J Mater Sci: Mater Med (2006) 17: 447–454
DOI 10.1007/s10856-006-8472-y

Biodegradable and semi-biodegradable composite hydrogels as bone substitutes: morphology and mechanical characterization

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Received: 28 June 2004 / Accepted: 7 July 2005

Abstract Biodegradable and semi-biodegradable composite hydrogels are proposed as bone substitutes. They consist of an hydrophilic biodegradable polymer (HYAFF 11) as matrix and two ceramic powders (α-TCP and HA) as reinforcement. Both components of these composites have been of great interest in biomedical applications due to their excellent biocompatibility and tissue interactions, however they have never been investigated as bone substitute composites. Morphological and mechanical analysis have shown that the two fillers behave in a very different way. In the HYAFF 11/α-TCP composite, α-TCP is able to hydrolyze in contact with water while in the HYAFF 11 matrix. As a result, the composite sets and hardens, and entangled CDHA crystals are formed in the hydrogel phase and increases in the mechanical properties are obtained. In the HYAFF11/HA composite the ceramic reinforcement acts as inert phase leading to lower mechanical properties. Both mechanical properties and microstructure analysis have demonstrated the possibility to design hydrophilic biodegradable composite structures for bone tissue substitution applications.

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1. Introduction

Generally, human bone may be considered as a composite material which by weight contains about 60% of mineral phase, 30% of organic phase and 10% of water [1]. The mineral phase is Calcium Deficient Hydroxyapatite (CDHA) that is a non stoichiometric calcium phosphate compound in which some Ca^{2+} ions are substituted by other ions such as Mg^{2+}, Na^{+}, K^{+}, etc. and some phosphate ions are substituted by carbonated ions. Collagen is the organic phase: it is organized as fibrils and acting as matrix holding together the needle like CDHA crystals. The resulting structure is highly organized making bone an anisotropic material [1], [3]. Ideally, an artificial implant, temporarily replaces the function of the damaged bone and subsequently induces a regeneration of the natural tissue [3]. This behavior can be achieved by designing the artificial bone using biocompatible materials which degrades slowly after implantation as the body heals itself, and which contains biologically active phases and/or molecules that stimulate the regenerative tissue growth.

In order to design a biodegradable bone substitute which fulfils the complex requirements, in this work two materials have been chosen: a Benzyl Ester of Hyaluronic Acid (HYAFF11) as the organic component and two ceramic powders α-Tricalcium Phosphate α-$Ca_3(PO_4)_2$, (α-TCP), and Hydroxyapatite $Ca_6(PO_4)_{10}(OH)_2$, (HA) as reinforcements.

Hyaluronic Acid (HyA) is a natural polymer found in many human tissues such as synovial liquid, skin, umbilical cord and many soft tissues. It appears to play a crucial role in many biological processes as hydration, cellular differentiation and proteoglycan organization [4]. Since it was isolated in 1934 [4], it has been used as a biomaterial in many applications because its presence in the human

body. Due to the esterification process, it is possible to modify the chemical-physical properties of HyA to obtain a class of polymeric materials with a wide range of viscoelastic properties and degradation behaviors, the Hyaffs. HYAFF11 has shown a good degradation rate and, above all, the products of degradation are non toxic and non carcinogenic [5].

From a mechanical point of view, the Hyaffs, like most hydrogels, have too low mechanical properties for bone substitution. To improve the mechanical properties of the HYAFF11, the calcium phosphate ceramic particles have been added to the hydrophilic polymer. In general, it is well known that calcium phosphates, have osteogenic properties stimulating natural bone replacement. As reported in many studies [6], [8], the most attractive characteristic of the calcium phosphates is their ability to form a strong direct bond with the host bone resulting in a strong dynamic interface compared to bioinert or biotolerant materials which form a fibrous interface [12].

Additionally, in the 1980's the idea of a new injectable bone substitute material was put forward based on calcium phosphate, in the form of "Calcium Phosphate Bone Cement" (CPBC). These ceramic biomaterials have the advantages of calcium phosphates and could be used as a cement [9]. Some advantages of these materials are their moldability, injectability and complete filling of a cavity *in situ* [13]. The host bone tissue also takes benefits from initial setting characteristics of the material which gives, in a clinically acceptable time, suitable mechanical strength for short term tissue functional recovery. Further advantages relate to the ability of CPBCs to activate the osteoclastic and osteoblastic functions of bone regeneration, which then contribute to the transformation of the cement material to an organized structure characteristic of newly formed bone [9].

