Immunomodulation Potential of a Novel Bilayer Hybrid Biomaterial for Oral Regeneration

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Introduction

A big challenge in oral surgery includes the bioengineering of biomaterials that simultaneously promote soft and hard tissue regeneration, while stimulating a pro-regenerative immune phenotype to support tissue remodeling. A strontium-rich hybrid system was developed, composed of Sr-doped HAp microspheres, delivered in an alginate vehicle. Herein a bilayer system based on the latter was developed, aiming to promote both gingival and bone tissue regeneration. This system was further enriched with decellularized fetal membranes (dFMs).

Objectives

Evaluate the immunomodulatory potential of a bilayer strontium-hybrid system doped with dFMs.

Materials and Methods

A triton-X-based decellularization was performed. The physico-chemical integrity and absence of nuclei was analyzed by histology, electronic microscopy, atomic force microscopy and Fourier transform infrared spectroscopy analysis. Macrophage inflammatory response was evaluated by flow cytometry and ELISA assays. Statistically analysis was performed using Kruskal-wallis test. .

Key-Words

Decellularization, Fetal Membranes, Alginate, Strontium, Nano-Hydroxyapatite, Inflammation, Macrophages

Bilayer injectable hybrid polymeric-ceramic doped with fetal membranes

Biomaterial Preparation

VEHICLE PREPARATION FOR BOTH LAYERS

NATURAL BIOMIMETIC ECM

STRONTIUM ALGINATE HYDROGEL

Sr^{2+}

SOLUTION TRIGGER

D-(+)-GLUCONIC ACID Δ-LACTONE

PEPTIDE

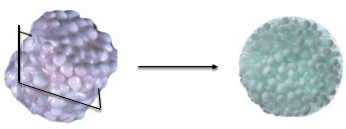
RGD (ARG-GLY-ASP)

BIOACTIVE FACTORS

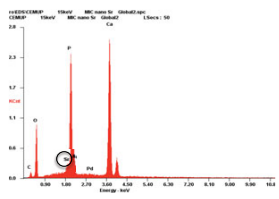
FETAL MEMBRANES PARTICLES

Sr-hybrid system for sustained Sr^{2+} local delivery – bone reinforcement

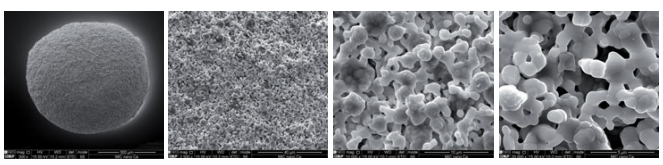
Sr^{2+} release from Alginate and Microspheres



Uniform pores between particles (ca. 220 µm)

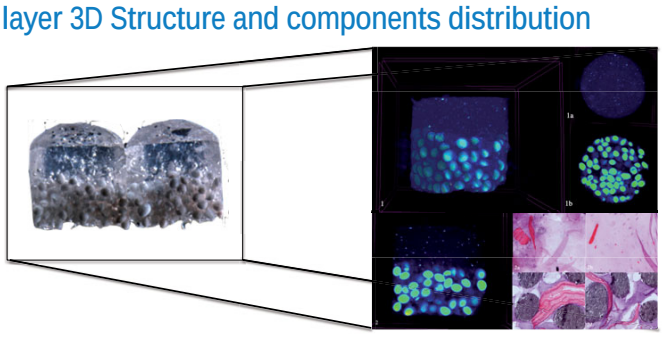


Sr^{2+} NANO-HIDROXYAPATITE MICROSPHERES



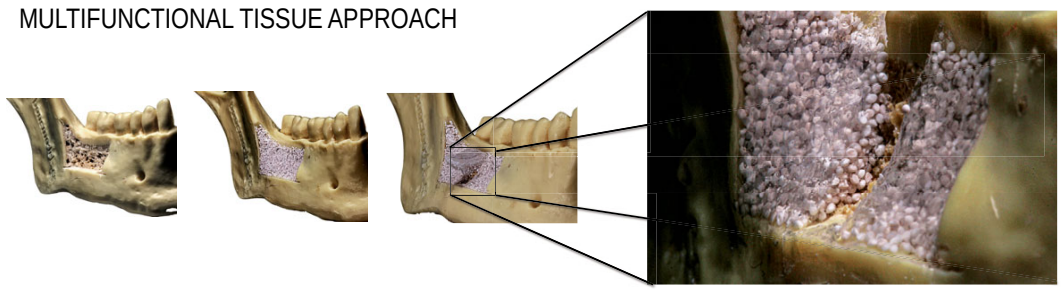
Stimulates osteoblastogenesis; inhibits osteoclastogenesis; immunomodulatory properties

