**GELATINE AND GELATINE/ELASTIN NANOCOMPOSITES FOR
VASCULAR GRAFTS:
PROCESSING AND CHARACTERISATION**

Dimitris Lamprou^a, Peter Zhdan^b, Fatima Labeed^a and Constantina Lekakou^b

^aCentre for Biomedical Engineering

^bCentre for Materials, Surfaces and Structural Systems

University of Surrey

Guildford

Surrey GU2 7XH

ABSTRACT

This study involves the preparation, microstructural, physical, mechanical and biological characterization of novel gelatine and gelatine/elastin gels for their use in the tissue engineering of vascular grafts. Gelatine and gelatin/elastin nanocomposite gels were prepared via a sol-gel process, using soluble gelatine. Gelatine was subsequently crosslinked by leaving the gels in 1% glutaraldehyde. The crosslinking time was optimized by assessing the mass loss of the crosslinked gels in water and examining their mechanical properties in dynamic mechanical tests. AFM (atomic force microscopy) studies revealed elastin nanodomains, homogeneously distributed and embedded in a bed of gelatine nanofibrils in the 30/70 elastin/gelatine gel. It was concluded that the manufactured nanocomposite gels resembled natural arteries in terms of microstructure and stiffness. The biological characterization involved the culture of rat SMCs (smooth muscle cells) on tubular gelatine and gelatine/elastin nanocomposite gels, measurements of the scaffold diameter and the cell density as a function of time.

1. INTRODUCTION

Synthetic vascular grafts are widely used nowadays for the replacement or bypass of diseased parts of the vascular system although they might suffer various short- or long-term problems. Their major limitation is that they cannot be used for arteries of less than 6 mm diameter due to the high risk of thrombosis rapidly after implantation and later intimal hyperplasia [1,2]. For replacing small arteries, e.g. coronary artery bypass, autologous veins or arteries are used instead. However, this might not be possible for many patients as there are problems of availability, double surgery and morbidity of original site. Autogogous grafts from donors carry the additional risks of pathogen transmission, rejection and, hence, the need for continuous medication to suppress rejection. Xenografts from animals carry not only the risk of cross-species transmission of pathogens but both themselves and umbilical cord vein grafts have local mechanical weaknesses which might lead to aneurisms [3-7].

For these reasons, there has been a huge effort towards research in tissue engineering of vascular grafts where the focus is to reproduce the structure, mechanics and biology of natural arteries. The arteries of the human body are divided into four different groups: elastic arteries, muscular arteries, small arteries and arterioles. Elastic arteries have a diameter of 10 to 20 mm and a collagen to elastin ratio of about 0.6 [8]. Muscular arteries have a diameter in the range of 3 to 10 mm, a collagen to elastin ratio in the range of 2-4 and more smooth muscle cells (SMCs) than the elastic arteries [8].

Arteries consist of three layers: the tunica intima, media and adventitia [9]. The tunica intima features mainly endothelial cells (ECs) attached on the lumen and oriented in the axial direction of the artery. The tunica media is the thickest layer of the three, containing collagen fibres in the axial direction, embedded elastin plates, and SMCs oriented in the circumferential direction. Tunica media is a laminate of up to 40 SMC layers for muscular arteries and up to 5 layers for arterioles. SMCs play a very important role in providing the contractile and relaxation response [10-11] and the muscle tone [12], especially for muscular arteries. Tunica adventitia is the outer layer of the arterial wall and consists of loose connective tissue, some SMCs, fibroblasts and a network of small blood vessels which support the wall.

Collagen and elastin are the major proteins of the extracellular matrix of arteries. Elastin can undergo a large stretch ratio of 1.6 and has a Young's modulus of 0.4-1 MPa whereas collagen is stiffer with a Young's modulus of 5-10 MPa [9-17].

