THE COMBINATION OF 3D PRINTING AND NANOFIBERS FOR TISSUE ENGINEERING OF ARTICULAR CARTILAGE

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Abstract

This study presents a combination of 3D print and nanofibers. The aim of the work is to create a scaffold for tissue engineering of articular cartilage. The combination mentioned provides a suitable synergic effect using specific characteristics supplied by the employed technologies. The load-bearing part was made by 3D print. This part provides necessary mechanical qualities and imparts the shape to the scaffold. The integrated and modified nanofiber layers with a specific pattern then provide a space for the adhesion and proliferation of the applied cells.

The study is divided into several basic parts. In the introduction, the most frequently employed methods for the fabrication of the scaffolds are described. The next chapter is dedicated to the materials and production technologies used in the course of experiment. In the technological part, there is a description of the creation of 3D structure and its subsequent testing. The results obtained are summed up in the conclusion. On the basis of these results, the combination of the applied technology is compared with other methods.

Keywords: 3D print, needleless electrospinning, tissue engineering, articular cartilage, cell cultivation

1. INTRODUCTION

The main purpose of articular cartilage is to provide a smooth, near frictionless articulating surface and act as a mediator for load transfer to the underlying bone. But, the regenerative capacity of damaged articular cartilage was to remain limited compared with other tissues such as bone and or muscle. Regardless of the various causes include joint disease to global health problems. Joint pain and loss of mobility resulting from damage to articular cartilage and associated secondary effects are among the most common causes of impairment in middle-aged and elderly populations. An important issue in tissue regeneration and repair is the fabrication of biodegradable three-dimensional scaffolds such that they mimic the extracellular matrix sufficiently closely to encourage cells to grow functional tissues and allow the diffusion of nutrients, metabolites and soluble factors. Scaffolds must also have suitable mechanical properties, which should retain during the process [1]. But the optimal scaffolds combinating of good mechanical properties and good adhesion, migration and cell proliferation is not suitable still.

To date, rapid prototyping technologies with layer-by-layer construction provide a very powerful tool to fabricate intricate 3D scaffold or cell/tissue constructs with precisely controlled macro- and micro-features. The technique boasts high reproducibility, with well-defined pore size and shape, and can fabricate structures with a high degree of pore interconnectivity. However, pore sizes created by rapid prototyping are very large for cell seeding. In addition, rapid prototyping cannot create nanoscale features such as those present in natural extracellular matrices. Therefore, finding a reliable method is necessary by which to control and modify, the microstructure of porous scaffolds used in tissue engineering applications. [2][3] To improve the cell adhesion/attachment, we used electrospinning method and nanofiber layer to improve cell adhesion and proliferation (Fig. 1).

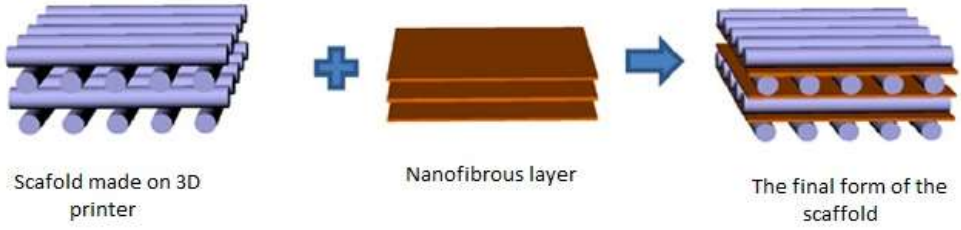

Fig. 1 Schematic view of making a scaffold [2]

2. MATERIALS AND METHODS

2.1 Materials

Biodegradable poly-ε-caprolactone (PCL) with an average molecular weight of 45,000 [Mn] was purchased from Sigma-Aldrich Inc. This polymer dissolved in chloroform/ethanol mixture (9:1 by weight) using a magnetic stirrer for 24 hours. And the polymer solution with 16 wt% concentration was used for the production of nanofibers. The same polymer was also used in 3D printers as the melt for printing.

2.2 Methods

Electrospinning: Electrospinning is an electro-hydrodynamic process is a versatile and promising platform technology for the production of nanofibrous materials consisting of diverse polymers and polymer composites. This platform process can provide bio or synthetic based polymer nanofibrous materials for the fabrication of innovative biomedical devices and for the fabrication of new technical applications [4].

Rapid prototyping: Rapid prototyping technology is a completely automatic system taking CAD data directly and producing 3D physical prototypes in only a few minutes or hours with high accuracy. Accurate and functional parts can be produced with no operator intervention. Rapid prototyping is widely used across a number of industry sectors from design, manufacturing, medical architecture and even art due to the competitive advantage provides [2].

