Chitosan Effects on Bioactive Glass for Application as Biocopmosite Biomaterial

Hassane Oudadesse, Xuan- Vuong. Bui, Yann. Le Gal, Amani Mostafa and Guy Cathelineau

Abstract— The objective of this work was to synthesize biocomposite composed by bioactive glass (BG) and chitosan (CH) as biomaterials for medical applications. 17 wt% of chitosan polymer was added to the glass matrix by using an original method to obtain BG/CH biocomposite. The obtained biocomposite was investigated by using numerous physico-chemical methods. The homogenous distribution of bioactive glass particles within the matrix of chitosan polymer was clearly showed in this study. "In vitro" assays without cells were employed to evaluate the effect of chitosan polymer addition on the glass matrix by studying the chemical reactivity and bioactivity of the BG and BG/CH composite after different times of soaking in SBF solution. Ionic exchanges and kinetic of bioactivity were highlighted. Also, "in vitro" assays with presence of celle were studied for the cytotoxicity evaluation. The obtained results showed the formation of a biological active hydroxyapatite (HA) layer and highlighted the bioactivity of bioactive glass, particularly after addition of chitosan polymer. The composite based on bioactive glass and chitosan polymer has excellence ability to form an apatite layer on its surface. SEM analyses showed a dense layer of HA on the surface of BG/CH biocomposite. ICP-OES highlighted the effect of chitosan on the dissolution of the glassy network after different times of soaking. The results indicated that the presence of chitosan polymer delayed the silicon release from glassy network toward the synthetic physiological solution. The chitosan acts as capping agent.The non toxic character of this biocomposite was confirmed.

***Keywords*—**Bioactive glass, chitosan, freeze-drying, hydroxyapatite, ''in vitro'', osteoblast.

X. V. Bui is with the University of Rennes 1, UMR CNRS 6226, 263 Avenue du General Leclerc, 35042 Rennes Cedex, France (e-mail: hassane.oudadesse@univ-rennes1.fr).

H. Oudadesse is with the University of Rennes 1, UMR CNRS 6226, 263 Avenue du General Leclerc, 35042 Rennes Cedex, France (e-mail: hassane.oudadesse@univ-rennes1.fr).

Y. Le Gal is with the University of Rennes 1, UMR CNRS 6226, 263 Avenue du General Leclerc, 35042 Rennes Cedex, France (e-mail: hassane.oudadesse@univ-rennes1.fr).

G. Cathelineau is with the University of Rennes 1, UMR CNRS 6226, 263 Avenue du General Leclerc, 35042 Rennes Cedex, France (e-mail: hassane.oudadesse@univ-rennes1.fr).

A. Mostafa is with the National Research Centre NRS, Gizza, Egypte (e-mail: amanieg1@yahoo.com).

I. Introduction

BIOACTIVE glasses are a group of bioactive ceramic materials. They are surface reactive biomaterials used as implant materials in the human body to repair and replace diseased or damaged bone. They were first discovered by Hench and Co-workers in 1969 [6]. Bioactive glasses are composed mainly of: SiO_2, Na_2O, CaO and P_2O_5. When immersed in a physiological solution, these bioactive materials can form an amorphous calcium phosphate layer and then crystallize to hydroxyl carbonate apatite (HCA) which has a similar chemical composition and structure with the mineral phase of human bone. This phenomenon is observed too during ''in vivo'' experiments. The formation of apatite layer allows a chemical bonding between implant biomaterials and bone tissues [1-4].

Biocomposites based on biodegradable polymers and bioactive ceramics have been developed for applications in bone repair and reconstruction. Several polymers such as polylactic acid (PLA), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA), gelatine, alginate and chitosan (CH) are widely used for this purpose because of their proven biocompatibility and complete bioresorbability [5-11].

Among the biodegradable polymers, chitosan has recently gained interests due to its special physiological properties. Chitosan is a natural polymer, it can be obtained by partial deacetylation of chitin, which was extracted from crustacean. It is considered as a suitable functional material for biomedical applications due to its good biocompatibility, biodegradability, non-antigenicity, anti-tumor activity, anti-inflammatory effect, protein adsorption properties and ability of accelerating wound healing. Chitosan plays an important role in the attachment, differentiation and morphogenesis of osteoblasts, the bone forming cells, because of its structural similarities with glycosaminoglycans, a major component of bone and cartilage [12-16].

The microsphere biocomposite has the advantage that it can be formulated and fused together to form complex shapes like scaffolds. This also would allow custom grafts to be designed to fit any site. The microporosities of these microsphere biocomposites increase the surface of contact between the biomaterial and the biological fluid. This enhances their interfacial reactions and consequently the bioactivity of material [17-18]. A combination of the polymer to calcium phosphate by lyophilization technique has been used to form microspheres [18].

The aim of this work was combination of dense material (bioactive glass) and biodegradable polymer (chitosan) to synthesize the bone-enhancing microsphere biocomposite which has the optimised porosity and surface area to induce fast bone ingrowth and good osteointegration. The bioactive glass prepared by melting method is incorporated into chitosan polymer to synthesize the BG/CH microsphere biocomposite by using lyophilization technique. Characterizations and "in vitro" assays of these two biomaterials were employed to highlight their association and particularly the effect of chitosan on the bioactivity and biocompatibility of bioactive glass.

II. MATERIALS AND METHODS

A. Preparation of bioactive glass (BG)

The BG used in this study has a chemical composition: 46% SiO_2, 24% CaO, 24% Na_2O and 6% P_2O_5 (wt %). It was synthesized by melting method [19]. The reagents used in the preparation of the glass were calcium silicate ($CaSiO_3$), sodium metasilicate (Na_2SiO_3) and sodium phosphate ($NaPO_3$). Batch mixed well then melted in a platinum crucible up to 1300° and kept during 3 hours. Afterward, the melted bioactive glass was poured into brass moulds and annealed at the glass transition temperature (about 536°C) in a regulated muffle furnace, to remove the residual mechanical constraints. The muffle furnace was cooled to room temperature at a rate of $1°C.min^{-1}$. Then, the bulk glasses were ground to obtain the bioactive glass particles with size less than 40 µm.

