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Polysaccharide nanofibrous scaffolds as a model for in vitro skin tissue regeneration

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Abstract Tissue engineering and nanotechnology have advanced a general strategy combining the cellular elements of living tissue with sophisticated functional biocomposites to produce living structures of sufficient size and function at a low cost for clinical relevance. Xylan, a natural polysaccharide was electrospun along with polyvinyl alcohol (PVA) to produce Xylan/PVA nanofibers for skin tissue engineering. The Xylan/PVA glutaraldehyde (Glu) vapor cross-linked nanofibers were characterized by SEM, FT-IR, tensile testing and water contact angle measurements to analyze the morphology, functional groups, mechanical properties and wettability of the fibers for skin tissue regeneration. The cell-biomaterial interactions were studied by culturing human foreskin fibroblasts on Xylan/PVA Glu vapor cross-linked and Xylan/PVA/Glu blend nanofibrous scaffolds. The observed results showed that the mechanical properties (72 %) and fibroblast proliferation significantly increased up to 23 % ($P < 0.05$) in 48 h Glu vapor cross-linked nanofibers compared to 24 h Glu vapor cross-linked Xylan/PVA nanofibers. The present study may prove that the natural biodegradable Xylan/PVA nanofibrous scaffolds have good potential for fibroblast adhesion, proliferation and cell matrix interactions relevant for skin tissue regeneration.

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1 Introduction

Skin is the largest organ in the body covering a surface area of about 1.8 m^2 and occupies 8 % of the total body mass of an adult. The functions are foremost as a barrier, preventing pathogens entering into the body and acts as a regulator for retention of water and heat loss. Dermal wound healing is a complex process necessitating coordination of several biological processes, including ingrowth of cells, organization of extracellular matrix (ECM), and rapid wound coverage to prevent infection [1]. Traditionally, autografts and allografts have been used to treat burns and other full thickness skin defects. Autograft transplantation has higher success rate but the difficulties are limited supply and may cause donor site morbidity. Allografts are abundant but always presented a risk of disease transmission and immunological rejection [2]. Tissue engineering emerged as a promising alternative to treat skin injuries and/or defects. Bioengineered skin substitute are not only replace the major physiological functions by providing rapid and reliable cover for wound defect but also easily applicable under routine use to reduce pain and discomfort of patients at the time of wound healing. Additionally, the biomaterials used for skin reconstruction should be biodegradable, repairable and also able to support the renewal of normal tissue with similar physical and mechanical properties of the skin it replaces. Bioengineered skin has a great advantage, if these substitutes are cost-effective, readily available, user-friendly and possess a long shelf life.

Electrospinning technology has tremendous potential to fabricate nonwoven nanofibrous scaffolds from biological and synthetic polymers for tissue engineering applications [3–6]. Synthetic polymers specifically engineered (both physically and chemically) to aid tissue regeneration and it is known that the surface microstructure and chemistry of

these engineered substrates can influence the ability of cells and tissues to attach, grow and function normally [7]. Natural collagen scaffolds works well for wound healing but processing and sterilization are essential to control infections for patients [8]. Moreover, most of the natural biopolymers are electrospun using organic solvents which may prove toxic for tissue engineering applications. Small amount of organic solvent may contain the scaffolds that could affect the cell growth and proliferation during cell culture. To avoid this problem, electrospinning of biocompatible polymers using aqueous solvents are highly desirable for cell culture and tissue engineering. Hemicelluloses are polysaccharides that occur together with cellulose in most plant tissues. The predominant hemicellulose present in hardwoods such as birch and aspen is an *O*-(4-*O*-methylglucurono) Xylan [9–11] and these are abundant in naturally occurring polysaccharides representing a large and cost-effective source of polymeric materials in tissue engineering applications [12]. In the present study, we have evaluated the efficacy of these biodegradable Xylan/PVA nanofiber matrices as dermal substitutes by seeding fibroblasts for skin tissue regeneration.

2 Materials and methods

2.1 Fabrication of nanofibrous scaffolds

Xylan (beech wood) and polyvinyl alcohol (PVA) (Mw 85,000 g/mol) were purchased from Sigma (USA). Xylan (15 wt%) was dissolved in 1 N NaOH solution at room temperature overnight and aqueous PVA solution (10 wt%) was prepared at 60 °C for 5 h. The Xylan/PVA electrospinning solution was prepared at room temperature with different weight percentage of Xylan content before electrospinning and stirred slowly for 30 min to avoid the bubble formation in the polymer solution. Xylan/PVA and Xylan/PVA-Glu blend polymer solution was loaded separately into a syringe (Becton–Dickinson, BD, NJ, USA) and a high voltage of 20 kV (DC high voltage power supply from Gamma High Voltage Research, Florida, USA) was applied to draw the fibers from the spinneret (27G1/2 needle) onto the collector plate (10 cm). The spinneret was first grounded to give a flat tip in order to produce continuous and uniform nanofibers. A constant feed rate of 0.75 ml/h was applied using a syringe pump (KD Scientific Inc., MA, USA) for the collection of nanofibers. Electrospun Xylan/PVA nanofibers were cross-linked with glutaraldehyde (Glu) vapors at 24, 48 h and then the fibers were cross-linked at 60 °C for 6 h. These nanofibers were subsequently dried under vacuum oven overnight for the characterization and cell culture studies of skin tissue engineering.

