**ELECTROSPINNING OF NANOCOMPOSITE FIBRILLAR TUBULAR AND FLAT
SCAFFOLDS WITH CONTROLLED FIBRE ORIENTATION**

A.A.Salifu, B.D.Nury and C.Lekakou

Centre of Materials, Surfaces and Structural Systems

Faculty of Engineering and Physical Sciences

University of Surrey

Guildford, Surrey GU2 7XH

Email of corresponding author: C.Lekakou@surrey.ac.uk

Tel.no. ++44 1483 689622

Abstract

Electrospinning was used in innovative electrospinning rigs to obtain tubular and flat fibrous structures with controlled fibre orientation with the aim to be used as scaffolds for biomedical applications, more specifically in the tissue engineering of vascular and orthopaedic grafts. Gelatine and hydroxyapatite (HA)-gelatine solutions of various compositions were tried and electrospinning of continuous fibres was maintained for gelatine and up to 0.30 g/g HA-gelatine solutions in 2,2,2-trifluoroethanol (TFE). Small diameter tubular scaffolds were electrospun with axial fibre orientation and flat scaffolds were cut from fibre mats electrospun around a wired drum substrate. The fibrous mats were crosslinked using a glutaraldehyde solution and subjected to image analysis of SEM micrographs, water swelling tests, and mechanical testing. Fibre diameter in the electrospun scaffolds could be varied depending on the feed solution concentration and composition whereas fibre orientation was affected by the processing conditions. After crosslinking, the 0.30 g/g HA-gelatine scaffolds absorbed the minimum amount of water after 48 h soaking and they had the highest Young's modulus, 60 MPa, and highest strength, 3.9 MPa.

Keywords: Vascular; orthopaedic; scaffolds; gelatine; hydroxyapatite; microstructures, water absorption; mechanical properties

1. INTRODUCTION

Fibrous scaffolds from biomaterials are very important in biomedical engineering as the biomaterials mimic the main components of tissues and offer great contributions in biointegration *in vivo*. It is most important that the microstructure of the scaffolds mimics that of tissues so that cells in the organism are guided to adhere and proliferate by the appropriate microstructure according to which they have been programmed to respond. Biomimetic architectures include a tailored structural hierarchy [16], a certain fibre orientation depending on organ [16], and a certain pore size so that large human cells, such as osteoblasts, may be able to migrate and are well distributed throughout the structure during tissue engineering *in vitro*. Gelatine is a key biomaterial used by the authors of this paper, as it is a low cost widely available material based on collagen, the main component of soft tissues and the matrix of bone. Gelatine-elastin nanocomposite gels have been characterised [15,16] and proved to have similar properties as soft tissues, being good candidates for vascular grafts. Furthermore, Vidyarthi et al [36] devised a sol-gel process to form in situ hydroxyapatite (HA) nanoparticles in gelatine. Studies so far [15-16,36] have yielded gelatine nanocomposite gels, which proved highly bioactive in the proliferation of rat smooth muscle cells [36] but less successful in the proliferation of human osteoblasts, mainly because these large cells were not able to migrate needing a porous scaffold. Electrospun PLLA/HA scaffolds of a fibre diameter of about 5 μm have been used successfully for the proliferation of osteoblasts [28] where the presence of hydroxyapatite nanoparticles has been seen beneficial for the adhesion of osteoblasts in experiments in rabbits [28]. On the other hand, hydrophilicity has been found important for the attachment and proliferation of cells in a study [29] in which PCL (polycaprolactone-co-lactide) scaffolds have been coated with collagen I. Studies of osteogenic differentiation of marrow stromal cells on random and

aligned PLLA fibres [21] showed that the aligned fibre scaffolds induced cell alignment and extensions in the fibre direction and tripled the extent of mineralisation after 3 weeks.

Hence, the present work aims at the fabrication of porous gelatine-based scaffolds of fibrous morphology with controlled fibre orientation. Two types of scaffolds have been targeted: tubular scaffolds for applications such vascular grafts; flat scaffold sheets for other types of applications. In both cases, the aim has been to achieve unidirectional fibre orientation, in particular in the axial direction for tubular scaffolds, as is the case of fibre orientation in tunica media in arteries [6,19]. Various techniques have been used in the past for the fabrication of porous scaffolds, such as drawing[26], phase separation [31], template synthesis [7], self-assembly [37], particulate leaching [12], foaming [24], freeze-drying [23], and electrospinning [4,20,34,39]. Electrospinning has been used for the fabrication of the fibrous scaffolds in this study, followed by crosslinking of gelatine in glutaldehyde solution [15-16,36].

