 10 μm , only 1/1,000 of the total needle volume), which is deflected to one side during the test, and is not creating any significant resistance to the compression (Figure 2.2).

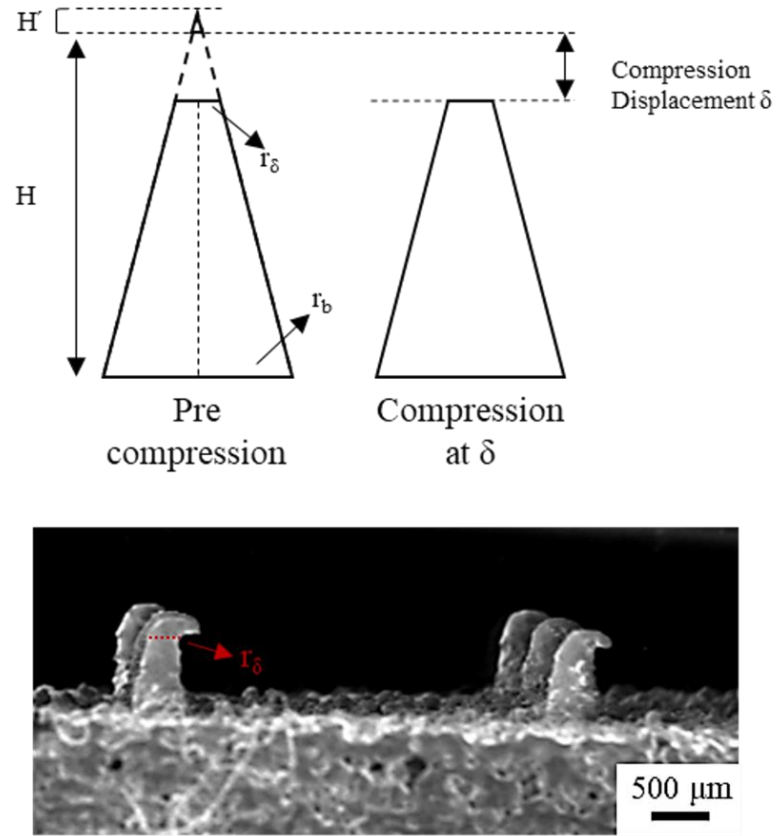


Figure 2.2: Compression Test Analysis – Schematic representation of the cross-sectional area utilised for calculating the compressive strength.

2.4. Results

2.4.1. Physical Properties of 316L Stainless Steel Powder

The particle morphology of 316L stainless steel powder exhibited a uniform and spherical shape, with a circularity value of 0.99 ± 0.01 (Figure 2.3a) as defined by Image J analysis of 6 different powder images. The particle size distribution indicated a range within the micro size dimension. Quantitative analysis using a particle analyser presented a normal distribution, with recorded values of $D_{10} = 20 \pm 0.5 \mu\text{m}$, $D_{50} = 30.5 \pm 0.1 \mu\text{m}$, $D_{90} = 46.1 \pm 0.7 \mu\text{m}$, and a bulk density of 7.97 g/cm^3 (Figure 2.3b).

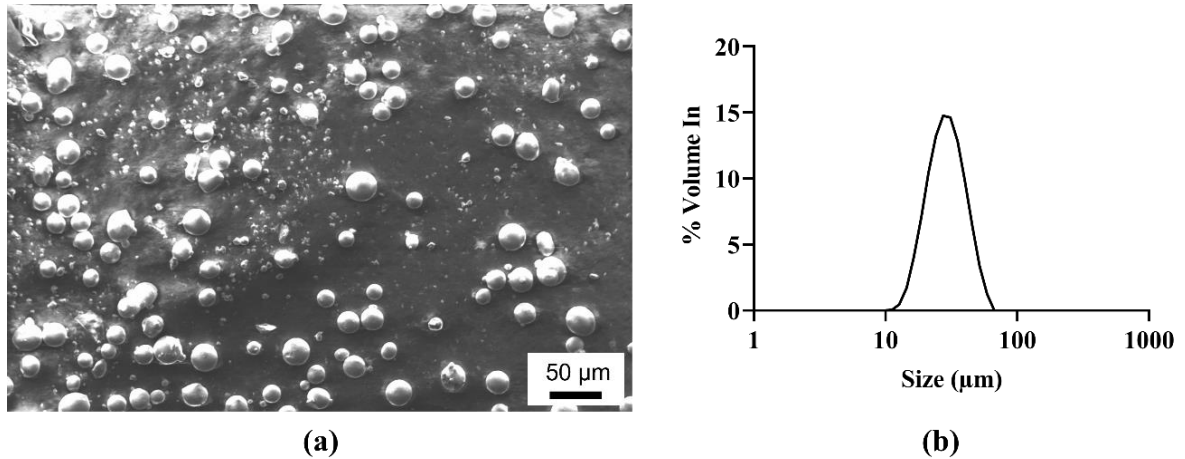


Figure 2.3: SEM and Particle Size Analysis – a) SEM image of the 316 L stainless steel powder morphology demonstrated spherical-shaped particles with ~99% circularity and b) normal percentage volume distribution profile of the particle size distribution of the powder.

2.4.2. Analytical Assessment of MNs for Printing DoE study

2.4.2.1 Geometry of MNs: Height – Tip Diameter – Base Diameter

The MN arrays were 3D printed as per the DoE runs, and their needle height, base diameter, and tip diameter were measured and analysed as the initial responses. As expected, there were disparities between the MN dimensions in the CAD file and the actual 3D printed MNs. A consistent observation for the majority of the 3D printed MN arrays was the reduction in needle height and base diameter by an average of $30 \pm 15\%$ compared to CAD model dimensions. Specifically, the needle height was consistently reduced by an average of $28 \pm 16\%$ for most runs. For most runs the base diameter was also reduced by $10 \pm 5\%$ (Figure 2.4a). However, for certain runs, an increase in base diameter of $50 \pm 5\%$ compared to the CAD file was observed (Figure 2.4b.) This led to a base diameter of $375 \mu\text{m}$ from $250 \mu\text{m}$, height of either $560 \mu\text{m}$ or $880 \mu\text{m}$ from $1,000 \mu\text{m}$. This finding are in line with earlier research that also identified variability in printed structures against their initial designs, primarily due to printing parameters, thermal effects and material properties [169]. Furthermore, the presence of MNs exhibiting an increased base diameter indicates a level of

inconsistency in the printing process, originating from possible anomalies in powder distribution, laser focusing, or other contributing factors [170].

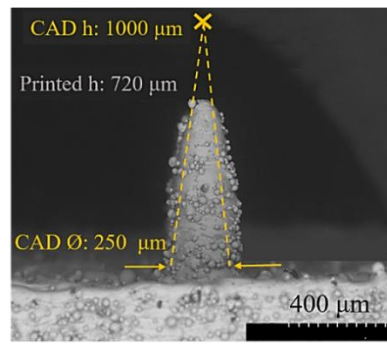
The analysis of the geometrical properties (height, base and tip diameter) obtained a significant linear model ($p\text{-value} < 0.01$, $R^2 = 92\%$) based on the Fit summary. Both the ANOVA and percentage contribution analyses indicated that laser power was the factor that significantly ($p\text{-value} < 0.05$) influencing the geometrical properties of the MNs. Specifically, increasing the laser power from 30 W to 70 W significantly ($p\text{-value} < 0.001$) increased the needle height from 600 μm to 850 μm , the base diameter from 200 μm to 350 μm and the tip diameter from 50 μm to 150 μm (Figure 2.4c). While the highest laser power (70 W) achieved a needle height closer to the initial CAD file dimensions ($h = 1,000 \mu\text{m}$), the resulting tip diameter and base diameter did not meet design requirements. For instance, MNs printed with the use of high laser power led to tip diameter greater than 50 μm limiting the ability of the needle to penetrate the skin [171]. Therefore, it is crucial to strike a balance between these dimensions when optimising the printing parameters to meet clinical standards.

In addition to the significant influence of laser power, the DoE analysis demonstrating a noteworthy interaction between laser speed and trace width ($p\text{-value} < 0.001$) concerning the needle height (Figure 2.5a). Specifically, when the laser speed was set at lower levels ($< 550 \text{ mm/s}$) and the trace width was reduced to the min level (*i.e.* 0.09 mm), the needle height demonstrated an increase within the range of interest (700 - 800 μm). Conversely, to achieve increased needle height while raising the laser speed to values exceeding 650 mm/sec, a high trace width of 0.12 mm was necessary. These results indicate a complex relationship between laser speed, trace width, and needle height as can be seen in Figure 2.5a, providing crucial guidance for optimising the printing process of MNs.

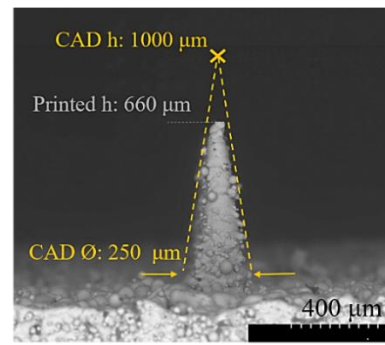
The DoE analysis further presented an additional interaction between laser speed and laser power ($p\text{-value} < 0.001$) concerning the needle tip diameter (Figure 2.5b). Specifically, it was

observed as can be seen in graph in Figure 2.5b that a smaller tip diameter ($<55\text{ }\mu\text{m}$) was achieved when the MNs were printed using low levels of laser power ($<50\text{ W}$) and laser speed of 500 mm/s . On the other hand, to achieve a reduced tip diameter, on laser power greater than 60 W a constant laser speed of 700 mm/s is required. Careful consideration of these interdependent factors is crucial for precise control over the tip diameter during the printing process.

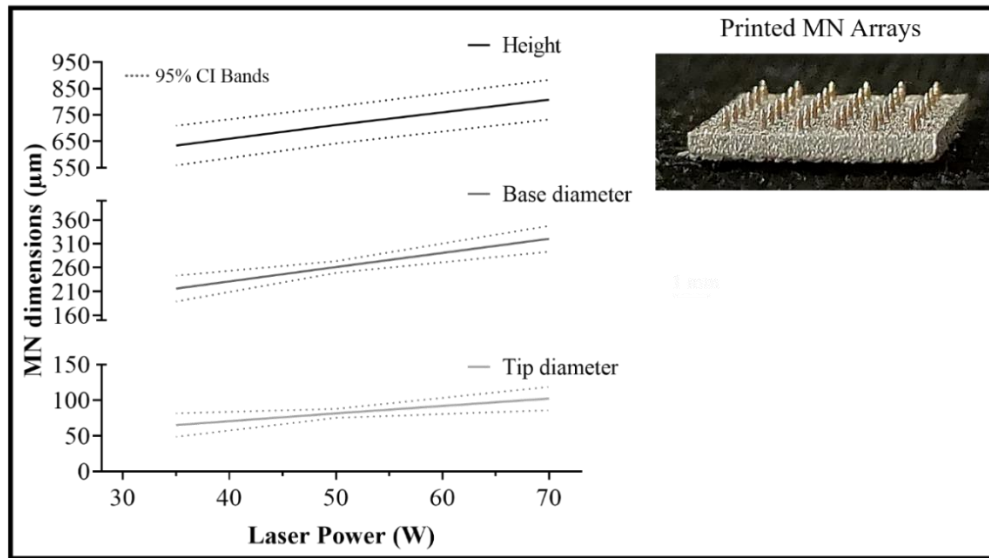
In addition, the laser speed was found by ANOVA study to interact significantly with trace width ($p\text{-value}<0.01$) when analysing the tip diameter (Figure 2.5c). Increasing the laser speed while maintaining a consistent high trace width (0.12 mm) can be seen from the graph that resulted in an increase in the tip diameter during the printing process. Conversely, maintaining a constant trace width at a lower value (0.09 mm) had a negative impact, resulting in a high tip diameter at high laser speeds closer to 700 mm/sec .



(a)



(b)



(c)

Figure 2.4: SEM Imaging and DoE Analysis of MNs Geometry – SEM images of needle side view show the comparison of needle height and base diameter deviations from the CAD file, indicating (a) a reduction in general but (b) with certain runs exhibiting an increase in base diameter. (c) The DoE ANOVA analyses demonstrated that laser power significantly influences the base diameter, needle height, and tip diameter of the MNs when laser speed is 600 mm/sec and trace of 0.11 mm. 95% CI=confidence interval.

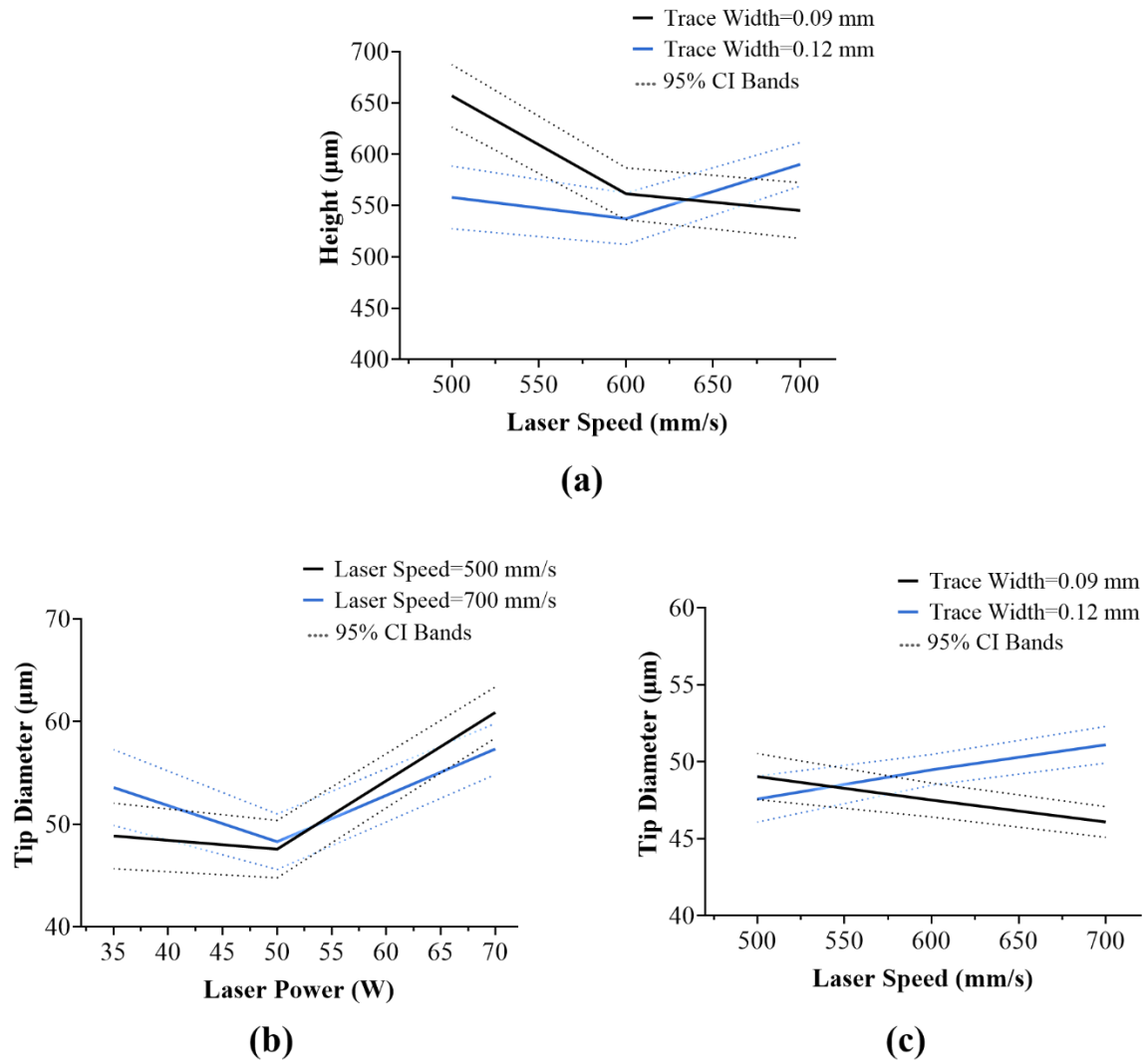


Figure 2.5: DoE Interactions of MN Arrays Geometry – The DoE analysis identified the significant influence of (a) the laser speed and trace width interaction on the resulting needle height, (b) the laser power and laser speed interaction and (c) laser speed and trace width interaction showing their combined influence on the resulting tip diameter. 95% CI=confidence interval.

2.4.2.2 Surface Roughness

Surface roughness (R_a) ranging between 15 μm to 38 μm were calculated for the different printed MNs depending on the print parameters used. The ANOVA analysis conducted on the roughness from the DoE study yielded a significant model ($p\text{-value} < 0.01$). This significance was attributed to the presence of significant interactions between laser power and trace width ($p\text{-value} < 0.01$) (Figure 2.6a), as well as between laser speed and trace width ($p\text{-value} < 0.05$) (Figure 2.6b). The interaction between laser power and trace width was found to be particularly significant at high levels of laser power ($> 60\text{ W}$). For instance, to achieve a low roughness on the MNs, it was necessary to print the MNs with a laser power greater than 60 W and a trace width of 0.12 mm. Conversely, the significant interaction between laser speed and trace width indicated that the roughness could be kept at low levels by printing the MNs at low levels of laser speed and high levels of trace width. These interactions, especially between laser power and trace width at high power levels, play a crucial role in determining the surface topography of the MNs. Similar results were found when a laser-based method was used for the fabrication of polymeric MNs by Donnelly *et al.* where, laser properties led to different surface roughness due to melt overlap [172].

SEM analysis confirmed a relationship between laser power, speed and trace width, and the structure of the MN arrays, highlighting that the roughness changes due to the presence of unmelted particles (Figure 2.6c, d) post-printing. At a laser speed of 700 mm/s and laser power of 70 W the overlap width between solidified microstructures decreases, resulting in visible gaps or holes in the microstructure (Figure 2.6c). In contrast, when the laser speed is decreased to 500 mm/s while keeping the laser power and trace width consistent, the solidified structure appears uniform and dense with no obvious pores or unmelted particles (Figure 2.6d). Furthermore, with the increase of laser power from 35 W to 70 W the unmelted particles of the MN decreased significantly when the trace width was constant at 0.12 mm. Insufficient

laser power resulted in incomplete powder melting, leading to structural defects. Conversely, when the laser power was increased and the trace width was set at 0.09 mm, a high number of unmelted particles were observed on the MNs, along with a non-uniform structure, thus compromising the quality of the final specimen. SEM analysis provided valuable insights into the relationship between laser power, speed, trace width, and the resulting microstructure of the MN arrays, highlighting the importance of optimising these parameters to achieve desired structural integrity and density.

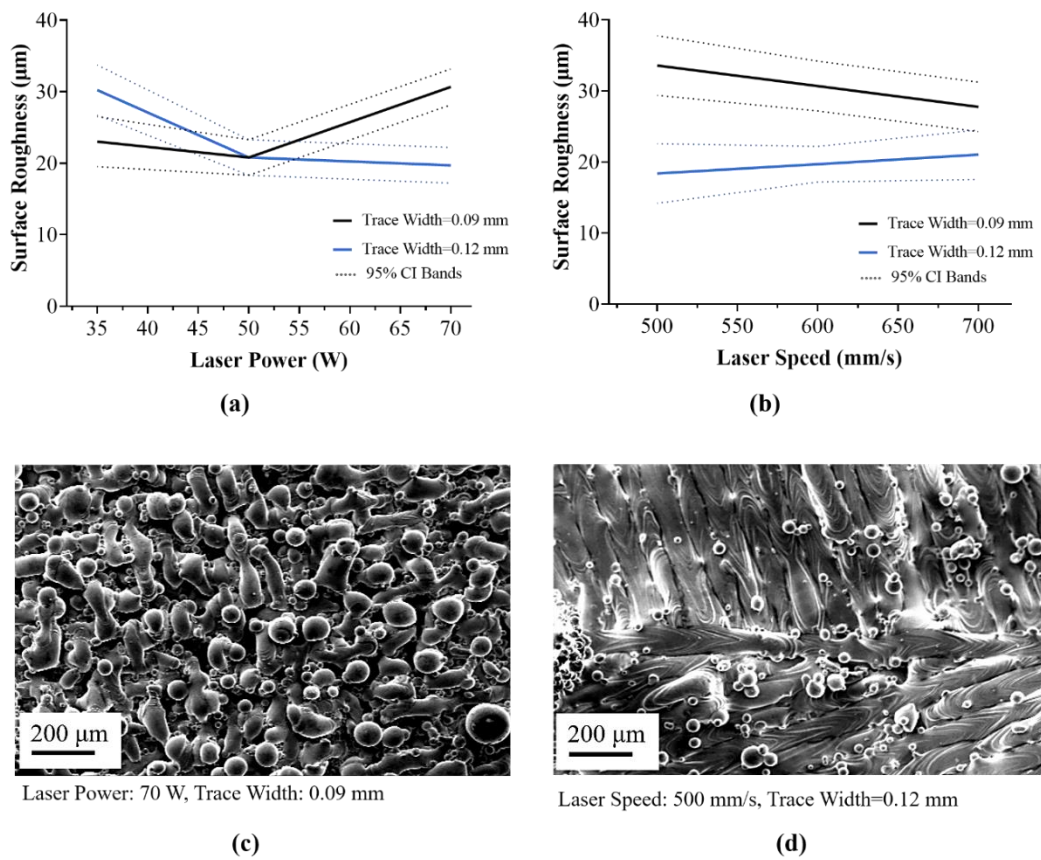


Figure 2.6: DoE Analysis of Surface Roughness and SEM Imaging – Interactions between (a) laser power and trace width and (b) laser speed and trace width influencing the surface roughness of the MN arrays. SEM image presenting the printed MN arrays at (c) low trace width and high laser power (70 W) and speed (700 mm/s) showing visible gaps or holes in the microstructure and (d) high trace width and low laser speed (500 mm/sec) and high laser power (>50 W) showing uniform structure without unmelted particles. 95% CI= confidence interval.

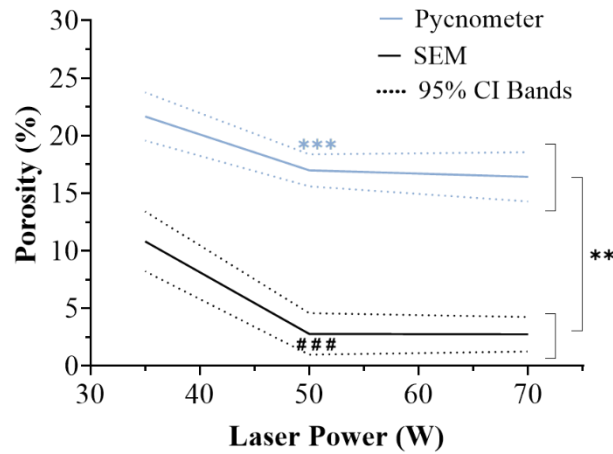
2.4.2.3 Porosity

The SEM analysis presented a porosity percentage ranging between 3-10%, while the pycnometer method indicated a porosity between 16-25%. The results indicate a significant decrease in porosity across all the different groups when assessed using SEM compared to the pycnometer method (Figure 2.7a). SEM analysis of the different surfaces of the MNs provided valuable insights into the porosity within their microstructure. The images captured post-3D printing demonstrated the presence of amorphous porosity. These pores appeared as irregular voids dispersed throughout the microstructure (Figure 2.7b) and were also denoted as amorphous as result of imperfections or defects during the printing process. Their presence can significantly impact the mechanical and functional properties of the MNs. Additionally, some SEM images showed porosity arising directly from the 3D printing process, leading to microporosity (Figure 2.7c). These pores were relatively larger in size and exhibited a more defined and organised structure.

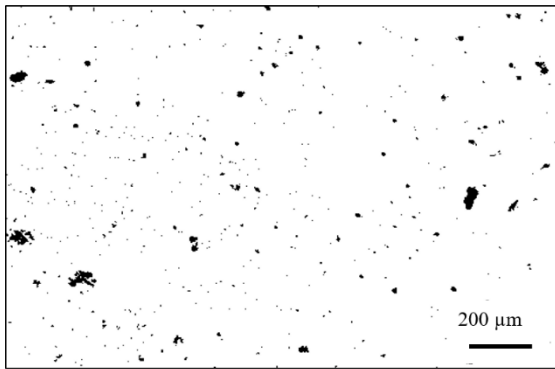
Although differences were observed on the range of porosity between the two methods (SEM and pycnometer), the DoE study for both demonstrated that laser power was the only factor that significantly ($p\text{-value} < 0.001$) influenced the porosity during the 3D printing process. Specifically, increasing the laser power resulted in a significant reduction in the porosity from both the SEM and pycnometer methods (Figure 2.7a). Furthermore, increasing the laser power beyond 50 W did not yield any additional effect on the porosity, suggesting a linear and stable behaviour.

The DoE analysis of the porosity obtained from pycnometer method also demonstrated a significant interaction ($p\text{-value} < 0.01$) between laser power and trace width during the printing process (Figure 2.8). Specifically, when the laser was operated at the lowest speed of 500 mm/s to achieve minimal porosity in the printed structures, it was necessary to utilise high laser power levels, approaching 70 W. Conversely, when printing at the higher speed of

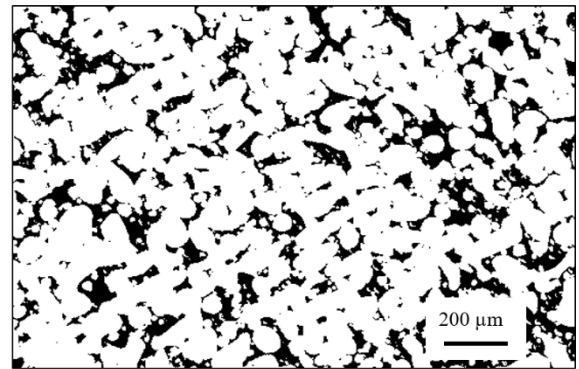
700 mm/s, lower laser power settings were required to obtain MNs with a uniform structure and controlled porosity. The inverse correlation observed between laser power and trace width suggests that higher laser power enables faster printing speeds without affecting the uniformity of the MNs.



(a)



(b)



(c)

Figure 2.7: DoE Study of MNs Porosity – (a) DoE graphs presenting the behaviour of MNs porosity, determined with pycnometer and SEM micrographs, increasing the laser power during the printing. Micrographic cross-sections of samples printing (b) at low laser speeds corresponding to low porosity rates and (c) at high laser speeds and trace width. Statistical significance denoted as ***p-value<0.001, ###p-value<0.001 and **p-value<0.01 and 95% CI= confidence interval.

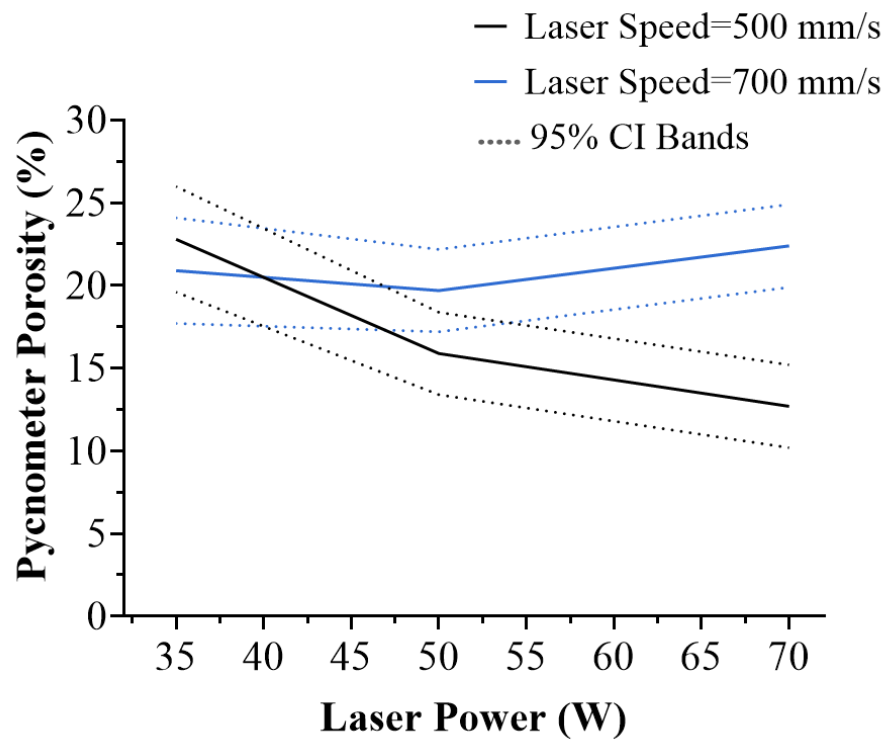


Figure 2.8: DoE Porosity using Pycnometer Methodology – Interaction between laser power and laser speed obtained from DoE analysis after analysing the percentage of porosity using a pycnometer. 95% CI= confidence interval.

2.4.2.4 Mechanical Properties

The effective compressive strength values reported for the 3D printed metallic MNs, ranging from 0.4 GPa to 1 GPa, which differ from those of the bulk stainless steel material due to several factors inherent to the 3D printing process and the microscale dimensions and structure of the MNs. Stainless steel bulk materials typically exhibit higher and more uniform mechanical properties 1.5 to 2.5 GPa, depending on the heat treatment. The DoE study indicated that the effective compressive strength was significantly ($p\text{-value}<0.001$) influenced by all three factors. It was observed that increasing the laser power from 30 W to 50 W resulted in a two-fold increase in effective compressive strength, reaching approximately 0.8 ± 0.2 GPa (Figure 2.9a). However, no further increase in effective compressive strength was achieved by increasing of laser power above 50 W. The effective compressive strength was also affected by the laser speed and the trace width. An increase in effective compressive strength was also achieved by increasing the trace width reaching 1 GPa at 0.12 mm trace width (Figure 2.9b). In contrast to laser power and trace width, the increase of laser speed during the 3D printing process led to a reduction in effective compressive strength of 0.6 ± 0.2 GPa (Figure 2.9c). This is consistent with the results documented by a previous study, where the solidification process of metal structures became more thorough with lower energy input, resulting in reduced mechanical properties [173]. According to the DoE study a significant interaction between trace width and laser speed ($p\text{-value}<0.01$) was observed. At a trace width of 0.12 mm increasing the laser speed at 700 mm/s led to an increase in effective compressive strength to approx. 0.9 ± 0.2 GPa, while for a lower laser speed of 500 mm/s the effective compressive strength was 0.6 ± 0.2 GPa (Figure 2.9d). Conversely, for a trace width of 0.09 mm increasing the laser speed led to a reduction in effective compressive strength. The results of the DoE study indicated that printing MNs with high mechanical properties requires either low trace

width and high laser speed (*i.e.* 0.09 mm and 700 mm/s, respectively) or high trace width with low speed (*i.e.* 0.12 mm and 500 mm/s respectively). At a laser speed of 600 mm/s, similar effective compressive strength values of 0.8 ± 0.2 GPa were obtained for a trace width of 0.09 mm and 0.12 mm, respectively.

Overall, the difference in effective compressive strength is related with the different MN dimensions impacting the mechanical strength of 3D printed metallic MNs. In particular, variations in needle height, and tip diameter account for strength differences to a notable degree. Specifically, needle height affects the compressive strength by influencing the overall stability and buckling resistance of the MNs. Taller needles are more prone to buckling under compressive loads, which can significantly reduce their mechanical strength. Conversely, shorter needles exhibit greater resistance to buckling, enhancing their compressive strength. The tip diameter also plays a critical role; a finer tip diameter improves the MNs' penetration efficiency but can compromise structural integrity. Needles with larger tip diameters distribute compressive loads over a larger area, thereby increasing their strength and reducing the likelihood of tip fracture during application. These dimensional characteristics are crucial for ensuring the MNs can effectively penetrate the skin while maintaining the necessary mechanical robustness for reliable drug delivery.

The failure primarily looked like buckling of the MNs. The bent and deformed appearance of the MNs indicates they failed under buckling, where the compressive loads exceeded the critical buckling load. Given the nature of 316L stainless steel, known for its excellent ductility and toughness, significant plasticity is evident from the smooth, continuous deformation seen in the MNs. This suggests that the material underwent plastic deformation before failure, as opposed to a brittle fracture. The absence of clear fracture surfaces supports the observation that the failure mode was ductile.