The first tissue engineered (TE) vascular graft [18] was produced by casting a mixture of ECs and SMCs on a synthetic Dacron[®] scaffold but it was found to be too weak. This and other similar studies demonstrated that mechanical integrity and performance are key issues in tissue engineering. L'Heureux et al [19] produced TE grafts of improved strength by culturing SMCs and fibroblasts on a tubular support. Various types of scaffolds can be used for the attachment and growth of cells. However, since one of the main points of TE is for the cells to produce extracellular matrix (ECM), the process should be greatly enhanced and accelerated if the cells are attached onto scaffolds resembling the materials, composition and structure of ECM in vascular grafts.

Some studies use collagen gels [19-24] or networks of collagen nanofibres [25] as the basis for the TE engineered grafts since collagen is a major matrix component in arteries. Gelatine has also been used [26], presumably as a low cost alternative source of soluble collagen. Collagen and gelatine generally favour the attachment, proliferation and retention of cells and, hence, they have been used in several studies to coat other materials used for scaffolds, such as polycaprolactone [27].

The purpose of this study is to prepare and validate nanocomposite gels mimicking the composition, structure and properties of natural arteries. For this reason, it was thought that the gels should be from collagen or a blend of collagen and elastin. Gelatine was used as a source of soluble collagen. Gelatine is denatured collagen [28], soluble in water and is proposed here as a low cost alternative to soluble collagen. A sol-gel preparation process was followed to achieve the nanocomposite structure, resembling that of natural arteries. Finally the gels needed to be crosslinked to stabilize the obtained collagen structure. The crosslinking time was optimized to obtain stable gels with the best mechanical behaviour, resembling that of the natural arteries. AFM and DMA tests were used to investigate the nanostructure and viscoelastic behaviour of the innovative gels, respectively. Finally, the optimized gels were assessed biologically by culturing SMCs: the resulting vascular graft specimens were characterized by measuring their dimensions to ascertain the contractile effects of SMCs, measurements of cell density to assess their growth and proliferation in the prepared scaffolds and fluorescence microscopy.

2. EXPERIMENTAL PART

2.1 GEL PREPARATION AND PHYSICAL CHARACTERISATION

Soluble animal gelatine and powder of insoluble E1625 elastin from Sigma Aldrich were the key materials used to make biomimetic gels, resembling the structure of the artery and intended to be used subsequently as a base for the attachment and proliferation of SMCs and ECs. Two types of gels were produced: gelatine and gelatine/elastin nanocomposite gels at 10/90, 20/80 and 30/70 elastin/gelatine compositions.

The main materials were used in the following quantities, depending on the type of gel: 160 mg gelatine powder for the gelatine gels; 144 mg gelatine powder and 16 mg elastin powder for the 90/10 gelatine/elastin gels; 128 mg gelatine powder and 32 mg elastin powder for the 80/20 gelatine/elastin gels; 112 mg gelatine powder and 48 mg elastin powder for the 70/30 gelatine/elastin gels. The preparation procedure was as follows: The gelatine powder was dissolved in 20 ml 0.01 N acetic acid under magnetic stirring. Then the elastin powder was added under stirring to produce a colloidal suspension of elastin in the gelatine solution for the gelatine/elastin blends. 0.01 NaOH was added to change the pH to 12, in which case gelatine also formed a colloidal suspension [29]. The suspensions were left to rest in cold store for 15 min, when the gelatine and elastin particles precipitated. Then the clear liquid above the precipitation was removed with a micro-pipette and the precipitate was placed on aluminium discs and left in cold store for 21 h to gel. Finally, the gels were placed in 1% glutaraldehyde in cold store where it is expected that the glutaraldehyde would effect crosslinking between the gelatine chains.

Gels were prepared with a crosslinking time of 2, 4, 6, 8 and 16 h and were tested in dynamic mechanical tests (DMTA) and whether they would dissolve or swell in water for different periods of time. The best gels were examined under the atomic force microscope (AFM) to study their micro- and nano-structure. An AFM micro-/nanoscope III, RICA, USA was used in tapping mode, which is the most appropriate mode for examining soft samples without damaging them.

