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Living fibres: 3D hydrogel fibres for tissue engineering

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Abstract

To use rapid fibre spinning technologies for the creation of 3D constructs for cell delivery, tissue engineering and the study of 3D cellular interactions. This study describes the fabrication of biosynthetic soft gel fibers containing myoblasts and myogenic growth factors and their use for restoration of dystrophin expression in dystrophic mdx mouse muscle.

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Living Fibres: 3D Hydrogel Fibres for Tissue Engineering

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PURPOSE

To use rapid fibre spinning technologies for the creation of 3D constructs for cell delivery, tissue engineering and the study of 3D cellular interactions. This study describes the fabrication of biosynthetic soft gel fibers containing myoblasts and myogenic growth factors and their use for restoration of dystrophin expression in dystrophic *mdx* mouse muscle.

INTRODUCTION

Tissue engineering approaches generally involve cell seeding onto biological or synthetically based scaffolds, prior to implantation or analysis *in vitro*.^[1] We have combined the synthesis of hydrogel biomaterials with cell seeding in one process to create cell laden hydrogel fibres, amenable to transplantation as well as for study of cellular interactions and behaviour in 3D.

Cell transplantation for replacement of lost muscle tissue has shown limited potential to date and we propose that this can be improved by controlled pre-engineering of proliferative differentiation-resistant muscle cellular constructs *ex vivo* prior to their implantation as biosynthetic myoregenerative scaffolds.^[2,3]

This study shows that use of alginate rods as delivery vehicles for myoblasts may improve the transplantation potential and remodelling of dystrophic muscle.

APPROACH

Wet Spinning of myogenic cell-laden fibres

Primary myoblasts (muscle precursor cells) were prepared from transgenic 129S4Gt(ROSA)26 mice, backcrossed to a C57Bl10 background (St Vincent's Hospital Melbourne #013/11), using methods previously described by our laboratories.^[4] Purified myoblasts were resuspended in Alginate (1-2%) (NovaMatrix) containing a cocktail of defined cytokines, prior to wet spinning. Myoblasts were seeded into alginate solution (2%) at a density of approximately 3×10^6 cells/ml. Non crosslinked alginate-cell preparations were then spun through a syringe into a coagulation bath (1M length) containing 150mM NaCl, 10mM HEPES, 110mM CaCl₂. Fibres were deposited on a rotating collection drum and immediately placed in cell media or buffer for analysis of cell viability and/or transplantation.

In Vitro and In Vivo analysis of Cell Laden Fibres

Immediately after wet spinning, cell laden fibres were stained with Calcein AM (Life Technologies) and Propidium Iodide (Sigma) to identify viable and non-viable cells. For *in vivo* transplantation, alginate fibres

were prepared containing a cytokine cocktail designed to improve cell survival. Fibres (approximately 500µm in diameter) were prepared as described above and washed briefly in PBS, before transplantation into the gastrocnemius muscle of *mdx* mice. Fibres were left for 4, 8 and 12 weeks before analysis. Gastrocnemius muscles were notexin treated, to induce a regenerative environment, prior to fibre implantation.

Muscles were harvested and snap frozen in liquid nitrogen-cooled isopentane and cryosectioned for analysis. Hematoxylin and Eosin staining was performed to determine tissue reaction to the fibres. Sections were stained for ROSA transgene activity, as well as dystrophin expression to identify donor cells.

RESULTS AND DISCUSSION

Myoblast laden alginate fibres could be spun at diameters ranging from 100µm to 800µm, depending on the spinning method and parameters employed. Analysis of cell viability in alginate fibres demonstrated at least 92% cell survival *in vitro* (Fig. 1) after the spinning process.

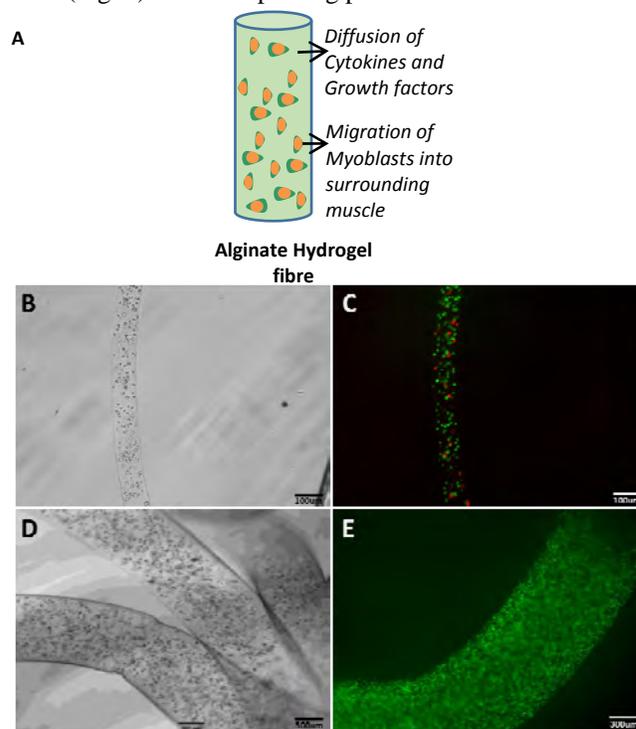


Fig. 1: Primary myoblasts wet spun into Alginate hydrogel fibres loaded with cytokines (A, B), were assessed for survival (C: calcein, green, alive and propidium iodide; red, dead) showed a survival rate of > 92% (C & E). Fibre diameter ranged between 100 µm (B & C) and 800 µm (D & E). These fibres were implanted into the dystrophic muscles of *mdx* mice (Fig 2)

In vivo analysis of cell delivery to muscle demonstrated survival of cells within alginate rods at 2, and 4 weeks with migration of donor cells from alginate fibres to surrounding muscle seen at 4, 8 and 12 weeks (Fig. 2). Inclusion of cytokines into alginate fibres showed some improvement in myoblast survival in a number of animals, and a decline in donor cell number seen over time in the treated animals, was proposed to be due to a lack of innervation of the newly-formed muscle.

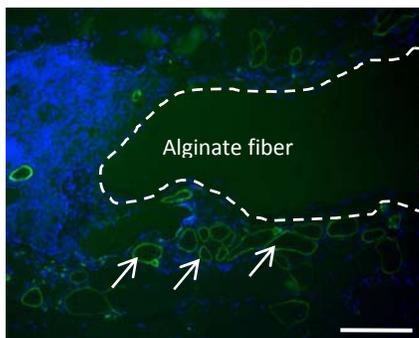


Fig. 2: Fluorescent image showing newly formed dystrophin expressing muscle fibres (arrows) at 12 weeks post-transplant of LIF and myoblast containing alginate rods. Cell nuclei are shown in blue. Arrows indicate donor cells. Scale bar is 100 μ m.

CONCLUSIONS

Cell laden hydrogel fibres can be used for cell delivery as well as for the delivery of cytokines and biofactors to tissues. In addition, these fibers can be used to expand cell numbers prior to and post transplantation.

FUTURE WORK

An ongoing study is currently underway to expand upon the results observed in this study. Ongoing experiments involve the construction of 3D scaffolds using fiber spinning technologies as well as other fabrication methods including printing and electrospinning that incorporate biofactors to promote innervation of the newly formed muscle *in vivo*. Future experiments will utilize these fabrication methods for cell transplantation and delivery of biofactors to muscle and nerve tissues.

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