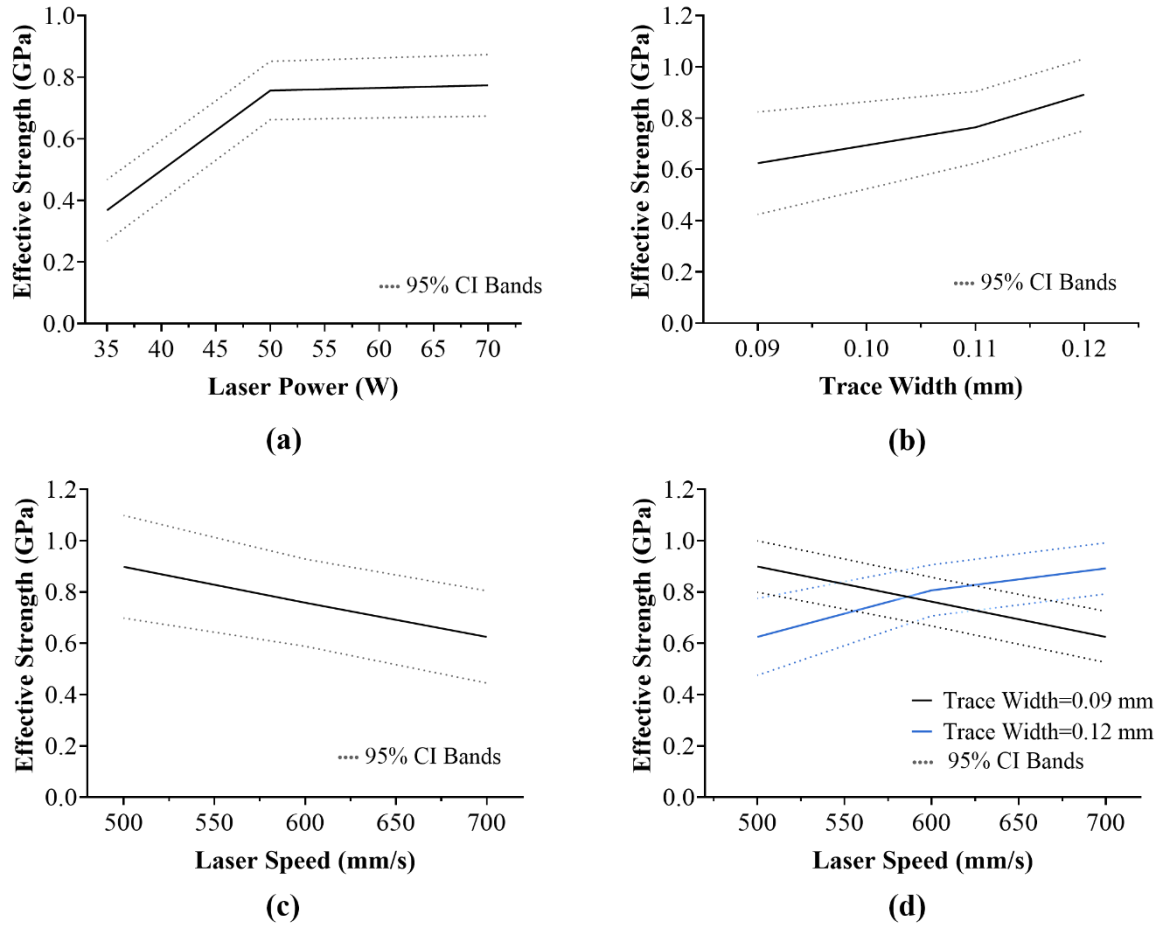


Figure 2.9: DoE Analysis of Compressive strength – DoE study demonstrating the behaviour of the effective compressive strength while considering the influence of the three key printing parameters: (a) laser power, (b) trace width and (c) laser speed as well as the (d) interaction between laser speed and trace width. 95% CI= confidence interval.

2.4.3. DoE Optimisation and Validation

Upon completing the analysis of the DoE study, the optimal properties for printing processes were determined. Considering the clinical requirements and goals related to the MN

properties, the DoE predicted the optimal printing parameters to be as follows: laser power of 65 W, laser speed of 650 mm/s, and trace width of 0.09 mm through a desirability rating of 92%, indicating a successful optimisation process. The predicted results determined from DoE optimisation met the clinical requirements for the needle height, porosity and effective compressive strength met the clinical requirements. However, the tip diameter, base diameter and the surface roughness were predicted to be outside the clinically required ranges. MN arrays were fabricated according to the parameters identified from the optimisation and the accuracy of the DoE models was assessed by comparing the actual experimental response values to the predicted response values using a t-test (Table 2.3).

The t-test analysis demonstrated a non-significant difference (p -value=0.1) between the values, with a non-significant percentage of difference between the values validating the accuracy of DoE model. The experimental results, as illustrated in Figure 2.10, show that the mean tip diameter and mean height of the MNs fall within the desired clinical ranges, indicating effective optimization of these parameters. However, the mean surface roughness and mean base diameter exceed the design requirements, suggesting that the current optimization process was insufficient in achieving the required smoothness and base dimensions. This excess in surface roughness can affect the uniform application and adherence of drug coatings, while an oversized base diameter can affect the mechanical stability and insertion capability of the MNs. Therefore, additional post-processing steps, such as electropolishing, are essential to refine these aspects. Electropolishing will help to smooth the surface and reduce the base diameter, ensuring that the MNs meet all clinical requirements for effective performance and usability.

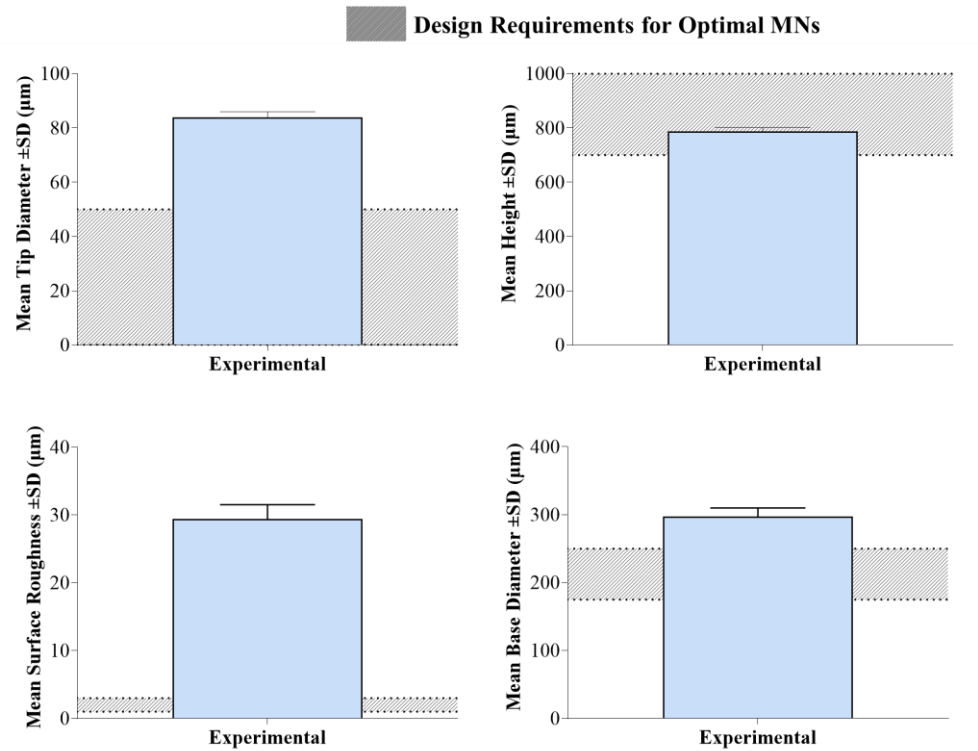


Figure 2.10: Experimental Results of MN Properties Post-Printing DoE Optimisation Compared to Design Requirements.

Table 2.3: DoE optimisation of the geometrical, structural and mechanical properties of MN arrays and validation of the DoE model accuracy comparing the actual experimental values with the predicted values obtained from the optimisation of DoE analysis.

<u>DoE 3D Printing Optimal Levels of the different Factors</u>				
Laser Power = 65 W		Laser Speed = 650 mm/s		Trace Width = 0.09 mm
Responses	Design Requirements	DoE Prediction	Experimental Values	Absolute Difference (%)
Tip diameter	< 50 μm	$88.5 \pm 0.3 \mu\text{m}$	$83.9 \pm 2.1 \mu\text{m}$	5.3
Needle height	$700 \mu\text{m} < h < 1,000 \mu\text{m}$	$745.4 \pm 2.5 \mu\text{m}$	$787.0 \pm 4.3 \mu\text{m}$	5.4
Base diameter	$175 \mu\text{m} < B < 250 \mu\text{m}$	$281.8 \pm 1.5 \mu\text{m}$	$297.4 \pm 2.5 \mu\text{m}$	5.4
Surface Roughness	$\sim 1.25 \mu\text{m}$	$26.2 \pm 2.1 \mu\text{m}$	$29.4 \pm 2.1 \mu\text{m}$	11.5
Porosity (SEM)	minimise	$3.5 \pm 0.5\%$	$4.1 \pm 0.3\%$	16.3
Porosity (Pycnometer)	minimise	$15.9 \pm 2.2\%$	$18.2 \pm 2.5\%$	13.5
Effective compressive strength	maximise	$0.52 \pm 0.2 \text{ GPa}$	$0.44 \pm 0.6 \text{ GPa}$	16.7

2.5. Discussion

In this chapter, we employed a DoE study to analyse and optimise the 3D printing parameters to achieve metallic-based MNs with the desired geometrical, structural, surface and mechanical properties for use as TDD systems.

A consistent observation across most 3D printed MN arrays was the reduction in needle height and base diameter by an average of $30 \pm 15\%$ compared to CAD model dimensions. This finding is in line with earlier research that also identified variability in printed structures against their initial designs, primarily due to printing parameters, thermal effects and material properties [169]. Furthermore, the presence of MNs exhibiting an increased base diameter indicates a level of inconsistency in the printing process, originating from possible anomalies in powder distribution, laser focusing and existing variation within the process [170]. These findings highlight the need for careful optimisation of printing parameters to achieve the desired dimensional accuracy for MNs.

The DoE study during the printing process demonstrated that laser power was the most significant factor influencing the MN arrays dimensions. This significant influence on MN geometry is consistent with findings from earlier research, where laser sintering parameters played pivotal roles in shaping the final microstructure [174,175]. Specifically, increasing the laser power from 35 W to 70 W significantly increased the geometrical characteristics needle height, base diameter, and tip diameter. Higher laser power generally increases the size of the melt area. A larger melt area can lead to a slight increase in the dimensions of printed features due to greater flow and spread of the material before solidification. On the other hand a higher scanning speed can lead to a shorter interaction time between the laser and material, resulting in less material being melted and a thinner layer being deposited per pass, thus reducing the overall height of the fabricated structure. For instance, Gill *et al.* obtained that keeping the MNs length to values lower than 1,000 μm reduces pain [92]. For

that reason, the needle height of $\sim 700\text{ }\mu\text{m}$ is considered sufficient for the MN's to efficiently breach the *stratum corneum* and reach the desired depth without reaching the nerves for effective painless drug delivery or fluid extraction [176–178].

Laser power was also indicated as the most significant factor influencing the MN surface and structural quality (porosity and surface roughness) of MNs. MNs printed with high laser power exhibited reduced porosity, which was maintained mainly within the patch, resulting in higher mechanical properties and improved structural integrity [179]. Increasing the laser power leads to higher energy input per unit area, which enhances the melting of the powder particles. This more complete melting reduces porosity by allowing the melt pools to coalesce more fully, filling in potential gaps between particles which could otherwise form voids. Another vital aspect is the roughness of the MNs, where the significant interactions between laser power, laser speed, and trace width were observed. If the scan speed is high, the energy input per unit length decreases, potentially leading to insufficient melting at the edges of the scan tracks. This can result in narrower features if the powder does not fully melt and spread. However, if the trace width is adjusted appropriately, it can compensate for the faster speed by ensuring sufficient overlap and melting. These interactions, especially the one between laser power and trace width at high power levels, play a crucial role in determining the MN surface's topography. Similar results were found when a laser-based method was used for the development of polymeric MNs by Donnelly *et al.* where, low laser power and high trace width led to higher surface roughness due to melt overlap [172]. A smooth surface or low number of unmelted particles and structural voids is essential for ensuring comfortable skin penetration [180] and even drug coating and loading [181]. Furthermore, to achieve MNs with high mechanical properties, the DoE study suggested using high laser power or a combination of low trace width and high laser speed. High laser power increases the energy input into the material, creating a larger and more stable melt pool. This enhanced melt pool

facilitates better fusion of the metal powder particles, resulting in a denser and more cohesive printed structure contributing to the higher mechanical strength of the MNs. Additionally, the larger melt pool helps to smooth out any irregularities and ensures a more uniform structure, further enhancing the mechanical properties. Using a low trace width in combination with high laser speed optimises the precision and control over the printing process. A low trace width (i.e., a narrower laser beam) allows for finer details and sharper features in the printed MNs. This precision is crucial for maintaining the structural integrity and achieving the desired geometric accuracy of the MNs. High laser speed, on the other hand, reduces the dwell time of the laser on any given point, minimising heat accumulation and potential thermal distortion. This rapid scanning also helps in maintaining a smaller and more controlled melt pool, which is essential for producing fine features without compromising the overall strength. This approach balances the need for fine geometric details with the requirement for strong and cohesive material properties, resulting in microneedles that are both sharp and robust. These findings are consistent with the existing literature, where lower laser powers have been shown to result in significantly reduced strengths due to the presence of unmelted powder within cross-sections [173].

The MNs demonstrated high compression strength ensuring their ability to effectively penetrate the skin without experiencing tip fracture during the penetration process. Compared to the broader MN literature, metallic MNs demonstrate higher mechanical properties over commonly investigated polymeric MNs [172,182]. In particular even if the compressive strength of polymeric MNs can vary based on the type of polymer used and the design of the MNs. In a study comparing different types of polymeric MNs, the cryomicroneedles were found to have a compressive strength of 24.7 ± 3.8 MPa [183]. Another study by Du *et al.* reported the compressive strength of polymeric MNs to be in the range of 72–75 MPa. However, both are significantly lower than the effective compressive strength of 1 GPa

obtained from the metallic 3D printed MNs [168]. The mechanical strength of MNs is frequently evaluated by different research teams using axial force tests [127,159,172]. For instance, Davis *et al.* [184], similarly to Zahn *et al.* [185] and Khann *et al.* [186], determined the compressive strength using axial force tests however their research focused on the mechanical characterisation of individual MNs.

As with the effect of MN density on insertion, as discussed, the failure force of a MN array cannot be assumed to be the same as that of a single MN. In contrast, this research measured effective compressive strength on arrays of 36 MNs offering a more precise and relevant representation of mechanical strength because it better simulates the actual conditions under which MN arrays are used in practical applications. In real-world scenarios, MNs are not used individually but rather as part of an array, which distributes forces and stresses differently than a single MN would. By testing an array, factors such as the interactions between needles, the collective structural integrity, and the potential for failure at weak points within the array can be also observed. This approach provides a more accurate assessment of how the entire MN array will perform under compressive forces, ensuring that the findings are directly applicable to their intended use in medical applications. Moreover, most studies tend to measure the reduction in needle height rather than directly assessing their strength [187]. Consequently, there are limited studies analysing or determining the compressive strength. By measuring the effective compressive strength of an array rather than just the reduction in needle height offers a more direct evaluation of the material's mechanical robustness and failure thresholds. Furthermore, the ability to determine effective compressive strength significantly enhanced the reliability and accuracy of the DoE for MN optimisation. While effective compressive strength may not be the most important response, its measurable and improvable nature provided a consistent mechanical property to evaluate. This allowed for more precise predictions and refinements during the DoE process, ultimately leading to

better-designed and more reliable MN arrays. The MNs bent rather than broke apart, indicating they deformed plastically under the compressive load. The behaviour observed is consistent with the properties of 316L stainless steel, which tends to exhibit high resistance to fracture and good plastic deformation capabilities, especially in the form of 3D-printed structures that may have unique microstructural characteristics due to the layer-by-layer construction process. The DoE study demonstrated that laser scan parameters significantly impact the properties of 3D printed stainless steel 316L MNs. Specifically, laser power, laser speed, and trace width were found to have substantial effects on the geometrical, structural, and mechanical properties of the MNs. For instance, the interaction between laser power and laser speed notably influenced the tip diameter, with lower laser power and moderate speed achieving smaller diameters, critical for effective skin penetration without causing damage. Furthermore, laser power was a key factor in determining the base diameter and needle height, with higher power levels increasing these dimensions, though not always within clinically acceptable ranges. Surface roughness, an essential factor for comfortable skin penetration and drug loading, was also significantly affected by interactions between laser power and trace width, highlighting the need for precise control over these parameters to achieve optimal MN performance. Thus, within the explored range, laser scan parameters were crucial, and their careful optimisation was necessary to meet clinical and functional requirements of the MNs.

Overall, the DoE study led to a suitable optimisation of the printing parameters with a desirability of 92%. Specifically, the optimal conditions as determined by DoE were laser power of 65 W, laser speed of 650 mm/s, and trace width of 0.09 mm. However, post process is required on the MNs in order to achieve improved surface roughness and reduced tip diameter closer to 50 μm . Low tip diameter is required to ensure that the MN arrays can be easily inserted into the skin without causing any damage on the skin, while the lowest value

obtained after printing MNs using different parameters was greater than 55 μm . Furthermore, the successful penetration of the *stratum corneum* depends also on the height to base ratio of the MNs, for that reason a base diameter lower than 250 μm is required to obtain a ratio of 4:1 taking into account the height of 700 μm achieved. In terms of surface roughness values ~ 1.2 μm are required, indicating low percentage of unmelted particles and sufficient MN penetration and drug loading [188].

To further reduce the tip, base diameter and surface roughness, a second DoE study is required focusing on the analysis and optimisation of the post processing of the MNs such as the electropolishing. Overall, a greater understanding of the process-structure-property relationship and optimisation using the DoE approach was achieved while the developed DoE model is providing a robust framework for quickly determining necessary printing parameters to meet different clinical standards.

Chapter 3: Design and Optimisation of the Electro-polishing Process for the Development of MN Arrays as Drug Delivery System

3.1. Introduction

Following the comprehensive analysis and optimisation of 3D printing parameters, stainless steel MN arrays with desirable geometrical and structural properties were developed within Chapter 2. However, the precise fabrication of small tip diameters and smooth surface finish are limited due to the limitations of the 3D printing technique. Addressing the final geometrical and physical properties, such as surface finishing of devices manufactured using AM is critical for their successful application in the field of TDD. Post-treatments have proven to be critical in achieving the desired surface characteristics for objects fabricated using AM processes. Traditional methods such as grinding, lapping, and mechano-chemical polishing have been widely used [189,190]. However, they have limitations in shaping complex objects with high precision and may introduce residual stress, compromising the durability of components [191].

Electropolishing on the other hand, has shown promise in improving geometrical and morphological properties. The process involves an electrochemical process extensively utilised to enhance the surface finish of AM components by removing material from the surface resulting in smoother surfaces that meet the requirements for various applications. The majority of research in the area of electropolishing has been completed on 316L stainless steel [192,193], although metals such as titanium, copper and nickel have also been studied [194–196]. The electropolishing process reduces surface roughness, surface irregularities (such as unmelted particles and pitting) and non-metallic inclusions without introducing residual stresses into the component, improving the corrosion resistance and functionality of medical implant materials [197–200]. Several studies have used electropolishing to enhance the biocompatibility of cardiovascular stents, predominantly made of 316L stainless steel [201–203]. Specifically, electropolishing was used by Scheerder *et al.* to decrease thrombogenicity and neointimal hyperplasia of coronary stents [204,205]. Moreover, this

method promotes the formation of a more compact and chemically homogeneous passive oxide film, acting as a barrier to the release of cytotoxic ions from the material's surface improving biocompatibility [206,207].

The choice of appropriate process parameters, such as temperature, time and current, is crucial for achieving desirable outcomes in electropolishing. Pendyala *et al.* emphasised the importance of monitoring surface roughness throughout the electropolishing process, providing insights into optimising process parameters for improved results [208]. Similarly, Maciąg *et al.* conducted a comprehensive evaluation of various factors influencing the electropolishing process, such as process duration and the applied current [209]. They assessed the impacts of these parameters on the surface roughness and overall visual appearance of the final products. In a different study, Jin *et al.* investigated the effects of varying temperature and mixing on polishing rate, surface roughness, topography, and chemical composition, enhancing the effectiveness of the electropolishing process [210].

The optimisation of the 3D printing process described in Chapter 2 did not achieve a tip diameter from 1 mm to 25 μm and surface roughness $\sim 1.2 \mu\text{m}$ which are considered as the values acceptable for the design requirements [211,212]. Overall, a reduction in tip diameter and surface roughness is required without negatively impacting on the other parameters such as needle height, etc. Furthermore, post-processing and robustness assessment were performed on the MN arrays to ensure they meet the design criteria and exhibit consistent results across different batches of MN arrays. Moreover, a comprehensive understanding was conducted of how the metallic microstructure and other structural parameters impact the properties and performance of the needles after electropolishing.

3.2. Chapter Aim

This chapter investigates the application of electropolishing to enhance the surface morphology, structural and geometrical properties of 3D printed 316L stainless steel MNs. The overall aim of this chapter is to design, analyse, and optimise the electropolishing parameters of the 3D printed solid metal-based MN arrays in order to meet the design requirements as an efficient TDD system. This was linked to the 3D printing process parameters and conditions and a process–structure–property map was developed.

The specific objectives of this chapter are:

- To determine the influence of electropolishing parameters, specifically time and current, on the geometrical and surface properties of the optimal 3D printed MN arrays from Chapter 2.
- Optimisation of MN geometry and surface/structural morphology using DoE, as well as validation of DoE studies using t-test analysis to compare the optimal predicted values with the actual experimental properties.

3.3. Material and Methods

3.3.1. Electropolishing Process

316L stainless steel MN arrays were 3D printed using the optimised printing parameters as described in Section 2.4.3. Following printing, the MN arrays were further processed using electropolishing to improve their geometrical and surface properties by removing any unmelted particles and reducing the surface roughness (Figure 3.1). Without electropolishing, the surface roughness remains pronounced, which can impede the uniform application and adherence of the drug coating. Also, this roughness can lead to inadequate coverage and potential issues with the functionality of the MNs. Electropolishing not only smooths the surface but also enhances capillary action, ensuring that the coating solution spreads

uniformly across the MNs, providing consistent and effective coverage. Thus, electropolishing is essential to achieve the desired surface quality and coating uniformity, which polymer coatings alone cannot accomplish.

For the electropolishing process the ESMA stainless steel electropolisher (ESMA E1085-1S, IL, USA) was employed to surface treat each MN arrays. During the e-polishing process, the 3D printed samples (anodes) were placed centrally, while the cathodes were located on the outer sides of the anodes at a 20 mm distance. A stainless-steel cathode was employed to withstand the acidic environment of the electropolishing solution and ensure effective polishing. Both anode and cathode were placed within a solution consisting of a mixture of phosphoric acid (75-85%) and sulfuric acid (15-25%). The process was carried out by applying a voltage of 6 volts. The temperature remained constant at 120–125 °C during the e-polishing process, while the time and current ranged from 60 s to 120 s and 3 A to 7 A respectively. The specific ranges for time and current were chosen to cover a broad spectrum of conditions that could potentially optimise the electropolishing process. In particular the range for time was selected taking into account the surface area of the MNs based on previous literature[213,214]. The geometry of the MN arrays, including features such as tip sharpness, base diameter, and overall height, can significantly influence the electropolishing process. On the other hand the range for current was selected taking into account the maximum capacity of the electropolisher.

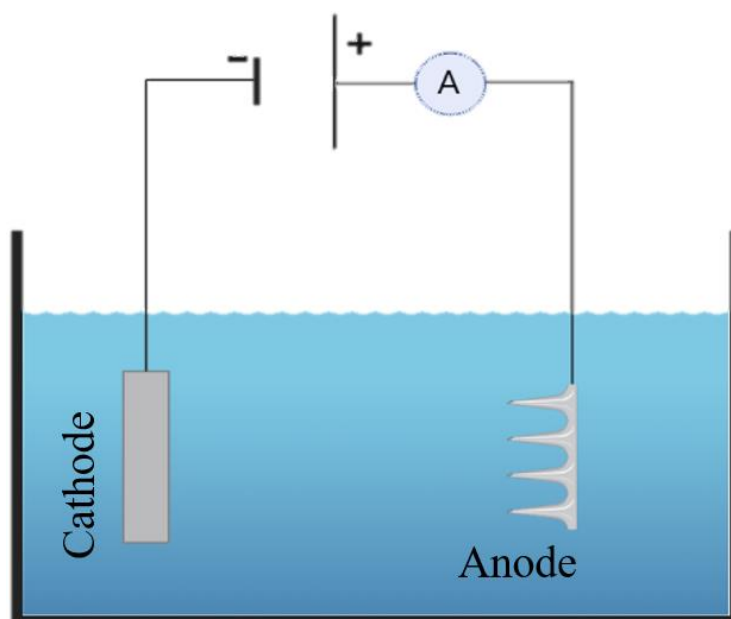


Figure 3.1: Schematic representation of the electropolishing process for MNs– Electropolishing process includes placing a cathodic electrode in the solution and exerting an electric current to cause a reaction between the object surface (anode) and solutions to remove the surface irregularities and impurities.

3.3.2. Analytical Characterisation of the MNs for the Electropolishing DoE Analysis

3.3.2.1 Geometrical Properties: Height, Tip, Base Diameter

The analysis of the geometrical characteristics (needle height, tip and base diameter), of the electropolished MN arrays was conducted using the Keyence VHX–700 ImageJ software as per Section 2.3.4.1. For each MN patch, 24 needles out of 36 were measured from the two outer and the two middle rows of each MN patch.

3.3.2.2 Surface Roughness

To determine the surface roughness (Ra) a non-contact optical surface Bruker Profilometer was employed as per Section 2.3.4.2 for each sample (n=12 needles per patch), which were randomly selected, and average value $\pm$ SD were recorded.

3.3.2.3 Pitting and unmelted particles

For the assessment of the surface quality, quantification of the number of unmelted particles and pitting after electropolishing was conducted. SEM analysis was completed to quantify the number of unmelted particles and the extent of pitting on the surface of the MNs. Sufficient electropolishing is required to remove unmelted particles (Figure 3.2a), but excess electropolishing will result in pitting (Figure 3.2b) on the surface. SEM images (n=12 per array, from three MN arrays) at a magnification of x100 were captured after completing electropolishing according to DoE study.

ImageJ, an open-source image processing software, was used for this quantification. The process involves several steps: first, the SEM images of the MNs are imported into ImageJ. The images are then converted to grayscale to enhance the contrast between the unmelted particles and the rest of the surface. Using the thresholding tool, the particles are isolated based on their grayscale intensity values. Once isolated, the "Analyze Particles" function is used to count and measure the particles, providing data on their number and size distribution. For pitting analysis, the same thresholding and particle analysis techniques are applied to identify and quantify the pits on the surface. Finally the percentage of pitting and unmelted particles on the MN surface was calculated using the (Equation 3.1) and (Equation 3.2). This method allows for precise and reproducible quantification of surface defects, facilitating a detailed assessment of the material's quality and the effectiveness of the 3D printing process.

Equation 3.1: Percentage of pitting

$$\% \text{ of pitting} = \frac{\text{Total Area } (\mu\text{m}^2)}{\text{Area of MNs covered with pitting as per Image J } (\mu\text{m}^2)} 100\%$$

Equation 3.2: Percentage of unmelted particles

$$\% \text{ of unmelted particles} = \frac{\text{Total Area } (\mu\text{m}^2)}{\text{Area of unmelted particles from Image J } (\mu\text{m}^2)} 100\%$$

3.3.2.4 Straightness

SEM images were also used to determine the straightness of the MNs. Straightness is a dimensional tolerance commonly used in manufacturing to ensure that a surface or feature of a part conforms to a perfectly straight line. The straightness is identified as (–) and is calculated as the distance between two parallel lines on either side of a surface line where the surface must lie (2D) (Figure 3.2c) [215]. A straightness of 0 indicates a straight line, while values >0 indicates lower straightness. Image J analysis of the SEM images was used to quantify the straightness in millimetres (mm). Using the "Freehand Line" tool a line was drawn following the outline of the MN, starting from the base to the tip. From the profile plot, points that correspond to the edges of the MN (where the intensity changes indicating the transition from MN to background) were identified. The distance was measured between the two points corresponding to the red lines (Figure 3.2c) at various points along the MN's length, the maximum distance is recorded as the straightness of MNs.

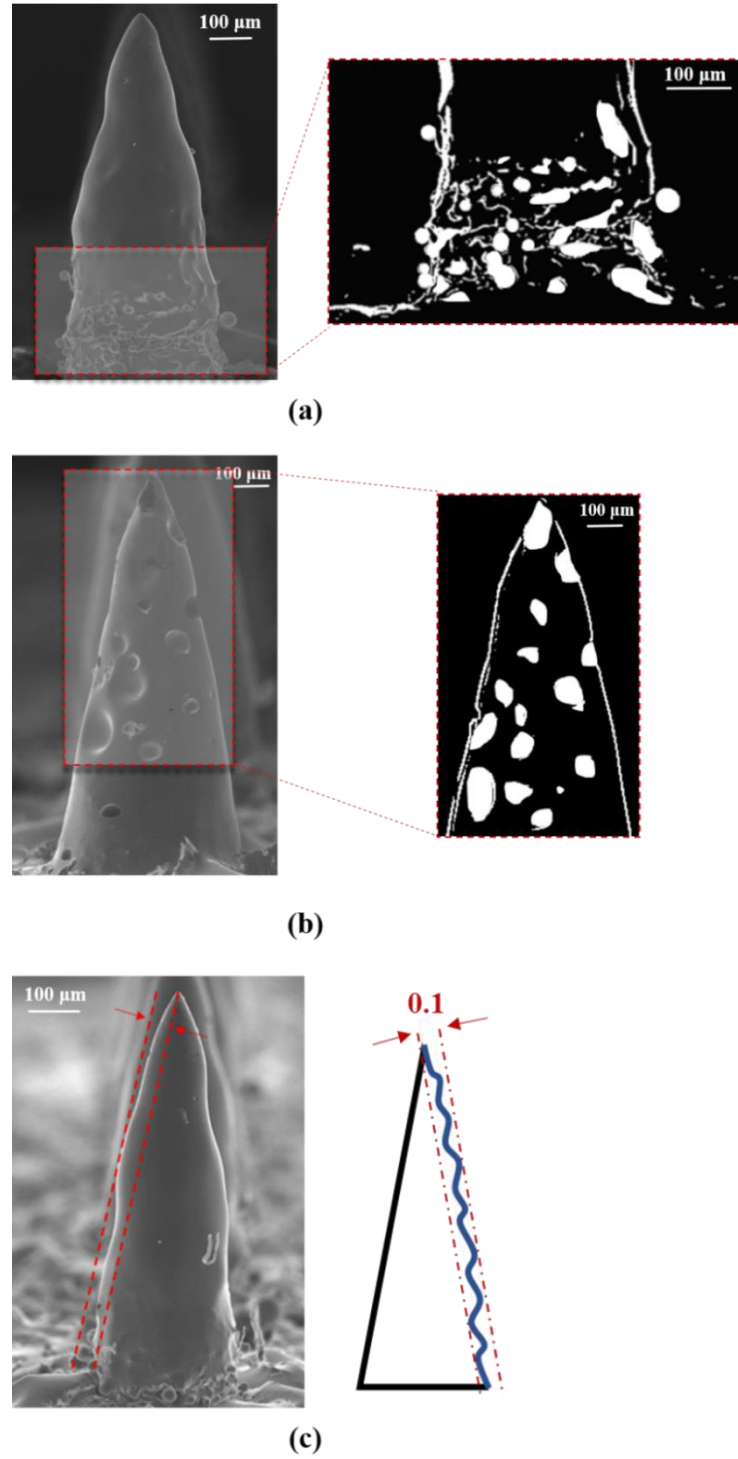


Figure 3.2: Morphological and Structural Analysis – SEM images displaying the percentage of the (a) unmelted particles surrounding the base of the needle and (b) pitting on the surface of the MNs. Zoomed-in images were generated using ImageJ. Unmelted particle and pitting areas are represented in white for visual clarity. (c) ImageJ analysis of MN straightness and a schematic representation of the reference used for the straightness calculation.

3.3.3. Parametric Study

The DoE software (Design Expert V5 software (Stat-Ease Inc. USA) was used to explore the influence of e-polishing parameters on the geometrical and structural properties of the MN arrays. The critical factors affecting the electropolishing process are time and current. A two-level Central Composite Design (CCD) was therefore used, including two factors, time and current (Table 3.1). The CCD resulted in 13 experimental runs, consisting of eight unique design points and five centre points (Appendix 3.1). To effectively optimise the electropolishing process it was necessary to complete an in-depth assessment of the surface properties of the MNs, therefore, six responses were included for the DoE of electropolishing (i) tip diameter, (ii) needle height, (iii) base diameter (iv) surface roughness, (v) unmelted particles and pitting and (vi) straightness. Numerical optimisation was also carried out as described in Section 2.3.1 by assigning each response a level of importance as described in Table 3.2.