In this study was used a special patterned nanofibrous layer that was produced by usage of Nanospider™ Production Line NS 1WS500U made by Elmarco. This is the smallest nanofiber production equipment that delivers sufficient output for small volume manufacturing. Another important part was special patterned wire collector (described in [5]), which caused the specific distribution of the fibers in the layer for good cell proliferation (Fig. 2).

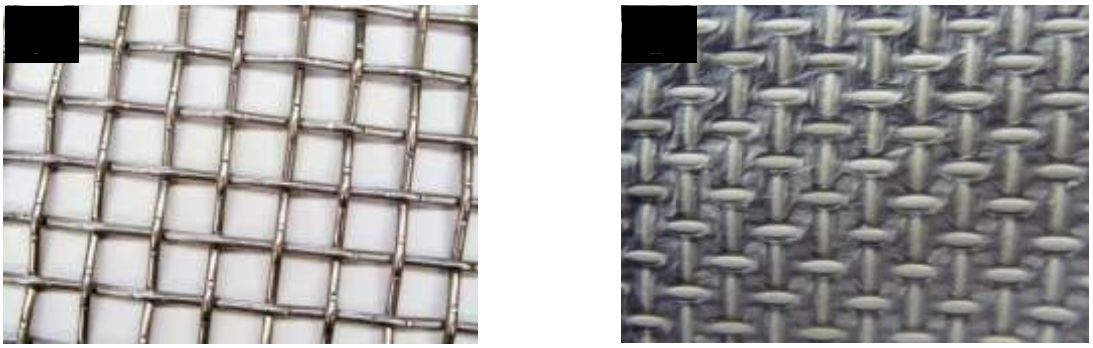

Fig. 2 A photo of the special wire collector (a) used for production of patterned nanofibrous layer (b)

The perpendicular laid structure produced by 3D printing, which ensuring the shape and necessary mechanical properties was realized by 3D printer produced by the team members especially for this application (Fig. 3).

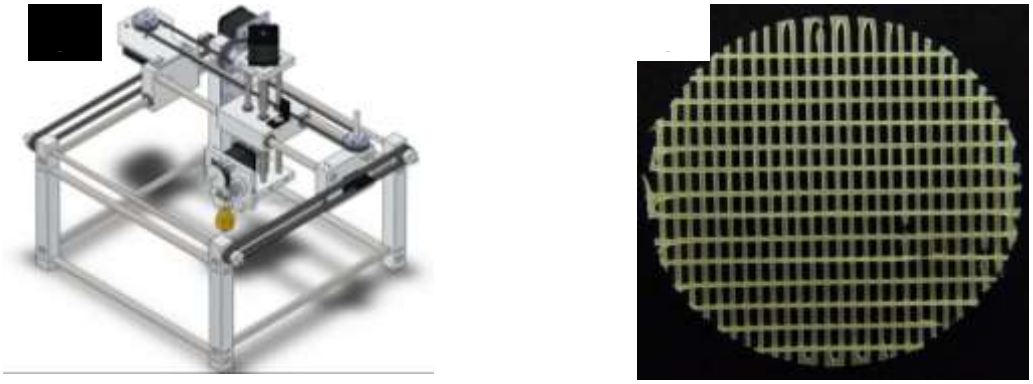

Fig. 3 A photo of the special 3D printer (a) used for production of perpendicular laid structure

The final vision of the scaffold was created by gluing layers of nanofibrous layer and printed parts. Three nanofibrous patterned layers and the four 3D printed layers were bonded by fourty spots of poly-ε-caprolactone solution (30 wt%) uniformly disturbed onto 3D printed layer facing the nanofibers (Fig. 4).

Fig. 4 A photo of the special 3D printed layer (a), special patterned nanofibrous layer (b) and final scaffold – diameter 16mm, thickness 4mm (c)

Dry scaffolds were characterized in term of morphology. Samples were sputter-coated by gold and then observed by a scanning microscopy (SEM, Tescan Vega 3SB Easy Probe). Samples were also tested in biological laboratory to confirm their non-toxicity and the observation of cell adhesion and proliferation.

In-vitro testing: For biological testing were used rabbit chondrocytes. Concentration of cells seeded in particular wells was $1*10^5$ in serum-free medium (EMEM). The samples were maintained in special incubator at 37°C, 5% CO_2. Medium was changed three times a week. Cell viability and proliferation was measured by MTT assay and microscopy analysis (SEM and fluorescence).

