

Silk–PVA Hybrid Nanofibrous Scaffolds for Enhanced Primary Human Meniscal Cell Proliferation

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Received: 1 May 2016 / Accepted: 29 September 2016 / Published online: 11 October 2016
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Abstract In this study, silk fibroin nanofibrous scaffolds were developed to investigate the attachment and proliferation of primary human meniscal cells. Silk fibroin (SF)–polyvinyl alcohol (PVA) blended electrospun nanofibrous scaffolds with different blend ratios (2:1, 3:1, and 4:1) were prepared. Morphology of the scaffolds was characterized using atomic force microscopy (AFM). The hybrid nanofibrous mats were crosslinked using 25 % (v/v) glutaraldehyde vapor. In degradation study, the crosslinked nanofiber showed slow degradation of 20 % on weight after 35 days of incubation in simulated body fluid (SBF). The scaffolds were characterized with suitable techniques for its functional groups, porosity, and swelling ratio. Among the nanofibers, 3:1 SF:PVA blend showed uniform morphology and fiber diameter. The blended scaffolds had fluid uptake and swelling ratio of 80 % and 458 ± 21 %, respectively. Primary meniscal cells isolated from surgical

debris after meniscectomy were subcultured and seeded onto these hybrid nanofibrous scaffolds. Meniscal cell attachment studies confirmed that 3:1 SF:PVA nanofibrous scaffolds supported better cell attachment and growth. The DNA and collagen content increased significantly with 3:1 SF:PVA. These results clearly indicate that a blend of SF:PVA at 3:1 ratio is suitable for meniscus cell proliferation when compared to pure SF-PVA nanofibers.

Keywords Silk fibroin · PVA · SF-PVA nanofiber · Meniscal cells

Introduction

Meniscus, a fibrocartilaginous tissue present in the knee joint, plays major roles in body weight distribution, lubrication, and in providing nutrition to the knee joint. Meniscus is prone to damage during excessive physical activities, sports, accidents or in old age. This often leads to surgical removal of the entire or injured part of the meniscus ultimately leading to osteoarthritis in long run. The healing of the damaged meniscus is difficult, especially in its inner zones due to its avascular nature. A suitable biodegradable scaffold seeded with patient's meniscus cell can offer a viable solution for this problem (Chambers and El-Amin 2015). Till the cells attach, proliferate, migrate, and form a functional tissue, scaffold can take the load and reduce the friction between the femur and tibia in the knee joint. Researchers have tried using different types of natural biomaterials like eggshell membrane (Pillai et al. 2015a), silk (Gruchenberg et al. 2015) and synthetic biomaterials like polycaprolactone (PCL) (Gopinathan et al. 2015), polyvinyl alcohol (PVA) (Puttawibul et al. 2014) for meniscal scaffold preparations.

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Among the natural biomaterials, silk is a biodegradable, widely researched, FDA (Food and Drug Administration)-approved material for tissue engineering applications (Musson et al. 2015). The naturally occurring silk fiber is composed of two kinds of proteins, namely fibroin and sericin. Fibroin is highly hydrophobic, filament core protein that accounts for 72–81 % of silk (Zhang et al. 2009). Silk fibroin has an edge over the others as a scaffolding material, due to its properties like high mechanical strength, flexibility, biocompatibility, and controlled biodegradability which is suitable for load bearing (Motaghtalab et al. 2015). Fibroin contains 43 % glycine, 30 % alanine, and 12 % serine which contribute to its highly crystalline β -sheet structure. It has a heavy chain (\sim 390 kDa) and a light chain (25 kDa) linked by disulfide bonds and a glycoprotein P25 (25 kDa) (Koh et al. 2015). Silk fibroin (SF) has a good compatibility on living organisms and good oxygen permeability (Altman et al. 2003) that can enhance cell attachment and proliferation. Efficiency of SF as a biomaterial has been proved by various researchers using different types of cells like mesenchymal stem cells (Chen et al. 2014), osteoblast cells (MG63) (Varkey et al. 2015), articular chondrocytes (Wang et al. 2006), etc. Mandal et al. (2011) fabricated silk fibroin-based porous scaffold, mimicking the native meniscus architecture. SF has RGD (Arg-Gly-Asp) sequence in its structure that can accelerate cell adhesion and proliferation (Koh et al. 2015). However, the major limitation of SF is its brittle nature in dry state (Luangbudnark et al. 2012). In order to address this issue, scientists have blended SF with various polymers like polyvinyl alcohol (PVA), especially for load-bearing scaffold applications (Li et al. 2001; Bhattacharjee et al. 2015; Li et al. 2015). PVA is a FDA-approved biomaterial used commonly for wound dressings and skin tissue engineering (Kenneth et al. 2014). It is a non-toxic, water-soluble polymer having high mechanical strength, flexibility (Ha et al. 2005), and is often blended with other synthetic or natural polymers such as polylactic acid (PLA) (Abdal-hay et al. 2016), chitosan (Sharma et al. 2016), and silk (Bhattacharjee et al. 2015) for tissue engineering applications. The main problem faced with PVA-based scaffolds is its poor cell attachment property (due to its high hydrophilic nature) (Liu et al. 2009) which can be overcome by blending silk having RGD tripeptide. Such blending not only increases its mechanical property but also cell adhesion and proliferation. Hence, there is a need to optimize the blend ratio between the SF and PVA to obtain optimum cell attachment and mechanical properties. In this study, SF-PVA hybrid nanofibrous scaffold has been investigated for their potential application in human meniscal cell attachment and growth. To the best of our knowledge, there

are no studies on use of SF-PVA hybrid scaffold for meniscal tissue engineering.

