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REVIEW ARTICLE

Engineering Porosity in Electrospun Nanofiber Sheets by Laser Engraving: A Strategy to Fabricate 3D Scaffolds for Bone Graft Applications

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Abstract | Nanofiber features in a scaffold provide favorable niche for cellular attachment, proliferation, and differentiation propelling their interest in tissue engineering. However, the inability of seeded cells to infiltrate inside 3D structures of electrospun nanofibers has remained a persistent bottleneck for their greater applicability. In the present work, an approach to address this problem is presented. Macro-pores are designed in common graphic software created by a laser-engraving machine on electrospun nanofiber sheets composed of a bioinspired material-*N*-methylene phosphonic chitosan for facilitating cellular infiltration into 3D scaffold. Effect of laser pulse energy and pulse per inch on pore morphology are investigated and FTIR spectrum is examined to preclude the degradation of material due to laser-engraving process. Furthermore, the micro-fabricated nanofiber sheets with multi-scalar porosity are rolled up to form a 3D scaffold as graft through biomimetic approach for bone-tissue engineering applications. Culture of MG-63 cells on rolled up nanofiber sheets containing laser-engraved macro-porous 3D scaffolds demonstrated no cytotoxicity induced by the scaffolds from MTT assay, while cellular migration into the sheets was evident from scanning electron microscopy. It is concluded that combined micro-fabrication-rolling approach may be simple, rapid way to design 3D bone grafts based on 2D electrospun nanofiber sheet of natural/semi-synthetic polymers for better osteoconductivity.

Keywords: *Electrospinning, 3D scaffold, Macro-pores, Laser engraving, Rolling, Bone scaffold, MG-63*

1 Introduction

An ever-growing number of bone diseases and injuries have placed high demand on bone grafts and substitutes which is not completely met by autograft or allograft procedures. On the other hand, synthetic bone grafts currently in clinical use have not been able to match osseointegration performance of autografts. This has driven an interest to design grafts inspired from the architecture, composition, and function of natural bone. One such important structural aspect considered for the designing of scaffolds is the multi scale nano- and micro-level hierarchical arrangement of body tissue. Nano-features of scaffolds mimicking extracellular matrix properties of native bone are ascribed to increase cellular adhesion as processes[1]. On the other hand, the presence of macro-pores is considered essential for the infiltration of cells inside 3D structures and facilitates efficient transport of nutrients and metabolites[2]. However, engineering scaffolds with these multi-scalar features are still a critical challenge for successful application in bone-tissue engineering.

Amongst various nano-scale scaffold fabrication techniques, electrospinning has shown

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considerable promise for bone-tissue engineering[3]. Electrospun scaffolds have been shown to increase the adherence of osteoblasts as well as increase their differentiation and mineralization of the anchorage-dependent cells[4]. Furthermore, electrospinning process is applicable to processing of polymer and ceramic materials which mimic the viscoelastic and osteoconductive nature of natural bone extracellular matrix[3]. Nevertheless, clinical success of electrospun nanofibers is limited by inadequately smaller nano-scale pore size which vastly restricts their ability to allow cell infiltration (or osteoconduction)[5, 6]. In this manner, they essentially represent a 2D fiber sheet compared to a 3D scaffold essential in tissue engineering. Thus, several authors have documented their efforts to increase pore size in electrospun nanofibers by employing modified or post-electrospinning techniques. Some to achieve this objective ranged from porogen leaching techniques (porogen can be a salt or sacrificial polymer)[7], cryogenic electrospinning[8], ultra-sonication[9], phase separation with non-solvent[10], or hybridizing of electrospinning with 3D-printing techniques[11]. However, most of these methods are controlled by thermodynamic parameters and allow limited control over time of fabrication or to obtain scaffolds of designed pore sizes. Some other techniques used have been mechanical expansion to obtain pore size of 206 μm, showing positive bone healing in vivo[12]. Li et al. recently employed a needle punching technique to improve cellular infiltration in nanofiber membranes[13], whereas Pal et al. demonstrated the feasibility of using emulsion electrospinning to fabricate nano–micro fibrous architecture scaffolds[14]. Recently, there has been a spurt of interest in generating pores, arrays, and 3D scaffolds based on structuring of nanofiber mats by laser ablation or irradiation[15–19]. However, these studies have described the culture of cells on the pore-patterned surfaces, but have not produced any strategy for translating the porous network into a 3D scaffold for bone-tissue engineering. The advantages of employing laser for the purpose include quick fabrication, stability of structure in unirradiated regions, and control over design and parameters. In this work, we present an approach that rolling up of the patterned electrospun membranes can be a facile strategy to obtain 3D bone scaffolds. To the best of our knowledge, the feasibility of this method for a natural polymer-based nanofiber scaffold and its biocompatibility has not been yet reported.

