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Title:

Automated fabrication of 3D chondrocyte-laden anisotropic scaffolds for articular cartilage

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ABSTRACT:
Tissue engineering (TE) strategies for repairing and regenerating articular car-tilage face critical challenges to approximate the biochemical and biomechanical microen-vironment of native tissue, particularly regarding collagen fibril depth-orientation and chondrocyte distribution. Here, a recently developed electromechanically 3D electrospin-ning platform was employed to develop three-dimensional (3D) anisotropic electrospun scaffolds in a fully automated manner with simultaneous chondrocyte incorporation. As expected, the 3D scaffolds possessed an arcade-like fibrous configuration with a uniform chondrocyte distribution. Overall, the results suggest that this combined approach has po-tential for cartilage TE.

1. Introduction

Articular cartilage self-repair is limited because the intrinsic regenerative ability of the tissue is low and cartilage lesions due to trauma or diseases tend to progressively intensify. Moreover, current treatment options usually lead to long-term unsatisfactory results because the tissue formed lacks the native anisotropic configuration, particularly the collagen fibres alignment, progressing from parallel in the superficial region, to random in the middle zone, and finally orientating perpendicular in the deep zone, as well an appropriate chondrocyte distribution, resulting in inferior biochemical and biomechanical properties (Correa 2017). Given these limitations, there is an increasing demand for tissue engineering (TE) approaches to develop tissue constructs with these characteristics. Several reports have pointed out the relevance of the electrospinning as a scaffold fabrication technique for cartilage TE due to similarities between the resultant polymer-based fibres and the collagen fibres in the extracellular matrix of the native tissue (Correa 2017). Still, despite the extensive research, few studies have assessed the importance of a controlled collagen fibril depth-orientation and chondrocyte distribution on the biochemical and biomechanical properties of engineered cartilage (McCullen 2012; Steele 2014; Munir 2020; Girão 2018, 2020; Semitela 2021). This mainly stems from the difficulty to create three-dimensional (3D) electrospun scaffolds with specific fibre alignment as well as a uniform chondrocyte distribution throughout these structures. Substantial progress has been made recently through the development of 3D electrospun scaffolds by means of post-processing strategies of the electrospun meshes (Girão 2018, 2020; Semitela 2021). However, these strategies are not automated, resulting in high sample variability, and the respective chondrocyte infiltration is limited, hindering the formation of functional engineered cartilage. This work is a preliminary effort to develop in a fully automated

manner chondrocyte-laden 3D anisotropic tissue constructs using a newly developed electromechanically 3D electrospinning platform.

2. Materials and Methods

All experiments were performed in a recently developed electromechanically 3D electrospinning platform (Completo 2020) (Figure 1a and b), thoroughly cleaned with 70 % (v/v) ethanol aqueous solution (ChemLab) beforehand and sterilized with UV radiation. The remaining used instruments were already sterile or autoclaved at 121 °C beforehand. All the electrospinning systems were assembled and cleaned prior to use with a phosphate-buffered saline (PBS; Sigma-Aldrich) supplemented with 1% (v/v) penicillin/streptomycin (P/S; Grisp) and 2.5 µg/mL solution of Amphotericin B (Sigma-Aldrich).

2.1. Preparation of the polymer solution

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 60:40 with 0.2% (v/v) of acetic acid (Sigma-Aldrich). The resulting solution was filtered-sterilized using a 0.45 µm filter and poured into a 5 mL plastic syringe.

2.2. Preparation of the the chondrocyte suspension

The immortalized human chondrocyte cell line, C28/I2 (kindly provided by Prof. Mary Goldring, Hospital for Special Surgery, New York and Harvard University), was 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 (F-12) 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) P/S (Grisp) and 0.25 µg/mL Amphotericin B. The medium was refreshed twice a week. Chondrocytes were harvested at pre–confluence using trypsin/EDTA solution (0.05%/0.02%, Sigma-Aldrich) and 70,000 chondrocytes were mixed with 3 wt% alginate (Sigma-Aldrich) solution in culture medium, previously filter-sterilized.