Materials and Methods

Materials

Raw SF fibers, from *Bombyx mori* cocoon, were procured from local dealers in Kanchipuram district, Tamil Nadu, India. PVA (Molecular weight: 160,000) was purchased from Hi-Media Laboratories Ltd., Mumbai, India. All other reagents used for dissolution, crosslinking, and cell culture were obtained from Hi-Media Laboratories Ltd., Mumbai, India.

Degumming and Dissolution of SF

SF was extracted directly from raw silk threads reeled from cocoons. Raw SF threads were degummed using 0.5 M Na_2CO_3 solution at 100 °C for 1 h and washed thrice with distilled water in order to remove sericin from the surface of the SF fibers. The degumming process dissolved the sericin which resulted in a weight loss of up to 24 %. The degummed SF fibers were dissolved in Ajisawa's reagent (Calcium Chloride (CaCl_2)/ethanol ($\text{CH}_3\text{CH}_2\text{OH}$)/water (H_2O) in a molar ratio of 1:2:8) solvent at 60 °C until a clear solution was obtained. This solution was dialysed against distilled water for 2 days, followed by dialysis against polyethylene glycol (PEG) for 1 day to remove salt and to concentrate the SF solution, respectively (Varkey et al. 2015). The solution was dried and stored at 4 °C until further use.

Electrospinning of Nanofibrous Scaffolds

From the dried SF, 10 % (w/v) solution was prepared in 98 % formic acid. PVA (10 % (w/v)) was dissolved in water at 80 °C to prepare a clear solution. SF-PVA blends were prepared in the ratios of 2:1, 3:1, and 4:1 (v/v) by mixing both the solutions at 60 °C for 1 h. To avoid phase separation of SF and PVA, the solutions were prepared freshly before each experiment. The electrospinning of SF-PVA solution was optimized by varying several parameters such as voltage, concentration, flow rate of the polymer solution, and distance between collector and the syringe tip. In the electrospinning process, a high-electric potential (Glassman High Voltage Inc., India) of 25 kV was applied to the tip of a syringe needle (19 gauge) with a flow rate of 0.3 ml/h. The electrospun nanofibers were collected on a

grounded aluminum foil placed at a distance of 17 cm from the syringe tip. Pure and blended nanofiber mats were exposed to 25 % (v/v) glutaraldehyde vapors (12 h) for crosslinking.

Characterization

The surface morphology of electrospun nanofiber mats was observed using atomic force microscopy (AFM) (NT-MDT, Russia). A silicon probe tip with a 5-nm curvature radius was used to generate the images through the intermittent contact mode. The fiber diameter was calculated using ImageJ software. Functional groups present in the different nanofibrous scaffolds were analyzed using Fourier transform infrared spectroscopy (FTIR) (Shimadzu (IRaffinity-1), Japan) at a spectral range of 4000–400 cm^{-1} .

The porosity of different nanofibrous scaffolds was measured using liquid displacement method (Nazarov et al. 2004). Nanofibrous (NF) scaffolds of known weights and dimensions were directly immersed in a known volume (V_1) of hexane (non-solvent for both SF and PVA) for 30 min inside a glass cylinder (graduated). After 30 min, the total volume was recorded as volume (V_2) and the remaining volume (V_3) was recorded after removal of the scaffold from the beaker. The following formula was used to calculate the percentage porosity (ε) of the NF scaffolds.

$$\varepsilon = (V_1 - V_3)/(V_2 - V_3) \quad (1)$$

Nutrient uptake ability of a scaffold can be indirectly calculated from the swelling ratio tests. Scaffolds of known size (10 × 10 mm) were weighed and immersed in a 50-ml beaker containing 10 ml of distilled water. The scaffolds were removed from the beaker after immersing it for 24 h and the excess water on the scaffold surface was removed by placing the scaffolds on the filter paper. Then, the scaffolds were weighed again in the swollen state to determine the water uptake ability of the scaffolds. The swelling ratio of the scaffolds was calculated using the following formula:

$$\text{Percentage swelling} = [(W_t - W_0)/W_0] * 100 \% \quad (2)$$

where W_0 is the initial weight and W_t is the weight of the swollen sample.