2.2 GRAFT PREPARATION AND BIOLOGICAL CHARACTERISATION

Tubular grafts were prepared by casting in a mould consisting of an external plastic tube of internal diameter of 10 mm and a glass rod insert of 5 mm diameter placed at the centerline. The colloidal suspension of gelatine or gelatine and elastin particles was prepared as before and poured into the tubular mould. After 15 min rest in cold store, precipitation of particles had occurred, and the clear liquid at the top was removed with a micro-pipette. The moulds were left in cold store for 21 h for the gels to form. Afterwards, the external plastic tube was cut and removed and the tubular gels were removed by sliding them gently over the glass rod. They were then left in 1% glutaraldehyde for 8 h to effect crosslinking between the collagen chains. Rat smooth muscle cells A7R5 clone CRL-1444 from ATCC were cultured, had their density measured and seeded on these gel scaffolds. The gel scaffolds with the SMCs were then examined under a fluorescence microscope (Nikon Elipse E400, Japan), had their cell density measured and the tubular gel grafts had their diameter measured as a function of time for four days. All the preparation and cell culture work was performed inside laminar flow hoods of class II and high level of sterile conditions was maintained throughout the experiments.

The cell culture medium was prepared as follows: it consisted of 500 ml of DMEM (D6171 Dulbecco's modified Eagle's medium from Sigma-Aldrich) in which there were added 50 ml FBS (F7524 fetal bovine serum from Sigma-Aldrich), 10 ml L-glutamine (G7513 L-glutamine from Sigma-Aldrich) and 5.6 ml penicillin-streptomycin solution (P7539 from Sigma-Aldrich).

The rat smooth muscle cells A7R5 clone CRL-1444 from ATCC arrived frozen inside DMEM. Cells were defrosted by warming to 37°C in a water bath and were added to 5ml of cell culture medium. The mixture was then mixed thoroughly by re-pipetting and centrifuging at 1500rpm for 3 minutes (Fisher Scientific, Accuspin 400, Loughborough, UK) to obtain a pellet of cells. The unwanted medium was removed and 5ml of fresh cell culture medium was added. After re-suspending the cells in the fresh medium, the suspension was transferred to a sterile culture flask, followed by the addition of 5ml fresh cell culture medium. The cells were then incubated in an incubator (CO₂ incubator, IMCO 17AIC) with an atmosphere of 95% air and 5% carbon dioxide for 1-2 days [30]. Cells were inspected frequently throughout the study to ensure that only healthy cells were used for the growth and centrifugation trials. Frequent feeding is important for maintaining the pH balance of the medium and for eliminating waste products. The cells must go in fresh media about every 2-3 days. For the cells, feeding pre-warmed media must be used and cells must be out of the incubator for as little time as possible. In each subsequent passage, the old cell culture medium was removed directly from the culturing flask and disposed. Then sufficient Trypsin-EDTA solution (4ml of T4049 Trypsin-EDTA solution from Sigma-Aldrich) was added to cover the bottom of the culture

vessel and incubated for 4 minutes to detach cells from the surface of the culture flask; cells were monitored under a microscope: cells are beginning to detach when they appear rounded. As soon as cells were in suspension, immediately 4ml of fresh cell culture medium was added to neutralise the effect of Trypsin-EDTA. The suspension was then centrifuged at 1500rpm for 3 minutes to obtain a pellet of cells. After centrifugation, the old solution was removed and 5ml of fresh cell culture medium was added. After complete re-suspension of cells in the fresh medium, the solution was transferred to a new culture flask and put back in the incubator. The same procedure was followed until the cells were ready to be seeded at the scaffold.

