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BIOMINERALIZATION PROCESS OF CHITOSAN/BIOGLASS ® MEMBRANES

Sofia G. Caridade, Esther G. Merino, Natália M. Alves and João F. Mano
3B's Research Group
E-mail: sofia.caridade@dep.uminho.pt

KEYWORDS
Bioactive membranes; composites; mineralization; dynamic mechanical analysis.

ABSTRACT
Nowadays, processing composites of biodegradable polymers with bioactive ceramics has become one of the strategies used for orthopaedic applications including in hard tissue regeneration due to the improvement on mechanical properties and bioactivity. The evolution of the biomineralization process only has been monitored in terms of bioactivity but never in terms of mechanical properties. In this sense, membranes of chitosan (CTS) and composite membranes of CTS with Bioglass® (BG) were prepared by solvent casting and characterized by Scanning Electron Microscopy (SEM). *In vitro* bioactivity tests were performed in both types of membranes, and their capability to induce the precipitation of apatite upon immersion in simulated body fluid (SBF) was monitored. The results showed that the addition of BG provided an osteoconductive character to the membranes. The biomineralization process was followed by measuring the variation of the viscoelastic properties of the composite membranes immersed in SBF, both online and offline. The change in the storage modulus, *E'*, and the loss factor, tan δ, were measured as a function of the immersion time using non-conventional dynamic mechanical analysis (DMA) tests.

INTRODUCTION
Over the years many efforts have been made to encounter biomaterials for improving life quality, namely in bone regeneration. The fabrication of composites comprising biodegradable polymers and bioactive glass becomes a suitable option to fulfill the requirements of bioactivity, degradability and mechanical strength (Marelli et al. 2010). When designing composites for bone regeneration it is very important that they fulfill the requirements above mentioned but also to understand the mechanisms that lead to a composite suitable for biomedical applications (Alves et al. 2010). The description of the biomineralization process in real time may provide information about the calcification kinetics and mechanism and could describe the evolution of the properties of the biomaterial. However until now, just few studies have followed the biomineralization process *in situ* (Leonor et al. 2003). In this study, CTS was combined with BG to produce membranes by solvent casting. *In vitro* bioactivity tests were performed in both types of membranes, where their capability to induce the precipitation of apatite upon immersion in 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
CTS was combined with BG to produce membranes by solvent casting. *In vitro* bioactivity tests were performed by immersing the membranes in SBF for different period of time. The materials prepared were characterized by SEM and EDS. Non-conventional DMA experiments were carried out at 37ºC and after the membranes were equilibrated in a bath of SBF. 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 *E'* and tan δ for 24 hours) and off-line (DMA tests after different immersion times in SBF until 7 days).

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. After immersion in SBF for 1 day one could detect the deposition of apatite in the CTS/BG membrane. No sign of bioactivity was seen in pure CTS membrane. Figure 1 shows SEM image and EDS of both CTS and CTS/BG membrane after immersion in SBF.

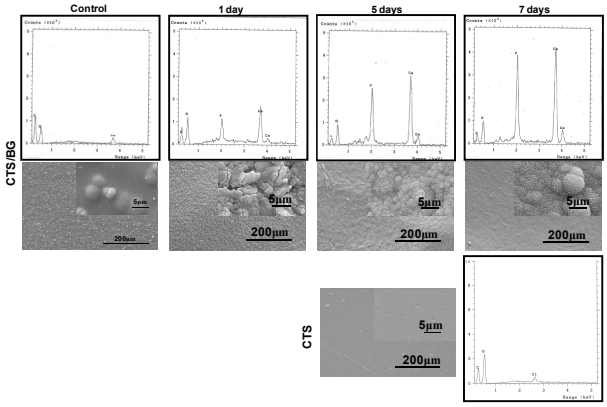

Figure 1: SEM image and EDS of the CTS and CTS/BG membranes after being soaked in SBF for a period of 7 days.

Offline experiments were carried out for long immersion periods: after soaking the membranes for a given time period in SBF. For the CTS membrane (Figure 2A) we can conclude that *E'* was not dependent on the previous immersion stage in SBF up to 7 days: all the scans are quite superimposed. A completely different behavior is observed for the CTS/BG membrane (Figure 2B). Before the static soaking stage in SBF the storage modulus of the CTS/BG membrane is higher than the one of the pure CTS membrane (Figure 2A), that could be explained by the reinforcement of the BG particles in the composite material. With increasing soaking time in SBF, it is clear that *E*' increases significantly. This stiffening effect induced by the immersion of the CTS/BG membranes in SBF should be a result of the development of an apatite layer over the two surfaces of the samples.

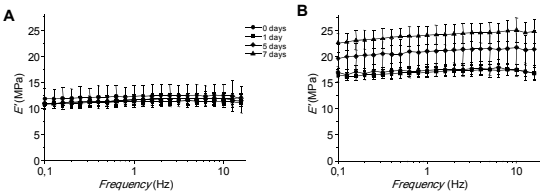

Figure 2: DMA scans obtained under immersion of A) CTS membranes and B) CTS/BG composite membranes in SBF at 37 ºC after being previously immersed in SBF for different time periods: ● - 0 days; ■ - 24 hours; ◆- 5 days; ▲ - 7 days.

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 SBF (Figure 3). For the CTS membranes no changes were observed with the immersion time, indicating that, as expected, during this period no calcification, degradation or other process that could change the mechanical behavior, took place. However, for the CTS/BG a decrease in the *E'* for the first 5h was observed indicating that the BG is continuously dissolved. After that, the stiffness starts to recover due to the deposition of a stiff apatite layer.

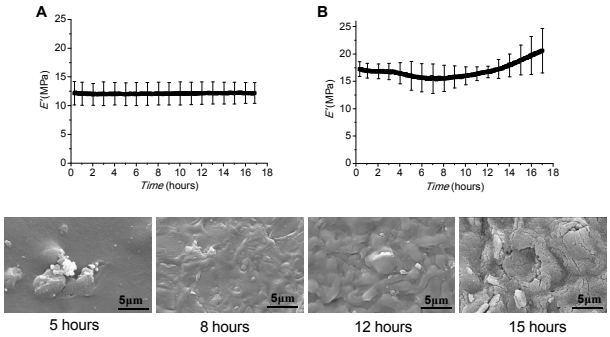

Figure 3: *E'* of CTS (A) and CTS/BG (B) obtained from online DMA measurements at 1 Hz on the membranes while immersed in SBF for the first 24 hours. SEM images on the surface of the CTS/BG membranes taken after short immersion times in SBF (5h, 8h, 12h and 15h) are also shown.

CONCLUSIONS

The CTS/BG membrane showed improved mechanical properties and excellent apatite forming ability than the pure CTS membrane. The enhanced properties of the CTS/BG composite membranes were attributed to the incorporation of the BG particles into the CTS.

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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AUTHOR BIOGRAPHY

SOFIA G. CARIDADE was born in Châlons-Sûr-Marne, France. She graduated in Materials Engineering by University of Minho, in 2007. She developed her masters research project at 3B's Research Group, under the supervision of Prof. João Mano, and continued her scientific interest in materials science for her PhD project. She is now working in collaboration with Prof. Catherine Picart at the Laboratoire des Matériaux et du Génie Physique, Minatec, France. Email:sofia.caridade@dep. uminho.pt.