Biodegradability is an essential requisite for the nanofiber mat to be used as a scaffold inside the human body. To study this phenomenon in vitro, the nanofibrous scaffolds were incubated in simulated body fluids (SBF), which mimics the ion composition of human blood plasma, though devoid of the essential proteins. The SBF was prepared by dissolving salts in deionized water according to Kokubo et al. (1990) and the pH of the solution was adjusted to 7.4. The solution was then

sterilized in an autoclave at 121 °C and 15 lbs for 20 min. The nanofiber mats were cut into samples of size 10 × 10 mm, weighed, and soaked in 30 ml of SBF. This was incubated at 37 °C for 28 days. The samples were removed every 7 days, dried, and the weights of the samples were determined. The percentage weight loss was determined according to Eq. 3. All experiments were performed in triplicate.

$$\text{Percentage weight loss} = [(w_0 - w_t)/w_0] \times 100 \% \quad (3)$$

where w_0 is the initial weight and w_t is the weight of the degraded sample.

Cell Culture Studies

Cell Isolation from Human Meniscus Samples

Human primary meniscal cells were isolated as per Pillai et al. 2015b, from surgical debris obtained after partial or total meniscectomy from PSG Institute of Medical Sciences and Research, Coimbatore, and Ortho One hospital, Coimbatore, India, with the consent of the patients (Institutional Human Ethical committee clearance no: 12/193). In brief, meniscal tissue was washed with 70 % ethanol followed by phosphate-buffered saline (PBS) twice, dissected into small pieces, and trypsinized for 1 h. The samples were digested using 1 % (w/v) collagenase enzyme for 3 h. Following this, the cells were washed with PBS twice and re-suspended in Dulbecco's modified Eagle's medium (DMEM) with 10 % (w/v) fetal bovine serum (FBS), 100 units/ml penicillin, and 100 mg/ml streptomycin. These cells were incubated at 37 °C with 5 % humidified carbon dioxide (CO_2) throughout the study. The cells were observed under inverted phase-contrast microscope for attachment onto the polystyrene petri plate. After reaching 100 % confluency, the cells were detached by using Trypsin–EDTA solution (2.5 % w/v) and used for further studies. Passage 2 (P2) meniscus cells were used to seed the scaffolds.

Meniscal Cell Seeding on Nanofibrous Scaffolds

Scaffolds of known size (1 × 1 cm) were sterilized by immersing in series of ethanol (100, 90, 80, 70, and 60 %) for 30 min followed by washing with sterile PBS. After repeatedly washing in sterile-distilled water and UV radiation exposure for 3 h (Shearer et al. 2006), nanofibrous scaffolds were placed in 24-well plates. The sterilized scaffolds were incubated for 48 h at 37 °C and 5 % CO_2 in DMEM and checked periodically for signs of contamination. Cells were seeded onto the fibrous scaffolds at a density of 3×10^4 and further studies were carried out.

MTT Assay

The cytocompatibility of the scaffolds was analyzed using 3-(4, 5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) assay for 1–5 days (Ghasemi-Mobarakeh et al. 2008). In a 24-well plate, 1 × 1 cm-sized scaffolds were finely chopped manually, treated with 1×10^4 cells attached to the culture plate, and incubated at 37 °C with 5 % CO₂. After incubation period, the medium along with the scaffolds was removed and the cytotoxicity of the exposed cells attached to the culture plate was determined using MTT assay. Human meniscal cells seeded on tissue culture plate was maintained as a control. 20 µl of MTT was added and incubated for 3.5 h at 37 °C. The medium was removed carefully and 150 µl of dimethyl sulfoxide (DMSO) was added followed by agitating in an orbital shaker for 15 min. Optical density of each well was read at 590 nm using 96-well microplate reader (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer). The cell viability percentage was calculated by comparing the absorbance of cells cultured on NF scaffolds to that of control.

DNA Estimation Using Hoechst Stain

DNA-binding Hoechst 33258 stain was used to determine the DNA content (Kim et al. 1988). The attached cells on to NF scaffolds were detached periodically using Trypsin–EDTA solution, pelleted, and treated with 5 ml of Hoechst stain and PBS. The samples were read at 356 nm for excitation and 492 nm for fluorescence emission in Fluoroskan Ascent™ Microplate Fluorometer (Thermo Scientific™). The total DNA content was calculated at different time intervals (6, 12, and 18 days) by comparing the absorbance of detached cells from different scaffolds to that of 6th day control.

Estimation of Extracellular Matrix (ECM) Production

The total amount of glycosaminoglycans (GAG) and collagen secreted can be quantified from the culture medium at regular time intervals (Whitley et al. 1989). This method helps us to analyze the impact of scaffolds on ECM secretion of the cells. The culture medium taken from the cells seeded on polystyrene plates were used as control. 1,9-dimethylmethylene blue (DMMB) assay was used to quantify total GAG content. Aliquots periodically taken from scaffolds were treated with DMMB dye and absorbance was measured at 525 nm in Multiskan™ GO Microplate Spectrophotometer (Thermo Scientific, USA). The chondroitin sulfate A sodium salt was used as a standard for GAG estimation. The total collagen content secreted in the medium was quantified using Sirius red

method (modified Hride Tullberg–Reinert method) (Reinert and Jundt 1999). Calf collagen was used as standard for collagen estimation. Sirius red dye was prepared in 0.5 M acetic acid solution and samples were agitated for 5 s. The samples were incubated at room temperature for 30 min, centrifuged (1500 rpm for 10 min), and supernatant was discarded. To remove the excess dye, the pellet was washed with 0.01 N hydrochloric acid and again suspended in 0.1 N potassium hydroxide. Absorbance was measured in a microplate reader at 540 nm.

