Compliance properties of a composite electrospun fibre–hydrogel blood vessel scaffold.

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Abstract

Compliance properties are considered critical to the long term functionality of vascular grafts and tissue engineered blood vessels *in vivo*. This study considers the compliance characteristics of vessels constructed from an inner layer of electrospun PVA-SbQ fibres incorporated in a PVA/Gelatin cryogel. The effect of the reinforcement on mechanical compliance in pulsatile flow is characterised over a wide range of mean pressure values (40-140 mmHg). Both the reinforced and non-reinforced vessels exhibit pressure-dependent compliance, a property shared with natural blood vessels. While the non-reinforced cryogel is excessively compliant, the incorporation of electrospun fibre reinforcement results in a compliance profile similar to that found in the literature for a human superficial femoral artery.

1 Introduction

Mechanical properties play a significant role in the function of many tissues and also in the performance of implanted grafts and tissue engineering scaffolds. In particular, there is convincing evidence that the dynamic mechanical compliance properties of vascular bypass grafts influence their long term prospects of becoming re-occluded with plaque or thrombus (Fig. 1) [1]. Compliance relates vessel dilation to changes or fluctuations in internal pressure, and it is known to affect the hemodynamics of pulsatile blood flow and hence the mechanical regime experienced by cells in the endothelium and vessel wall [2].

For natural arteries, compliance is found to depend in a non-linear fashion on the mean pressure of the pulsatile blood flow (e.g. femoral artery, Fig. 2(a) [3]). This non-linear response modulates the elastic response of the artery in response to altered or heightened pressure regimes with progressively reducing compliance. This characteristic is not, however, reflected in many synthetic grafts which exhibit relatively constant compliance over a wide range of physiological or pathological mean blood pressures (e.g. PTFE graft, Fig. 2(a, b)) [1,3].

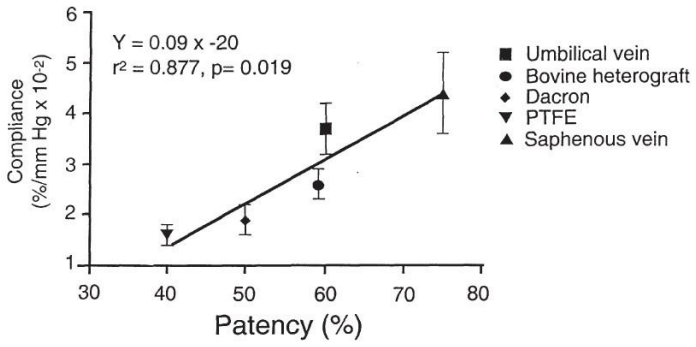

Fig. 1 Correspondence between compliance of selected grafts and their long term patency [1].