The cell density was measured using a Neubauer Haemocytometer (Weber Scientific International, Teddington, UK). When the cells in the culture medium reached a density of 0.5×10^6 cells/ml, they were seeded at the gel scaffold. Before the cells are put at the gel, the gel must be sterilized. After the gels were removed from the 1% glutaraldehyde, they were further disinfected by immersing them in 6% hydrogen peroxide for 15 min. Then they were washed with autoclave water to remove traces of the sterilizing agents. Two types of gel scaffolds were produced: disc shaped grafts and tubular grafts. The gel discs were placed at a well plate and were ready to be used for the cell culturing. Each well had one of the above disc-shaped gel scaffolds and two wells were the control wells (containing only cells), so it could be tested if the cells were growing well outside the scaffold and what happened at the same time in the same cells at the gel scaffold. Finally, the rat SMCs were added at the top of the disc-shaped gel scaffolds with 0.5ml of medium and a cell density of 0.5×10^6 cell/ml and then put in the incubator for 4 hours to

allow for the cells to attach. Then 2ml cell culture medium was added and the plates were placed again inside the incubator for 4 days.

With regards to the tubular grafts, they were placed in a vessel with the suspension of rat SMCs at a cell density of 0.5×10^6 cell/ml and were rotated around their axis at 25 rpm for 4 hours, while the whole system was in the incubator. Then the cell culture medium was added and the system was left in the incubator for 4 days.

The cells to be examined under the fluorescence microscope must be dyed. The V12883 Vybrant® CFDA SE Cell Tracer Kit from Molecular Probes was used to dye the cells. The hydrolysed product of CFDA SE has excitation and emission peaks at 492 and 517 nm, respectively. Cells labeled with CFDA SE can be visualized on a fluorescence microscope using standard fluorescein filters. In the experiments, the dye concentration was maintained as low as possible to maintain normal cellular physiology and reduce potential artefacts from overloading. The dye was prepared as follows: 10mM CFDA SE stock solution was prepared immediately prior to use by dissolving the contents of one vial of component A (lyophilized powder of carboxy-fluorescein diacetate, succinimidyl ester) in 90 μ l of DMSO (component B-dimethyl sulfoxide). Then the stock solution was diluted in 1ml of phosphate-buffered saline (PBS) to the desired working concentration of 5 μ M. The plates with the scaffold-cells were removed from the incubator and the entire cell culture medium was removed. The dye was added at the top of the gel scaffold and put in the incubator for 15 minutes. Finally, the gel scaffold was removed from the incubator and the loading solution was replaced with fresh cell

culture medium and incubated again for another 30 minutes. Now the gel scaffolds were ready to be examined under the fluorescence microscope.

3. RESULTS AND DISCUSSION

3.1 SWELLING AND MASS LOSS STUDIES IN WATER

Samples were placed in sterilized water at 37 °C. They were removed every 10 min, their weight was measured and were placed back in the water. Fig.1 displays the results up to 80 min for samples of different crosslinking times. In general, an initial weight loss of 5-10% was experienced by all samples, possibly because some mass can dissolve in water or some water is actually desorbed from the gel due to the crosslinking. In the case of the samples with 4 h crosslinking the mass loss seems to be reversed for a while after 40 min in water, possibly due to the fact that the gel absorbed water and had some swelling up to 70 min total in water, thereafter showing mass loss possibly due to some disintegration of the gel. The highly crosslinked sample with 24 h crosslinking time was very brittle and its increasing weight loss after 80 min was due to the fact that it was breaking into small fragments at the edges. It is noticeable that the sample with 8 h crosslinking time had its weight stabilized after 40 min; the sample was in fact left in sterilized water at 37 °C for 5 days and showed no further weight loss, which indicates that the crosslinking was adequate to render it insoluble to water for this time duration.

3.2 RESULTS OF DMA TESTS

An RDA II Rheometrics was used for the dynamic mechanical testing in torsion mode at 37 °C. The samples under torsion were subjected to a dynamic frequency sweep test with frequencies, ω , in the range of 1 to 79 Hz, for different strains. Data of elastic shear modulus, G' , and loss modulus, G'' , were assembled. The elastic modulus increased with frequency for all gels, as expected in a viscoelastic material. Table 1 presents the maximum G' at different strains for gelatine gels with different crosslinking times. It is obvious that the crosslinking time of 8 h yields the highest G' and, taking also into account the mass loss data, this has been selected as the optimum crosslinking time for the collagen of gelatine in 1% glutaraldehyde.