Past studies have concluded that the organic solvent trifluoroethanol (TFE) is appropriate for the electrospinning of gelatine [10]. Solvent concentration and processing parameters are important in electrospinning to ensure the formation of continuous nanofibres and avoiding bead formation [17]. In this study, fibre orientation is another concern and this is governed not only by the geometry of the electrospinning substrate electrode(s) [34] but also by the processing parameters and nanocomposite composition and properties, as it will be demonstrated in this study. The gelatine-based nanocomposite fibrous and porous scaffolds were characterised in scanning electron microscopy (SEM), water swelling studies, and mechanical testing.

2. MATERIALS AND METHODS

Gelatine (Sigma Aldrich) was dissolved in 2,2,2-trifluoroethanol (TFE) at two concentrations, 10% w/v and 12.5% w/v gelatine in TFE. The solutions were electrospun into tubular and flat structures. After electrospinning was completed, the fibrillar structures were left under vacuum at room temperature for 24 hours for the evaporation and removal of TFE. The product was then placed in a sealed dessicator with 15 ml 25% glutaraldehyde in a Petri dish for 3 days at room temperature to effect crosslinking of gelatine, so that the fibrillar structure would not dissolve in an aqueous medium if used as scaffold for biomedical applications [15-16]. The crosslinked products were air dried in a fume cupboard for 3 hours for the removal of glutaraldehyde.

For the preparation of “hydroxyapatite”(“HA”)-gelatine nanocomposite fibrillar structures, CaHPO_4 salt was produced as HA precursor by reacting calcium hydroxide $\text{Ca}(\text{OH})_2$ and phosphoric acid H_3PO_4 at an optimised molar ratio $\text{Ca/P} = 0.43$ [36]. More specifically, 41.3 mL of 0.1 M $\text{Ca}(\text{OH})_2$ was added to 9.6 mL of 1 M H_3PO_4 and the pH of the resulting solution was increased from 2 to 9 by the addition of 1 M NaOH to precipitate out the CaHPO_4 salt (HA) formed. The solution was filtered afterwards using a Whatman #2 filter paper and the precipitates were left to dry for 48 hours. Different weights of HA dry precipitates and gelatine were directly mixed to achieve 0.70, 0.50, 0.40, 0.30, 0.20 and 0.10 g/g “HA”/gelatine weight ratios and dissolved in TFE to prepare 10% w/v of composite to TFE solutions (for the 0.70, 0.50 and 0.40 g/g composites) and 12.5% w/v solutions (for the 0.30, 0.20 and 0.10 g/g composites). After the electrospinning of flat fibrillar structures, the products were dried and crosslinked in the same manner as the gelatine products.

Figure 1 presents the electrospinning set-up devised in this study for the electrospinning of flat fibrillar mats with fibre orientation intended in the circumferential drum direction. The feed system consisted of a 5 mL syringe serving as the reservoir for the viscoelastic polymeric solution to be electrospun, and placed in a syringe pump (Model A-99.FMZ, Razel Scientific Instruments, Inc., Stamford, CT, USA). A stainless steel blunt end hypodermic needle (18 gauge x 38 mm long) was fitted to the tip of the syringe and 1.75 m PTFE microbore tubing was fitted to the tip of the needle, with the other end of the tubing fitted to supply an 1mL syringe. The 1mL syringe with the second stainless steel needle was held vertical by a specially-made adjustable clamp above the fibre collector. The cable from a high voltage power supply (30 kV capacity, Model PS/EH30R03.0-22, Glassman High Voltage, Inc., Whitehouse Station, NJ, USA) was attached to the needle via an insulated crocodile clip. The collector designed for the electrospinning of flat fibrillar structures was a rotating copper wire-framed drum. It was made from two circular non-conducting Perspex disks 13 cm in diameter with a hole in the centre for the central stainless steel shaft insulated with a surrounding PVC pipe. Around the circumference of each disk, 3 mm deep notches, spaced 1 cm apart, were made on the top and a 7 cm-diameter conducting aluminium disk was fitted at the centre. Threaded holes, spaced 1 cm apart, were made around the circumference of the aluminium disks. Copper wires were stretched between the notches on opposite disks and screwed onto the aluminium disks through the threaded holes. The drum was rotated by means of a 15V DC motor at 2 rpm and was electrically grounded.