In the present work, a simple method to design nanofiber scaffolds with engineered macro-pores using a laser-engraving machine is presented. Nanofibers are based on *N*-methylene phosphonic chitosan, a chitosan derivative known to possess high osteoconductive and osteoinductive potential[20, 21]. Corel draw application was used for laser-engraving machine to create patterns on nanofiber sheets. Effect of laser process on scaffold properties was investigated by means of FTIR, mechanical, and cell-culture studies. Furthermore, we propose a roll-up approach after the laser micro-fabricated nanofiber sheets (i.e., after the fabrication of holes in the nanofibers) to generate 3D electrospun nanofiber grafts, which can be used for future implantation. Roll-up approach for generating scaffolds is also gaining some interest, though it has not been extensively applied to obtain bone grafts based on nano/micro-fabricated scaffolds[22]. The whole process of obtaining macro-pores containing electrospun nanofibers as schematically represented in Fig. 1 is simple, rapid, and amenable for processing large number of scaffolds at any given time.

2 Materials and Methods

2.1 Synthesis of Polymer

1 g chitosan (Marine Chemicals, Cochin, India, Grade > 90% DA) was dissolved in 30 ml 0.5 M acetic acid and one part by weight formaldehyde and phosphorous acid were added to reaction mixture, as reported in the literature[23]. Reaction was carried out at 70 °C for 14 h. Product was precipitated in ethanol, washed several times to remove unreacted molecules, and dried in vacuum for 3 days. Presence of phosphorous in product was ascertained by means of elemental analysis by an EDX spectrometer (Inca PentaFET-3, Oxford Instruments, UK).

2.2 Electrospinning of NMPC

A conventional electrospinning setup was used to fabricate nanofiber sheets of NMPC. For this purpose, 80 mg/ml NMPC dissolved in deionized water (conductivity 18 MΩ) was blended with equal volume of same concentration polyvinyl alcohol (Goshenol 17R, NiponGhosei, Singapore) solution to obtain a final solution with 8% w/v total polymer content. Blend solution was stirred for 3 days for thorough mixing. This solution was then fed into a 2.5-ml syringe equipped with blunt needle and placed on a syringe pump (KD Scientific, USA). A copper ground wrapped with aluminum foil located at a distance of 8 cm from needle tip was used as collector. For electrospinning, flow rate used was 5 μl/min and an applied voltage of 2.5 kV/cm. After electrospinning,

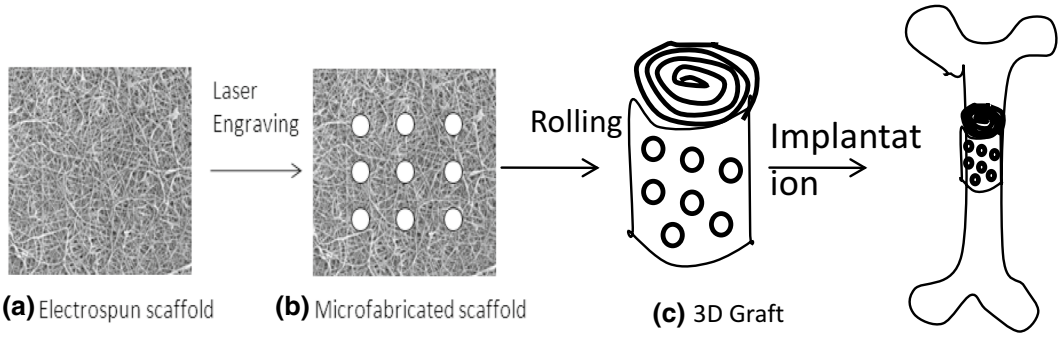

Figure 1: Schematic representation of the process of designing bone grafts based on **a**, **b** laser micro-fabrication of electrospun nanofiber scaffolds and **c**, **d** subsequent roll up for implantation.

aluminum foil was unwrapped and the fibrous sheet deposited was peeled off and immersed in a solution of 0.1 M HCl–50 mM glutaraldehyde acetone for crosslinking of the mats. Sheets were observed under scanning electron microscope (EVO 100, Carl Zeiss, Germany) after coating with gold. Thickness of sheets was measured by digital micrometer (Mitutoyo Corporation, Japan) at five random points.

2.3 Laser Micro-fabrication on Electrospun 2D Sheet

Electrospun nanofiber sheets were placed onto a Universal Laser-Engraving Machine VLS2.30 (Universal Corporation, USA) which utilizes a CO_2 laser for micro-fabrication. Designs for through and through cutting of holes in the sheets were fed to the laser engraver by Corel Draw Software. A High-Density Power Focusing Optics (HDPFO) lens was used for executing the process. Designing was carried out by CorelDraw software and macro-porosity of 300 μm diameter designed as circles were placed at inter-pore distance of 500 μm. Laser engraving was carried out at varied power from 3 to 6 Watts, 50–150 pulse per inch, and scan speed parameter of 15%. After micro-fabrication, electrospun sheets were inspected under Olympus Stereozoom microscope.

2.4 FTIR Analysis

FTIR spectra of NMPC powder, crosslinked nanofiber sheet (NF), and laser micro-fabricated nanofibers (LMNF) were acquired by a GX spectrophotometer (Perkin Elmer, USA) equipped with ZnSe horizontal ATR attachment at scan resolution of 4 cm^{-1}. 24 scans were acquired for each sample and analyzed by Spectrum software.