Cell Adhesion Studies

Primary human meniscus cell's adhesion to the scaffolds was monitored using DNA-binding Hoechst (33258) fluorescence staining (Latt and Stetten 1976). Staining of meniscal cell-seeded scaffolds and control sample (cells seeded on polystyrene plate) were carried out at different time intervals (6, 12, and 18 days). For staining, DMEM was removed from the 24-well plate aseptically and washed with PBS. The scaffolds with cells were treated with Carnoy's fixative (1:3 acetic acid:methanol) for 10 min and were repeated twice under same conditions. After that, Hoechst stain was added to all the samples and incubated for 30 min. Excess unbound dye was removed by washing with deionized water. The samples were allowed to air dry for 1 h and observed under inverted phase-contrast Epifluorescence microscope (Nikon Eclipse Ti-S, Japan) fitted with a filter having a wavelength range of 460–490 nm. The images were captured using a Nikon CCD camera attached to the microscope.

Statistical Analysis

All the experiments were carried out in triplicate and the data were expressed as mean ± standard deviation. One-way ANOVA with Tukey's multiple comparison tests was performed for the statistical significance ($p < 0.05$) of the data using Origin 8.0 software.

Results and Discussion

Scaffold Preparation and Characterization Studies

Nanostructure is very important for successful cell attachment and proliferation (Gittens et al. 2011). Xu et al. (2004) reported that the cell attachment and proliferation were higher in uniform fibers with lesser diameters. The lower diameter will yield higher available surface area required for efficient cell attachment and proliferation (Gopinathan et al. 2015). Figure 1a–e show the AFM images of nanofiber mats. The inset in Fig. 1 shows the