Figure 2 presents the set up for the electrospinning of tubular fibrillar structures to achieve axial fibre orientation along the tube. In this case, the collector was custom made, comprising a 5 mm diameter fused quartz rod. It was rested on two V shaped holders (as part of a transparent plastic frame) allowing free rotation. The rod was rotated at 2 rpm via the

15V DC motor. The positive electrode was made of a stainless steel blade with the feed steel needle resting on it on the longitudinal axis of the blade with the tip of needle exceeding the lower edge by about 2-3mm. This steel blade was 1.4mm thick, 40mm wide and 38mm long, with the lower edge being knife edged. This positive electrode was fixed to the plastic frame at 45° with respect to the horizontal plane, with a vertical distance of 12cm from the surface of the collector. It was placed in such a way that the collector would be central to its width. The feed steel needle was anchored by a crocodile clip that was connected to the positive lead of the power supply. Thus, both the needle and the blade were connected to the high voltage power supply. The ground electrode was also made of a stainless steel blade of 75mmx10mmx1.4mm with the upper edge along the width being knife-edged. It was placed at 45° with the horizontal plane, at a 25mm vertical distance from the collector, so that the collector would be central along its width. Furthermore, the upper surfaces of both blade electrodes were in the same imaginary plane, at a distance of 21.2cm between the knife edges of the two electrodes.

3. RESULTS

First of all the results of the electrospinning of the tubular structures are presented. Table 1 summarises the processing parameters for their fabrication via electrospinning. The duration of the electrospinning run affected the mass and thickness of the produced tubular scaffold, as expected. Optical microscopy and SEM revealed that in all scaffolds fibres were not always straight, while there was an orientation distribution for the fibres. Scaffold TG.1 with the higher gelatine concentration had the thickest fibres (average fibre diameter = 3.2 μm), while

the solutions of 10% w/v gelatine concentrations produced on average fibres with submicron diameter (average fibre diameter = 0.64 μm for TG.2 and 0.67 μm for TG.3).

Given that only the solutions of 10% w/v gelatine concentration yielded nanofibres, these electrospun structures (scaffolds TG.2 and TG.3) were crosslinked. Figures 3 and 4 present SEM photographs of scaffolds TG.1 (uncrosslinked), TG.2 (crosslinked) and TG.3 (crosslinked). Beading is most visible in TG.2, while TG.3 has the best fibre orientation and the most regular structure. Hence, it is interesting that although the same electrospinning rig was used for all scaffolds, only TG.3 had regular fibre orientation. In this study, the fabrication of TG.3 included the highest feed rate and the highest DC voltage. Initially, the fibre orientation in TG.3 was at a small angle with respect to the axis of the rod collector due to the slow rotation of the collector. This was corrected by turning the feed needle at the same angle opposite to the rotation direction to counteract the rotation of the collector.

Figures 5 and 6 present flat scaffolds produced by electrospinning on the large wired drum collector (Figure 1). In all cases of electrospinning of fibres, aligned fibres appeared on the collector with the alignment perpendicular to the wires; as the electrospinning continued and the thickness of the fibrous scaffold increased, fibre misalignment increased with HA-gelatine fibres being laid randomly after 1 hour of electrospinning.

Figure 7 presents the produced flat uncrosslinked scaffolds after 1 hr of electrospinning. The 10% w/v gelatine solution produced fibres of 0.49 μm average diameter with relatively good alignment after 1 hr of electrospinning whereas the 12.5% w/v gelatine solution produced thicker fibres (0.8 μm average diameter) as in the case of tubular scaffolds. The HA-gelatine scaffolds had submicron diameter fibres (see Table 2) and some beading on the fibres, while

fibres were laid at random orientation after 1 hr of electrospinning. As the content of produced HA was increased, the diameter of the electrospun fibres decreased. Solutions of 0.50g/g and 0.70 g/g HA-gelatine could not form fibres by electrospinning. Figure 8 presents an SEM photograph of a flat 0.30 g/g HA-gelatine electrospun and crosslinked scaffold: the scaffold had a pore diameter of $3.56 \pm 1.43 \mu\text{m}$.