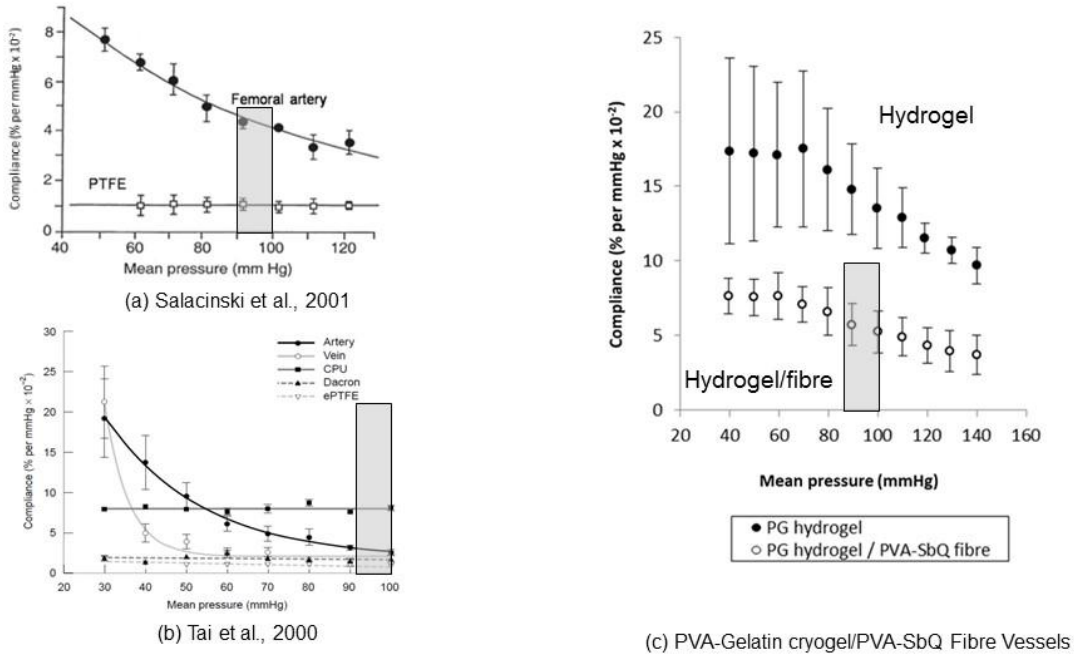

Fig. 2 (a) Non-linear compliance characteristic of human femoral artery as compared to PTFE graft (modified from [1]), (b) Compliance versus mean pressure curves for vessels and CPU, Dacron and ePTFE grafts (modified from [3]), (c) Compliance profiles with respect to mean pressure for PVA/Gelatin cryogels (1 freeze thaw cycle) and PVA/Gelatin cryogels reinforced with electrospun PVA-SbQ fibres.

Compliance mismatch between an implanted arterial bypass graft and contiguous natural vessel can alter blood flow, potentially increasing the risk of thrombosis and intimal hyperplasia. Salacinski *et al.* have summarized the compliance of grafts and their patency rates [1]. Their study showed a clear relationship between compliance and patency rates (Fig. 1), which suggests that better patency rates can be obtained when graft compliance characteristics are carefully tailored to match that of the host artery.

Synthetic grafts made from Dacron and variants of PTFE exhibited low compliance that remains constant across mean blood pressures between 30 and 100 mmHg [3]. A more compliant vascular graft fabricated from poly(carbonate)polyurethane (CPU) showed a

steady compliance value of the order of 8.1 % per mmHg x 10^{-2} over the same mean pressure range [3]. Significant compliance mismatch would therefore arise at mean pressures both above and below 50 - 60 mmHg, including at physiological blood pressures.

Hydrogels typically exhibit non-linear stress versus strain responses of a similar character to those of soft tissues. However, they also suffer from inherent strength and stiffness limitations which impede their suitability for some applications. Electrospinning, on the other hand, can produce fibres that are similar in morphology to the collagen fibres present in the ECM of various tissues, and can also offer some control over fibre structure and alignment. However, most electrospinning processes result in flat or tubular constructs, composed of densely arranged fibres with limited thickness, that have good strength but do not exhibit the non-linear "J-shaped" stress versus strain response of vascular tissue. There is, therefore, currently much interest in the reinforcement of suitable hydrogels to impart mechanical properties comparable with those of fibrous tissues [4, 5, 6].

A number of such studies target the development of vascular grafts or tissue engineered blood vessel scaffolds. In uniaxial testing, non-linear J-shaped responses have been obtained from ring segments fabricated from electrospun elastomer mesh (PEUUR) infiltrated with a hydrogel matrix of fibrin, with fibre-gel bonding aided by PEGDA grafted to the gel [7]. Static compliance values comparable with natural vessels have been reported for a hydrogel (PEG based) - electrospun fibre (polyurethane) vascular graft subjected to a ramped (but not pulsatile) internal pressure (0-150 mmHg) [8]. Another recent study utilised a physically cross-linked polyvinyl alcohol (PVA) cryogel as a middle layer between two electrospun layers of polycaprolactone (PCL), reporting uniaxial tensile, suture retention and burst pressure tests to confirm satisfactory mechanical properties [9]. While these studies underscore the promise of the fibre-gel approach, the feasibility of matching the non-linear compliance response of typical natural vessels over a range of pressures is as yet unexplored.

The present study addresses this question by investigating the effect on compliance characteristics of incorporating PVA-SbQ electrospun fibres in a PVA/gelatin cryogel vessel compared with the unreinforced case. PVA/gelatin cryogel samples have been extensively mechanically and biologically characterised in terms of suitability for vascular tissue engineering applications [10, 11]. PVA-SbQ (Polyvinyl alcohol with styrylpyridinium pendent groups) is a photosensitive polymer which becomes water insoluble when photocrosslinked, and is widely used in photolithographic processes [12]. Parameters for electrospinning of PVA-SbQ have previously been determined [13, 14].

2 Material and Methods

2.1 Electrospinning

PVA-SbQ was sourced from Polysciences Inc. (USA) with a concentration of 13.3%, 45,000 molecular weight and 4.1 mol% SbQ content. PVA-SbQ fibres were electrospun onto a flat plate using a Gamma High Voltage Research power supply and process parameters of voltage 10 kV, distance 10 cm and feed rate 0.3 mL/h for 20 min.

2.2 Gels

The PVA/Gelatin solution was prepared as per previous studies [10, 11]. Briefly, for the preparation of gels, PVA (average molecular weight (Mn) of 78,400 g/mol) was obtained from Vassar Brothers Medical Center (New York, USA). Gelatin, type B from bovine skin, was supplied by Sigma-Aldrich, Germany (Bloom strength ~225 g). 9% (w/v) poly(vinyl alcohol) and 1% (w/v) gelatin was dissolved in deionized water in an autoclave at 121°C for 1 h. Solutions were stirred until cooled.

