

Hydroxyapatite precipitation on sand dollar skeleton coated by bacterial cellulose

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Abstract – In this work, the skeleton of Sand Dollar was coated by bacterial cellulose (BC) produced by *Gluconacetobacter hansenii* and subsequently coated with hydroxyapatite. The skeleton of sand dollar is composed of magnesian calcite ((Ca,Mg)CO₃) and exhibits a hierarchically porous structure with interconnected porosity. After coating the small-sized pores were partially covered by cellulose microfibrils, where HAp particles were homogeneously deposited. The pore geometry of sand dollar is adequate for bone regeneration, and allows cell migration through its large cells and permit vascularization through the small pores. Moreover, BC/HAp coating offers a bioactive surface for cell adhesion.

In bone tissue engineering, scaffolds serve as bioinert or bioactive supports that allow surrounding cells to migrate into them, and regenerate the damaged tissue. In addition to the chemical nature the microarchitecture of a scaffold is decisive for new tissue formation. Scaffolds must exhibit adequate porosity and pore geometry to allow cell migration and tissue formation. Several biomaterials have been investigated for this purpose, including bacterial cellulose (BC), hydroxyapatite (HAp) and combination of both, due to their biocompatibility and osteoconductivity [1,2]. The skeleton of sand dollar (*Clypeaster subdepressus*, Echinodermata: Echinoidea) exhibit interconnected porosity with pore size gradients, high surface area, thermal stability and low weight. Interconnected pores also provide a framework for bone ingrowth and ensure the nutrition and blood supply for growing bone [2]. Araiza et al [3] transformed an Echinoderm calcite skeleton into porous hydroxyapatite by treatment with phosphated boiling solutions to change its composition, maintaining the porous microstructure. In this work, sand dollar skeleton was used as templates for BC coating and *in situ* deposition of HAp. The process combines the bioactivity of BC and HAp with the mechanical strength of sand dollar structures. Cubic samples of the skeleton were immersed in the culture medium of the bacteria *Gluconacetobacter hansenii* in an orbital shaker at 30°C. BC-coated scaffolds were immersed in synthetic body fluid (SBF) during 48 h for hydroxyapatite precipitation (CB/HAp). The microstructure of the scaffolds was evaluated by scanning electron microscopy (SEM), which revealed that the microarchitecture of sand dollar is composed of hierarchically structured pores with a bimodal pore size distribution (Figure 1), where small pores of approximately 50 μm form the walls of larger ducts (~500 μm). X-ray diffractometry revealed that sand dollar is composed of CaMg(CO₃)₂ (Figure 2). X-ray dispersive energy spectrometry confirmed the presence of Ca and P with a ratio Ca:P of 1.47, which is lower than the Ca:P-ratio for stoichiometric hydroxyapatite (1.67). The small-sized pores (50 μm) were partially coated with cellulose microfibrils, where HAp particles were homogeneously deposited over the surface (Figure 3). The pore geometry of sand dollar is adequate for bone regeneration, and allow cell migration through the large cells and permit vascularization through the small pores. Moreover, BC/HAp coating offers a bioactive surface for cell adhesion.

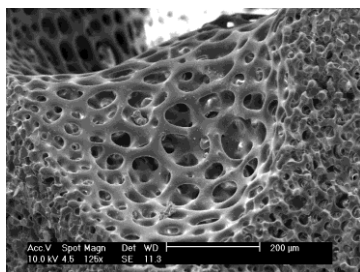


Figure 1: SEM micrograph of a sand dollar skeleton.

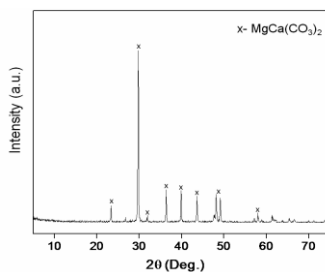


Figure 2: XRD spectrum of a sand dollar.

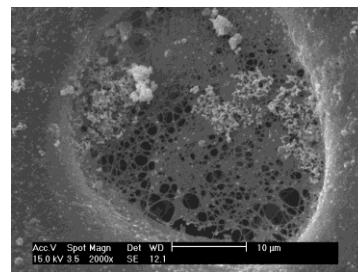


Figure 3: Micrograph of pore coated with BC/HAp.

References

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