B. Preparation of chitosan solution (CH)

The chitosan solution was prepared by dissolving of 0.4 (g) of chitosan polymer with a medium molecular weight in 40 (ml) of 1% acetic acid solution. This solution was stirred at room temperature, and then filtered to get rid of air bubbles and to obtain a homogeneous viscous solution.

C. Preparation of microsphere bioactive glass/chitosan biocomposite

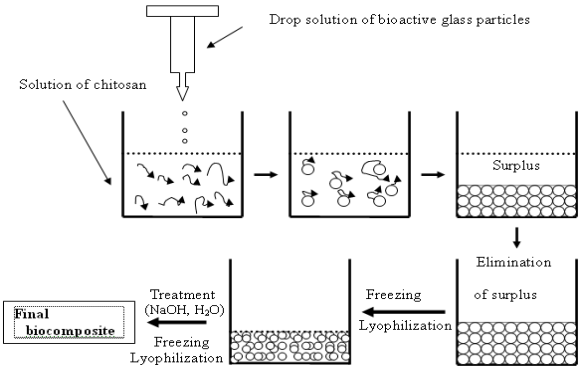

Fig. 1 Preparing protocols of BG/CH biocomposite microsphere

Biocomposite based on bioactive glass and chitosan polymer was elaborated by freeze-drying method. First, the bioactive glass particles was suspended in the previously prepared chitosan solution and stirred during 2 hours by using magnetic agitation at 1200 (rpm). After removal of surplus solution, the mixture of bioactive glass and chitosan polymer was solidified by liquid azotes and transferred immediately into a freeze-drying at -60°C, for 24 hours to remove solvents. To neutralize the radicals of acetic acid, the obtained biocomposite was immersed in NaOH solution, and then washed with deionised water. These steps are necessary to avoid the cell toxicity caused by the excess of acetic acid during "in vitro" bone cell examination. At the end, the biocomposite of bioactive glass and chitosan polymer was frozen and freeze-dried again for 24 hours to remove solvent totally [16-18, 20-25]. The biocomposite contained 17 wt% of chitosan polymer was elaborated according to the steps presented in figure 1. It was named: BG/CH biocomposite microsphere.

D. ''In vitro'' bioactivity evaluation in simulated body fluid

''*In vitro*'' bioactivity of the materials was determined by soaking them in a simulated body fluid (SBF). This solution was prepared by dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$ and $CaCl_2$ in deionised water and buffered with $(CH_2OH)_3CNH_2$ and HCl (6N) to adjust the pH value at 7.4, following the method described by Kokubo [26-27]. The composition of SBF solution is similar to the one of human blood plasma as shown in Table I. Both powder samples of BG and of BG/CH biocomposite microsphere were immersed in SBF solution for 0 (control), 1, 2, 3, 5, 7, 15 and 30 days at body temperature at 37°C. At the end of soaking time in SBF solution, the powder samples removed and rinsed with deionized water to stop the exchange reactions, finally rinsed with absolute alcohol. The powder samples dried and stored for further investigation of the formation of HA layer on the surface of powder samples. This was verified by using FTIR and SEM. The SBF solutions were stored in fridge to evaluate the ionic concentrations by using ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry).

Table 1: Concentrations of the SBF solution

Ion	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}
SBF	142.0	5.0	2.5	1.5	148.8	4.2	1.0
Plasma	142.0	5.0	2.5	1.5	103.0	27.0	1.0

E. Cell culture assays

The used culture medium was standard medium DMEM (Sigma Chemical Co., St. Louis, MO) with 15 mM HEPES, 2 mM L-glutamine, 10% FBS (Fetal Bovine Serum), 100 UI/ml penicillin and 100 µg/ml streptomycin. Osteoblast like cells $SaOS_2$ were cultivated in DMEM at 37°C in a humidified incubator with 5% CO_2 and 95% humidity. The cytotoxicity was determined using the colorimetric MTT assay. MTT assay measures the reduction of the tetrazolium component MTT by viable cells. Therefore, the level of reduction in MTT into formazan can reflect the level of cell metabolism.

F. Physico-chemical characterization

The powder samples of BG and of BG/CH biocomposite microsphere before and after soaking in SBF solution were investigated by using several physico-chemical techniques. The Fourier Transformed Infrared Spectroscopy (FTIR) (Bruker Equinox 55) was employed to identify the functional groups of the prepared BG and BG/CH biocomposite. Fine powders of the samples mixed with KBr powder in the ratio 1:100 and the mixture subjected to a load of 10 tons/cm^2 to produce disc. FTIR collected spectra were taken in the range 400 and 4000 cm^{-1} with resolution of 2 cm^{-1}. The morphological surfaces were studied by using scanning electron microscopy (SEM) (Jeol JSM 6301). The powder samples were coated by gold-palladium layer. Inductively coupled plasma-Optical Emission Spectrometry (ICP-OES) method possessed a high sensitivity less than 1 ppm was employed to evaluate the elemental ion concentrations of SBF before and after soaking of biomaterials.

III. RESULTS AND DISCUSSIONS

A. Characterizations

A1. FTIR analysis

Figure 2 shows IR spectra of bioactive glass (BG), of chitosan (CH) and of BG/CH biocomposite microsphere. For bioactive glass, the spectrum shows the characteristic bands of silica network. It reveals four broad absorption bands. First band about 503 cm^{-1} is characteristic to angular deformation vibration of Si-O-Si bond between SiO_4 tetrahedrons in silicate network. Three other characteristic bands at 745, 932 and 1036 cm^{-1} attributed to stretching vibrations of Si-O bond in each SiO_4 tetrahedron are detected. In addition, only slight band at 590 cm^{-1} is characteristic for bending vibration of O-P-O group. It highlights the presence of a small amount of phosphate linked to the silica network [28-31].

