New chitosan/Bioglass® composite membranes for guided tissue regeneration

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Introduction

A number of combinations of biodegradable natural-based polymers and bioactive ceramics have been proposed for orthopedic applications including in hard tissue regeneration [1,2]. It has been reported that this kind of composites impart strength and bioactivity to the system. Guided tissue regeneration (GTR) is a well known technique that repairs tissue defect or reconstructs new tissue by using a barrier membrane to protect the defect site from invasion of other tissue, such as fibrous connective tissue. If processed in the form of membranes, composite containing natural-based polymers could be very useful in (GTR) for the regeneration of periodontal tissues, bone around natural teeth and dental implants.

In this study, chitosan (CTS) was combined with Bioglass® (BG) to produce membranes by solvent casting. *In vitro* bioactivity tests were performed in composite membrane, where their capability to induce the precipitation of apatite upon immersion in simulated body fluid (SBF) was monitored. The process of biomineralization was also followed, for the first time, by dynamic mechanical analysis, DMA, measuring the variation of the viscoelastic properties of the composite membranes immersed in SBF, both on-line and off-line.

Materials and Methods

Chitosan (medium molecular weight) was purchased from Sigma-Aldrich, Germany, and was purified prior to use. For the composites membranes, 30%wt of BG was added to the chitosan (CHT). The solutions (1 %wt of material in 1 %wt aqueous acetic acid) were cast in Petri dishes and left to dry at room temperature. After drying, both chitosan (CTS) and composite membranes (CTS/BG) were peeled off and neutralised in a 0.1 M NaOH solution, washed thoroughly with distilled water and dried again. *In vitro* bioactivity tests were performed by immersing the membranes in simulated body fluid (SBF) for different period of time.

The materials prepared were physico-chemically characterized by SEM, EDS, FTIR and XRD. Non-conventional DMA experiments were performed using a TRITEC2000B DMA from Triton Technology (UK), equipped with the tensile mode [3]. The measurements were carried out at 37ºC and after the membranes were equilibrated in a liquid bath (SBF or PBS). DMA spectra were obtained during a frequency scan between 0.1 and 40 Hz. The calcification of the membranes in SBF was performed both online (continuous lecturing of the stora modulus and loss factor for 24 hours) and off-line (DMA tests after different immersion times in SBF until 5 days.

Cytotoxicity of the membranes was evaluated by culturing mouse lung fibroblast cell line (L929) with leachables from the materials and cultures of osteobloast-like cells (SaOs2) onto the membranes were performed to evaluate the biological response of the membranes.

Results and Discussion

Homogeneous membranes were produced using the solvent casting protocol. When BG was added, the particles were seen to disperse homogeneously over the composite membranes. Biologic studies were performed and indicated that the membranes didn't exhibit significant levels of cytotoxicity. After immersion in SBF for 1 day one could detect the deposition of apatite in the CHT/BG membrane. Both density and thickness of the apatite layer increased with increasing soaking time in SBF. So sign of bioactivity was seen in pure CHT membrane. Figure 1 shows SEM image of both CHT and CHT/BG membrane after 7 days of immersion in SBF.

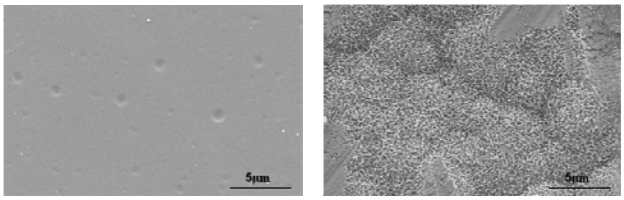

Figure 1: SEM image of the surface of the CHT (left) and CHT/BG (right) membranes after being soaked in SBF for 7 days.

DMA tests indicated for the first time that this technique could be used to follow *in situ* the evolution of the mechanical properties when the membranes were immersed in PBS or SBF. In the first case a clear reduction is seen in the storage modulus indicating that the BG is continuously dissolved. In SBF this process is observed in the first 500 min, but then the stiffness starts to recover due to the deposition of a stiff apatite layer. The changing in the viscoelastic properties, modelled using a simple mechanical model, was found consistent with the evolution of apatite formation as seen by SEM and by ICP analysis of the liquid medium.

In conclusion new chitosan/Bioglass® membranes were proposed in this work to be used in GTR. This work showed that innovative mechanical tests could be used to characterize the mechanical performance of composites under meaning full physiological conditions, including during the process of biomineralization.

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References: [1] J.F. Mano, et al., Comp. Sci. Tech. 64, 789 (2004); [2] J.F. Mano, et al. J.R.Soc. Interface, 4, 999 (2007); [3] S.G. Caridade, et al. Carbohyd. Polym. 75, 651 (2009)