Modification and mechanical properties of electrospun blended fibermat of PLGA and siloxane-containing vaterite/PLLA hybrids for bone repair

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Abstract. Multi-syringe electrospinning has been successfully employed to produce a blended fibermat composed of poly(lactic-glycolic acid) (PLGA) fibers and a composite fiber for bone repair. The composite fiber, siloxane-containing vaterite (SiV)/poly(L-lactic acid) (PLLA), donated as SiPVH has the ability to release soluble silica species and calcium ions at a controlled rate. The SiPVH fibermats have demonstrated excellent bone regeneration ability *in vivo* at the front midline of the calvaria of rabbits. However, they are brittle and have low tensile strength resulting from the large particulate SiV (60 wt%) content. In this study, co-electrospinning of PLGA with SiPVH was performed in the hope of achieving a blended fibermat with improved mechanical properties. The co-electrospun fibermats showed good homogeneous blending of the PLGA and SiPVH composite fibers that had excellent flexibility. The blended PLGA-SiPVH fibermats had significantly improved mechanical properties compared to the SiPVH fibermats, where more than 20 times higher elongation to failure was achieved on comparison to the SiPVH fibermat. As well as strength, high porosity and large pore size are vital for the migration of cells into the centre of the graft. This was accomplished by heating the PLGA-SiPVH fibermats at 110°C for a fixed time, which induced the softening and flow of PLGA towards the more stable SiPVH fibers. Heating had successfully produced PLGA-SiPVH fibermats with large open pores and inter-fused SiPVH fibers, which also had better tensile mechanical properties than the SiPVH fibermat.

Keywords: biopolymers, biocomposites, electrospinning, blended fibermat, mechanical properties

1. Introduction

Large maxillofacial defects are often regenerated with barrier membranes [1–3]. Barrier membranes function by containing the regeneration site from ingress of soft tissue into the defect site, which is often filled with bone harvested from the patients and/or biological agents such as bone forming cells and growth factors [3]. These, also called guided bone regeneration (GBR) membranes are required to be biocompatible, integrate with host-surrounding tissue and have high mechanical properties and flexibility to maintain and support defect site and should also be easy to handle.

Fibrous membranes are promising as GBR materials due to their high and breathable interconnected porosity [3–8]. This allows efficient transport of nutrient and oxygen into and metabolic waste out of the regeneration site. The fibrous structure enhances cell attachment [9], therefore promotes soft tissue growth along the membrane while the porosity can be designed to inhibit in-growth. Electrospinning is a simple and versatile method to produce fibermats with fiber diameters ranging from nano-micrometer [10–12]. Electrospun materials are very promising for the regeneration of tissues and organs [13–15] due to the possibility of mimicking some of the

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structural features of natural extra cellular matrix (ECM) [16]. Numerous studies show the enhanced cellular attachment and proliferation on the fibrous materials [9, 17, 18]. Several studies have also shown the higher mechanical properties, especially the elongation to failure, of the electrospun fiber mats [19–22] and blended fiber mats [23]. The mechanical properties of electrospun fibers depends greatly on the degree of alignment of the fibers within the mat, fiber lay-ups and interface properties of fiber-fiber contact [20, 24]. Therefore, the versatility of electrospinning presents a promising method of fiber production for biomedical application.

The current authors have previously employed electrospinning to fabricate bi-layered GBR membranes that were shown to be bioactive, biocompatible and mechanically strong while being flexible [25]. The bi-layered membranes consists of two layers, a PLLA fiber mat layer and a siloxane-containing vaterite (SiV) / PLLA composite fiber mat layer (SiPVH) [25, 26]. The PLLA layer acted as the soft tissue barrier membrane, where the interconnected porosity was optimised by tailoring the fiber diameter to inhibit cell migration into the depth of the fiber mat. The SiPVH fiber mat layer provides space for bone formation. Both, the PLLA and the SiPVH layers were bonded together by hot-pressing with a stainless steel mesh that was placed on top of the PLLA layer. Whereby, at regions on the fiber mat in contact with the steel mesh fiber-fiber fusion was induced [26]. Early experiments on SiPVH dip-coated membranes showed MC3T3-E1 cells proliferate on the membrane and more so on the hydroxyl carbonate apatite (HCA) coated SiPVH [5]. Therefore, the authors had also coated the bi-layered membranes with a bone mineral-like apatite layer by soaking in simulate body fluid (SBF). The authors then performed *in vivo* experiments on New Zealand rabbits by placing the bi-layered GBR membranes at the 8 mm defects in the front midline of the calvaria of the rabbits [25]. They found enhanced bone formation on the SiVPVH composite layer at the sites where depressions (regions of fiber-fiber fusion) were made by stainless steel mesh. Their study of the fiber mat morphology showed a unique melted structure which they proposed could have influenced the cells to proliferate and lay-down new bone faster at those sites [25, 26]. They also noted that the cells could only migrate through the non-

