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INTERACTION STUDIES OF MIXED MATRICES OF CHITOSAN-POLY- ε -CAPROLACTONE AND ALENDRONATE FOR BONE TISSUE ENGINEERING

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INTRODUCTION

Tissue engineering actual tendencies leads to the development of biocompatible matrices with accurate physical and mechanical properties in bone reconstruction. As a regeneration of a new tissue is achieved, the scaffold is no longer needed and so it is reasonable to use biodegradable scaffolds [1]. The rate of degradation must be in parallel with the tissue regeneration, and is very important to provide long term construct biocompatibility, because only natural tissue will remain in the body–a neo-organ. In this context one of the most common compound used is the natural polymer chitosan, whose mechanical properties can be improved by adding synthetic polymers [2]. The great interest in this macromolecule is due to its proved biocompatibility and biodegradation properties [3].

Matrix also requires the capacity to transport osteogenic agents which enhance bone regeneration. Bisphosphonates are a new class of synthetic compounds structurally related to pyrophosphate, an endogenous modulator in homeostasis of calcium, and they are clinically used for various metabolic bone disorders such as Paget's disease, hypercalcemia of malignancy, bone metastasis and osteoporosis [4]. The reduced targetability of some bisphosphonates in relationship to the dose increased and its hepatosplenic accumulation has been reported [5]. It is due to high precipitability with divalent ions in the circulation in blood plasma, which may be taken up by reticuloendothelial system as foreign substances [6]. Therefore, new drug delivery systems are needed to overcome these problems.

The aim of our work is the development of a scaffold for tissue engineering based in chitosan/poly-ε-caprolactone blend which contains an adequate concentration of alendronate (a nitrogen bisphosphonate) for osteoblastic bone growth without toxic effects.

MATERIALS and METHODS

Materials. High molecular weight Chitosan (Ch) and poly-ε-caprolactone (PECL) were purchased from Aldrich. The viscometric average molecular weight of Ch is 780 kD and 21% DA (determined by FTIR). Weight average molecular weight and polydispersity index of PECL is 65000 and 1.4 respectively, as indicating by the manufacturing. Tissue culture flasks and dishes were purchased from Corning (Princeton, NJ, USA); Dulbecco's

Modified Eagles Medium (DMEM) and trypsin-EDTA were purchased from Gibco (Gaithersburg, MD, USA). Fetal bovine serum (FBS) was obtained from Gen (Buenos Aires, Argentina).

Matrices preparation. Blend of PECL and chitosan were prepared by solvent casting according to Sarasam et al [7]: 9 ml of a polyester solution in acetic acid (0.3 % w/w) were added to 3 ml of 0.5% w/v of chitosan in dilute acetic acid (3 % v/v). Blends with different concentrations of alendronate were prepared (1, 2, 5, 10 % w/w), designed as ChEA1, ChEA2, ChEA5 and ChEA10 respectively. The solution of the blend were poured onto glass Petri dishes (5 cm diameter), and the solvent was allowed to evaporate at room temperature, then under vacuum until constant weight, and stored in desiccators until use.

Matrix characterization. Scanning electronic microscopy (SEM) was employed to observe the morphology of the matrices .The surfaces of the matrices were coated with gold and their morphology was examined using scanning electron microscopy (SEM, Phillips505, Holland), with a accelerating voltage of 20 kV, and the images were analyzed by Soft Imaging System ADDAII. FTIR spectra of films were recorded on a Nicolet 380 FTIR between 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans of accumulation. EZ-OMNIC software was used by analyzed the spectra.

Biocompatibility studies. Matrices of the blends were sterilized with UV radiation before cells seeding. UMR106 osteosarcoma rat cells in the culture flask were grew to be confluent in DMEM containing 10 % FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37ºC in a 5 % CO_2 atmosphere [8]. Cells were sub-cultured using trypsin-EDTA. The UMR106 cell line has been shown to conserve certain characteristics of differentiated osteoblastic phenotype [9]. For the different experiments, cells were seeded on polymeric matrix-coated dishes and incubated in 10% FBS medium for 24 hours.

Cell growth was estimated by staining the cells with Giemsa [10].

Cellular differentiation was investigated after 24 o 48 h culture by means of collagen production (Sirius red stain) and osteoblast marker Alkaline phosphatase (ALP). For ALP marker cells were lysed with 0,1% Triton – X100. We determined protein content (by Bradford Method) and ALP specific activity (by p-nitrophenyl phosphate to p-nitrophenol hydrolysis) with the extract aliquots.