2.2 Material characterization

The surface morphology of electrospun nanofibrous scaffolds was studied under Scanning Electron Microscope (JEOL JSM-5600LV) at an accelerating voltage of 10 kV, after gold coating (JEOL JFC-1200 fine coater, Japan). For measuring the fiber diameter of electrospun fibers from the SEM images, $n = 10$ fibers were chosen at random on each of the scaffolds. The average fiber diameter was then calculated along with standard deviation (SD) using image analysis software (Image J, National Institutes of Health, USA). Functional groups present in the scaffolds were analyzed using fourier transform infrared (FTIR) spectroscopic analysis in Avatar 380, (Thermo Nicolet, Waltham, MA, USA) over a range of 400–4,000 cm^{-1} at a resolution of 2 cm^{-1}. The hydrophobic or hydrophilic nature of electrospun nanofibers was measured by sessile drop water contact angle measurement using video contact angle (VCA) optima surface analysis system (AST products, Billerica, MA). Tensile properties of nanofibrous membrane were measured at normal room temperature using an Instron 5845 Microtester (USA), at a cross-head speed of 5 mm/min. Rectangular specimens were cut from the electrospun membranes of 20–30 μm thickness and used for mechanical property studies. The rectangular specimens were mounted vertically on mechanical gripping units of the tensile tester and a load of 10 N was applied for tensile measurements.

2.3 Cell culture

The Xylan/PVA nanofibers collected on round glass cover slips of 15 mm diameter and then placed in a 24-well plate with a stainless steel ring to prevent lifting of nanofibers. The nanofibers were sterilized under ultraviolet (UV) light for 2 h, washed thrice with phosphate buffered saline (PBS) for 15 min each in order to remove any residual solvent and subsequently immersed in complete medium overnight before cell seeding. Human foreskin fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum (FBS) and 1 % antibiotics solution in a 75 cm^2 cell culture flask. Cells were incubated in CO_2 incubator at 37 °C at 5 % CO_2 and medium was changed twice every week. The confluent cultured cells were detached by adding 1 ml of 0.25 % trypsin containing 0.1 % EDTA. Detached cells were centrifuged, counted by trypan blue staining using hemocytometer and then the fibroblasts seeded on the sterilized scaffolds at a seeding density of 7,000 cells per well for skin tissue engineering.

2.4 Cell proliferation

The scaffolds were separated into Xylan/PVA (Xylan 1) 48 h cross-linked, Xylan/PVA (Xylan 2) 24 h cross-linked and Xylan/PVA/Glu blend (Xylan 3) nanofibrous scaffolds. Cell proliferation on these nanofibrous scaffolds was determined using the colorimetric MTS assay (CellTiter 96 Aqueous One solution, Promega, Madison, WI). The reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2(4sulfophenyl)-2H tetrazolium salt (MTS) to form purple formazan crystals by the dehydrogenase enzymes secreted by mitochondria of metabolically active cells formed on the basis of this assay. The formazan dye showed absorbance at 492 nm and the amount of formazan crystals formed was directly proportional to the number of cells. After 5 days of cell seeding, the media was removed from 24-well plate and the scaffolds were washed in PBS. The cell-scaffolds were then incubated in a 1:5 ratio mixture of MTS reagent and serum free DMEM medium for 3 h at 37 °C in 5 % CO_2 incubator. After the incubation period, samples were pipetted out into 96 well plates to read at 490 nm using a microplate reader (Fluostar Optima, BMG Lab Technologies, Germany).

2.5 Expression of CMFDA dye

Fluorescent dye expression was observed in the cultured fibroblasts using 5-chloromethylfluorescein diacetate (CMFDA), which on cleavage of its acetates by cytosolic esterases produces a brightly fluorescent CMFDA derivative. After 15 days of culture, the cell culture medium was removed followed by the addition of 180 µl of DMEM medium and 20 µl CMFDA (25 µM) to the cells and incubated at 37 °C for 2 h. The CMFDA medium was then replaced by the addition of complete medium and cells were incubated overnight in CO_2 incubator. Subsequently, the culture medium was removed and the cells were washed with PBS and after addition of serum-free medium observed under an inverted Leica DM IRB laser scanning microscope (Leica DC 300F) at 488 nm.

2.6 Sirius red staining for collagen

Sirius red staining was used for analyzing the presence of collagen in the cell matrix. It is a strong anionic dye whose sulfonic acid groups interact with the basic groups of collagen, staining into red. The cells were first fixed with 10 % formaldehyde, stained with Harris haematoxylin to distinguish the nucleus of cells and washed three times with deionized water. This was followed by 0.1 % sirius red F3B staining in a saturated aqueous solution of picric acid for 1 h. The cells were washed with mild acidified water followed by 100 % ethanol and viewed under a Leica BM IRB microscope. Collagen was stained red on a yellow background in the nanofibrous scaffolds.