Table 3.1: Factors and their levels used for the development of the Central Composite Design for the analysis and optimisation of the electropolishing parameters of MN arrays.

Studies	Factors	Levels
E-Polishing DoE	Current (A)	3, 5, 7
	Time (s)	60, 90, 120

Table 3.2: Requirements including acceptable levels for the DoE numerical optimisation as well as the order of importance.

Responses	Requirements	Importance
Needle height	maximise	**
Tip diameter	minimise	*****
Base diameter	<250 μm	**
Surface Roughness (Ra)	$\sim 1.2 \mu\text{m}$	****
Pitting	minimise	***
Unmelted Particles	minimise	***
Straightness	maximise	***

3.3.4. Analytical Characterisation of the optimal Electropolished MNs

3.3.4.1 Porosity

To determine the physical properties and to investigate whether the electropolishing affected the structural properties of the optimal 3D printed and electropolished MNs, the porosity was determined. Specifically, Nanotom computed tomography (Nanotom CT) (Phoenix Nanotom® M, Texas, USA) scanning was used to quantify the porosity of metallic MNs. Porosity analysis is essential to assess the structural integrity and suitability of MNs for drug delivery applications. The metallic MN sample were secured onto the sample holder, ensuring it was immobilised and oriented appropriately for scanning followed by the placement of the holder within the Nanotom CT scanner. The analysis was conducted within the X-ray energy range of 70-120 kV, with a voxel size in the range of 10-30 μm . As the sample is rotated (360-degree rotation) the CT scan is initiated to acquire a series of X-ray projection images.

Specifically, the images were extracted from Nanotom CT and saved as image stacks. These image sequences were then imported into ImageJ software for the quantification of the volume of porosity for MN array. The process begins by importing the image stack into ImageJ and converting it to grayscale if necessary. A thresholding technique is applied to distinguish between the solid material and the pores. Once the threshold is set, the "3D Objects Counter" plugin can be used to analyze the image stack in three dimensions, providing volumetric data on the porosity. In this binary representation, the black voids within the object correspond to the regions of porosity. The extent of porosity was measured across all the images within the stack using ImageJ's analytical tools.

3.3.4.2 Mechanical Properties

The effective compressive strength of the optimised 3D printed and electropolished MNs was determined to assess if there was a negative effect following adoption of the electropolishing process. Mechanical testing was conducted using the Zwick Materials Testing machine as reported in Section 2.3.4.4. A total of six MNs were compressed and the average strength $\pm$ SD were determined.

3.4. Results

3.4.1. Analytical Characterisation of the MNs for the Electropolishing DoE Analysis

3.4.1.1 Geometrical Properties: Height, Tip, Base Diameter

The geometrical properties after electropolishing ranged as follow: needle height = $770 \pm 70 \mu\text{m}$, base diameter = $290 \pm 50 \mu\text{m}$ and tip diameter = $40 \pm 20 \mu\text{m}$ achieving a reduction of 6.45% for base diameter and 60% for tip diameter while the needle height remained constant. Electropolishing is influenced by several key factors, the primary factors include current density, electrolyte composition, time, and geometry. Current density, the amount of current passing through the material surface, directly affects the rate of material

removal. Higher current densities generally increase the rate at which material is dissolved. The chemical composition of the electrolyte solution, containing acids like phosphoric and sulfuric acid, determines the efficiency and uniformity of the polishing process. Additionally, the duration for which the current is applied influences the extent of material removal; longer times can result in more significant material removal but must be controlled to avoid excessive erosion.

Lastly, the geometry of the MNs, including surface area and shape, plays a critical role. Complex geometries might result in uneven material removal due to varying current distribution which is also the reason that the tip of the MN becomes sharper while the height remain the same. Due to the nature of the electropolishing process, removal of material from protruding areas (like the tip) is more efficiently than from recessed areas. This phenomenon occurs because the electric potential field is more concentrated at sharp points and edges. This concentration results in a higher current density at the tip compared to the rest of the surface, leading to more rapid material removal at these points. While the overall surface area might be high, the sharp tip has a much higher local current density compared to flat or less pronounced areas. This higher local current density causes material to be removed more efficiently from the tip, leading to a sharpening effect rather than a blunt erosion. The ANOVA analysis after electropolishing produced significant linear models for the different geometrical variables investigated. Both time and current were found to have a significant ($p\text{-value} < 0.1$) impact on the MN dimensions. Specifically, a reduction in all the geometrical characteristics was observed after increasing electropolishing time (Figure 3.3a) and similarly with increased current (Figure 3.3b). When the current is increased from 3 A to 7 A, the electropolishing of MNs resulted in the smallest possible tip diameter of $22.7 \pm 9.2 \mu\text{m}$ after 90 sec. Furthermore, the reduction in base diameter of the MNs achieved a height:base diameter ratio closer to 4:1, thus achieving geometrical characteristics that meet

the clinical requirements, as demonstrated in the SEM images (Figure 3.3c). Due to the smaller dimensions, the electropolishing improved also penetration efficacy and success rate during transdermal applications [216–218]. For instance, according to Gill *et al.* the decrease of MNs length and the sharper tip of MNs reduces pain [92].

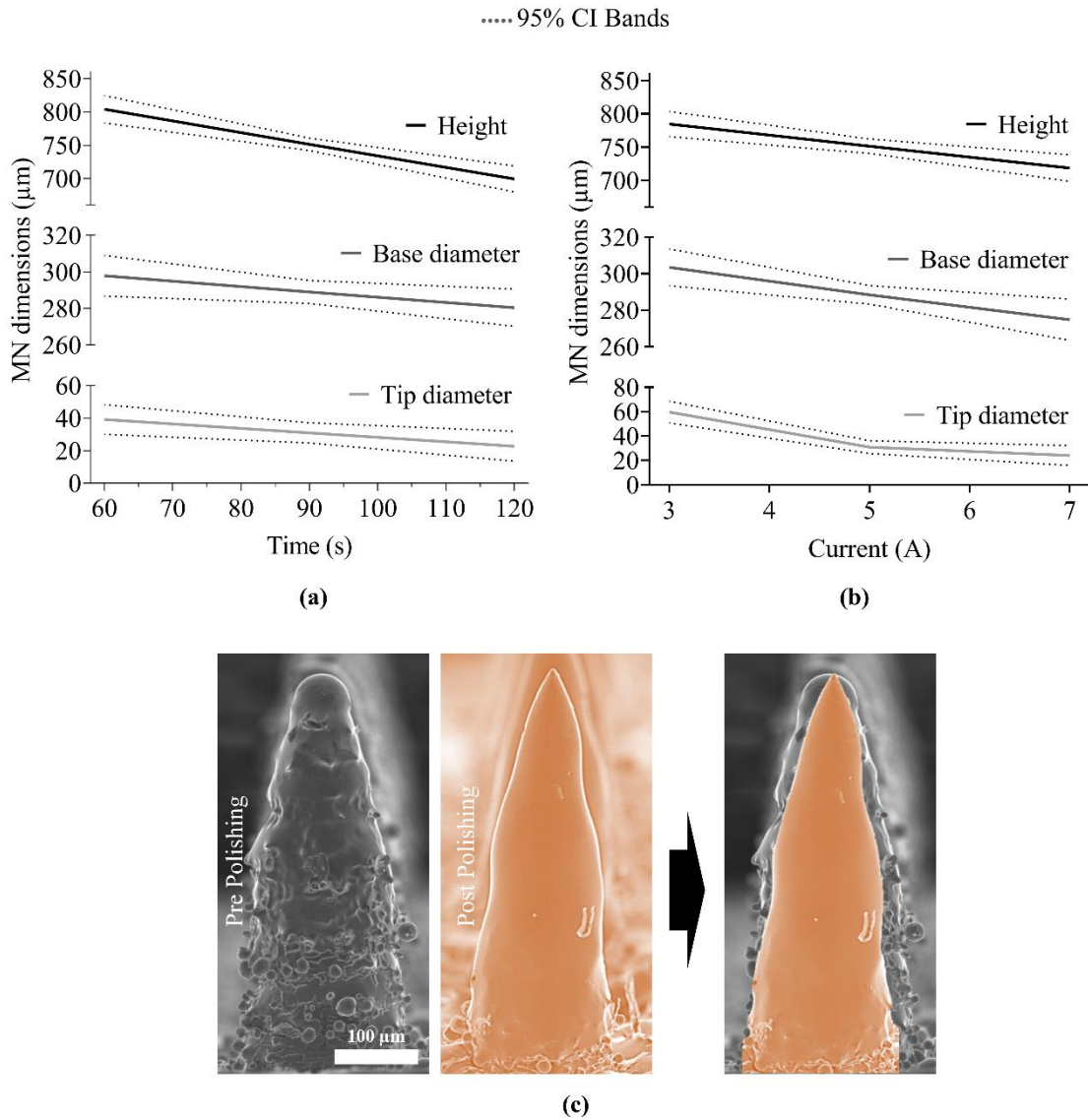


Figure 3.3: DoE Individual Factors Analysis for the Geometrical Properties - The ANOVA analyses demonstrated that both (a) time and (b) current significantly influence the needle height, base diameter and tip diameter of the MNs. (c) SEM images comparing the needles pre and post electropolishing presenting the decrease of the tip and base diameter. 95% CI= confidence interval.

The DoE analysis demonstrated significant interactions between time and current applied during the electropolishing process influenced the resulting height (p-value<0.001), tip (p-value<0.01) and base diameter (p-value<0.01) of the MNs. Figure 3.4a shows the impact of different current levels on MN height. When MNs were electropolished using the lowest current setting (3 A), no significant effect on height was observed, as it remained relatively constant at $781.4 \pm 11.2 \mu\text{m}$. However, utilising the highest current setting (7 A) led to a significant reduction in MN height over time, decreasing from $815.7 \pm 25.3 \mu\text{m}$ after 60 s to $621.2 \pm 23.1 \mu\text{m}$ after 120 s. Additionally, the interaction between time and current had a noteworthy influence on the tip diameter (Figure 3.4b) and base diameter (Figure 3.4c) of the MNs. Electropolishing at 3 A resulted in a tip diameter of $64.3 \pm 14.2 \mu\text{m}$ and a base diameter of $300.2 \pm 12.5 \mu\text{m}$, whereas employing a current of 7 A led to a substantial reduction in both the tip diameter ($24.5 \pm 10.5 \mu\text{m}$) and the base diameter ($275.3 \pm 13.1 \mu\text{m}$) of the MNs. These findings highlight the importance of considering the interactions between different factors in the electropolishing process, as they significantly influence the dimensional characteristics of the MNs. Such insights are valuable for optimising the electropolishing parameters to achieve desired MN dimensions.

3.4.1.2 Surface Roughness

In the present study, the surface roughness of the fabricated metallic MNs reported an average roughness value of $\sim 1.23 \mu\text{m}$ after electropolishing. The DoE analysis demonstrated that both time and current affected significantly (p-value<0.01) the surface roughness. As the electropolishing time increases from 60 s to 120 s, there is a consistent decrease in surface roughness as can be seen in Figure 3.5a. This suggests that longer electropolishing durations result in smoother surfaces. The surface roughness value decreases from approximately $4.8 \mu\text{m}$ at 60 s to roughly $3.6 \mu\text{m}$ at 120 s. A decrease in surface roughness is also observed as the applied current increases (Figure 3.5b), indicating that a higher current tends to produce

a smoother surface finish. Specifically, at a current of 3 A, the surface roughness stands at approximately $4.6\text{ }\mu\text{m}$, which decreases to around $3.5\text{ }\mu\text{m}$ when the current is set to 7 A. The evaluation of surface roughness is a pivotal aspect in the characterisation of metallic MNs, as it significantly influences their functional and performance attributes. This low value refers to a notably smooth surface profile (Figure 3.5c).

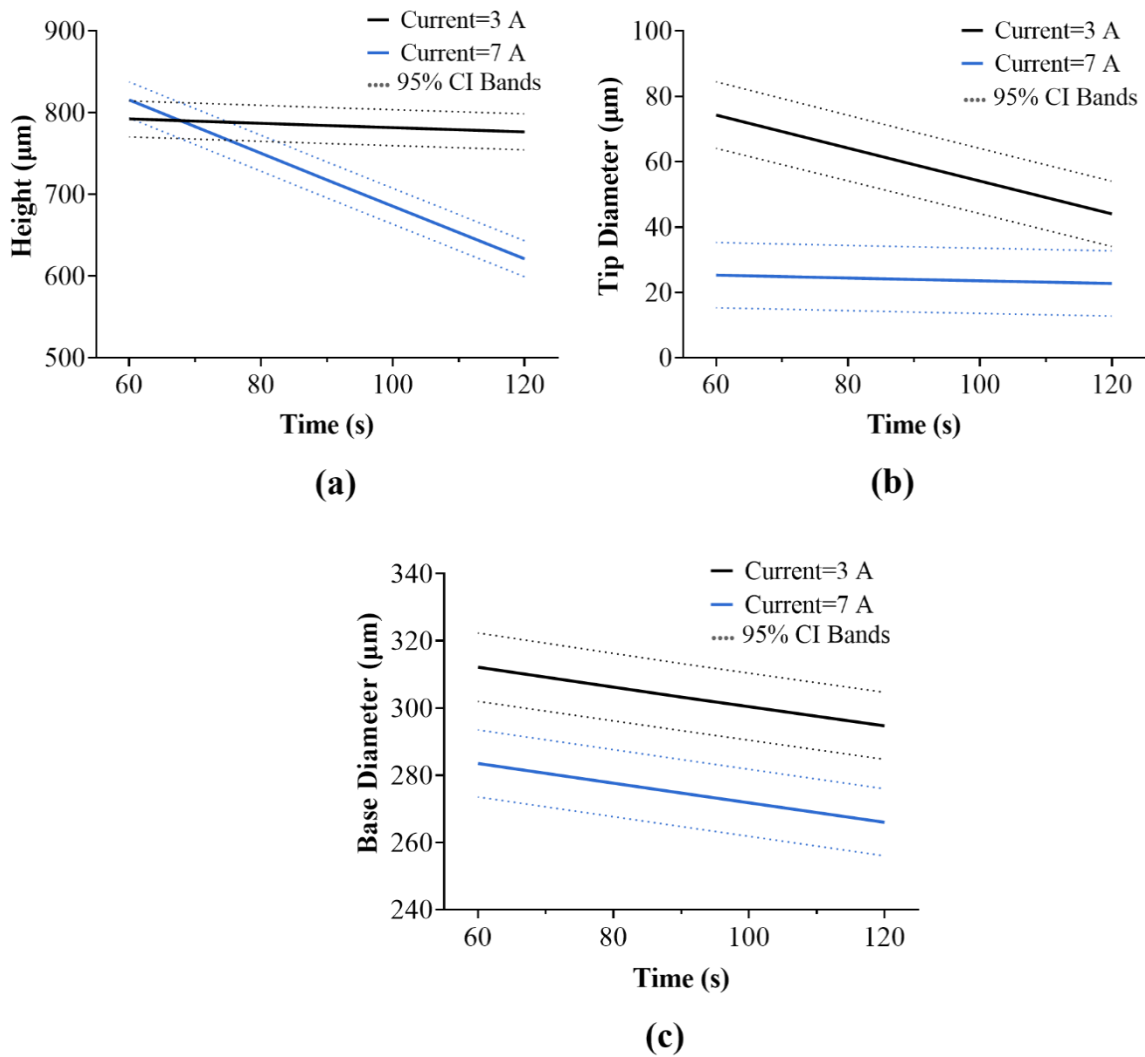


Figure 3.4: DoE Interactions of MN Arrays Geometry – The DoE analysis identified the significant influence of the time and current interaction on the resulting (a) needle height, (b) tip diameter and (c) base diameter showing their combined influence. 95% CI=confidence interval.

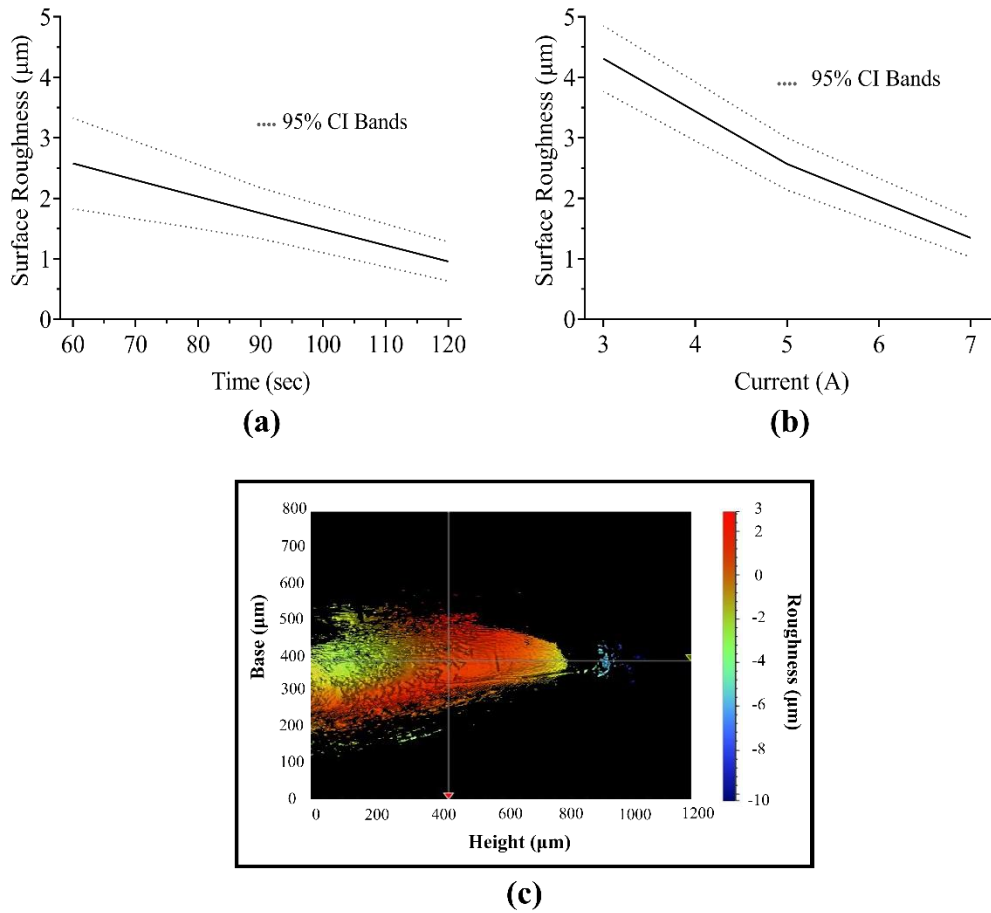


Figure 3.5: DoE Surface Roughness – Analysis of the impact of electropolishing (a) time and (b) current in surface roughness and (c) roughness distribution on the MN surface, indicating a notably smooth surface. 95% CI=confidence interval.

3.4.1.3 Pitting and Unmelted Particles of MN Arrays

The surface quality was assessed by quantifying the presence of pitting and unmelted particles on the MN surfaces. As the electropolishing time and current increases, the percentage of pitting was found to range between 1.99-4.98%, while the percentage of unmelted particles ranged from 5.9-12.1%. Applying electropolishing for longer durations and at higher currents clearly reduces the number of unmelted particles on the surface.

The results from the DoE study indicated that both time and current significantly affect ($p\text{-value}<0.001$) the surface quality of the MN arrays. A correlation can be observed between these two responses, where an increase in time and current led to decreased unmelted particles while increasing pitting on the surface (Figure 3.6a and Figure 3.6b). Improved surface finishing and quality were observed after electropolishing for 120 s at 7 A with only 5% pitting observed.

In addition to the individual effect of each factor, the DoE analysis demonstrated a significant interaction between time and current, impacting both the percentage of pitting and the presence of unmelted particles on the MNs. Figure 3.7a demonstrates that when MNs underwent electropolishing under the highest current condition (current=7 A), the occurrence of pitting and defects on the MN surface increased significantly, covering approximately 10% of the MN surface. Conversely, electropolishing at lower current levels caused less damage to the MNs, resulting in less pitting. Additionally, it was observed, as expected, that electropolishing at high current (7A) can significantly reduce the number of unmelted particles (Figure 3.7b) with only approximately 5% of unmelted particles remaining. By considering these two interactions, an expected interrelationship was obtained between the percentage of pitting and the presence of unmelted particles following the electropolishing process on the MNs.

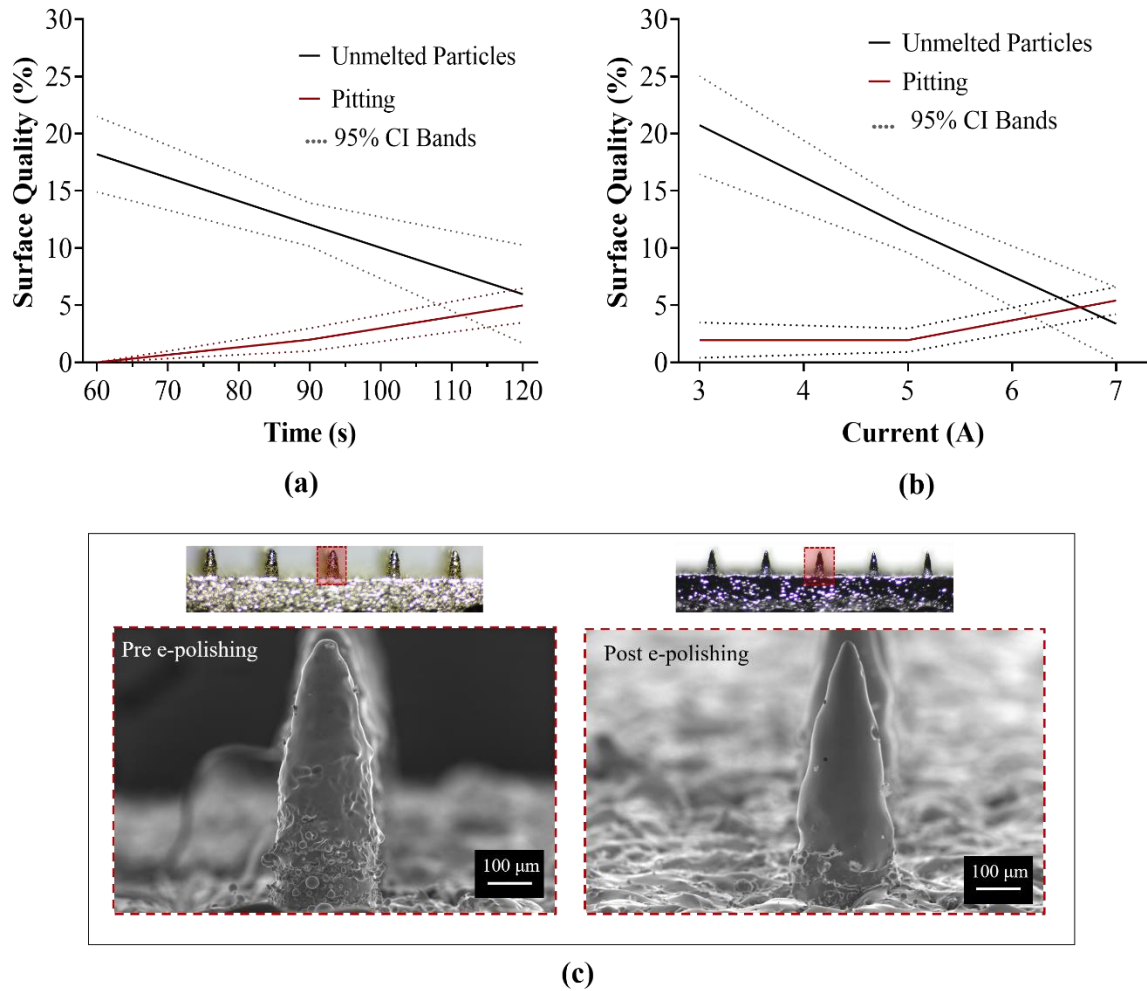


Figure 3.6: DoE Individual Factors Analysis for the Surface Quality – Influence of (a) time and (b) current on surface quality of MNs, including percentage of unmelted particles and pitting. (c) SEM images illustrating reduction of unmelted particles and surface roughness improvement due to the electropolishing process. 95% CI: confidence interval.

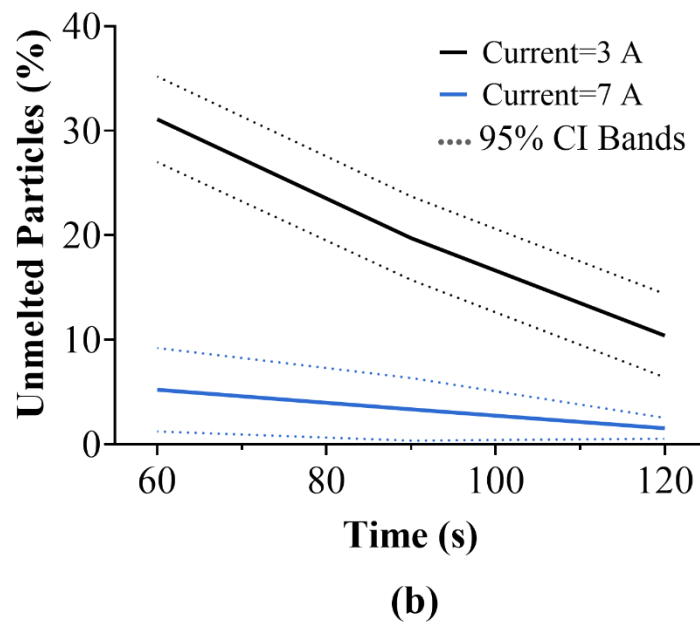
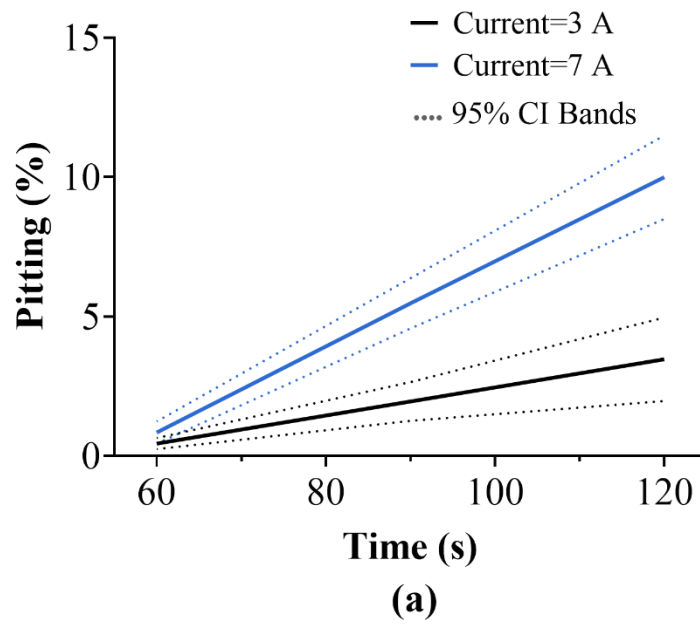


Figure 3.7: DoE Interactions of MN Arrays Surface Quality - DoE analysis identified the significant influence of the time and current interaction on the resulting percentage of (a) pitting, (b) unmelted particles on the surface of the MN. 95% CI=confidence interval.

3.4.1.4 Straightness of MNs

Straightness results as a function of the electropolishing parameters ranged from 0.03-0.06 mm. DoE analysis demonstrated that only current affected the straightness significantly ($p\text{-value} < 0.01$). Furthermore, electropolishing under a current higher than 5 A improved the straightness of the needle as indicated by the straightness value being closer to zero as per Figure 3.8.

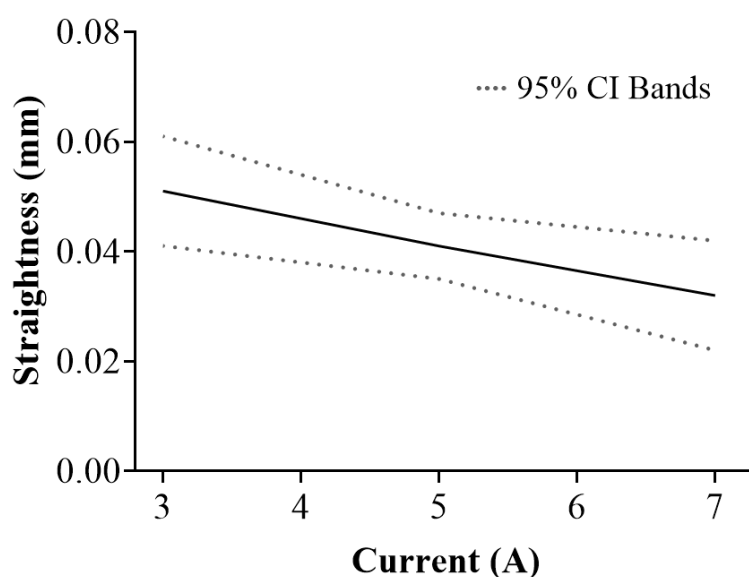


Figure 3.8: DoE Individual Factors Analysis for the Straightness of the MNs – DoE for electropolishing parameters demonstrating the impact of the most significant factor current on the straightness of MNs. 95% CI: confidence interval.

3.4.2. DoE Optimisation and Validation

The results for the 316L stainless steel electropolished MNs are presented, focusing on key parameters such as pitting, unmelted particles, and straightness. These factors are essential in evaluating the performance of MNs for skin penetration and drug delivery, ensuring their effectiveness and safety. Taking into account the design requirements and desired outcomes for MN quality (Table 3.3) the DoE presented the optimal parameters as duration of 60 s and a current of 7 A, yielding a desirability rating of 100%, signifying a successful optimisation

process. To validate the accuracy of the DoE models, the predicted response values from the optimisation phase were compared with the actual experimental values (Table 3.3). A non-significant difference (p-value=0.1) between the values, and the percentage of difference between the values was also found to be non-significant. This validates the accuracy of the DoE model.

The resulting values for height ($766.4 \pm 10 \mu\text{m}$), base diameter ($283.6 \pm 11.9 \mu\text{m}$), and tip diameter ($24.4 \pm 5.7 \mu\text{m}$) were within the design ranges, indicating that the MN array is suitable for painless and easy application as a TDD system. Overall, both the geometric values and the SEM images, which demonstrate surface and structural quality post-printing and electropolishing, confirm that the MN arrays possess micro-sized dimensions.

The SEM images also highlight the ability of electropolishing to enhance surface finish and quality. Pitting refers to the formation of small holes or pits on the surface of the material, which can potentially compromise its structural integrity and performance [219]. Specifically quantitative analysis demonstrated percentage of pitting $\sim 0.64 \pm 0.1\%$, indicating a relatively low level of corrosion for the material. The unmelted particles in the MNs were determined to be $4.47 \pm 0.51\%$, however the unmelted particles were mainly found on the patch of the MNs, which indicates that the needles are smooth. Furthermore, a correlation can be observed between these two responses, where an increase in time and current led to decreased unmelted particles while increasing pitting on the surface. The obtained straightness value of $0.036 \pm 0.01 \text{ mm}$ indicated that the MNs have a low level of deviation from the perfectly straight shape. This property holds particular significance for applications that require accurate and efficient penetration of the skin.

Overall, as illustrated in Figure 3.9 the geometrical and structural parameters of the MN arrays, obtained under the optimal electropolishing levels identified by the DoE, demonstrated

values within the desired design criteria. The mean tip diameter and mean height of the MNs fall within the desired clinical ranges, indicating that the optimisation process did not influence these critical dimensions. Specifically, the mean tip diameter is well within the required range, ensuring sharpness suitable for penetration, while the mean height meets the target, crucial for effective drug delivery. Furthermore, the mean surface roughness and mean base diameter also met the design requirements after DoE and electropolishing optimization. This indicates that the electropolishing, was successful in achieving the smooth surface and appropriate base diameter necessary for optimal MN performance. The surface roughness is sufficiently low to ensure the uniform application and adherence of drug coatings, and the base diameter is within the acceptable range to provide mechanical stability and ease of insertion. This confirms the efficacy of the optimisation process and underscores the successful application of DoE in enhancing the precision and quality of the electropolishing process for MN fabrication.