On the basis of the G' data at 79 Hz, a plot of shear stress versus strain was constructed and is presented in Fig.2 which shows an almost linear stress-strain relationship for the gel after 8 h of crosslinking time similarly to natural arteries at such low strains [8]. By using the relation

$$E = 2 (1+\nu) G \quad (1)$$

a Poisson's ratio $\nu = 0.5$ and $G = 0.60$ MPa for 5% strain, a Young's modulus $E = 1.7$ MPa was calculated for the gelatine gel of 8 h crosslinking time, which is comparable to the Young's modulus of iliac and femoral arteries [13].

DMA tests were also performed for gelatine/elastin gels of 90/10, 80/20 and 70/30 compositions after crosslinking in 1% glutaraldehyde for 8 h. The samples were in dynamic torsion at 37 °C and were subjected to a frequency sweep in the range of 1

to 79 Hz at different strains. They showed an increase of the elastic shear modulus with frequency, indicating viscoelastic behaviour for all gelatine/elastin gels. Using the shear elastic modulus at 79 Hz, a stress-strain graph was constructed for all gels, shown in Fig.2. In this, it is demonstrated that the presence of elastin lowers the modulus for all compositions compared to 100% gelatine. This is expected as elastin has about 10 times lower modulus than collagen [9, 13-17]. However, it is rather interesting that the lowest modulus is for 10% elastin content, then the 20/80 elastin/gelatine gel has a slightly higher modulus and the 30/70 elastin/gelatine gel has the highest modulus of all tested elastin/gelatine gels. This was attributed to the fact that when a small amount of elastin is added, it disrupts the gelatine structure by adding severe inhomogeneities that lower the modulus more than when 30% elastin is added that might be distributed more homogeneously.

By applying equation (1) to the 70/30 gelatine/elastin gel at 5% strain, a Young's modulus of 1.15 MPa has been estimated. Both the composition and Young's modulus of the 70/30 gelatine/elastin gel are similar to those of the abdominal, iliac and femoral arteries [13].

3.3 AFM RESULTS

Fig.3 presents the surface height AFM mapping of the 100% gelatine gel sample after being crosslinked in 1% glutaraldehyde for 8 h. The gel surface looks like a bed of homogeneously distributed, parallel nanofibrils of a diameter in the range of 1.5 to 7 nm, which is a typical collagen nanostructure.

Fig.4 presents the surface height mapping and phase angle mapping of a sample from the 70/30 gelatine/elastin gel after being crosslinked in 1% glutaraldehyde for 8 h. It is clear that the gel is a nanocomposite of elastin flat domains of about 200 nm lateral dimensions and 50 nm thickness, dispersed in a gelatine matrix. Fig.5 shows a further magnified AFM graph of surface height and phase angle mapping of the same gel. This reveals further smaller features of the gelatine matrix, more specifically the bed of collagen nanofibrils of up to 7 nm diameter, homogeneously distributed and parallel to each other. Fig.5 shows that this bed is split into flat bundles of about 50 nm width each due to the embedment of elastin nanodomains. This is very similar to the structure of tunica media of natural arteries, where elastin plates are embedded in the matrix of collagen fibres [9]. It is clear that the homogeneously distributed nanodomains of elastin give the 70/30 gelatine/elastin gel its good mechanical integrity and properties compared to the gelatine/elastin gels of lower elastin contents.