RESULTS and DISCUSSION

Blending Ch/PECL with different concentration of alendronate allowed us obtaining slightly porous matrices with well interconnected pores as shown by SEM images in Figure 1(A and B).

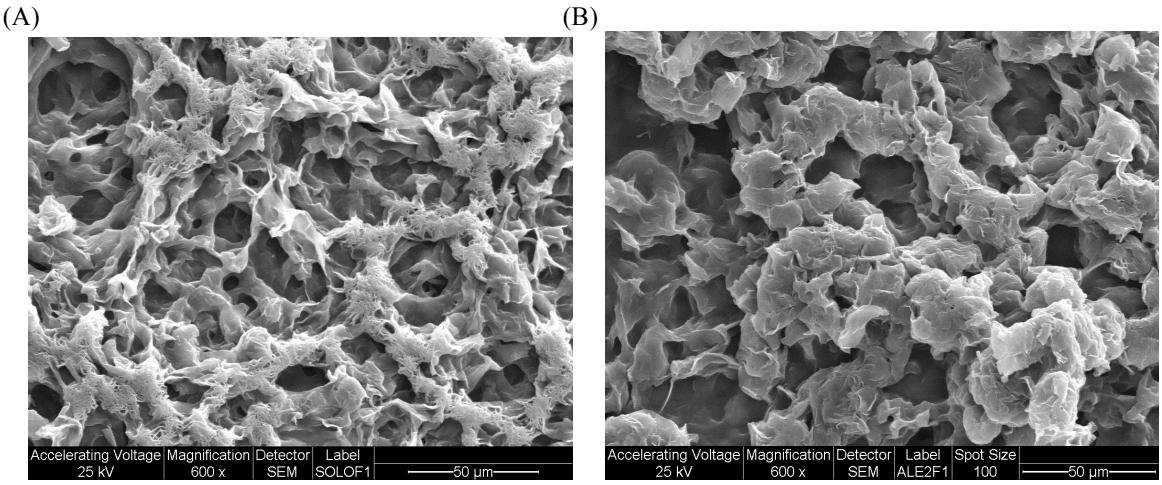

Figure 1. ChE (A) and ChEA (B) matrix surface. Magnification x 600.

The superficial characterization of the ChE and ChEA matrices showed a change in micromorphology as the amount of alendronate in the blend increases. The ChE matrix (Fig. 1A) shows more irregular surface morphology than that of the ChEA. It is quite different in the case of the matrices containing alendronate, with ChEA2 (Fig. 1B) presenting a skeletal structure composed of small platelet-balls.

The FTIR spectra of Ch, ChE and ChEA2 are shown in Figure 2 between 1900 and 400 cm^{-1}. Chitosan spectrum show two typical bands at 1640 and 1556 cm^{-1} attributed to amide I and amide II respectively. The spectra of blends exhibited a characteristic band of carbonyl group of PECL at 1725 cm^{-1} and a shoulder at about 1690 cm^{-1}. This shoulder band is attributable to the hydrogen bonded carbonyl groups with hydrogen-donating groups (–OH and $–NH_2$) of Ch, as was observed by other researches [11]. In addition, the bands at 1315 cm^{-1} (stretching vibration of C-N of Ch) and 1377 cm^{-1} (bending vibration of O-H of Ch) showed shifted to lower wave numbers in the blends, 1294 and 1369 cm^{-1} respectively. These observation can also be attributed to hydrogen bond and demonstrate that physical interaction are present in the chitosan-poly-ε-caprolactone blends. The area analysis of 1556 and 1640 cm^{-1} bands (data not show) evince a significant change related with the chitosan-alendronate ionic interaction.

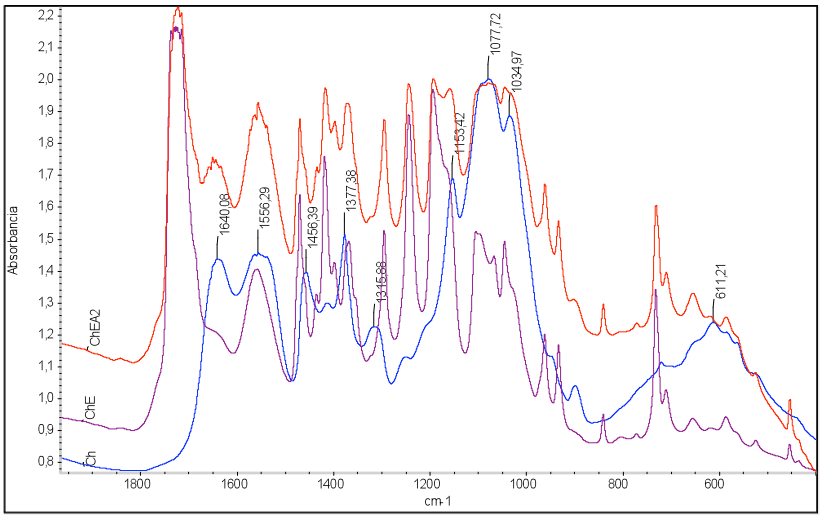

Figure 2. FTIR of Ch, ChE and ChEA2.

