Development of nanostructured 3D matrices to direct mesenchymal stem cells behaviour

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Introduction

New strategies for bone regeneration therapies evoke the development of improved biomaterials that reproduce key functions of the natural extracellular matrix (ECM), which serves both as a structural support for cells and as a dynamic biochemical network that directs cellular activities.
In this study, chemical functionalization of alginate hydrogels with an osteogenic signaling peptide was investigated. The selected peptide is based in the C-terminal sequence of OGP (Osteogenic Growth Peptide), known to increase bone mass and fracture healing in vivo, and to regulate cell proliferation and osteogenic differentiation in vitro [1]. Generally designated by OGP_{10-14} (Tyr-Gly-Phe-Gly-Gly), this five amino-acid sequence retains full bioactivity, being responsible for binding to the OGP receptor [1]. OGP-alginate was further combined with alginate modified with a cell-adhesion peptide (RGD-alginate), previously shown to enhance the viability and osteogenic differentiation of cells in the entrapped state [2] and with alginate modified with a protease-sensitive peptide (PVGLIG-alginate) to allow cells to partially remodel the hydrogel and spread within the matrix. Alginate hydrogels containing the three peptides were used as a 3D matrix for culturing human mesenchymal stem cells (hMSC), and the effect of these multifunctional microenvironments in cell behaviour, namely on osteogenic differentiation was investigated.

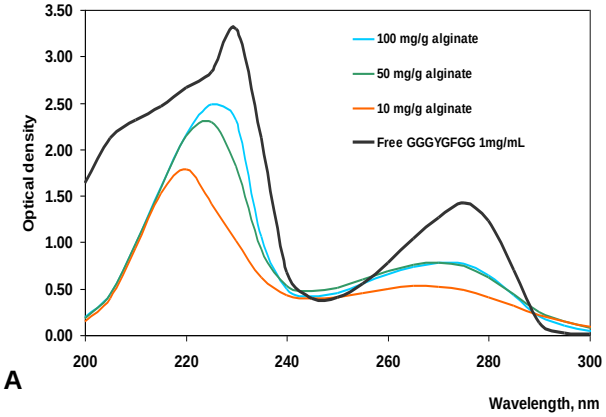
Materials and Methods

Alginate (Protanal LF 20/40, FMC Biopolymers was bulk-functionalized with the peptide sequence GGGYGFGG (OGP10-14 with a poly-glycine spacer, GenScript) using standard aqueous carbodiimide chemistry [2]. Different amounts of peptide (10, 50 and 100 mg) per gram of alginate were used. The amount of coupled peptides was analyzed by UV spectroscopy and by using the bicinchoninic acid (BCA) assay. 3D cultures of hMSC within multifunctional alginate hydrogels were established by combining cells with gel precursor solutions prior to polymerization.
Cells inside the discs were cultured under basal and osteogenic induction conditions for up to 16 days. Osteogenic differentiation was analyzed by assaying alkaline phosphatase activity (ALP) using a cytochemical staining in situ.

Results and Discussion

UV spectroscopy and the BCA acid assay showed that the osteogenic peptide sequence was effectively grafted to alginate, and that the coupled amount increased with the amount of peptide initially available for reaction. The reaction yields varied from 84±3% (10 mg/g alginate) to 46±4% (100 mg/g alginate) (Figure 1). hMSC were cultured within RGD-alginate, PVGLIG/RGD-alginate and OGP/PVGLIG/RGD-alginate discs. Cells entrapped within alginate discs were only able to remodel the hydrogel matrix and spread when PVGLIG-alginate was used in combination with RGD-alginate.
The osteogenic differentiation of 3D cultured hMSC was analysed through ALP activity along the time of culture in cells entrapped in RGD-alginate, PVGLIG/RGD-alginate and OGP/PVGLIG/RGD-alginate discs, under basal and osteogenic induction conditions. In figure 2 is possible to observe that ALP activity increase along the time, especially under osteogenic induction conditions and in the presence of OGP_{10-14}. Moreover, under the same conditions, the degradation of the hydrogels was accelerated;

