Advances and Current Trends in Biomechanics - Belinha et al. (Eds)

Chapter 41 - Page 182-186, 2021

eBook: ISBN9781003217152, CRC Press

DOI: doi.org/10.1201/9781003217152-41

https://www.taylorfrancis.com/chapters/edit/10.1201/9781003217152-41/bilayered-arcade-like-scaffolds-articular-cartilage-repair-semitela-couto-capit%C3%A3o-mendes-marques-completo?context=ubx&refId=5bd6a57c-158e-42f2-b8e9-03cd2e145ad2

Title:

Bilayered arcade-like scaffolds for articular cartilage repair

Â. Semitela, J. Couto
Centre of Mechanical Technology and Automation (TEMA), Department of Mechanical Engineering, University of Aveiro, Portugal

A. Capitão, A. F. Mendes
Centre for Neuroscience and Cell Biology, University of Coimbra, Portugal

P. A.A.P Marques, A. Completo
Centre of Mechanical Technology and Automation (TEMA), Department of Mechanical Engineering, University of Aveiro, Portugal

*** Corresponding Authors:**

E-mail addresses: completo@ua.pt (A. Completo)

ABSTRACT:

Articular cartilage is a highly organized tissue that it is adapted to the complex mechanical loading in joints. Given the limited self-healing of this tissue, tissue engineering (TE) strategies have explored the development of 3D anisotropic fibrous scaffolds with the implementation of specific mechanical stimulus. However, this functionally is dependent on the ability to recreate the depth-dependent collagen fibre alignment on the 3D fibrous scaffolds, which has been considerably challenging. In this work, bilayered structures with different fibre orientations were fabricated via polycaprolactone and Gelatin electrospinning and polyethylene glycol (PEG) particles electrospraying. After PEG removal, large interfibre spaces were created, that were compatible with chondrocyte migration The *in vitro* studies confirmed the biocompatibility of the scaffolds and their ability to guarantee cell attachment and migration through the scaffold. The mechanical stimulation applied through unconfined compression substantially improved chondrocyte response. These results confirmed the potential of the developed 3D bilayered scaffolds for articular cartilage TE.

1. Introduction

Articular cartilage is a highly specialized tissue of semi-rigid consistency with outstanding properties of deformability, resistance to mechanical loading and low-friction gliding, essential to joint function. However, this tissue does not possess vascularization or neural networks which will prevent its intrinsic ability to self-regenerate (Correa and Lietman, 2017). Current clinical treatment strategies have varying success rates, but average long-term results are unsatisfactory (Correa 2017). Due to these limitations, tissue engineering (TE) strategies have been explored as an alternative solution to develop tissue constructs capable of mimicking the depth-dependent organization of the fibrous collagen network of the native articular cartilage tissue, progressing from parallel in the superficial region, to random in the middle zone, and finally orientating perpendicular in the deep zone, by combining electrospinning as a scaffold fabrication technique and mechanical stimulation. Indeed, electrospinning has proved to be capable of creating anisotropic scaffolds by sequentially electrospinning different fibre sizes and orientations in a continuous construct to mimic the structural, topographic organization and mechanical properties of the articular cartilage (McCullen 2012; Steele 2014). Due to nearly two-dimensional nature of these scaffolds, post-processing strategies have attracted substantial interest due to their high flexibility in creating the desired final scaffold shape. In fact, our group has recently developed a new methodology to fabricate bilayered electrospun scaffolds with an arcade-like fibrous organization, that was able to induce depth-dependent chondrocyte attachment and migration *in vitro* (Girão 2020). To improve the bio-inductive properties of the scaffolds, Gelatin (GEL) has been incorporated within the polycaprolactone (PCL) system, in this work, to improve the bio-inductive properties, as previously demonstrated (Semitela 2020). Additionally, a specific mechanical stimulus was applied through cyclic

unconfined compression, which has been known to accelerate ECM production and, consequently, increase the elastic moduli of the tissue formed (McCullen 2012).

2. Materials and Methods

2.1. Fabrication of the bilayered fibrous electrospun scaffolds

PCL (Sigma-Aldrich) and GEL (Sigma-Aldrich) were dissolved separately in 2,2,2-trifluoroethanol (TFE; TCI) at a concentration of 10% (w/v), stirred vigorously at room temperature (RT) overnight, and mixed at a ratio of 50:50 with 0.2% (v/v) of acetic acid (Sigma-Aldrich). A PEG (Sigma-Aldrich) solution (1.5 g/mL) in chloroform (ACROS Organics) was prepared at 50 °C, cooled at RT, and then poured into a 10 mL plastic syringe to be electrosprayed simultaneously with the electrospinning of the PCL + GEL solution following the specifications described elsewhere (Semitela 2020, 2021), in a NANON-01 A electrospinning setup (MECC). Rectangles with 5 by 40 mm were cut from this mesh, rolled into a spiral – deep layer – and placed in the collector for a new deposition of PCL+GEL fibers and PEG microparticles – superficial layer. The resulting scaffolds were then washed in a graded series of ethanol aqueous solutions and distilled water to dissolve the PEG microparticles and freeze-dried for further structural, mechanical and biological characterization.