2.5 Cell-Culture Study

Biocompatibility and/or leaching of any toxic products during process of laser engraving were evaluated by means of cell-culture study with human osteoblast such as MG-63 cells. MG-63 cells are convenient to handle that cell lines are supposed to behave like normal osteoblast garnering popularity for testing of biomaterial scaffolds. Detailed culture conditions of cells can be found in our previous work[21]. NF and LMNF were sterilized in 70% ethanol, rinsed with PBS, and soaked in medium overnight. Cells were seeded at concentration of 3×10^4 cells/cm^2 of scaffold in 24-well tissue culture plates. Cell proliferation was assessed by MTT assay following procedure reported in the literature for pores containing electrospun scaffolds. After 7 days of incubation, scaffolds were removed from media, washed with PBS to remove all unadhered cells, fixed in glutaraldehyde solution for 4 h followed by gradient ethanol drying, and observed under SEM.

2.6 Rolling Up of Laser Micro-fabricated Electrospun Nanofiber Sheet (RLMNF)

The nanofiber sheets after micro-fabrication were rolled up and fixed by acrylate glue. RLMNF were then subjected to cell seeding at the same density as mentioned above. Micrographs were acquired by an Olympus Stereozoom microscope, while SEM pictures were captured after gold coating by EVO 100 SEM.

2.7 Statistical Analysis

Statistical significance in different study groups was determined with one-way analysis of variance (ANOVA) test. The statistically significant value set was at $p < 0.05$ and $p < 0.01$.

3 Results and Discussion

3.1 Fabrication of Electrospun Nanofibers of NMPC

Phosphorylated derivatives of chitosan are reported to possess many properties suitable for bone regeneration. These include high gene delivery efficacy, amphotericity, osteoinduction, and metal chelating capacity leading to biomineralization. Effects of phosphate grafting of chitosan on cell response are observed in soluble form as well as in other physical forms such as surface phosphorylation of membranes, hydrogels, and bio-composite rods[24–26]. Keeping in view these results, NMPC was selected as the material for the fabrication of LMNF scaffolds. Laser fabrication on electrospun scaffolds made of natural polymers has not received much attention, although they received some interest with polycaprolactone. The modification of chitosan to introduce phosphate pendant groups was performed as per literature reports available. In the method, formaldehyde was added to act as a coupling agent between primary amine groups of chitosan and phosphonic moieties furnished by phosphorous acid. After 14 h of reaction, elemental composition of the product was found to be C (27.1%), N (6.79%), O (57.87%), and P (8.24%), which confirmed grafting of phosphorous onto chitosan backbone. Further chemical confirmation of product was offered by FTIR analysis which is discussed later. Interestingly, chemical modification yielded a derivative soluble in water, and thus, precluded use of strongly acidic solvents otherwise needed for electrospinning of chitosan.

Fabrication of nanofiber scaffold by electrospinning technique is dependent upon polymer physicochemical properties and polymer–solvent interactions. As such chitosan is difficult to electrospin, ascribed to strong intermolecular forces of attraction in the polymer chain. This entails the use of solvents such as strong acetic acid or trifluoroacteic acid to prepare chitosan nanofibers. However, phosphorylated derivative of chitosan was soluble in water, and hence, electrospinning was attempted from aqueous NMPC solutions. NMPC solutions demonstrated more of a splashing phenomenon under applied electric field and the as-spun deposits had appearance of nanoparticles. This necessitated blending of NMPC with equal concentration of polyvinyl alcohol as an electrospinning aid for the fabrication process. As reported in previous work, PVA was found to effectively decrease the solution conductivity while increasing chain entanglements but without affecting the viscosity of the solutions, thereby facilitating nanofiber formation[23].

Process parameters for electrospinning were then optimized to 5 µl/min flow rate, 8 cm collector distance, and 20 kV voltage. To yield stability to nanofiber mats, crosslinking was performed and unreacted glutaraldehyde was washed off. Electrospun nanofiber sheets so produced were 0.15 mm in thickness with unimodal fiber diameters of 200 nm counted from 5 frames of 100 points each by Image J software. These sheets were used for laser micro-fabrication.

3.2 Engineering Macro-pores in Electrospun Sheets by Laser Engraving

To design macro-pores in nanofiber sheets, Corel Draw was used to create a frame containing spheres of 300 µm diameter with inter-object distance of 500 µm and printed with Universal Laser Engraver VLS 3.0. During the process of conversion of design fed into the UCP software to fabricated scaffold, two process parameters—pulse energy and pulse per inch—were found to play a crucial role in determining the architectural characteristics of the pores created by laser irradiation.