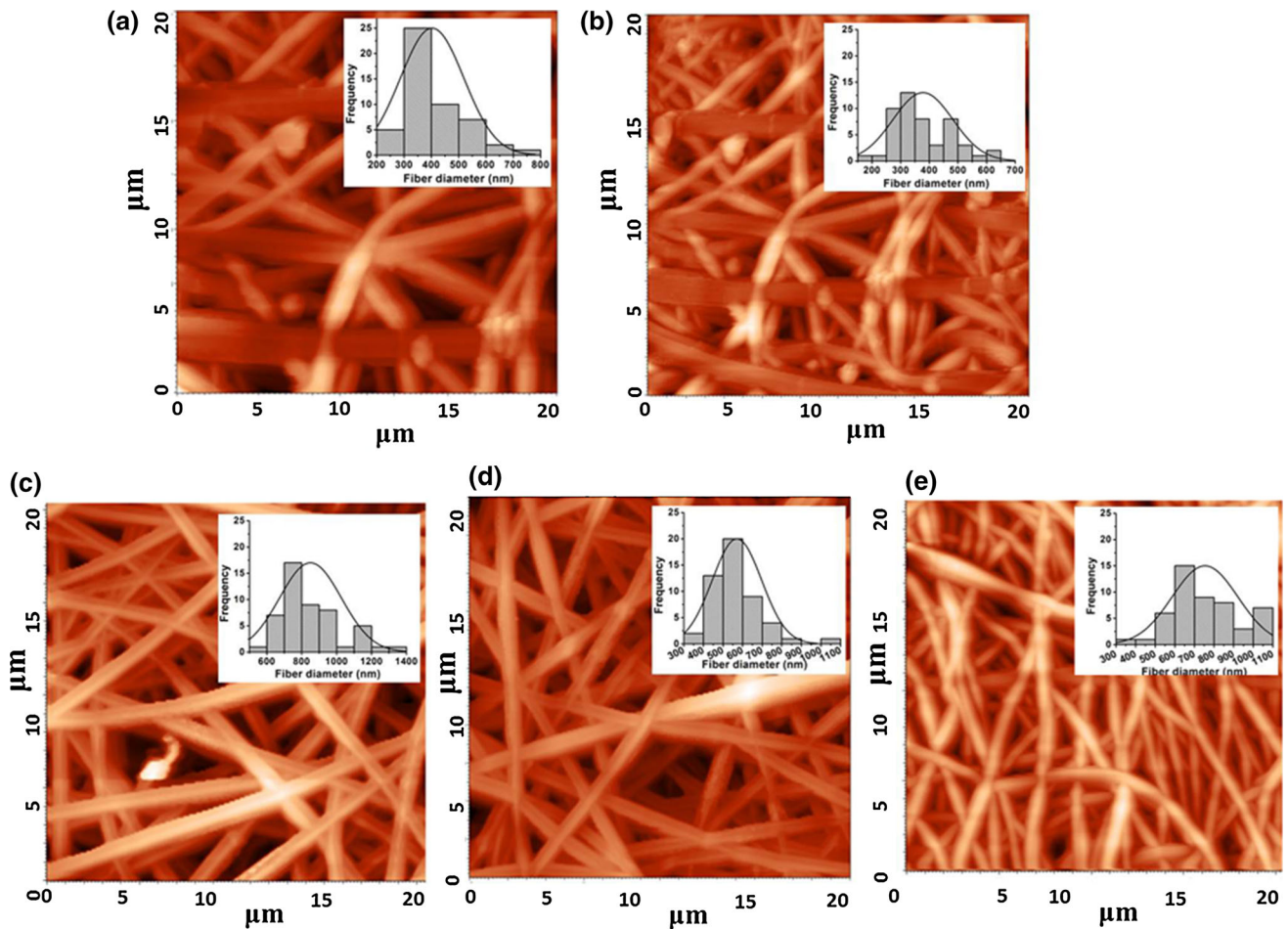


Fig. 1 AFM images of NF scaffolds **a** Silk NF, **b** NF PVA, **c** 2:1 SF:PVA, **d** 3:1 SF:PVA, **e** 4:1 SF:PVA

overall diameter distribution. The control PVA and SF nanofibers had a diameter of 378 ± 105 nm and 403 ± 118 nm, respectively. However, on blending PVA and SF, the nanofiber diameter increased considerably under same processing conditions. The diameters of 2:1, 3:1, and 4:1 nanofibers were 850 ± 177 , 571 ± 126 , and 754 ± 167 nm, respectively. 3:1 SF-PVA blend nanofiber scaffold showed less diameter which indicated better processability compared to other two blends. Along with increased surface area, porosity of the scaffold plays a vital role in tissue engineering. The porous scaffolds provide good cell attachment, penetration, growth, tissue formation, better nutrient diffusion, and waste by-product transport from the scaffold (Nguyen et al. 2013). An ideal scaffold should have 76–81 % of porosity (Nakamatsu et al. 2006; Hollister 2005). The overall porosity of the nanofibrous scaffolds was calculated using liquid displacement method (Fig. 2a). There was no significant difference in porosity between blended nanofibrous scaffolds (~ 80 %), while pure PVA scaffold was found to be

slightly higher in porosity (~ 85 %). Among all the developed scaffolds, pure SF nanofibrous scaffold had the lowest porosity (~ 75 %). Figure 2b shows the swelling ratio of nanofibrous scaffolds after 24 h of incubation in distilled water. The blended nanofibrous scaffolds showed significantly ($p < 0.05$) higher swelling ratio with an average of 458 % when compared to the pure SF (282 %) and PVA (364 %) scaffolds. Such increase in swelling ratio may be attributed to the difference in the degree of crosslinking in a blended nanofibrous matrix. The efficiency of glutaraldehyde crosslinking is expected to be the highest in the case of pure PVA as it contains more hydroxyl groups when compared to SF. Similar type of observation has been made in FTIR spectrum (Fig. 3) where the hydroxyl group intensity was found to be the highest in pure PVA and the intensity decreased gradually with an increase in SF ratio. Such variation in hydroxyl group may contribute directly to the swelling ratio of the blended scaffolds. The FTIR spectra also showed the

Fig. 2 **a** Porosity and **b** swelling ratio of nanofibrous scaffolds ($p < 0.05$)

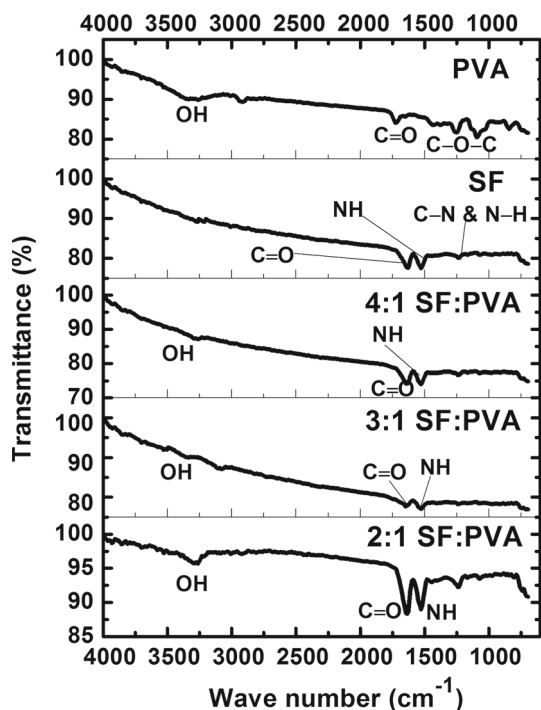
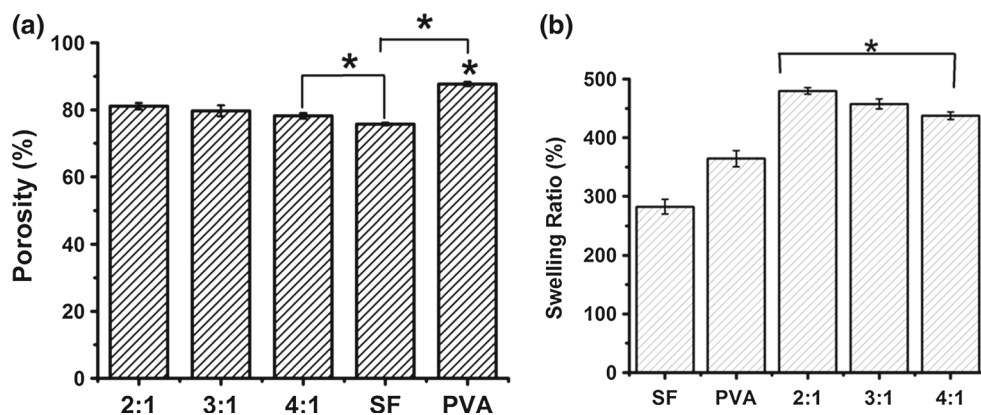