2.7 Cell morphology

The fibroblast morphology was analysed using SEM after 15 days of seeding cells on the scaffolds. The media was removed from cell culture wells and the samples were fixed with 3 % Glu in PBS for 3 h. The scaffolds were then rinsed with distilled water for 15 min and then dehydrated with a series of ethanol gradients starting from 30, 50, 75, 90 and 100 % (v/v). Subsequently the samples were dried with hexamethyldisilazane (HMDS, sigma) solution and allowed to air-dry at room temperature in the fume hood. The samples were then gold coated to observe the fibroblast morphology using scanning electron microscope.

2.8 Statistical analysis

The data presented are expressed as mean ± standard deviation. Statistical analysis was done using student's *t* test and significant level of the data was obtained. $P \leq 0.05$ was considered to be statistically significant.

3 Results and discussion

3.1 Characterization of nanofibrous scaffolds

The chemical composition, physical structure and biologically functional moieties are all important attributes for fabricating scaffolds in skin tissue engineering. Principally, the scaffolds should be designed by mimicking the structure and biological functions of the native ECM. Figure 1 showed uniform beadless Xylan/PVA nanofibers produced by electrospinning after cross-linked with Glu vapors for 24, 48 h and fiber diameter was obtained around 745 ± 60 nm, 1090 ± 88 nm respectively. The Xylan/PVA/Glu blend nanofiber diameter of 465 ± 42 nm (Fig. 1c) and these fibers are not stable for long time cell culture in skin tissue engineering. Thus the fiber diameter has increased with increased degree of cross-linking density and time period of the nanofibrous scaffolds. The FT-IR spectra of Xylan, PVA and Xylan/PVA cross-linked nanofiber scaffolds were presented in Fig. 2. The broad region appears at 3,300–3,550 cm^{-1} corresponding to O–H vibration in Xylan molecule. The absorption at 1,651 cm^{-1} is principally associated with absorbed water. The prominent band at 1,042 cm^{-1} was attributed to C–O, C–C stretching or C–OH bending in hemicelluloses [13, 14]. The band at 1,093 cm^{-1} corresponds to C–OH bending strongly influenced by the degree of branching. PVA

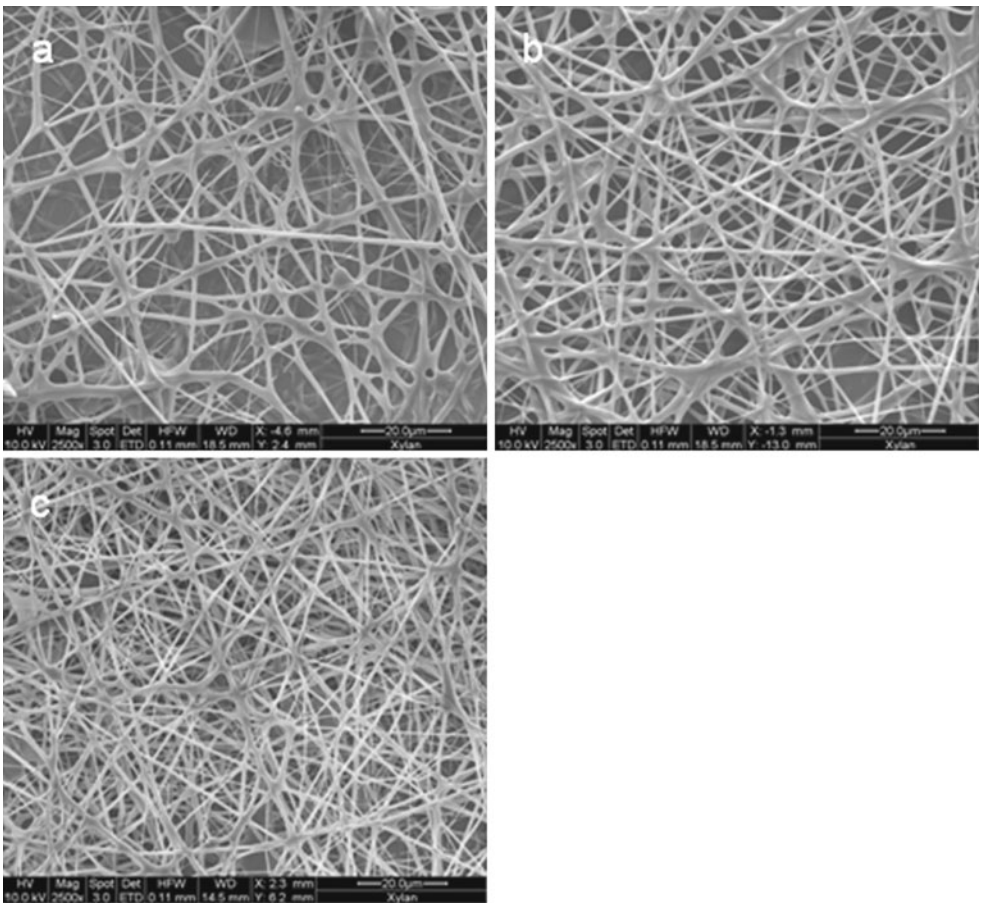

Fig. 1 Scanning electron microscopic images of Xylan/PVA nanofibers cross-linked with Glu vapors and Glu blend nanofibers. **a** Xylan/PVA (24 h Glu), **b** Xylan/PVA (48 h Glu), **c** Xylan/PVA (1 % Glu blend)