2.2. Characterization of the bilayered fibrous electrospun scaffolds

Scanning electron microscopy (SEM) was used to visualize the topography of the bilayered scaffolds with and without the PEG microparticles using Hitachi TM4000 plus (5 kV). PEG microparticle diameter and pore size was determined using Image-Pro Plus software ($n > 50$).

The water absorption capacity of the scaffolds was determined by swelling of freeze-dried scaffolds (with known weights) in distilled water for 24 hours at 37 °C.

The compressive properties of the bilayered scaffolds were measured under quasi-static unconfined compression in wet conditions at a rate of 0.5 mm/min, using a Shimadzu MMT-101 N (Shimadzu Scientific Instruments) with a load cell of 100 N. The compressive moduli of the samples were calculated through the tangent modulus of the linear portion of the stress–strain curve at low strain.

An immortalized human chondrocyte cell line C28/I2 (kindly provided by Prof. Mary Goldring, Hospital for Special Surgery, New York and Harvard University) was used. Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air, in Dulbecco's Modified Eagle Medium (DMEM)/Nutrient Mixture F-12 Ham 1:1 (v/v) (DMEM: Gibco, Life Technologies; F-12: Sigma-Aldrich) supplemented with 10% (v/v) non-heat-inactivated fetal bovine serum (Gibco, Life Technologies), 1% (v/v) penicillin/streptomycin (Grisp). Medium refreshments were performed two times a week. Cells were harvested at pre-confluence using trypsin/EDTA solution (0.05%/0.02%, Sigma-Aldrich). The scaffolds were disinfected in 70% (v/v) ethanol aqueous solution. Then, 0.25×10^6 cells were seeded on the bottom and top of each scaffold followed by incubation at 37 °C for 2 hours. After an initial attachment

period of 5 days, 5 cell-laden scaffolds were placed in a patented bioreactor (Bandeiras 2015) and subjected to unconfined cyclic compression for 12 days, following a specific stimulation regime: 2 hours of sinusoidal compression (0.5 Hz, 0 – 10 % strain) and 4 hours of rest; repeated 4 times a day. 5 static controls were considered. Medium was refreshed two times a week.

Cell viability was calculated based on the metabolic activity of the chondrocyte-laden scaffolds after 1, 7, 14 and 17 days of culture measured by a resazurin method. Resazurin solution (0.1 mg/mL in phosphate buffered saline (PBS; Sigma-Aldrich); ACROS Organics) was added to fresh medium at a final concentration of 10 % (v/v). Scaffolds were incubated in this solution at 37°C for 4 hours in the dark, after which 100 µL per well was transferred to a 96-well plate and absorbance (Abs) at 570 and 600nm was measured in a microplate reader (Synergy HTX, BioTek). For each day, final absorbance values for each sample were calculated as the ratio Abs570/Abs600nm minus the Abs570/ Abs600 nm ratio of a negative control (scaffold without cells). The absorbance values of cells incubated in the tissue culture polystyrene on the first time point were taken as 100% and the percentage of viable cells was calculated from these control values.

After the final time point, chondrocyte morphology and distribution within the scaffolds was assessed by SEM and haematoxylin and eosin staining. The dynamically stimulated scaffolds and the respective static controls were fixed in 4 % (w/v) paraformaldehyde in PBS, embedded in frozen embedding medium (Cryomatrix, Thermo scientific) and sectioned transversally in a cryostat (mev, SLEE cryostat). The scaffolds' cross-sections were dehydrated with graded concentrations of ethanol aqueous solutions, treated with hexamethyldisilane (TCI), kept in a fume hood for air drying and visualized by SEM. Several scaffolds' sections were also stained with hematoxylin and eosin solutions to highlight in purple-blue the cells in the bilayered scaffolds.

