Controlled release of calcium and phosphate ions from nanogels for in situ mineralization in bone tissue engineering

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Abstract

Introduction:

Biological apatites, the major inorganic component of bone mineral, differ from pure hydroxyapatite (HA) in stoichiometry, composition, crystallinity and other physical and mechanical properties that explain their special behaviour in bone remodelling cycles. Simulated or synthetic body fluids (SBF) are prepared in accordance with the chemical analysis of human body fluid. They are metastable buffered solutions supersaturated towards apatite crystals. When supersaturation degree increases (because of the addition of calcium and phosphate salts), mineralization is induced. Once apatite is nucleated, it can grow spontaneously incorporating ions from the SBF (Na^+, Mg^{2+}, CO^{2-}_3, etc.) into the structure (so differing from the formula $Ca_{10}(PO_4)_6(OH)_2$ and 1.67 of Ca/P molar ratio of synthetic ones) and such precipitation is similar to biological mineralization [1].

The term nanogel refers to spherical, nanometric, crosslinked polymer matrix that forms colloidally stable dispersions. Due to their small sizes and high surface-volume ratio, they have fast swelling–deswelling properties [2]. They can be charged with drugs and used as controlled release systems [3,4]. Hydroxyapatite precursor salts (calcium chloride and sodium hydrogen phosphate) can be encapsulated into nanogels to the aim of controlling the hydroxyapatite reaction kinetics. Moreover, nanogels could act as nucleation sites of hydroxyapatite. In this work advantages from the precipitation in SBF and from nanometric hydrogel properties have been combined. When heated to physiologic temperature, above the lower critical solution temperature (LCST) of the poly(N-Isopropylacrylamide-co-acrilic acid) nanogels, both Ca and P ions-loaded nanogels become hydrophobic and release their contents into the SBF solution, where the ions react to form hydroxyapatite mineral.

Materials and methods:

Poly(NIPAAm-co-AA) nanogels were synthesized by precipitation radical polymerization in water as previously described [5]. After dialysis and freeze-drying nanometric hydrogels of sizes close to 100nm were obtained.

Nanogels were dispersed in highly concentrated aqueous solutions of $CaCl_2$ and Na_2HPO_4 and after several hours they were recovered by ultrafiltration and freeze-drying. Then, the SBF solution was prepared and the load and controlled release of sodium and phosphate ions was measured. It is known that a Ca/P molar ratio of 1.80 added to SBF guaranties a good biomimetic hydroxyapatite, for this reason, it is necessary to control the behaviour of nanogels in the presence of these ions. Different concentrations of salts and nanogels were used to study the load of the salts into the nanogels (data not shown). The amount of salt loaded into the nanogels was characterized by ICP-AES (Inductively coupled plasma atomic emission spectroscopy, axial, model VISTA-MPX from VARIAN).

For the study of the controlled release, loaded nanogels were introduced in dialysis bags with deionized water at 37ºC instead of SBF in order to avoid any precipitation and to determine the Ca or Pi released without the influence of any other salt. The controlled release of Ca or Pi was analyzed at different time points by ICP.

For the synthesis of biomimetic hydroxyapatite powders in SBF, both Ca-loaded (1000 mg/ml; 400:3) and Pi-loaded nanogels (90 mg/ml; 12:1), in a ratio of 2:1, were placed in SBF solution for five days at 37ºC. Powders were recovered by centrifugation, washed and dried, and characterized by XRD (D8-Advance, Bruker), FTIR spectroscopy and ICP-AES.

Results and discussion:

Results demonstrated that the higher the amount of salt in the solution, the bigger amount the nanogels can load, but this loaded amount does not depend on the amount of nanogels. It could be due to electrostatic interactions that only let the nanogels load with salt until a limit, when the limit is reached, no more nanogels absorb salt.

Figure 1 shows the accumulated concentration of Ca or Pi released from the nanogels to water along the time. The release almost reached equilibrium at the fourth day. Concentration of Ca or Pi released depends on the amount of loaded nanogels and initial solution concentration used for the load. The

bigger the amount of loaded nanogels, the larger the amount of salt released. Ca/P molar ratios between the Ca and Pi released were estimated from the curves and, as the ideal Ca/P molar ratio to form a biomimetic hydroxyapatite in SBF must be close to 1.80, the combined release from 100 mg of Ca-nanogel and 200 mg of Pi-nanogel was selected for the study of the hydroxyapatite synthesis.

XRD spectra of the hydroxyapatite powders recovered after five days incubation of salt-loaded nanogels in SBF solution had got peaks that corresponded to the standard for stoichiometric HA (International Centre of Diffraction Data (ICDD), JCPDS 09-0432) confirming the apatitic nature of the sample. Figure 2 shows FTIR spectra of the powders obtained. The broad band beyond 3000 cm−1 corresponds to the OH groups. The little band at 1650 cm−1 corresponds to adsorbed water. Phosphate groups are seen in the region between 550 and 600 cm−1 and the strong band close to 1000 cm−1. Carbonate vibration bands are observed at 1400–1550 cm−1 confirming the carbonate apatite nature. The percentage of elements found in the sample determined by ICP-AES (%Na 0.37, %K 0.15, %Mg 0.32, %S 0.04) confirmed the substitutional inclusion of different amounts of ions from the SBF solution in the apatitic structure as in natural hard tissues.

Conclusions

Poly(NIPAAm-co-AA) nanogels were capable to charge calcium and phosphate ions and provide a controlled release of them for several days. The released amount and kinetics depends on the initial salt concentration and nanogel/salt ratio, which can be fixed depending on the final result we want to obtain. The approach proposes a method to use the nanogels as controlled release systems and nucleation agents for the in situ precipitation of biomimetic hydroxyapatite from scaffolds.

References

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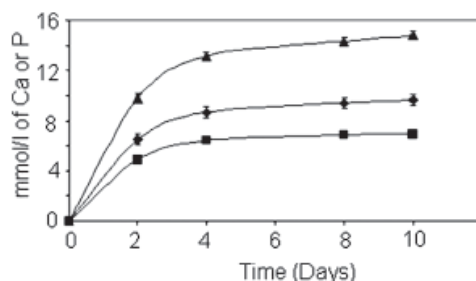I. Obieta, J. Nanosci. Nanotechnol. 10 (2010), 2826

Figures

Figure 1. Concentration of the released Ca and Pi in water from nanogels at 37 ºC. (▲) 60 mg of Ca-nanogel (1000 mg/ml; 400:3), (♦) 40 mg of Ca-nanogel (1000 mg/ml; 400:3) and (■) 100 mg of Pi-nanogel (90 mg/ml; 12:1).
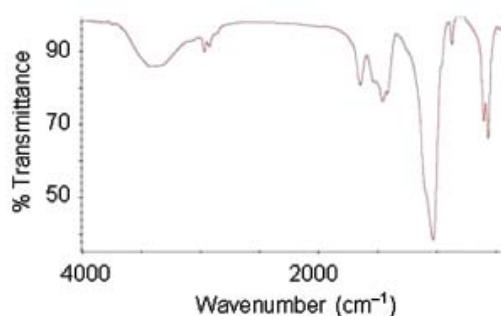

Figure 2. FTIR spectra of the biomimetic hydroxyapatite powder obtained.