Fig. 3 FTIR spectrum of nanofibrous scaffolds

characteristic peaks for SF and PVA. The peaks of SF in the region of 1527 cm^{-1} (Amide-II, secondary NH bending) and at 1627 cm^{-1} (amide I, C=O stretching) were due to the presence of β -sheet in the SF (Chen et al. 2001). C–N and N–H functionality peaks were also found in $1270\text{--}1230\text{ cm}^{-1}$ region of FTIR spectrum (Fig. 3). For PVA, the characteristic peaks were observed in the range of ~ 3260 , ~ 1720 , and $1250\text{--}1090\text{ cm}^{-1}$ corresponding to OH group, C=O group, and C–O–C group, respectively (Sudhamani et al. 2003). Similar characteristic peaks for SF and PVA were observed in all blended scaffolds (2:1, 3:1, and 4:1), which confirm the presence of both SF and PVA. The major peaks in the range of ~ 1630 and $\sim 1530\text{ cm}^{-1}$ in all blends confirmed the structural stability of β -sheet in the SF (Chen et al. 2001).

In Vitro Degradation Studies

An ideal scaffold must be biodegradable so that the natural extracellular matrix can replace it in the scaffold (Bhattacharjee et al. 2015). The crosslinking of these nanofibers improved the mechanical stability of the scaffolds and prevented it from fast degrading by hydrolysis when compared to non-crosslinked nanofibrous scaffold. However, within the crosslinked nanofibrous scaffold, the degradation rate was not statistically significant ($p > 0.05$). In vitro biodegradation studies were carried out in SBF for all scaffolds used in this study up to 35 days (Fig. 4). The nanofibrous scaffolds showed an initial sharp decline in weight (8–12 % weight loss) up to 7 days, followed by a slow dissolution rate. On further incubation, the weight of the scaffold reduced gradually ($\sim 16\text{--}20\%$) till the end of experiment. Bhattacharjee et al. (2015) also reported similar degradation profile of SF and PVA nanofibrous scaffolds in PBS up to 20 days. The biodegradation rate of SF–PVA blend mainly depends upon PVA concentration, as PVA has more –OH groups. In this study, PVA concentration was constant (10 %) in all samples that may be attributed to the similar degradation rate.