Table 2 presents a summary of the properties of the flat electrospun scaffolds. Apart from measurements of the fibre diameter of the electrospun fibrillar scaffolds, the crosslinked electrospun scaffolds were subjected to water swelling tests and tensile testing. In the water swelling tests, the samples of the scaffolds were soaked in distilled water for 48 hr. Gelatine is a hydrogel absorbing water, as a result the higher the gelatine content in the scaffold, the higher was the percentage of swelling. Furthermore, the scaffolds were kept in water for a total of 5 days without any weight loss (after the first swelling by water), indicating that there was no free or degraded uncrosslinked gelatine to dissolve in water during this time duration. Mechanical tensile testing of the crosslinked scaffolds under a crosshead speed of 10 mm/min revealed the reinforcing effects of the HA nanoparticles, which resulted to an increase of the Young's modulus of the porous scaffolds to about 60 MPa for the 30g/g HA-gelatine nanocomposite fibre scaffold. The presence of HA nanoparticles in gelatine reduced the tensile strength of the fibrous porous scaffolds to start with for the compositions of 0.10 and 0.20 g/g HA-gelatine. The reason for this might have been that the HA nanoparticles disrupted the gelatine matrix and introduced discontinuities. However, the 30g/g HA-gelatine composition proved excellent for the nanocomposite fibrous porous scaffold and yielded the highest strength.

4. DISCUSSION

This study has focused on the fabrication and testing of tubular and flat fibre scaffolds with the aim of using them in the tissue engineering of biomimetic vascular and orthopaedic grafts, respectively.

Current synthetic vascular grafts cannot be used to replace narrow arteries with a diameter smaller than 6 mm due to the high risk of thrombosis rapidly after implantation and later intimal hyperplasia [5, 30, 38]. In such cases, a saphenous vein graft is used instead. This approach of using an autologous saphenous vein has, in fact, been used by surgical teams for also replacing larger prosthetic grafts in the area of the groin that were infected [3, 8, for example]: on this occasion, given that a large diameter graft was needed, two parts of an opened great saphenous vein were sutured axially to be joined together as well as at either end to be joined with the main artery. Such a procedure involved surgical operations on delicate material, requiring great surgical skill. However, suitable saphenous vein might not be always available for all patients. Consequently, there has been extensive research on the development of tissue engineered grafts for which the scaffold is a critical part from a structural, biocompatible and anti-thrombogenic, and cell proliferation favouring points of view. Natural biomaterials close to the materials of natural arteries are ideal to avoid thrombosis and gelatine in this study fits this requirement as it is collagen-derived, collagen being the main material component of natural arteries. The second specification set in this study is to fabricate gelatine fibrillar, tubular scaffolds with axial fibre orientation to mimic the collagen fibre orientation in the tunica media of the walls of natural arteries [6, 19, 40]. Finally, the fabricated scaffolds should have sufficient mechanical strength, where it was measured [9] that the ultimate tensile strength of the tunica media in the human coronary artery is about 1.3 ± 0.7 MPa.

Electrospinning onto a rotating rod of 5 mm diameter directed fibre deposition in axial orientation via two inclining steel blade electrodes. Maximum fibre orientation was achieved at the maximum feeding rate of 1.5 ml/hr and maximum applied voltage of 30 kV DC after 50 min of electrospinning. The crosslinked scaffold with high fibre orientation (TG.3) had still oriented structure but with short transverse crosslinks and elongated pores in the axial direction. The fibre diameter increased with increased gelatine concentration of the feed solution. After crosslinking in the vapour of 25% glutaraldehyde for 3 days, immersing the scaffolds in water at room temperature for 48 hr resulted in about 20% w/w water absorption, indicating the high degree of crosslinking of the gelatine fibres. Mechanical testing of the crosslinked scaffolds revealed a high Young's modulus of 33.8 MPa in the axial direction, which is expected to reach about 0.8 of this value after 20% water absorption, i.e. about 27 MPa, where natural collagen has a Young's modulus of about 5-10 MPa [2,11,18,19,22,27,32,35,40]. The tubular crosslinked scaffolds had excellent tensile strength in the axial direction of 2.9 MPa compared with the corresponding axial tensile strength of the tunica media of the human coronary artery of 60 kPa [9]. The strain to failure of the crosslinked gelatine scaffolds is 11.7 % which lower than the strain to failure of arteries at about 35% in the axial direction [9]: this is due to the fact that the tunica media of arteries also contains elastin that lowers the modulus and increases the strain to failure. The beneficial role of elastin in this case has been recognised in a previous study by our group [15] of gelatine-elastin gels.

