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

P170

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INTRODUCTION

Bone defects, consecutive to traumas or diseases, are not always repaired by phyisioloical 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

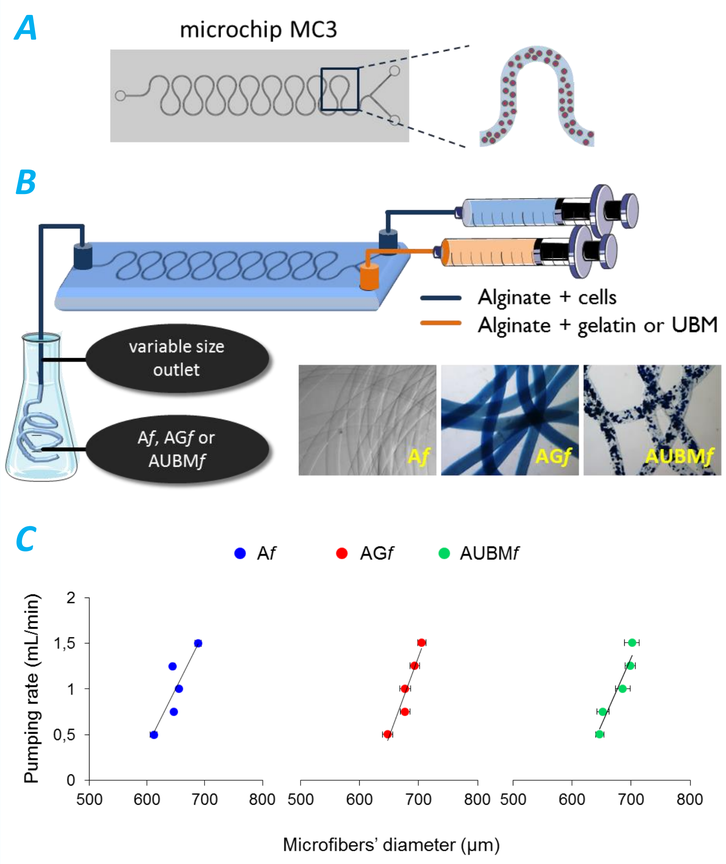
- To produce **alginate-ECM microfibrous** scaffolds by a **highly controlled microfluidic procedure**;
- To assess composite microfibers for **cell embedding**;
- To evaluate the **osteogenic potential** of entrapped cells inside engineered microfibers.

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

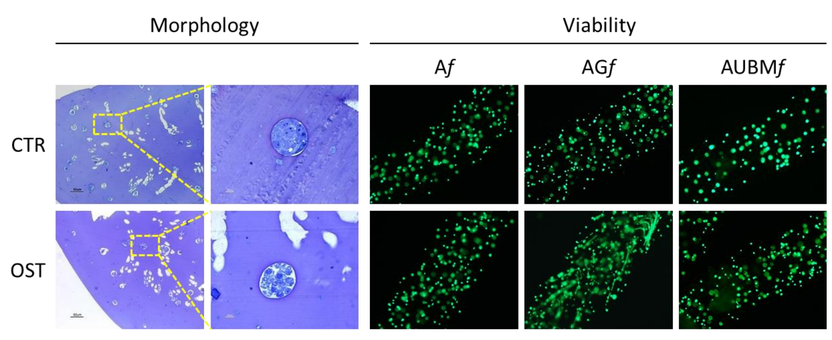
1. Microfluidic chip and microfibers' preparation



A) Schematic representation of the microchip employed for the microfluidic production of composite microfibers. At the right side a magnification of the micromixing snake is shown: thys system allows an homogeneous distribution of particles. ***B)*** The microfluidic procedure for the microfibers' production and cell embedding. At the down left side photomicrographs of alginate (A*f*), alginate plus gelatin (AG*f*) and alginate plus UBM (AUBM*f*) microfibers are reported after Coomassie Blue staining. **(C)** Dimensional analysis on microfibers' diameters in relation to the flow rate of alginate suspension.

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 anotehr 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 $BaCl_2$ bath were the alginate stream was gelled to produce composite microfibers ***(B)***. The MC3 channel geometry allows an optimal and homogeneous distribution of cells or ECM-particles along alginate fibers 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 morphological and dimensional 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 diameteres, not affecting structural and morphological characteristics of the microfibrous scaffolds.