Essentially, the effect of laser irradiation on nanofiber sheets was a burning-cum-evaporation of polymer materials at coordinates as designated by designing software. However, the accuracy of pore size and shape is dependent upon the resolution of printing process which in turn is governed by minimum spot size of laser-focusing lens, speed of laser engraving, pulses per inch, and pulse energy. In this study, we took an HPDFO lens with a minimum spot size of 75 µm to explore the feasibility of laser-beam-enabled printing process for creating pores in nanofiber matrix. Second, the speed of laser printing was kept constant at 15% of the instrument value. Thereafter, as shown in Fig. 2, the sequential process of engraving was elucidated by gradual increment in laser power. Initially, at low power values (5 W), it can be seen that polymer burn off was incomplete in the designated area and structures created on fibrous sheets were disjointed spots. As the laser power was increased to 10 W, heating up combined by start of vaporization process resulted in the appearance of a blown up structure, though complete etching did not occur even at this power. This indicated that laser fabrication process for NMPC-based nanofibers involved both photochemical and photothermal processes[27]. Upon further increasing the pulse

energy, an increase in polymer burn out was evident as more area of electrospun scaffolds was found to be now etched out and presented better edge retention. Apart from pulse energy, dimensions of created pores by laser irradiation were also found to be dependent upon pulses per inch parameter (Fig. 3). There existed a direct correlation between the number of pulses emitted and smoothness of surface of the created pores. In the study, it was seen that at 50 pulses per inch by machine, holes created were not exactly spherical, but had a club-shaped appearance, i.e., pores with very high indentation depths. Then, as pulse per inch increased; pores exhibited more serrated structures with edges of grooves now showing more indentation with less indent depth. At the optimized conditions, micropores with smooth edges were created. In addition, SEM images were acquired to ascertain changes in morphology or possible damage to nanofiber structure in the immediate vicinity of laser irradiation.

3.3 FTIR Analysis After Laser Micro-fabrication

FTIR analysis was performed on electrospun nanofiber sheet before (NF) and after micro-fabrication (LMNF) and is depicted in Fig. 4. Spectrum of NF exhibited a broad absorbance in the range 3600–3000 cm^{-1} due to –O–H and –N–H_2-stretching vibrations, and methylene stretches at 2917 cm^{-1} and at 1729 cm^{-1} attributed to acetoxy groups of PVA. Characteristic vibrations of *N*-methylene phosphonic chitosan were detected at 1643 cm^{-1} (secondary amine), 1535 cm^{-1} (amide bonds in chitosan), and phosphate vibrations contributing to signals at 1249 (–P=O), 933 and 849 cm^{-1} (–P–O–H) [23, 28]. The spectrum matched the frequencies associated with this compound reported previously in many studies. Inspection of the spectrum of electrospun sheet after micro-fabrication demonstrated that all the major peaks' assignments made in case of sheets before electrospinning could be found for LMNF also. For example, absorbance at 3600–3000 cm^{-1}, 2917 cm^{-1}, 1729 cm^{-1} , and 1643 cm^{-1} was found similar to that obtained for NF as also phosphate frequencies at 1243 cm^{-1}, 942 cm^{-1}, and 849 cm^{-1}. Small shifts were observed for amide vibration at 1535 cm^{-1} from 1545 cm^{-1} after laser irradiation and 1089 cm^{-1} vibration frequencies. These small shifts may be attributed to some photochemical changes through short pulse interaction of laser focused on polymer matrices induced in the surrounding

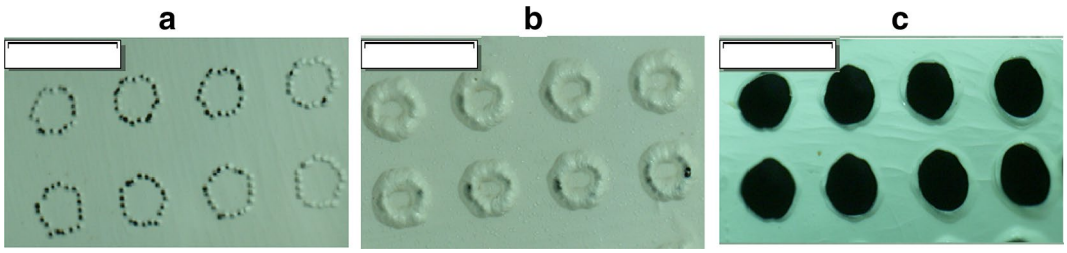

Figure 2: Effect of laser irradiation on macro-pores created in electrospun scaffolds under different pulse energies of **a** 1.5 W, **b** 3 W, and **c** 6 W at 150 pulse per inch. Scale bar denotes 800 μm.

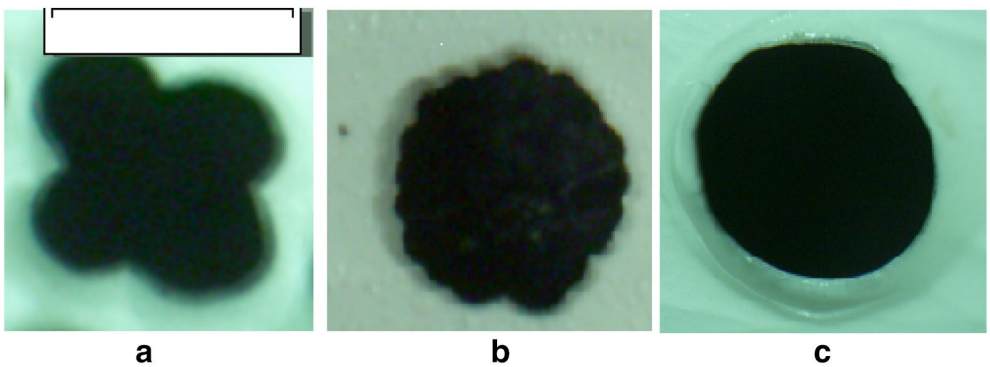

Figure 3: Effect of laser irradiation on morphology of macro-pores created in electrospun scaffolds by varying pulse per inches from **a** 50, **b** 100, and **c** 150 at 6 W energy. The scale bar denotes 350 μm.

