Biological evaluations of silicate or carbonate-substituted hydroxyapatite ceramics with various microstructures for bone regeneration

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Calcium phosphate ceramics are commonly used in clinical applications for bone repair, neverthelesscurrent researches aim at designing bioceramics which would have a better bioresorbability competence to favorbone regeneration and healing. In this context theinfluence of the microstructure and chemical composition of hydroxyapatiteceramics on cellular behavior is of high interest.

Thereby, three different chemical compositions of hydroxyapatite were synthesized by aqueous precipitation: stoichiometric hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (HA), silicated hydroxyapatite $Ca_{10}(PO_4)_{5,6}(SiO_4)_{0,4}(OH)_{1,6}$ (SiHA) and carbonated hydroxyapatite $Ca_{9,45}(PO_4)_{5,5}(CO_3)_{0,55}(OH)_{0.99}(CO_3)_{0.23}$ (CHA).For each chemical composition,ceramics with dense or porous microstructurewere produced by different sintering profiles. Physic-chemical characteristics of sintered pellets were determined by XRD, FTIR, zeta potential, specific surface area measurements, SEM, surface topography analysis, mercury intrusion porosimetry and elemental analyses.Afterwards, bioceramic samples were evaluated in comparable conditions with a pre-osteoblastic cell lineMC3T3-E1 that synthesize bone and a monocytes/macrophages cell line RAW 264.7precursors of osteoclasts that resorb bone. Cell proliferation, differentiation, viability, adhesion and morphology were studied as well as the physic-chemical dissolution of bioceramics.

Results revealed that irrespective of the microstructure, CHA samples behave differently of the others samples. A significant higher biocompatibility for carbonated pellets was observed by osteoclast andosteoblast cell culture tests. Stronger osteoclastic proliferation but a lower differentiation while higher osteoblastic proliferation but the same rate of differentiation on CHAsampleswere observed compared to the other compositions. Conversely, for the same chemical composition, porous HA and SiHA showed less osteoblastic proliferation, but higher differentiation while cellsexhibited a higher osteoclastic proliferation on these samples, but a lower osteoclastic differentiation compared to the dense pellets. These biological results can be connected to the physic-chemical dissolution of materials, which in turn depends widely of the specific surface area, grain size and open porosity of bioceramics which impacts the calcium and phosphorus release in the culture medium. Regarding the silicon, its amount always increases in the culture medium indicating that it does not precipitate contrarily to the released calcium and phosphorus. Around 1 wt.% of total silicon present in porous pellets was released after 24h of immersion in the cell culture medium. Its presence influences cell adhesion as the surface area of cells was largeronto the silicated pellets but more focal adhesions were observed onto dense microstructure whatever the chemical composition suggesting that the porosity, the pore size and the hydrophobic character of the ceramic surface is crucial for strong cell adhesion.