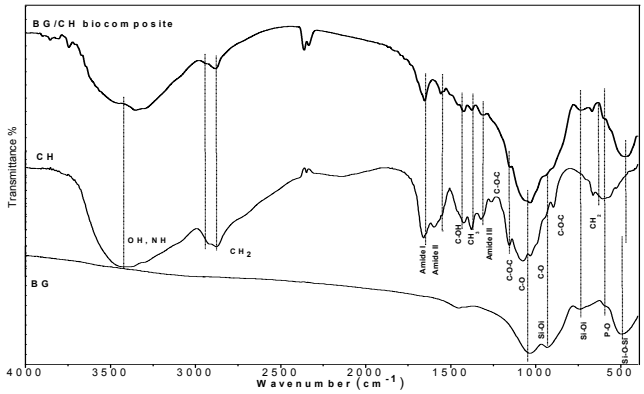

Fig. 2 IR spectra of bioactive glass (BG), Chitosan (CH) and BG/CH biocomposite

The infrared spectrum of chitosan shows absorption band at 3000-4000 cm^{-1} to stretching vibration of –NH_2 and –OH groups. The characteristic bands at 1657, 1597 and 1320 cm^{-1} assign to the amide one, amide two and amide three absorption bands, respectively. The bands at 2926, 2880 and 665 cm^{-1} correspond to the -CH- bending vibrations. Characteristic band at 1380 cm^{-1} attributes to stretching vibration of methyl group present in the residual acetylamido groups of the chitosan, due to incomplete deacetylation of the parent chitin. The band at 1422 cm^{-1} is characteristic of bending vibration of C-OH group. The characteristic bands at 1075 and 1033 cm^{-1} assign to the skeletal vibrations of C-O stretching. Two absorption bands at about 1153 and 890 cm^{-1} correspond to stretching vibrations of C-O-C groups in saccharide structure of chitosan [32-37].

The IR spectrum of BG/CH biocomposite microsphere shows the characteristic bands of both chitosan and bioactive glass. However, several characteristic bands are shifted, deformed or disappeared. This attributes to some chemical interactions between the bioactive glass and the chitosan. For the chitosan, the absorption band of amide two is shifted a little to the short wave. The characteristic bands of C-O bonds in chitosan skeleton at 1153, 1075, 1033 and 890 cm^{-1} are observed with slight intensity or disappeared. This evidences that the groups of these bands have taken part in bonding with bioactive glass. For the bioactive glass, two bands at 932 and 1036 cm^{-1} (Si-O-Si) are deformed. The characteristic band at 503 cm^{-1} (Si-O-Si) is shifted a little to the short wave. This is an indication of participation in bonding of glass with chitosan polymer. Thank to these links, bioactive glass particles are well combined into the matrix of chitosan polymer to form the microspheres of BG/CH biocomposite.

A2. Microstructure and morphological examination

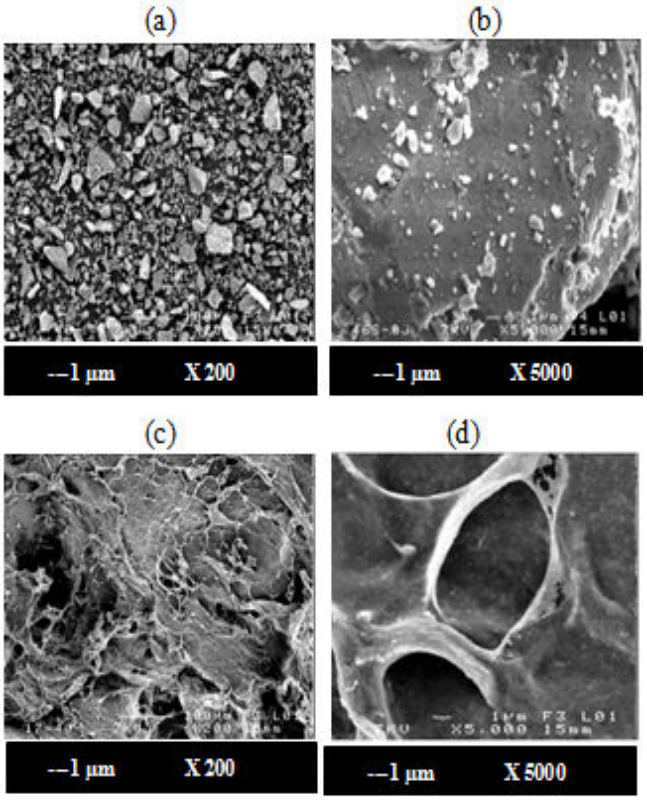

Fig. 3 SEM images of (a, b) bioactive glass (BG) and (c, d) BG/CH biocomposite

Figure 3 shows the SEM images of the BG and BG/CH biocomposite microsphere. The bioactive glass presents a heterogeneous structure, it consists of random-size particles with sharp edges as shown in figure 3a. The photograph of figure 3b is of higher magnification shows inhomogeneous particles on the surface of bioactive glass. The BG/CH biocomposite microsphere displays a porous membrane structure with interconnecting open pores as shown in figure 3c. The figure 3d with higher magnification for the microsphere shows the presence of smooth surface spreading of particles in various areas proving coating of bioactive glass particles with chitosan. There are no characteristic particles embedded and dispersed onto the composite surface, denoting homogeneity and compatibility between two materials. The porous structure of the obtained biocomposite microsphere increases the loading capacity of the physiological fluid into biomaterials which favour the interfacial reactions between biomaterials and physiological solution. Consequently, the bioactivity of bioglass can be enhanced. In addition, the porosity of biocomposite microsphere is necessary in bone tissue engineering for the easy migration of fluid, organic matter, biological cells and then proliferation of osteoblasts and mesenchymal cells, as well as vascularisation [16-18].