3.4 RESULTS OF THE BIOLOGICAL CHARACTERIZATION

Observations of the rat SMCs under the microscope demonstrated that the rat SMCs seem to be about 7 μm long and 3 μm wide, when suspended in the culture medium, whereas they expand and spread when they attach to the glass surface of the flask. When the cell density reached 0.5×10^6 cells/ml in the culture medium, they were seeded at the different types of gel scaffolds and the cell density on the gel as well as the control cell suspension was measured regularly for 4 days; the results are presented in Fig.6. The control cell suspension displays an approximately linear increase of cell density from 5×10^5 cells/ml on day 0 to 12×10^5 cells/ml on day 4 and the rate of growth is the same as that measured for the cells in the culture

medium, from the first passage through the subsequent passages. Regarding the cells seeded at the gel scaffolds, their density drops initially on day 1 from 5×10^5 cells/ml to $3\text{--}4 \times 10^5$ cells/ml. This might be due to the fact that some of the cells might not have been attached to the gel and remained in the surrounding culture medium, although a large percentage, 60–80% of cells attached at the gel. The best cell attachment on day 1 is observed in the gel scaffold of 90% gelatine/10% elastin. After day 1, the cells in all gel scaffolds multiply at approximately similar rate as those in the control cell suspension, demonstrating that all gel scaffolds provide a favourable environment for proliferation of rat SMCs. The gel of 70% gelatine and 30% elastin displays particularly faster growth of cell density.

Fig.7 shows the change of the diameter of three types of tubular gel grafts as a function of time, after they were immersed in the SMCs culture. It is clear that the tubular gel scaffolds shrink within one day after being placed in the SMCs culture, as soon as the SMCs are attached to the gels. The diameter of the elastin/gelatine gel scaffolds shrinks the most to 78% of its original size, as expected due to the larger compliance of the gels with elastin because of the soft elastin nanodomains. The gelatine gel scaffold shrinks only to 94% of its original size due to the attachment of rat SMCs, as the gelatine gel has been found to have a higher modulus than the elastin/gelatine gels. No significant further contraction is observed in all tubular gel grafts after day 1 of cell culture seeding, which is consistent with the observations in a previous study [22] on a tubular Dacron® graft coated with collagen and seeded with porcine SMCs. In the present study, most of the contraction of all tubular gel grafts was measured 3 h after the seeding of SMCs, which means that it is due mainly to the attachment of SMCs.

The fluorescence micrographs in Fig.8 show abundant evidence of rat SMCs on the different types of gels, after 4 days proliferation. Fig.9 is a fluorescence micrograph on a through-thickness section of one of the gels and demonstrates that the rat SMCs are sufficiently small and able to permeate the gel structure and be embedded between the gelatine or elastin microfibrils, where they grow and multiply successfully.

4. CONCLUSIONS

The series of studies presented in this paper demonstrated that innovative gelatine and gelatine/elastin nanocomposite gels were prepared successfully so that they mimic natural arteries in composition, nanostructure and stiffness. Low cost soluble gelatine was used as the basis, so that it can be dissolved and form the desired gel structure in a sol-gel process. Crosslinking of collagen in 1% glutaraldehyde for 8 h was found to be the optimum time both in terms of being stable and insoluble in water for up to five days (after an initial mass loss of about 13% in the first 40 min) and providing the highest elastic shear modulus, without breaking into fragments, as in the case of 24 h crosslinking time.

AFM studies revealed that gelatine gels had the surface nanostructure of collagen in arteries, consisting of a bed of homogeneously self-assembled, parallel nanofibrils of about 1.5 to 7 nm diameter. 70/30 gelatine/elastin gels mimic the composition of thoracic, iliac and femoral arteries as well as their nanostructure: AFM graphs showed flat elastin nanodomains homogeneously distributed and being embedded in

the bed of gelatine nanofibrils, in the same way as in tunica media in natural arteries [9]. This proves that the 30/70 elastin/gelatine gel was a nanocomposite and seemed to have superior handling ability than the 100% gelatine gels. The 30/70 elastin/gelatine gel was also more flexible than the 100% gelatine gel with an estimated Young's modulus of 1.15 MPa compared to 1.7 MPa for the latter, both comparable to the Young's modulus of natural arteries [10]. The 10% and 20% elastin gels showed much inferior properties, suspected to be due to the inhomogeneous distribution of such small amounts of elastin causing weak points in the gelatine structure. In any way, the lowest elastin content in natural arteries is 25% [8] which is close to the successful nanocomposite gel with 30% elastin.