References
[1] Chen Y-C et al. J Peptide Res 2000;56:147-156;
[2] Evangelista MB et al. Biomaterials 2007;28:3644-3655;



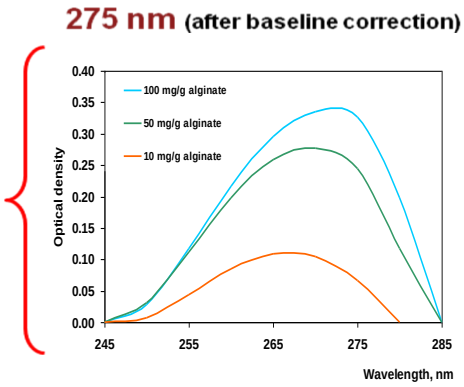


Table 1.OGP concentration in 1%wt alginate solution.

Available, mg/mL	Immobilized, mg/mL	Yield, %
1.000	0.379	46±4
0.500	0.165	50±4
0.100	0.039	84±3

B

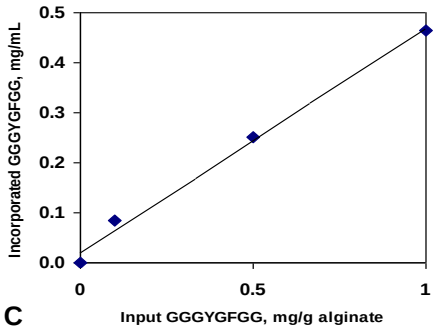


Figure 1. (A) The presence of peaks around 230/275 nm was observed, which indicates that the peptide was effectively grafted to the polymer backbone. (B) Table 1. lists the amount of immobilized OGP10-14 obtained using different initial amounts of peptide and the respective reaction yields. (C) A linear relationship between the amount of immobilized peptide and that initially available for reaction was obtained.

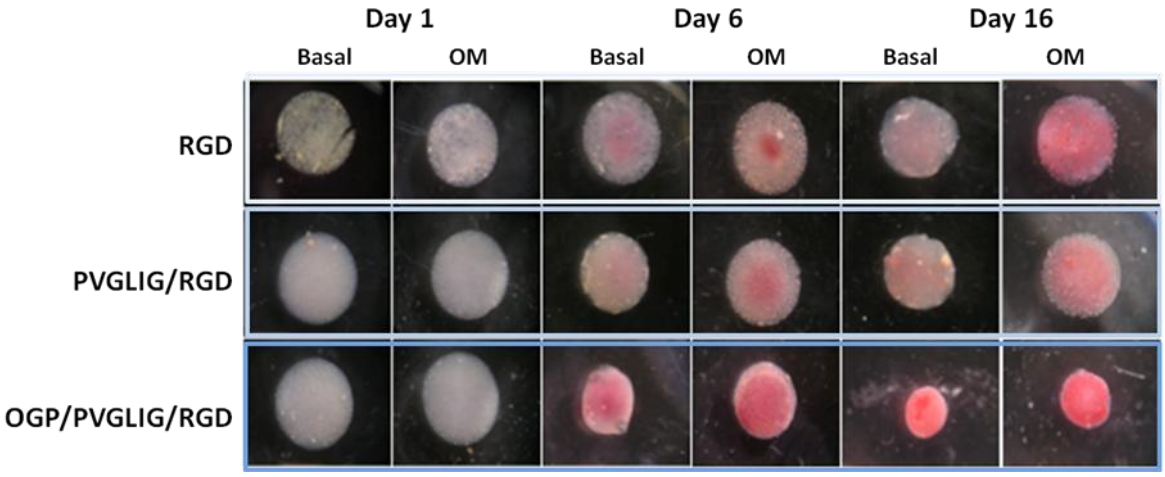


Figure 2. Expression of ALP activity along the time in cells entrapped in RGD-alginate, PVGLIG/RGD-alginate and OGP/PVGLIG/RGD-alginate discs, under basal and osteogenic induction conditions. ALP activity increase along the time, especially under osteogenic induction conditions, and in the presence of OGP_{10-14}. Moreover, in the presence of OGP_{10-14} the degradation of the hydrogels was faster (original magnification 25x).