B. ''In vitro'' bioactivity assays

B1. FTIR structural analysis of apatite layer

The FTIR spectra of bioactive glass (BG) and of bioactive glass/chitosan biocomposite (BG/CH biocomposite) after 3 days and 15 days of soaking in SBF solution are shown in figures 4-5. The IR spectrum of synthetic hydroxyapatite is used as references to evaluate the structural evolution and the bioactivities of the prepared biomaterials [38]. After soaking in SBF solution, the initial characteristic bands of BG and also of BG/CH biocomposite are modified strongly because of the interfacial reactions between these two biomaterials and the SBF physiological solution. Consequently, the spectra of these biomaterials reveal new bands.

In detail, the spectrum of BG/CH biocomposite microsphere shows three new well-defined phosphate bands at 565, 603 and 1039 cm^{-1} after 3 days of soaking in physiological solution. They are assigned to stretching vibrations of PO_4^{3-} group in phosphate crystalline phases. This result confirms the formation of a calcium phosphate layer. In addition, the carbonate bands at 874 and 1420 cm^{-1} are also observed. The first band is characteristic of a bending vibration while the second band attributes to a stretching vibration of the C-O liaisons in carbonate groups. The presence of carbonate bands indicates the formation of a layer of carbonated hydroxyapatite on the surface of BG/CH biocomposite. After 3 days of soaking in SBF solution, the spectrum of BG does not present the characteristic bands of apatite layer. The obtained results highlight the rapid formation of apatite layer on the surface of BG/CH biocomposite.

After 15 days of soaking in SBF solution, the characteristic bands of hydroxycarbonated apatite (PO_4^{3-}: 565, 603, 1039 cm^{-1} and CO_3^{2-}: 874, 1420 cm^{-1}) are well observed in the spectrum of BG and also in the spectrum of BG/CH biocomposite. These characteristic bands are being observed well in the spectrum of BG/CH biocomposite. This confirms the well crystallization of the apatite layer on the surface of BG/CH biocomposite. In addition, the spectra of BG and of BG/CH biocomposite reveal three Si-O-Si bands at 470 cm^{-1} (bending vibration) , 799 cm^{-1} (bending vibration) and 1075 cm^{-1} (stretch vibration). These confirm the presence of a silica gel [19]. The apparition of apatite mineral and a silica gel highlight the interactions between the biomaterials and the physiological solution as described by Hench et al. This mechanism could be explained through the following steps: (a) rapid exchange of protons H_3O^+ from the physiological solution with Ca^{2+} , Na^+ ions in bioglass to form the Si-OH groups, (b) loss of soluble silica as $Si(OH)_4$ by breaking of Si-O-Si bridging links and subsequent formation of surface silanol groups in the process, (c) condensation and repolymerization of surface silanols to form SiO_2-rich surface layer, (d) migration of Ca^{2+} and PO_4^{3-} through the surface silica-rich layer and formation of a Ca-P rich layer on the surface of bioglass, (e) incorporation of OH^-, CO_3^{2-} from the solution and subsequent crystallization of the Ca-P layer to form HCA [1-4]. The obtained results confirm the bioactivity of BG and also of BG/CH biocomposite. Especially, they highlight the positive effect of chitosan polymer on the bioactivity of bioactive glass. The BG/CH biocomposite shows a rapid formation of well crystallized apatite layer on its surface and in a short time in comparison with pure bioactive glass.

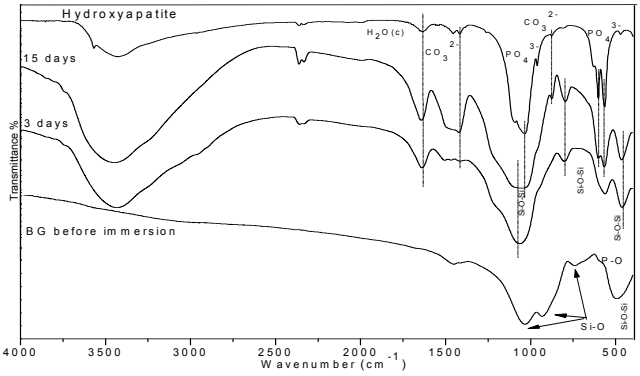

Fig.4 IR spectra of bioactive glass (BG) after 3 and 15 days of soaking in SBF solution and Hydroxyapatite

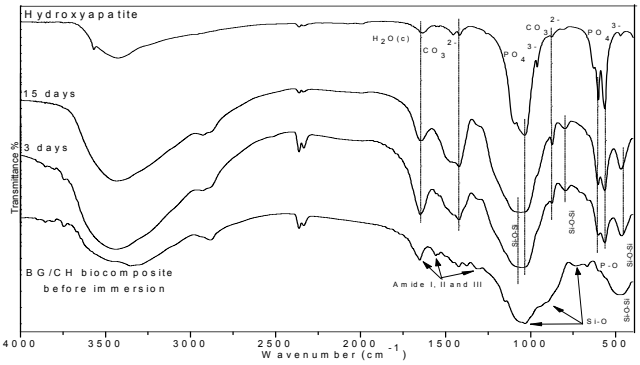

Fig.5 IR spectra of BG/CH biocomposite after 3 and 15 days of soaking in SBF solution and Hydroxyapatite