2.3. Fabrication and characterization of the chondrocyte-laden 3D scaffolds

PCL+GEL solution, placed in the electromechanically 3D electrospinning platform (Figure 1a and b), was fed at a controlled flow rate of 2.5 mL/h to a blunt needle with a tip diameter of 0.4 mm (21 G), with an applied positive voltage of 15 kV. The polymer jet was ejected onto at the rotating collector at 1500 rpm, with an applied negative voltage of – 8 kV using a needle to collector distance of 13 cm. The fibres were then placed on the support according to a specific programmed alignment (Figure 1c). After each layer of PCL+GEL fibres, a drop of chondrocyte

suspension was placed on top of the fibres. This process was repeated eight times. The resulting 3D scaffolds were then removed from the support, placed in 48-well plate and cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air in culture medium for 7 days.

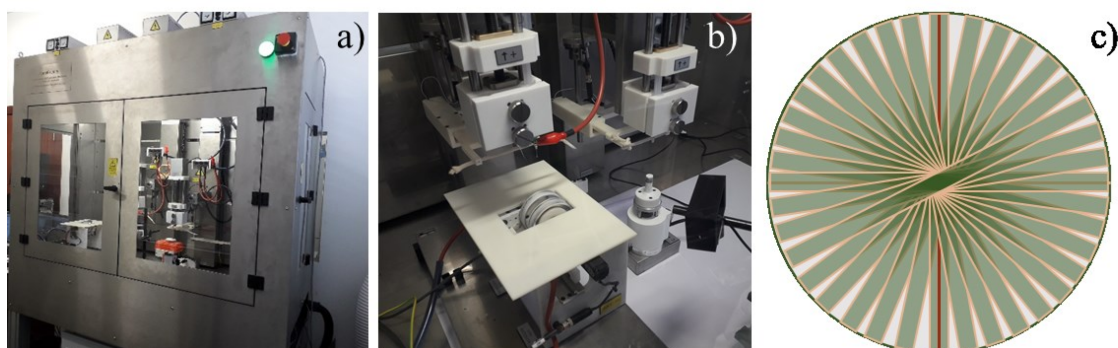

Figure 1. Developed electromechanically 3D electrospinning platform (a and b) and the programmed fibre alignment (b).

Chondrocyte viability was calculated based on the metabolic activity of the chondrocyte-laden scaffolds after 1 and 7 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 seventh day of culture, chondrocyte morphology and distribution within the scaffolds was assessed by SEM and haematoxylin and eosin staining. The 3D scaffolds 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 haematoxylin and eosin solutions to highlight in purple-blue the cells in the bilayered scaffolds.

3. Resuls and Discussion

Chondrocyte-laden 3D scaffolds were successfully fabricated (Figure 2a), electromechanically 3D electrospinning platform.