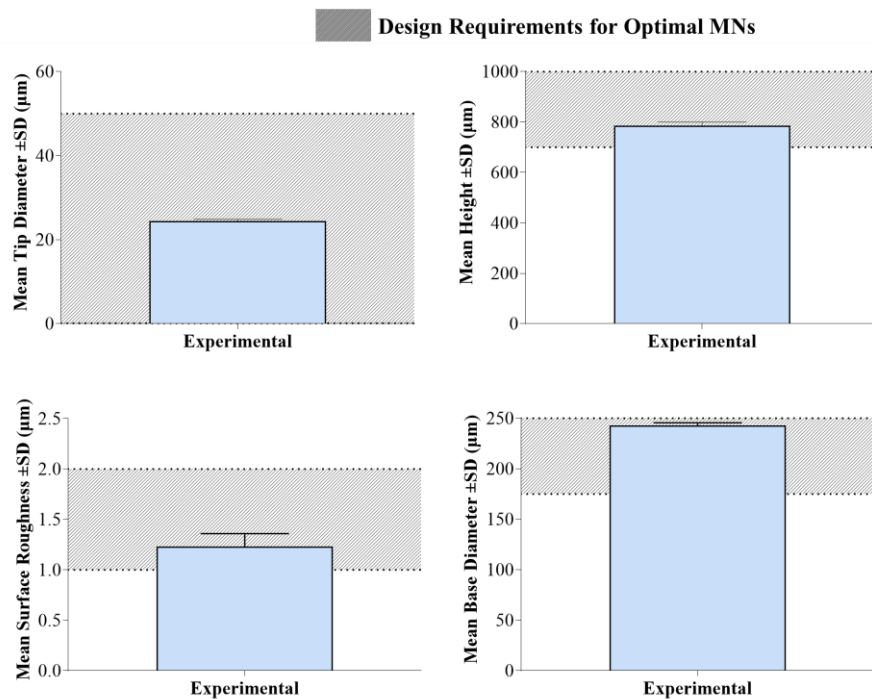


Figure 3.9: Experimental Results of MN Properties Post-Electropolishing DoE Optimisation Compared to Design Requirements.

3.4.3. Analytical Characterisation of the optimal Electropolished MNs

3.4.3.1 Porosity

In addition to the ability of the electropolishing process to effectively enhance the material's structural properties, such as reduced surface roughness, it was also observed that it did not adversely affect the porosity of the material. This aspect is crucial, as maintaining solid MNs is essential for the intended applications. The measured porosity was determined to be 9.40% predominantly within the patch (bottom area that the MNs are embedded) (Figure 3.10a). A non-uniform distribution of voids was observed, with the majority being concentrated within the patch of MNs. On the other hand, as expected the MNs exhibited a solid structure without any observable porosity (Figure 3.10b). This outcome highlights the effectiveness of the manufacturing process in producing a coherent and dense network of material in the needle regions.

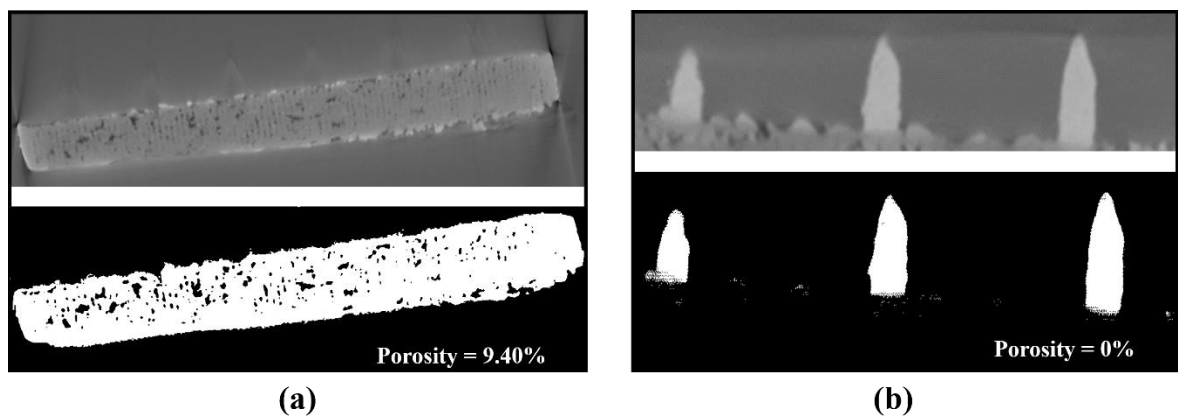


Figure 3.10: Porosity of Optimal MNs – Percentage of porosity within (a) the structure of the patch and (b) MNs showing porosity only in the patch.

Table 3.3: DoE optimisation of the geometrical and structural properties of MN arrays and validation of the DoE model accuracy comparing the actual experimental values with the predicted values obtained from the optimisation of DoE analysis.

<u>DoE Electropolishing Optimal Levels of the different Factors</u>				
Electropolishing Time=60 s		Electropolishing Current=7 A		
Responses	Clinical Requirements	DoE Prediction	Experimental Values	Percentage Difference (%)
Tip diameter	< 50 μm	$25.5 \pm 0.5 \mu\text{m}$	$24.36 \pm 0.5 \mu\text{m}$	4.57
Needle height	$700 \mu\text{m} < h < 1,000 \mu\text{m}$	$811 \pm 3 \mu\text{m}$	$786 \pm 5 \mu\text{m}$	3.08
Base diameter	$175 \mu\text{m} < B < 250 \mu\text{m}$	$263 \pm 2 \mu\text{m}$	$243 \pm 2 \mu\text{m}$	7.94
Surface Roughness	Minimise (approx. 1.2 μm)	$1.52 \pm 0.71 \mu\text{m}$	$1.23 \pm 0.43 \mu\text{m}$	21.09
Pitting	minimise	$0.84 \pm 0.05\%$	$0.64 \pm 0.1\%$	27.03
Unmelted Particles	minimise	$5.2 \pm 0.2\%$	$4.47 \pm 0.51\%$	15.09
Straightness	minimise (goal=0 mm)	$0.032 \pm 0.01 \text{ mm}$	$0.036 \pm 0.01 \text{ mm}$	11.76

3.4.3.2 Mechanical Properties

The impact of electropolishing on the effective compressive strength was assessed and no significant changes were detected post-polishing because the process primarily affects the surface layer without altering the bulk properties of the material. Structural integrity was maintained, with the material exhibiting an effective compressive strength of approximately 0.8 ± 0.2 GPa. Electropolishing smooths the surface by removing a thin layer of material, typically around 10-20 μm , which is insufficient to impact the internal microstructure and mechanical properties significantly. The effective compressive strength is largely determined by the bulk material characteristics, such as grain size, phase composition, and overall height to base ratio, which remain unaffected by the electropolishing process. This suggests that the material's mechanical properties remain consistent, highlighting its potential for applications that require a balance between surface finish and structural strength.

The optimal metallic MNs demonstrated high effective compression strength ensuring their ability to effectively deliver drugs into the skin without experiencing tip fracture during the penetration process. Compared to the broader MN literature, metallic MNs demonstrate a mechanical superiority over commonly investigated polymeric MNs [172,182]. For example, a study by Du *et al.* reported the compressive strength of polymeric MNs to be in the range of 72–75 MPa, significantly lower than our observed values [168]. This highlights the potential of metallic MNs, especially in applications that require higher mechanical durability, such as tissue penetration without the fracture of the tip. The low percentage of pitting and unmelted particles, coupled with a high mechanical properties indicated the ability of the MNs to withstand the mechanical stress associated with skin penetration, without fracture [220].

3.5. Discussion

This chapter presents a comprehensive investigation into the optimisation and validation of the electropolishing process for 3D printed MN arrays, with a primary focus on their surface

geometry and structural properties, and their clinical relevance in TDD applications. Electropolishing was explored as a potential solution, known for its capability to reduce surface roughness, enhance corrosion resistance, and improve biocompatibility of medical implants [197–199]. Specifically, a DoE study was conducted to explore the influence of current and time during electropolishing on the geometrical and surface properties of the optimised 3D printed MN arrays from Chapter 2 and to optimise the electropolishing process to improve the geometrical and surface properties of the MNs and achieve MNs that meet the clinical requirements.

While the optimised 3D printing protocol successfully met several clinical requirements, certain geometrical and structural characteristics of the MN arrays demanded additional analysis and optimisation. Thus, the responses explored in this study focused on the geometrical and surface properties of the MN arrays. The study aimed to maintain the tip, base diameter and surface roughness within the clinical ranges as achieved from the optimised 3D printing protocol while achieving the required tip diameter improving the surface properties of the MNs.

The further post-processing step of electropolishing and the investigation of its influence on the surface quality and geometrical characteristics of the MNs presented valuable insights. The results from the DoE study demonstrated that both time and current during electropolishing enhanced the overall quality, geometrical characteristics (*e.g.*, base and tip diameter) and performance of 3D printed metallic MNs, aligning them with clinical requirements. Specifically, subjecting the MNs to electropolishing for 120 sec at 7 A enabled the manufacture of MN at their smallest dimensions, featuring a needle height of $699.3 \pm 19.5 \mu\text{m}$, tip diameter of $22.7 \pm 9.2 \mu\text{m}$, and base diameter of $280.4 \pm 10.2 \mu\text{m}$. Due to the smaller dimensions, improved MN's penetration efficacy and success rate of TTD applications can be achieved [216–218]. In addition to the reduced MN dimensions the

surface quality was also improved. Assessment of the surface quality quantified the number of unmelted particles and pitting after electropolishing demonstrated a correlation, wherein an increase in both time and current led to reduced surface roughness and improved needle straightness. Even if, a small number of pitting appeared after electropolishing, the amount was limited so no effect was indicated on needle quality, mechanical properties or wettability [221,222]. Also, it was found that the electropolishing process did not influence the mechanical properties while improved the morphological and surface properties. Specifically, effective compressive strength of ~ 0.8 GPa was obtained for MNs pre and post electropolishing which is significantly higher ($p\text{-value} < 0.05$) compared to commonly used polymeric MNs with values ranging from 72 to 75 MPa [168,172,182].

The low percentage of pitting and unmelted particles, coupled with a high compression strength indicated the ability of the MNs to withstand the mechanical stress associated with skin penetration, without fracture [220]. Moreover, the minimal deviation from the ideal straight configuration ensures the accuracy and efficiency in skin penetration, effectively reducing insertion force and minimising tissue damage.

Both DoE studies were designed to ensure the optimal MN arrays met the clinical performance criteria and provided a robust and repeatable manufacturing process. The electropolishing process parameters were linked back to the parameters and conditions inherent to the 3D printing process in the development of a comprehensive process-structure-property map. The predicted optimal levels for 3D printing were a laser power of 65 W, laser speed of 650 mm/s, and trace width of 0.09 mm. In parallel, the optimal electropolishing parameters were duration of 60 sec and a current of 7 A. Subsequently, the optimised MNs exhibited improved surface finish and quality, providing MN arrays with clinically acceptable micro-sized dimensions for easy and potential painless application [223,224].

In conclusion, this chapter successfully addressed its primary aim, which was to contribute to a more comprehensive understanding of the electropolishing factors affecting the responses of the metallic MNs. Optimisation through DoE allowed the identification and development of optimal 3D printed metallic MNs, aligning with design requirements for geometrical mechanical and structural properties. Essentially, the adaptability of a DoE model extends its relevance beyond single-use optimisation. As design requirements change, the developed DoE model can be recalibrated appropriately, eliminating the need to start the analysis from scratch. Thus, it not only facilitates in-depth initial analysis and understanding of the key process parameters affecting the final properties of MNs, but also creates a standard tool for development of 3D printed MNs.

These studies can be considered as a preliminary design to understand the factors and levels that had the greatest impact on fabrication of the MNs. Given the promising properties, the optimal printed and electropolished MNs were selected for further analysis including their ability to penetrate the skin and deliver drug. This will confirm its suitability for clinical use as a TDD system.

Chapter 4: Development and Characterisation of Optimal MN Arrays as a Drug Delivery System

4.1. Introduction

Having optimised the 3D printing and post-processing processes for the fabrication of MNs that meet the design requirements for TDD, the next stage in the development process was to evaluate the ability of MNs to penetrate the skin without excessive forces through functional testing. This evaluation combines critical aspects like skin penetration, dry drug elution, and their potential to enhance efficacy, efficiency, ease of penetration (potentially painless), as well as the loading and rapid release of drugs.

TDD systems offer a non-invasive alternative to traditional drug administration methods. The development of MNs has enhanced the efficiency of TDD by enabling the delivery of therapeutic agents across the skin's *stratum corneum*, which is the primary barrier to drug penetration [47]. A fundamental advantage of MNs is their ability to bypass the skin's outermost layer, creating micro-channels that facilitate the enhanced delivery of drugs [51,225]. The efficacy of this penetration is typically assessed through drug elution studies or *in vitro* and *in vivo* skin penetration studies, which often employ techniques like confocal microscopy to visualise the depth and distribution of drug penetration on different type of skin [13,226]. For instance, porcine ear skin is commonly used as a human skin substitute providing reliable and ethical means for conducting investigations, particularly in drug delivery, dermatology, and wound healing research [13,227,228]. Furthermore, the use of Parafilm® as a skin substitute has proven to be a valuable approach in various biomedical studies and experimental settings [229]. The use of Parafilm ® allowed for the use of a reliable and consistent skin-like substitute, which could be adopted for MN insertion tests, drug delivery studies, and other investigations requiring a skin-simulating medium. SynTissue® also is a synthetic adult skin tissue designed to closely resemble human tissue in terms of mechanical and anatomical properties.

Drug loading in MNs is pivotal as it determines the therapeutic dose suitable for transdermal delivery. Various strategies, such as coating the MN surface with the drug, encapsulating the drug within dissolvable MNs, or integrating the drug into hydrogel-forming MNs, have been investigated [230]. Once the MNs penetrate the skin, the release of the loaded drug depends on the diffusion, dissolution, or a combination of both. Factors affecting drug release include MN material, drug molecule size, and the surrounding skin environment [231]. Release kinetics studies are essential to ensure sustained and controlled drug release, maintaining therapeutic drug concentrations in the systemic circulation.

While some clinical requirements of MNs are met using optimised fabrication protocols, specific geometric and structural characteristics need further analysis. It is important to achieve the desired drug loading without compromising other MN properties like mechanical strength or penetration efficiency.

4.2. Chapter Aim

The aim of this chapter was to conduct a comprehensive analysis of the functional properties exhibited by the optimal metallic-based MN arrays, as identified in Chapter 2 and Chapter 3. Specifically, skin penetration and dry drug elution were determined to investigate whether they meet the clinical requirements as an efficient TDD system.

The specific objectives of this chapter were the:

- Analytical assessment of the functional characteristics of optimal MNs using penetration testing into different types of synthetic and natural skin.
- Development of a drug loaded MN array and assessment of drug elution.

4.3. Material and Methods

4.3.1. Fabrication of optimal MN Arrays

Solid metallic MN arrays consisting of a square patch (10x10 mm) with 36 conical solid MNs per patch were 3D printed as per the optimal 3D printing and electropolishing parameters defined in Chapter 2 and Chapter 3. The properties of these MNs are as follows: average needle height of $786.3 \pm 5.8 \mu\text{m}$, base diameter of $263.1 \pm 2.5 \mu\text{m}$ and tip diameter of $25.5 \pm 0.5 \mu\text{m}$. To provide visual insight, the optimised 3D printed MN arrays showing the arrangement, alignment, and structural integrity was assessed using optical microscopy (Figure 4.1).

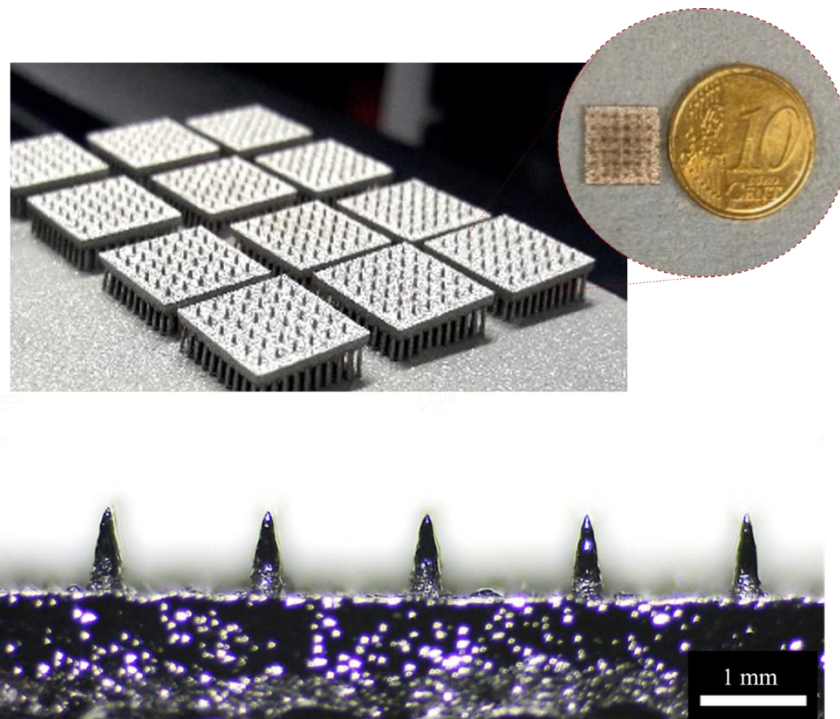


Figure 4.1: Optimal 316L-SS MN Arrays – 3D printed metallic MNs post-3D printing (upper image) and post electropolished as visualised under an optical microscope (lower image). Conical MNs were fabricated with the geometrical characteristics meeting the clinical specifications. The polishing process enhances the surface smoothness and clarity of the MNs.

4.3.2. Porcine Skin and Skin Substitutes Preparation

To assess the functional properties of the optimal MN arrays penetration testing was conducted using synthetic and natural porcine skin types. Specifically, three different skin types were used: (i) multilayer Parafilm® (M Laboratory Wrapping Film, Fisher Scientific, UK), (ii) synthetic skin (SynTissue®, SynDaver Labs, Tampa, Florida, USA) and (iii) pig skin (Local Butcher, Dublin, Ireland).

During the preparation steps, 20 square layers of Parafilm®, each measuring 20 mm x 20 mm x 2 mm, were carefully cut and stacked on top of each other to create a multi-layered sample. The layers were securely fixed using forceps, ensuring minimal movement during experimentation as per literature [229]. In terms of the preparation the SynTissue® samples, rectangular sections measuring 20 mm x 20 mm x 2 mm were carefully cut from the bulk material. The sections were then treated to remove any imperfections or air bubbles, ensuring uniformity and consistency. Prior to experimentation, the SynTissue® samples were hydrated by soaking them in phosphate-buffered saline (PBS) for 24 h to achieve an optimal physiological state. Finally, porcine ears skin sections were carefully excised to remove hair and subcutaneous fat. Skin samples of 20 mm x 20 mm x 2 mm were collected from each ear of the pig. After microscopic examination to ensure the absence of any remaining hair, any residual hair was meticulously removed using a scalpel. The skin samples were then preserved by soaking in PBS and stored in foil-wrapped containers in a freezer. Prior to experimentation, the frozen skin samples were thawed in PBS in a refrigerator for 24 h to ensure full defrosting and rehydration. The samples were then touch dried with tissue before being used for the penetration tests.

4.3.3. Functional Properties: Penetration test

The penetration test was conducted to determine the functional properties of optimal MNs to analyse and quantify the ability of the patch to penetrate the *stratum corneum* of different

synthetic and natural skin types [229]. The Zwick Machine equipped with a 500 N load cell was used to insert the MN arrays into three different skin types, *i.e.*, multi-layered stacks of the Parafilm®, SynTissue®, and the porcine skin. The test samples (n=6) were placed on the base probe and the MNs were attached to the load cell and inserted into the skin at a rate of displacement of 0.5 mm/min. To ensure insertion, into the skin samples, a max force of 20 N was applied for 30 s on the MNs as per previous literature [229] (Figure 4.2). After the loading period, the MN patch was removed, and both needle and skin sample were examined for any visible damage or signs of penetration. The force at penetration was recorded and identified as the first peak on the stress-strain graph, representing the maximum force required for the successful penetration of the MNs into the skin.

The penetration depth was measured using the white-light Keyence 3D digital microscope (KEYENCE Ltd, UK). Initially for parafilm, each layer was examined to confirm the presence of holes, indicating successful penetration. The number of layers penetrated provided a measure of depth. For synthetic and porcine tissue, cross-sectional samples were prepared post-penetration. These samples were then examined under Keyence microscopy to accurately measure the penetration depth.

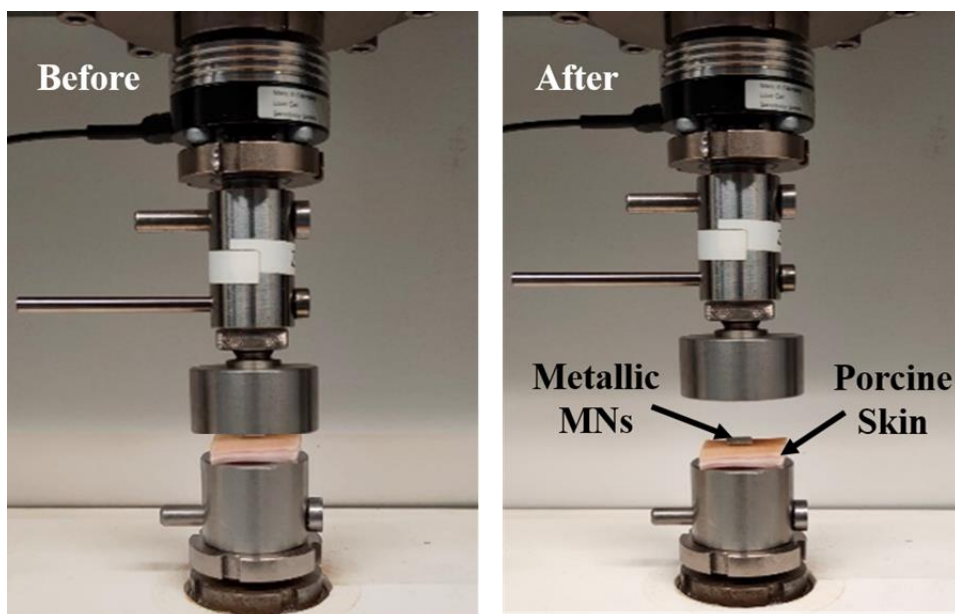


Figure 4.2: Penetration Test on Porcine Skin – Set up of 500 N load cell, for the insertion of MN arrays in the porcine skin by applying a max force of 20 N for 30 s (Before) achieving skin penetration (After).

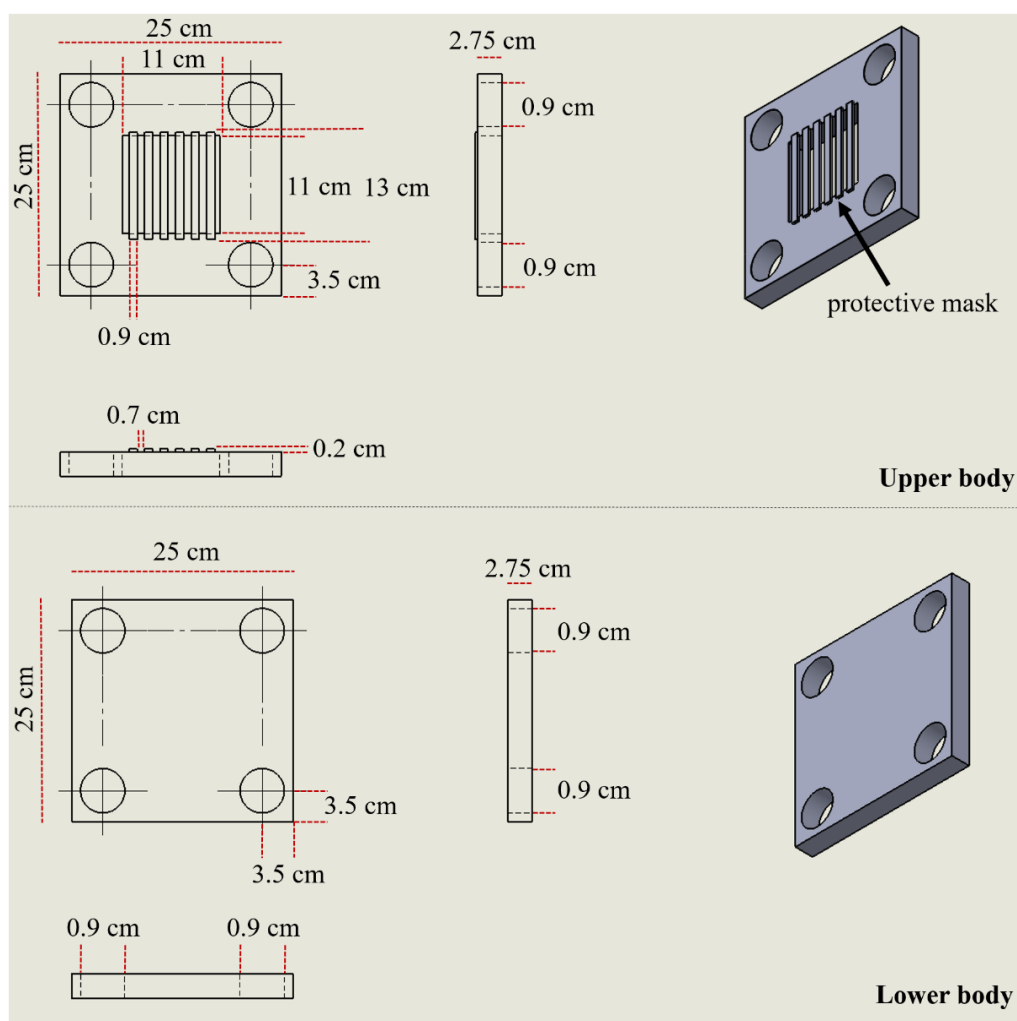
4.3.4. Dry Drug Elution

Initial drug loading tests were conducted by applying the dip coating technique using a shallow tray (volume=1 cm³), which contained the 0.2% solution of Rhodamine B ((RhoB), molecular weight of 479 g mol⁻¹, Merck, Ireland) loaded in coating solution. The MNs were then carefully immersed into the solution and removed to ensure a thin layer of drug-loaded solution to adhere to each MN. However, it was found that the drug did not remain on the MNs but instead covered the patch. To permit direct contact of the MNs with the drug-loaded coating while effectively preventing the flat substrate of the MN patch from coming into contact with the drug-loaded coating, a protective mask was designed and developed. A protective poly-lactic acid (PLA) mask was custom-designed, and 3D printed. The mask was specifically tailored to accommodate the array of MNs by creating six blank spaces precisely aligned with the locations of the MNs. The height of the protective mask was set at 200 μm, ensuring an appropriate distance between the patch and the coating solution (Figure 4.3a). This design ensured precise and controlled application of the coating solution on the MNs,

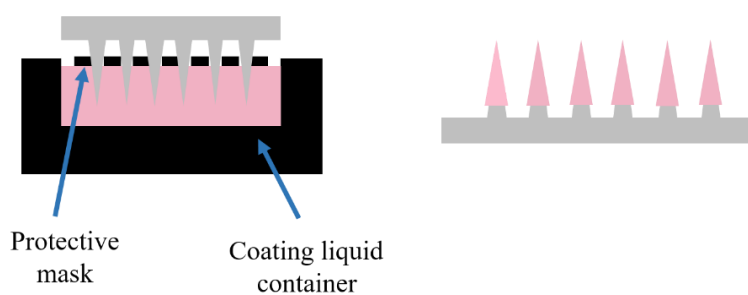
facilitating optimal coating adherence and uniformity. The protective mask was positioned between the patch (base substrate) and the drug-loaded coating interface during the coating process following the MNs dip coating on the model drug (Figure 4.3b). The drug-coated MNs were left to dry in an oven for a minimum 30 min at 37 °C.

To confirm the successful MNs insertion and drug delivery, a qualitative tape stripping test was conducted. Initially, the drug-coated MNs with RhoB solution were manually applied to porcine ear skin and affixed with a 2 Kg weight for 20 min. Following insertion, the porcine skin was cleaned using PBS to remove any debris. During the tape stripping test a piece of double-sided sticky tape was applied to the porcine skin and gently rubbed. The tape was then pulled off and placed onto a clean white sheet of paper. This process was repeated a total of 10 times until the tape remained clean, indicating that the *stratum corneum* had been entirely removed by the eighth strip. Additionally, to ensure that the RhoB marker had penetrated beyond the *stratum corneum*, a scalpel was used to scrape away further layers of the epidermis.

To analyse and quantify the ability of MNs to absorb and store a model drug, drug elution tests were completed. The rate of elution of the RhoB from the drug-coated patch was determined by placing the patch in 20 mL of 25% ethanol solution and quantifying the rate of drug elution over time. The extraction (300 µL) was transferred to a 96-well plate and the 96-well plate containing the eluate samples was scanned for absorbance at 565 nm (Infinite 200 PRO plate reader, TECAN, Switzerland).



(a)



(b)

Figure 4.3: Protective Mask for Drug Loading – (a) Design and (b) application of the PLA mask, tailored with void spaces aligned to MN positions, enables direct contact with coating solution, ensuring precise and controlled application. A 200 μm height maintains ideal patch-coating distance, enhancing adherence uniformity.

4.4. Results

4.4.1. Functional Properties: Penetration Test

The optimal metallic-based MNs successfully penetrated the three different types of skin, including synthetic and natural tissue types (*i.e.*, Parafilm®, SynTissue®, and pig skin). The graphs in Figure 4.4a illustrate the average force-displacement curves obtained for each skin type, highlighting the peaks that were identified as the penetration forces. Importantly, during these penetration tests, the MNs demonstrated robustness by not fracturing, and the tip remained intact without bending. This resilience underscores the suitability of the selected MN design for effective skin penetration across various tissue types.

The required penetration force (Figure 4.4a) and depth (Figure 4.4b) varied depending on the skin type. However, in all cases successful penetration was achieved without the fracture of the needle. For instance, the average force to penetrate the pig skin was 4.4 N and resulted in a penetration depth of ~ 0.7 mm, while lower forces and penetration depths occurred when penetrating synthetic tissue ($F = 2.5 \pm 1.5$ N and depth = 0.4 ± 0.1 mm) and parafilm ($F = 3.5 \pm 0.5$ N, depth = 0.5 ± 0.08 mm), indicating a painless penetration. The forces required for the penetration were lower than the force applied by a human thumb (average of 35-60 N), obtaining the ability of MNs to easily penetrate the *stratum corneum* under thumb pressure [232]. Microscopic images of the Parafilm® (Figure 4.5a), SynTissue® (Figure 4.5b), and pig skin (Figure 4.5c) show the uniform distribution of the penetration marks highlighting the consistency of MN insertion. The distinct punctures further validate the capability of the MNs to successfully penetrate diverse skin types. Providing the closest mimicry to human skin, the porcine skin section displays deeper and broader penetration sites. The distribution and depth of these punctures emphasises the effectiveness of the MNs in a biologically relevant setting and indicates their potential for successful use in transdermal delivery.

Even if MN removal after skin penetration can pose several mechanical challenges, due to the tissue adhesion and friction during the insertion, the design and material properties of the developed MNs influence their performance. Specifically, the MNs maintained their sharpness and did not fracture or deform upon removal (Figure 4.5d). The post-processing techniques, such as electropolishing, further improved the surface finish and robustness of the MNs, facilitating easy and safe removal from the skin without causing any tip failure or leaving any fragments behind. This indicates that the carefully optimised fabrication parameters were effective in producing MNs that are not only efficient in penetrating the stratum corneum but also reliable in terms of their removal.

