J Mater Sci: Mater Med (2008) 19:2551–2554
DOI 10.1007/s10856-007-3242-z

Experimental validation of a new approach for the development of mechano-compatible composite scaffolds for vascular tissue engineering

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Received: 28 February 2007 / Accepted: 16 July 2007 / Published online: 4 October 2007

Abstract The clinical need for the design of small-diameter vascular substitutes with high patency rates has never been so urgent as nowadays. Mechano-compatibility is widely known as one of the main key parameter for the design and the development of highly-patent vascular substitutes independently of their nature, i.e., arterial prostheses, arterial grafts or tissue-engineered blood-vessel. In this work, we attempt to target mechano-compatibility of cylindrical scaffolds for vascular tissue engineering by a computational model based on the composite theory associated with finite element and genetic algorithm. Then, cylindrical composite scaffolds were fabricated from gelatine (matrix) and silk (reinforcement) to experimentally validate theoretical results obtained by the implemented computational model. Finally, the compliance of the scaffolds was measured by an in-house developed specific device. Results show that the computational predictions from numerical simulation are in good agreement with the measurements obtained form the experimental tests. Therefore, the proposed computational model represents a valid tool to assist biomaterial scientists during the design of composite scaffolds, and especially in targeting their mechanical properties.

1 Introduction

Cardiovascular diseases represent one of the main causes of mortality throughout the globe [1]. Atherosclerosis, a degenerative disease which progressively attacks the arteries, is a common factor in most of these deaths [2]. During the last few decades, marked progresses have been made to clinically replace damaged arteries using synthetic prostheses or autologous grafts. However, especially for medium and low-diameter arteries (less then 6 mm), the patency rates of these substitutes rarely exceeds 50% after 10 years [3]. In this challenging context, tissue engineering represents a high-potential alternative for the development of small vascular substitutes with enhanced patency. In 1986, Weinberg et al. firstly proposed the use of a collagen gel to support and promote the growth of vascular cells in a controlled environment [4]. However, reconstituted collagen gels generally exhibit mechanical properties lower than native arteries and intrinsically need to be reinforced to increase manipulability (including suturability) and to avoid rupture during implantation. The importance of mechano-compatibility has also been considered regarding its implication toward cell activity. Mechanical stimuli such as physiological stresses and strains are part of the physiological environment of cells and are known to influence cells through complex mechano-transductive mechanisms [5, 6]. Furthermore, from a functional point of view, compliance mismatch has been depicted as one of the main reason for clinical failure of all kind of vascular substitutes [7]. In this context, improving the mechanical properties of collagen-based scaffold is important and one of the strategies considered to achieve this goal is to include reinforcement into the collagen matrix. Previous work focused on the design of a collagen/silk mechano-compatible composite scaffold [8]. Silk fibroin, being a

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mechanically strong natural fibre susceptible to proteolytic degradation in vivo and showing acceptable biological performances, represents an interesting reinforcing candidate. However, the design of a mechano-compatible composite material is not a trivial task. Several parameters such as the geometry of the scaffold and the proportion of reinforcing fibres have to be carefully chosen in order to mimic the behaviour of a native artery. Presently, there are no simple tools to assist biomaterial scientists in designing composite scaffolds, especially for targeting their mechanical properties. Therefore, in this work, a computational approach based on finite element methods and genetic algorithms were implemented for the design of cylindrical composite scaffolds with targeted mechanical properties.

2 Materials and methods

2.1 Preparation of the cylindrical composite scaffolds

Composite scaffolds were fabricated from gelatine and silk. Gelatine was selected as a valid substitute for collagen and considered in this study mainly for the inherent easiness in experimental manipulation and for pecuniary reasons Gelatine cylinders ($n = 3$) were fabricated from porcine skin type A gelatine (Sigma-Aldrich Co., lot 32K0047) with a proportion of 7%w (control) in deionized water. Specimens were left at room temperature during a period of 24 h in a cylindrical mould for gelification. The mould was then opened, the tubes removed and mounted on a compliance device for further mechanical tests. Composite cylinders ($n = 3$) were fabricated from gelatine (7%w) and silk (Bombyx silk, Treenwaysilks, Canada) (2%v) by adding short (2 mm) silk fibres randomly oriented within the matrix. The compliance of the specimens was then measured and compared to the values obtained theoretically from the computational approach.

2.2 Computational approach

The computational approach was described in details elsewhere [8]. Briefly, a finite element model was combined with composite theory and genetic algorithm to design a tubular scaffold which mimics the experimentally measured compliance of a native artery using MATLAB. Minor modifications were performed to the original algorithm to be coherent with the method of fabrication. First, the composite theory was modified to consider the random orientation of short fibres within the gelatine matrix. The stiffness of the samples ($n = 3$) was measured using a tensile test (Instron 5848 Microtester, Norwood, MA) with respect to the volume fraction of silk. Tensile tests were conducted on flat samples of dimension 15 × 10 × 3 mm (Fig. 1). Finally, the volume fraction of silk fibres in the matrix and the thickness of the tubes walls were numerically optimized to obtain a structure which mimics the compliance of a native artery.

