PREPARATION OF ALGINATE MICROFIBERS FOR CELL ENTRAPMENT USING A MICROFLUIDIC DEVICE

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ABSTRACT
This paper describes a method for the production of multi-compartmental alginate microfibers using a glass microfluidic device. The key parameters, which critically influence the formation of microfibers and their geometries, were identified and their interplay was investigated with a statistical approach by means of a “design of the experiments” optimization and screening. Finally, the processes impact on cell viability was considered. It is here demonstrated that this development provides a controllable approach for the production of alginate microfibers with controlled shape and content, which could be further developed for scaling up and working towards FDA approval.

KEYWORDS: Microfiber, Multi-compartmental, Cell Encapsulation

INTRODUCTION
A key aspect of tissue engineering is the development of scaffolds that support, stimulate, and direct the growth of specific cells. Biomaterial based scaffolds in a fibrous form are potential appealing for tissue engineering since they can enable the guided cell growth, alignment, and migration [1]. Recently, microfluidic based techniques has been conveniently applied for the production of alginate microfibers [2-3]. The applied techniques rely on the use of a coaxial flow device or on the use of a roller assisted microfluidic system. With regard of the coaxial flow approach, the dimensions of the fibers were successfully controlled by regulating the flow rate within the channels, thereby controlling the dimension of the central Na-alginate stream [2]. Alternatively Su and colleagues [3] reported the generation of alginate microfibers with a roller-assisted microfluidic system. This approach relies on the use of a microfluidic strategy with a simple design for the generation of alginate gel fibers that are collected with a rotor. By controlling the flow rate of the alginate dispersion within the microfluidic device and the velocity of the roller the authors were able to control the dimension of the produced fibers.

However, dimensional characteristics are not the only crucial aspects for the possible implantation of such a tissue engineering scaffold, thereby, in spite of these interesting results, the authors believe that some questions still remain to be answered for the final in vivo applications of fibrous, lab-on-a-chip generated, scaffolds. Despite the important role played by the morphological characteristics of the fibers on their in vivo applicability [4], the dimensional homogeneity and the surface smoothness of the produced scaffold is not evident from the previous mentioned papers. In order to address this issue, a simple but milder microfluidic production strategy, that does not imply the use of either sheath flow or rotating roller, was applied for the generation of alginate based uniformly-sized with a smooth surface microfibers with different diameters. Another important issue to address in the design of a successful scaffold for tissue engineering is related with the increase of the encapsulated cell viability and the reduction of the foreign body response. Recent findings demonstrate that co-encapsulation of cells and drug delivery systems (DDS) in a multi-compartmental scaffold offers a rational alternative to enhance the effectiveness of the tissue engineering construct [5].

In this respect, the current paper describes the effect of chip geometry and experimental set-up on the morphological and functional characteristics of multi-compartmental microfibers for cell transplantation. The presented microfluidic technique permits the production of engineered microfibers containing cells and drug delivery systems and also to change the fibers diameter and content (amount of cell and drug delivery system) on demand. For these reasons, the produced scaffolds, named multifunctional alginate microfibers, could find many applications in the field of tissue engineering as small vascular grafts, nerve conduits, artificial kidney tubules, as well as drug release vehicles.

EXPERIMENTAL
A pholithography-wet etching procedure was used for the fabrication of glass microchips [6]. Teflon tubes with different internal diameter (from 210 to 700 μm) were connected with the outlet.

Figure 1: Schematic representation of the general preparation strategy (A) and microchip geometries (B).
Fluidic reagents were introduced into the microfluidic network from glass gastight syringes (Hamilton, Reno, NV) by syringe pumps (model KD100, KD scientific Inc., Holliston, MA). Two types of glass chips were produced for this study, namely, Chip #1 and Chip #2 (Figure 1). Chip #1 had a relatively simple design with a single inlet and a straight channel. The channel had an approximately semicircular section with a depth of 115 µm and a width of 250 µm. Chip #2 consisted of three inlets and three micromixers in series. Channels in Chip #2 had the same dimension as Chip #1. Each mixing chamber (10 mm long, 2 mm wide) in Chip #2 contained an array of cylindrical pillars (750 µm in diameter). “Empty” and “multifunctional” (containing cells, drug delivery systems or both) barium alginate microfibers were produced with Chip #1 and Chip #2 (Figure 1), respectively. Using Chip #1, sodium alginate dispersion (1.5-2.5%, w/v) (used as main constituent of the microfibers) was introduced into the inlet of the microchip at appropriate flow rates (i.e. 15-35 µL/min). In Chip #2, a sodium alginate dispersion and two sodium alginate suspensions were delivered via the three inlets. The two suspensions contained different amounts (10-40 mg/ml) of either DDS (i.e. lipospheres) or cell suspensions (Wharton’s Jelly Mesenchymal Stem Cells, WJMSCs, at concentration of 2.9 x 10⁶ cells/ml). The output from the outlet of the two chips was transferred via Teflon tubes (with different internal diameter), into a BaCl₂ solution (0.5-3.0%, w/v) where the Na alginate flow stream was gelled to produce the final Ba-alginate consolidated microfibers.