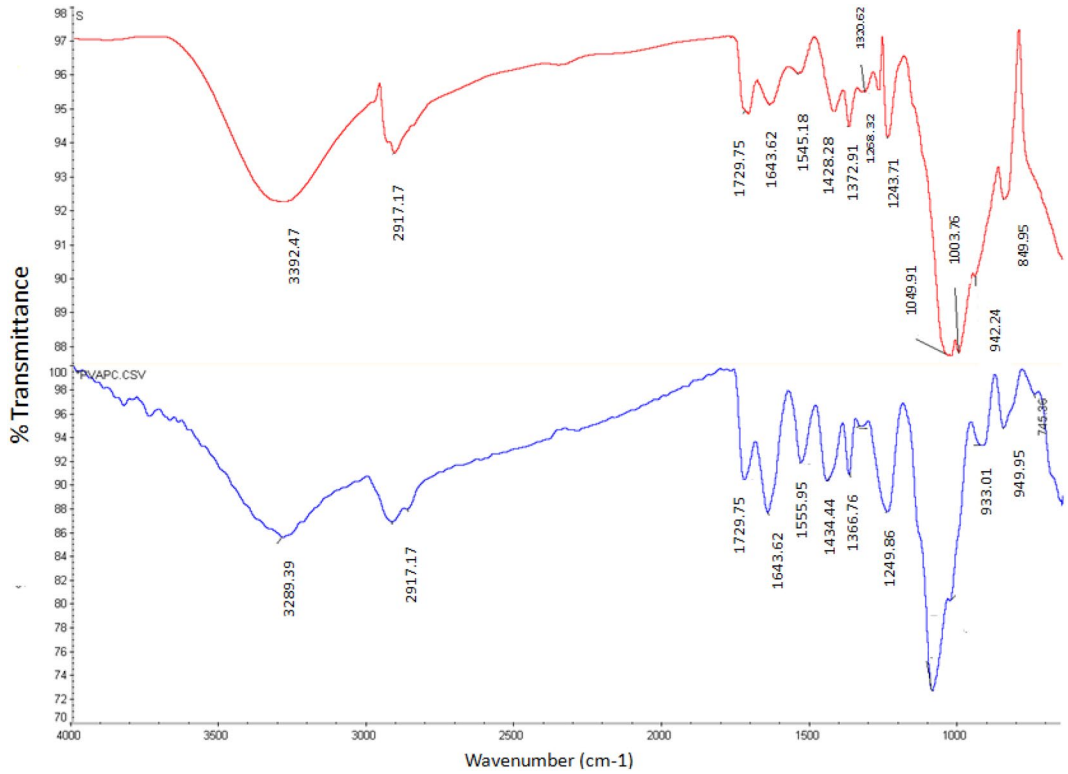

Figure 4: FTIR spectrum of electrospun nanofiber scaffolds of *N*-methylene phosphonic chitosan/polyvinyl alcohol before and after laser micro-fabrication.

areas due to heat diffusion. However, the importance of FTIR experiment lies in demonstrating that laser irradiation does not result in any significant degradation product in the left out scaffold as any new peaks or disappearance of any present characteristic peaks was not observed. It also points out to the accuracy of the laser-engraving process, wherein selective spots/areas of the scaffold predetermined by the software were only removed without any chemical destruction of the surrounding scaffold.

3.4 Cell Culture on Electrospunnanofibers, Micro-fabricated Nanofibers, and Rolled Micro-fabricated Nanofibers

To appreciate the importance of micro-fabrication-cum-rolling process for electrospun fibers, SEM microscopic images of cells cultured on a typical electrospun nanofiber sheet without any micro-fabricated pores for 8 h and after 5 days are represented in Fig. 5a, b. These micrographs manifest cells can interact with a nanofiber matrix through extension of processes similar in dimensions to fibers resulting in cell–matrix adherence. However, it is also evident that nano-scale porosity in fiber matrix is vastly smaller to size of individual cells (~10 to 30 μm) to enable cellular infiltration inside the sheets. This essentially makes NF a 2D matrix on which cells proliferate and form a cell-fiber sheet in two dimensions with limited or no penetration in vertical direction, as shown in Fig. 5b.