One of the main reactants in CPBC is α-TCP. In presence of water α-TCP hydrolyses through a dissolution-precipitation reaction, giving rise to the formation of an entangled network of calcium deficient hydroxyapatite (CDHA) crystals:

$$3Ca_3(PO_4)_2 + H_2O \Rightarrow Ca_9(HPO_4)(PO_4)_5OH$$

As a consequence of this reaction, the initially plastic paste sets and hardens to a solid body. Ginebra *et al*. [10] demonstrated that after 24 hours of immersion in water, the compressive strength of this cement was about 25 MPa and after 64 hours the reaction was complete, reaching its maximum value. The high reactivity with water makes the α-TCP very interesting as reinforcement of an hydrophilic polymer such as HYAFF11. The composite can be prepared in two stages: in the first stage the composite is paste-like and it can be kept in the unreacted form until it is needed. In the second stage, it becomes stronger simply by immersing it in water or absorbing water from the surrounding tissue.

Another material used as reinforcement in this study has been hydroxyapatite, which has been extensively used as bone substitute in many clinical applications, because of its biocompatibility and osteoconduction [11]. The use of these two different inorganic components, α-TCP as reactive system and HA as non-reactive reinforcing phases, will define their final influence on the mechanical properties of the composite materials [16]. The combination of a ceramic material as α-TCP or HA with an organic phase is aimed to not only improve the mechanical properties of HYAFF11, but also introduce a new bioactive element to promote the osteointegration, that is increasing the bioactivity of the composite [1].

2. Materials and methods

2.1. Materials preparation

HYAFF 11 (supplied by F.A.B. s.r.l., Abano Terme, Italy) is an hydrophilic semi-synthetic polymer obtained by the fully esterification of the Hyaluronic Acid (HyA) carboxylic groups with benzyl alcohol which makes it water insoluble [5]. A polymer solution of 8% by weight of HYAFF11 was obtained by dissolving the HYAFF11 powder in Dymethylsulfoxide (DMSO, Aldrich Chemical Company) by stirring at room temperature.

The α-TCP was prepared by heating in air at 1300°C for 15 h and then quenching to room temperature in air an appropriate mixture of $CaHPO_4$ (Merck 02144) and $CaCO_3$ (Merck 2076). The powder was milled in an agate ball mill for 1 h, the powder size distribution obtained is shown in Fig. 1 and was measured by laser diffraction (α-TCP $\oslash_{Av}$ = 5 micron). Phase purity was assessed by X-ray diffraction. Grade P205 hydroxyapatite (HA:$Ca_{10}(PO_4)_6(OH)_2$) (P205 grade) was obtained from Plasma Biotal, Tideswell, Derbyshire, UK. The particle size distribution is given in Fig. 1b showing a bimodal distribution with a spherical morphology ($\oslash_{range}$ = 4–11 micron, $\oslash_{0.5}$ = 4.02 micron).

HYAFF11/α-TCP composites were prepared with two different weight fractions of filler: 93 wt.% α-TCP and 86 wt.% α-TCP, by mixing the HYAFF11 polymer solution with the powder until it became an homogeneous fluid. The paste obtained was injected in cylindrical glass moulds (D = 6 mm, L = 12 mm), and then immersed in Ethanol to extract the DMSO. After 24 hours, the specimens were removed from the glass cylinders: some of them were dried at room conditions, the others were immersed in distilled water at 37 ± 1 °C for 24, 48, and 96 hours. HYAFF11 and HYAFF11

