

Microcharacterization of a new injectable polymer/ceramic composite as bone substitute in spine surgery

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The results of microscopic and spectroscopic analysis of a mixture of biphasic calcium phosphate (BCP) powder and cellulose ether aqueous sol are reported in this study. This new composite is injectable and is aimed to be used as bone substitute for spine surgery. The influence of the polymer on the physico-chemical properties of BCP was investigated by complementary techniques such as scanning electron microscopy (SEM), high-resolution transmission electron microscopy (HR-TEM), X-ray diffraction (XRD), infrared and Raman spectroscopies and X-ray photoelectron spectroscopy (XPS). When brought into contact with the polymer solution, the ceramic is modified in its granulometry, crystallinity, and surface chemistry. A dissolution/precipitation reaction occurs, leading to a phosphorus-rich and amorphous outer layer of the ceramic surface. Moreover, the bonding state of the composite involves new features that could be assigned to calcium complexes (calcium carbonate or calcium hydroxide).

1. Introduction

Among the biomaterials used in spine surgery, biphasic calcium phosphate has proven to be a good biodegradable bone substitute [1], which promotes spine fusion. The future challenge to perform percutaneous surgery leads to the need for injectable bioactive materials. In this aim, a composite based on osteoconductive BCP powder and hydroxypropylmethylcellulose (HPMC) was developed. HPMC was chosen as polymer vehicle because of its hydrosolubility, its rheologic behaviour and its biocompatibility [2].

As biocompatibility and bioactivity depend strongly on the physico-chemical material properties, the aim of this study was to evaluate the possible changes initiated in the ceramic because of its association with the polymer carrier. The characterization of such composite material remains limited if only conventional analytical techniques are used. The study of fine changes initiated in the ceramic structure as well as the analysis of the ceramic/polymer interface require sensitive techniques, such as high resolution transmission electron microscopy (HR-TEM) and X-ray photoelectron spectroscopy (XPS).

2. Materials and methods

The material was composed of 60% BCP powder (60% hydroxyapatite, 40% β -tri-calcium phosphate) and 40% HPMC water solution (2% w/w) which was steam sterilized (121 °C, 20 min).

This composite material was microcharacterized using scanning electron microscopy (SEM) (Jeol, JSM 6300) supported microprobe analysis and with HR-TEM (Jeol, JEM 200CX), and compared to BCP powder. Moreover, the sterile composite, as well as its separate components were analysed by X-ray diffraction (XRD) (Siemens, D5000), by FT-infrared and conventional Raman spectroscopies (Bruker IFS-28, Jobin Yvon T 64000) and by X-ray photoelectron spectroscopy (Leybold ESCA LH12).

3. Results and discussion

3.1. SEM analysis

SEM investigations of reference BCP powder show clusters of size 80–200 μm , made up of 1 μm individual micrograins. Energy dispersive X-ray analysis gives a mean Ca/P weight ratio of 2.3.

SEM observations of the composite paste reveals a non-homogeneous repartition of the ceramic phase in the polymer phase. At places of high ceramic density, the polymer is stretched as bonds between the BCP grains. It seems to be in a flocculated form. Otherwise, it appears as a film spacing film BCP grains out and creating a kind of macroporous structure in the composite paste (Fig. 1). The size of loosened BCP clusters, found randomly dispersed in the polymer film, lies between 1 and 5 μm . This decrease of granulometry indicates a partial dissociation of the initial BCP agglomerates and thus that the

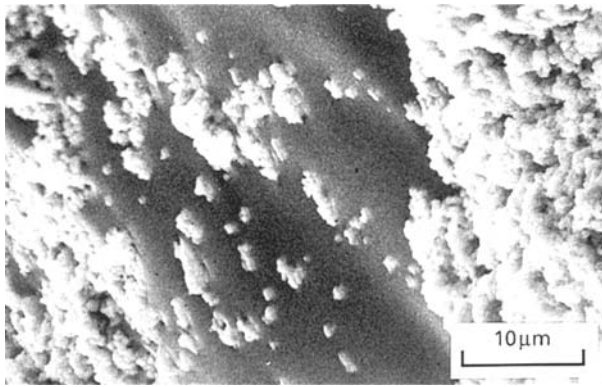


Figure 1 SEM micrograph showing the composite morphology.