MTT assay was carried out to evaluate the biocompatibility of LMNF (Fig. 6). Both NF and LMNF were cultured with MG-63 cells for 7 days and metabolic activity of cells monitored on days 1, 3, and 7 to determine the effect of micro-fabrication on electrospun nanofiber scaffolds. As shown in Fig. 6, the optical absorbance values on NF were 0.834 ± 0.069, 1.53 ± 0.147, and 2.06 ± 0.211 on days 1, 3, and 7, respectively. On laser micro-fabricated scaffolds, readings recorded were 0.621 ± 0.027, 0.956 ± 0.055, and 1.62 ± 0.11 for the same days. As the absorbance value significantly increased ($p < 0.05$) from days 1 to 7, it can be said that both the matrices are not cytotoxic and support cell proliferation. In comparison with LMNF, NF exhibited significantly ($p < 0.05$) higher absorbance value. This may be due to the reduced solid surface area caused

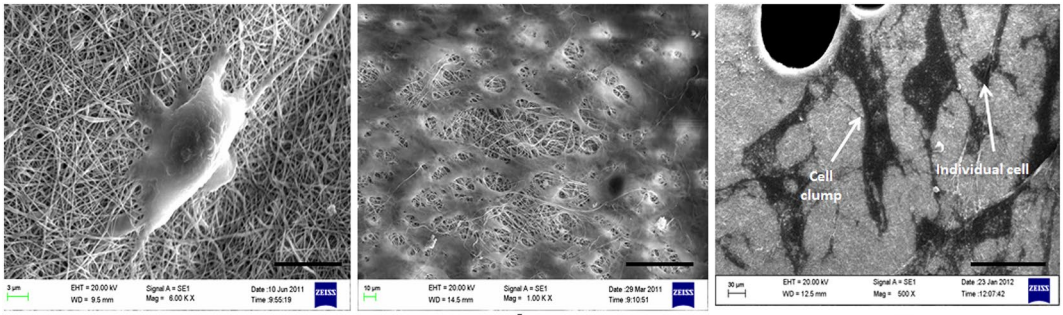

Figure 5: SEM images of **a** single cell view of MG-63 cells adhering to a typical electrospun nanofiber matrix of NMPC illustrating the cellular processes (p) extended by cells to interact with matrix and difference in scales of pores in nanofibrous matrix (h) and individual cell size limiting cell infiltration inside surface of matrix (scale bar represents 9 μm), **b** MG-63 cells typically forming a two-dimensional cell sheath on nanofiber matrix after 7 days of culture (scale bar represents 45 μm), and **c** cell images acquired at edges of macro-pores created on electrospun matrix by laser micro-fabrication on nanofiber sheets showing cytocompatibility of process (scale bar represents 120 μm).

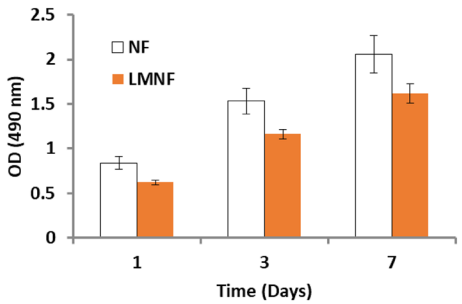

Figure 6: MTT assay to evaluate biocompatibility of laser-engraving process on different days of MG-63 cell culture.

due to reduction in nanofiber mass after micro-fabrication. Obviously, areas which were burnt out due to laser irradiation were not available for cell attachment. However, it can also be seen that a reduction in the difference of optical absorbance was observed between NF and LMNF on day 7 compared to day 3 which may be attributed to gradual ingrowth of cells inside the matrix of LMNF compared to NF. MTT assay thus demonstrated that laser micro-fabrication process did not create any cytotoxic surface for cells to attach or proliferate and may thus be considered a biocompatible process. Images of cells seeded on LMNF surfaces were acquired at the edges of laser-fabricated macro-pores on day 3 that also showed MG-63 cells to grow at the edge area providing further evidence of the cytocompatibility, as displayed in Fig. 5c.

3.5 Rolling Up of Electrospun Sheets to Obtain 3D Graft

Figure 7 displays the scaffolds formed after rolling up of electrospun nanofiber sheets micro-fabricated by laser-engraving machine. The micropores present throughout the depth of rolled up sheet is expected to provide channels for cellular infiltration inside of 3D nanofiber scaffolds. To test the applicability of the concept for the purpose, MG-63 cells were seeded on the upper surface of an RLMNF scaffold, and after 7 days of incubation, cells were examined by SEM on a cross section of the scaffold. A top view of such cross section is represented in Fig. 7b, while Fig. 7c shows the edges of the cross section just after fabrication without any cell incubation. In addition, Fig. 7d shows the florescent image of cells cultured on the 3D nanofibrous scaffold obtained in a semi-confocal apotome microsope. The presence of cell nuclei in association with cytoskeletal fibers along the length of the scaffolds provides further evidence of the potential of the method to fabricate 3D nanofibrous scaffolds from 2D sheets. This also indicates that the creation of pores can be a viable strategy for increasing cell infiltration in electrospun nanofiber meshes, while roll-up approach can be promising for electrospinning as well bioprinting [29].