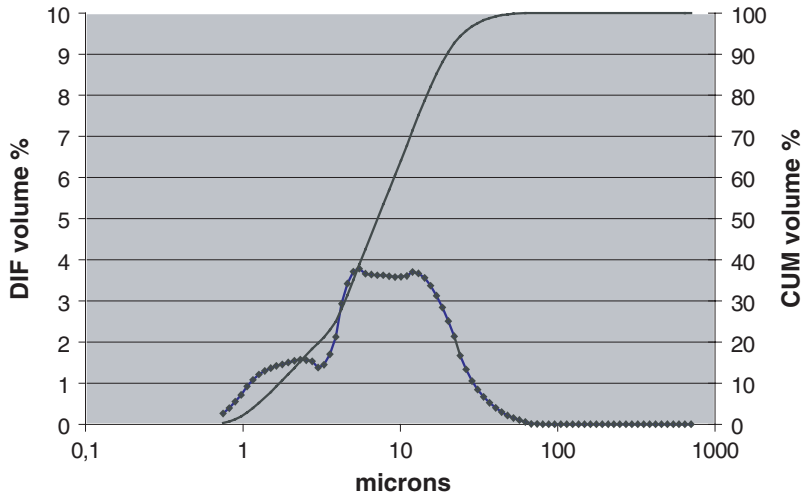

Fig. 1 Particle size distribution of α-TCP (a) and HA (b) Particle size distributions for P205 Hydroxyapatite.

with 86 wt.% of HA were prepared by following the same procedure.

α-TCP specimens were prepared, mixing the α-TCP powder (P) with a water solution (L) of Na_2HPO_4 (2.5%wt) in order to obtain the rate L/P = 0.35. Cement paste was injected in a Teflon mould and the mould was immersed in a water solution of Na_2HPO_4 (2.5%wt) at 37 $\pm$ 1°C for 96 hours. After this period of time, the specimens were dried for 24 hours at room conditions and then they were extracted and tested.

2.2. Scanning electron microscopy

Microstructures and their evolution were investigated on gold-coated fracture surfaces using a Scanning Electron Microscopy (Mod Leica 420) at 20 kV excitation voltage. Scanning Electron Microscopy HYAFF11/α-TCP was performed to follow the evolution of the composite structures due to the setting and hardening of the α-TCP included in the polymeric phase.

2.3. Compressive mechanical properties

Cylindrical specimens of HYAFF11, HYAFF11/α-TCP and HYAFF11/HA, with a diameter of 6 mm and length of 12 mm, were tested in compression after different periods of immersion in water.Before testing, all the materials were dried for 24 hours at room conditions.

The testing regime followed ASTM D 695, using an INSTRON 4204 testing machine at a cross-head speed of 1 mm/min. T-Student Test statistical analysis ($p < 0.05$) was performed.