The biological characterization of gelatine and 90/10, 80/20, 70/30 gelatine/elastin nanocomposite gels demonstrated that they are all suitable scaffolds for the attachment and proliferation of rat SMCs. Measurements of the scaffold diameter displayed the presence of contractile action of SMCs at a maximum of 78% of the original scaffold diameter for the gelatine/elastin scaffolds, after 3 h of cell seeding, which has been concluded to be the time for cell attachment. After this time of cell attachment, cell density on the scaffolds increased at approximately the same rate as in the control cell suspension. Fluorescence microscopy showed that rat SMCs were able to penetrate the microstructure of the gel scaffolds, which means that it is most suitable for them to be seeded on the gel scaffolds at a separate stage. These are very encouraging results for the use of the proposed novel gelatine/elastin nanocomposite gels in the tissue engineering of vascular grafts.

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Table 1. Maximum elastic shear modulus, G' in MPa, of gelatine gels of different crosslinking times in dynamic torsion frequency sweep tests at 37 °C and different strains. (The results for each crosslinking time were determined from four repeat experiments using four different gel samples, respectively, and had an overall mean standard deviation of ± 0.015 MPa)

Crosslinking time (h)	0.1% strain	1% strain	2% strain	3% strain	4% strain	5% strain
2	0.28	0.22	0.22	0.24	0.18	0.19
4	0.27	0.19	0.33	0.23	0.38	0.39
6	0.52	0.18	0.38	0.28	0.30	0.44
8	0.46	0.59	0.56	0.56	0.58	0.60
16	0.19	0.23	0.19	0.25	0.29	0.29

Fig.1. Mass loss as a function of time for gelatine gels in water at 37 °C after they have been crosslinked in 1% glutaraldehyde for different crosslinking times (The data points for each crosslinking time were determined as the mean from four experiments using four different gel samples, respectively, and the error bars represent the standard error with respect to the best fit, where the best fits are represented by solid lines).

Fig.2. Shear stress-strain relationship for gelatine and gelatine/elastin gels of 90/10, 80/20 and 70/30 compositions after being crosslinked in 1% glutaraldehyde for 8 h (The data points for each type of gel were determined as the mean from four experiments using four different gel samples, respectively, and the error bars represent the standard error with respect to the best fit, where the best fits are represented by solid lines; reproducibility errors were very close to the corresponding standard error with respect to the best fit).

Fig.3. AFM surface height mapping of gelatine gel after 8 h of crosslinking in 1% glutaraldehyde.

Fig.4. AFM surface height mapping and phase angle mapping of 70/30 gelatine/elastin gel after 8 h of crosslinking in 1% glutaraldehyde; sample area: 659x659 nm.

Fig.5. AFM surface height mapping and phase angle mapping of 70/30 gelatine/elastin gel after 8 h of crosslinking in 1% glutaraldehyde; sample area: 199x199 nm.

Fig.6. Graph of cell density against time over 4 days
 (The data points for each type of gel were determined as the mean from four specimens using four different gel samples, respectively, and the error bars represent the mean reproducibility range for each type of gel).

Fig.7. Graph of the diameter of the tubular scaffolds as a function of time over the 4 days of the scaffolds in the SMCs culture (Each point is the mean of four measurements from different locations in the scaffold, where the maximum variation was 5%).

Fig.8. Fluorescence micrographs of rat SMCs after 4 days proliferation in the gel scaffolds: (a) 100% gelatine gel (b) 70/30 gelatine/elastin gel (c) 80/20 gelatine/elastin gel (d) 90/10 gelatine/elastin gel

Fig.9. Fluorescence micrograph of rat SMCs after 4 days proliferation in the 90/10 gelatine / elastin gel: through thickness section