

## Cells adhesion and proliferation in chitosan membranes with hydroxyapatite coating

A. F. Fraga<sup>(1)\*</sup>, H. S. Tavares<sup>(2)</sup>, E. C. S. Rigo<sup>(3)</sup>

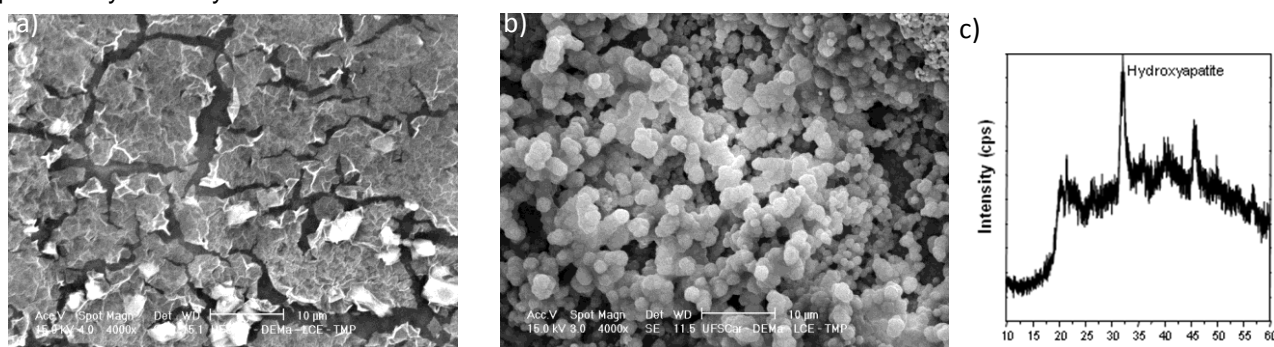
(1) Universidade Federal de São Carlos – UFSCar – Departamento de Engenharia de Materiais – São Carlos, SP, Brazil, email: [affraga@yahoo.com.br](mailto:affraga@yahoo.com.br)

(2) Universidade Estadual Paulista – UNESP – Instituto de química – Departamento de Biotecnologia – Araraquara, SP, Brazil.

(3) Universidade de São Paulo – USP – Faculdade de Zootecnia e Engenharia de Alimentos – Departamento de Ciências Básicas, Pirassununga, SP, Brazil.

**Abstract-** The aim of this work was to obtain membranes of chitosan coated with hydroxyapatite by biomimetic process using solution of sodium silicate as nucleation agent. The membranes were characterized by scanning electron microscopy (SEM), X ray diffraction (XRD) and infrared Fourier transformed (DRIFT). The solution of sodium silicate as nucleation agent was effective to prepare a dense well-defined layer of hydroxyapatite. After coating the membranes was submitted the culture cells with human stem cells. The membrane presented cell adhesion and proliferation and don't present cytotoxicity.

The hydroxyapatite-HA should be highlighted by the main constituent of mineral phase of calcified tissues. Dense grains or porous form of HA is used as filling of cavities and also as coating of metallic implants and periodontal membranes. This work describes a new methodology to produce bioactive coatings on the surface of chitosan membrane by biomimetic method using sodium silicate solution with nucleating agent. Prior to immersion in SBF, the membrane is subjected to soaking in sodium silicate solution (SS) [1]. This treatment creates a number of calcium phosphate precursor sites over the surface, which will eventually create favorable condition for the nucleation and growth of HA. Figure 1a shows the surface of chitosan membrane, the morphological analysis showed an irregular structure, derived from the natural characteristics of the polymer [2]. The sample presents dense structure without porous formation and also with high roughness surface. Figure 1b shows the surface obtained after HA coating in membranes previously treated in silicate solution. As it can be observed, a dense and effective coating was obtained in the membrane surface. This morphology was found by several authors and is an indication of the predominance of hydroxyapatite phase [3]. Figure 2c indicates a trend of HA more crystalline. This behavior was expected due to the time of exposure in solutions of sodium silicate and 1.5 SBF. During that time, there was a solubilization and new precipitation of HA observed by SEM, that provided the slow nucleation and thus the growth of crystals, resulting in a crystalline phase of HA. This results indicates that sodium silicate solution play an important role in the HA deposition from SBF solution. After coating the membranes was submitted the culture cells with human stem cells. The membrane presented cell adhesion and proliferation and don't present cytotoxicity.



**Figure 1** – SEM of the superficial area of chitosan membranes. a) Chitosan membranes without coatings b) Chitosan membranes immersed in sodium silicate and coating in 1,5 SBF solution. c) XRD of Chitosan membranes immersed in sodium silicate and coating in 1,5 SBF solution.

[1].RIGO, E.C.S, BOSCHI, A.O., YOSHIMOTO, M,et al. Materials science & engineering. C., p.647 - 651, (2004).

[2] SIGNINI R., CAMPANA FILHO, S.P., Polímeros: Ciência e Tecnologia, v. 11, n. 2, p. 58-64, (2001).

[3] ABE, Y.; Kokubo, T.; Yamamuro, T. Journal of materials science-materials in medicine., v. 1, p. 536-540, (1990).