B2. Surface morphology by SEM

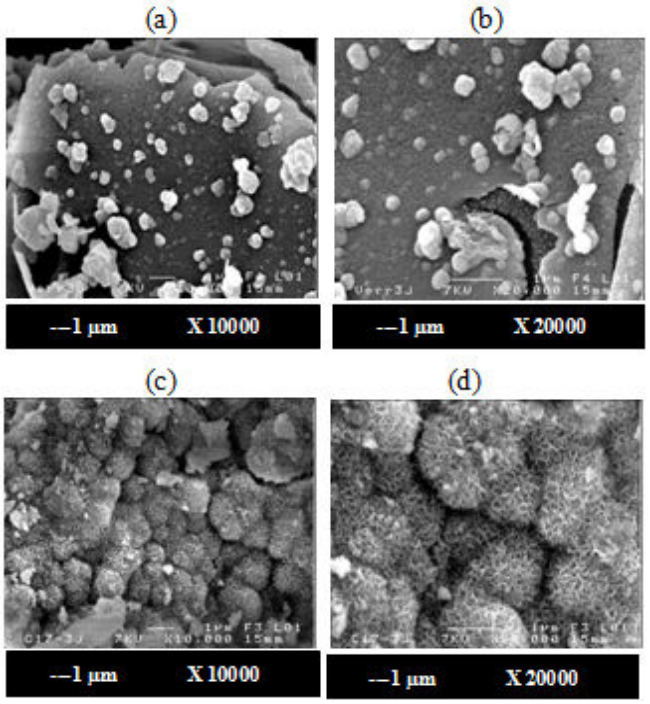

Fig.6 SEM images of (a, b) bioactive glass (BG) and (c, d) BG/CH biocomposite after 3 days of soaking in SBF solution (X 10000 and 20000)

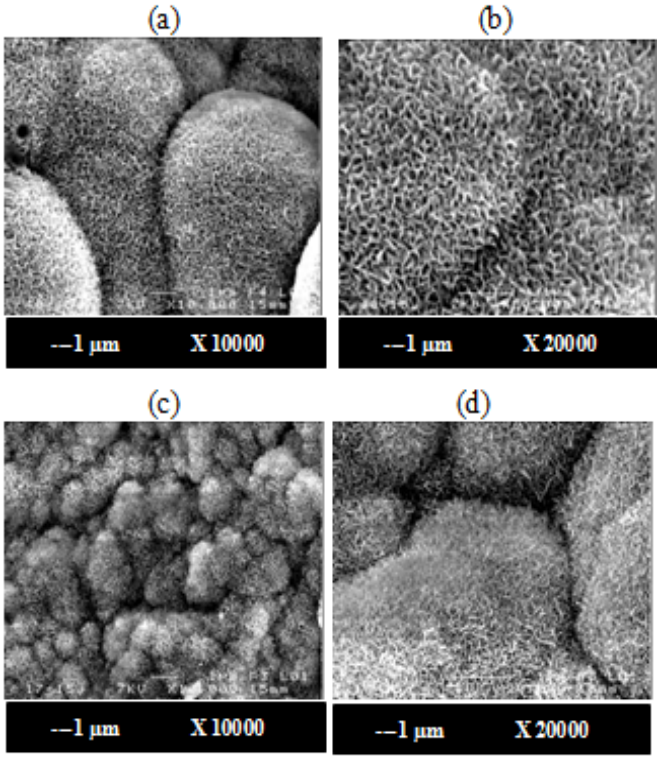

Fig.7, SEM images of (a, b) bioactive glass (BG) and (c, d) BG/CH biocomposite after 15 days of soaking in SBF solution (X 10000 and 20000)

Figure 6 shows the SEM micrograph of the surface of BG and of BG/CH biocomposite microsphere after 3 days of soaking in SBF solution. The hydroxyapatite crystals begin to form but the surface of bioactive glass (BG) is not modified yet. While the surface of BG/CH biocomposite covers almost by apatite crystals. This result confirms the rapid formation of an apatite layer on the surface of BG/CH biocomposite.

Figure 7 shows the surface morphologies of BG and of BG/CH biocomposite after 15 days of soaking in synthetic physiological liquid SBF. The hydroxyapatite layer is observed on the surface of BG and also of BG/CH biocomposite. This layer consists of small crystals which are continuous with identical form. The surfaces of the investigated samples are almost completely covered by the apatite crystals, especially on the surface of the BG/CH biocomposite where thick layer of apatite is being observed.

It is recognized that the hydroxyapatite layers formed on the surfaces of BG/CH biocomposite is more dense and visible than the one over the BG. This confirms the well crystallization of apatite layer on the surface of BG/CH biocomposite. It looks better than the one over pure bioactive glass. The association of chitosan with bioactive glass creates the positive effects to improve the formation and the crystallization of hydroxyapatite layer. It is considered that the porous structure of BG/CH biocomposite microsphere should be responsible for the formation of a dense apatite layer. The BG/CH biocomposite with a high surface area to volume can facilitate the transport of Ca^{2+} and PO_4^{3-} ions from the physiological fluid onto the surface of BG/CH biocomposite to form a dense apatite layer.

B3. ICP-OES analysis

The concentrations of calcium and phosphorus ions in SBF solution versus soaking times are linked to the formation of hydroxyapatite layer. The phosphocalcic ratio Ca/P is a high indicator of the purity of the hydroxyapatite matrix. The measurements of Ca, P concentrations can evaluate the rate of biomineralization of biomaterials after immersion in physiological solution.

