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## Injectable Bone Substitute using a hydrophilic polymer

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**Abstract:**

We studied a new injectable biomaterial for bone and dental surgery consisting of a hydrophilic polymer as matrix and bioactive calcium phosphate (CaP) ceramics as fillers. This material is composed of complex fluids whose flow is determined by the laws of rheology. We investigated the macromolecular effects on this composite in a tube. The stability of the polymer and the mixture is essential to the production of a ready-to-use injectable biomaterial. These flow properties are necessary to obtain CaP bioactivity in a dental canal or bone defect during percutaneous surgery. Macromolecules provide spaces between CaP ceramic granules and facilitate the role of the biological agents of bone substitution.

Keywords: Biomaterial, Calcium Phosphate, Injection, Bone substitute.

## **Introduction:**

Current approaches to the reconstruction of bone tissue in orthopedic surgery, stomatology and dental applications rely on calcium-phosphate (CaP) ceramics. CaP biomaterials are used in bone repair, substitution or augmentation, as osteoconductive fillers to achieve bone coalescence [1-4]. CaP are the principal raw materials used for bone substitutes in the elaboration of granules or blocks [2]. The macro porosity of CaP blocks facilitates the penetration of cells and biological growth factors into the implant, allowing the osteogenetic process to occur within the inner surface of pores.

Two types of injectable bone substitutes (IBS) are presently under development in laboratories. The first consists of ionic hydraulic cements which set in vivo after injection. Their components need to be mixed before injection, and hardening is achieved by recrystallization of the mineral phase. For instance, Norian SRS® [5], a new ionic cement that hardens in physiological conditions, is a blend of monocalcium phosphate monohydrate (MCPM) with  $\alpha$ -tricalcium phosphate (TCP) and calcium carbonate (CC). The hardening reaction occurs when a sodium phosphate solution is added and mixed with the powder. The main limitations with these materials are the need to mix the components before use and the existence of a dense structure after hardening which cannot be readily colonized by cells. [6].

The second type consists of CaP ceramic suspensions in carrier phase which are ready to use. Injectable biomaterial has recently been developed [7], whose combines a polymeric water solution viscous phase (non-ionic cellulose ether) with bioactive CaP ceramic granules [8]. Ionic polymers are not used because of their spontaneous ionic reticulation in the presence of CaP in water, causing a volumetric contraction of the composite mixture. Instead, cellulosic non-ionic hydrophilic derivatives have been used. This formulation can be modified to provide ready-to-use sterilized injectable material that decreases the risk of infection since the product does not need to be prepared during surgery. The fillers are maintained in viscous phase at the surgical site. The mechanical strength of this material results from early osteoconduction and bone substitution [9].

## **Materials and Methods**

The polymer used for this study was hydroxypropylmethylcellulose (HPMC; Benecel® MP 824 from Aqualon France). The polymer was dissolved in bidistilled water (polymer concentration: 2% w/w), and the solution was stirred for 3 days. The CaP fillers used were composed of biphasic calcium phosphate (BCP; 60% HA and 40%  $\beta$ -tricalcium phosphate) prepared in our laboratory in three ways:

- A commercial BCP is produced by a sintering method (Triosite®, Zimmer Company, France).

- This first material was modified by brief washing in deionized water and sintering at 900°C to remove impurities. Analyses of some BCP lots showed a high extractable pH in water due to the presence of calcium oxide which can be transformed into calcium carbonate or calcium hydroxide.
- BCP prepared in our laboratory according to the LeGeros method (precipitation of calcium-deficient apatite and sintering) [10].

Fillers were powdered and sieved between 80 and 200 µm.

The composite was prepared under an air atmosphere by mixing CaP powder with polymer solution. The mixture was then placed in a 12-ml glass bottle sealed at time T<sub>0</sub> and sterilized in an autoclave for 20 min at 121°C according to pharmacopeial recommendations

To study the influence of polymer on the suspension, apparent viscosity (Brookfield® RDV1+, 1RPM) and flow measurements through a hole and a tube (texture analysis TA XT2® Rheo Company, France ) were performed with different formulations, steam sterilization and storage.

### **Results and discussion:**

Mineral fillers need to pass through narrow channels (syringe tube, dental root canal) without alteration or demixing of phases. Apparent viscosity measurements and extrusion tests have indicated that macromolecules are most suitable for this purpose. Figure 1 shows the changes in apparent viscosity for two suspensions of CaP granules diluted differently. The mixture remains liquid up to a concentration of 58% of BCP and then suddenly becomes plastic, increasing slightly in viscosity. Beyond 60% of mineral phase, the aqueous suspension becomes solid because the different composite strata cannot slide. Measurements become impossible since the viscometer only records friction between the heavy mixture and the analysis spindle. The addition of a polymer allows the viscosity of the suspension to increase rapidly and substantially. The plasticity zone in which the material remains injectable is considerably enlarged. In an extrusion experiment, a study of molecular weight and polymer concentration showed that the best carrier had a heavy molecular weight (Fig. 2). However, the degree of viscosity was limited since friction with the needle wall became too strong (Fig. 3).