Cells grew well into the matrices showing normal morphology, being able to adhere and proliferate well on all the investigated matrices.

Cells growth well on the ChE film and were included diaper into the matrix (Figure 3A). The addition of alendronate in the ChE matrix induced a dose-dependent inhibition in the cell proliferation (Figure 3B and 3C).

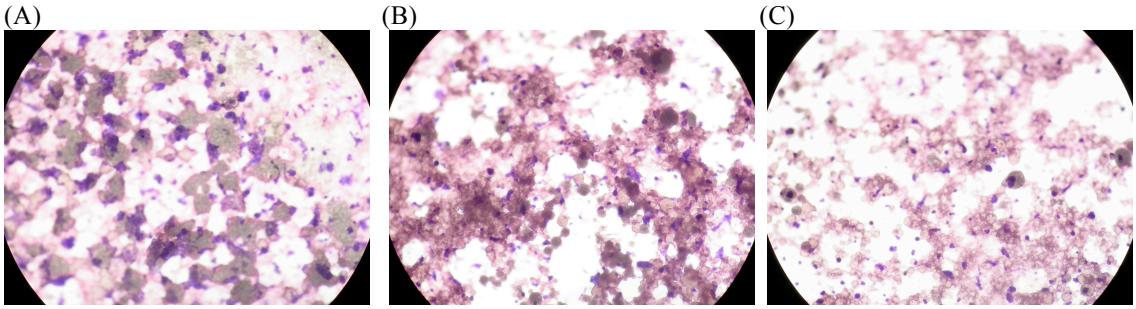

Figure 3. Microphotograph of UMR106 cells growing on ChE (A), ChEA2 (B) and ChEA5 (C), stained with Giemsa. Obj. 10X

We also evaluated the osteoblastic differentiating capacity of cells growing on different matrices. Figure 4 shows the expression of ALP, an osteoblastic marker, after 48h of culture. As can be seen, alendronate induce a dose-dependent reduction in this marker.

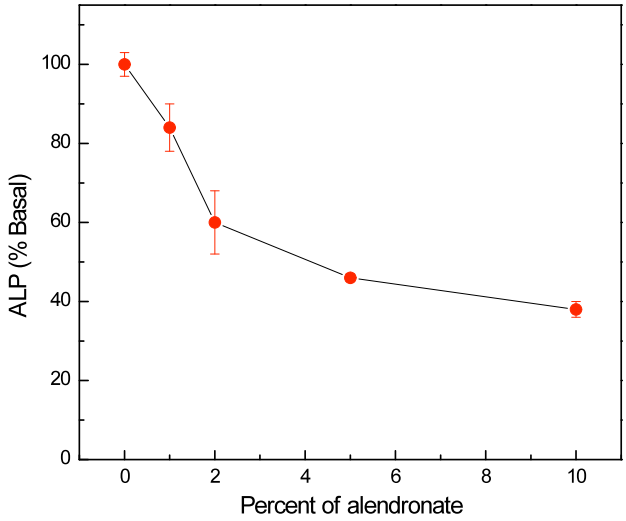

Figure 4. Effect of alendronate on ALP activity of UMR106 cell growing on different matrix

This observation suggests that although the polymeric films were able to support the progression of the cells, the alendronate probably released in the medium was exerting a cytotoxic effect. We have previously shown a similar inhibitory effect when UMR106 cells were cultured in the presence of 10^{-5}-10^{-4} M alendronate, suggesting a toxic effect [12]. Although we do not directly evaluate the alendronate released into the medium, it could be estimated that the ChEA2 matrix should release at less 10^{-5} M alendronate after for 48 hours of culture.

Our results, although preliminary, shows that mixed matrices prepared starting of chitosan and poly-ε-caprolatone blends presents slightly porous structure with good interpolymeric interactions and biocompatibility. The inclusion of alendronate exerts a dose-dependent inhibitory effect on the cell progression. With regard to this aspect, further in vitro experiments are in progress in order to investigate and develop controlled release system of alendronate.

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