Bilayer 3D Structure and components distribution



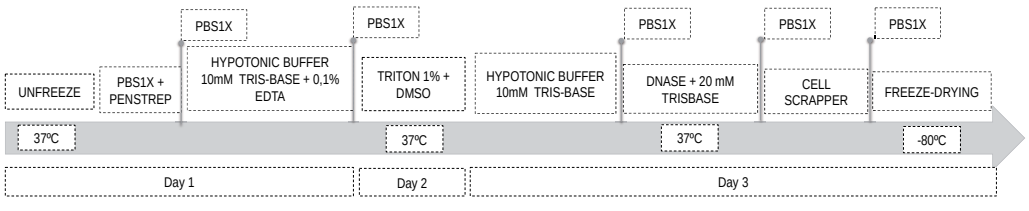
MicroCT and Histological analysis show a uniform dispersion of microspheres and dFMs.

MULTIFUNCTIONAL TISSUE APPROACH



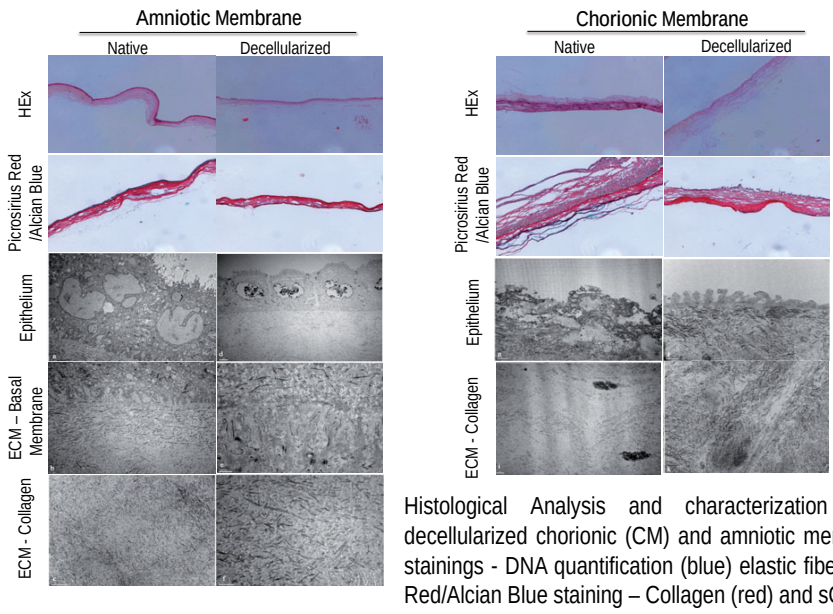
Fetal Membranes – Amniotic and Chorionic membranes

