Composite ECM-alginate microfibers produced by microfluidics as scaffolds with biomineralization potential

Angelozzi M1, Miotto M2, Penolazzi L1, Lolli A1, Mazzitelli S2, Badylak SF3, Piva R4, Nastruzzi C2

1 Department of Biomedical and Specialty Surgical Sciences, University of Ferrara, Ferrara, Italy
2 Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy
3 McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA

e-mail: nglmrc1@unife.it, nas@unife.it; website: www.biomaterials.it

INTRODUCTION

Bone defects, consecutive to traumas or diseases, are not always repaired by physiologocal healing and tissue engineering has emerged as the new approach for tissue restoration, overcoming limitations linked to classical graft procedures. The success of a tissue engineering strategy depends from a perfect combination of biomaterials, cells and bioactive factors in order to obtain a construct with an optimal mineral density and mechanical characteristics. For this purpose, the choice of the appropriate biomaterial, but also the fabrication's technique are two essential requisites for creating an osteoconductive and osteoinductive scaffold with ideal morphological and structural characteristics. In this context we propose an optimized system based on microfluidic to produce bio-inspired microfibers, where the structural properties of alginate were combined with bioactive features of ECM-derived materials (gelatin and UBM).

AIMS

• To produce alginate-ECM microfibrous scaffolds by a highly controlled microfluidic procedure;
• To assess composite microfibers for cell embedding;
• To evaluate the osteogenic potential of entrapped cells inside engineered microfibers.

HIGHLIGHTS

The microfluidic procedure allowed a precise control of the dimensional and morphological characteristics of the microfibers, favourable influencing the viability and function of the embedded cells.

METHODS and RESULTS

1. Microfluidic chip and microfibers’ preparation

The microfluidic chip MC3 with two inlets, a snake micromixing channel and variable outlet tube was employed for composite microfibers production (A). An alginate solution (2%) containing cells and anotahi within gelatin (2.25%) or UBM (0.5%) were delivered via the two inlets of the microchip at a total flow rate of 1.5 mL/min. The output was transferred through a 700 µm (diameter) outlet tube into a 45°C bath, where the alginate stream was gelled to produce composite microfibers (B). The MC3 channel geometry allows an optimal and homogenous distribution of cells or ECM-particles along alginate fibres as shown in the channel magnification (A) or as evaluable in the photomicrographs of composite microfibers after Coomassie Blue staining (B). The microfluidic procedure positively influenced also the dimensional and morphological characteristics of the microfibers. Dimensional analysis (C) demonstrated that increasing flow rates corresponded to larger microfibers’ diameters. Notably the even distribution of ECM-components caused only a slight shift towards higher diameters, not affecting structural and morphological characteristics of the microfibrous scaffolds.

2. Cell embedding in composite microfibers

SaOS-2 cells resulted evenly distributed within the composite microfibers and acquire a round shape morphology without substantial changes after osteogenic treatment (A). Notably microfluidic embedding procedure sustained cell functions and viability, with embedded SaOS-2 cells still viable also after 14 days of culture in basal or osteogenic medium (B).

3. Osteogenic potential of embedded cells

Monolayered SaOS-2 cells displayed signs of mineralization not earlier than day 12-14, while Alcian blue staining indicated the presence of mineralized matrix. (B) Gene expression analysis of osteogenic markers (Col1A1 and Runx2) confirmed the osteogenic potential of embedded cells: Alcian blue staining of SaOS-2 cells displayed higher levels of Col1A1 and Runx2 respect monolayered cells, even in absence of osteogenic inducers. However embedded cells maintained responsiveness to osteogenic medium, showing a further increase in Col1A1 and Runx2 expression levels.

CONCLUSIONS and FUTURE PERSPECTIVES

• Alginate-ECM microfibers with highly controlled dimensional and morphological properties were produced by a microfluidic approach;
• Composite microfibers can be used for cell embedding, sustaining cell functionality and viability;
• Microfibers’ environment support and enhance osteogenic potential of SaOS-2 cells;
• These devices can be employed as platform to test osteogenic regenerative ability of different cellular models, such as mesenchymal stem cells (MSCs).

The authors declare no conflict of interest.