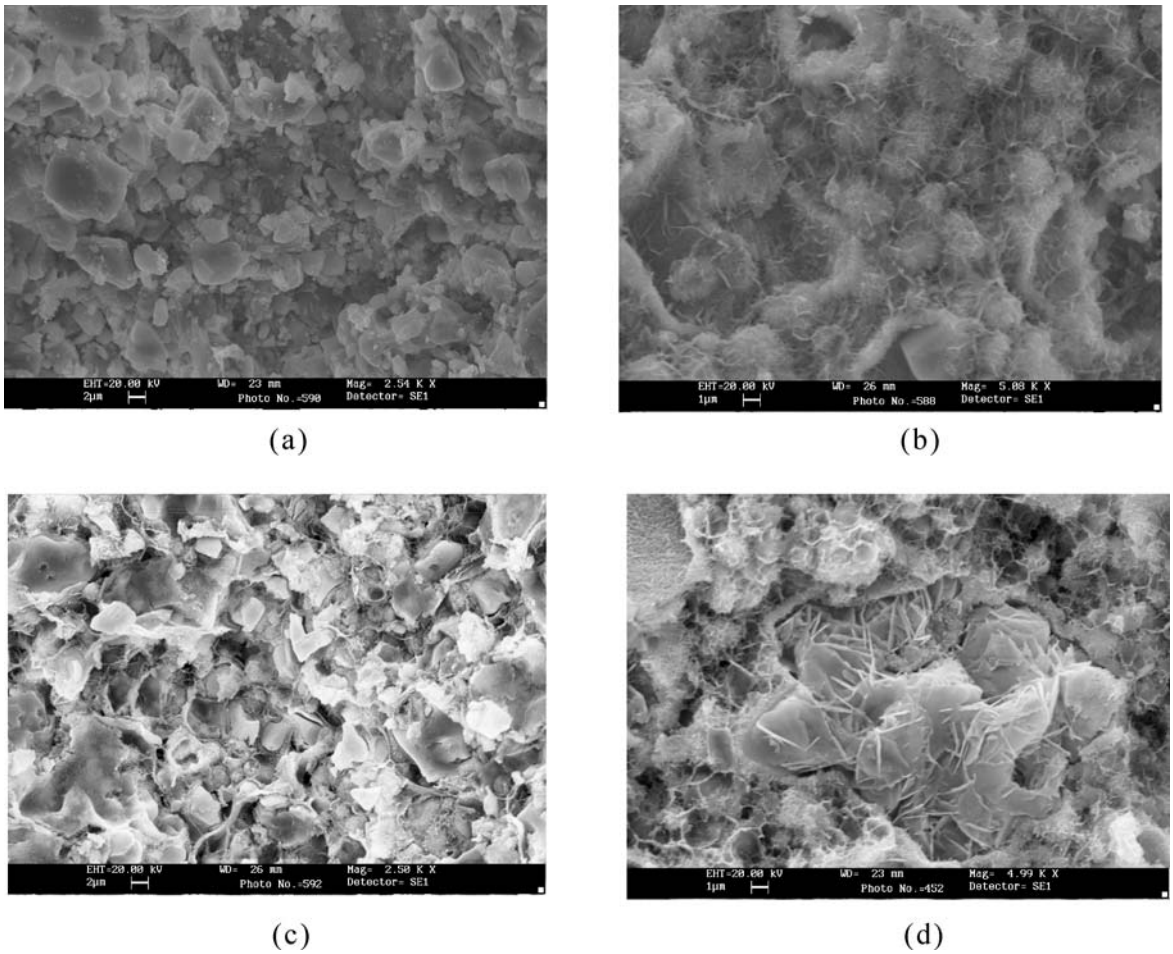

Fig. 2 S.E.M. of Hyaff 11 reinforced with 93%wt of α-TCP: (a)no reaction, after (b) 24 h, (c)48h (d) 96h of reaction in water at 37°C.

3. Results and discussion

3.1. Microstructure evolution

S E M micrographs of the composite HYAFF11/α-TCP (7/93) fracture surfaces at different reaction times are shown in Fig. 2. The differences between the various stages in the evolution of the microstructure can be distinguished, corresponding to different times of immersion in water. In Fig. 2a, showing HYAFF11/α-TCP without immersion in water, the round particles of α-TCP can be seen in the polymer matrix without any specific orientation or differentiation. In Fig. 2b it is shown how the composite structure has evolved after just 24 h of immersion in water: the smaller α-TCP particles have dissolved completely whereas the larger ones have been surrounded by a layer of small needle crystals presumably of CDHA.

After 48 hours immersion in water, Fig. 2c, the remaining α-TCP particles have continued to dissolve, although at a lower rate. The presence of some laminar crystals of CDHA within the interstices are clear. These crystals are larger than the first CDHA crystals precipitated. At the end of this stage, it is difficult to detect any remaining round particles of α-TCP, although the rims of small crystals formed in the second stage can still be distinguished, the space previously occupied by the α-TCP is now occupied by bigger laminar crystals. In the final stage (Fig. 2d), after 96 hours in water, radial or parallel orientations of crystals occur with a more compact aspect, due to crystal growth leading to a more homogeneous material.

In Fig. 3 the same sequence is shown for the composite HYAFF11/α-TCP (14/86). Similarly for this composite it is possible to observe the microstructural evolution during the setting and hardening mechanism. In Fig. 4 the fracture surface of HYAFF11/HA (14/86) composite is shown. In particular Fig. 4a shows the fracture surface of HYAFF11/HA (14/86) before the immersion in water. The microstructure is similar to the unreact HYAFF 11/α-TCP, (Fig. 3a) where the rounded HA agglomerates are immersed in HYAFF11 matrix. No appreciable morphological changes or transformation can be observed when the materials are immersed for 96 hours in water at 37 °C (Fig. 4b) comparing to the non-immersed sample, since the HA particles act as inert phase.