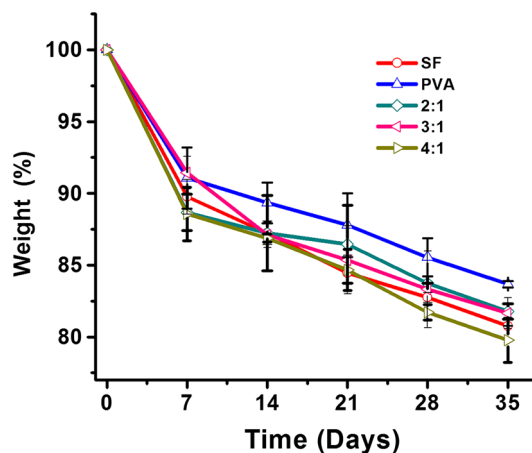


Fig. 4 Biodegradation studies of NF scaffolds in SBF

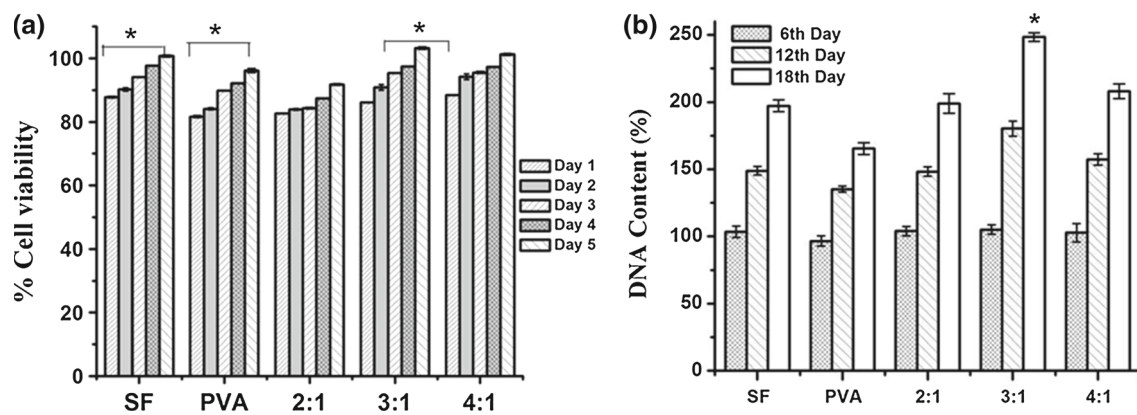


Fig. 5 **a** MTT assay. **b** DNA estimation of meniscus cells seeded on NF scaffolds

In Vitro Cell Attachment Studies

MTT assay was carried out to test the cytotoxicity of the scaffolds. From Fig. 5a, it is evident that all the scaffolds were non-toxic, supported cell growth, and showed increase in cell viability with days. This increase in viability is attributed toward the initial time taken by cells to adapt to the nature of scaffold followed by the increase in cellular proliferation (Gopinathan et al. 2015). Among the various blended scaffolds prepared, 3:1 SF-PVA showed the highest viability (103.2 ± 0.4 % at day 5), although there was no much difference among the scaffolds. Figure 5b shows the overall DNA content estimated during the cell culture experiment in vitro. DNA content increased significantly in all scaffolds as incubation days increased when compared to 6th day control. On 6th day, all scaffolds showed similar growth rate as reflected in the MTT assay. After 12 days, the DNA content increased up to 1.5-folds more in all scaffolds, which showed that the meniscus cells adapted to the new environment well. Among the different scaffolds used, 3:1 showed the highest DNA content, 2.5 times higher on 18th day than the control sample ($p < 0.05$). The increase in DNA content can be directly correlated with the amount of meniscal cells proliferated in the scaffolds which show the ability of the scaffolds to support the cells.

Biomaterial surfaces play a vital role in cell adhesion and proliferation which can be analyzed using cell adhesion studies in vitro (Schacht and Scheibel 2015). Hoechst staining is one of the simplest and efficient methods for visualizing attached cells on opaque scaffolds. Figure 6 shows the Hoechst-stained images of the human meniscal cells seeded on scaffolds up to 18th day. Human meniscal cells showed good cell attachment, proliferation, and growth in all nanofibrous scaffolds. Compared to the control SF and PVA, all blended

scaffolds showed higher cell attachment and proliferation. This may be due to the disintegrating or brittle nature of SF (Luangbudnark et al. 2012) and poor cell attachment property of PVA because of its high hydrophilic nature (Liu et al. 2009). Due to these problems of pure PVA and SF, researchers have blended them for obtaining better cell attachment (Li et al. 2001; Bhattacharjee et al. 2015; Li et al. 2015). Such blending not only increases cell adhesion and proliferation but also increases its mechanical properties. Among the blends, 3:1 SF-PVA showed the highest meniscal cell attachment and proliferation, which is evident from Fig. 6. The nanofibrous scaffolds were biocompatible and showed the presence of distinct nucleus on their surfaces indicating the attachment of viable cells. These results clearly indicate that 3:1 SF-PVA blend is a potential scaffold for meniscal tissue engineering when compared to other combinations used in this study. To our knowledge, this is the first study to prove that blending of SF with PVA at 3:1 ratio supports enhanced meniscal cell attachment and proliferation.

ECM Estimation (Collagen and GAG)

Figure 7 shows the collagen and GAG estimation for the cell-seeded scaffolds. ECM secretion can be directly correlated by estimating the collagen and GAG. The aliquots of medium taken from the plates were used for the estimation. The secretion of collagen and GAG was increasing throughout the study. 3:1 SF-PVA nanofibrous scaffold showed significantly higher collagen (9.27 ± 0.02 $\mu\text{g/ml}$) and GAG secretion (9.80 ± 0.04 $\mu\text{g/ml}$) when compared to all other blended and control scaffolds (Fig. 7a, b), supporting our earlier observations. The ECM secretion was higher than meniscal cells grown in raw eggshell membrane (Pillai et al. 2015a) and polycaprolactone nanofibers (Gopinathan et al. 2015).