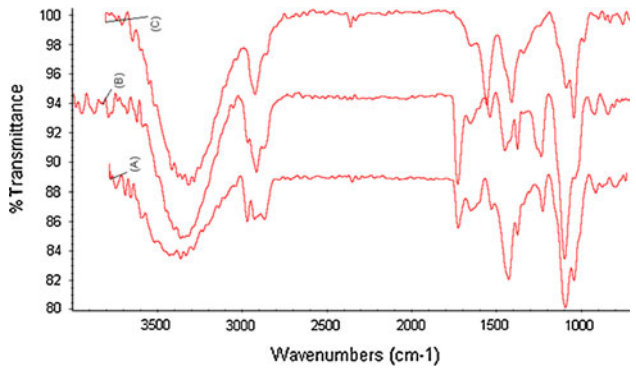

Fig. 2 FT-IR spectra of *a* Xylan, *b* PVA, and *c* Xylan/PVA cross-linked nanofibers

showed O–H corresponding peaks at 3,363 cm^{-1} (broad region) and the prominent peak at 1,096 cm^{-1} was attributed to C–O stretching. The peak observed at 1,728 cm^{-1} corresponds to the C = O stretching from the unhydrolyzed ester groups in the poly (vinyl acetate). After cross-

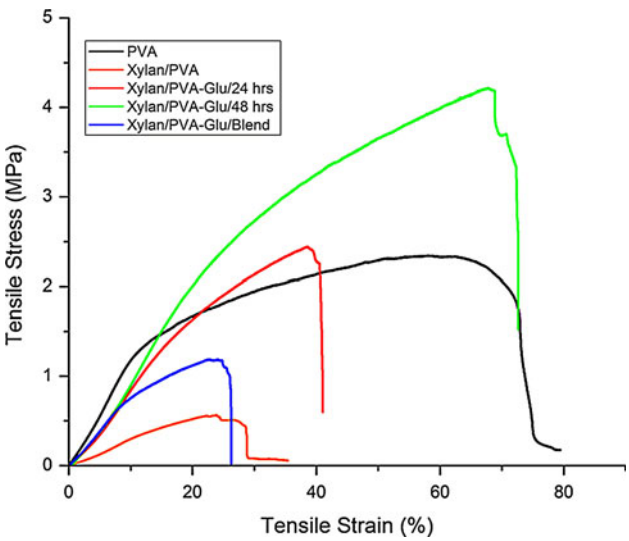

Fig. 3 Tensile properties of the electrospun Xylan/PVA nanofibrous scaffolds. The mechanical properties of 48 h Glu cross-linked Xylan/PVA nanofibers increased 72 % compared to 24 h Glu cross-linked fibers

Table 1 Tensile properties of electrospun nanofibrous scaffolds

Electrospun nanofibers	Tensile stress (MPa)	Tensile strain (%)	Young's modulus (MPa)
PVA	2.32	63.15	6.2
Xylan/PVA	0.54	24.14	1.7
Xylan/PVA/24 h Glu	2.44	38.74	7.00
Xylan/PVA/48 h Glu	4.21	68.02	9.18
Xylan/PVA/Glu blend	1.19	23.09	1.44

linking Xylan/PVA scaffolds, the intensity of OH groups from Xylan and PVA are reduced. At the same time, the samples were stable in cell culture medium demonstrating that the scaffolds are Glu cross-linked. The contact angle values of the scaffolds vary depending on the cross-linking density of nanofibers. Contact angle values for Glu vapor cross-linking at 24 and 48 h values were observed 47.2° for 24 h and 51.2° at 48 h, this is due to increased cross-linking density of the Glu. The Glu solution cross-linking only showed 18.5° due to low cross-linking density. This indicates that the vapor cross-linking gives more hydrophobic nature and stable scaffolds than solution cross-linking. The hydrophilic nature of the construct for skin tissue engineering is very important because the electrospun nanofibrous membrane should meet the requirements

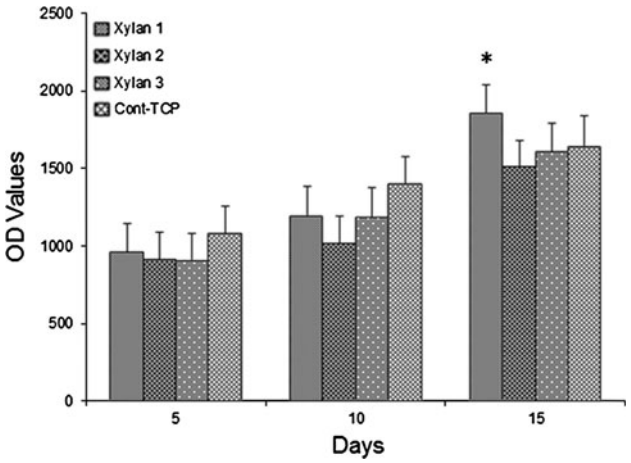

Fig. 4 Proliferation of fibroblast by MTS assay. Xylan 1 (48 h cross-linking), Xylan 2 (24 h cross-linking), Xylan 3 (Xylan/PVA/Glu blend) and TCP

such as higher gas permeation and protection of wound from infection and dehydration for scarless wound healing [15].