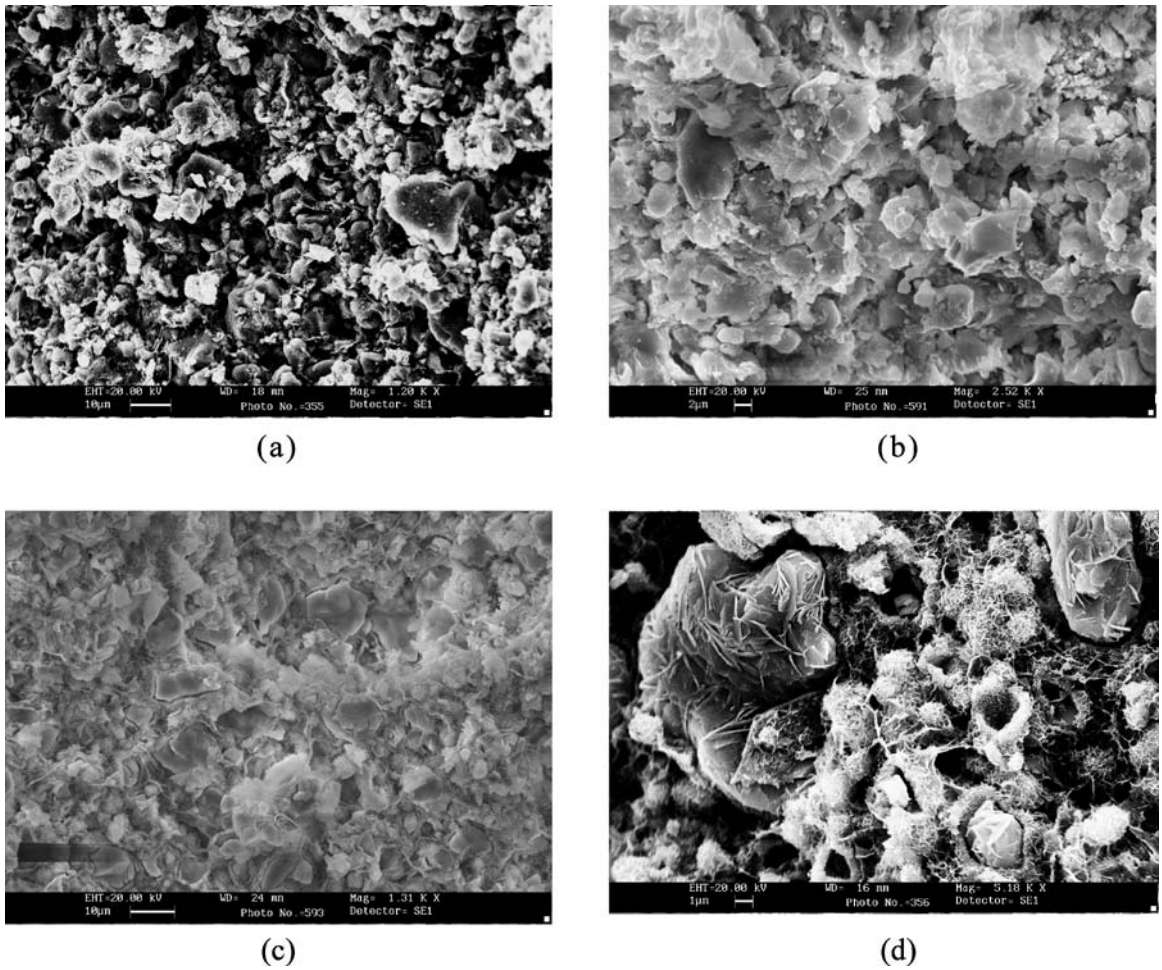

Fig. 3 S.E.M. of Hyaff 11 reinforced with 86%wt of α-TCP: (a) no reaction, after (b) 24 h, (c)48h (d) 96h of reaction in water at 37°C