depressed areas; therefore porosity for cell migration was important [25].

From an enhancement of bone regeneration point of view; it is highly desirable to obtain the melt-fused morphology throughout the fiber mat, however the hot-pressing process closes majority of the pores, which are critical for cell migration. The hot-pressing process that leads to the desired unique morphology is also the major drawback in this material. Therefore innovative processing methods are required to overcome this hurdle in achieving the unique morphology. Here, in this study one such strategy is explored. The fabrication of the unique melted structure throughout the fiber mat was attempted by electrospinning a blended fiber mat and applying a heating procedure that induced partial melting and fusion of the poly(lactic glycolic acid) (PLGA) fibers. In addition to the melt-fused morphology, by co-spinning PLGA and SiPVH, the blended fiber mat was expected to have improved mechanical properties, which is important for ease of handling, shaping to specific shape and finally to support the biological moieties and cells during the process of bone regeneration.

2. Experimental section

PLLA (Purasorb®; PURAC, The Netherlands) and PLGA (53% lactic, 47% glycolic) (Purasorb®; PURAC, The Netherlands) with average molecular weight (M_w) of 260 kDa and 160 kDa, respectively were used. Chloroform was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

2.1. Preparation of SiV particles

The silicon-doped calcium carbonate (SiV) powders were prepared by a carbonation process as described by Yasue *et al.* [27] and later modified by Kasuga *et al.* [28]. Briefly, calcium hydroxide ($\text{Ca}(\text{OH})_2$) and aminopropyltriethoxysilane (APTES) were mixed together in methanol, followed by a constant bubbling of CO_2 gas for 75 min at a rate of $2 \text{ l} \cdot \text{min}^{-1}$. The resulting slurry was dried at 110°C , resulting in the formation of SiV powder with average particle size of $1 \mu\text{m}$.

2.2. Preparation of PLGA-SiPVH blended fiber mats

The SiPVH fiber mats were prepared according to the procedure described by Obata *et al.* [25]. Briefly,

SiV particles containing fibers, SiPVH, was produced by electrospinning a composite solution of 60 wt% SiV and 40 wt% PLLA. The composite solution was prepared by melt blending SiV and PLLA at 200°C for 10 min with a kneader and dissolved in chloroform with 10 wt% polymer concentration. Electrospun fibers of PLGA were produced after dissolving in chloroform with 10 wt% polymer concentration. Samples were then electrospun on the Nanofiber Electrospinning Unit (NEU, Kato Tech Co, Japan). The mixtures for electrospinning were loaded into a glass syringe and pushed out at a flow rate of $0.50 \mu\text{l}\cdot\text{s}^{-1}$ through a metallic needle (22 gauge for SiPVH and 19 gauge for PLGA) that was connected to a +20 kV electric field. The fibers were collected on a rotating drum that was positioned 15 cm from the tip of the needle. Blended fibermats were produced by electrospinning two separate solutions side-by-side. Blended fibermats containing fibers of PLGA-SiPVH were produced with a final thickness of 230 μm after spinning for 210 min.