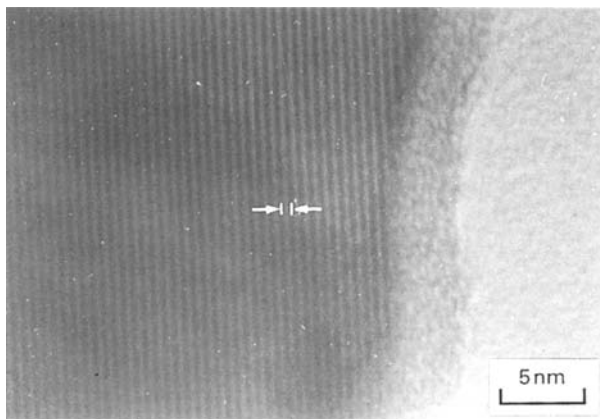


Figure 2 HR-TEM image of a hydroxyapatite grain in the composite, showing the light fringe at its surface and the (100) reticular plane.

grain boundary does not withstand the interaction with the cellulose ether. The elemental chemical analysis gives a Ca/P ratio unchanged for the ceramic phase, while it reveals an excess of calcium in the polymer phase (Ca/P about 7).

3.2. TEM observations

TEM observations show a light-contrasted fringe at the surface of BCP grains in the composite section. The fringe thickness is about 60 nm. HR-TEM reveals this fringe to be an amorphous layer appearing at the surface of the crystalline ceramic grain, which has been in contact with the polymer solution. Fig. 2 shows no discontinuity between the reticular planes (100) of a hydroxyapatite grain and this amorphous surface layer. This could indicate a dissolution/reprecipitation process initiated by the presence of polymer aqueous solution at the ceramic surface. Such an interfacial reaction has been described by other authors [3, 4].

3.3. XRD analysis

Analysis of the XRD diagram of composite (not shown) reveals broader peaks than those found in BCP, demonstrating the effect of the polymer on the loss of crystallinity of the ceramic phase.

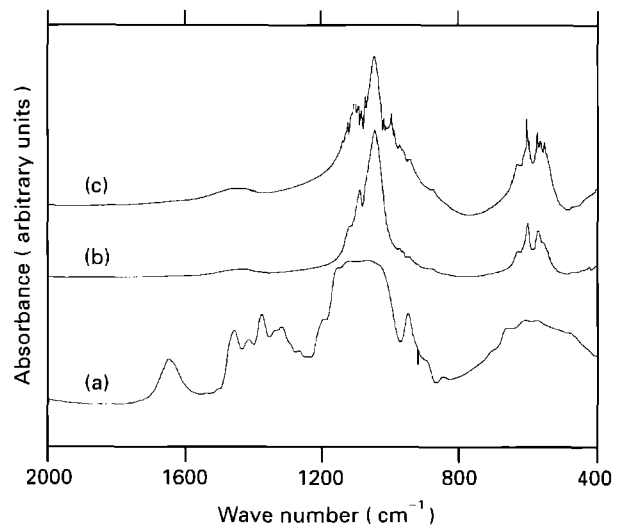


Figure 3 FT IR spectra of: (a) HPMC polymer; (b) BCP; (c) composite.

Moreover, calculation of the β -TCP/HA ratio (based on the intensities of the (2 1 1) HA peak and the (0 2 10) β -TCP peak) indicates a decrease of the β -TCP content in the ceramic phase of the composite.

3.4. Infrared measurements

The IR absorption bands of the polymer, ceramic and composite samples are shown in Fig. 3. The spectrum of the polymer film has four main bands in the frequency range from 700 to 1700 cm^{-1} (curve a). The most intense band centred at 1100 cm^{-1} and is assigned to C–O vibrations [5]. The bands located at 950 and 1650 cm^{-1} are identified as pyran group and water, respectively. The large band spreading from 1320 to 1450 cm^{-1} contains many features which are attributed to carbon–hydrogen groups (CH and CH_2).

In the BCP ceramic sample, pronounced peaks are found at 570–600 and 1050 cm^{-1} (curve b). These bands are assigned as stretching and bending of PO_4 group [6]. Shoulders at 550 and 1120 cm^{-1} are also observed which are identified as characteristic of β -TCP phase. The presence of the OH group in HA phase is detected by the peak situated at 630 cm^{-1} . We note that a shoulder as well as a band appearing, respectively, at 870 cm^{-1} and 1450 cm^{-1} indicates a small calcium carbonate contamination of the sample [6].