The aim for the orthopaedic grafts was to mimic the natural bone as much as possible in materials, composition and orientation, although one has to recognise that the tissue engineered graft to be implanted in the organism needs to be of lower stiffness than mature bone to integrate well in the fractured bone site with the existing bone, simulating young and

growing “soft” bone. Gelatine was also used in this case as a collagen substitute as well as CaHPO_4 salt as hydroxyapatite precursor resulting in a nanocomposite material referred to as “hydroxyapatite” (“HA”)-gelatine nanocomposite. Popular materials used in the research for orthopaedic grafts include poly(lactic acid) /calcium metaphosphate composites, which will be compared to the scaffolds produced in this study.

Electrospinning of “HA”/gelatine solutions in TFE took place onto a rotating wire drum aiming at achieving circumferential fibre orientation. Increasing the content of “HA” decreased the fibre diameter and at the high “HA” contents of 0.50g/g and 0.70 g/g “HA”-gelatine compositions no fibres were formed, instead there was only spraying. Electrospinning of fibres took place for the 0.10 g/g, 0.20 g/g and 0.30 g/g “HA”-gelatine compositions using feed solutions of 12.5 w/v nanocomposite concentration, where the fibre diameter decreased with increasing “HA” content, being for example 480 ± 70 nm in the case of 0.30 g/g “HA”-gelatine nanocomposite fibres. Electrospinning of pure gelatine solutions in this manner onto the wire drum resulted in circumferential fibre orientation after 60 min of electrospinning but orientation was more difficult to achieve in the “HA”-gelatine scaffolds. In general, there was excellent “HA”-gelatine nanocomposite fibre orientation after 5 min of electrospinning, with some small orientation irregularities after 15 min of electrospinning, whereas multilayer orientation appeared after 30 min of electrospinning. Dense crosslinking of the electrospun 0.30 g/g “HA”-gelatine scaffold after 60 min of electrospinning (Figure 8) still indicates a dominant fibre orientation with regular large pores present. Immersion of the crosslinked scaffolds in water for 48 h resulted in a relatively small uptake of water, by 9% w/w for the 0.30 g/g “HA”-gelatine scaffold, indicating the large extent of crosslinking in the scaffolds and the “HA” content. This scaffold displayed a very good level of Young’s modulus of about 60 MPa and a very good strength of 3.9 MPa. Poly(lactic acid)-

metaphosphate scaffolds prepared by solvent casting exhibited a much lower tensile strength of 0.1 MPa [13] and the same material type of scaffolds prepared by a novel sintering method displayed a tensile strength of only 0.7 MPa [13]. We believe that the reasons for the excellent mechanical properties of our scaffolds are their fibrillar crosslinked structure with high degree of orientation and the nanodispersed “HA” particles in the gelatine matrix of each fibre [16,36]. In fact, the tensile strength of our scaffolds is comparable to the tensile strength of poly(lactic acid)-hydroxyapatite composite with 35% hydroxyapatite crystal fibres fabricated by hot pressing [14]. Our results are also comparable with the mechanical properties of oriented electrospun scaffolds of poly (lactide-co-glycolide) (PLGA) (with a tensile modulus of 40 MPa and a tensile strength of 6 MPa in the orientation direction [33]). On the other hand, Mouthouy et al [25] fabricated PLGA/Collagen-HA scaffolds by electrospinning and found out that while the increase of HA content leads to an increase in the Young’s modulus of scaffolds similarly as in this study, it leads to a decrease of the tensile strength. Furthermore, results of low mechanical properties were obtained for electrospun nanofibrous PVA-collagen-HA scaffolds with a maximum tensile strength of 0.2 MPa for 10 wt.% HA [1].

Addition of smaller amounts of HA, 0.10 and 0.20 g/g HA-gelatine, brought some decrease of the tensile strength of gelatine scaffolds from 2.9 MPa to 1.8 MPa, due possibly to interruptions of the gelatine matrix continuity by the HA particles, phase separation and possible inhomogeneities in fibres. However, the 0.30 g/g HA-gelatine scaffold was superior in all mechanical properties, including modulus, strength and tensile strain at fracture, over the rest of HA-gelatine scaffolds.