3.6 Summary

In summary, the present study illustrates the applicability of a process for obtaining bone grafts by laser micro-fabrication on electrospun nanofibers and subsequent rolling up of sheets. As demonstrated, cell ingrowth is increased

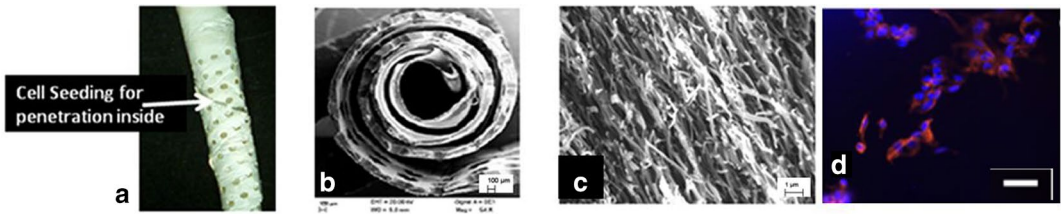

Figure 7: Digital view of **a** rolled up laser micro-fabricated nanofiber scaffold based on phosphorylated chitosan derivative (scale bar represents 100 μm), **b** cross-sectional scanning electron microscopic image of the scaffold, **c** SEM image of the laser cut edges in electrospun scaffolds (scale bar represents 1 μm), **d** apotome florescent image of MG-63 cells cultured on electrospun nanofibers (scale bar represents 60 μm).

through the created pores into the inside layers. Though it is a very preliminary study and further in-depth optimization would be required to establish this approach, the initial results show the potential of the method to generate 3D structures based on 2D electrospun sheets.

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References

1. Rampichová M, Košťáková Kuželová E, Filová E, Chvojka J, Šafka J, Pelcl M, Daňková J, Prosecká E, Buzgo M, Plencner M, Lukáš D, Amler E (2018) Composite 3D printed scaffold with structured electrospun nanofibers promotes chondrocyte adhesion and infiltration. Cell Adhes Migr 12:271–285. https://doi.org/10.1080/19336918.2017.1385713
2. Wu J, Hong Y (2016) Enhancing cell infiltration of electrospun fibrous scaffolds in tissue regeneration. Bioact Mater 1:56–64. https://doi.org/10.1016/j.bioactmat.2016.07.001
3. Arslan A, Çakmak S, Gümüşderelioğlu M (2018) Enhanced osteogenic activity with boron-doped nanohydroxyapatite-loaded poly(butylene adipate-co-terephthalate) fibrous 3D matrix. Artif Cells Nanomed Biotechnol. https://doi.org/10.1080/21691401.2018.1470522
4. Whited BM, Whitney JR, Hofmann MC, Xu Y, Rylander MN (2011) Pre-osteoblast infiltration and differentiation in highly porous apatite-coated PLLA electrospun scaffolds. Biomaterials 32:2294–2304. https://doi.org/10.1016/j.biomaterials.2010.12.003
5. Gao J, Luo J, Xiong J (2017) A facile method for tailoring the three-dimensional porous nanofibrous scaffolds by the dual electrode electrospinning. Mater Lett 209:384–387. https://doi.org/10.1016/j.matlet.2017.08.032
6. Zhong S, Zhang Y, Lim CT (2011) Fabrication of large pores in electrospun nanofibrous scaffolds for cellular infiltration: a review. Tissue Eng Part B Rev 18:77–87. https://doi.org/10.1089/ten.teb.2011.0390
7. Wright LD, Andric T, Freeman JW (2011) Utilizing NaCl to increase the porosity of electrospun materials. Mater Sci Eng, C 31:30–36. https://doi.org/10.1016/j.msec.2010.02.001
8. Leong MF, Chan WY, Chian KS, Rasheed MZ, Anderson JM (2010) Fabrication and in vitro and in vivo cell infiltration study of a bilayered cryogenic electrospun poly(D, L-lactide) scaffold. J Biomed Mater Res, Part A 94:1141–1149. https://doi.org/10.1002/jbm.a.32795
9. Alsberg E, Bae MS, Lee JB, Kim CH, Heo DN, Yang DH, Kwon IK, Jeong SI (2011) Highly porous electrospun nanofibers enhanced by ultrasonication for improved cellular infiltration. Tissue Eng Part A 17:2695–2702. https://doi.org/10.1089/ten.tea.2010.0709
10. Yang H, Wang L, Xiang C, Li L (2018) Electrospun porous PLLA and poly(LLA-: Co -CL) fibers by phase separation. New J Chem 42:5102–5108. https://doi.org/10.1039/c7nj04970f
11. Sooriyaarachchi D, Minière HJ, Maharubin S, Tan GZ (2019) Hybrid additive microfabrication scaffold incorporated with highly aligned nanofibers for musculoskeletal tissues. Tissue Eng Regen Med 16:29–38. https://doi.org/10.1007/s13770-018-0169-z
12. Lee SJ, Park YJ, Shim IK, Park WH, Seol YJ, Kim KH, Jung MR (2010) Novel three-dimensional scaffolds of poly(L-lactic acid) microfibers using electrospinning and mechanical expansion: Fabrication and bone regeneration. J Biomed Mater Res Part B Appl Biomater. 95:150–160. https://doi.org/10.1002/jbm.b.31695
13. Li H, Ding Q, Chen X, Huang C, Jin X, Ke Q (2019) A facile method for fabricating nano/microfibrous three-dimensional scaffold with hierarchically porous to enhance cell infiltration. J Appl Polym Sci 136:1–8. https://doi.org/10.1002/app.47046