The produced microfibers were then examined by an optical stereomicroscope (Nikon SMZ 1500, Japan) with imaging analysis software (Eclipsen et version 1.16.5, Laboratory Imaging s.r.o. for Nikon B.V.) to determine their dimensions. Mean diameters (± SD) were obtained by taking 9 measurements along the (10 mm) length of the samples at equal intervals. To study the effect of different experimental parameters including alginate concentration, BaCl₂ concentration and flow rate on the size and size distribution of the produced alginate microparticles, a randomized central composite face-centered design (CCF) consisting of 17 runs was used. The experimental design and its evaluation were performed by the PC software MODDE 8.0 (Umetrics AB, Sweden), followed by multiple linear regression (MLR) algorithms. Cell viability was assessed using a double staining method with propidium iodide (PI) and Calcein-AM before and after cells were encapsulated in alginate microfibers.

RESULTS AND DISCUSSION

Initial experiments were carried out using Chip #1. Fig. 2 shows microfibers obtained using different outlet tubes with internal diameters ranging from 200 to 700 µm. The corresponding diameter of the produced microfibers spanned from 180 to 500 µm (Figure 2 A-C) demonstrating the capability of this system in controlling microfibers size. It should be considered that varying the diameter of the outlet tube, thinner or thicker microfibers could also be easily produced (data not shown). It was observed that, in all cases, the obtained microfibers possessed a uniform shape (i.e. constant diameter through the entire microfiber length) and a highly smooth surface.

The effect of three experimental parameters namely “alg” (i.e., alginate concentration), “BaCl₂” (i.e., BaCl₂ concentration) and “pump” (i.e., flow rate) were studied using a randomized central composite face-centered (CCF) design of the experiment. The results of the design of the experiments (DoE) analysis are plotted in Figure 2 as three-dimensional graphs showing the responses of both microfiber diameter and relative standard deviation to the three key factors. The surface plots show the inverse relationship between microfiber diameters and both BaCl₂ concentrations and pumping rate. Conversely, the diameter of the produced microfibers increased when the alginate concentration was increased. However, the diameter reached is maximum for an alginate concentration of 2.2 % (w/v). A further increase in alginate concentration caused the diameter of the microfibers to decrease. The results provided by DoE analysis represent a clear
guideline for selecting appropriate experimental conditions in order to obtain microfibers with desired dimensions and mechanical and biological properties. It should be noted that the variation of alginate and barium solutions are related to the porosity and mechanical strength of the produced microfibers.

After investigation of the factors and their interactions on the responses, the validity and the significance of the model was estimated by analysis of variance (ANOVA). All the data obtained fitted well in the model determining a good reproducibility of the studied model, namely 0.99. We got a large regression coefficient $R^2$ (i.e. 0.97) that results a necessary condition for a validity model with a significant power of prediction of the model $Q^2$ (i.e. 0.81).

![Figure 3: Optical stereo photomicrographs of multifunctional alginate microfibers, containing DDS and WJMSCs (A) and Calcein-AM and propidium iodide viability assessment, at day 0 (B). Bar corresponds to 200 µm.](image)

As stated in the introduction, another important goal of the current study is to produce biomaterials based scaffolds for the integrated immunoprotection of the embedded cells. To this aim we produced newly designed microfibers basically formulated with the well-validated polymer alginate, but containing multiple functional compartments (i.e. drug delivery systems) that can interact with the embedded cells. Adjusting the relative flow rates of the three independent pumps, one pumping plain alginate solution (pump #1), the others pumping alginate containing DDS or cells (pump #2 and #3 respectively), it was possible to exert a control in the amount of particulate systems within the final produced engineered alginate microfibers. The photomicrographs of the obtained microfibers (reported in Figure 3 A) demonstrated that the presence of both DDS and cells does not alter the shape, dimension and surface of the microfibers. In addition, they maintain a highly uniform diameter distribution throughout the entire fiber length. Furthermore, the viability of WJMSC encapsulated in alginate microfibers was investigated. The fluorescent photomicrographs recorded immediately after the embedding procedure (reported in Figure 3 B) indicate that the cells were highly viable (>95%) confirming that this approach could be conveniently applied for the encapsulation of living cells.

**CONCLUSION**

In summary, a simple, cost-effective, well-controlled and biological compatible process has been developed enabling the production of uniform alginate microfibers with controlled size and content, possibly capable of scaling up, working towards FDA approval. This process could be used to produce multi-compartmental microfibers based on a wider range of biomaterials specially designed for tissue engineering.

**REFERENCES**


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