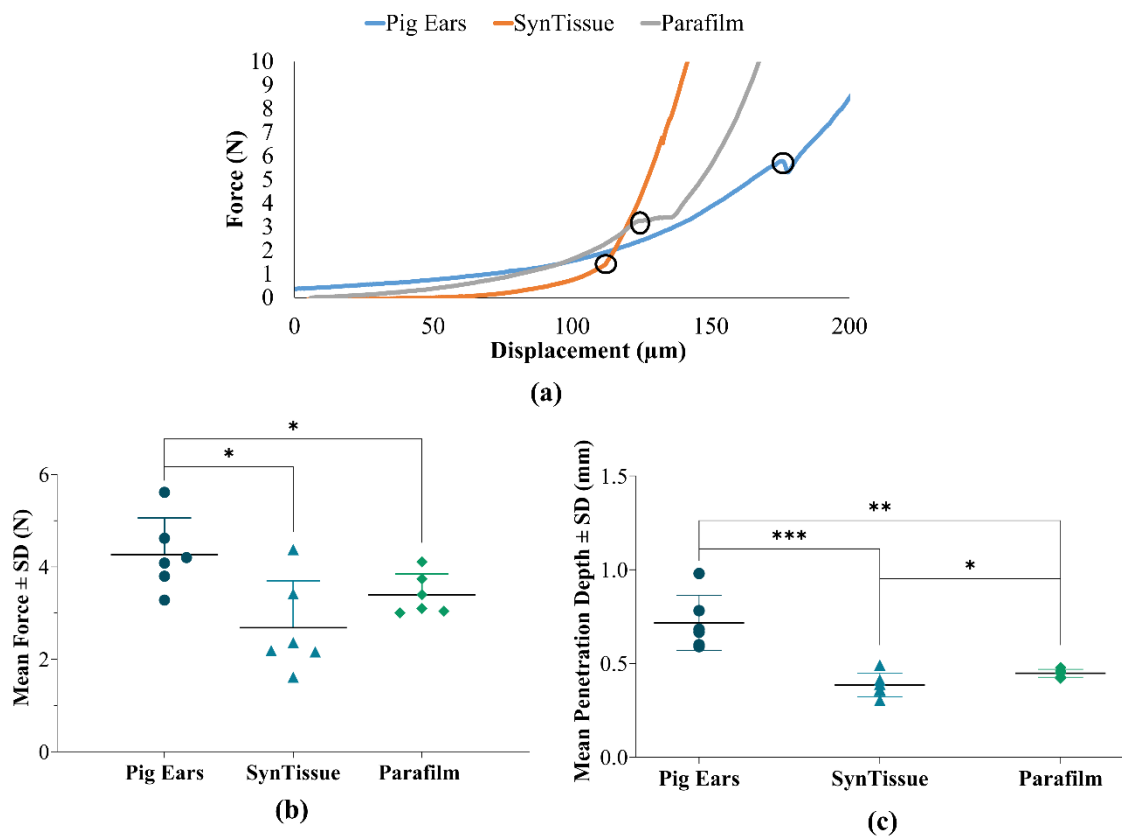


Figure 4.4: Penetration Force and Depth – Optimal MN arrays after penetration test displaying the (a) average force-displacement curves for each skin type (peaks marked on

each curve represent the maximum forces at the point of insertion), (b) mean penetration forces and (c) mean penetration depths of three different materials (*i.e.* Parafilm®, SynTissue® and Pig skin). Statistical significance denoted as *p value<0.05, **p-value<0.01 and ***p value<0.001.

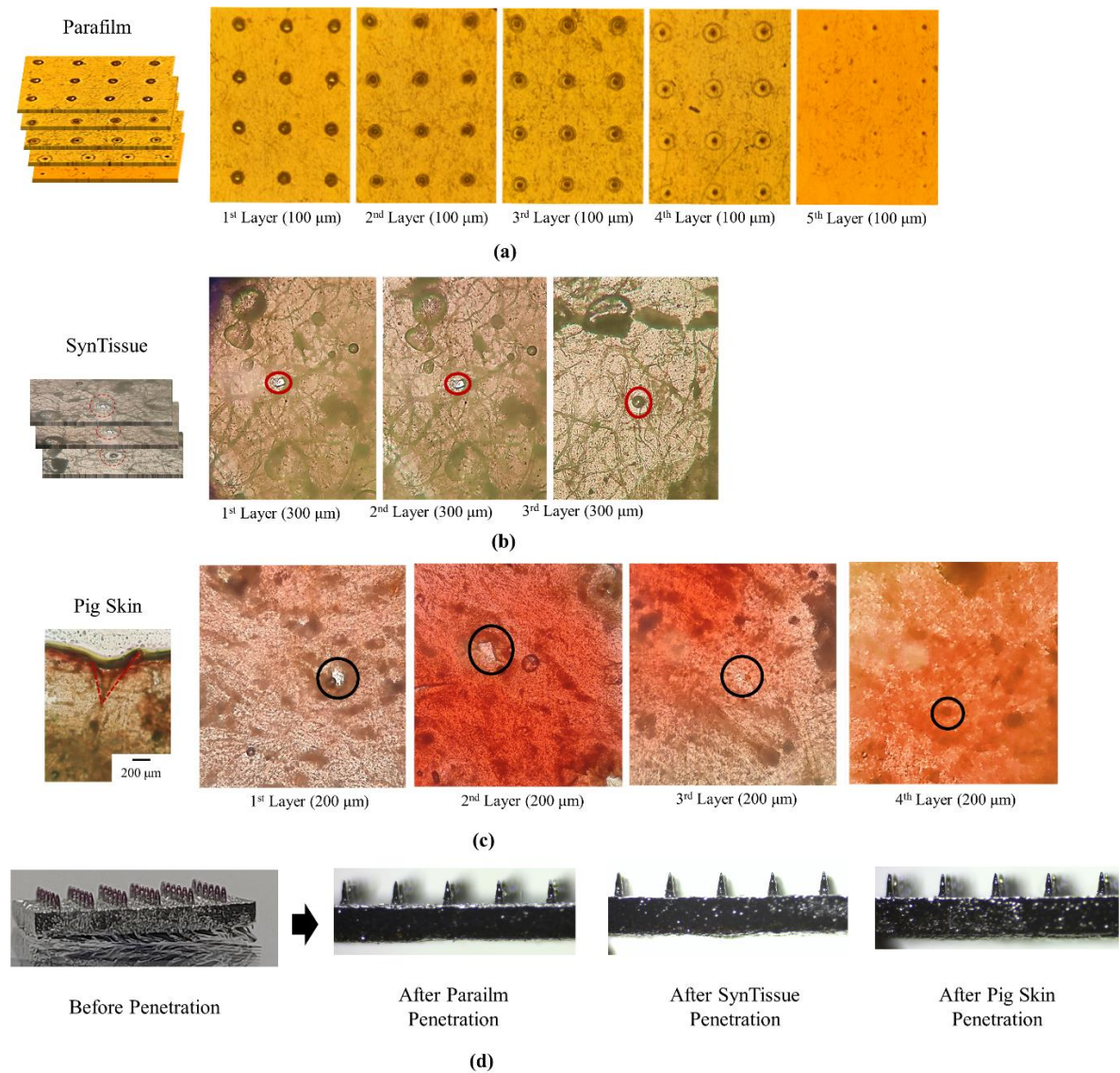


Figure 4.5: Penetration Site of the Different Skin Types – Microscopic images obtained after the penetration of the (a) Parafilm®, (b) SynTissue®, (c) pig skin for further validation of the penetration depth and (d) MNs before and after penetration showing no signs of deformation.

4.4.2. Dry Drug Elution

The evaluation of the capacity of MNs to adsorb small molecules and the subsequent distribution of the marker RhoB were assessed using confocal imaging (Figure 4.6a). The cumulative quantity of RhoB loaded per MN array was approximately $1.95 \pm 0.3 \mu\text{L}$ however, most of the drug was stored and adsorbed within the patch, with a comparatively lower concentration on the needle surfaces.

The elution process of the RhoB from the MN arrays into an ethanol solution exhibited an initial rapid drug release, observed as a bolus, within the first minute of the elution test (Figure 4.6b and 4.6c). The release profile indicated approx. $73 \pm 6\%$ elution during the 1 min and a total of $88 \pm 3\%$ elution within the first 10 min. The remaining drug exhibited an elution rate of $0.20 \pm 0.06 \text{ mM/h}$.

Upon insertion into porcine skin, RhoB was found in the deeper layers of the skin, which was verified from the micrographic images (Figure 4.7a) after sectioning the skin on the MN insertion site. An insertion depth of $700 \pm 32 \mu\text{m}$ was measured, confirming the penetration of the *stratum corneum*. Furthermore, verification of insertion and delivery was ensured by tape stripping, which removed the *stratum corneum*, showing the penetration of the RhoB within the skin (Figure 4.7b). RhoB formulation loaded onto the MN patches was effectively eluted upon application and subsequently absorbed into pig skin, as illustrated in Figure 4.7c.

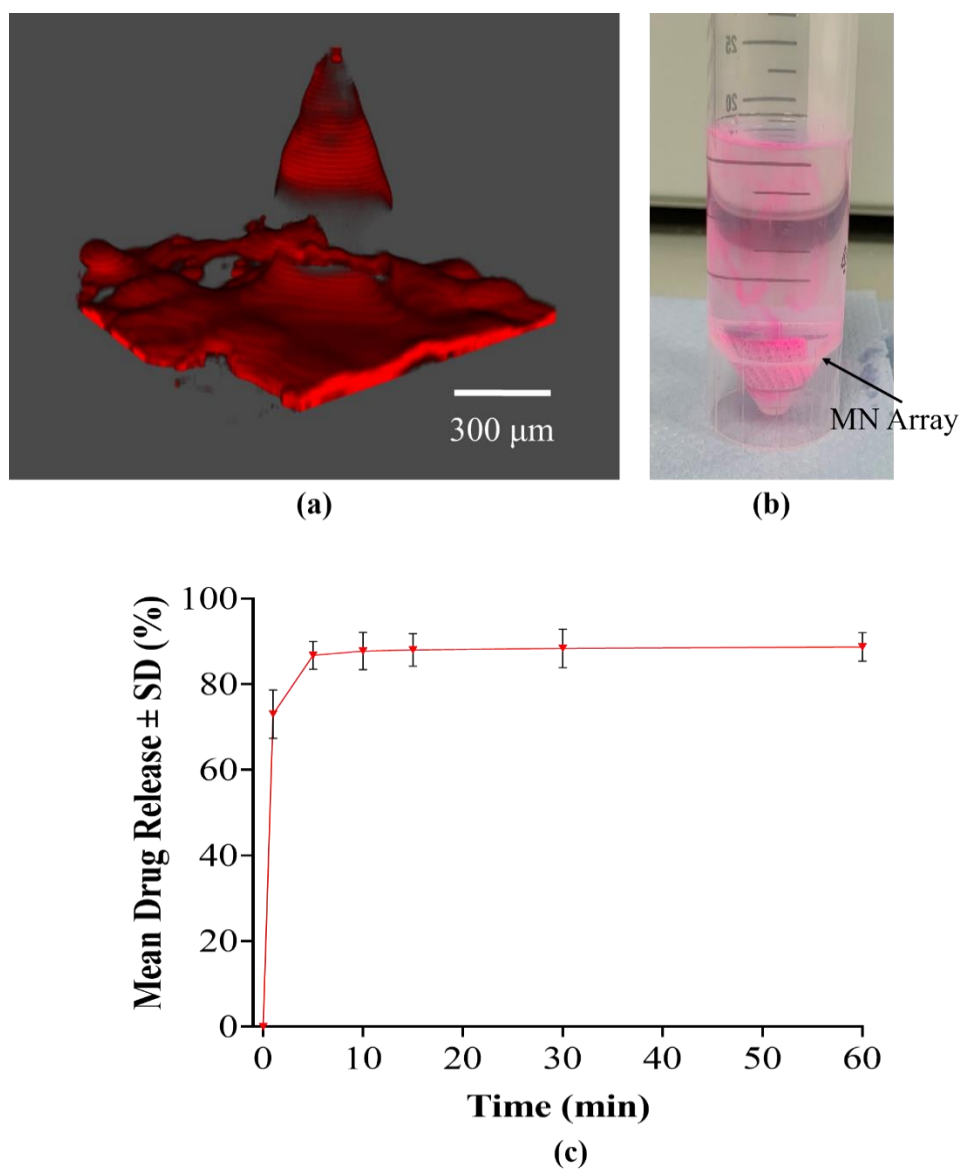
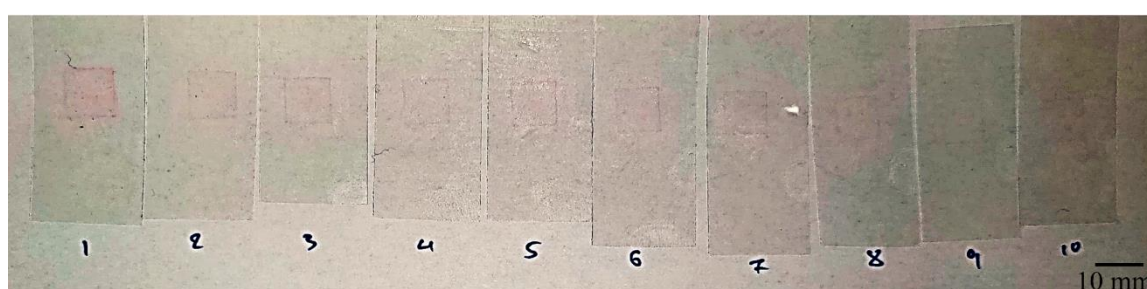


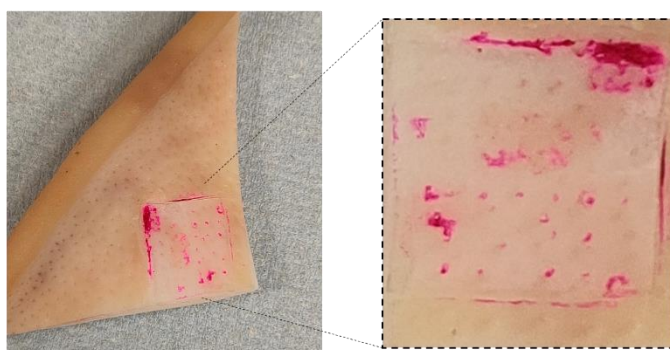
Figure 4.6: Drug Absorbance and Elution of RhoB from MN Arrays – (a) Confocal image showing the RhoB distribution on the surface of a MN and (b) solution with RhoB eluting from the MN patch as well as (c) the percentage of drug eluted within 1 h.



(a)



(b)



(c)

Figure 4.7: Insertion and Delivery of Dry Drug into Porcine Skin – (a) Micrographic image of the sectioned porcine skin following MN insertion (black arrow show pink staining at each insertion site) and (b) tape stripping to the insertion site, verifying the insertion of the RhoB within the skin layers. (c) Epidermal layers of skin were scraped away using a scalpel to show the RhoB delivered.

4.5. Discussion

The optimised MNs exhibited improved surface finish and quality, providing MN arrays with clinically acceptable micro-sized dimensions for painless and easy application [223,224]. The chapter aimed to investigate the ability of optimal metallic MNs to penetrate the skin without presenting any type of fracture. While at the same time the ability of MNs to absorb and deliver a model drug was determined.

The ability of MNs to penetrate different skin types (Parafilm®, SynTissue® and Pig skin) without fracturing or bending demonstrates a significant advantage over traditional MNs, especially considering the challenges in ensuring consistent penetration across varying skin properties. Additionally, the design and development of these MNs ensure they are easy to remove, minimising the risk of additional trauma or residue left in the skin, thereby enhancing their practicality and safety in real-world applications. The difference in the required penetration force across different skin types ($F_{\text{pig skin}}=4.4 \pm 1.6 \text{ N}$, $F_{\text{SynTissue}}=2.5 \pm 1.5 \text{ N}$ and $F_{\text{parafilm}}=3.5 \pm 0.5 \text{ N}$) likely arises from differences in skin thickness and distinct mechanical properties, including density and elasticity [233]. The force required for penetration varied depending on the skin type yet remained below the minimum threshold of 10 N suggests that the MNs can be effectively and easily used in a self-administered manner, enhancing their practicality for transdermal drug delivery. This force threshold represents the average force typically applied by a human, ensuring ease of application through the *stratum corneum* without the need of any applicator and without the risk of MN fracture or tissue damage [232]. The minimal force needed for the MNs to penetrate the skin confirms their ability to easily penetrate the *stratum corneum*. Furthermore, the penetration depth across all skin types indicates that the MNs are likely to be painless, as they avoid reaching nerve endings typically located deeper in the skin [232]. This is consistent with other studies that emphasise the importance of controlling penetration depth for painless application [234]. Microscopic

analysis showing uniform penetration marks, especially in the biologically relevant porcine skin model, underscores the consistency and reliability of the MNs. This uniformity is crucial for ensuring consistent drug delivery across the application area.

The ability of MNs to absorb and subsequently distribute molecules of RhoB, offers a unique and effective mechanism for TDD. In particular, rapid initial release followed by a plateau elution release kinetics of the RhoB was observed in an ethanol solution. This aligns with the growing interest in developing MNs that can provide tailored release profiles for various therapeutic needs [235]. Even if a protective mask was used to enhance coating control, limiting undesirable spreading of the coating onto the patch, most of the drug was stored and adsorbed within the patch, with lower concentrations on the needle surfaces. It is essential to note that this method does not entirely eliminate the possibility of contact between the coating and the patch but rather reduces the likelihood of unwanted spreading. The burst release can be attributed to the high concentration gradient between the MN and the solvent, leading to rapid drug diffusion [236], indicating a need for optimisation in drug loading techniques.

Overall, these optimal metallic MNs hold the potential to enhance efficacy and efficiency, ensuring easy and potentially painless penetration, with ability to be drug-loaded, and rapid drug release. This is particularly relevant given the increasing demand for non-invasive and patient-friendly drug delivery systems. However, further research and optimisation, particularly in drug loading techniques and long-term skin interaction, would be necessary to maximise the efficiency of drug delivery via the MNs and fully realise their potential in real-world applications [237]. One approach to prevent the spread of a drug-loaded coating onto the patch while at the same time control drug release involves the use of a polymer coating with increased viscosity, attainable through alterations in the molecular weight or concentration.

Chapter 5: Development of an Optimal Coating Method to Improve Drug Loading of 3D Printed Metallic MN Arrays

5.1. Introduction

Several enhancement approaches have been used to improve TTD using different MN systems [47,92,172,238–241]. Four different modes of MN-based drug delivery have been investigated. These modes are: (i) piercing an array of solid MNs into the skin followed by application of a drug patch at the treated site [124]; (ii) coating drug onto MNs and inserting them into the skin for subsequent dissolution of the coated drug within the skin [242]; (iii) encapsulating drug within biodegradable, polymeric MNs followed by insertion into skin for controlled drug release [243]; and (iv) injecting drug through hollow MNs [244].

Among these approaches, coated MNs are attractive since they can be adapted to achieve a tailored delivery of a range of therapeutic agents into the skin and can be implemented as a simple system for self-administration. This method involves applying a drug formulation onto solid MNs, which are then inserted into the skin, allowing for subsequent drug dissolution within the skin. The approach provides benefits such as potentially long-term stability of drugs on MNs. In addition, MNs can be cleaned and recycled after their administration because the coated formulation is dissolved in the skin and no damage is caused on the MNs. thus there is potential for them to be reused [245,246]. The reuse of MNs is possible, but still there is need for the development of a system for reloading the drugs. [247].

Previous research has demonstrated the *in vivo* delivery of peptides and proteins using dip coated MNs [59,242]. Moreover, robust immune responses against model antigens, such as ovalbumin, have been generated in guinea pigs through this method [248]. In the first publication on coated MNs [249], it was reported that the MN patches were coated by dipping the MNs in a coating solution. This resulted in coating of the entire MN array, including the base substrate [144,237]. A few years later, to improve delivery efficiencies and to reduce drug wastage, more precise coating methods were developed to restrict coatings to just the MN shafts; one such method was published in 2004 [250]. Furthermore, Gill and Prausnitz

developed a micron scale dip coating process to produce uniformly coated MNs [237]. This process relies on coating MN shafts with a thick layer of coating and not the base substrate using a highly viscose formulation to decrease surface tension. More recently, Hu *et al.*[251] presents dip-coated solid and hollow MNs with controllable lengths and tip shapes. The MNs produced were tested for drug delivery using porcine skin, demonstrating their ability to pierce the skin and deliver drugs effectively while *in vitro* tests confirmed the successful delivery of drugs without significant contamination on the skin's surface.

Various polymers have been explored extensively for dip coating solid MNs to improve the storage and controlled delivery of drugs within the skin, with PVA (polyvinyl alcohol), PVP (polyvinylpyrrolidone), and CMC (carboxymethylcellulose) emerging as notable candidates [89,252,253]. The selection of a specific polymer coating solution depends on various factors, including the physicochemical properties of the drug, desired release kinetics, and the intended application. For instance, PVP has a relatively fast degradation when is in contact with tissue fluid, which could facilitate rapid release of drugs thus increasing bioavailability. PVP coatings can also enhance the mechanical strength and the adhesion of the drug on the MNs surface, ensuring efficient drug-loading [253]. It forms a robust film on MN surfaces, achieving greater drug stability and controlled release while improving penetration into the skin [254–256]. The biocompatibility and adhesive strength of PVP make it particularly suited for TTD applications [237]. Another water-soluble polymer widely employed for its biocompatibility and film-forming properties is PVA, demonstrating a high drug-loading capacity and controlled drug release [257]. Its hydrophilic nature also facilitates rapid dissolution upon insertion into the skin, promoting efficient drug diffusion. The versatility of PVA makes it suitable for a range of therapeutic compounds and molecular weights. CMC, a cellulose derivative, is recognised for its biocompatibility and ability to form viscoelastic gels [144,258]. When applied as a coating to MNs, CMC has been reported to provide a sustained

drug release by storing the drug within its gel-like structure [237,259]. This controlled release profile is advantageous for maintaining therapeutic drug levels over an extended period, reducing the need for frequent administration.

Although, coated MNs have been used to deliver proteins and drugs into the skin, detailed studies of the coating process itself have not been reported. Furthermore, greater understanding of this process is necessary to enable the coated MNs to be tailored for different drug delivery applications. The micron-scale dimensions of the MNs require specialized coating techniques to achieve uniform coverage and precise control over the specific regions of the MNs to be coated. This is largely because the effects of surface tension, capillarity and viscous forces become more prominent at these small length scales. Therefore, a single coating formulation may not be universally applicable to coat all therapeutic compounds and proteins [237]. Unfortunately, the complex physics of dynamic wetting is not fully understood, which makes theoretical predictions of coating uniformity and thicknesses difficult [260,261]. The interaction between the liquid coating material and the microneedle surface involves intricate processes such as surface tension, capillary action, and fluid dynamics, all of which can vary based on the properties of the liquid and the microneedle material. These factors create difficulties in developing theoretical models that can reliably forecast how evenly the coating will spread and how thick it will be across different regions of the microneedles. Consequently, achieving consistent and precise coatings often requires extensive experimental optimisation. This chapter aims to address these limitations and investigate the performance of different polymer-based materials as coating solutions on solid metallic MNs using the dip-coating process to coat MNs.

5.2. Chapter Aim

In this chapter the process of coating MNs for TTD systems was investigated and analysed. The mechanisms, challenges, and parameters that influence the uniformity and effectiveness of the coating process were assessed. This chapter aims to analyse and enhance the adaptability and efficiency of coated MNs in delivering various therapeutic compounds through the skin, promoting the advancement of non-invasive, pain-free, and controlled drug delivery technologies.

The specific objectives of this chapter were the:

- Analysis and characterisation of the different polymer-based coating formulations.
- Comprehensive analysis of the characteristics, performance and efficiency of the coated 3D printed solid metal-based MNs.
- Optimisation of the polymer formulations and the number of coating layers for enhanced drug delivery and functionality of the MN arrays as TDD system.
- Analytical assessment of the performance of coated MNs in terms of their ability to store and release drugs efficiently.

5.3. Material and Methods

5.3.1. Preparation of Different Polymer Solutions

Carboxymethyl Cellulose Solutions

Carboxymethyl cellulose (CMC) with Mw ~90,000 was purchased from Merck Life Sciences Ltd (Ireland) to prepare CMC polymer solutions. Initially, the CMC powder (0.1 g, 0.4 g and 0.8 g) was weighed using a precision balance and then added into 10 mL of DI water for the development of, 1%w/v, 4% w/v, and 8% w/v CMC respectively. To ensure homogenous mixing, a magnetic stirrer was employed to agitate the solution for a duration of 1 h at room

temperature (22 °C). The mixed CMC solution was transferred into centrifuge tubes and subjected to centrifugal forces for 2 min at a rotation speed of 2,000 RPM. This step aimed to minimise the presence of bubbles within the solution, thus enhancing its overall quality. The homogenised CMC solution was then stored at 4 °C.

Polyvinyl-alcohol Solutions

Polyvinyl alcohol (PVA), with an 88% degree of hydrolysis and Mw ~85,000-120,000 was purchased from Fisher Scientific (Ireland). The study involved the synthesis and subsequent analysis of PVA solutions across varying concentrations—specifically, 1%, 4%, 8%, 10% (w/v), and 20% w/v. For the preparation of 1% w/v PVA, 0.1 g PVA were slowly added into 10 mL of DI water. Similarly, 1 g and 2 g of PVA was dissolved in 10 ml of DI for the development of 10% w/v PVA and 20% w/v PVA, respectively. Subsequently, the resulting mixture was subjected to a hotplate magnetic stirrer operating at 250 RPM and a temperature of 80°C was applied during mixing to break up the crystal structure, thereby facilitating its dissolution within the solvent. This dynamic stirring and heating process was carried out for 2 h to ensure a homogenous PVA solution. As per CMC solutions, the homogenous PVA solution was subjected to a centrifugation step (2 min at a rotation speed of 2,000 RPM) and stored in the fridge (4 °C) until its intended application in subsequent phases of experimentation.

Polyvinylpyrrolidone Solutions

Polyvinylpyrrolidone (PVP) was purchased from Merck Life Sciences Ltd and solutions of varying concentrations—namely, 1%, 4%, 8%, 10%, and 20% (w/v)—were prepared. The preparation process involved dissolving the appropriate quantity of PVP in DI water. Subsequent to the procedure applied for CMC solutions, the PVP solutions followed a similar protocol, including continuous mixing (250 RPM) at room temperature (22 °C) for 2 h. The

mixed solution was transferred into centrifuge tubes and subjected to centrifugal forces for 2 min at a rotation speed of 2,000 RPM. This step aimed to minimise the presence of bubbles within the solution, thus enhancing its overall quality. The homogenised PVP solution was then stored at 4 °C.

5.3.2. Preparation of Coating Solution Formulations

Coating formulations named CMC-RhoB, PVA-RhoB and PVP-RhoB were developed incorporating the selected model drugs (0.2% RhoB solution for small molecules) with all the different concentrations of polymer solutions (1%, 4%, 8% (w/v) CMC and 1%, 4%, 8%, 10%, 20% (w/v) PVA and PVP) at the Polymer:RhoB ratio of 2:1 by volume. This ratio was selected to ensure a sufficient loading while maintaining the structural integrity and adhesive properties of the coatings. This step aimed to prepare MNs with an optimal polymer coating infused with the model drug, ready for subsequent experimental evaluations and analyses.

5.3.3. Dip Coating of MN Arrays

Arrays of 36 MNs were coated with CMC, PVA and PVP coating solutions using the micro-scale dip coating process employing a protective mask as described in Chapter 4. Initially, the different coating solution formulations (CMC-RhoB, PVA-RhoB and PVP-RhoB) at required concentrations were transferred within the base of the protective mask. MNs were then dipped multiple times (each dip represents the application of one layer) into the coating solutions, creating different number of coating layers. For consistent coating the MNs were kept within the coating solution and removed after 5 sec for each layer from the moment of dipping. Specifically, in this study MNs with 1, 5 and 10 layers of coating solution were prepared and assessed. The coating was allowed to air-dry for 5 min between the layers while after the completion of coating MNs were allowed to air-dry for 24 h before use.

5.3.4. Analytical Assessment of Polymer Solutions

5.3.4.1 Viscosity

The viscosity analysis was conducted (n=3) using a rheometer (Physica MCR 501, Anton Paar, Austria), equipped with a conical spindle (CP50-2-SN23384; d=0.208 mm). The rotation analysis technique used provided valuable insights into the flow behaviour and viscoelastic properties of the different polymer solutions. In this study, the focus was on evaluating the viscosity change of the samples changing the temperature from 10°C to 60°C with a consistent linear increase at a rate of 5 °C/min. Viscosity affects the coating process's uniformity and efficiency, influencing how well the solution spreads and adheres to the stainless steel MNs. A consistent viscosity is essential for achieving a uniform coating thickness and ensuring precise control over the regions being coated. By understanding how viscosity changes with temperature, optimal coating conditions can be identified, ensuring that the coating solution performs effectively during the application process. Each polymer solution (0.098 mL) was loaded onto the cylindrical rheometer's sample chamber with inner diameter of 50 mm following the spindle immersion into the solution until a normal force of 0.5 N is achieved, maintaining consistent and reliable measurements throughout the analysis. The rheometer imposed a shear rate of 0.063 s^{-1} and a strain of 1% on the sample, ensuring a controlled deformation that allowed the rheometer to capture the sample's flow behaviour accurately and effectively assessed the dynamic viscosity response.

5.3.4.2 Degradation Study

Cylindrical-shaped specimens of Section 5.3.1 (n=6) were prepared using polytetrafluoroethylene (PTFE) moulds with a diameter of 10 mm and height of 1 mm from each of the polymer solutions at the different concentrations. The main purpose of this test was to investigate the degradation rate of each polymer and composition to understand the differences in their degradation profiles. It is important to note that the samples used for

degradation testing were freeze-dried, while the MN coatings were air-dried. This difference in drying methods affects the sample structure, leading to variations in the total percentage of degradation and the degradation time. However, despite these differences, the overall behaviour and rate of degradation observed in the tests will still be representative of each coating's characteristics. All specimens were freeze dried at -40 °C over a duration of 48 h and weighed to obtain their initial weight (W_0). Following the freeze-drying phase, each sample was immersed in approx. 0.8 mL of 0.1 M PBS at a pH 7.4 to ensure proper immersion for the degradation study, simulating physiological environments. Incubation of the samples within a temperature of 37°C was conducted while during incubation the PBS was replaced to maintain a consistent degradation environment. At specific time intervals, pre-set at every 5 min up to a 3 h timeframe, the polymer samples were removed from the PBS and underwent another freeze-drying cycle before being subjected to re-weighing (W_t). To quantify the extent of degradation, the percentage of weight loss ($W\%$) for each sample was calculated using the formula from Equation 5.1. Furthermore, the kinetics (K_{PVA} , K_{CMC} and K_{PVP}) of the degradation rate for each composition was calculated as the slope of the degradation line.

Equation 5.1: Calculation of the percentage of weight loss over time to determine degradation rate.

$$W\% = \frac{(W_0 - W_t)}{W_0} \times 100\%$$

5.3.5. Analytical Assessment of Coated MN Arrays

5.3.5.1 Qualitative Analysis of Coating

Coating solutions at different concentrations were prepared, specifically targeting the range of concentrations relevant to the study's objectives, including the 1%, 4%, 8% (w/v) CMC

and 1%, 10%, and 20% (w/v) PVA and 1%, 10%, and 20% (w/v) PVP solutions. The coating solutions were used for the dip coating of the MN arrays. Qualitative analysis was carried out by assessing microscopic images of the coated needles, captured using the Keyence microscope. This analysis provided insight into the coating's uniformity, and overall quality. The microscopic images (n=6) were systematically compared across the various concentrations.

5.3.5.2 Quantitative Analysis of Coating

The optimal concentrations of polymer solutions (8% w/v CMC, 20% w/v PVP and 10% w/v PVA) as defined by the qualitative observation were mixed with RhoB at a 2:1 volume ratio, leading to Solution 1: 8% w/w CMC-RhoB, Solution 2: 20% w/v PVP-RhoB, and Solution 3: 10% w/v PVA-RhoB. The resulted coating solution formulations were used to dip coat MN arrays using different number of layers (1, 5 and 10 layers) followed by air drying for 24 h. This analysis aimed to determine the optimal number of layers per coating solution formulation.

The confocal microscope (Leica TCS SP8 Confocal, USA) at a wavelength of 595 nm was used to visualise the coated MNs (23 needles per sample, n=3), facilitating clear differentiation between the coating and the substrate. For imaging an objective lens of 10x was selected to achieve the appropriate magnification. Z-stack imaging was performed by acquiring a series of optical sections along the vertical axis of the MNs, allowing for a comprehensive visualisation of the entire coating structure, accounting for any potential variations in thickness along the length of the MNs.

Dimensions relating to the coating length and thickness on the MNs were obtained using Image J to further supplement the qualitative assessment. The images were calibrated using a known reference size to establish an accurate scale. Subsequently, image processing

techniques, including thresholding and edge detection, were applied to segment the drug-coated regions on the MNs. The software's measurement tools were utilised to obtain the length of the segmented drug coatings. This measurement was conducted along the longitudinal axis of each MN. For each coating MN array, multiple MNs were measured to ensure statistical significance (23 needles per sample, $n=3$).

5.3.5.3 Drug Loading on MNs and Elution

MN arrays were dip-coated using the optimal number of layers as defined by the quantitative analysis of the coating. Coating solutions such as Solution 1: 8% w/v CMC-RhoB, Solution 2: 20 % w/v PVP-RhoB, and Solution 3: 10% w/v PVA-RhoB in a ratio of 2:1 volume was used for the dip-coating in order to determine the cumulative quantity of model drug loaded per coated MN array. The drug loading technique onto solid metallic MNs involves both qualitative observation and quantitative analysis to ensure accurate drug dosing and uniform distribution. In the qualitative phase the microscopic examination was conducted to observe the presence and distribution of the drug. For quantitative analysis, multiple batches of MNs were weighed before and after drug loading, and the mass of the drug loaded was calculated. This mass was used to determine the concentration of RhoB on the MNs by considering the surface area of the MNs.