5. CONCLUSIONS

Gelatine and HA-gelatine nanocomposite fibres were electrospun successfully with the possibility to control fibre orientation using specially designed rigs and appropriate processing conditions. In general, fibre diameter was from submicron (nanometers) to a few microns, whereas the diameter depended on the concentration of the solution under electrospinning: the higher the concentration of gelatine, the larger was the fibre diameter. Increasing the content of HA, on the other hand, decreased the fibre diameter. High feed rates and high DC potential, which still maintained continuous fibre formation, resulted in the best fibre orientation. Two different configurations of the electrospinning rig were designed to achieve (a) small diameter tubular grafts with axial fibre orientation via the use of a rotating glass rod substrate and the guidance of two razor edged blades at appropriate orientation; (b) very large diameter tubular grafts around a rotating wired drum substrate aiming at circumferential fibre orientation, where the grafts could be cut to obtain flat fibrous sheets with continuous fibres mostly oriented in one direction. As the electrospinning proceeded to longer process times and the thickness of the fibrous product around the rotating wired drum increased, electrospinning could not yield oriented fibres any more.

The products were crosslinked using a glutaraldehyde solution to yield scaffolds with generally homogeneous mesopore size. The scaffolds were subjected to tensile testing and the 0.30 g/g HA-gelatine scaffold yielded the highest modulus and strength, as expected; this was also the composition with the highest content of HA that could be electrospun into continuous fibres without fibre break up during processing. The small diameter tubular scaffolds with axial fibre orientation are recommended for vascular grafts whereas the 0.30 g/g HA-gelatine flat scaffolds are recommended as starting scaffolds for orthopaedic applications.

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Table 1: Processing parameters for the electrospinning of tubular fibrillar gelatine structures

Scaffold	Gelatine concentration (% W/V)	Voltage (kV)	Feeding rate (ml/hr)	Duration the electrospinning run (min)
TG.1	12.5% W/V	27KV	1.5ml/hr	90min
TG.2	10% W/V	27KV	1.0ml/hr	60min
TG.3	10% W/V	30KV	1.5ml/hr	50min

a

Table 2. Properties of the flat electrospun fibrous scaffolds

	Gelatine (from 10w/v solution)	Gelatine (from 12.5w/v solution)	0.10g/g HA- gelatine (from 12.5w/v solution)	0.20g/g HA- gelatine (from 12.5w/v solution)	0.30g/g HA- gelatine (from 12.5w/v solution)
Fibre diameter (mean±std.dev) (nm)	490±30	810±70	650±70	540±50	480±70
%w/w water absorption after 48 hr soaking of crosslinked scaffolds	22±1	18±1	16±1	11±1	9±1
Young's modulus of crosslinked scaffolds (MPa)		33.8	41.2	54.9	59.8
Tensile strength of crosslinked		2.90±0.05	1.80±0.05	1.80±0.05	3.90±0.10

scaffolds (MPa)					
Strain to failure (%) of crosslinked scaffolds		11.7	8	4	9.4

Figure 1. Electrospinning set up for the electrospinning of flat sheets of fibrillar structures

Figure 2. Electrospinning set up for the electrospinning of tubular fibrillar structures

Figure 3. SEM (at low magnification) of samples of the tubular gelatine fibrillar scaffolds TG.1 (uncrosslinked), TG.2 (crosslinked) and TG.3 (crosslinked).

Figure 4. SEM (at high magnification) of samples of the tubular gelatine fibrillar scaffolds TG.1 (uncrosslinked), TG.2 (crosslinked) and TG.3 (crosslinked)

Figure 5. SEM of samples of the flat gelatine uncrosslinked fibrillar scaffolds produced from 12.5% w/v gelatine solutions for different durations of electrospinning

Figure 6. SEM of samples of flat 0.30g/g HA-gelatine uncrosslinked fibrillar scaffolds for different durations of electrospinning

Figure 7. SEM of samples of flat uncrosslinked fibrillar scaffolds fabricated after 60 min of electrospinning: (a) gelatine from 10%w/v gelatine solution; (b) 0.10 g/g HA-gelatine from 12.5% w/v solutions; (c) 0.20 g/g HA-gelatine from 12.5% w/v solutions; (d) 0.30 g/g HA-gelatine from 12.5% w/v solutions

Figure 8. SEM of sample of flat crosslinked fibrillar scaffolds fabricated after 60 min of electrospinning: 0.30 g/g HA-gelatine from 12.5% w/v solution