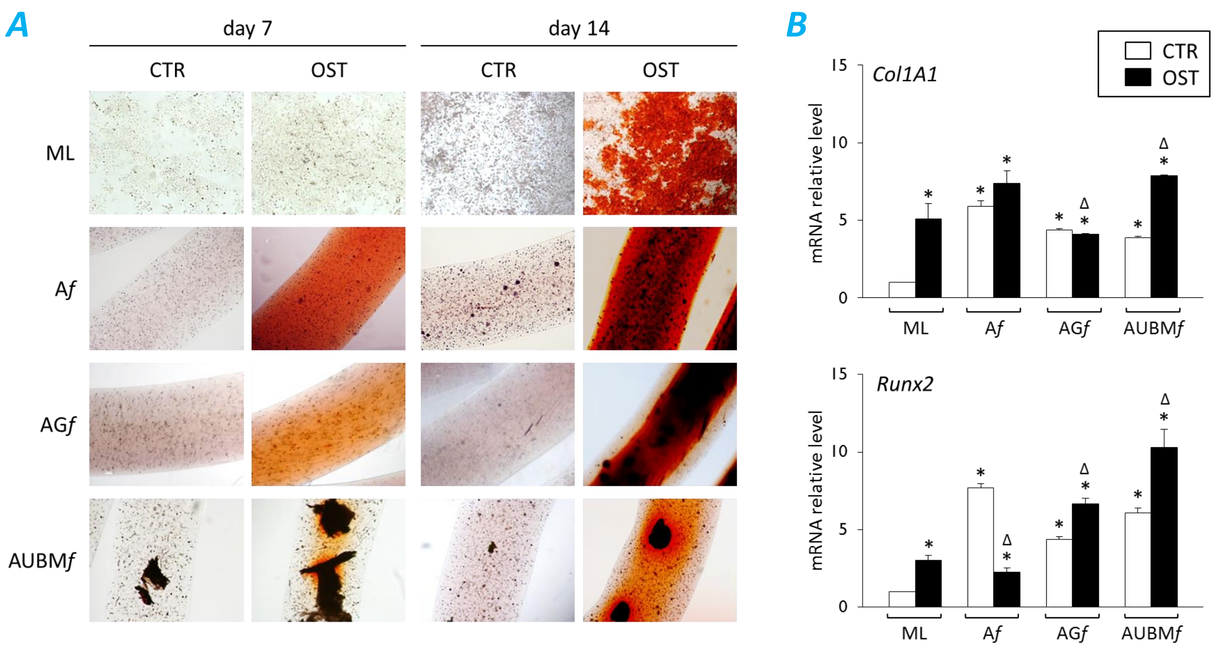
2. Cell embedding in composite microfibers



Morphology and viability analysis of SaOS-2 embedded cells inside microfibers after 14 days in basal (CTR) or osteogenic (OST) medium. For morphology microfibers were embedded in acrylic resin and specimen were cut into 5 µm slices and stained with toluidine. Viability assay was performed by calcein-AM (green)/propidium iodide (red) double staining.

SaOS-2 cells resulted evenly distributed within the composite microfibers and aquire 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 A*f*, AG*f* and AUBM*f* embedded cells began to produce mineralized matrix since day 7 and progress up to day 14. In AUBM*f* the deposition of mineral matrix appears to be co-localized with UBM particles, whose surface is strongly positive to the Alizarin Red staining ***(A)***. Also gene expression analysis of two osteogenic markers (Col1A1 and Runx2) confirmed the osteogenic potential of embedded cells: A*f*, AG*f* and AUBM*f* embedded 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 ***(B)***.

(A) Alizarin Red Staining of SaOS-2 cells in monolayered (ML) or embedded condition after 7 or 14 days in basal (CTR) or osteogenic (OST) medium. Red areas indicated the presence of mineralized matrix. **(B)** Gene expression analysis of collagen type 1 (Col1A1) and Runx2 in monolayered (ML) or embedded SaOS-2 cells after 14 days of culture in basal (CTR) or osteogenic (OST) medium. Statistical analysis was performed for each condition versus monolayer (*) or for osteogenic conditions versus relative control (Δ). p-values ≤ 0.05 were considered statistically significant.

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