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Precision wet-spinning of cell-impregnated alginate fibres for tissue engineering

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Abstract

The selective assembly of functionalised fibres produced by wet-spinning into implantable three dimensional contructs presents attractive prospects for the field of medical bionics[1]. In particular, the incorporation of biological factors and large numbers of cells within biocompatible and macroporous fibres is expected to deliver improvements to drug delivery platforms as well as to tissue engineering biotechnology[2, 3].

Keywords

tissue, engineering, cell, fibres, spinning, wet, alginate, precision, impregnated

Disciplines

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Precision Wet-Spinning of Cell-Impregnated Alginate Fibres for Tissue Engineering

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INTRODUCTION

The selective assembly of functionalised fibres produced by wet-spinning into implantable three dimensional contructs presents attractive prospects for the field of medical bionics[1]. In particular, the incorporation of biological factors and large numbers of cells within biocompatible and macroporous fibres is expected to deliver improvements to drug delivery platforms as well as to tissue engineering biotechnology[2, 3].

Hydrogel class materials, in particular chitosan and sodium alginate, are suitable for soft tissue engineering applications due to their compliance with the physical properties of target tissues and permissive degradation profile that elicits minimal inflammatory response [4, 5]. The wet-spinning process allows for these materials to be deposited, woven or knitted into defined structures [6]. Thorough optimisation of novel processes for biomaterial fabrication is paramount, as the resulting devices and conduits become both more effective and reproducible with precision assembly[7].

OBJECTIVE

This study assessed wet-spinning technology as a method for generating three dimensional soft gel fiber geometries biologically functionalized by incorporation of live cells. This was achieved through the analysis of process parameters, fibre dimension, as well as cell-loading, viability, position and alignment.

Sodium alginate of varying concentrations was selected as a matrix material for the fibres. Primary myoblasts from C57BL10-ROSA mice were used for fabrication of regenerative muscle constructs, exploiting geometric compatibility between muscle structure linearity and wet-spun fiber output. This process was developed into a an optimised methodology for reproducible production of fibres using this model, and a three dimensional myo-regenerative conduit developed.

APPROACH

Materials. A buffer solution of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, Biochemical, #L505388) was made up to a concentration of 20mM in a 308mM sodium chloride (NaCl) solution. Alginic acid with sodium salt (Sigma, #035K0205) was made to concentrations of 2% and 4% (wt/v) at 60°C under stirring. A ROSA mouse myoblast cell line was grown to 80% confluence in a series of thirty T-75 flasks in 20mL of Dulbecco's Modified Eagle's Medium (DMEM) at 37°C. The cells were then trypsinised and centrifuged at 280g for 2 minutes to pellet the cells. The cells were then resuspended in HEPES solution to concentrations of 20, 40 and 60 millions cells/mL. Mixing with corresponding alginate solutions resulted in cell concentrations of 10, 20 and 30 million cells/mL for both 1% and 2% (wt/v) alginate concentration.

Wet Spinning. A wet spinning arrangement as described in Figure 1, was used to fabricate cell-impregnated alginate fibres. An aqueous coagulation bath (L=1m) of 2% calcium chloride (Sigma, #48H0106) was utilised in cross-linking the fibres. Solutions were extruded into a coagulation bath using a high precision KD Scientific KDS-410 constant volume syringe pump at a rate of 0.03mL/min (32G nozzle - EFD Nordson). A rotating drum was operated at a fixed velocity of 2cm/s for the collection of fibres following coagulation.

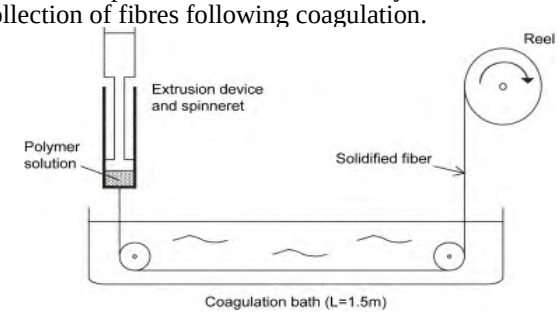

Figure 1: Basis of the wet-spinning process, adapted from Ruijter et al.[8]

Analysis. For viability , fibres were collected into HEPES containing 1µM calcein AM in DMSO and 1µg/mL of propidium iodide (PI). An Olympus IX70 fluorescence microscope was used at 10x to observe cell viability and fibre dimension (n>3). For cryoslicing, fibres were embedded into Tissue-Tek Optimal Cutting Temperature Compound and placed into a slurry of dry ice and ethanol. These were stored at -70°C before being sliced using a LEICA CM1900 Rapid Sectioning Cryostat. Four independent experiments were carried out to ensure the repeatability of the fabrication process. Finally, several fibres were collected into both proliferation and differentiation media for observation.

RESULTS AND DISCUSSION

Each of the cell loadings tested were successfully fabricated repeatedly into fibres of both 1% and 2% alginate matrices (Figure 2). Through optimisation of the material feed rate and handling methods, large quantities of cell laden fibres could be manufactured. While there were minor differences in the fibre diameters observed (~130-150µm), the alginate concentration and cell loadings demonstrate no correlation to any significant differences in diameter (Figure 3).

