Marine-based bioactive ceramics for tissue engineering approaches

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Statement of Purpose: The ocean can be regarded as vast source for new materials and molecules that is still greatly unexplored. The biodiversity that characterizes the marine environment represents an enormous potential for the acquisition of novel microstructures. Marine sponges have morphologies with a wide variety of complex and hierarchical structures from nano to the macro-scale, making it potential candidates for new structured materials. Today, ca. 60% of the synthetic bone graft substitutes available involve ceramic materials, either alone or combined in a composite structure. Ceramic-based biomaterials comprise calcium phosphate ceramics, bioactive glasses and glass ceramics. They are characterized by their bioactivity and unique bone bonding properties, which are usually related to their surface chemistry [1]. The use of biostructures and bioceramics derived from the marine environment for their application as biomaterials is very recent. For instance, several authors have proposed in the last years, the use of different marine species like coral skeletons, sea urchins and sponges as three dimensional biomatrices [2-4].We have focused on the potential of bioceramics obtained from three marine sponges, *Petrosia ficidormis, Agelas oroides* and *Chondrosia reniformis*, for biomedical applications. In vitro bioactivity studies promote the precipitation of crystals of calcium phosphate (e.g. hydroxyapatite) on the surface of marine derived bioceramics suggesting these as a new source of bioactive ceramics for tissue engineering and regenerative medicine (TERM) applications.

Methods: Sponge samples (*Petrosia ficidormis, Agelas oroides* and *Chondrosia reniformis*) were collected in Mediterranean Sea, namely in Spanish east coast (*Petrosia* sp. And *Agelas* sp.) and Israeli coast.. Bioceramics were obtained, after sponge calcination in a furnace at 750ºC for 6 hours. *In vitro* bioactivity of the bioceramics was evaluated by immersion in simulated body fluid (SBF), for 14 and 21 days. The structures were observed by SEM and the chemical composition was evaluated by energy dispersive x-ray spectroscopy (EDS) and fourier transform infrared spectroscopy (FTIR). Cytotoxicity studies were executed and included a comparison between the novel bioceramic structures with Bioglass®.

Results: The bioceramic structures obtained after calcination present different morphological and chemical compositions, as observed by SEM-EDS. *Petrosia ficidormis* skeleton is a 3D architecture, mainly composed of silicon and oxygen groups. On the other hand the inorganic part of *Agelas oroides* and *Chondrosia reniformis* is a powder. Besides silicates, *Agelas oroides* also contains calcium and magnesium, while *Chondrosia reniformis* is mainly composed of calcium carbonates and magnesium. The microscopic observation of the ceramics crystals after immersion in SBF solution for 14 and 21 days did not reveal any crystal precipitation in case of *Petrosia ficidormis* derived bioceramic structures suggesting no inherent bioactivity. In case of *Agelas oroides* and *Chondrosia reniformis* we observe newly formed crystals on their surface, with the typical cauliflower-like shape characteristic of hydroxyapatite (Figure 1). These crystals are composed of Ca, P and Mg as demonstrated by EDS analysis. FTIR analysis confirmed the presence of characteristics peak of carbonates and phosphates of hydroxyapatite, $\nu_x CO_3$ and $\nu_x PO_4$, after immersion of the marine origin ceramics in SBF solution. XRD analysis confirms the crystallographic planes of hydroxyapatite and some intermediate crystals, which demonstrate the bioactivity of the marine derived bioceramics. Finally, *in vitro* test results demonstrate that marine bioceramics from these sponges are non-cytotoxic.

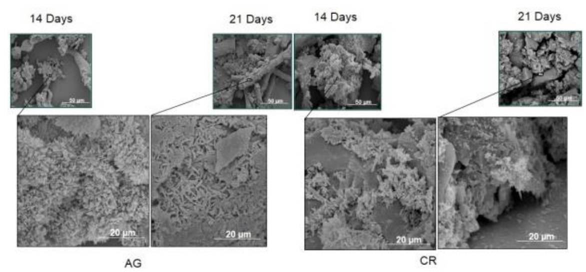

Figure 1. SEM micrographs of bio-ceramics after immersion in SBF solution.

Conclusions: In this study, the bioactivity of ceramics resulting from calcination of three marine sponges,*Petrosia ficidormis, Agelas oroides and Chondrosia reniformis* was evaluated. The results show that *Petrosia ficidormis* has no inherent bioactivity on the contrary of *Agelas oroides* and *Chondrosia reniformis*, which present, after 14 days and 21 days of immersion in SBF solution, hydroxyapatite crystals on surface. Observing the chemical composition of the sponges after calcination, bioactivity can be explained by the presence of calcium and magnesium groups, which allow nucleation of crystals. The analysis of FTIR and XRD proved the existence of this type of crystals. *Chondrosia reniformis* and *Agelas oroides ceramics* show, hereafter a bioactive behavior that suggests its use for tissue engineering and regenerative medicine applications.

References: (Low, K.L., J. Biomed. Mater. Res. Part B Appl. Biomater, 2010. 94B(1): p. 273-286.),(Kim S-K. Food Res Int 2006;39:383-93), (Silva TH, Int Mat Rev;57:276-306), (Abramovitch-Gottlib, L., 2006. Tissue Eng Vol.12 [4], pp.729-739),(Green, D. 2003,Tissue Eng Vol.9, pp. 1159-1166).