The tensile strength and Young's modulus of PVA, Xylan/PVA (24 h), Xylan/PVA (48 h), Xylan/PVA/Glu blend and Xylan/PVA noncross-linked nanofibers were shown in Fig. 3; Table 1. The mechanical stability of the

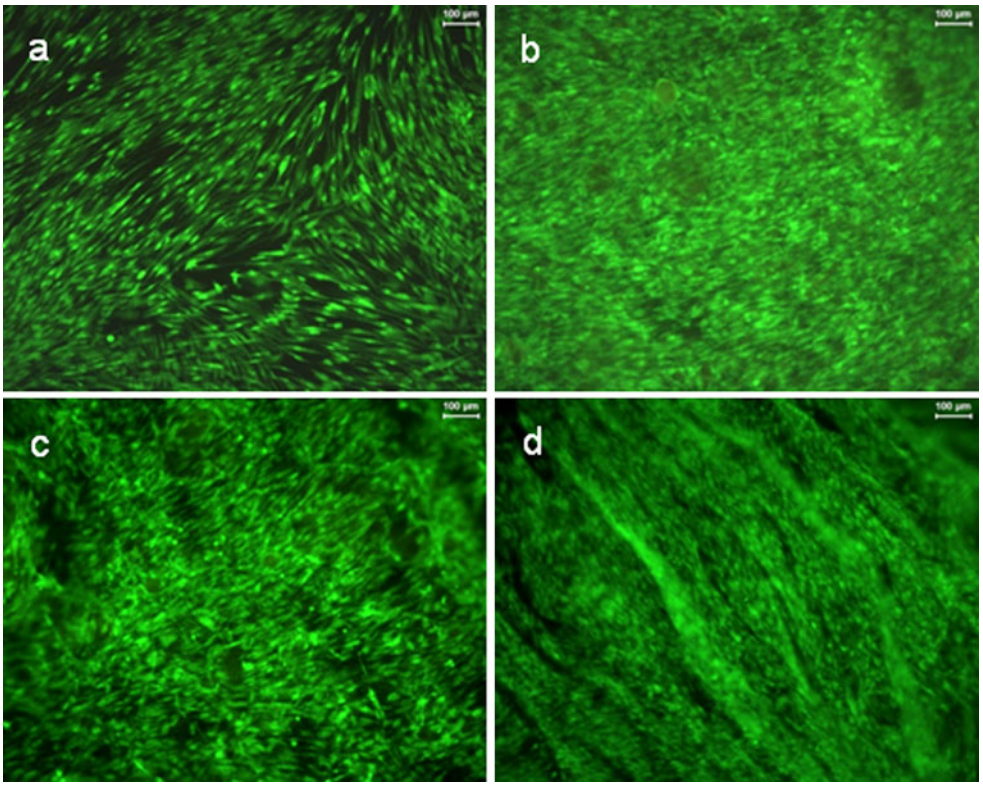

Fig. 5 Morphology of cultured fibroblasts on day 15 (CMFDA). **a** TCP (control), **b** Xylan/PVA (24 h Glu), **c** Xylan/PVA (48 h Glu), **d** Xylan/PVA/Glu blend nanofibers