2.3. Modification of blended fibermats

PLGA-SiPVH fibermats were cut into circular membranes with a diameter of 17 mm. These were heated in a drying oven at 90, 110 and 130°C for 15, 20, 25 and 30 min to obtain the heat-fused morphology of the PLGA-SiPVH fibermats. Previous work has demonstrated the enhanced cellular proliferation of MC3T3 cells on an HA coated SiPVH fibermat in comparison to an uncoated SiPVH fibermats [26]. Therefore, cut fibermats before and after heating were soaked in a solution containing ions with 1.5 times the concentration of conventional simulated body fluid (SBF) to obtain the HA coating. The 1.5 SBF solution consisting of 3.75 mM Ca^{2+} , 213.0 mM Na^{+} , 2.25 mM Mg^{2+} , 7.5 mM K^{+} , 222.45 mM Cl^{-} , 6.3 mM HCO_3^{-} , 1.5 mM HPO_4^{2-} , 0.75 mM SO_4^{2-} , 50 mM $(\text{CH}_2\text{OH})_3\text{CNH}_2$ and 45.0 mM HCl was prepared using reagent grade NaCl, NaHCO_3 , KCl, $\text{K}_2\text{PO}_4\cdot 3\text{H}_2\text{O}$, $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$, HCl, CaCl_2 , NaSO_4 and $\text{NH}_2\text{C}(\text{CH}_2\text{OH})_3$. After immersion in 1.5 SBF solution for 24 h the membranes were washed with distilled water and dried at room temperature.

2.4. Characterisation of blended fibermats

Samples for scanning electron microscopic (SEM, JSM-6301F, JEOL, Japan) observations were coated with amorphous osmium using an Os coater (Neoc, Meiwafoysis, Japan) and observed under 5 kV accelerating voltage and 15 mm working distance.

Tensile tests were performed on the AGS-G, Shimadzu, Japan machine equipped with a 50 N load cell. Rectangular samples with dimensions of 5 mm $\times$ 40 mm were cut out of the fibermats and tested with a grip-to-grip separation of 20 mm at a strain rate of 5 mm/min. Samples were tested until complete failure and SEM observations were performed on the failed samples.

3. Results and discussion

Figure 1 shows the SEM images of the PLGA-SiPVH blended fibermats. The SiPVH on comparison to PLGA fibers has a much rougher fibrous surface due to the presence of SiV particles in the SiPVH fibers. The SiPVH fibers also have a large average fiber diameter than the PLGA, this was due to the higher viscosity of the SiPVH (5312 mPa·s) electrospinning solution than PLGA (793 mPa·s). The fiber diameters measured from the SEM images of PLGA ranged from 1.5–10.0 μm and the average diameter of SiPVH was measured to be $18\pm 5 \mu\text{m}$. The fiber diameter of SiPVH was found to be more homogeneous than that of PLGA. The cross-sectional image of the PLGA-SiPVH fibermat (Figure 1b) shows that the polymer and SiPVH fibers were very well blended.

3.1. Heating of PLGA-SiPVH blended fibermats

Optical microscopy was performed on the samples heated at 90, 110 and 130°C for 15 and 30 min (data not shown). The PLGA-SiPVH fibermat heated at 90°C for 30 min showed only a very small change in original morphology. On the other hand, at 130°C complete melting and large run off of the PLGA fibers were observed at both 15 and 30 min of heating. Figure 2 shows the SEM images of the PLGA-SiPVH fibermats after heating at 110°C for 15, 20, 25 and 30 min. All the PLGA-SiPVH fibermats heated at 110°C showed PLGA softening and flow;

Figure 1. Surface (a) and cross-sectional (b) SEM images of the PLGA-SiPVH fibermat

this lead to fusion with the SiPVH fibers. An identical morphology was observed after heating for 15 and 20 min, here the initial as-spun PLGA fibrous network is still vaguely visible, more so in the 15 min heated sample. However, more coalescence

of the PLGA was observed on the 20 min heated sample. On the other hand, heating for 30 min produced a large amount of flow where almost all the PLGA has aggregated towards the SiPVH fibers. However, heating for 25 min seems to have pro-

Figure 2. SEM images of the PLGA-SiPVH fibermat after heating at 110°C for (a) 15, (b) 20, (c) 25 and (d) 30 min

duced enough flow of PLGA fibers that has resulted in a fibermat morphology that has open pores of the order of 100 μm and a substantial proportion of PLGA still bridging the SiPVH fibers. This morphology could be beneficial for cell attachment, spreading and migration within the fibermat.