MTT assay for the cell proliferation: Cell viability and proliferation was measured by MTT assay at day 1, 3, 7, 14, 21 and 28 after cell seeding. A 250 µl of MTT solution and 750 µl of EMEM was added to all samples and was incubated for three hours at 37°C, 5% CO_2. The formazane crystals were dissolved in acidic isopropyl alcohol and the absorbance was measured at 570 nm with the reference at 650 nm.

Microscopy analysis (SEM and fluorescence): After 1, 3, 7 and 14 days of cell seeding all scaffolds were processed for microscopy analysis. All samples were washed with PBS prior to fixation to remove unattached cells. Scaffolds for SEM and fluorescent microscopy were fixed by 2.5% glutaraldehyde and

dehydrated with upgrading concentration of ethanol (60%, 70%, 80%, 90%, 95% and 100%). After drying, samples were sputter-coated by gold and then observed by scanning electron microscope (SEM, Tescan VEGA3 SB easy probe). For fluorescent microscopy, samples were washed with PBS after fixation and incubated for 15 minutes with propidium iodide in the dark at room temperature. After incubation period, samples were washed with PBS and analysed by fluorescent microscope (NICON Eclipse Ti-E).

Figure 4 shows SEM images of the scaffolds created with 3D printing microfibers and nanofibrous layer before performing the biological testing.

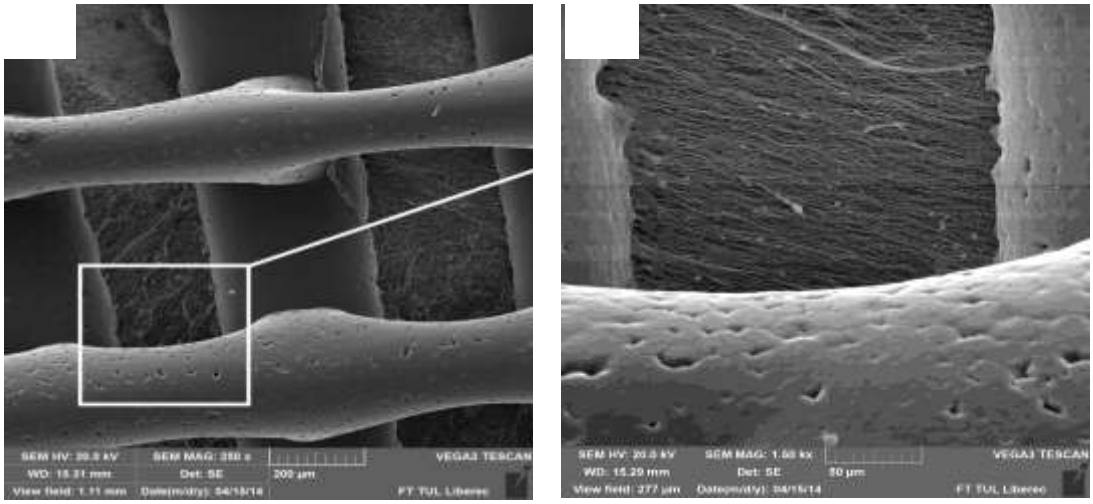

Fig. 4 SEM image of top view onto scaffold combinating 3D printing and patterned nanofibrous layers (a) and detail the marked area (b)

3. RESULTS AND DISCUSSION

Biological testing confirmed that the selected polymer and production method are suitable for the fabrication of this scaffolds. Results from scanning electron microscope and fluorescent microscope showed that the scaffolds support the cell adhesion, proliferation and very good viability. The images of both microscopies proving this claim are in Fig. 5 and 6. The images were taken fourteenth day of testing. The subsequent separation of individual layers of scaffold confirmed the cell migration into complete scaffold.