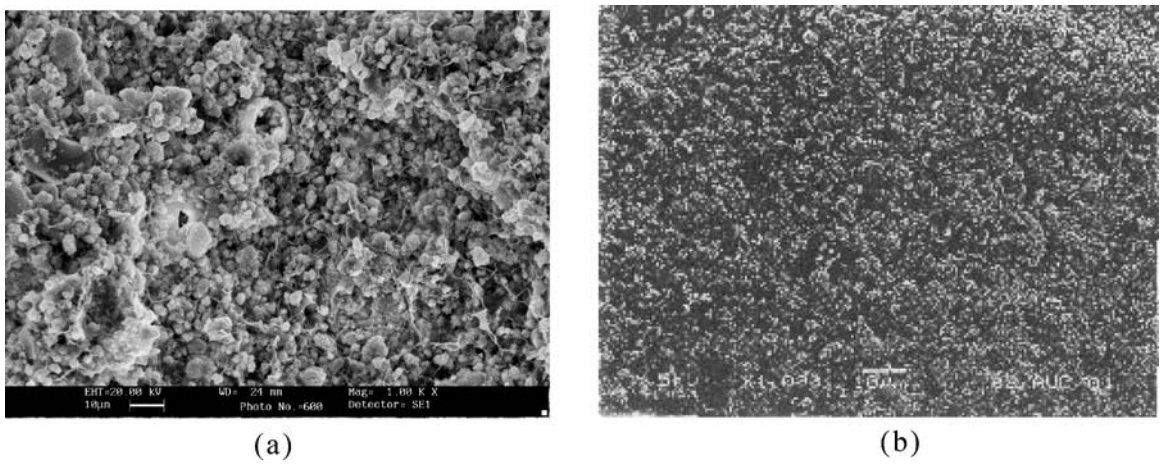

Fig. 4 S.E.M. of Hyaff 11 reinforced with 86%wt of HA (a) no immersion in water, (b) 96 h reaction in water at 37°C

3.2. Compressive properties

Compressive test data have been used to evaluate the main mechanical properties such as the elastic modulus (E), the maximum compressive strength (σ_{max}) and the maximum deformation (ε_{max}) as function of the weight content of α-TCP, the setting and hardening time related to the different immersion time in water, and type of the reinforcement.

Figure 5 shows the stress-strain curves of HYAFF11/α-TCP (7/93 percent by weight) at different times of reac-

Table 1 Compressive properties of HYAFF 11/α-TCP(7/93 %wt) at different reaction times in water at 37°C.

Materials	E (MPa)	σ_{max}(MPa)	ε_{max} (mm/mm)
HYAFF 11/TCP(7/93)-no reaction	190 ± 10	8.5 ± 2.0	0.09 ± 0.01
HYAFF 11/TCP(7/93)-24h of reaction	290 ± 10	11.0 ± 2.5	0.07 ± 0.01
HYAFF 11/TCP(7/93)-48h of reaction	210 ± 40	9.0 ± 0.5	0.07 ± 0.01
HYAFF 11/TCP(7/93)-96h of reaction	380 ± 20	10.0 ± 2.0	0.05 ± 0.01

Table 2 Compressive properties of HYAFF 11/α-TCP(14/86 %wt) at different reaction times in water at 37°C.

Materials	E (Mpa)	σ_{max}(MPa)	ε_{max} (mm/mm)
HYAFF 11/TCP(14/86)-no reaction	150 ± 40	12.0 ± 1.0	0.26 ± 0.05
HYAFF 11/TCP(14/86)-24h of reaction	200 ± 80	19.0 ± 2.0	0.12 ± 0.05
HYAFF 11/TCP(14/86)-48h of reaction	260 ± 40	18.0 ± 1.5	0.13 ± 0.02
HYAFF 11/TCP(14/86)-96h of reaction	260 ± 30	17.0 ± 1.0	0.16 ± 0.01

tion (immersion in water), performed on specimens dried at 25 °C for 24 hours, the related values of mechanical parameters are reported in Table 1. It can be seen that the maximum compressive strength does not increase in an uniform manner as the reaction proceeds, although the elastic modulus of the composite increases as function of immersion time in water. As expected a decrease in the maximum deformation with the hardening of the structure is observed.

Figure 6 and Table 2 show the mechanical properties of the HYAFF11/α-TCP (14/86 percent by weight) composite. The maximum compressive stress and the elastic modulus increase as function of the water immersion time, reaching their maximum values after 24 hours of reaction. Obviously, the maximum deformation decreases as the structure hardens.

Considering the mechanical properties of both composites after 96 hours of immersion in water, it is clear that increasing the polymeric phase (HYAFF11) content increases the maximum deformation and the maximum compressive stress, contributing to the improvement in the composite toughness, decrease in the elastic modulus is obtained. The presence of the polymer (HYAFF11), in the compositions investigated, reduces the presence of micropores found in the α-TCP alone [18]. rendering the composite structure more homogenous. The increase of the α-TCP content led to an increase in the elastic modulus of the composite leading to a more brittle material.