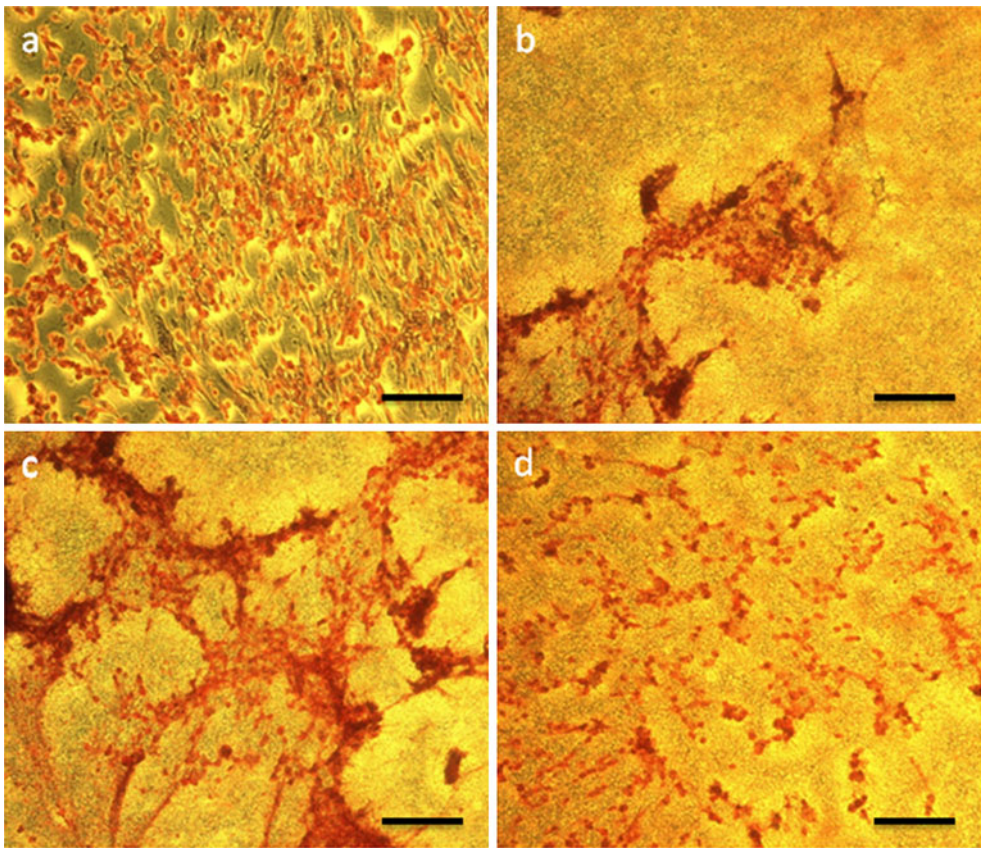

Fig. 6 Sirius red staining for collagen. **a** TCP (control), **b** Xylan/PVA (24 h Glu), **c** Xylan/PVA (48 h Glu), **d** Xylan/PVA/Glu blend nanofibers (*scale bar* 100 μm)

substrate plays an important role, as very high tensile strength may result in the substrate remaining on the wound bed for long time period after regeneration, thus obstructing new tissue development; while a weaker membrane may not support cell growth for the required time period of tissue regeneration. Figure 3 showed that the PVA uncross-linked sample had a Young's modulus of 6.2 MPa and Xylan/PVA noncross-linked sample had the least Young's modulus of 1.7 MPa. Xylan/PVA Glu vapor cross-linked (24 h) scaffold showing mechanical strength was lower (2.44 MPa) compared to 48 h cross-linked fibrous scaffold of 4.28 MPa and percentage of increase up to 72 %. Xylan/PVA noncross-linked nanofibers are not stable in in vitro cell culture and also the mechanical properties are significantly lesser than 48 h cross-linked nanofibers than 24 h cross-linked fibrous scaffolds. The elastic modulus showed better in 48 h vapor cross-linked scaffold 38.74 % compared to 24 h vapor cross-linked nanofibers of 68.02 %. Xylan/PVA 48 h Glu vapor cross-linked nanofibrous scaffolds providing stability for long term culture of fibroblasts for generating dermal substitute for wound healing.

3.2 Interaction of Xylan/PVA nanofibers and fibroblasts

Electrospun nanofibrous membranes showed good and immediate adherence to wet wound surface and the membrane attains uniform adherence to the wound surface without any fluid accumulation [16, 17]. Nanofibrous wound dressing may achieve the controlled evaporative water loss, excellent oxygen permeability and promote fluid drainage ability due to the porosity and inherent property of Xylan/PVA nanofibrous membrane. Electrospinning of water soluble Xylan/PVA nanofibers cross-linked with Glu provides a stable nanofibrous membrane for the adhesion and proliferation of fibroblast and secretion of ECM for normal wound healing. The rate of epithelialization increases and the dermal fibroblasts were well organized in electrospun nanofibrous membrane and thereby provide a good support for wound healing.

Xylan is a natural polysaccharide showing good wettablity for the adhesion and proliferation of fibroblast and secretion of ECM may initiate the wound bed for the migration of keratinocytes and fibroblast for normal

scarless wound healing. The wound healing process was characterized predominantly by fibroblast proliferation and ECM synthesis and remodeling into a highly organized architecture [18]. The results showed that the fibroblast proliferation significantly ($P < 0.05$) increased up to 23 % in Xylan 1 compared to Xylan 2 and 3 nanofibrous scaffolds (Fig. 4). Surface roughness of these Xylan/PVA nanofibrous scaffolds was desirable for better cell attachment, growth and proliferation and also enhanced by the presence of functional groups and surface hydrophilicity. The cell morphology was also observed by CMFDA dye on day 15 in all three samples of the Xylan/PVA nanofibrous scaffolds. Xylan 1 showing better cell morphology and even distribution of fibroblasts on the nanofibrous scaffolds (Fig. 5b). The observed results showed that the Xylan 2 and 3 samples are not stable after 15 days in fibroblast culture (Fig. 5b, d). Xylan/PVA (48 h) served as a potential nanofibrous scaffolds for fibroblast proliferation and interactions with the fibers to form a dermal substitute for skin tissue regeneration.