3. Resuls and Discussion

The bilayered scaffolds were successfully fabricated, in a first stage still with the PEG particles, with an average diameter of 34.82 ± 8.21 µm (Figure 1a), that after their removal leave large interfibre spaces with a higher percentage of sizes ranging from 10 to 20 µm (Figure 1b), which are suitable for chondrocyte migration. The scaffolds were able to absorb almost immediately eleven times their weight in water, demonstrating their hydrophilic character, mainly attributed to the presence of GEL in the fibres (Figure 1c) (Semitela 2021). The stress-strain curves of the compression assays (Figure 1d) clearly depicted an elastic behaviour with a compression modulus of nearly 2 kPa. This value was significantly lower than the ones reported for articular cartilage (89.5 ± 48.6 kPa) (McCullen 2012). Nevertheless, there are reports that scaffolds with lower compression moduli should be favourable to chondrogenesis by avoiding the stress-shielding effect, and consequently, allowing mechanical stimulation of the chondrocytes (Steele 2014).

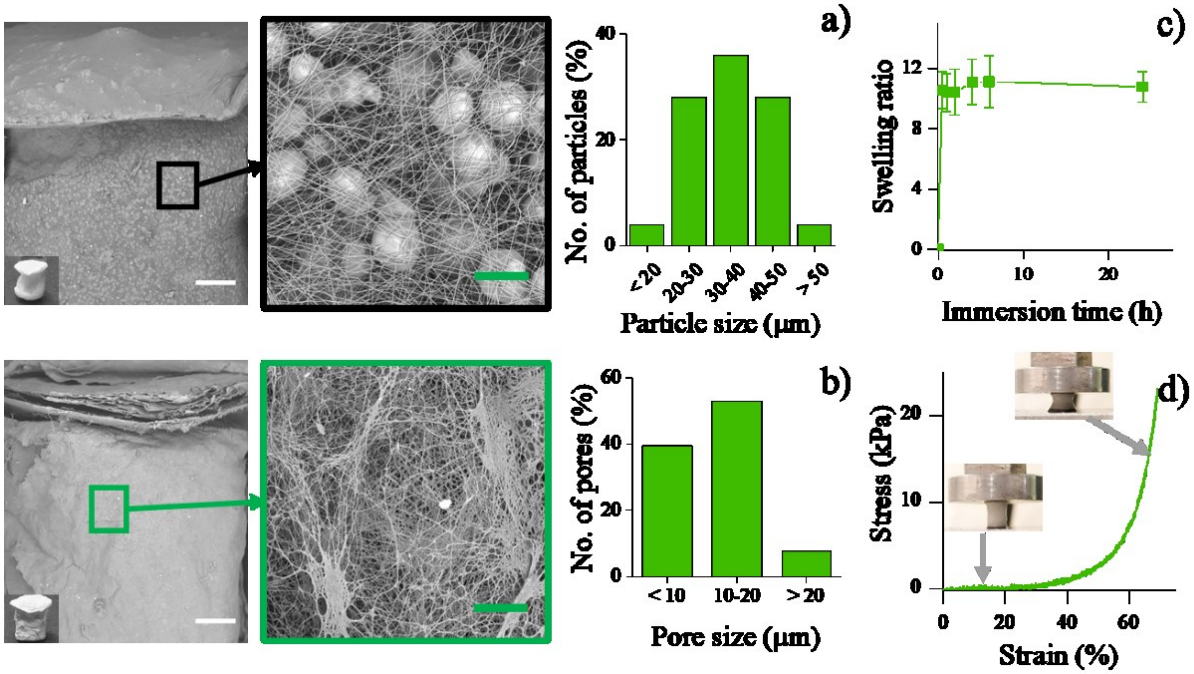

Figure 1. Structural and mechanical characterization of the bilayered scaffolds: Topography of the scaffolds with PEG microparticles and the respective PEG particle size distribution (a); and after PEG removal and the respective pore size distribution (b); swelling ratio up to 24 hours of immersion (c) and mechanical response to compression (d). Scale bars: white: 500 µm, black: 100 µm and green: 50 µm.

The *in vitro* studies revealed that these scaffolds allowed substantial chondrocyte attachment and proliferation, not only due to the arcade-like fibrous configuration, but also due to GEL incorporation and enlarged porosity provided by the PEG removal (Semitela 2020). The dynamically compressed environment seemed to be extremely beneficial to chondrocyte proliferation and migration, as the cell-seeded scaffolds subjected to compression displayed higher percentage of viable chondrocytes (Figure 2a) as well as evidence of chondrocyte migration between the layers (Figure 2c), which was not visible under static conditions (Figure 1b).