The variations of calcium ions concentrations in SBF solution as a function of soaking time are shown in figure 8. For BG, calcium ions concentration in the analyzed SBF solution increases very strongly from 100 ppm to 153 ppm during the first day of immersion. This increase is coherent with the release of available calcium content from bioactive glass in the desalkalization process. The calcium ions concentration increases gently till the second day, and then it decreases very strongly till the last day of immersion (30 days). This decrease corresponds to the precipitation of calcium ions on the surface of bioactive glass to form the apatite layer. For BG/CH biocomposite, calcium ions concentration increases strongly during the first day of immersion from 100 ppm to 148 ppm. The increase is less than the one of BG. This could be attributed to the presence of chitosan polymer that prevent the calcium ions dissolution, or one part of calcium amount can be released already in the synthesis process of BG/CH biocomposite, or as soon as the first day, the large quantity of calcium ions is transferred to the surfaces of BG/CH biocomposite to form the apatite layer. Then, the calcium ions concentration decreases very strongly from the first day till the last day of immersion. The calcium consumption by BG/CH biocomposite is faster than that of 46S6 bioactive glass. This confirms the rapid formation of an apatite layer on the surface of bioactive glass/chitosan biocomposite.

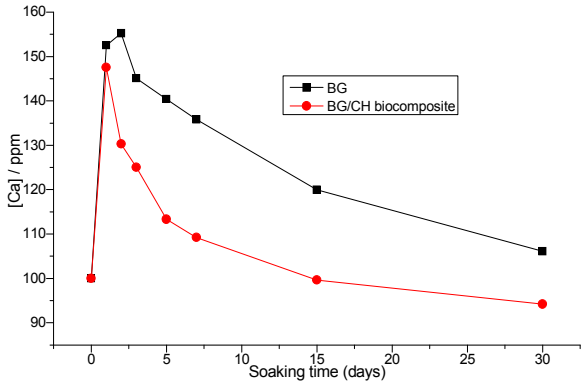

Fig.8 Evolution of elemental concentrations of Ca in SBF solution measured by ICP-OES, versus soaking times

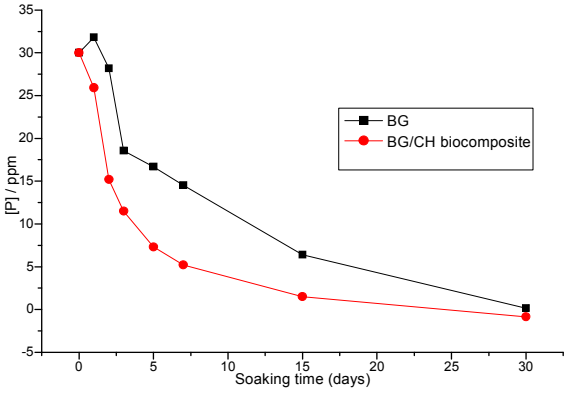

Fig.9 Evolution of elemental concentrations of P in SBF solution measured by ICP-OES, versus soaking times

Figure 9 shows the variations of phosphorus ions concentrations in SBF as a function of soaking time. For BG, the phosphorus ions concentration augments a small quantity from 30 ppm to 32 ppm after the first day of immersion. It corresponds to dissolution of a small quantity of phosphorus from the matrix of bioactive glass. Furthermore, the phosphorus ions concentration decreases very fast till the last day of immersion because the phosphorus is used to form the layer of calcium phosphate on the surface of pure bioactive glass. After 30 days of immersion, the phosphorus ions concentration in SBF solution is 0.1 ppm. It verifies that all of the phosphorus ions present in SBF and in vitreous matrix of

bioactive glass is used to form the hydroxyapatite layer. For BG/CH biocomposite, there is a no increase of the phosphorus ions concentration after the first day of immersion as in BG. It is probably due to the presence of chitosan that retard the dissolution of bioglass from the matrix of BG/CH biocomposite. As soon as the first time, the phosphorus ions concentration decreases strongly. This could be attributed to consumption in the formation of hydroxyapatite layer on the surface of BG/CH biocomposite. At 15 days of immersion, the BG/CH biocomposite utilizes almost all the phosphorus ions in the SBF solution to form the apatite layer. At this time, the phosphorus ions concentration is of 1.5 ppm while this concentration of BG is 6.5 ppm. After 30 days of immersion, the phosphorus ions concentration is zero in SBF solution. All of the phosphorus ions are consumed to form the hydroxyapatite layer. The phosphorus consumption of BG/CH biocomposite is faster than the one of BG. This confirms the rapid formation of an apatite layer on the surface of bioactive glass/chitosan biocomposite.

The obtained results are in a good agreement with the analyses carried out by FTIR and SEM methods. They confirm precisely the excellent bioactivity of BG/CH biocomposite.

C. Cytotoxicity and cellular viability activity by MTT assay

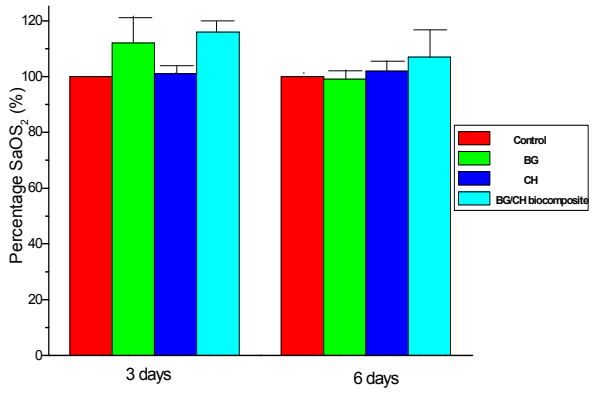

Fig.10, The cell viability of $SaOS_2$ cells on the bioactive glass (BG), Chitosan (CH) and BG/CH biocomposite after culturing for 3 and 6 days

The cell viability, bioactivity and cytotoxicity of conditioned media of BG, CH and BG/CH biocomposite are determined by a colorimetric MTT assay. The results are presented in histogram (Fig. 10). After three days of contact with conditioned media, the cell viabilities are 112, 101 and 116% for BG, CH and BG/CH biocomposite respectively. The viability of cells without contact with biomaterials is fixed as control (100%). After six days, there is a more moderate increase of cell proliferation: 99% for BG, 102% for CH and 107% for BG/CH biocomposite. These results show good cell viability compared to the control and the absence of toxicity on our materials. It is noted that the cells proliferation on BG/CH biocomposite is better than that on bioactive glass (BG). These results demonstrate the superiority of cell viability on BG/CH biocomposite with an increase of 16% for three days and 7% for six days of contact with conditioned media. The introduction of chitosan in the bioactive glass improves the osteoblasts cells proliferation. Accordingly, the results can support fairly well the discussion of the stages of biomineralization mechanism in the investigated glasses.