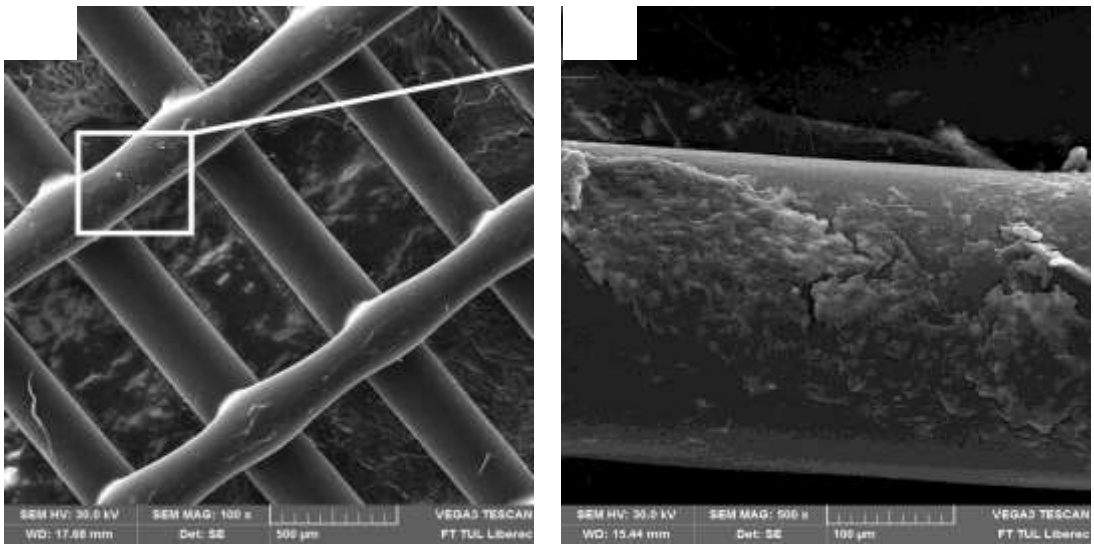

Fig. 5 SEM image at day 14 – top view onto complete scaffold (a) and detail the marked area (b)

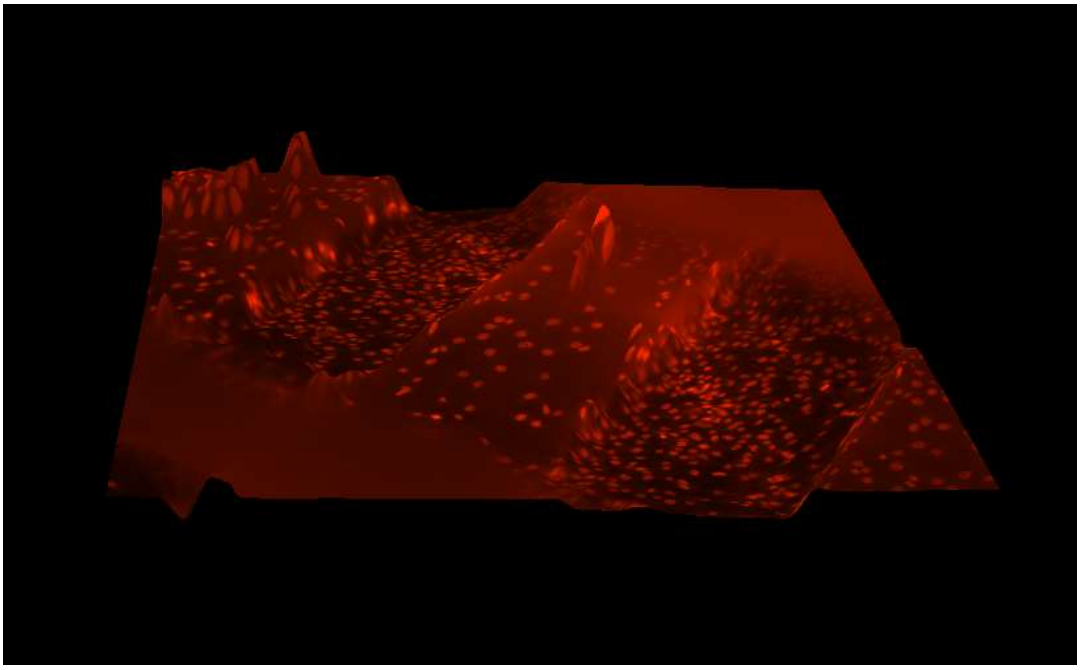

Fig. 6 3D image from fluorescence microscope proving the cell adhesion and viability on the nanofibrous layer and the printed parts

CONCLUSIONS

The production of materials based on 3D printing and electrospinning is very interesting by point of cell proliferation into bigger depth. Combined scaffold for tissue engineering was developed by combining a 3D printing with an electrospinning. The combined scaffold was composed of microfibers of a regular structure with an interconnected pore network and high nanofiber surface area for optimal cell attachment. In vitro tests confirmed that the hybrid scaffold improved initial cell attachment, cell proliferation, and differentiation relative to only printed scaffold. This study proves successful possibility of combination of 3D printing and special patterned nanofibrous layer for knee cartilage regeneration. The in-vivo tests are in process no

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