The secretion of collagen proved that the electrospun nanofibrous scaffolds have good potential for wound healing through skin tissue regeneration. It was noticed that the secretion of collagen was predominant in Xylan/PVA (48 h; Fig. 6c) scaffolds compared to TCP (Fig. 6a) and other nanofibrous scaffolds (Fig. 6b, d). Similarly the cell-biomaterial interactions are also analyzed using SEM as shown in Fig. 7. The cultured cells on nanofibers (Fig. 7b, c, d) were found to show better interactions with the *milieu* compared to TCP (Fig. 7a). Xylan/PVA 24 h and 48 h Glu vapor cross-linked nanofibrous scaffolds, cells were found to migrate within the nanofibers and communicate with adjacent cells via extension of filopodia. Usually fibroblast adhesion and cytoskeletal reorganization occur within few hours of cell culture and cells are normally expected to start proliferation within 24 h and secrete collagen to act as a surface active agent and demonstrates its ability to penetrate lipid free interface. During granulation tissue formation, fibronectin provides a temporary substratum for migration and proliferation of

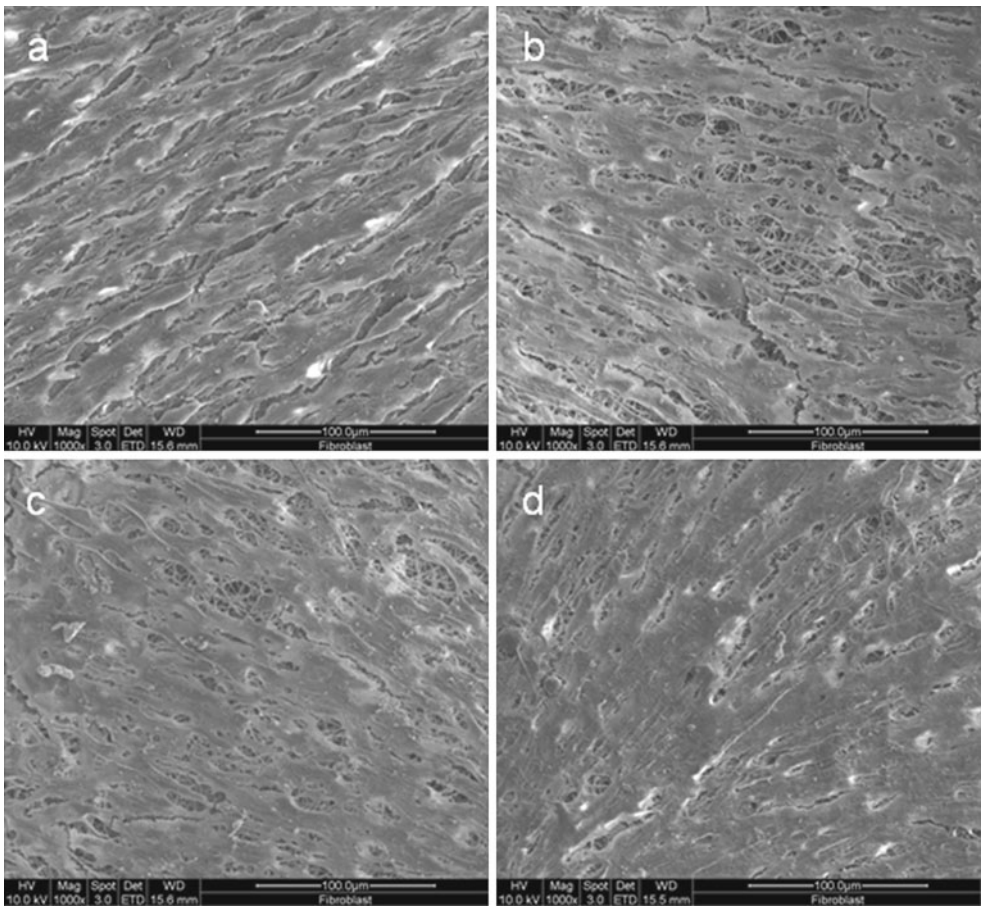

Fig. 7 Morphology of fibroblasts in SEM. **a** TCP (control), **b** Xylan/PVA (24 h Glu), **c** Xylan/PVA (48 h Glu), **d** Xylan/PVA/Glu blend nanofibers

cells and provides a template for collagen deposition, which increases stiffness and tensile strength of healing tissues [19]. The fibroblasts showing normal morphology and also confluent on Xylan 1 nanofibers within the period (15 days) compared to all other scaffolds (Figs. 5, 7). This is because of the desirable porous structure and suitable mechanical properties of these scaffolds which guide cells entering into the substrate by their amoeboid movement [20].

Allogeneic skin substitute composed of both keratinocytes and fibroblast would be an ideal tissue engineering strategy but the manufacturing cost is very high. The objectives of fabricating Xylan/PVA nanofibers were used as a cost effective natural biomaterial to fabricate allogenic dermal substitute for wound healing. During the earliest stages of wound healing, activated dermal fibroblasts migrate from the wound edges to physically close the gaps between wound margins. Furthermore, the cell migration into the fibrous mat and deposition of ECM throughout the scaffold would be beneficial for normal dermal wound healing. Dermal fibroblasts play a key role in skin extracellular protein turnover, ECM interaction, cell–cell communication, etc. [21], which are closely related functions. Our results showed robust interaction between cells and Xylan/PVA nanofibrous scaffolds suggesting that they could be used for treating chronic or trauma wounds for normal skin tissue regeneration.