2.3 Measurement of the compliance

An experimental device built in-house was previously developed to measure the diameter compliance of vascular grafts and arteries [9]. Briefly, samples were subjected to a static pressure while the resulting diameter was measured using an optical micrometer (Serie 183 B, LaserMike inc., Dayton, Ohio). The diameter compliance was then computed using the following formula:

$$C = \frac{1}{D}\frac{dD}{dP} \tag{1}$$

where: "D" represents the external diameter of the tube and "P" the pressure in the tube.

3 Results and discussion

The relationship between the stiffness of the matrix and the volume fraction of silk fibres was measured on flat samples by a tensile test. Elastic modulus was calculated from the stress-strain relationship (not shown) and results are presented in Fig. 1. The relationship between elastic modulus and volume fraction of silk appears to be "J" shaped. One physical interpretation of this phenomenon could be that for low volume fraction of silk, the composite material is

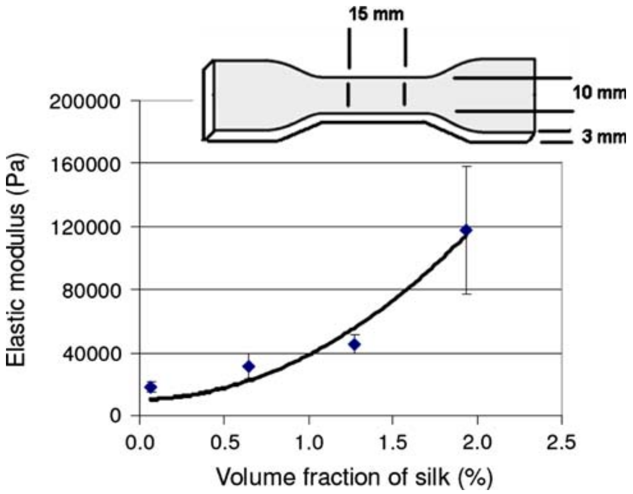

Fig. 1 Elastic modulus as a function of volume fraction of short silk fibres in gelatine-reinforced scaffolds

best described by the series model of mixtures [10]. However, this model cannot explain alone such a variation in the slope of the curve; then, one could hypothesis that the material follows the parallel model for higher volume fraction of silk [10]. In fact, the parallel model for a composite of gelatine and silk predicts increases in stiffness larger than those experimentally measured. This could be explained by the experimental observation that silk fibres slip in the gelatine matrix during tensile tests, therefore suggesting that the interfacial adhesion between silk fibres and gelatine matrix is lacking, and should be optimized (for example by surface modification of the silk fibres).

Once integrated in the computational model, this stiffness-volume fraction of silk relationship allowed finding an optimal solution: A cylinder with an inner diameter of 5 mm, an outer diameter of 11 mm (wall thickness of 3 mm), reinforced with 2%v of silk. This was found from the elastic modulus by using finite element methods to relate the properties of the material with the compliance of the tube. Then, a genetic algorithm was implemented to converge toward an optimal solution by minimizing the difference between the compliance of the model and the compliance of the native artery. In brief, this means that, if compared with an artery in the physiological range of pressure (between 70 and 130 mmHg), the expected (FEM) optimal mechanical behaviour will be as close as possible to the compliance of the artery. This could be achieved by minimizing the squared deviations between the computational model and the native artery. These numerical results were then used as input for the fabrication of reinforced and not-reinforced cylindrical scaffolds. Compliance was then measured for these scaffolds by applying a pressure inside the tubes and measuring its diameter. A typical pressure-diameter relationship and the relative compliance curves are presented in Fig. 2 for both, not-reinforced and reinforced cylindrical scaffolds. It can be observed that results from the computational model are in good agreement with the experimental results (Fig. 2A). This confirms the capacity of prediction of the FEM. Furthermore, it is interesting to observe that silk-reinforced tubes show smaller deformation than not-reinforced ones, for the same internal pressure. At low pressures (up to 50 mm Hg), the compliance of not-reinforced scaffolds is similar to that of native artery (Fig. 2B), while at physiological pressures, their expected compliance is much higher. The addition of 2%v of silk fibres within the matrix stiffens the material and lowers its compliance (Fig. 2B). At low pressure, the reinforced scaffold compliance is comparable to that of synthetic prosthesis, while at higher pressure, their expected compliance is more like that of native arteries. Although rupture of reinforced scaffolds was observed at 50 mm Hg, experimental results confirmed that their stiffness increases with the volume fraction of silk (Fig. 1). This demonstrates that the addition of silk fibres could predictably modify the mechanical behaviour of the scaffolds. At 2%v, the expected stiffness of the composite is around 100 kPa, about 10 times less than the circumferential stiffness of a native artery (~1 MPa) [11]. Therefore, modifying volume fraction of silk up to 2%v is not sufficient to achieve (predicted) mechano-compatible behaviour at physiological pressures. For that reason, the computational model also allowed optimising the compliance of the scaffolds through thickness modulation: The resulting scaffold thickness was 3 mm. This value is higher than that of a small or medium diameter artery, which is around 1 mm [12], but increasing the thickness of the scaffold's wall contributes to lower its compliance. Therefore, the predicted (FEM) compliance of the scaffold decreased close to the compliance of a native artery for