2.3 Composite Vessel

The PVA-SbQ fibrous mat was manually wrapped around a stainless steel mandrel (diameter 6 mm). The covered mandrel was then placed into a cylindrical casting mould (diameter 10

mm) and PVA/Gelatin solution was poured into the cavity. The closed mould was then subjected to 1 freeze thaw cycle (-20°C for 12 h followed by 21°C for 12 h). In a further step the hydrogel/fibre construct was submerged in a constantly stirred coagulation bath of 7.5% KOH and 1M Na_2SO_4 for 1 h as per previous studies [10, 11]. The fibrous layer is located on the inner surface of the vessel, while the gel is present through the entire wall thickness. PVA/Gelatin cryogel vessels without fibre reinforcement were also produced in the same manner.

2.4 Compliance Tests

Compliance tests were conducted using a custom apparatus [15], consisting of a pulsatile flow pump (Harvard Apparatus, USA), a pressure sensor (DTX, BD, NJ, USA), an amplifier and monitoring device (TA-100, CWE, PA, USA) to measure intraluminal pressure in real time and a video extensometer for external diameter measurement.

The compliance of a vessel is defined as follows [15]: $C = \frac{D_s - D_d}{D_d(P_s - P_d)} \times 10^4$

where D_s and D_d are the systolic and diastolic external diameters of the grafts, and P_s and P_d are systolic and diastolic pressures respectively. In the test, $P_s - P_d$ is maintained as 60 mmHg. The unit of compliance is % per mmHg x 10^{-2}. Each experiment was conducted three to six times. All quantitative data are presented as Mean ± Standard deviation.

3 Results

Fig. 2(c) shows the compliance values of the PVA/Gelatin (PG) hydrogel and the PVA/Gelatin hydrogel/fibre composite with 1 freeze-thaw cycle and 1 h static coagulation. The compliance values are graphed with respect to the mean pressure of the pulsatile pressure signal, over a range from 40 mmHg to 140 mmHg. The maximum and minimum pressures are + and – 30 mmHg with respect to the mean values. The unreinforced PVA/Gelatin (PG) hydrogels have a linearly decreasing compliance with respect to increases in mean pressures.

The compliance values among these samples are spread in a wide range from 26.6 %/mmHg x 10^{-2} at 140 mmHg mean pressure to 8 %/mmHg x 10^{-2} at 40 mmHg mean pressure. The distribution of compliance values at each mean pressure was confined to a smaller range as mean pressure increased.

Similarly, the PG hydrogel/fibre samples showed decreasing compliance values in response to increases in mean pressure, mimicking the natural artery characteristic. At low mean pressure (40 mmHg), the compliance reached as high as 9.1 %/mmHg x 10^{-2}. It gradually decreased to as low as 2.5 %/mmHg x 10^{-2} at the high mean pressure of 140 mmHg. The dynamic compliance changes observed in response to mean pressure increases are comparable to published values for a human artery (Fig. 2(a) and (c)).

4 Discussion

The PVA/Gelatin hydrogel single layer graft was highly compliant over the whole pressure range (Fig. 2(c)). When the PVA-SbQ fibres were present on the luminal surface of PVA/Gelatin hydrogel, the graft exhibited a close match to the femoral artery compliance characteristic over the whole mean pressure range (Fig. 2(c)). One of the most significant properties is that this hydrogel/fibre graft exhibits a progressively reducing compliance as mean pressure is raised. Decreased compliance can be found at higher pressure, which modulates strain response and restricts over-dilation. The mean compliance of the fibre-hydrogel vessels at 100 mmHg (physiologically representative) is 5.2 %/mmHg x 10^{-2}, which is in the favourable range for patency of the grafts depicted in Fig. 1. The fibre-gel vessel in this study incorporates randomly aligned electrospun fibres located in the innermost layer of the composite, whereas the fibre orientation in native vessels is typically aligned and features helically arranged layers through the thickness of the arterial wall.

In the preparation of the fibre-gel vessel, two physical cross-linking processes (freeze-thaw cycles and coagulation) were used to form the gel on and around the fibre structure. No

chemical crosslinking step was implemented to specifically promote bonding between the fibres and the hydrogel. However, the cryogelation (freeze-thaw) process involves PVA crystal formation assisted by hydrogen bonding between hydroxyl groups on the PVA chain, so it is possible that some similar physical crosslinks exist between the fibres and the gel.

5. Conclusions

Hydrogel-fibre blood vessel constructs can reproduce the compliance characteristics of a natural vessel over a range of mean pressures. This property is important in terms of the ability of a vessel to adapt to mean blood pressure fluctuations and changes as per the native vessel, and the potential implications for long term vessel patency.

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