4 Conclusions

Currently available tissue engineered products for skin substitution including dermal and epidermal constructs, although not perfect; occupy a specific niche within a complex approach to treat full-thickness extensive burns, improving patient's survival rates and quality of life after injury. Tissue engineering and nanotechnology have advanced a general strategy combining the cellular elements of living tissue with sophisticated functional composites to produce living structures of sufficient size and function to improve patient's life. These carriers need to be relatively inexpensive to manufacture on a large scale, which would facilitate their commercial clinical application. Xylan/PVA nanofibrous substrates provided better fibroblasts adhesion, proliferation and favorable cell-biomaterial interactions for preparing allogenic dermal substitute for skin tissue regeneration. Fabrication of such natural nanofiber based wound care products are able to demonstrate that their products are more cost effective and may provide better outcome to serve people.

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References

1. Parenteau-Bareil R, Gauvin R, Berthod F. Comparative study of bovine, porcine and avian collagen for the production of a tissue engineered dermis. Acta Biomater. 2010;7(10):3757–65.
2. Supp DM, Boyce ST. Engineered skin substitute. Clin Dermatol. 2005;23:403–12.
3. Venugopal J, Zhang YZ, Ramakrishna S. In vitro culture of human dermal fibroblasts on electrospun polycaprolactone collagen nanofibrous membrane. Artif Organs. 2006;30:438–44.
4. Chandrasekaran AR, Venugopal J, Sundarrajan S, Ramakrishna S. Fabrication of a nanofibrous scaffold with improved bioactivity for culture of human dermal fibroblasts for skin regeneration. Biomed Mater. 2011;6:015001.
5. Ravichandran R, Liao S, Ng CCH, Chan CK, Raghunath M, Ramakrishna S. Effects of nanotopography on stem cell phenotypes. World J Stem Cells. 2009;1(1):55–66.
6. Ravichandran R, Venugopal JR, Sundarrajan S, Mukherjee S, Ramakrishna S. Poly(glycerol sebacate)/gelatin core/shell fibrous structure for regeneration of myocardial infarction. Tissue Eng Part A. 2010;17(9–10):1363–73.
7. Cima LG, Vacanti JP, Vacanti C, Ingber D, Mooney D, Langer R. Tissue engineering by cell transplantation using degradable polymer substrates. J Biomech Eng. 1991;113(2):143–51.
8. MacNeil S. Biomaterials for tissue engineering of skin. Mater Today. 2008;11(5):26.
9. Dinand E, Vignon MR. Isolation and NMR characterization of a (4-*O*-methyl-D-glucorono)-D-xylan from sugar beet pulp. Carbohyd Res. 2001;330:285–8.
10. Habibi Y, Mahrouz M, Vignon MR. Isolation and structure of D-xylans from pericarp seeds of opuntia ficusindica prickly pear fruits. Carbohyd Res. 2002;337:1593–8.
11. Ebringerova A, Alfoldi J, Hromadkova Z, Pavlov GM, Harding SE. Water soluble *p*-carboxybenzylated beechwood 4-*O*-methylglucuronoxylan: structural features and properties. Carbohyd Polym. 2000;42:123–31.
12. Jeong L, Yeo IS, Kim HN, Yoon YI, Jang DH, Jung SY, Min BM, Park WH. Plasma-treated silk fibroin nanofibers for skin regeneration. Int J Biol Macromol. 2009;44:222–8.
13. Kacurakova M, Capek P, Sasinková V, Wellner N, Ebringerova A. FT-IR study of plant cell wall model compounds: pectic polysaccharides and hemicelluloses. Carbohyd Polym. 2000;43:195–203.
14. Unlu CH, Günister E, Atici O. Synthesis and characterization of NaMt biocomposites with corn cob xylan in aqueous media. Carbohyd Polym. 2009;76:585–92.
15. Venugopal J, Ma LL, Ramakrishna S. Biocompatible nanofiber materials for the engineering dermal substitute for skin regeneration. Tissue Eng. 2005;11:847–54.
16. Khil MS, Cha DI, Kim IS, Bhattarai N. Electrospun nanofibrous polyurethane membrane as wound dressing. J Biomed Mater Res. 2003;67B:675–9.
17. Bhattarai SR, Bhattarai N, Yi HK, Hwang PH, Cha DI, Kim HY. Novel biodegradable electrospun membrane: scaffold for tissue engineering. Biomaterials. 2004;25:2595–602.
18. Barnes MJ, Morton LF, Bennett RC, Bailey AJ, Sims TJ. Presence of type III collagen in guinea pig dermal scar. Biochem J. 1976;157:263–6.

19. Yannas IV, Burke JF, Orgill DP, Skrabut EM. Wound tissue can utilize a polymeric template to synthesize a functional extension of skin. Science. 1982;215:174–6.
20. Venugopal JR, Low S, Choon AT, Kumar AB, Ramakrishna S. Nanobioengineered electrospun composite nanofibres and osteoblasts for bone regeneration. Artif Organs. 2008;32:388–97.
21. Lerman OZ, Galiano RD, Armour M, Levine JP, Gurtner GC. Cellular dysfunction in the diabetic fibroblast: impairment in migration, vascular endothelial growth factor production, and response to hypoxia. Am J Pathol. 2003;162:303–12.