14. Pal P, Srivas PK, Dadhich P, Das B, Maulik D, Dhara S (2017) Nano-/microfibrous cotton-wool-like 3D scaffold with core-shell architecture by emulsion electrospinning for skin tissue regeneration. ACS Biomater Sci Eng 3:3563–3575. https://doi.org/10.1021/acsbiomaterials.7b00681
15. Johnson JK, Woon Choi H, Nam J, Farson DF, Lannutti J (2007) Structuring electrospun polycaprolactone nanofiber tissue scaffolds by femtosecond laser ablation. J Laser Appl 19:225–231. https://doi.org/10.2351/1.2795749
16. Chiussi S, Cordero D, Rebollar E, León B, Neves NM, Martins A, Reis RL (2010) Improvement of electrospun polymer fiber meshes pore size by femtosecond laser irradiation. Appl Surf Sci 257:4091–4095. https://doi.org/10.1016/j.apsusc.2010.12.002
17. Jenness NJ, Wu Y, Clark RL (2012) Fabrication of three-dimensional electrospun microstructures using phase modulated femtosecond laser pulses. Mater Lett 66:360–363. https://doi.org/10.1016/j.matlet.2011.09.015
18. Wu Y, Vorobyev AY, Clark RL, Guo C (2011) Femtosecond laser machining of electrospun membranes. Appl Surf Sci 257:2432–2435. https://doi.org/10.1016/j.apsusc.2010.09.111
19. Lim YC, Wu Y, Choi HW, Lannutti JJ, Fei Z, Farson DF, Johnson J, Lee LJ (2010) Micropatterning and characterization of electrospun poly(ε-caprolactone)/gelatin nanofiber tissue scaffolds by femtosecond laser ablation for tissue engineering applications. Biotechnol Bioeng 108:116–126. https://doi.org/10.1002/bit.22914
20. Datta P, Thakur G, Chatterjee J, Dhara S (2014) Biofunctional phosphorylated chitosan hydrogels prepared above pH 6 and effect of crosslinkers on gel properties towards biomedical applications. Soft Mater. 12:27–35. https://doi.org/10.1080/1539445X.2012.735315
21. Datta P, Ghosh P, Ghosh K, Maity P, Samanta SK, Ghosh SK, Das Mohapatra PK, Chatterjee J, Dhara S (2013) In vitro ALP and osteocalcin gene expression analysis and in vivo biocompatibility of n-methylene phosphonic chitosan nanofibers for bone regeneration. J Biomed Nanotechnol 9:870–879. https://doi.org/10.1166/jbn.2013.1592
22. Liu Y, Li X, Qu X, Zhu L, He J, Zhao Q, Wu W, Li D (2012) The fabrication and cell culture of three-dimensional rolled scaffolds with complex micro-architectures. Biofabrication. https://doi.org/10.1088/1758-5082/4/1/015004
23. Datta P, Dhara S, Chatterjee J (2012) Hydrogels and electrospun nanofibrous scaffolds of N-methylene phosphonic chitosan as bioinspired osteoconductive materials for bone grafting. Carbohydr Polym 87:1354–1362. https://doi.org/10.1016/j.carbpol.2011.09.023
24. Kapat K, Rameshbabu AP, Maity PP, Mandal A, Bankoti K, Dutta J, Das DK, Dey G, Mandal M, Dhara S (2019) Osteochondral defects healing using extracellular matrix mimetic phosphate/sulfate decorated GAGs-agarose gel and quantitative micro-ct evaluation. ACS Biomater Sci Eng 5:149–164. https://doi.org/10.1021/acsbiomaterials.8b00253
25. Mao C, Pan H, Shao C, Chen C, Gu T, Tang R, Zheng B, Gu X, Sun J (2019) Phosphorylated chitosan to promote biomimetic mineralization of type I collagen as a strategy for dentin repair and bone tissue engineering. New J Chem 43:2002–2010. https://doi.org/10.1039/c8nj04889d
26. Zhang G, Lau CPY, He K, Wang XH, Kumta SM, Zheng LZ, Qin L, Tang T, Patrick Y, Xie XH, Wang XL (2011) Effect of water-soluble P-chitosan and S-chitosan on human primary osteoblasts and giant cell tumor of bone stromal cells. Biomed Mater 6:015004. https://doi.org/10.1088/1748-6041/6/1/015004
27. Aguilar CA, Lu Y, Mao S, Chen S (2005) Direct micropatterning of biodegradable polymers using ultraviolet and femtosecond lasers. Biomaterials 26:7642–7649. https://doi.org/10.1016/j.biomaterials.2005.04.053
28. Lebouc F, Dez I, Madec PJ (2005) NMR study of the phosphonomethylation reaction on chitosan. Polymer (Guildf). 46:319–325. https://doi.org/10.1016/j.polymer.2004.11.017
29. Shulunov V (2019) A novel roll porous scaffold 3D bioprinting technology. Bioprinting. 13:e00042. https://doi.org/10.1016/j.bprint.2019.e00042

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