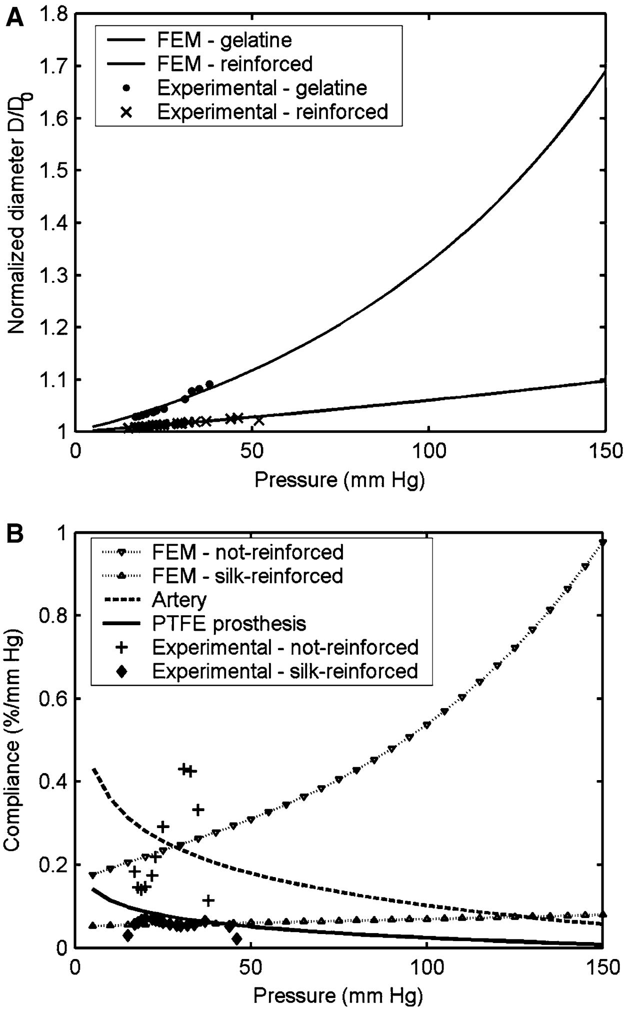

Fig. 2 The evolution of the diameter (**A**) and the compliance (**B**) of not-reinforced and silk-reinforced gelatine scaffolds as a function of the applied luminal pressure for experimental and computational approaches

physiological pressures. This confirms that the computational method converged toward the design of a mechano-compatible solution. Finally, all analyses were conducted hypothesizing perfect elasticity. However, it is also now well known that arteries exhibit viscoelastic behaviour once submitted to internal physiological pressure [13]. Therefore, it would be interesting to extend the proposed approached to design compatible viscoelastic materials for tissue engineering.

This paper main focus is devoted to present an interesting tool for the design of mechano-compatible materials. Gelatine and silk were especially selected in this work to easily demonstrate the interest of FEM and GA to predict the behaviour and design scaffolds for tissue engineering. Now that this interest was demonstrated, we believe that this approach could be reasonably extended to more complex composite structures, for example, collagen matrix reinforced by silk (ongoing works). Therefore, the fact that this proposed simple composite structure (gelatine/silk) did not resist to physiological pressures is not dramatic, mainly for the two following reasons. First, the relevant results of this work mainly concerns the method proposed to design composite biomaterials (for which collagen, poly-glycol-acid, hydroxyapatite for bones, Poly-caprolactone, and several others could be interesting candidates) rather than the selection of a final candidate showing physiological mechanical properties for direct use in vascular tissue engineering. Second, the final mechanical properties of the construct (i.e., the scaffold after colonization by cells) result from the mechanical properties of the scaffold and those resulting from the progressive organization of the extra-cellular matrix (ECM) due to cell-material interactions. Therefore, it is theoretically reasonable to imagine that the organization of cells and the ECM around the scaffold will provide (after maturation in a bioreactor) the final properties required for further clinical implantation [14].

The strategy followed in this work was to introduce a computational model for the prediction of the compliance of vascular scaffold as a function of the volume fraction of silk dispersed in the matrix and the thickness of the tubes walls. In the future, this approach could be applied for optimising the mechanical properties (including compliance) of a composite (for example, collagen/silk) scaffold. This approach is particularly suitable for assisting biomaterial scientists or tissue engineers in targeting mechanical properties during the first steps of the scaffold design. This is expected to introduce a systematic approach and also has the intrinsic potential to minimize the number of experiments required for the development of a scaffold showing mechano-compatibility.

Acknowledgements We acknowledge Professor Martin King, from North Carolina State University, USA, for help and guidance, and for providing the silk. This work was partially supported by the Natural Science and Engineering Research Council of Canada. FC was awarded of a Post-Graduated Scholarship (M.Sc. level).

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