This material is a suspension. The stability of a suspension is explained by many laws, and sedimentation occurs with gravity. Sedimentation controls and viscosity measurements can change some formulation parameters. The polymer concentration decreases the kinetics and not the ratio of sedimentation (Fig. 4). Without polymer, the sedimentation is heavier, and granule compaction is maximal. With polymer, each granule is separated from another by the macromolecular gel. This suspension can be compared with interconnected macroporous CaP

block ceramics. *In vivo* osseous studies of this injectable material have confirmed its high permeability to cells and biological fluids, which allows early osteoconduction and bone substitution (Fig. 5).

The composite must remain stable and injectable after storage to provide a ready-to-use IBS requiring no modifications by the surgeon. Studies of apparent viscosity (Brookfield® RDV1+, 1Rpm) after steam sterilization (121°C, 21 min) and storage for a year indicated the contribution of the medium in stabilizing the mixture. When BCP synthesis introduced calcium oxide impurities into the ceramic, calcium hydroxide was produced and extractable pH (1 g of BCP in 20 ml of bidistilled water) increased, thereby decreasing composite viscosity after sterilization. When the extractable pH was nearly neutral, the viscosity of the mixture remained relatively stable for one year (Fig. 6). To analyze these behavioral modifications, changes in chemical degradation and macromolecular conformation were studied by infrared spectroscopy [11-12] and light scattering. The results showed that the medium and type of CaP ceramic filler could modify cellulose polymer after sterilization and long-term storage. Thus, the polymer/CaP filler composite was not just a simple mixture of organic (polymer) and inorganic (CaP fillers) phases but a complex reaction between the two phases.

Degradation appeared to be slight since most FT-IR spectra displayed low absorption bands or no absorption bands indicative of chemical modification. The greatest degradation occurred in alkaline conditions and with oxidizing agents. With BCP without calcium oxide, FT-IR analysis detected no chemical modifications of the polymer, whereas FT-IR microspectroscopy revealed very small changes on a few dry particles of extracted polymer from the IBS. However, a long-term rabbit bone response to IBS induced with HPMC and washed BCP showed that “the association of cellulosic ether carrier with bioactive ceramic particules does not detrimentally affect the bony incorporation of BCP particles” [13]. All our *in vivo* and *in vitro* experiments with this mixture have demonstrated its biocompatibility and biofunctionality [14-16]. Further experiments with IBS in rabbit bone in the same conditions using a new HPMC showed that bone ingrowth occurred faster than in experiments with the first HPMC [13] or with macroporous ceramic blocks [17]. The macromolecular parameters influencing bone growth kinetics are currently unknown and require further investigation.

### **Conclusion:**

The physical measurements and experiments reported here facilitate the choice of molecular weight and the degree of substitution suitable for specific medical indications. However, the flow behavior of a mixture cannot be predicted with mathematical precision because of the many parameters that influence IBS rheology. Mixture designs have been used to adapt IBS formulations to surgical technology [18]. The greatest challenge is to

understand the implications of each polymer parameter in order to provide more adequate bone substitution than with dense materials and to produce functional IBS.

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Figure 1: Apparent viscosity (Brookfield® RDV1+, 1 RPM) of two calcium-phosphate water suspensions as a function of BCP ratio in weight. One of the suspensions was performed only with water, and the other with aqueous polymer solution ( 1.5% of HPMC in water w/w).

Figure 2: Extrusion of the BCP mixture (53% to 65% in weight, particles 80-200  $\mu\text{m}$ ) with water or water polymer solution containing 1.5% or 2% of HPMC through a 4-mm hole at a rate of 0.1 mm per second. Apparent composite viscosity was adjusted to about 1 to 1.2 million Mpa s (Brookfield® RDV1+, 1 RPM). Compression strength measurements on the piston of an injection syringe were recorded with a computer to obtain this profile.

Figure 3: Injection of the BCP mixture (50% in weight, particles 40-80  $\mu\text{m}$ ) with water or aqueous polymer solution (HPMC 1.5% or 2%) through a 0.8X25 mm needle at a rate of 0.1 mm per second. Apparent composite viscosity was 40,000 Mpa s for the water suspension, 200,000 Mpa s for the 1.5% HPMC in water suspension and 600,000 for the 2% solution (Brookfield® RDV1+, 1 RPM). Compression strength measurements on the piston of an injection syringe were recorded with a computer to obtain this profile

Figure 4: Stability of suspensions to 33% (W/W) of BCP granules sifted between 200 and 500  $\mu\text{m}$  in different aqueous solutions of HPMC polymers with a concentration ranging from 0 to 2.5% in weight/volume.

Figure 5: Scanning electron Microscopy (backscattered electrons) of two femoral implantations in rabbit bone of ceramics granules (a) or injectable bone substitutes (b) after 3 weeks. (Original magnification X 10).

Figure 6: Viscosity variation as a percentage of initial viscosity (before sterilization) of the composite mixtures. The 2% HPMC polymer in water was mixed with different BCP concentrations synthesized and treated by different processes in order to obtain different extractable pH (1 g of powder in 20 ml of bidistilled water). These measurements were performed for up to 12 months of storage using a Brookfield RDVI+ Viscometer (1 rpm).