To determine the rate of drug (RhoB) release from the MNs, elution tests were conducted. During these tests, the coated MN array was immersed in 20 mL PBS, allowing the polymer to gradually dissolve over time and release the model drug. The resulting eluate, containing the released model drug, was transferred to a 96-well plate after 1, 5, 15, 30, 60, 90 and 120 min. Subsequently, the 96-well plate containing the extraction of the samples was subjected to absorbance measurements at 565 nm using a plate reader. The absorbance at this wavelength corresponds to the presence and concentration of the released model drug, enabling the quantification of drug release. This experimental setup aimed to determine the

effectiveness of the various polymer coatings in encapsulating the model drug, aiming to the understanding of the release kinetics of each solution when coated onto solid metallic MNs.

5.3.5.4 Adhesion Forces between Coating Solution and MNs

To evaluate the ability of the coating solutions to adhere to the MNs during insertion into the skin a shear adhesion study was conducted. Stainless steel cubes with 10 mm x 10 mm dimensions were used as substrates after electropolished for 1 min at 7 A, as per optimal levels for MNs electropolishing, to ensure the same roughness between stainless steel cubes and MNs. The surface of the cubes was evenly covered with 0.2 mL polymer solution, forming a uniform layer. The coated cubes were then left to air dry at room temperature for 24 hours to ensure solution adherence to the substrate. The universal mechanical testing machine (Zwick Roell, UK) equipped with a 500 N load cell and a sharp pin was used to gradually apply a force from the pin to the interface (Figure 5.1), with a crosshead speed of 1 mm/min, causing the solution-coated surface to detach from the substrate. The force required for the detachment process was recorded as the adhesion force (n=6) [262,263].



Figure 5.1: Shear Adhesion Test Set-up – Sample type and experimental arrangement used to examine the coating solutions adhesion forces on stainless steel surfaces.

5.3.5.5 Penetration Depth into Porcine Skin–Optical Coherence Tomography

The coated MNs with coating Solution 1, 2 & 3 were inserted into porcine ears (10 x 10 x 2 mm) and were scanned both topside (MN patch) and bottom side (skin) to identify the exact penetration depth using optical coherence tomography (OCT) (EX1301 VivoSight®, Michelson Diagnostics Ltd, Kent, UK) analysis. Dissolvable MNs (PVA with a molecular weight of 9–10 kDa and PVP with molecular weights of 58 kDa and 360 kDa) with similar height of ~700 μ m produced and provided by Queens University of Belfast were selected as a control [264]. A laser wavelength of 1300 ± 15.0 nm was used, facilitating real-time high-resolution imaging of the upper skin layers was selected. The skin and the MN patch were scanned at a frame rate of up to 15 scans per second (scan width ~ 2.0 mm).

5.3.5.6 Histological Analysis

The samples were prepared before the cryosectioning process as can be seen in Figure 5.2. The coated MNs with coating Solution 1, 2 or 3 and three control samples coated with only RhoB were penetrated porcine ear tissue samples (10 x 10 x 2 mm). The MNs were held in place using a weight of 2 Kg for 30 min to ensure the release of the drug. After the removal of the MNs, the skin samples were placed into the moulds containing OCT mounting media (Xylene-based, low viscosity, VWR International Ltd., Belgium), that simplify the flow and inhibits the formation of air bubbles, ensuring that the sectioning area facing upwards. The moulds were then placed into liquid nitrogen to freeze following the transfer to the cryostat chamber at -20 °C. Skin sections of 10 μ m thickness were obtained using a cryostat microtome (Leica CM3050 S Research Cryostat, UK). Sectioning of skin (n=12) were placed on top of a glass slide to assess the penetration depth and drug distribution using the optical and fluorescence microscopy.

5.3.5.7 *In Vitro* Drug Delivery into Skin

The elution test was performed on porcine skin following the insertion of MN arrays. The MNs were applied to the skin for varying durations (1, 30, 60, and 120 min) to analyse the rate of drug delivery. The skin samples were immersed in 20 mL of PBS and incubated for 2 h to fully elute the complete drug dosage from the skin. The amount of drug eluted was determined using a plate reader as per Section 5.3.5.3

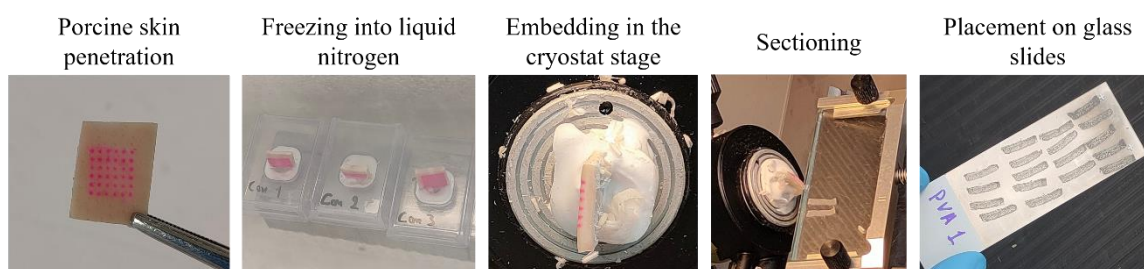


Figure 5.2: Histological Analysis Process - The sequential steps included in the histological analysis process conducted for studying MN penetration into porcine skin tissues. Each step is crucial for the precise examination and visualisation of MN interactions with the skin.

5.3.5.8 *In Vitro* Permeation through the Skin

Franz diffusion cell system was used to assess the permeation or diffusion rate of RhoB through porcine skin (Figure 5.3a). A Franz diffusion cell system is used to study the rate of drug permeation through a biological membrane or synthetic barrier. It consists of two chambers: a donor chamber where the test substance is applied, and a receptor chamber filled with a solvent that simulates body fluids. The two chambers are separated by the membrane being tested. Over time, samples are taken from the receptor chamber to analyse the amount of substance that has diffused through the membrane, allowing the evaluation of the permeability and release kinetics of drugs or other compounds. This system is widely used in pharmaceutical and cosmetic research to assess transdermal drug delivery and skin absorption.

For this study, a polylactic acid (PLA) Franz cell was 3D printed using original Prusa i3 (Original Prusa i3 MK3S 3D, Prusa Research, Czech Republic) [265]. Unlike a conventional Franz cell, which is typically made of glass and comes in a standardised shape and size, 3D printing provided the flexibility to customise the cell to accommodate our specific MN design. This modification was important to align the cell with the requirements for our research, enabling us to effectively evaluate drug permeation or diffusion rates through tissue using the metallic MN arrays. As shown in Figure 5.3a, the donor chamber is at the top of the cell. The tissue of interest, in this case porcine skin (thickness of 1 mm), was cleaned of extraneous tissues and pre-hydrated in PBS for 2 h. The prepared skin was clamped epidermal-side-up between the donor and receptor chambers of the Franz cell (Figure 5.3b). The receptor compartment was filled with 10 ml of PBS, maintained at 37 °C using a circulating water bath and a magnetic stirrer to ensure homogeneity (Figure 5.3c). Each cell was wrapped with parafilm to avoid drying of the tissue. The coated MN was inserted into the skin with a force of 20 N held for 5 s, following the placement of the coated MNs into the donor compartment. The donor and receptor chambers of the Franz diffusion cell system were securely held together using clamps to ensure a tight seal and prevent leakage. Samples were periodically collected from the receptor chamber (*i.e.*, 1 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h and 24 h) and the volume was replaced with PBS at every time point.

The concentration of RhoB in each sample was quantified using a plate reader at an emission wavelength of 565 nm. Furthermore, the permeability coefficient (K_p) for each coated-MN at different time points was determined using Fick's first law of diffusion (Equation 5.2), considering the steady-state flux (dQ/dt), the active diffusion area of the Franz cell (A), and the initial drug concentration in the donor receptor (C_0).

Equation 5.2: Permeability coefficient (K_p)

$$K_p = \frac{dQ}{dt} \times \frac{1}{A \times C_0}$$

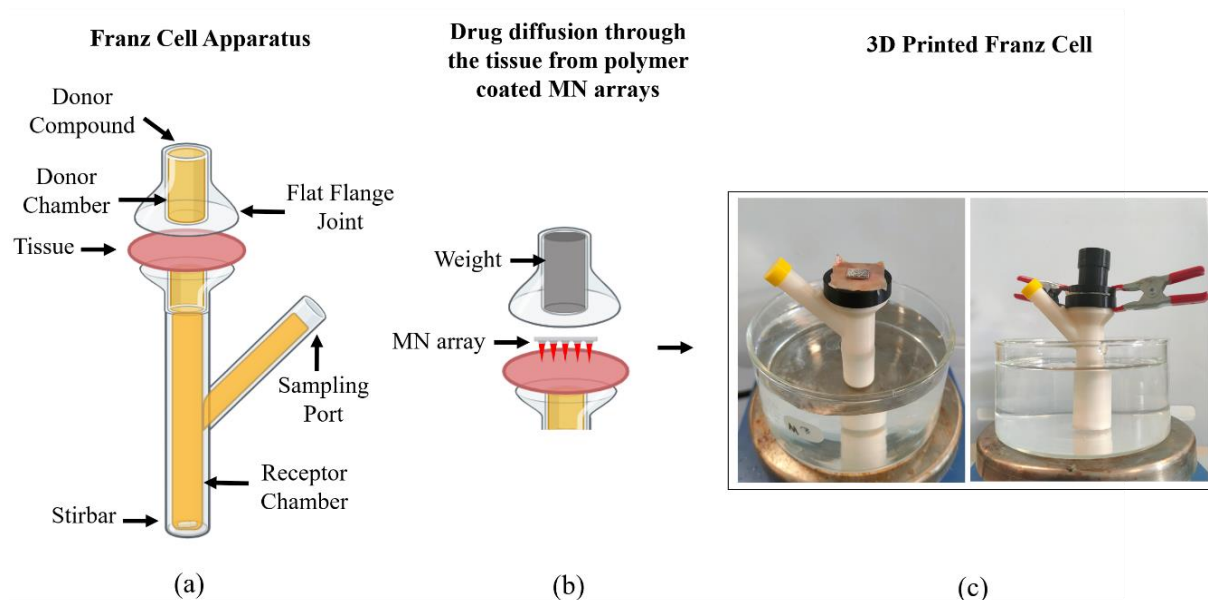


Figure 5.3: Franz Diffusion Cell – (a) Detailed components of the Franz cell apparatus. (b) Illustration showing the precise placement of skin and MNs within the apparatus. (c) In-house 3D-printed Franz cell design alongside the test setup configuration.

5.4. Results

5.4.1. Analytical Assessment of Polymer Solutions

5.4.1.1 Rheological Properties

The viscosity of CMC, PVA, and PVP solutions was analysed at different concentrations and temperatures, presented in Figure 5.4a (22 °C) and Figure 5.4b (37 °C). Increasing the concentration of these polymers (1% to 8% (w/v) for CMC and 1% to 20% (w/v) for PVA and PVP) resulted in higher viscosities at both temperatures, a change confirmed as statistically significant with p -values < 0.001 .

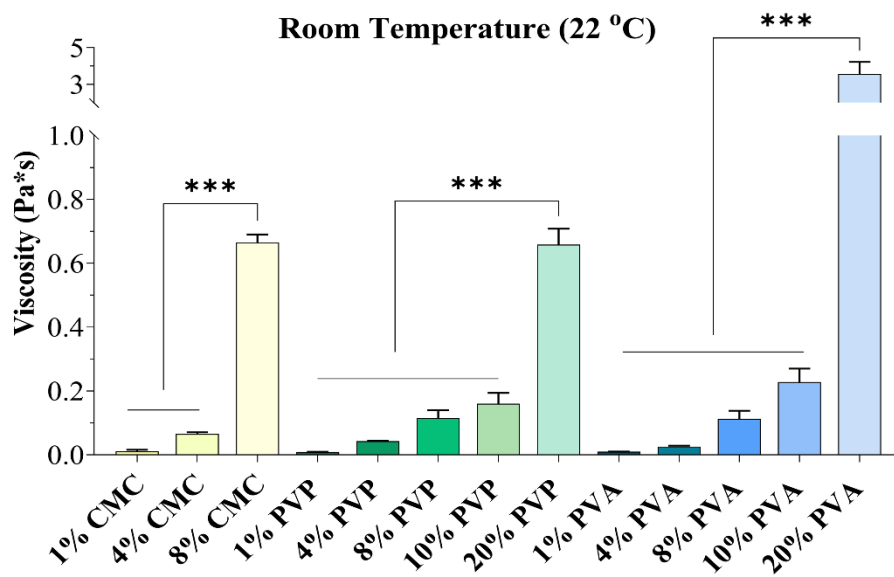
A temperature increase from 22 °C to 37 °C reduced the viscosity of all solutions. Specifically, 8% w/v CMC and 20% w/v PVP solutions showed a 20% decrease in viscosity (from 0.7 Pa*s to 0.5 Pa*s), while 20% w/v PVA demonstrated a minor 5% reduction. The 20% w/v PVA solution maintained the highest viscosity (3.2 Pa*s).

Polymer concentration positively correlates with viscosity, and a temperature rise to 37 °C generally lowers it, varying by polymer type and concentration. Understanding the viscosity of the coating solutions at both room temperature (22 °C) and skin temperature (37 °C) is related to the practical application of MNs and the effective release and absorption of the drug by the skin. At room temperature (22 °C), where the coating process typically occurs, the viscosity of the solutions determines how easily and uniformly the polymers can be applied to the MNs. A higher viscosity at this temperature ensures that the coating adheres well to the MNs without dripping, resulting in a consistent and uniform layer of the drug-loaded polymer. Once the MNs are applied to the skin, they are exposed to body temperature (37 °C) and since the increase in temperature can reduce the viscosity of the polymer solutions, it potentially alters the drug release dynamics. A lower viscosity at skin temperature facilitates faster drug diffusion and release from the MNs into the skin.

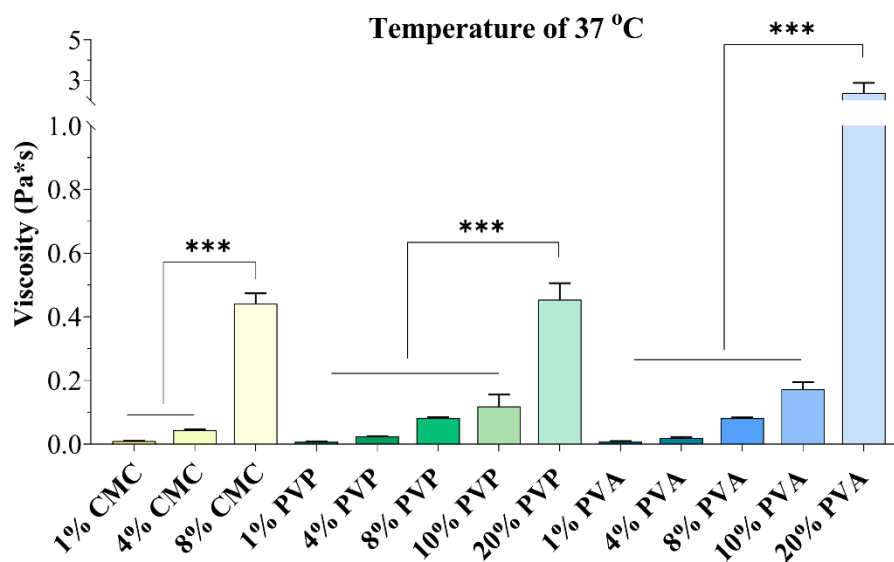
5.4.1.2 Degradation Properties

The degradation rates of CMC, PVA, and PVP solutions at various concentrations were determined. Lower concentrations degraded faster: 1% w/v and 4% w/v CMC solutions (Figure 5.5a), alongside 1%, 4%, and 8% (w/v) PVP solutions (Figure 5.5b) completely degraded within 15 min. Higher concentrations degraded more slowly. For instance, 8% w/v CMC and 10% w/v and 20% w/v PVP solutions took 60-90 min for complete dissolution in PBS. PVA solutions, particularly at 10% and 20% concentrations (Figure 5.5c), showed the slowest degradation, with only 20-30% degradation in the first hour, followed by a steady decrease in the following hour.

The kinetics of mass loss for CMC, PVP, and PVA were investigated over a period ranging up to 4 h. The rate constants (K) for each polymer at varying concentrations were determined from the respective plots. For CMC, the observed rate constants decreased with increasing polymer concentration, suggesting a slower release at higher concentrations. Specifically, K_{CMC} values were found to be 100 min^{-1} , 5.82 min^{-1} , and 0.96 min^{-1} for 1%, 4%, and 8% (w/v) CMC solutions, respectively. In the case of PVP, a similar trend was observed. The rate constants K_{PVP} showed a decrease from 100 min^{-1} at 1% concentration to 1.53 min^{-1} at 20% concentration. Intermediate concentrations of 4%, 8%, and 10% (w/v) yielded rate constants of 8.89 min^{-1} , 5.24 min^{-1} , and 0.96 min^{-1} , respectively. For PVA, the decline in rate constants with increased concentrations was also evident. For instance, K_{PVA} values ranged from 3.07 min^{-1} at 1% to 0.44 min^{-1} at 20%.



(a)



(b)

Figure 5.4: Rheological Properties – Viscosity of CMC, PVP and PVA solutions increasing the solution concentration, leading to an increase at both (a) room temperature and (b) 37 °C. Statistical significance denoted as ***p value<0.001.

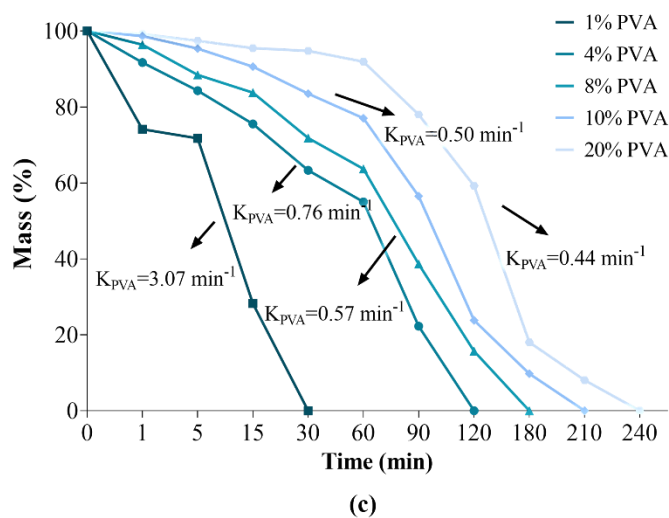
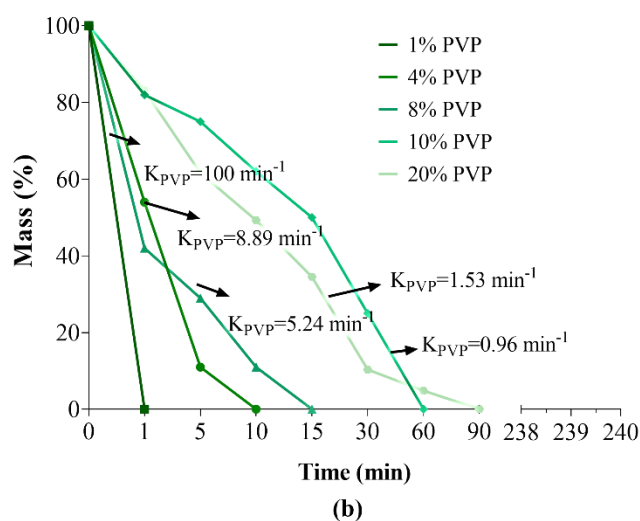
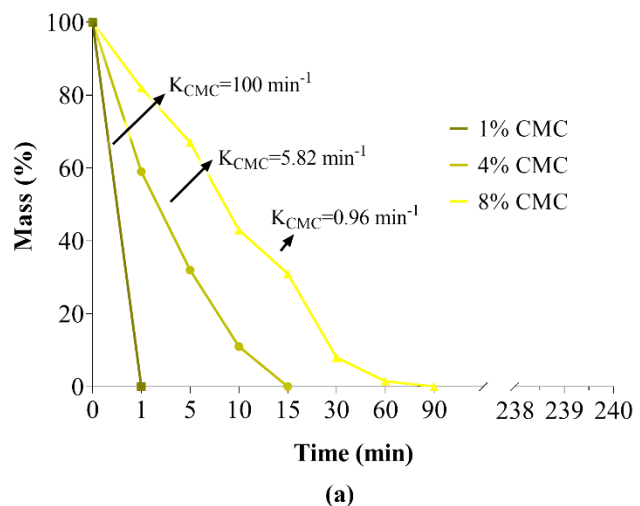


Figure 5.5: Degradation – Concentration-dependent degradation profile of (a) CMC, (b) PVP and (c) PVA solutions. Solutions with lower concentrations exhibit rapid degradation while higher concentrations degrading more gradually.

5.4.2. Analytical Assessment of Coated MN Arrays

5.4.2.1 Qualitative Analysis of Coating

The qualitative assessment of coating CMC, PVA, and PVP solution at different concentrations as mentioned in Section 5.3.5.1 was obtained. After using CMC-based coating solution for the dip coating of MNs, concentrations of 1% and 4% proved unsuitable for successful coating of the MNs. Instead of adhering to the MNs, the coating material dispersed across the entire patch of the MN array (Figure 5.6a). For the 8% CMC solution, coatings consisting of 1, 5 and 10 layers were successfully deposited on the MNs without dispersion onto the patch. Partial coating of the patch was observed for 1% PVA and 1% PVP, and 10% PVP solutions during the dip coating process (Figure 5.6b). On the other hand, higher concentrations of both PVA and PVP (10% and 20% respectively) enabled successful MN coating *via* dip coating. As can be seen from Figure 5.6b, a sequential immersion of MNs for 1, 5, and 10 cycles resulted in layer-by-layer deposition of coating solution solely on the MNs, with no dispersion of the coating on the patch. Uniform coating was achieved with 1 and 5 layers of coating, preserving the geometrical integrity of the needles. Finally, the high viscosity of the 20% PVA coating solution led to a distinct behaviour, whereby a droplet accumulated at the needle tips rather than the desired uniform distribution. Overall, successful MN coating without dispersion on the patch was achieved using 10% PVA, 8% CMC and 20% PVP, as demonstrated by layered deposition through sequential dipping (1, 5, and 10 layers).

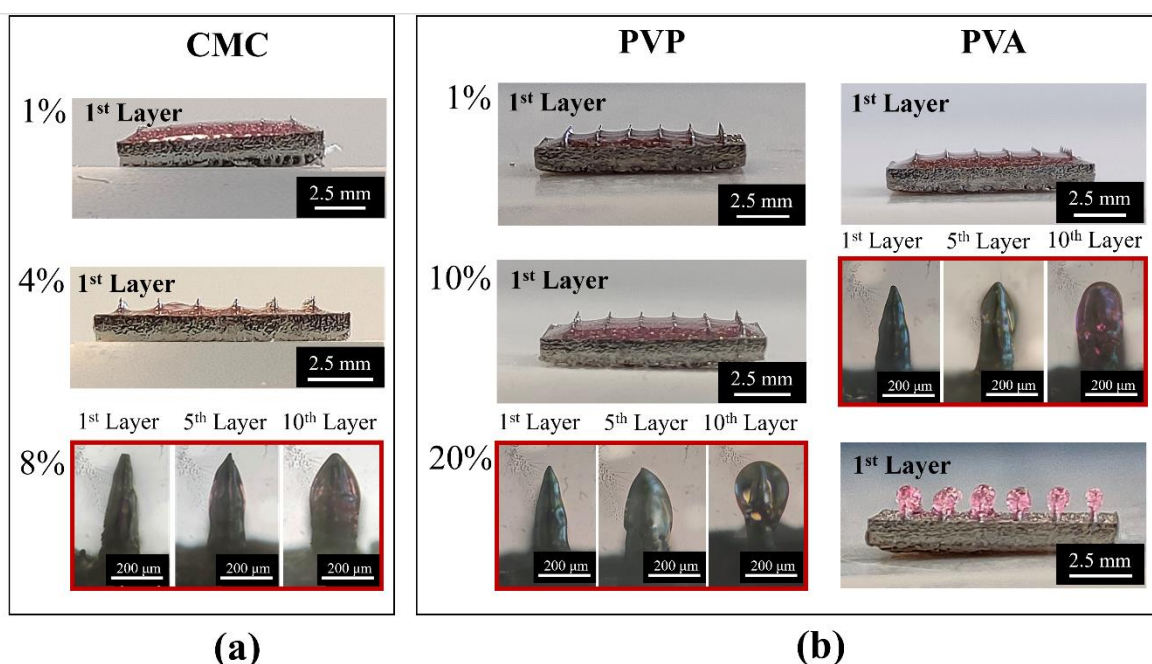


Figure 5.6: Qualitative Assessment of MN Coating Solutions – Qualitative depiction of the coating outcomes for MNs using (a) CMC and (b) PVP and PVA polymer solutions. Successful MN coating was achieved through layered deposition of 8% w/v CMC, 20% w/v PVP and 10% w/v PVA coating solution (marked with red boxes).

5.4.2.2 Quantitative Analysis of Coating

The investigation into optimal coating solutions focused on dip-coated MN arrays using varying numbers of layers (1, 5, and 10 layers) using Solution 1: 8% w/v CMC-RhoB, Solution 2: 20% w/v PVP-RhoB, and Solution 3: 10% w/v PVA-RhoB. This study aimed to determine the most suitable layer number for each coating solution, evaluating MN coating length and thickness at the midpoint of the total coating length using confocal microscopy. A consistent trend emerged across all coating solutions, with a significant increase (p -value < 0.05) in MN thickness after five layers of coating (5-layer coating) compared to one layer (1-layer coating) (Figure 5.7a). Furthermore, a further increase (p -value < 0.001) in total thickness was evident after 10 layers of coating compared to 1 and 5 layers, subsequently influencing the distribution of the coating across the MN surface. Parallel to the findings on

coating thickness, the analysis of coating height across the length of the needle demonstrated a decrease as the layers of coating increased. Particularly, for all coating solutions the 10-layer coating displayed significantly lower coating lengths (p -value <0.01 and p -value <0.001) compared to the 1-layer and 5-layer counterparts (Figure 5.7b). An average coating length of $\sim 400\ \mu\text{m}$ was calculated for the 10-layer coated MNs, compared to an average coating length of $\sim 550\ \mu\text{m}$ for the 1-layer and 5-layer coated MNs.

Assessment of the percentage of coverage per MN area coated with different solutions showed the highest coverage percentage after 5 layers of coating for all formulations (Figure 5.7c). Notably, the 8% CMC-RhoB solution achieved a 75% coverage after 5 layers. Confocal image analysis was used to assess the quality of coatings produced using Solution 1, 2, and 3 (Figure 5.7d). After a single layer of coating, non-uniform coating distribution was evident. Comparison of images after 5 and 10 layers of coating highlighted that the 10-layer coated MNs showed that the coating was predominant on the needle tips, resembling a bolus rather than being evenly distributed over the MN surface. Considering the coating quality, coverage percentage, and confocal images, the 5-layers coating provided the optimal balance between coating uniformity and coverage, providing the most consistent and desirable coating outcome without affecting the geometrical properties. In particular, as we can see from the graphs in Figure 5.7a and 5.7b the diameter and the height of the MNs was not affected significantly with the dimensions ranging within design requirements.

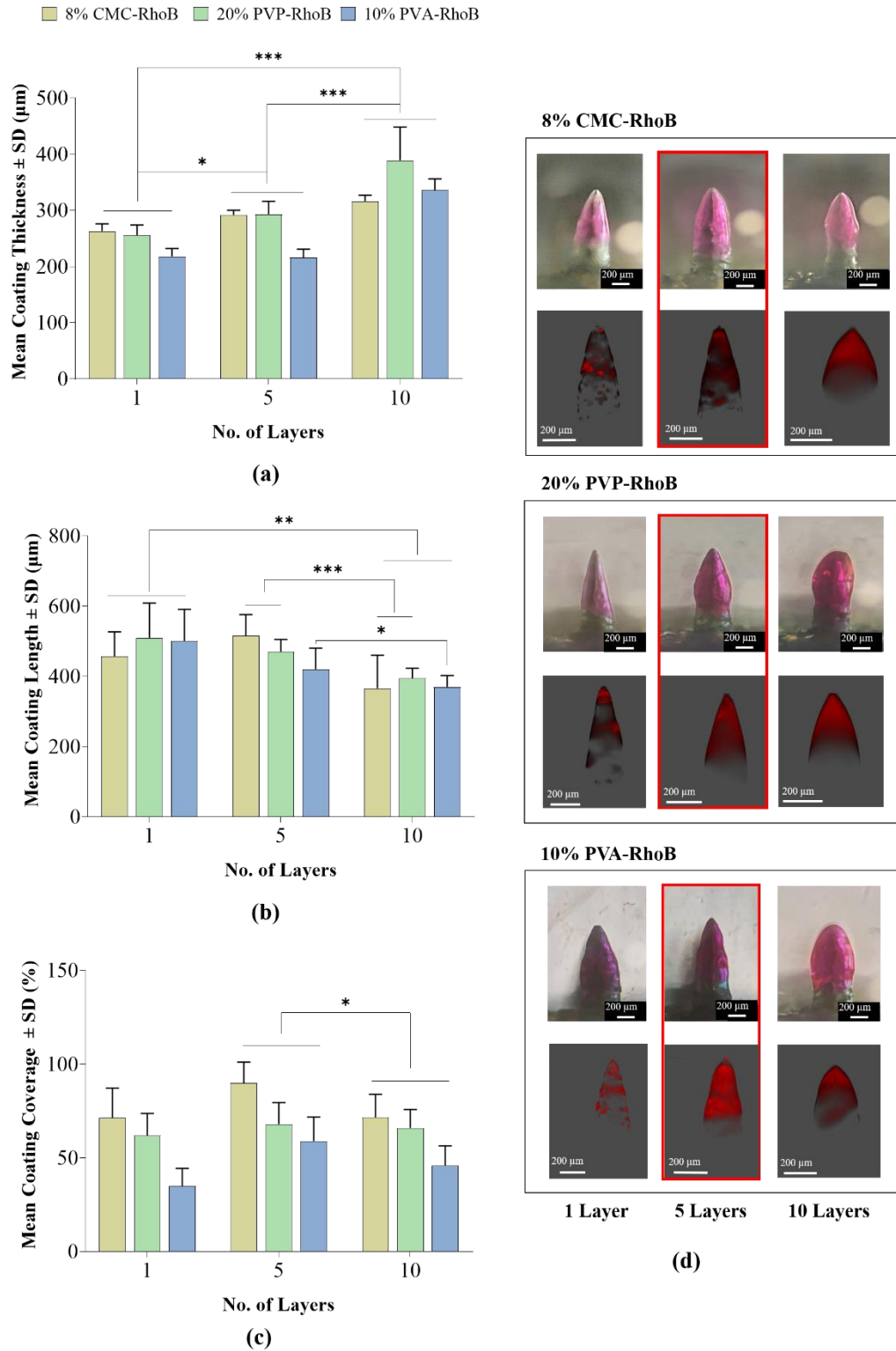


Figure 5.7: Coating Quality – Variation in coating (a) thickness (b) length and (c) percentage of coverage across the different coating solutions for varying numbers of coating layers. (d) Confocal and microscopic images providing a visual representation of the coating's distribution and behaviour with red boxes highlighting the optimal coating. Statistical significance denoted as *p value<0.05, **p value<0.01 and ***p value<0.001.

5.4.2.3 Drug Loading on MNs and Elution

The quantification of drug loading was conducted across the different coating solutions, denoted as 1, 2, and 3, each achieving successful drug incorporation of the small molecule RhoB onto the MN arrays. This yielded corresponding masses of RhoB per 36 MNs: approximately 12.5 μg for 8% w/v CMC-RhoB, 15 μg for 20% w/v PVP-RhoB, and 11 μg for 10% w/v PVA-RhoB (Figure 5.8a). Statistical analysis demonstrated a significant difference ($p\text{-value} < 0.01$) between the 20% w/v PVP-RhoB and 10% w/v PVA-RhoB groups with the 20% w/v PVP-RhoB solution demonstrating substantially higher drug loading and retention on the MNs, providing robust evidence of its enhanced drug loading efficiency.

Furthermore, investigations of drug release kinetics were conducted through analysis of the elution rates from the MNs within PBS. The 8% w/v CMC-RhoB and 10% w/v PVA-RhoB (2:1 volume rate) solutions showed a rapid elution within 1-5 min, while the 20% w/v PVP-RhoB solution (2:1 volume rate) exhibited complete elution over a 5 min timeframe (Figure 5.8b). No differences were observed between the rate of elution for the different groups, indicating comparable elution behaviour among the different coating solutions. Despite the slow degradation of the PVA a rapid elution was observed this can be attributed to the immediate solubility of the polymer coatings in the PBS solution. When the MNs are placed in PBS, the aqueous environment rapidly dissolves the polymer coatings, leading to different release of the drug. This solubility-driven elution process occurs before significant degradation of the polymer can take place, resulting in a fast release of the drug. It is important to consider that the degradation tests were conducted on freeze-dried samples, which typically exhibit a different structural integrity compared to their freshly coated counterparts. The freeze-drying process increases porosity, potentially slowing the degradation rate as the polymer matrix becomes more resistant to breakdown. In contrast, the elution tests were

performed on samples coated with the polymer in its viscous solution form, which retained a more homogeneous and soluble nature when exposed to PBS.