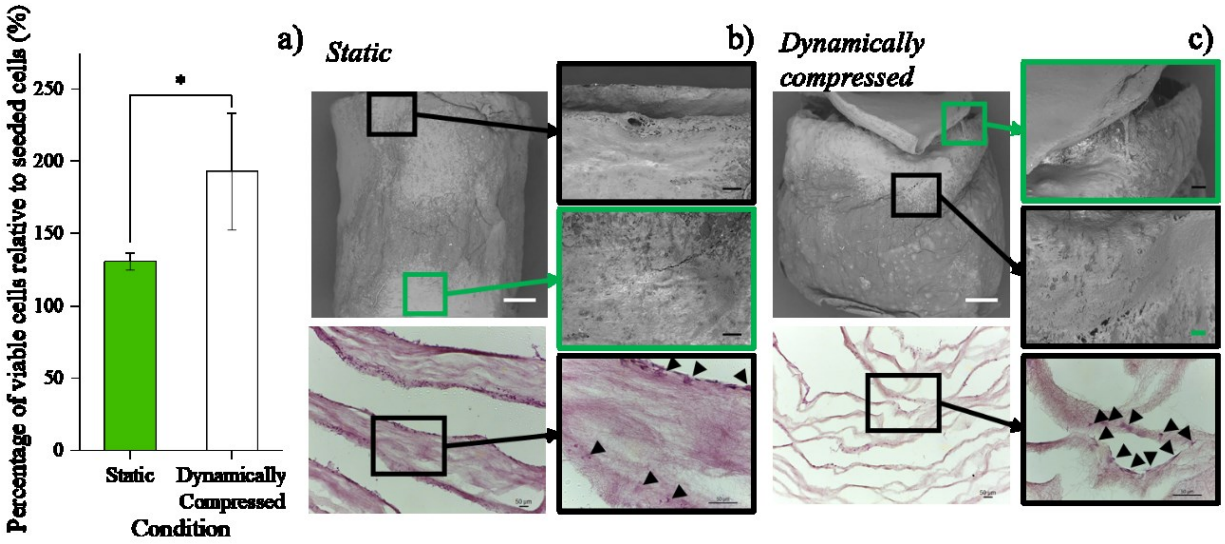

*Figure 2. In vitro characterization of the bilayered scaffold: percentage of viable chondrocytes in the scaffolds (a) and the respective SEM and haematoxylin and eosin staining images after 17 days of culture under static (b) and dynamically compressed conditions (c). Statistical analysis by One-way ANOVA followed by post hoc Tukey's test; *p<0.05, where * denotes statistically significant differences between culture conditions. Scale bars: white: 500 µm, black: 100 µm and green: 50 µm.*

4. Conclusions

Ultimately, these bi-layered scaffolds offer suitable attachment and mechanical support and promoted substantial chondrocyte proliferation and migration not only under dynamically compressed conditions, but also in static conditions, rendering them well-suited for articular cartilage TE applications.

5. AKNOWLEDGEMENTS

This work was supported by the Portuguese funding of Program COMPETE-FEDER, Programa Operacional Competitividade e Internacionalização through the projects POCI-01-0145-FEDER-028424 and CENTRO-01-0145-FEDER-022083. Also, by Fundação para a Ciência e Tecnologia I.P. (FCT, IP) through the projects PTDC/EME-SIS/28424/2017, UIDB/00481/2020 and UIDP/00481/2020. The authors thank to FCT for the PhD grant SFRH/BD/133129/2017.

6. REFERENCES

Bandeiras, C., et al. 2015. Tissue Engineered Cartilage in Unconfined Compression: Biomechanical Analysis. *Materials Today: Proceedings* 2: 355–364.

Correa, D., Lietman, S.A., 2017. Articular cartilage repair: Current needs, methods and research directions. *Seminars in Cell and Developmental Biology* 62: 67–77

Girão, A.F., et al. 2020. Microfabrication of a biomimetic arcade-like electrospun scaffold for cartilage tissue engineering applications. *Journal of Materials Science: Materials in Medicine* 31: 69

McCullen, S.D. et al. 2012. Anisotropic fibrous scaffolds for articular cartilage regeneration. *Tissue Engineering Part A* 18: 2073–2083.

Semitela, Â., et al. 2020. Electrospinning of bioactive polycaprolactone-gelatin nanofibres with increased pore size for cartilage tissue engineering applications. Journal of Biomaterials Applications 35:471-484.

Semitela, Â., et al. 2021. Boosting in vitro cartilage tissue engineering through the fabrication of polycaprolactone-gelatin 3D scaffolds with specific depth-dependent fiber alignments and mechanical stimulation. *Journal of the Mechanical Behavior of Biomedical Materials* 117: 104373.

Steele, J.A.M., et al. 2014. Combinatorial scaffold morphologies for zonal articular cartilage engineering. *Acta Biomaterialia* 10: 2065–2075.