3.2. Morphology of the PLGA-SiPVH blended fibermats soaked in 1.5 SBF

Figure 3 shows the SEM images of the PLGA-SiPVH fibermats (Figure 3a) after soaking in 1.5 SBF for 24 h, (Figure 3b, d, e) after heating at 110°C for 25 min then soaking in 1.5 SBF and (Figure 3c) after soaking in 1.5 SBF for 24 h then heating at 110°C for 25 min. Firstly, the SiPVH fibers in all of the samples after soaking 1.5 SBF for 24 h were completely coated with a thick layer of HA (as shown in Figure 3d). As well, several particles of HA were also observed on the surface of PLGA, especially in the case of fine PLGA fibers that were

observed to be completely coated (Figure 3e). Figure 3d, e shows the high magnification SEM images of Figure 3b; the HA particles on the SiPVH and PLGA surface have a globular cauliflower-like morphology. Secondly, the fibrous morphology of the as-spun-HA-coated (Figure 3a) and the heated and HA coated (Figure 3b, c) fibermats show a clear difference on the size of the pores in the fibermats. Where, from the SEM images the pore size seems to increase in the order as follows: as-spun-HA-coated (Figure 3a) < HA-coated-heated (Figure 3c) < heated-HA-coated (Figure 3b). The through-pore area fraction of the fibermats was calculated from the SEM images in Figure 3 using ImageJ. They were found to be 6.4, 16.2 and 9.2% for the as-spun-HA-coated, heated-HA-coated and HA-coated-heated, respectively. Although this not the true porosity of the fibermat but it gives an indication of the extent of porosity of each sample and demonstrates that the heating treatment signifi-

Figure 3. SEM images of the HA-coated PLGA-SiPVH fibermat (a) without heat application, (b) after heating and soaking, (c) after soaking and then heating and (d, e) high magnification images of (b)

cantly increases the porosity. The as-spun-HA-coated has the smallest pore space, this is expected since no heating was applied to result in the coalescence of the fine fibers of PLGA. While, in the case of the HA-coated-heated fiber mat (Figure 3c) the HA coating on the fine PLGA fibers, where several fine PLGA fibers with HA coating (circled in Figure 3c) are clearly visible in the SEM image, has hindered its flow at the post-heating stage. This has reduced the coalescence of PLGA and hence the required effect of heating was not achieved. However, the heated-HA-coated fiber mat (Figure 3b) has the desired morphology, where the SiPVH fibers are completely coated with HA and large interfiber distances can be seen. Therefore, for the blended PLGA-SiPVH fiber mats, the processing conditions of heating at 110°C for 25 min followed by soaking in 1.5 SBF for 24 h is proposed to be ideal in achieving the desired fiber mat morphology.

3.3. Mechanical properties of the PLGA-SiPVH blended fiber mats

Figure 4a shows the tensile stress-strain curves and Figure 4b ultimate tensile strength (UTS) and elongation to failure of the as-spun SiPVH and blended PLGA-SiPVH fiber mats and the heated PLGA-SiPVH fiber mats. Both, the UTS and elongation before failure of all the PLGA-SiPVH blended samples were higher than that of SiPVH fiber mats. The SiPVH fiber mat had low UTS of 0.16 MPa and a brittle failure with a very low elongation to failure of 1.44%. The as-spun PLGA-SiPVH blended fiber-

mat showed the largest elongation before failure of more than 30%. Both, as-spun and the 15 min heated samples had the highest UTS (~0.5 MPa), which then steadily decreased with heating time. Samples heated for 20 and 25 min at 110°C and then soaked in 1.5 SBF for 24 h were also tested in tension. A small decrease in the UTS was observed with soaking in SBF. This could be due to the degradation of PLGA in the 1.5 SBF solution, however the drop in UTS was minor.