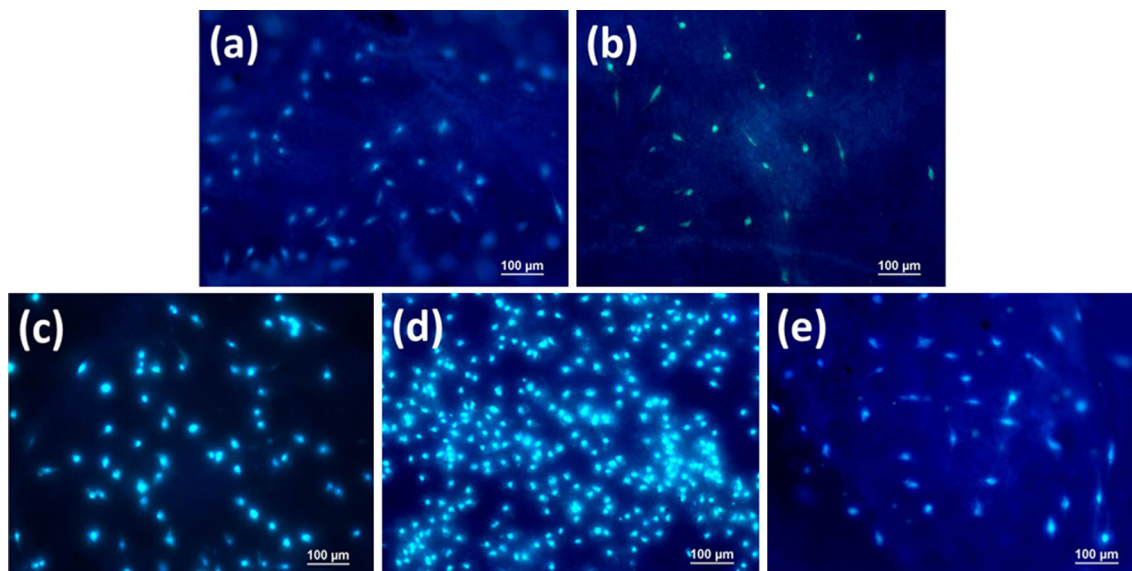
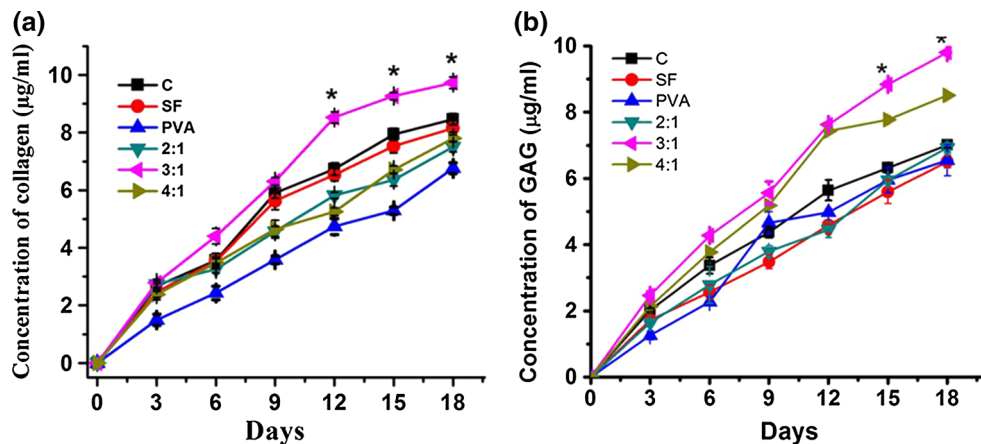


Fig. 6 Hoechst-stained images of **a** NF silk, **b** NF PVA, **c** 2:1 SF: PVA, **d** 3:1 SF: PVA, **e** 4:1 SF: PVA scaffolds

Fig. 7 **a** GAG and **b** collagen content estimation secreted by meniscus cells seeded on NF scaffolds



Conclusion

The present study evaluated the impact of blend ratios for increased primary human meniscal cell attachment and proliferation in SF-PVA-based scaffolds. The blend of SF fibroin and PVA showed uniform nanofibers which retained its structure even after crosslinking. Compared to all the other developed scaffolds, 3:1-blended scaffold supported enhanced cell attachment, growth, proliferation, and ECM (collagen and GAG) secretion. The attachment and proliferation rate of primary human meniscal cells can be further increased by incorporating appropriate biomolecules into the 3:1-blended SF-PVA scaffold. Such studies can be used to develop better scaffolds for meniscal tissue engineering in future.

Acknowledgments The authors like to express their deep gratitude to the management of PSG Institutions, Tamil Nadu, India and Tamil Nadu State Council for Science and Technology (TNSCST) for their

financial and other shapes of support to carry out this work. We appreciate the support, guidance, and contribution from Dr. P. Radhakrishnan, Director and Dr. T. Lazar Mathew, Advisor, PSG Institute of Advanced Studies, Dr. David V. Rajan, Ortho One Orthopaedic Speciality Centre, and Dr. Ramalingam, PSG IMS&R, Coimbatore.

Compliance with Ethical Standards

Conflict of Interest None of the authors have a conflict of interests including direct or indirect financial relations with any of the trademarks and companies mentioned in this paper.

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