Decellularization Protocol



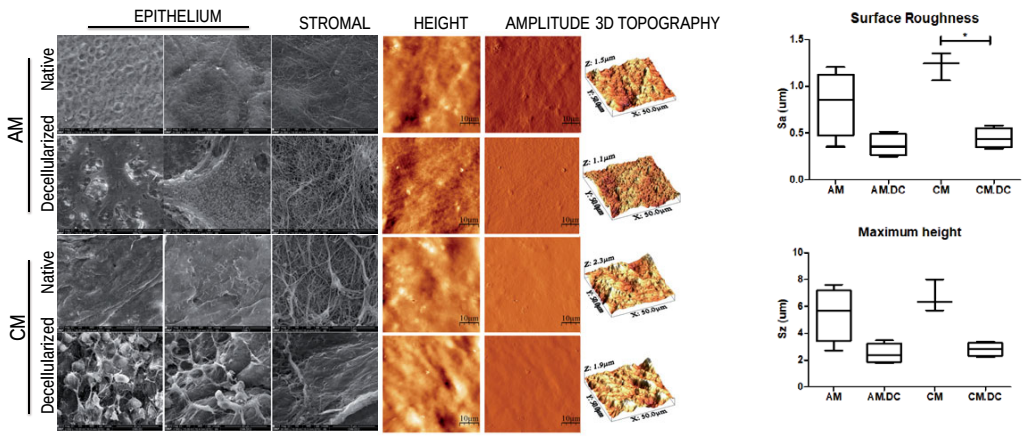
Results and Discussion

Evaluate the effect of the decellularization in the fetal membranes



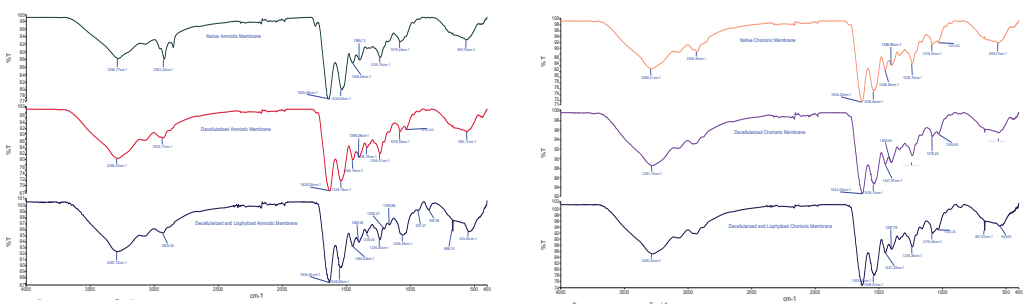
Histological Analysis and characterization of the resulting decellularized chorionic (CM) and amniotic membranes (AM). H&E stainings - DNA quantification (blue) elastic fibers (red) - Picrosirius Red/Alcian Blue staining – Collagen (red) and sGAGs(blue)

Physical Characterization of dFMs –SEM-EDS and Atomic Force Microscopy



SEM results confirmed the efficacy of the decellularization method used showing empty nucleous and the morphology of the membranes preserved. AFM analysis showed significant differences in roughness (*p< 0.05) between CM and CM native and decellularized samples (Sa).

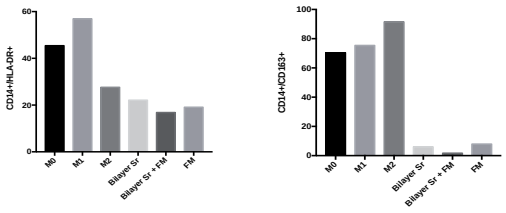
Chemical Characterization of dFMs - Fourier Transform Infrared Spectroscopy analysis (FTIR)



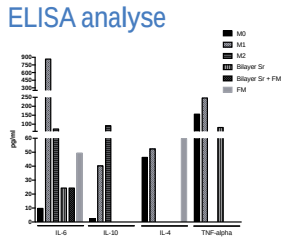
Bands at approximately 2960 cm^{-1} that are assigned to an assymmetric stretching mode of CH3 group decrease after decellularization possible due to cells loss in the membrane matrices.

Local immunomodulatory response (preliminary studies)

Flow Cytometry Analysis



ELISA analyse



Expression of cell surface markers of macrophages differentiation on Scaffolds (Bilayer Sr, Bilayer Sr + FM, FM) and the control groups (M0, M1, M2). Stimulation with LPS and IL-10 in control groups M1 and M2, respectively, were performed

Results

The effectiveness of the decellularization process was confirmed by the absence of nuclei and maintenance of its chemical structural integrity. The preliminary results indicated a low macrophage activation and a decrease of TNF-α, IL-4 and IL-6 secretion upon dFMs integration.

Discussion

The incorporation of dFMs into a biomaterial showed to be an interesting strategy for tissue regeneration. Preliminary results concerning immunomodulatory properties indicated low macrophage activation.

Conclusions

The dFMs incorporation into a biomaterial showed to be a promising multifunctional tissue approach. Further tests should be performed to explore the immunomodulation capacity of the biomaterial.

Clinical Implications

Concerning the innovative biomaterial design, the understanding of biological approaches to mitigate the foreign body response and drive the tissue inflammation into a pro-regenerative phenotype is essential..