The elongation to failure of the PLGA-SiPVH blended fiber mats decreased drastically after heating, however still higher than that of SiPVH fiber mat. Although, the 15 min-heated sample had relatively high UTS, the elongation before failure decreased to around 4%. Wei *et al.* [24] showed that the fracture energy decreases with increasing fiber-fiber fusion, where in this study longer heating time leads to higher fusion hence the elongation before failure is observed to decrease. Figure 5 shows the SEM images of the failure regions on the as-spun and heated samples of the PLGA-SiPVH blended fiber mats after tensile testing. In the as-spun fiber mat (Figure 5a–c) large plastic deformation before complete failure is evident, where several regions (circled in Figure 5a) of fiber stretching were observed on the whole fiber mat. A higher magnification image of the circled area in Figure 5a is shown in Figure 5b, where the SiPVH fibers were seen to be completely fractured while the PLGA fibers have thinned to various thicknesses. Complete fracture of the sample took place when the cir-

Figure 4. (a) Exemplary tensile stress-strain curves and (b) ultimate tensile strength and elongation to failure of the SiPVH fiber mat and PLGA-SiPVH blended fiber mats before and after heat treatment at 110°C for 15, 20, 25 and 30 min.

cle regions connected up along the width of the whole fibermat. Figure 5c shows a SEM image from the region of complete failure, which shows more thinned PLGA and fractured SiPVH fibers.

All the heated samples had a similar failure mechanism which was very different from that of the as-spun blended fibermats. Plastic deformation in the heated fibermats was observed to be very local and

Figure 5. SEM images of the (a–c) as-spun and heated (d) 15 min, (e) 25 min and (f) 25 min-heated and soaked in 1.5 SBF PLGA-SiPVH fibermats after performing tensile tests. In the images the regions of local deformation in the as-spun PLGA-SiPVH are shown with ellipses, box in (d) and (f) show the fractured SiPVH fibers stabilised by PLGA, black arrows point to the PLGA strands that had plastically deformed and white arrows in (f) indicate a segment of SiPVH fiber stabilised by melt-fused PLGA.

confined to the region of final fracture. Figure 5d–f shows the SEM images of the failure zones of the heated samples. There are several important observations to be made. First, the fiber morphology close to the fracture zone had minimal disturbance after testing, a clear example for this is seen in Figure 5f, where a part of SiPVH fiber shaped ‘U’ (indicated by white arrows in SEM image) was not pulled out during testing. This indicates that the failure was brittle-like and that the melt-fused PLGA-SiPVH structure was very stable. Second, small strands of PLGA, indicated by black arrows in the SEM images, are the only PLGA polymer to undergo plastic deformation. Thirdly, these strands of PLGA are observed to hold the fractured pieces of SiPVH fibers in place (square boxes in SEM images), this is advantages after implantation if the SiPVH fibers were to fracture off; they could be held in implantation site by the PLGA. Finally, the failure region and mode of failure of the heated-HA-coated PLGA-SiPVH fibermat (Figure 5f) was identical to the heated-only fibermats. Therefore, the melt-fused PLGA-SiPVH fiber morphology obtained by heating at 110°C for 20 or 25 min and HA coated by soaking in 1.5 SBF could be promising for cell adhesion, spreading and migration. In vitro cell culture and in vivo animal studies are required to confirm this.

4. Conclusions

An attempt to mimic the morphology of the hot mesh-pressed bi-layered fibermat structure via a double syringe electrospinning and heating process was performed. The SEM images show clearly that the double syringe electrospinning of PLGA and SiPVH results in a well-blended fibrous structure. The PLGA-SiPVH fibermats have more than 30% elongation before failure and ~0.5 MPa ultimate tensile strength. On heating the PLGA fibers soften and flow towards the SiPVH fibers and adhere to it. This is found to indirectly, increase the pore size of the fibermat and result in multiple triple-points that connect the SiPVH fibers. Heating at 110°C for 20 min and more so after 25 min was found to produce a microstructure with pores of the order of 100 µm. The melt-fused PLGA-SiPVH fibermats after heating have higher elongation to failure and ultimate tensile strength on comparison to SiPVH-only fibermats. The melt-fusing by PLGA was also

observed to provide stability to the SiPVH fibers while also increasing their strength.

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