IV. CONCLUSION

A bioactive biocomposite microsphere of bioglass and chitosan polymer were successfully designed and produced by freeze-drying technique. The obtained composite was investigated by several physico-chemical methods. The SEM examinations show that the bioactive glass particles are homogeneously distributed into the matrix of chitosan polymer. The SEM indicates also that the BG/CH biocomposite has a microsphere porous structure. The FTIR analyses confirm that the chitosan is well associated with bioactive glass by the bonds created between these two materials. The chitosan effects created some changes to silica network of bioactive glass.

The “in vitro” assays were carried out for both BG and BG/CH biocomposite by soaking of powdered samples in SBF solution. The obtained results confirm the formation of carbonated hydroxyapatite layer on the surfaces of these two biomaterials. Especially, the presence of chitosan polymer enhanced the bioactivity of bioactive glass. The BG/CH biocomposite showed an excellent capacity to form the apatite layer on its surface. After 3 days of soaking in SBF, the obtained results confirm the formation of this layer. The high bioactivity of BG/CH biocomposite can be explained by its porosity structure. This is the basic reason to induce the excellent capacity of formation of hydroxycarbonated apatite layer on the surface. The porosity facilitates contact between the biomaterials and the simulated body fluid (SBF). Because of the porous state of the microsphere, the ionic exchanges between the biomaterials and the SBF became easily, this improves the bioactivity of materials. The good interfacial bonding between chitosan and the powder of bioglass particles can result in an increase in the mechanical strength for further investigation and the good integration of bioglass particles. This may be able to prevent migration out of the chitosan matrix that cause inflammation and tissue damage. The ‘‘in vitro’’ experiments in presence of cells were realized too, the obtained results confirm the absence of toxicity and good cell viability for BG and also for BG/CH biocomposite.

In conclusion, the BG/CH biocomposite synthesized from the incorporation of bioactive glass particles into matrix of chitosan polymer using the lyophilisation technique is promising in term of its bioactivity, and may find potential applications for bone repair and tissue engineering. In bone surgery, this new biocomposite offer to surgeons more possibilities to adapt it when the bone metabolism activities are very dynamic and necessitate a rapid bioconsolidation at the bone-biomaterial interface. Other parameters like the age, gender, location site of implant have need of this kind of biomaterial.

REFERENCES

[1] L.L. Hench, R.J. Splinter, W.C. Allen, T.K. Greenlee, *Bonding mechanisms at the interface of ceramic prosthetic materials*, J Biomed Mater Res 1971, 5(6), pp. 117-141.

[2] H. Oudadesse, M. Mami, R. Doebez-Sridi, P. Pellen, F. Perez, S. Jeanne, D. Chauvel-Lebret, A. Mostafa, G. Cathelineau, *Study of various mineral compositions and their bioactivity of bioactive glasses*, Bioceramics 22 (2009), pp. 379-382.

[3] L. L. Hench, *The story of bioglass*, J Mater Sci: Mater Med 2006, Vol. 17, pp. 967-978.