Comparing the composite spectrum (curve c) to those of the BCP ceramic and the polymer film, we notice that the specific peaks of BCP are present in the composite while none of the polymer is retrieved. As the polymer concentration is weak, the corresponding IR features could be masked by those of BCP. Nonetheless, the band centred at 1050 cm^{-1} is somewhat broader in the composite spectrum than that found in BCP. Therefore, this band can be considered as resulting from the superposition of the corresponding bands in both ceramic and polymer components. We also remark that an enhancement in intensity of the band centred at 1450 cm^{-1} indicates that the composite contains a higher amount of carbonate than BCP does.

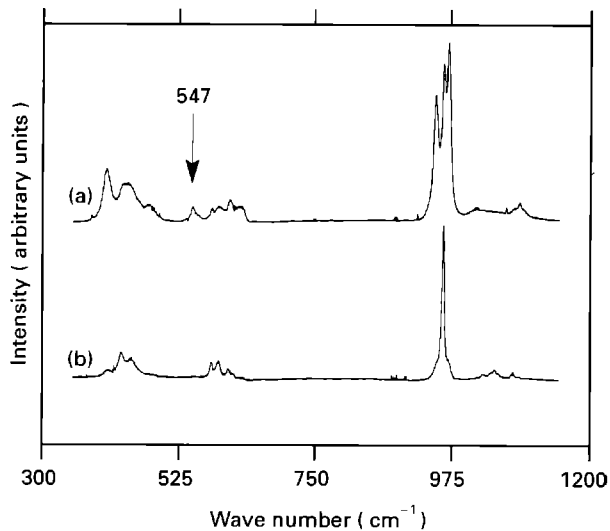


Figure 4 Raman spectra of: (a) BCP; (b) composite paste.

3.5. Raman measurements

Fig. 4 shows the Raman spectra of BCP and composite paste samples, obtained with laser excitation $\lambda = 676.4$ nm. Comparing the spectrum of the composite to that of BCP, we can see that the proportion of β -TCP has decreased in the composite (disappearance of the peak at 547 cm^{-1} and decrease in intensity of the peaks at 406 , 626 , 948 and 969 cm^{-1} [7]). Following this result, the diminution of β -TCP occurs after mixture of BCP powder in the polymer solution. This clearly shows that β -TCP is the most unstable part of the BCP ceramic. The partial dissolution of the powder leads to accumulation of ionic species (for instance Ca^{2+} , PO_4^{3-} , HPO_4^{2-} and H_2PO_4^-) at the polymer–ceramic interface.

3.6. XPS measurements

For the polymer film obtained after steam sterilization at 121°C , the C 1s line is observed at 285 eV , which is characteristic of hydrocarbon or carbon–carbon bonding (Fig. 5, curve a). Broadening in the high energy side of the spectrum suggests that other carbon bondings are also present. The decomposed spectrum shows four components with peak positions at 285 eV (C–H of C–C), 286.7 eV (C–O–R), 288.6 eV (C = O) and 290.8 eV ($\pi \rightarrow \pi^*$ shake-up transition). On the other hand, using the same technique, of O 1s line (not shown) has two components with peaks located at 531.8 eV (C = O) and 533.4 eV (C–O), in agreement with the deconvolution result of the carbon line. Therefore, XPS data reflect well the structure of the polymer.

For the XPS spectra of the composite sample, in addition to the Ca 2p, O 1s and P 2p lines, carbon C 1s spectrum is also observed. It is composed of four components with peaks located at 298.3 eV (carbonate), 285 eV (hydrocarbon contamination), 286.6 eV (single carbon–oxygen, probably from the polymer) and 281.3 eV (Fig. 5, curve b). The latter seems to be linked with the presence of $\text{Ca}(\text{OH})_2$ or of CaCO_3 [8].