In Table 3 and Fig. 7 the mechanical properties of HYAFF11, HYAFF11 reinforced with 86% by weight of α-TCP, HYAFF11 reinforced with 86% by weight of HA and 100% by weight α-TCP cement are shown. Considering the α-TCP cement, its brittle behaviour is evident with higher maximum compressive strength and elastic modulus, and lower deformation at break compared to those of the composites. It is also clear that the mechanical properties of the composites are intermediate between those of the matrix alone and the plain cement.

Table 3 Compressive properties of HYAFF 11, HYAFF 11/α-TCP(14/86 %wt), HYAFF 11/HA (14/86 %wt), and α-TCP after 96h of reaction times in water at 37°C

MATERIALS	E (MPa)	σ_{max}(MPa)	ε_{max} (mm/mm)
HYAFF 11	27 ± 4	3.0 ± 1.5	0.55 ± 0.10
HYAFF 11+ α-TCP (14/86)	260 ± 30	17.0 ± 1.0	0.16 ± 0.01
HYAFF 11+ 86%wt HA (14/86)	195 ± 40	12.0 ± 1.1	0.24 ± 0.08
α-TCP	800 ± 200	19.0 ± 2.5	0.02 ± 0.005

The compressive properties of the HYAFF11/α-TCP composite are higher than HYAFF11/HA due to the structural interaction between the CDHA needle crystals produced during the α-TCP hydrolysis, i.e., the setting reaction that takes place within the HYAFF 11 matrix. No setting reaction takes place in the HYAFF 11/HA composite due to the inert behaviours of HA. Statistical analysis performed by T-Student Test demonstrated that for the composite materials $p < 0.05$ while for Hyaff11 $p > 0.05$ (0.089).

From the mechanical analysis is possible to state that the hardening reaction up to 96 hours led to the improvement of the mechanical properties of HYAFF11/α-TCP composites, and varying the relative content of each component it is possible to obtain composite materials with a range of mechanical properties. The morphological and the mechanical properties analysis led to a full complementary information about the setting and hardening mechanism and of α-TCP particles in a hydrophilic matrix. With regard to the mechanism of crystal precipitation, it is clear from the micrographs, that the CDHA crystals are developed in the voids close to the α-TCP particles and on the reactant surface. This process is facilitated from the micro-pores obtained during the phase inversion process, enhancing the dissolution-precipitation of the crystals.

For the HYAFF 11/α-TCP, one of the most interesting features is that, after the extraction of the polymer solvent with ethanol, the rounded particles of α-TCP are entrapped in a three-dimensional structure of HYAFF 11 (paste-like material). Once the system is immersed in water or biological fluids, the reaction starts and hardening of the structure occurs. The α-TCP particles are included in the polymer, which forms a three-dimensional structure rich in voids, controlled by the processing conditions, that are necessary to allow the dissolution/precipitation processes. Moreover, HYAFF 11,

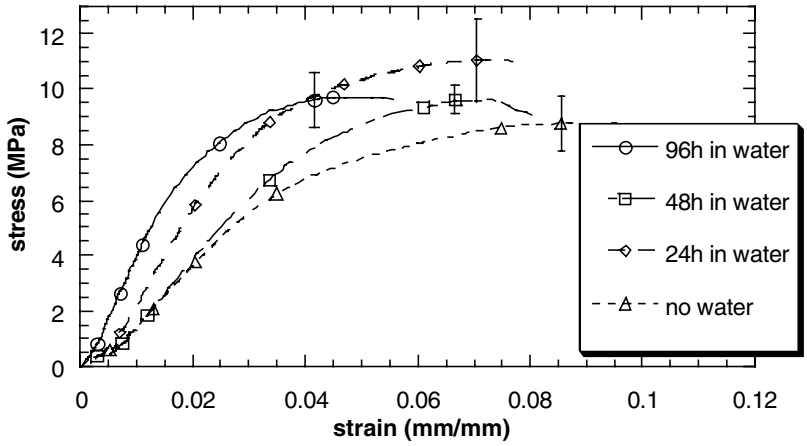

Fig. 5 Compressive properties of Hyaff 11 reinforced with 93%wt of α-TCP at different reaction times in water at 37°C.