[4] L.L. Hench and J.K. West, *Biological applications of bioactive glasses*, Life Chem Rep 1996, Vol. 13, pp. 187-241.
[5] J. Rich, T. Jaakkola, T. Tirri, T. Narhi, A. Yli-Urpo, J. Seppala, *In vitro evaluation of poly(epsilon-caprolactone-co-DL-lactide)/ bioactive glass composites*, Biomaterials 2002;23:2143–2150.
[6] D. Walsh, T. Furuzono, J. Tanaka, *Preparation of porous composite implant materials by in situ polymerization of porous apatite containing ε-caprolactone or methyl methacrylate*, Biomaterials 2001;22:1205–1212.
[7] Q. Qiu, P. Ducheyne, P. Ayyaswamy, *New bioactive, degradable composite microspheres as tissue engineering substrates*, J Biomed Mater Res 2000;52:66 –76.
[8] Y. Shikinami and M. Okuno, *Bioresorbable devices made of forged composites of hydroxyapatite (HA) particles and poly-L-lactide (PLLA). Part II: practical properties of miniscrews and miniplates*, Biomaterials 2001;22:3197–3211.
[9] J. Devin, M. Attawia, C. Laurencin, *Three-dimensional degradable porous polymer-ceramic matrices for use in bone repair*, J Biomater Sci Polymer Edn 1996;7:661– 669.
[10] X. Deng, J. Hao, C. Wang, *Preparation and mechanical properties of nanocomposites of poly(D,L-lactide) with Ca-deficient hydroxyapatite nanocrystals*, Biomaterials 2001;22:2867–2873.
[11] S. Rizzi, D. Heath, A. Coombes, N. Bock, M. Textor, S. Downes, *Biodegradable polymer/hydroxyapatite composites: surface analysis and initial attachment of human osteoblasts*, J Biomed Mater Res 2001;55:475– 486.
[12] G. Brandenberg, LG. Leibrock, R. Shuman, WG. Malette, H. Quigley, *Chitosan: a new topical hemostatic agent for diffuse capillary bleeding in brain tissue,* Neurosurgery 1984, Vol 15, pp. 9-13.
[13] R.A. Muzzarelli, F. Tanfani, M. Emanuelli, D.P. Pace, E. Chiumzzi, *Sul fated N-(carboxymethyl) chitosans: novel blood anticoagulants*, Carbohydrates Researches 1984, Vol 126, pp. 225-231.
[14] S. Hirano and Y. Yagi, *The effects of N-substitution of chitosan and the physical fonn of the products on the rate of hydrolysis by chitinase from Streptomyces griseus,* Carbohydrates 1980, Vol 8, pp. 103-108.
[15] L. Jiang, Y. Li, X. Wang, L. Zhang, J. Wen, M. Gong, *Preparation and properties of nano-hydroxyapatite/chitosan/carboxymethyl cellulose composite scaffold*, Carbohydrate Polymers 2008, Vol 74, pp. 680-684.
[16] M. Peter, NS. Binulal, S. Soumya, SV. Nair, T. Furuike, H. Tamura, R. Jayakumar, *Nanocomposite scallolds of bioactive glass ceramic nanoparticles disseminated chitosan matrix for tissue engineering applications*, Carbohydrate Polymers 2009.
[17] B.D. Boyan, G. Niederaur, K. Kieswetter, N.C. Leatherbury, *Biodegradable implant material comprising bioactive ceramic*, United states patent 1999.
[18] J.D. Bumgardner, B.M. Chesnutt, W.O. Haggard, Y. Yuan, T.M. Utturkar, B. Reves, *Chitosan/nanocrystalline hydroxyapatite composite microsphere-based scaffolds*, United states patent 2007.
[19] E. Dietrich, H. Oudadesse, A. Lucas-Girot, M. Mami, *"In vitro" bioactivity of melt-derived glass 46S6 doped with magnesium*, Journal of Biomedical Materials Research 2008, Vol 88A, pp. 1087-1096.
[20] E.J. Lee, D.S. Shin, H.E. Kim, H.W. Kim, Y.H. Koh, J.H. Jang, *Membrane of hybrid chitosan-silica xerogel for guided bone regeneration*, Biomaterials 2009.
[21] I.B. Leonor, E.T. Baran, M. Kawashita, R.L. Reis, T. Kokubo, T. Nakamura, *Growth of a bonelike apatite on chitosan microparticles after a calcium silicate treatment*, Acta-Biomaterialia, ScienceDirect 2008.
[22] S. Herman, M. Hermes, S. Costa, *Nanostructured poly(vinyl alcohol)/bioactive glass and poly(vinyl alcohol)/chitosan/bioactive glass hybrid scaffolds for biomedical applications,* Chemical Engineering Journal 2007.
[23] W.W. Thein-Han and R.D.K. Misra, *Biomimetic chitosan-nanohydroxyapatite composite scaffolds for bone tissue engineering*, Acta-Biomaterialia, ScienceDirect 2008.
[24] L. Kong, Y. Gao, W. Cao, Y. Gong, N. Zhao, X. Zhang, *Preparation and characterization of nano-hydroxyapatite/chitosan composite scaffolds*, Wiley InterScience 2005.
[25] F. Zhao, Y. Yin, W. William, J. Lu, C. Leong, W. Zhang, J. Zhang, M. Zhang and K. Yao, *Preparation and histological evaluation of biomimetic three- dimensional hydroxyapatite/chitosan-gelatin network composite scaffolds*, Biomaterials 2002, Vol 23, pp. 3227-3234.
[26] T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi, T. Yamamuro, *Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W*, Journal of Biomedical Materials Research 1990, Vol. 24, pp. 721-734.
[27] T. Kokubo and H. Takadama, *How useful is SBF in predicting in vivo bone bioactivity*, Biomaterials 2006, Vol. 27, pp. 2907-2915.
[28] I. Lebecq, *Etude de bioverres à base de SiO_2, CaO, Na_2O non dopés et dopés par le phosphore*, Thèse, Université de Valenciennes 2002.
[29] M. Sitarz, W. Mozgawa, M. Handke, *Rings in the structure of silicate glasses*, Journal of Molecular Structure 1999, Vol. 511, pp. 282-285.
[30] M. Handke, M. Sitarz, M. Rokita, E. Galuskin, *Vibrationnal spectra of phosphate-silicate biomaterials*, Journal of Molecular Structure 2003, pp. 651-653.
[31] S. A. MacDonald, C. R. Schardt, D. J. Masiello, J. H. Simmons, *Dispersion analysis of FTIR reflection measurements in silicate glasses*, Journal of Non-Crystalline Solids 2000, pp. 275-282.
[32] M. Guiping, Y. Dongzhi, J. F. Kennedy, N. Jun, *Synthesize and characterization of organic-soluble acylated chitosan*, Carbohydrate Polymers 2009, Vol. 75, pp. 390–394.
[33] M. Guiping, Y. Dongzhi, Z. Yingshan, X. Ming, J.F. Kennedy, J. Nie, *Preparation and characterization of water-soluble N-alkylated chitosan*, Carbohydrate Polymers 2008, Vol. 74, pp. 121–126.
[34] H.Y. Kweon, I.C. Um, Y.H. Park, *Structural and thermal characteristics of Antheraea pernyi silk fibroin/chitosan blend film*, Polymer 2001, Vol. 42, pp. 6651-6656.
[35] J. Wang, C. Liu, J. Wei, P. Chi, X. Lu and M. Yin, *Synthesis and properties of chitosan/polypeptide bioconjugate composite*, Biomed. Mater 2007, Vol. 2, pp. 32–38.
[36] Y. Baimark, P. Srihanam, Y. Srisuwan, *Effect of chitosan Molecular Weights on Characteristic of Silk Fibroin/Chitosan Blend Film*, Current Research in Chemistry 2009, Vol. 1, pp. 8-14.
[37] X.D. Liu et al, *A novel method for immobilization of chitosan onto nonporous glass beads through a 1,3-thiazolidine linker*, J Polymer 2003, Vol. 44, pp. 1021-1026.
[38] P. Luo et al, Methods of synthesizing hydroxyapatite powders and bulk materials, United States Patent 1999.