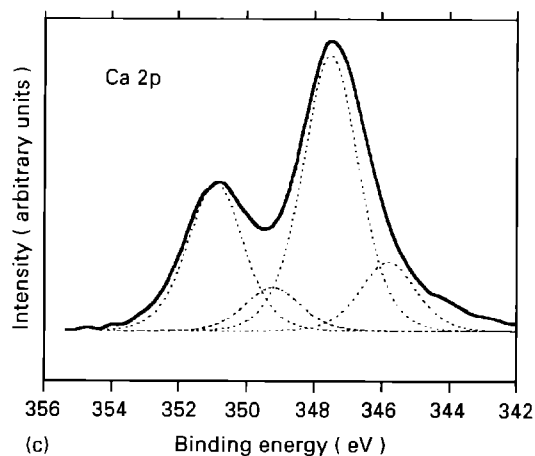
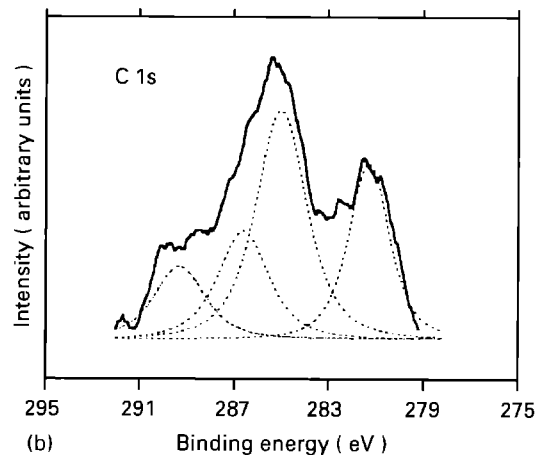
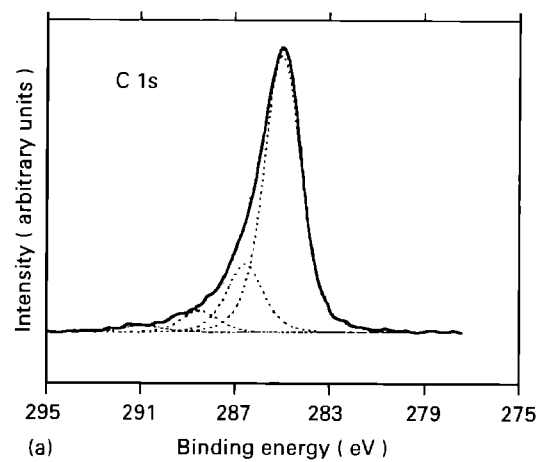


Figure 5 XPS spectra of (a) polymer film (C 1s line); (b) composite sample (C 1s line); (c) composite sample (Ca 2p line).

In fact, the calcium line is composed of two doublets (Fig. 5, curve c): the main $2p_{3/2}$ peak is located at 347.5 eV (Ca in CaPO_4) while the low energy binding peak at 345.8 eV may correspond either to $\text{Ca}(\text{OH})_2$ or to CaCO_3 , according to earlier results on calcium compounds [8]. This finding would be in good agreement with the IR interpretation. The appearance of calcium hydroxide and calcium carbonate in the composite shows that the polymer solution would favour the dissolution of BCP by creating an acidified aqueous micro-environment around the powder grains. Although not shown here, we note that the P 2p line has two peaks [9] located at 133.4 eV (PO_4 group) and 134.2 eV (HPO_4). On the other hand, the O 1s line

has one main peak at 531.3 eV (PO_4 group [10]) with components at 529.1 eV ($\text{Ca}(\text{OH})_2$ [11]) and 533.3 eV (HPO_4). Note that in the IR composite spectrum, HPO_4 species could not be detected (normally appearing at 865 cm^{-1}), suggesting that they would result from a surface modification. The observation of the HPO_4 species on the ceramic surface could be related to the amorphous surface layer detected in TEM investigations. The formation of these ions may be associated with a reprecipitation process on the ceramic surface [3].

4. Conclusions

In summary, the characterization of the composite by various techniques gives consistent results. Under the interaction with non-ionic cellulosic ether carrier, the BCP powder undergoes physico-chemical changes such as loss of crystallinity, carbonation and an interfacial dissolution/precipitation process. The latter reaction could be compared to the initial step of formation of carbonate apatite *in vivo* [6]. The macroporous structure given by the polymer presence might be appropriate to allow bone ingrowth, if the granulometry of BCP powder remains large enough to be used as scaffold for bone repair and not to stimulate inflammatory reaction.

An injectable composite that can be well controlled by complementary characterization techniques seems to be a good candidate as material for arthrodesis, giving a new alternative to BCP block implants for spine surgery.

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