

Cell proliferation of human fibroblasts on calcium phosphate based ceramics

J. Marchi^{(1)*}, C. Ribeiro⁽²⁾, A. H. A. Bressiani⁽²⁾, J. C. Bressiani⁽²⁾ and M. M. Marques⁽³⁾

- (1) CCNH (Centro de Ciências Humanas e Naturais), Universidade Federal do ABC, R. Santa Adélia, 166, Bangu, Santo André, 09210-170, SP, Brazil, e-mail: juliana.marchi@ufabc.edu.br
 (2) CCTM (Centro de Ciência e Tecnologia de Materiais) - IPEN, Instituto de Pesquisas Energéticas e Nucleares. Av. Prof. Lineu Prestes, 2242, 05508-000, São Paulo, SP, Brazil.
 (3) Departamento de Dentística, Faculdade de Odontologia, Universidade de São Paulo, SP, Brazil
 *Corresponding author

Abstract – The aim of this work was to evaluate the *in vitro* biocompatibility of calcium phosphate ceramics containing different proportions of β -tricalcium phosphate and hydroxyapatite in the presence of human fibroblasts (FMM1). The results showed that these materials were biocompatible and may promote and enhance bone regeneration.

In general, combination of hydroxyapatite (HAp) and β -tricalcium phosphate (β -TCP) leads to bioactive and resorbable calcium phosphate ceramic, so that potential use of these materials in bone tissue repair can be enhanced. These ceramics can show variations in surface texture or microtopography. Also, surface chemistry may affect the cellular response to an implant. Cell adhesion is an important parameter not only in biologic development, but is also crucial in tissue engineering. If tissue is to be repaired, the new cells must be aligned and positioned correctly.

In this study, the effect of different ratios of HAp and TCP phases in the surface roughness and biological responses, such as cellular attachment and proliferation was evaluated. Calcium phosphates sintered samples in different ratios (25%wt HAp, 50%wt HAp, 75%wt HAp) and monophasic compositions (HAp and TCP) were obtained to investigate the role of composition and surface characteristics in cellular behavior of fibroblasts derived from human gingival FMM1 cell line. Final density and roughness of each sample were determined after sintering. The cells were cultured in Dulbecco's modified Eagle medium supplemented by 10% fetal bovine serum and 1% antibiotic-antimycotic solution. The cells were maintained in an incubator at 37 °C and under a humidified 5 % CO₂ atmosphere. Cell adhesion and cell proliferation assays were carried out with 1.10⁵ fibroblasts plated on the top of each sample. Cultures were fed every other day. For the proliferation assay, the number of cells adhered to the ceramic samples of each experimental group, in triplicate, was counted one, two and three days after seeding after SEM observations. The cells were then counted using the Image Pro Plus for Windows Computational Image Software. The data were compared by ANOVA followed by the Tukey's test. This method, previously determined by Feist et al [1], is allowed for cell counting on the surface of each sample. Independently of the composition, the overall morphology of the FMM1 cells was similar (Fig.1). The cells appeared as elongated, flat with either bipolar spindle shape or dendritic morphology. Moreover, the cells were spread and well-attached to the surfaces. The counting provided data for plotting the cell growth graphs (Fig.2). The present study demonstrated that all compositions were non cytotoxic, biocompatible and may promote and enhance bone regeneration. Moreover, β -TCP presented higher cell proliferation, being observed confluence period after three days in culture.

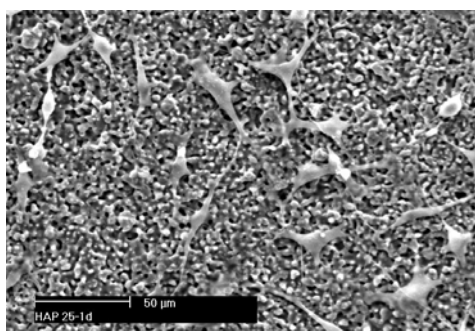


Figure 1: Typical scanning electron micrographs of cell growth on calcium phosphate substrates

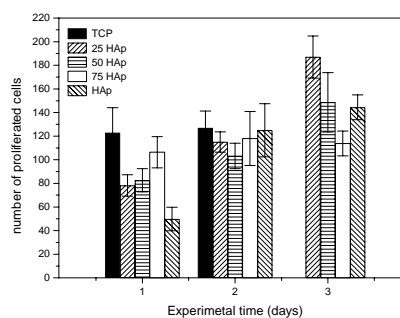


Figure 2: FMM1 cell growth on calcium phosphate substrates after 1, 2 and 3 days

References

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 [2] J. Marchi, CS Delfino, J.C. Bressiani, A.H.A. Bressiani and MM Marques, Int. J. App. Ceram Tech (2009), in press