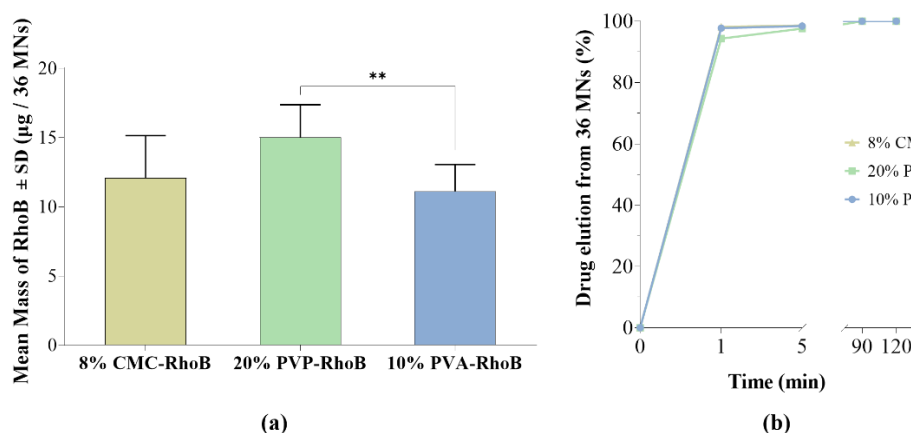


Figure 5.8: Elution – (a) Quantity of drug successfully loaded onto the MNs and (b) the rate of drug elution from the MN across different coating solutions. Statistical significance denoted as **p value<0.01.

Overall, this analysis highlights the complexities of drug retention and release kinetics from the coated MNs. The variations in drug loading quantities between coating solutions underscore the influence of formulation on drug incorporation efficiency.

5.4.2.4 Adhesion Forces between Coating Solution and MNs

To understand the durability, effectiveness and reliability between the three different coating solutions on the metallic MNs, adhesion forces at the interface between coating solution and stainless steel surface were determined. The obtained results consistently demonstrated adhesion forces exceeding 5 N, which is the force required for the penetration of MN arrays into porcine skin. This suggests that all tested coating solutions effectively adhered to the stainless steel surface, ensuring that no residual coating would remain on the skin during MN insertion. Specifically, the coating solutions exhibited notable adhesion forces, with values of 24.64 ± 5.3 N for 8% w/v CMC-RhoB, 37.82 ± 6.9 N for 20% w/v PVP-RhoB, and 18.93 ± 5.2 N for 10% w/v PVA-RhoB (Figure 5.9). These forces were significantly higher (p-value < 0.001) than the minimum required penetration force of 5 N. These findings affirm that the

high adhesion forces of the coatings resulted in a sufficient adhesion strength on the stainless steel substrate, facilitating their transfer to the skin during MN insertion.

When comparing the performance of the different coating solutions, it was observed that 20% w/v PVP-RhoB demonstrated the highest adhesion forces, with a statistically significant difference ($p\text{-value} < 0.001$) compared to the other coatings. In contrast, 8% w/v CMC-RhoB and 10% w/v PVA-RhoB exhibited similar adhesion forces. The enhanced adhesion properties of the 20% w/v PVP-RhoB coating can be attributed to the combined effects of PVP's inherent adhesive properties and the concentration of PVP in the solution.

The extent of deformation during the MN insertion depends on the adhesion properties of the polymer used for their coating. Polymers with lower stiffness and higher flexibility, such as PVA, are more likely to deform compared to stiffer polymers like PVP. As result of the high adhesion strength, obtained when PVP solution was used, is less likely to delaminate upon insertion. However, its higher stiffness may lead to less deformation, maintaining a more consistent surface area for drug diffusion. While PVA and CMC might exhibit higher deformation due to its lower adhesion properties. Deformed coatings present less surface area, potentially slowing the diffusion of the drug, influencing the drug elution rate.

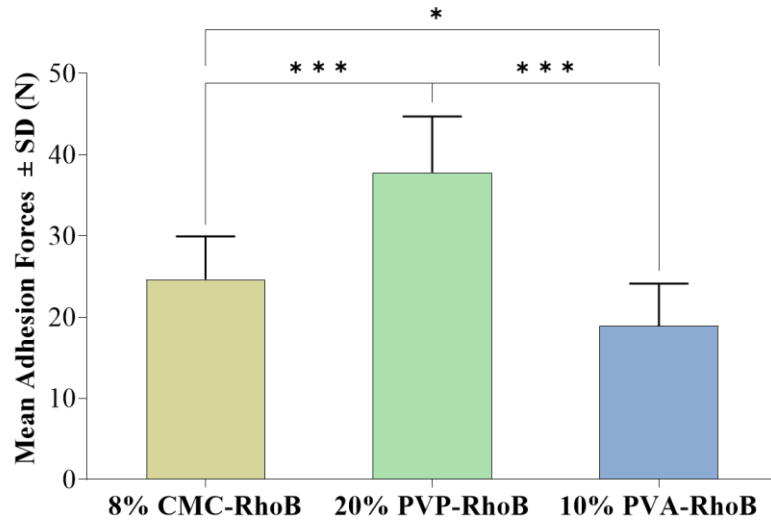


Figure 5.9: Coating Adhesion Forces – Adhesion forces achieved by various coating solutions (8% w/v CMC-RhoB, 20% w/v PVP-RhoB, and 10% w/v PVA-RhoB) when applied to a stainless steel surface. Between the solutions the 20% w/v PVP-RhoB exhibited the highest adhesion forces which indicate highest coating integrity. Statistical significance denoted as *p value<0.05 and ***p value<0.001.

5.4.2.5 Penetration Depth into Porcine Skin–Optical Coherence Tomography

Optical Coherence Tomography (OCT) presents a non-invasive and high-quality imaging method extensively employed for obtaining cross-sectional images of light-scattering media, particularly biological tissues. Operating on the principle of low-coherence interferometry, OCT generates pseudo-colour images of tissues based on the intensity of reflected light. OCT has demonstrated its effectiveness in providing detailed imaging of tissue structures and foreign object interactions such as when dissolvable MNs were analysed (Figure 5.10a) demonstrating the needle penetration site. However, we encountered a significant limitation related to the material of the MNs employed in this investigation. The fundamental limitation arose from the inherent property of OCT's operating principle; specifically, the OCT laser's inability to penetrate metals. Consequently, the images obtained through OCT failed to

capture any visual observations regarding the penetration depth of the metallic MNs into the skin as can be seen from Figure 5.10b.

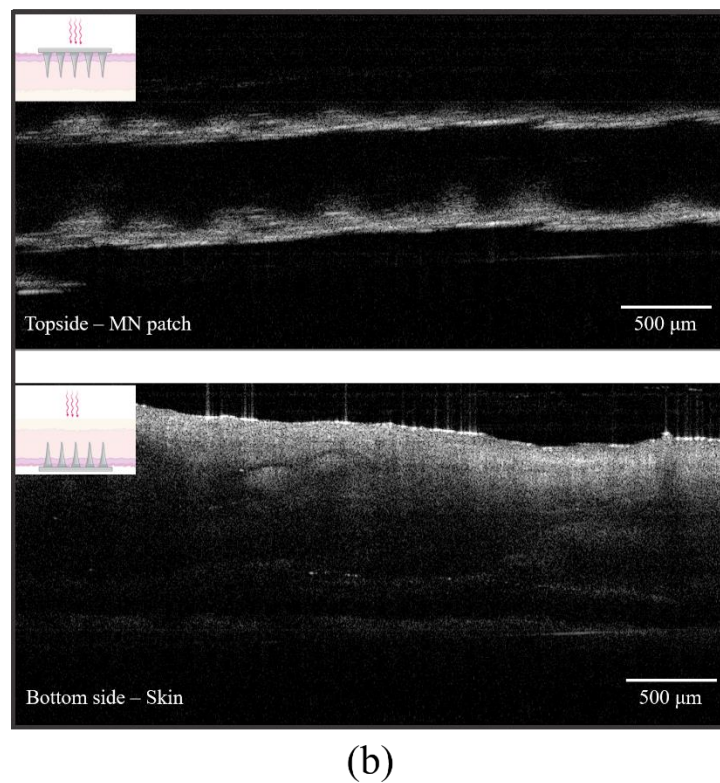
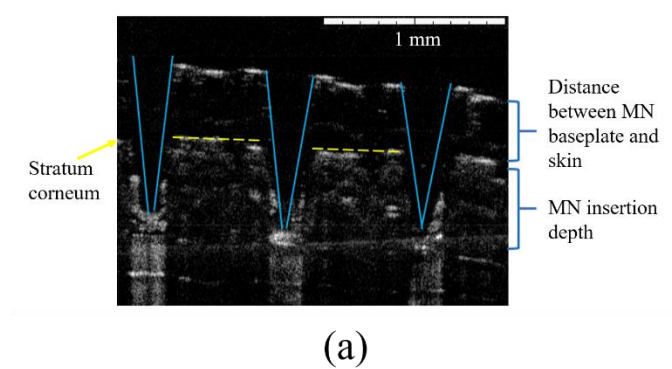


Figure 5.10: Optical Coherence Tomography - 2D images obtained topside and bottom side to visualise the penetration depth of MNs into porcine skin. The key limitation becomes apparent—MNs were constructed from stainless steel, which hindered their visualisation using OCT.

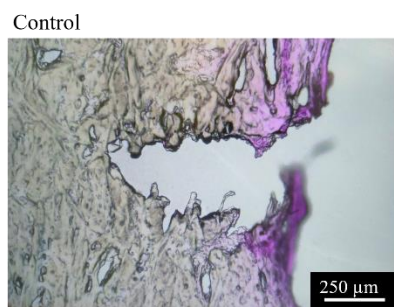
5.4.2.6 Histological Analysis

Histological analysis was conducted to measure the percentage of coated MNs inserted into the skin and to observe the model drug distribution within the skin. Fluorescence-field microscopy was used to visualise MN insertion and drug diffusion in the porcine skin model.

Optical microscopy images obtained from skin cross-sections after MN removal showed the successful MN insertion into full-thickness porcine skin for all coated MN groups (Figure 5.11a). The images demonstrated the depth of the micro-holes created by the MNs. The penetration depth achieved was approximately 600 μm across all groups with no significant difference between the coated MNs and dissolvable polymeric MN control (Figure 5.11b). The consistent achievement of this penetration depth across different coating solutions underscores the robustness and reliability of the MN system in facilitating drug delivery beyond the *stratum corneum*, thus enhancing the potential for successful transdermal drug administration.

The distribution of the model drug, RhoB, within the skin was captured through fluorescence images as can be seen in Figure 5.12. The model drug was predominantly observed within the inner layers of the skin, demonstrating successful diffusion and penetration beyond the *stratum corneum*. This finding supports the efficacy of the MNs in delivering drugs to deeper skin regions, a crucial aspect for achieving therapeutic outcomes in transdermal drug delivery. In addition, as observed from the histological analysis and degradation studies, different polymers exhibit distinct behaviours that affect their diffusion profiles. For instance, PVP exhibits high adhesive strength and rapid degradation upon contact with tissue fluid, which facilitates a swift release of drugs. PVP coatings tend to form robust films, enhancing mechanical strength and ensuring efficient drug-loading, but their rapid diffusion could limit sustained drug release. In contrast, CMC forms viscoelastic gels that provide a more controlled and sustained drug release. The high viscosity of CMC can lead to more significant

deformation, reducing the surface area and potentially slowing diffusion, as seen in the slower degradation rates of higher concentration solutions. PVA also demonstrates high drug-loading capacity and controlled release properties, with its hydrophilic nature promoting rapid dissolution and efficient drug diffusion. However, deformations in PVA coatings, particularly at higher viscosities, can result in non-uniform distribution and less efficient permeation. The deformation characteristics of these polymers, influenced by their viscoelastic properties and adhesion strength, are directly correlated with their diffusion and permeation profiles, as supported by histological evidence.



(a)

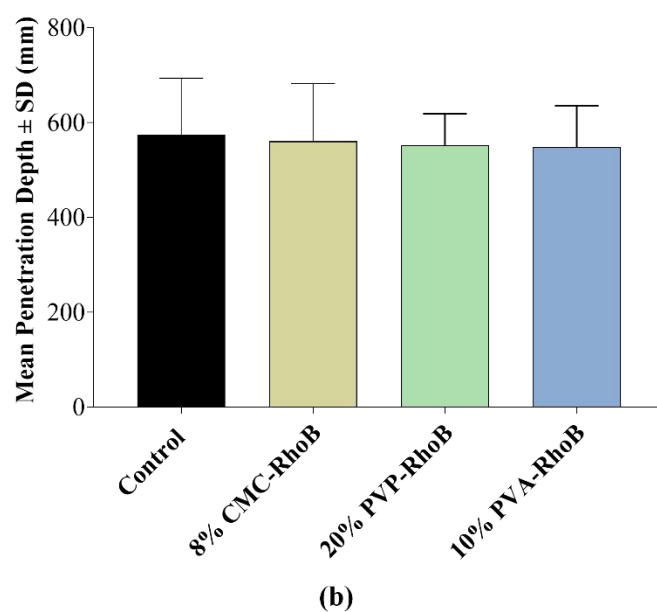


Figure 5.11: Histological Analysis – (a) Microscopic images of skin sections at the MN insertion sites and (b) maximum penetration depths achieved after histological analysis, providing a visual observation of their successful insertion into the skin.

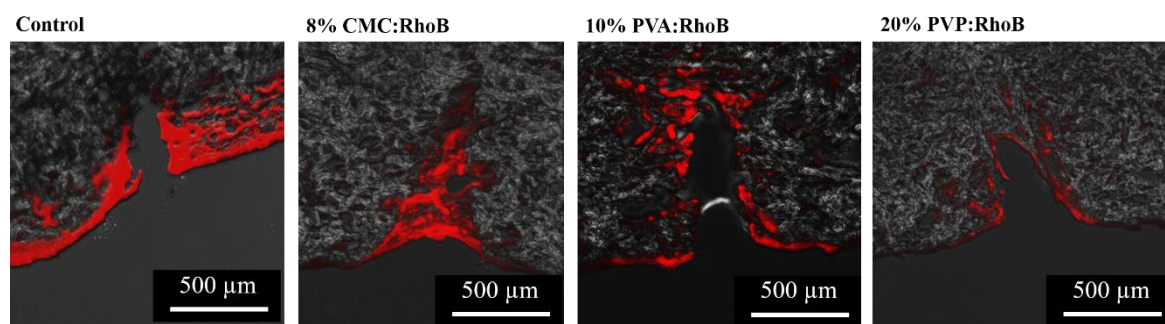


Figure 5.12: Fluorescence Microscopy of Penetrated Skin –Images obtained from skin cross-sections after MN removal, demonstrating insertion of coated MNs beyond the *stratum corneum* and drug dispersion of RhoB within the skin.

5.4.2.7 *In Vitro* Drug Delivery into Skin

In vitro drug loading analysis was conducted on the specimens of porcine skin post-penetration with coated MNs, employing RhoB as the model drug. The amount of drug absorbed from the skin post-MN insertion exhibited distinct variations among the different coating solutions as calculated by the slope of the absorbed drug. Specifically for this testing the MNs were held on the skin for different time points and the RhoB elution was calculated to investigate the required insertion time of each coated MNs to achieve optimal drug delivery. After 5 min of MNs insertion into the skin the average drug absorption was $6.7 \pm 1.8 \mu\text{g}/\text{cm}^2$ for CMC-RhoB, $12.2 \pm 1.8 \mu\text{g}/\text{cm}^2$ for PVP-RhoB, and $7.2 \pm 1.2 \mu\text{g}/\text{cm}^2$ for PVA-RhoB (Figure 5.13a). This indicates that 50-60% of drug loaded on CMC-RhoB and PVA-RhoB coated MNs was absorbed by the skin within 5 min of insertion. While for the PVP-RhoB coated MNs 80% of the drug loaded was absorbed by the skin (Figure 5.13b). Notably, an increase in the duration that the MNs remained in contact with the skin led to a progressive increase in the amount of drug absorbed. For instance, after 30 min, a substantial ~70% of the total drug content initially loaded within the CMC-RhoB and PVA-RhoB coated MNs was absorbed by the skin (Figure 5.13b). This translated to an average drug absorption ranging between $7\text{-}13 \mu\text{g}/\text{cm}^2$ across the various coating solutions, demonstrating the time-

dependent drug release capability of the MNs. Complete drug delivery from the MNs into the skin was achieved after 60 min for MNs with PVP-RhoB coating solution. In contrast, the PVA- and CMC- coated MNs required an extended duration of 2 h to fully deliver the total drug loaded on MNs into the skin.

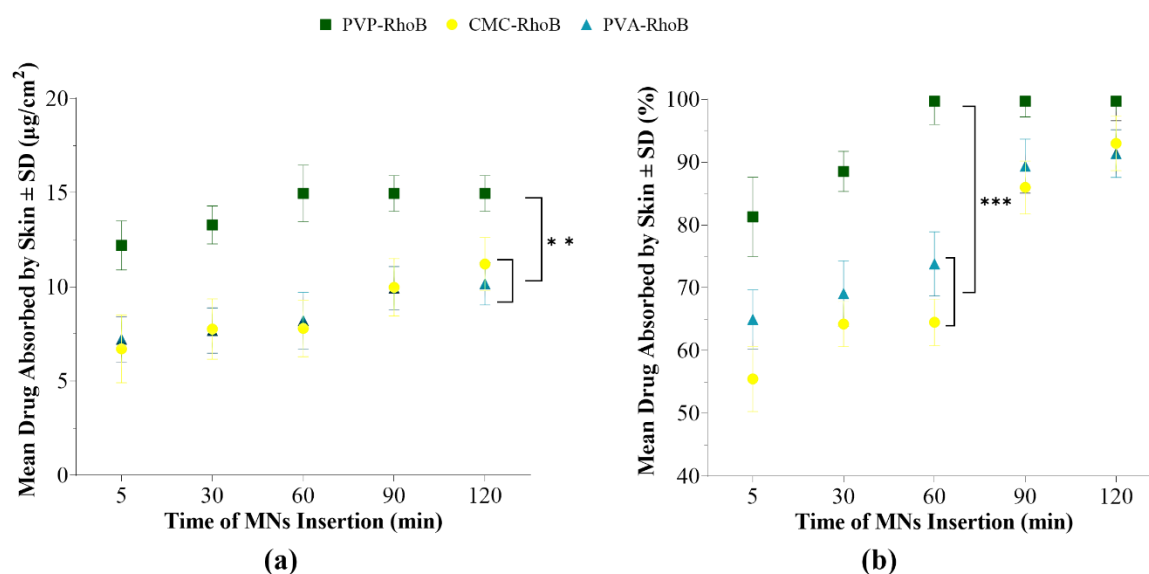


Figure 5.13: *In Vitro* Drug Delivery into Skin – (a) amount and (b) percentage of drug absorbed by skin for CMC-RhoB, PVP-RhoB, and PVA-RhoB following MNs insertion over time. Statistical significance denoted as **p value<0.01 and ***p value<0.001.

5.4.2.8 *In Vitro* Permeation through the Skin

The Franz cell test was conducted to assess the transdermal permeation profile of RhoB from the MN coated with PVP-RhoB, CMC-RhoB and PVA-RhoB.

MNs coated with CMC-RhoB presented a rapid permeation within 15 min leading to 20% of the loaded drug diffusing through porcine skin within a dynamic environment with continues liquid flow (Figure 5.14). This early rapid release might be advantageous for achieving immediate therapeutic concentrations. However, in the subsequent period between 15-120 min, a reduction in the rate of permeation was noted, culminating in an accumulative

permeation of 40%. This slower phase potentially indicates a more regulated drug release mechanism. A notable surge in drug diffusion occurred between 120 to 240 min when a total of 80% of the drug permeating the skin. This instant increase could be attributed to a secondary release phase or alterations in the skin's barrier properties. For instance, the secondary release phase is related to the multi-layered structure of the coating where the initial layer releases quickly, followed by a slower release from the underlying layers. Furthermore, as the outer layers deplete, the diffusion gradient changes, potentially slowing down the release rate. After a certain point, additional drug stored within the MN matrix become accessible, leading to a renewed increase in diffusion rate and a secondary release phase. Changes in the skin's barrier properties due to the continuous flow of liquid in the Franz cell can gradually increase the hydration of the stratum corneum (the outermost layer of the skin). The increased hydration can disrupt the lipid matrix, making the skin more permeable to the drug, enhancing permeability and as result increasing drug diffusion. The presence of MNs in the skin can also lead to skin alteration by creating microchannels in the skin, which may become more pronounced as the experiment progresses. These microchannels can facilitate greater drug permeation by providing direct pathways through the stratum corneum, bypassing the primary barrier function of the skin. Post-240 min until the 24-h mark, there was a slow permeation with a further 10% of the drug permeating. When compared to conventional drug delivery systems, the MNs coated with CMC-RhoB solution display a unique combination of both rapid and sustained release patterns. This dynamic release behaviour underscores its potential adaptability in diverse therapeutic scenarios.

The cumulative amount of the drug permeated, as plotted against time, provided an initial increase, followed by a constant release rate after approximately 120 min (Figure 5.14). The permeability coefficient (K_p^{CMC}), which represents the distance the drug permeates through the skin per unit time under a unit concentration gradient, was calculated. Specifically, the

permeability coefficient is calculated by determining the flux (J) as the slope from the linear portion of the drug permeation profile and dividing it by the drug concentration loaded onto the MNs. Using the data from the linear phase (between 1 min to 120 min), and knowing the area and the concentration of the drug loaded, the permeability coefficient (K_p^{CMC}) of the drug through porcine skin was calculated to be approximately 0.0015 cm/min. Within this initial stage the steady-state flux (J) drug permeated was found to be 0.018 $\mu\text{g}/\text{cm}^2/\text{min}$. The kinetics observed are indicative of a 40% release resulting from the drug molecules on the surface of the MNs or those in the outermost layers of the formulation. The rate was increased between 24-h of MN application leading to a J of 0.044 $\mu\text{g}/\text{cm}^2/\text{min}$. The subsequent linear phase represents the instant diffusion of RhoB from the MNs into the porcine skin.

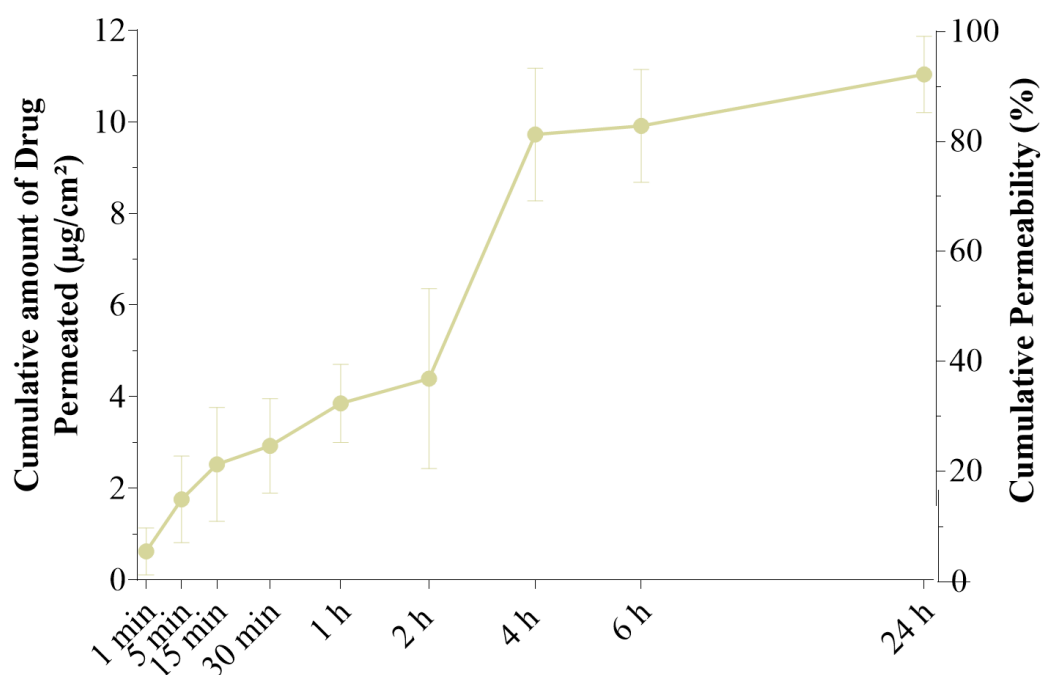


Figure 5.14: Time-dependent Permeation of CMC-RhoB loaded MNs—Graphical representation of the cumulative amount of drug permeated ($\mu\text{g}/\text{cm}^2$) and cumulative permeability (%) of RhoB delivered through porcine skin over a 24-hour period using CMC-RhoB loaded MNs. The curve illustrates an initial rapid increase followed by a plateau, indicating near saturation of drug permeation after 6 hours.

PVP-RhoB loaded MNs reached a plateau 1 h after the MN application achieving around 15 μg release, which corresponds to the total amount loaded on the MNs Figure 5.15. The graph shows an instant increase in the drug permeation from 0 to 60 min, suggesting rapid absorption or diffusion. However, after this 60-min mark, the rate of permeation plateaus since the total amount of loaded drug was already transferred. From the data, the flux of the drug can be determined as the slope of the linear portion of the quantity released vs. time plot. Specifically, the J derived from the linear portion of the curve (between 15 min and 60 min), where permeation appears to be consistent, was determined to be $J = 0.23 \mu\text{g}/\text{cm}^2/\text{min}$ with a permeability coefficient $K_p^{\text{PVP}} = 15.77 \times 10^{-3} \text{ cm}/\text{min}$. These results suggest that PVP-RhoB, when loaded on MNs, permeates rapidly through porcine skin within the initial 60 min.

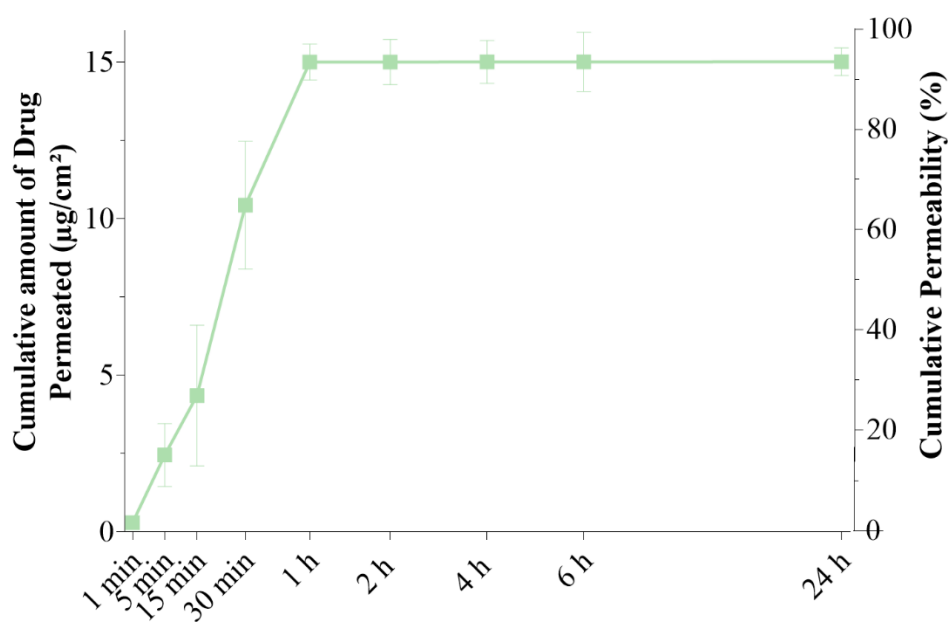


Figure 5.15: Time-dependent Permeation of PVP-RhoB loaded MNs - Graph showing the cumulative amount and percentage of drug over 24 h. The PVP-RhoB loaded MNs initial led to rapid increase in the measured amount, which then stabilises over time, maintaining a consistent level.

In term of PVA-RhoB loaded MNs, from the onset at the 1-min mark, extending up to a 24-h duration, the curve rapidly rises, indicating an increase in the cumulative amount of the drug that permeated through the skin Figure 5.16. A prominent characteristic of this curve is its tendency to plateau, especially after the 6-h mark, suggesting that the drug's permeation is approaching a saturation or equilibrium point at a total of $96 \pm 3\%$ drug released. Quantitative analysis yielded a J of $0.017 \mu\text{g}/\text{cm}^2/\text{min}$ with K_p^{PVA} $1.52 \times 10^{-3} \text{ cm}/\text{min}$ permeability coefficient for the PVA-RhoB loaded MNs. At the end of the 24-h period, a cumulative drug permeation of $11.12 \mu\text{g}/\text{cm}^2$ is recorded, closely mirroring the given total amount of $11.77 \mu\text{g}/\text{cm}^2$.

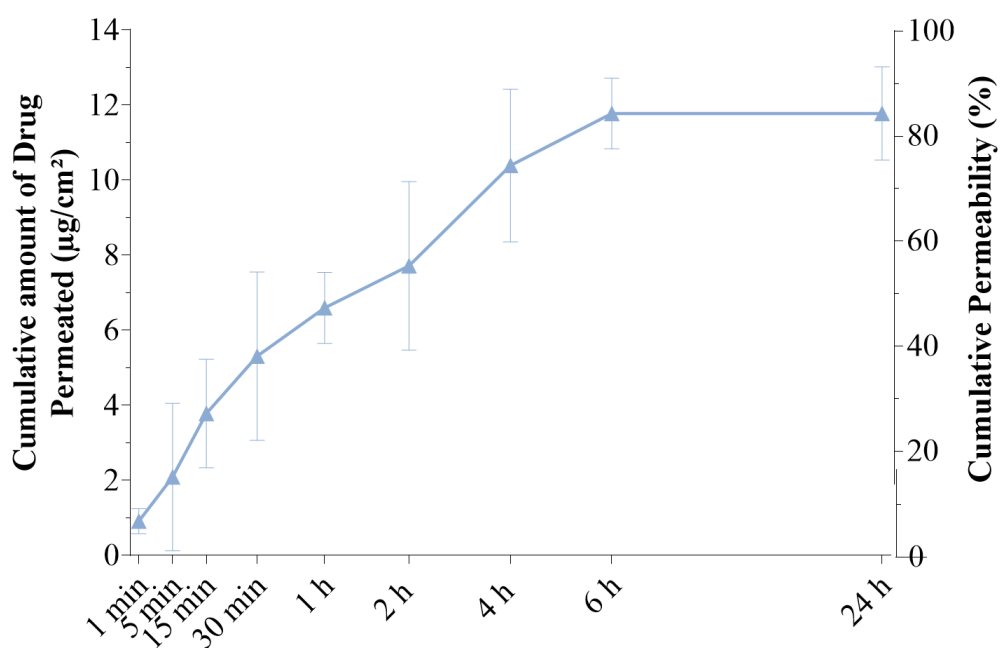


Figure 5.16: Time-dependent Permeation of PVA-RhoB loaded MNs – Progressive increase in drug permeation over a 24-hour period, showing the correlation between cumulative amount permeated and the cumulative permeability percentage.

Comparing the results, the CMC-RhoB MNs exhibited the most pronounced initial release, with 20% of the drug diffusing within the first 15 min. In contrast, PVP-RhoB MNs showed

a consistent release pattern, achieving full drug transfer within 60 min. PVA-RhoB had more moderated release within the initial phase. Furthermore, PVP-RhoB MNs displayed the highest permeability coefficient, indicating the most efficient diffusion through the skin, followed closely by CMC-RhoB and PVA-RhoB. CMC-RhoB MNs presented the highest flux between 2 h to 4 h of application, making it the most dynamic in drug release. PVP-RhoB MNs, on the other hand, maintained a consistent flux, while PVA-RhoB showed a more gradual release. Taken together, these observations highlight the varying release kinetics associated with different coating materials and underscores the importance of selecting an appropriate coating solution based on the desired drug delivery profile.

All coated MNs using the different polymer-based coating solutions, when tested using the Franz cell, exhibited release profiles that were slower than their respective degradation rates. This observation can be attributed to the differences in the experimental conditions and mechanisms changing drug release and polymer degradation. In the Franz cell setup, the drug release process is influenced by several factors, including the diffusion of the drug through the skin, the interaction of the drug and polymer with the skin's microenvironment, and the hydration of the polymer coating. The skin acts as a barrier, slowing down the permeation of the drug even as the polymer begins to degrade. Additionally, the degradation of the polymer coatings primarily occurs when the polymers are exposed to an aqueous environment, which leads to their breakdown and the subsequent release of the drug. However, in the controlled environment of the Franz cell, the release of the drug depends by the rate at which the drug can diffuse through the skin layers. This diffusion process is slower than the degradation of the polymer itself, as it involves not just the dissolution of the polymer but also the penetration of the drug molecules through the stratum corneum.

