Universidade do Minho
Escola de Engenharia

Semana da Escola de Engenharia
October 24 - 27, 2011

MICROPATTERNING OF BIOACTIVE GLASS NANOPARTICLES ON CHITOSAN MEMBRANES FOR SPATIAL CONTROLLED BIOMINERALIZATION

Gisela M. Luz[1,2], Luciano Boesel[3], Aránzazu del Campo[3], João F. Mano[1,2,a]

[1] 3B's Research Group, AvePark, 4806-909 Taipas, Guimarães, Portugal;
[2] IBB - Institute for Biotechnology and Bioengineering, PT Associated Laboratory, Guimarães, Portugal;
[3] Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany.

[a] jmano@dep.uminho.pt

KEYWORDS

Microcontact printing, micropatternings, bioactive glass, bioactivity, nanoparticles.

ABSTRACT

Bioactive glass nanoparticles (BG-NPs) capable of bone regeneration were patterned on biocompatible chitosan free-standing films by a microcontact printing technique, using a PDMS stamp. After "inking" the elastomeric stamp in a BG-NPs pad, it was pressed against the polymeric membrane substrate and then lifted-off, in order to transfer a perfectly defined bioactive micropattern.
The formation of the patterns was controlled by scanning electron microscopy (SEM). The mineralization of the bioactive glass patterns was induced in vitro by soaking the samples in simulated body fluid (SBF) over different time points up to 7 days. The confined mineralization was confirmed by Fourier Transform Infrared Spectroscopy (FTIR), Energy dispersive X-ray analysis (EDX) and SEM. Cell viability and attachment were studied through MTS test, fluorescence microscopy and SEM.

INTRODUCTION

Microcontact printing is a biocompatible technique, suitable to be used in biological applications. Exposed to the right surface chemistry and roughness and consequent hydrophicility, cells will adhere, proliferate, differentiate, and synthesize the proteins required to that process. (Anselme, Bigerelle et al. 2000; Prodanov, te Riet et al. 2010) Recognizing that the pattern of a surface may improve the cellular response makes it critical to control the surface pattern of a biomaterial in order to assure proper host tissue integration. In this work we developed chitosan membranes micropatterned with BG-NPs capable of inducing the growth of apatite. Due to their osteoinductivity, osteoconductivity, and osteointegration potential, bioactive glasses are excellent materials to template mineralization. (Seyedjafari, Soleimani et al. 2010)

RESULTS AND DISCUSSION

Bioactivity study

Bioactivity of the BG-NPs patterns was assessed *in vitro* by analyzing the ability of calcium phosphates formation upon immersion in SBF.
By performing EDX analysis, one can observe that Si decreases over time, while Ca and P values increases due to apatite growth, Fig. 1 (a).
FTIR analysis, Fig. 1 (c) presents the spectra related to the powder scratched from the printed membranes after 0 and 7 days of immersion in SBF. The amorphous peak in the control sample at around 600 cm^{-1}, after 7 days in SBF evolves to two peaks accusing the cristallinity of the hydroxyapatite phosphate groups. SEM confirms the formation at the end of seven days of study, of a cauliflower-like cluster, formed by needle-like crystals that are the tipical hydroxyapatite conformation. See evolution from day 0, Fig. 1 (b), till day 7, Fig. 1 (d). The mineralization occurred only in the spots that formed the initial BG-NPs pattern.
.

Semana da Escola de Engenharia
October 24 - 27, 2011

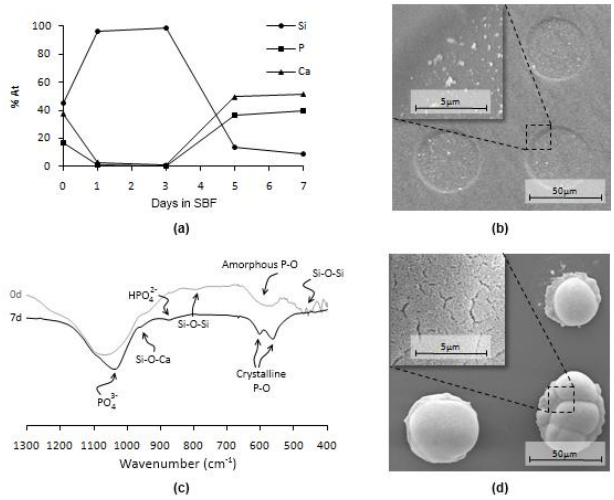

Figure 1: **(a)** EDX analysis of the BG-NPS patterned membranes soaked in SBF for 0, 1, 3 5 and 7 days. **(b)** Original pattern. **(c)** FTIR spectra of the powder scratched from the surface of the mCP membranes after 0 (control) and 7 days of immersion in SBF. **(d)** SEM images of the typical apatite clusters after 5 days of soaking of the patterned chitosan membranes in SBF.

Viability and cell attachment

In vitro tests confirmed that the viability of L929 cells is higher in the BG-NPs patterned membranes than in the plain chitosan surface. See MTS test results in Fig. 2 (c). Fluorescence images, Fig. 2 (a) and (b) show that the cells attach only in the areas printed with BG-NPs.

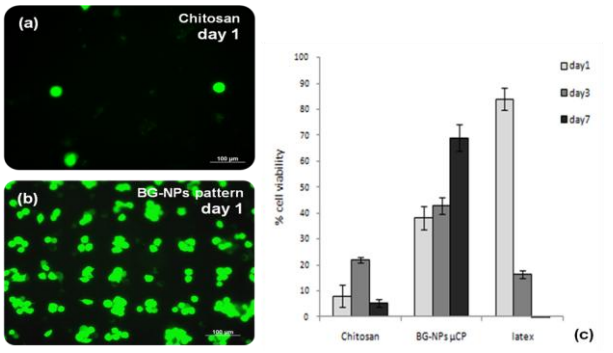

Figure 2: Fluorescent images of cell stained with Calcein AM after 1 day of culture in plain chitosan **(a)** and BG-NPs patterned membranes **(b)**; **(c)** Cell viability results from MTS test.

These results prove the potential of spatial controlled biomineralization at the cellular level.

CONCLUSIONS

Total control of spatial biomineralization was achieved through micropatterning of bioactive glass nanoparticles on chitosan membranes. Moreover, the produced membranes proved to be a suitable substrate for cell growth, being the BG-NPs a highly reactive surface able to bond with living cells.

By combining the remarkable osteoproductive and osteoconductive properties of BG-NPs and the excellent biocompatibility of chitosan, with this simple microcontact printing approach, it was proved that it is possible to control the cellular interactions with a bioactive substrate at the microscale. Due to their ability to promote guided tissue regeneration in the bone area and controlled soft tissue growth, this BG-NPs micropatterned chitosan membranes have the potential to integrate third generation materials and also to open new possibilities in the coating of non-planar medical devices.

REFERENCES

Anselme, K., M. Bigerelle, et al. 2000. "Qualitative and quantitative study of human osteoblast adhesion on materials with various surface roughnesses." *Journal of Biomedical Materials Research* 49(2): 155-166.

Prodanov, L., J. te Riet, et al. 2010. "The interaction between nanoscale surface features and mechanical loading and its effect on osteoblast-like cells behavior." *Biomaterials* 31(30): 7758-7765.

Seyedjafari, E., M. Soleimani, et al. 2010. "Nanohydroxyapatite-Coated Electrospun Poly(L-lactide) Nanofibers Enhance Osteogenic Differentiation of Stem Cells and Induce Ectopic Bone Formation." *Biomacromolecules* 11(11): 3118-3125.