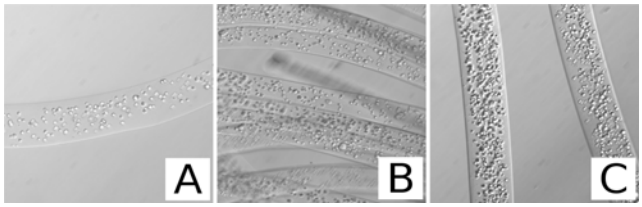

Figure 2: Alginate fibres (2% wt/v) impregnated with A) 10M cells/mL, B) 20M cells/mL, C) 30M cells/mL.

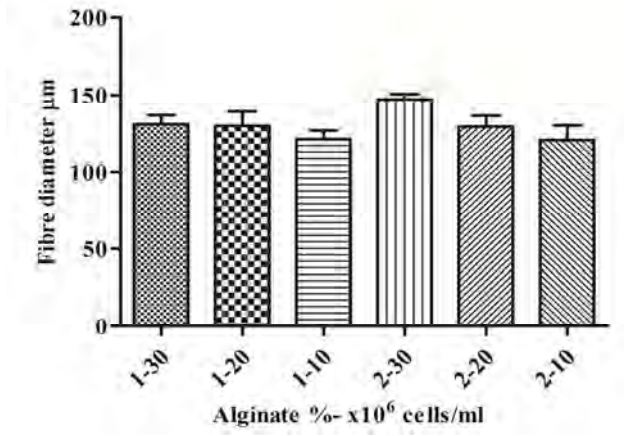

Figure 3: Average fibre dimension with respect to cell and alginate concentration.

Samples produced with 1% alginate and 20 million cells/mL showed the highest cell viability over the four experiements of 96 ± 1% (**Figure 4**).

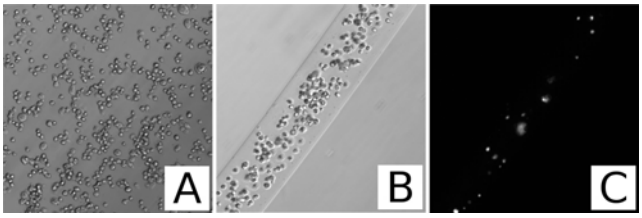

Figure 4: A) Cell formulation prespin. B) 1% alginate fibre containing 20M cells/mL. C) Same fibre under fluorescence, showing cell death through PI staining.

Fibres produced using 2% alginate as a matrix material, while displaying similar fibre diameters, demonstrated a lowered cell viability and consistency when compared to the 1% alginate fibres (Figure 5). Finally, in a preliminary attempt to produce a wet-spun, cell-laden three-dimensional construct, a 5-layer, 1% alginate, 20M cells/mL mesh was produced through the continual 90° reorientation of the mounted collection substrate (Figure 6).

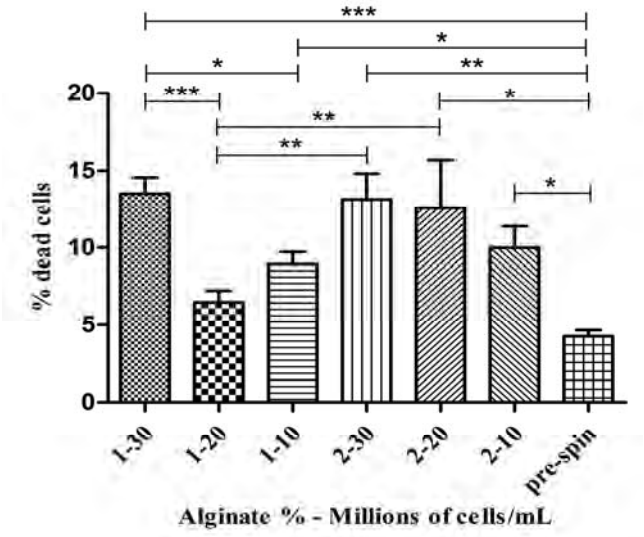

Figure 5: Comparison of cell viability for each tested subgroup over four independent experiments (n=4). A one-way ANOVA was applied to the data (*** for $p<0.05$, ** for $p<0.01$, * for $p<0.001$).

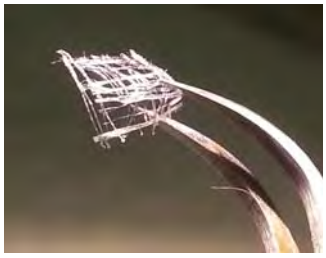

Figure 6: Multi-layered, wet-spun mesh of 1% wt/v alginate containing 20M cells/mL.

CONCLUSION

This work communicates the successful fabrication of cell-laden fibres at high cell concentrations. The cells remain viable post-fabrication and it was determined that the most efficient subgroup consisted of a 1% wt/v alginate matrix incorporating a cell concentration of 20M cells/mL. A cell-loaded, three dimensional wet-spun scaffold was successfully produced.

FUTURE WORK

Future experimentation will integrate slow release growth factors and other biologically relevant agents such as anti-inflammatory therapeutics into these systems. Cells of varying description should be fabricated into multi-layered structures to meet specific three-dimensional tissue engineering requirements, and *in vivo* studies be carried out in animal models to observe cell migration and engraftment in target tissues such as muscle and nerve.

KEYWORDS: Alginate, hydrogel, wet-spinning, fibre, fiber, microfiber, cell encapsulation, tissue engineering, additive fabrication.

ACKNOWLEDGEMENT

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