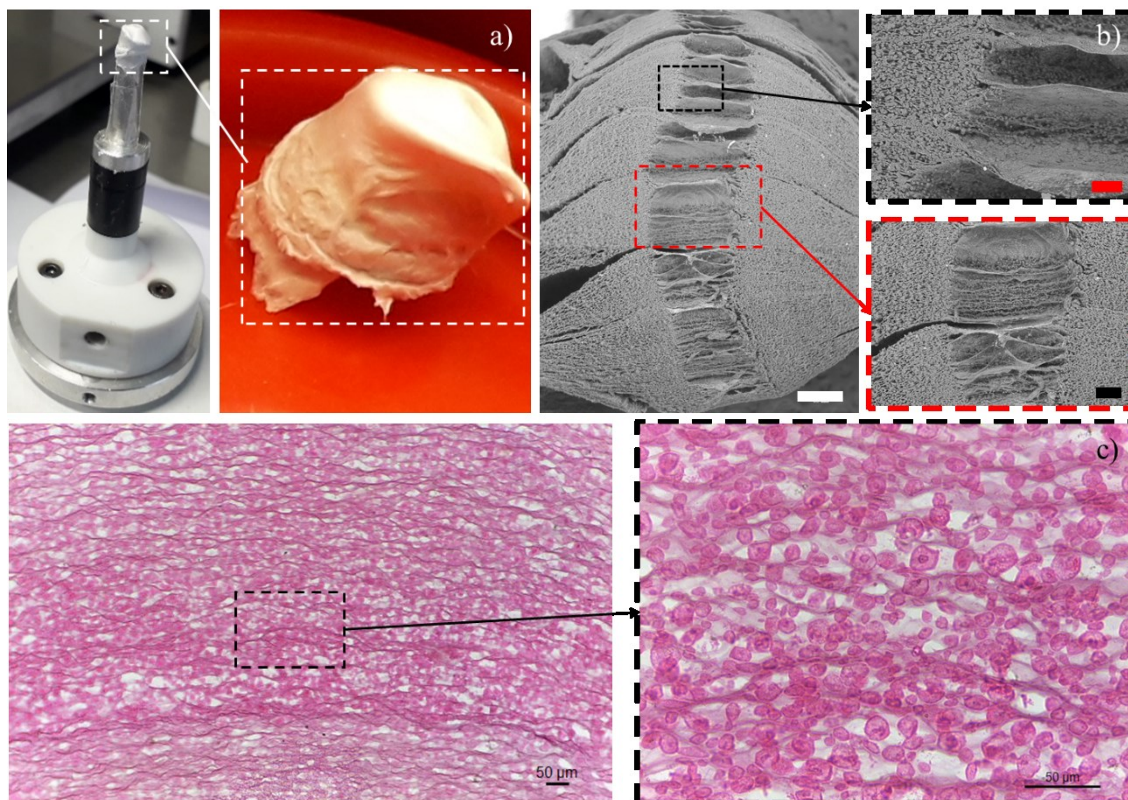

Figure 2. Chondrocyte-laden 3D scaffold (a); SEM images (b) and hematoxylin-eosin staining (c) of the 3D chondrocyte-laden scaffolds' sections after 7 days of culture. Scale bars: white: 500 µm, black: 200 µm and red: 100 µm.

Despite the fact that a substantial percentage of chondrocytes did not survive the layering process – only around 20 % of the seeded chondrocytes were viable after 1 day of culture –, these were able to proliferate over the 7-day culture period, as a 2-fold increase on the percentage of viable chondrocytes was observed. The low initial chondrocyte survival could be attributed to the detrimental effects of several factors: the toxic solvents used for the polymer electrospinning, chondrocyte dehydration on top of the fibres and the inadequate process environmental conditions (low CO_2 concentration and low temperature) (Canbolat 2011). Over time in culture, these factors no longer were significant, which was why the number of viable chondrocytes increased. Chondrocytes were effectively incorporated within the fibrous matrix, which was confirmed on the SEM images (Figure 2b) and haematoxylin-eosin staining (Figure 2c) of the 3D scaffolds' sections of the scaffolds. Additionally, chondrocyte distribution was fairly homogeneous throughout the height of the 3D scaffold.

4. Conclusions

Ultimately, even though optimization of the operational parameters should be performed, up to this point chondrocyte-laden 3D anisotropic electrospun scaffolds were successfully developed with a controlled fibre depth-orientation and chondrocyte distribution, showing the potential of this approach for cartilage TE.

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REFERENCES

Canbolat, et al. 2011. Mammalian cell viability in electrospun composite nanofiber structures. *Macromolecular Bioscience* 11: 1346–1356.

Completo, A., Marques, P.A.A.P. 2020. *Electrospinning system and process for large-scale manufacturing of aligned 3D fiber matrices*. European Patent Office (EPO)-EP3670714 (A1).

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. 2018. Mimicking nature: Fabrication of 3D anisotropic electrospun polycaprolactone scaffolds for cartilage tissue engineering applications. *Composites Part B: Engineering* 154: 99-107

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.

Munir, N. et al. 2020. Integrational Technologies for the Development of Three-Dimensional Scaffolds as Platforms in Cartilage Tissue Engineering. *ACS Omega* 5: 12623−12636

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 Behaviour 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.