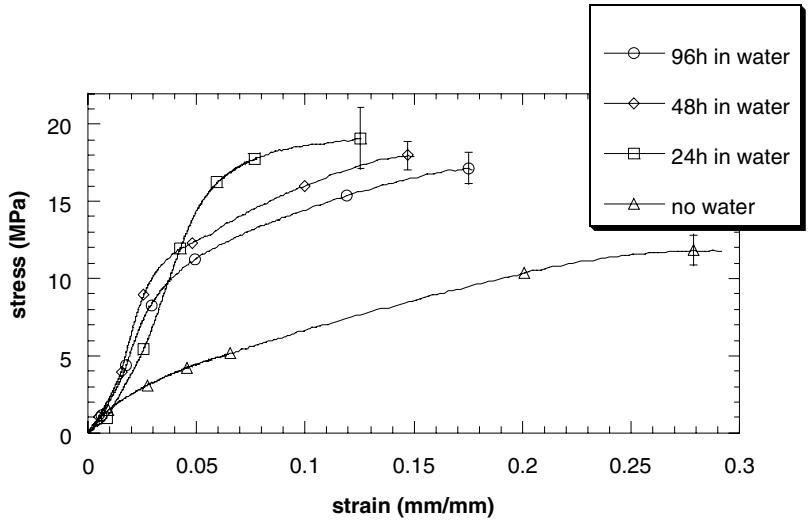

Fig. 6 Compressive properties of Hyaff 11 reinforced with 86%wt of α-TCP at different reaction times in water at 37°C.

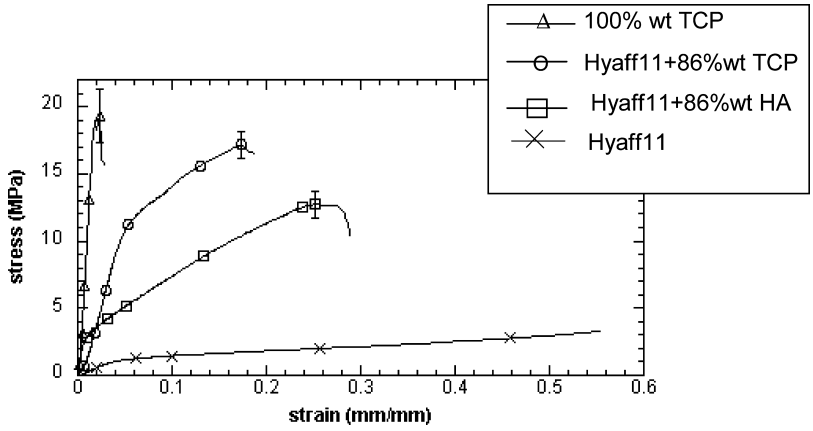

Fig. 7 Compressive properties of Hyaff 11, Hyaff 11/α-TCP(14/86 %wt), Hyaff 11/HA (14/86 %wt), and α-TCP after 96h of reaction in water at 37°C.

as an hydrophilic polymer, is capable of retaining water which is necessary for the hardening reaction characterised by the dissolution of calcium phosphate followed by precipitation of CDHA needle-like crystals which form a more dense structure.

4. Conclusions

Biodegradable (HYAFF11/α-TCP) and semi-biodegradable (HYAFF11/HA) composite hydrogels are proposed as bone substitutes, consisting of an hydrophilic biodegradable

polymer (HYAFF 11) as matrix with two ceramic powders (α-TCP and HA) as reinforcing phase. For the composite HYAFF 11/α-TCP the ceramic phase evolves from α-TCP to CDHA, after immersion in water, leading to higher mechanical properties than HYAFF11/HA composite. Mechanical properties are in the range of cancellous bone and consequently these composites may find interesting applications as bone fillers in orthopaedic and dental applications. The main features of the hydrophilic composites allow the possibility to modulate the mechanical properties and to deliver appropriate drugs to the application sites.

Acknowledgements The Authors gratefully acknowledge the support from 5FP Project "*Disc*" Contract No. G5RD-CT-2000–00267 and M.I.U.R.(Piani di Potenziamento Rete Scientifica e Tecnologica).

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