Furthermore, polymers coated on MNs might deform differently upon insertion into the skin due to their different mechanical properties and viscoelastic behaviours. For instance, PVP is

known for its flexibility and good film-forming properties. Upon insertion, PVP coatings may deform slightly but maintain integrity due to their elastic nature, retaining a consistent drug release profile. The rapid hydration of PVP in the presence of skin moisture facilitates swift drug elution, making PVP-coated MNs suitable for applications requiring immediate therapeutic effects, such as pain relief or acute treatments. PVA has moderate flexibility and can form strong films, exhibiting deformation upon insertion such as micro-channels in the coating, leading to an initially faster drug release followed by a more controlled release as the polymer gradually dissolves and hydrates in the skin environment. PVA coated MNs can offer a consistent drug delivery rate, making them potentially suitable for chronic disease management, such as for delivering insulin in diabetes, where the initial burst release quickly lowers blood sugar, and the sustained release maintains glucose levels. Finally, CMC is more rigid compared to PVP and PVA and forms highly viscous gels when hydrated. Upon insertion, CMC coatings might crack or fragment, especially under the mechanical stress of penetrating the skin. This behaviour can lead to a burst release of the drug from the fractured areas and a more sustained release from the remaining intact polymer coating. The dual-phase release profile provided by CMC is advantageous for chronic conditions where a prolonged therapeutic effect is desired.

5.5. Discussion

The main objective of this chapter was the analysis and optimisation of polymer solutions (CMC, PVA, and PVP) utilised in the coating of MNs to ensure the adequate loading of drugs. Throughout various experimental assessments, important findings were obtained, offering insights into the behaviour and performance of the employed polymer coatings under various conditions and formulations.

The increased polymer concentrations are synonymous with increased viscosity, aligning with established scientific examples in polymer science [266]. Enhanced viscosity is crucial

for improved adhesion and uniformity in the coating process, facilitating optimised drug loading into MNs [70,237,267]. The temperature dependence of the viscosity demonstrates a differential decrease at physiological temperatures (37 °C). This result is in tune with established thermodynamic principles, where elevated temperatures typically promote molecular mobility, thereby reducing viscosity [268,269]. Comparing the changes in viscosity of PVP, PVA, and CMC-based coatings at body temperature (37 °C) demonstrated the behaviour of each polymer under physiological conditions. At 37 °C, the viscosity of all three polymers decreases, but the extent of this reduction varies. For PVP, the decrease in viscosity at body temperature ensures good coating spread and adhesion, enhancing drug release upon skin contact. PVA also shows a reduction in viscosity, which facilitates uniform coating and efficient drug loading. CMC, known for its higher initial viscosity, experiences a significant viscosity drop at physiological temperatures, improving its ability to adhere to the MNs and release the drug effectively. By comparing these polymers, it becomes evident that the lower viscosity at body temperature is beneficial for all three, but the degree of change and its impact on coating performance differ. This comparison is essential for selecting the most appropriate polymer for specific drug delivery applications, ensuring optimal *in vivo* performance and therapeutic outcomes. Evaluating the degradation kinetics, lower concentration solutions exhibited accelerated degradation rates, a phenomenon previously observed in polymer-based drug delivery systems [270–272]. This behaviour can be attributed to the increased porosity and permeability of the coatings when applied at lower concentrations. When the polymer solution is less concentrated, the resulting polymer solution tends to be less dense. This structural characteristic allows water and other environmental factors to penetrate the polymer more easily, accelerating the degradation process. At lower concentrations, the polymer chains are less tightly packed, creating a more porous matrix. This porosity facilitates faster hydrolysis or other degradation mechanisms

within the polymer. In contrast, higher concentration solutions are more resistant to water penetration, thus slowing down the degradation rate. Such rapid degradation could affect drug release profiles, requiring careful concentration selection to tailor degradation kinetics to desired therapeutic targets.

Among various MN coating methods, some excel in achieving uniformity and selective coating of the MN while avoiding the base substrate. Precision techniques like dip-coating and inkjet-coating are particularly effective in this regard, focusing the coating specifically on the MN shafts and minimising waste. Coated MNs have been tested in humans. For example, coated MNs have been used to study treatment of warts [273], osteoporosis [274] and migraine [275]. The tolerability and acceptability of coated MNs in humans has also been evaluated. Griffin *et al.* [276] noted redness of the skin (erythema) in humans, which lasted day 3 to day 7 post– MN application. It was found that most of the subjects preferred MN patch application over needle and syringe [276].

This chapter systematically evaluated the effect of different layers (1, 5, and 10) using various coating solutions (8% CMC-RhoB, 20% PVP-RhoB, and 10% PVA-RhoB) on MN thickness, length, and coverage. The significant increase in MN thickness with more layers, especially after five layers, suggests an enhanced drug-loading capacity. This relationship between number of layers and coating thickness is also observed from other studies, obtaining that the greater the number of dips, the greater will be the coating thickness [237,277–279]. This increase in coating thickness is accompanied by a reduction in coating length, indicating a trade-off between thickness and coverage. This results in a thicker layer near the base, when the polymer coating is applied more thickly, but leaves less polymer available to cover the entire length of the MN. Consequently, the thicker the coating, the shorter the portion of the MN that remains covered. This trade-off highlights the need to balance coating thickness and coverage. While a thicker coating may enhance drug loading

capacity and ensure a more robust release, it can also reduce the effective length of the MN that can penetrate the skin and deliver the drug. The optimal balance was achieved with a 5-layer coating, as evidenced by the highest coverage percentage and uniform distribution. Because the dip-coating method provides control and precision in coating, the amount of drug loaded using the polymer-based coating solutions ranged from 11-16 μg for RhoB. The amount of drug loaded on the MNs is significantly higher than other studies. For instance, drug loading of 0.4 ng to 10 μg was achieved when loaded virus-like particles or bacteria [216,280,281]. In general, the approximate weight ranges of the different materials that have been coated are: peptides and proteins, 0.05 μg to 500 μg [282,283] and small molecules, 132 ng to 1.9 and 3.8 μg [284,285]. Overall, the capacity of the polymer-based coated MNs is particularly suitable for delivering small-molecule drugs, peptides, and vaccines that require precise dosing. For instance, MNs can be employed for transdermal delivery of insulin in diabetic patients, offering a pain-free alternative to traditional injections and ensuring better compliance. Additionally, the device can be used for administering vaccines, such as influenza or hepatitis B, enabling quick and efficient immunisation with minimal discomfort. The controlled and precise delivery enabled by the dip-coating method also makes MNs ideal for localised treatment of skin conditions, such as psoriasis or melanoma, where targeted drug delivery can enhance therapeutic outcomes while minimising systemic side effects.

The high adhesion forces exhibited by all tested coating solutions reinforce the efficiency of the MNs in ensuring consistent drug transfer through the skin. This observation is vital, given that adequate adhesion is critical for effective MN penetration and drug delivery [242]. The higher performance of the 20% PVP-RhoB in achieving superior enhanced forces could be attributed to PVP's inherent adhesive properties [267,268].

Delivery efficiency of a coated MN is an important and biologically relevant design parameter because it directly affects the dose of the active material that is administered *in*

vivo. The delivery efficiencies of MN arrays coated *via* the dip-coating method average around 70%–90% [286]. Delivery efficiency is defined as the fraction of the drug coated on a MN array that gets delivered into the skin. Although this is an important parameter, not all published studies have determined it. However, in this chapter the delivery efficiency is determined and has been reported to be in a range of 60% -90 % in porcine skin which is in line with the range of delivery efficiency of coated MNs from other studies when tested in skin [287–291].

The permeation studies further supported the observations obtained from the degradation and absorption evaluations. Of note is the observation that coatings that exhibited higher adhesion forces also exhibited encouraging penetration profiles, highlighting consistency in findings. For example, PVP-RhoB, with its high adhesive force, also presented high permeability coefficients and a steady drug release flux, in line with theoretical expectations and existing scientific claims. This connection suggests that the adhesive properties of the polymer coatings play a crucial role in the effective delivery of the drug through the skin. Higher adhesion forces ensure that the coating remains intact during the insertion of the MNs, allowing for a more efficient transfer of the drug into the skin layers. This ensures that a higher proportion of the loaded drug reaches the target site, which is particularly important for achieving the desired therapeutic effect. The combination of strong adhesion and effective penetration can also be advantageous for applications where precise dosing and controlled release are required. Similarly, in vaccine delivery, strong adhesion ensures that the antigen is effectively delivered to elicit a robust immune response. CMC-RhoB showed a combination of rapid and sustained release, a dynamic profile that could be advantageous for various therapeutic applications requiring immediate and prolonged drug effects. Given these promising results, MN drug delivery devices have a wide range of potential applications. For instance, they can potentially be used in chronic disease management, such as for delivering

insulin to diabetic patients, providing a painless and user-friendly alternative to traditional injections. The rapid and sustained release profiles observed, particularly with CMC-RhoB, suggest that these devices could maintain steady insulin levels, enhancing glycemic control. Similarly, MNs are suitable for administering vaccines, allowing for quick, efficient, and minimally invasive immunisations. Additionally, MNs can be employed in dermatology for localised treatment of skin conditions like acne, psoriasis, and melanoma, where precise drug delivery is essential for effective treatment. The ability to tailor the release profile by selecting appropriate coatings makes these devices versatile tools in personalised medicine, where treatments can be customized to the specific needs of patients. For instance, the rapid release phase of certain coatings could be ideal for immediate relief in acute pain management, while the sustained release phase could ensure prolonged therapeutic effects, beneficial for chronic conditions.

This study demonstrated the significance of a synchronised strategy where the degradation rate complements the desired drug release kinetics to optimise the therapeutic efficacy of MNs in transdermal applications, paving the way for innovative advancements in transdermal drug delivery systems. Future work should explore the release rate of larger molecule through the optimal polymer-based solution in order to investigate the effect of drug molecular weight on the release profile for therapeutic applications.

Chapter 6: Overall Discussion, Concluding Remarks and Future Perspectives

6.1. Overall Discussion

Transdermal drug delivery systems offer a non-invasive and patient-friendly approach, yet conventional methods often encounter limitations with nucleic acid and large drug molecule delivery, causing discomfort to patients. MN present a promising solution due to their painless and minimally invasive drug delivery capabilities. Recent progress in AM has enabled fabrication of metallic MN arrays with desirable therapeutic delivery properties.

This study uses the DMLS process to fabricate MN arrays from metallic powder, followed by electropolishing. To provide optimal design control, a DoE software was used to optimise the process parameters systematically and efficiently, generating statistically optimal experimental designs with minimal trials. The main aim was to develop coating 3D printing solid MN arrays suitable for clinical application as efficient TDD systems. Initially, the key parameters were determined for the development of ideal 3D printed and electropolished MNs. Then, MN coating solution properties were optimised to ensure effective coating. This involved strategies to lower the solution surface tension by using a protective mask while investigating optimal solution concentration and number of coating layers, thereby enhancing the wetting of the MN surface, and improving the uniformity of the coating. Finally, the optimal coated MNs were thoroughly assessed based on their functional properties. This evaluation combines critical aspects like skin penetration, dry drug elution, and their potential to enhance efficacy, efficiency, ease of penetration (potentially painless), as well as the loading and rapid release of drugs. Within this chapter, the key findings of this thesis are presented.

Successful development of solid metallic 3D printed MNs as TDD systems using DoE approach.

Two different DoE approaches were employed to analyse and optimise the 3D printing and electropolishing parameters, ensuring that the desired geometrical, structural, surface, and

mechanical properties of the MNs were achieved. The main finding from DoE was the identification of laser power during printing and time and current during electropolishing as the most significant factors influencing the dimensions and structural integrity of MNs. This insight is vital for the field of additive manufacturing, particularly for medical devices, as it underscores the need for precise control over the printing process and any required post-process to achieve products that meet clinical standards. Higher laser power tends to increase the melt pool size, enhancing the flow and fusion of metal powders, which in turn affects the overall dimensions and structural cohesion of the printed MNs. Conversely, laser power that's too high can lead to excessive material spread and thermal distortion, underscoring the need for careful optimization. During electropolishing, the time and current directly influence the rate of material removal and surface finish. Properly calibrated electropolishing parameters ensure minimal dimensional reduction while achieving a smooth, defect-free surface, crucial for painless skin penetration and drug delivery efficiency. These insights are vital for future work as they provide a clear direction for optimizing MN fabrication processes, ensuring consistency, and enhancing the functionality of MNs for various medical applications.

The development of both BBD and CCD design for factorial analysis not only clarifies the impact of each separate factor but also provides a reusable framework. This allows for various numerical optimisation studies to be conducted, adapting to new geometrical and structural properties based on the specific site of application. Furthermore, understanding the interaction between these factors allows for more targeted adjustments in the manufacturing process, improving the reliability and performance of the final product, and paving the way for further innovations in the design and application of MNs.

The electropolishing process parameters were linked back to the parameters and conditions inherent to the 3D printing process in the development of a comprehensive process-structure-

property map. The predicted optimal levels for 3D printing were a laser power of 65 W, laser speed of 650 mm/s, and trace width of 0.09 mm. In parallel, the optimal electropolishing parameters were duration of 60 s and a current of 7 A. These findings contrast with Krieger *et al.*'s previous research, which utilised a 55 W laser power, 600 mm/s speed, and 0.12 mm trace width for 3D printing, alongside electropolishing parameters of 5 A for 90 s which was primarily focused on the development and evaluation of microneedle electrodes (MNEs) for surface electromyography (sEMG) [83]. The research demonstrated that these parameters effectively produced MNs that could penetrate the skin without causing discomfort, significantly reduce electrode-skin impedance, and maintain signal quality comparable to traditional wet Ag/AgCl electrodes over prolonged periods. Krieger's *et al.* study, while informative, did not employ a comprehensive optimisation, exploring variations in the different parameters enhancing in this way the performance and application range. This contrast highlights the significance of this work in advancing the fabrication process for metallic MNs, demonstrating the benefits of systematic optimisation in enhancing the field's foundational methods. The optimal MNs after printing and electropolishing for 120 s at 7 A enabled the manufacture of MN at their smallest dimensions, featuring a needle height of $699.3 \pm 19.5 \mu\text{m}$, tip diameter of $22.7 \pm 9.2 \mu\text{m}$, and base diameter of $280.4 \pm 10.2 \mu\text{m}$.

Comparing the optimised MN arrays to metallic MN arrays developed by Krieger *et al.* for biosignal recording applications, it was found that MNs with the necessary geometrical and physical properties for therapeutic delivery applications could be achieved. Krieger *et al.* used a DMLS process with specific parameters: 55 W laser power, 600 mm/s speed, and 0.12 mm trace width for 3D printing, alongside electropolishing parameters of 5 A for 90 s [83]. The optimised MNs within this thesis after electropolishing, demonstrated refinement in both dimensions and precision while achieving a minimal height reduction of 0.08%. In contrast, Krieger's study reported larger tip diameters of $38 \pm 2 \mu\text{m}$ and a shorter needle height of

632 ± 24 µm after electropolishing, with a significant height reduction of 3.81%. Notably, despite both studies using similar CAD files for 3D printing the MNs, the results here highlight the effectiveness of the electropolishing process in achieving finer tip dimensions while preserving needle height. Furthermore, unlike Krieger's study, which, although informative, did not apply comprehensive Design of Experiments (DoE) optimisation, our approach underscores the importance of systematic multi-objective parameter optimisation. Subsequently, the optimised MNs exhibited improved surface finish and quality, providing MN arrays with clinically acceptable micro-sized dimensions for painless and easy application with a high potential of drug loading [223,224]. It is critical to indicate that electropolishing parameters, including time and current, should be tailored to the specific mass and surface area of the material in use. The mass and surface area significantly influence the rate at which material is removed during electropolishing. Larger surface areas require more uniform current distribution to avoid uneven material removal, which can lead to surface defects and compromised structural properties. Additionally, the time parameter must be adjusted in conjunction with the current to ensure that the material removal is uniform and controlled. Prolonged electropolishing time at an inappropriate current level can result in over-polishing, where excessive material is removed, leading to a loss of critical dimensions and potentially weakening the MNs. As result, these parameters might require optimisation for various MN array geometries due to the differences in mass and surface area. Such an approach underscores the importance of adapting the electropolishing process to the unique geometrical features of each MN design.

The optimal metallic MNs demonstrated suitable effective compression strength ensuring their ability to effectively deliver drugs into the skin without experiencing tip fracture during the penetration process. Compared to the broader MN literature, metallic MNs demonstrate a mechanical superiority over commonly investigated polymeric MNs [172,182]. However,

it's crucial to note that these strength values are not directly comparable to intrinsic material properties. Instead, they serve as indicative factors for assessing the general mechanical performance of 3D-printed and electropolished MNs under various conditions. These effective compressive strength values are primarily used in DoE studies to understand the interaction between mechanical and geometrical properties. By using compressive strength as a measurable outcome, the DoE approach helps identify how different 3D printing and electropolishing parameters influence the overall robustness of MNs. This interaction is important for optimising the manufacturing process to achieve MNs that meet dimensional specifications and possess the necessary mechanical properties for effective skin penetration and drug delivery. Optimal metallic MNs have been shown to penetrate various skin models effectively with a penetration force under 10 N. This level of force, which is commonly exerted in manual applications, ensures that MNs can be inserted through the *stratum corneum* easily without specialised tools, minimising the risk of breaking the MNs or damaging the tissue [232]. The required force for entry is modest, indicating the MNs' capability for straightforward skin penetration. Additionally, the penetration depth being less than 1,000 μm suggests a potential for pain-free application while maintaining high delivery efficiency [232].

Successful development of a 3D printed mask for masked dip coating technique to control MN coating.

A physical mask was introduced between the MN base substrate and the coating liquid interface. The mask is designed to allow contact of MNs with the coating liquid but to physically keep the flat substrate of the MN patch from touching the liquid. By focusing the coating on the MNs themselves, the mask helps in optimising the drug loading on the MNs. This targeted approach ensures that the active pharmaceutical ingredients are primarily

concentrated on the parts of the MNs that will penetrate the skin, thereby enhancing the efficiency of drug delivery. The mask concept was first used by Gill *et al.* [144], wherein the dip-holes in the mask were produced *via* laser machining, and later produced using microfabrication techniques by Cassie *et al.* [292]. The dip-coating technique with a mask has been used by other investigators [293]. Gill *et al.* laser machined a channel to hold the coating liquid and covered it with a masking plate with laser-drilled micrometre-sized holes. The holes were made to precisely correspond to the locations of the MNs in the array. By ensuring that only the MNs are exposed to the coating liquid, the mask facilitates a more uniform application of the polymer solution on the MNs. This uniformity is essential for consistent drug delivery and effective MN performance. Furthermore, the mask prevents the flat substrate of the MN patch from being coated with the polymer solution. This is important because coating the substrate could lead to unnecessary material usage, potential wastage, and complications during the application and penetration process. After proper alignment, the MN arrays were successfully dip-coated without contaminating the base substrate. The 100% dip coating of the 3D printed MNs was achieved without contaminating the base substrate of the MN patch. This coating process led to the fine control over the length of the MN that can be coated, these findings were in line with other studies [144,292] in which this fine control by coating from as little as 25% to almost 100% of the MN shaft was reported.

One main challenge when referring to the coating of MNs lies in the long-term stability and storage of MNs, particularly those loaded with biologically active compounds. Many drugs, especially biologics like peptides and proteins, are sensitive to environmental conditions such as temperature and humidity. Ensuring that MNs can maintain drug stability during storage and transportation is crucial for their commercial viability. This requires advancements in both the formulation of the drug-loaded coatings and the materials used for the MNs themselves.

Successful development of coated metallic MNs using polymer-based coating solutions for enhanced MN coating efficiency and controlled drug release.

The study indicated that achieving optimal coatings required a careful balance of polymer concentrations and number of layers. The concentration is important since polymers can be engineered to degrade at specific rates, allowing for controlled and sustained drug release over time. This ensures that therapeutic levels of the drug are maintained in the target area for an extended period, enhancing efficacy and reducing the need for frequent dosing. The uneven coating distribution, which can be result of the number of layers, on the MN surface could impact ability of the MNs to penetrate the skin and drug release kinetics. Furthermore, the drug loading efficiency varied between the polymers tested, with 20% PVP-RhoB emerging as a particularly effective candidate. These differences in drug loading efficiencies highlights the importance of polymer selection and optimisation in achieving the full therapeutic potential of MNs [263,264]. The release kinetics also varied among the coatings. While all coatings exhibited an initial burst release, as is commonly observed in transdermal delivery systems, the subsequent release profiles were notably distinct [64,67,265,266]. The PVP-RhoB coated MNs allowed a swift and almost complete elution of the drug within an hour, aligning with some studies that emphasise the efficacy of PVP in facilitating rapid drug release.

Histological analyses further corroborated the effectiveness of the MNs, demonstrating successful penetration and uniform drug distribution across skin layers. Comparative analysis of *in vitro* drug absorption provided PVP-RhoB coated MNs to have immediate drug absorption, with 80% drug absorption noted post-insertion. This aligned with its degradation behaviour, emphasising the synergistic effect of material composition on drug release kinetics. In contrast, CMC-RhoB and PVA-RhoB demonstrated time-dependent absorption

increases, emphasising the role of coating material in modulating drug release rates. MN coatings that exhibited a relatively rapid degradation profile exhibited faster drug release and higher permeation rates in the early stages. This suggests a plausible connection where degradation of the coating material appears to play a central role in facilitating the initial burst release of the incorporated drug.

The permeation studies further supported the observations obtained from the degradation and absorption evaluations. Of note is the observation that coatings that exhibited higher adhesion forces also exhibited encouraging penetration profiles, highlighting consistency in findings. Higher adhesion forces ensure that the coating remains intact during the insertion of the MNs, allowing for a more efficient transfer of the drug into the skin layers. This strong adhesion is particularly advantageous for precise dosing and controlled release applications, such as vaccine delivery, where robust antigen delivery is needed for a strong immune response. For example, PVP-RhoB, with its high adhesive force, also presented high permeability coefficients and a steady drug release flux, in line with theoretical expectations and existing scientific claims [294,295]. Furthermore, CMC-RhoB showed a combination of rapid and sustained release, a dynamic profile that could be advantageous for various therapeutic applications requiring immediate and prolonged drug effects. These findings suggest that MNs have wide-ranging potential applications, including chronic disease management, where they could provide a painless alternative to traditional injections. The observed release profiles, especially with CMC-RhoB, indicate that MNs can maintain steady drug levels, improving treatment outcomes.

The ability to tailor release profiles by selecting appropriate coatings makes MNs versatile tools in personalised medicine, where treatments can be customised to patient needs. For example, rapid release coatings could provide immediate relief for acute pain, while sustained release coatings ensure prolonged therapeutic effects for chronic conditions. Future research

should investigate the release rates of larger molecules through optimal polymer coatings to understand the impact of molecular weight on drug release profiles, further enhancing MNs' therapeutic applications.

Different permeation profiles were obtained for the different polymers, emphasising the intricate role of molecular interactions, degradation rates, and mechanical properties inherent in each coating material in modulating drug release rates. The variation in drug release and permeation profiles across the various MN coatings can be attributed to the molecular interactions, degradation rates, and mechanical properties inherent in each coating material. Overall, several challenges remain in the field of MNs. One major challenge is the variability in skin penetration depth due to differences in skin types and the mechanical properties of the MNs. Consistent and reliable penetration is essential for the effective delivery of drugs. Our study addressed some of these issues through the optimisation of MN geometries and material properties, but further research is needed to develop universally applicable solutions. Another significant challenge is the biocompatibility and long-term stability of the materials used for MNs. While stainless steel and titanium have shown promising results, concerns about potential cytotoxicity and the body's reaction to long-term exposure to these materials need to be thoroughly investigated. Advanced materials and coatings that can enhance biocompatibility while maintaining mechanical strength are areas of ongoing research.

6.2. Concluding Remarks

In conclusion, this study provided a comprehensive investigation into the optimisation of geometrical and structural features of metallic MNs, utilising a multi-objective parameter optimisation approach based on DoE. The critical analysis of the 3D printing and post-processing parameters, specifically laser power under DMLS and electropolishing conditions, demonstrated their highest influence on dimensional accuracy, mechanical robustness, and surface quality of MNs. In particular, the optimal metallic MNs exhibited

sufficient geometry and compressive strength, critical parameters, thus confirming their ability for efficient, painless penetration through the *stratum corneum* without reaching the pain receptors. The resulting MN arrays showed a significant reduction in penetration force in various skin types, highlighting their applicability and minimal invasiveness. In addition, the improved surface quality and reduced presence of unmelted particles and pitting potentially improved skin penetration and permeability behaviour and mechanical durability of MNs, crucial for successful transdermal drug delivery. Furthermore, this study emphasises the critical role of polymer concentration, layering, and properties in optimising MN coatings for TDD. The selection of polymers and their concentration significantly impacts drug loading efficiency, release kinetics, and adhesion strength, crucial for successful skin penetration and drug delivery. The contrasting release profiles of different polymers underscore the importance of tailored polymer selection based on specific therapeutic needs.

6.3. Limitations

In the optimisation of polymer coatings for MNs, several trade-offs must be carefully considered to balance drug delivery efficiency and mechanical properties. One significant trade-off involves the porosity and roughness of the coating material. Higher porosity can enhance drug delivery by providing more pathways for the drug to diffuse through the coating, thus enabling a quicker release. However, increased porosity typically results in lower mechanical strength, which might be acceptable if the coating can effectively smoothen the surface and enhance mechanical interlock between the coating and the substrate. This mechanical interlock could potentially compensate for the reduced strength by improving adhesion and stability of the coating. Additionally, while higher roughness might initially seem detrimental due to potential inconsistencies in drug distribution, it can actually benefit the coating process by ensuring better attachment of the polymer to the MNs, as seen in techniques like dip-coating which rely on capillary forces for uniform application.

Furthermore, higher roughness and non-electropolished MNs might be tolerated on materials like steel because the coating itself could potentially smoothen the surface, enhancing mechanical interlock between the coating and the substrate. This trade-off must be carefully managed to ensure that the MNs can effectively drug loaded and achieve sufficient drug permeation. Considering these aspects, the discussion on MN coatings should also address broader challenges in the field, such as the need for more efficient coating methods that minimise drug wastage and the difficulty of delivering large molecular weight therapeutics transdermally.

6.4. Future Perspectives

Future research on studying the release rates of drugs with varying molecular weights using MNs.

The challenge of delivering high molecular weight therapeutics like insulin transdermally lies in their size, which limits their passage through the skin. Future work can focus on modifying the MN coating formulations to accommodate these large molecules without compromising the structural integrity of the MNs. Comparison of different molecular weights of drugs (from small molecules to large biologics) can be conducted investigating their diffusion and release rates through MNs. Investigating also how various polymers interact with drugs of different sizes and structures, which could affect the encapsulation and release efficiency. Finally, evaluation of the efficacy and safety of the MNs for delivering drugs of varying molecular weights can be conducted, ensuring they are effective and biocompatible for clinical use. This research is crucial for broadening the applicability of MNs in delivering a range of therapeutic agents, from small-molecule drugs to large protein-based biologics.

Application of an automated precision coating techniques

Another prospective development is the automation of the MN coating process. The goal is to develop a method that selectively coats only the needles, to prevent unnecessary drug wastage and enhance the efficiency of the coating and delivery system. Efforts can be focused into optimising a dispensing technique that deploys a controlled amount of the drug-polymer solution onto the MN array with precision. Parameters such as the dispensing time, pressure, and nozzle dimensions can be optimised, potentially through computational models that simulate the coating dynamics. The introduction of a g-code-based system can streamline the process, allowing for rapid, reproducible, and scalable production.

Future work involving pre-clinical and clinical trials.

One area for further research is conducting *in vivo* studies using animal models to assess the long-term biocompatibility and pharmacokinetics of the drug release profiles. These studies could include various therapeutic applications such as chronic disease management (*e.g.*, diabetes, where insulin delivery could be tested for stability and efficacy), acute pain relief (assessing the immediate analgesic effects and sustained release), and obesity treatment (evaluating the effectiveness of weight-loss medications). Additionally, studies can explore the immunogenicity and systemic distribution of vaccines delivered via MNs.

Furthermore, future work involving clinical trials for MN-based TDD systems is essential to ensure their safety and efficacy for clinical use. These trials should focus on assessing the safety profile of the MNs, particularly regarding potential skin irritation or allergic reactions. Additionally, the therapeutic effectiveness of the MNs in delivering drugs must be compared with traditional delivery methods. Patient compliance and acceptability are also crucial, requiring feedback on the ease of use and comfort. It's important to monitor the

pharmacokinetics and pharmacodynamics of drugs delivered through these systems, understanding their absorption, distribution, metabolism, excretion, and therapeutic actions. Lastly, investigating any long-term skin changes or other effects from repeated use of MNs is vital for their sustained clinical application.

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Appendices

Appendix 2.1: BBD experimental matrix for the 3D printing process optimisation.

Run	Factor 1	Factor 2	Factor 3
	Laser Power (W)	Laser Speed (mm/s)	Trace Width (mm)
1	70	600	0.12
2	70	700	0.11
3	50	700	0.09
4	35	600	0.12
5	70	500	0.11
6	35	500	0.11
7	35	600	0.09
8	70	600	0.09
9	50	700	0.12
10	50	500	0.09
11	50	500	0.12
12	50	600	0.11
13	50	600	0.11
14	50	600	0.11
15	50	600	0.11
16	50	600	0.11
17	35	700	0.11

Appendix 2.2: Analytical Data of each response for BBD experimental matrix for the 3D printing process optimization.

R 1	R 2	R 3	R 4	R 5	R 6	R 7
Tip Diameter	Needle Height	Base Diameter	Surface Roughness	Effective Strength	Porosity (SEM)	Porosity (Pycnometer)
µm	µm	µm	µm	MPa	%	%
78.86	743.12	262.37	18.20	0.77	2.52	16.79
70.56	715.30	242.40	20.00	0.79	3.20	17.60
84.20	721.60	301.50	16.60	0.70	1.92	16.20
105.52	775.61	335.61	20.02	0.61	2.83	18.60
59.16	660.21	228.39	28.29	0.30	12.88	23.41
101.48	677.88	251.41	20.92	1.06	2.57	19.79
81.83	719.07	313.07	23.41	0.87	2.51	15.93
102.70	775.18	316.92	31.70	0.85	4.52	17.83
82.91	733.40	320.00	21.20	0.76	1.13	17.45
59.33	590.29	196.35	27.93	0.36	3.47	18.23
55.91	599.66	220.68	25.26	0.39	12.56	21.81
76.80	772.50	278.60	19.25	0.80	2.85	16.03
82.58	756.30	253.40	17.57	0.75	2.32	17.93
89.65	771.96	283.39	26.12	0.83	1.19	15.94
85.60	841.92	294.13	19.03	0.67	1.59	19.49
102.33	760.02	267.44	19.51	0.72	1.13	17.56
73.96	558.87	223.92	20.61	0.41	14.35	27.31

Appendix 3.1: CCD experimental matrix for the electropolishing process optimisation.

Run	Factor 1	Factor 2
	Time (s)	Current (A)
1	90	5
2	90	5
3	90	5
4	90	3
5	90	7
6	60	3
7	60	7
8	90	5
9	120	5
10	60	5
11	90	5
12	120	3
13	120	7

Appendix 3.2: Analytical Data of each response for CCD experimental matrix for the electropolishing process optimization.

R 1	R 2	R 3	R 4	R 5	R 6	R 7
Height	Tip diameter	Base diameter	Unmelted Particles	Straightness	Pitting	Surface Roughness
µm	µm	µm	%	mm	%	µm
749.18	27.97	283.25	10.96	0.04	1.44	1.30
770.24	35.13	296.52	11.14	0.03	2.30	1.63
725.01	19.65	270.64	8.93	0.04	0.58	0.98
798.38	66.22	309.42	24.82	0.04	2.66	1.27
745.06	23.67	279.53	9.27	0.04	6.81	2.65
791.62	70.72	311.81	31.25	0.06	0.00	1.74
808.42	25.42	283.31	4.50	0.04	0.06	0.97
759.65	29.34	290.57	10.41	0.04	0.71	1.36
709.14	31.01	293.48	5.05	0.04	6.29	1.34
802.73	47.83	307.13	19.30	0.06	0.54	4.51
732.28	25.39	276.32	7.68	0.03	1.73	1.53
770.10	40.65	291.98	11.59	0.05	3.18	1.85
608.14	23.05	264.29	1.82	0.02	9.37	1.40