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## EVALUATION OF A PDLLA/45S5 BIOGLASS COMPOSITE: MECHANICAL AND BIOLOGICAL PROPERTIES

Ginsac Nathalie<sup>1</sup>, Chevalier Jérôme<sup>1</sup>, Chenal Jean Marc<sup>1</sup>, Meille Sylvain<sup>1</sup>, Hartmann Daniel<sup>2</sup>, Zenati Rachid<sup>3</sup>.

1. Université de Lyon, INSA Lyon, Laboratoire Matériaux Ingénierie et Sciences (MATEIS), UMR CNRS 5510

Villeurbanne, France

2. Université de Lyon, Faculté de Pharmacie, Rockefeller, Laboratoire des Biomatériaux, Unité Réparation Tissulaire, Interactions Biologiques et Biomatériaux" (RTI2B)

Lyon, France

3. Noraker, Adresse, Villeurbanne, France

## ABSTRACT

The present study aims to characterize a composite made of poly-L,DL-lactic acid (P(L,DL)LA, Mv: 120 KDa) containing 30 wt% of 45S5 bioactive glass particles. Glass transition (around 52°C) of the polymer was assessed by differential scanning calorimetry (DSC). The mechanical properties of the neat polymer and the composite were evaluated by tensile and compressive tests. From these tests, it was confirmed that the addition of bioglass into the polymer matrix leads to a slight decrease in tensile strength and an increase in elastic modulus. In vitro bioactivity of this composite was evaluated by immersion in a simulated body fluid (SBF) at 37°C for different durations. Formation of hydroxyapatite crystals on the surface of the composite was recorded by scanning electron microscopy and confirmed by X-ray diffraction. Many hydroxyapatite crystals covered the surface of the composite after 14 days of immersion in simulated body fluid. Osteoblast cells MG 63 (human osteosarcoma cell line) were cultured in direct contact with the polymer and the composite. Cells morphology and attachment were analysed using SEM and MTT viability test. Scanning electron microscopy analysis showed the presence of cells at the surface of the composite. These results confirmed the biocompatibility of the composite and the positive effect of the bioglass on the osteoblast cells adhesion and proliferation on the composite.

## INTRODUCTION

The need of innovation in bone reconstruction is a major requirement. Currently the ultimate approach in bone reconstruction is the use of donated bone allograft, but it presents infections risks. Autograft bone does not transmit pathologies and does not create immune response, but the quantity of bone available is low. Synthetic biomaterials could offer an attractive approach. Bioabsorbable devices are the best alternative for internal fixation. Indeed during healing process, bioabsorbable devices maintain the fixation, decompose gradually, and the stresses are transferred gradually to the healing tissue so that no stress shielding will occur. Furthermore, bioabsorbable surgical devices do not require a removal operation, and so may reduce the total cost when compared to metallic devices. The copolymer P(L,DL)LA offers an attractive choice for different applications due to its wide range of properties. Since the discovery of bioglass by Hench<sup>1</sup>, the bioactivity of a variety of glass compositions has been well established. Bioglass, 45S5, is a particular commercial bioactive glass containing 45% SiO<sub>2</sub>, 24,5% Na<sub>2</sub>O, 24,5% CaO et 6% P<sub>2</sub>O<sub>5</sub> (%wt). The lack of in situ mould ability and the relative brittleness of bioactive glasses limit the range of their applications. A composite could combine the osteoconductive properties of bioactive glass with the processability of biodegradable polymer and overcome the brittleness of the bioglass. Bioactive glasses react in vitro in many solutions and in vivo in body fluids. Due to ion dissolution, a silica gel layer is formed on their surface during the first hours, and is followed by calcium phosphate precipitation and crystallisation, creating an apatite layer at the bioglass surface. Composites made of biodegradable polymers and bioactive glasses have been studied

for several medical applications that included non-load bearing applications<sup>2,3,4</sup>. The aim of the current study is to evaluate the mechanical properties of the polymer matrix and of the composite made of 30(wt%) bioglass and also to confirm its biocompatibility and bone bonding ability.

## EXPERIMENTAL

### Materials

P(L,DL)LA 70:30 copolymer (inherent viscosity = 5.5-6.5 l.mol<sup>-1</sup>, density = 1.27 g/cm<sup>3</sup>, Boehringer, Ingelheim, Germany) was used. This copolymer is filled with 45S5 bioglass particles. The composition of bioglass (quoted hereafter BG) is 45.0% SiO<sub>2</sub>, 24.5% Na<sub>2</sub>O, 24.5% CaO, and 6.0% P<sub>2</sub>O<sub>5</sub> by weight. The matrix and the composite with 30% (by weight) BG were prepared by a process protected by Noraker (patent n°WO2008116984 (A2)). Briefly, polymer was first dissolved in a solvent and mixed with ceramic powder (average diameter 3 µm), and then the solvent was evaporated. Plates, cylinders and dumbbell specimens of the matrix and the composite were obtained by injection molding.

### Thermal characterization

Differential scanning calorimetry (DSC) thermograms were recorded with a Pyris Diamond apparatus. Approximately 6 mg of the matrix and the composite were placed in aluminium pans. Samples were cooled to 20°C and held for 3 min prior heating from 20 to 160°C at a heating rate of 2°C/min. Then samples were cooled from 160 to 20°C at 10°C/min, prior a second similar heating run. Thermograms were analyzed to determine thermal transitions of the materials.

### Bioactivity study

The development of a surface Ca-P layer on the P(L,DL)LA/BG composite was evaluated in a simulated body fluid (SBF) with solution ion concentrations similar to those of blood plasma. Preparation of SBF solution was realized according to ISO23317 Standard. P(L,DL)LA and P(L,DL)LA/BG rectangular specimens (10x10x2 mm<sup>3</sup>) were incubated at 37°C in SBF for 7, 14 and 28 days. After each immersion period, samples were rinsed with distilled water and dried in a desiccator. The formation of Ca P layer on the surface of the samples was characterized using X-ray diffraction (Rigaku) and scanning electron microscopy (JEOL 840 A LGS).

### Mechanical characterization

The following mechanical properties were measured on both the polymer matrix and the composite.

Dog bone shape specimens having a length of 35 mm and a rectangular cross-section (2 x 5 mm<sup>2</sup>) were tested in tension with a MTS machine coupled with video extensometer according to NF EN ISO 527-2 standard. The tests were conducted using a cross-head speed of 0.35 mm/min (0.1% of the length). Tensile strength (St) and elastic modulus (Et) were calculated. Rational stress and strain were also calculated to account for the section modification during the test.

Cylinders with a diameter of 6 mm and a length of 11 mm were tested in compression with an INSTRON 8502 machine, using a cross-head speed of 1 mm/min according to ISO 604:2002 standard. Compressive strength (Sc), elastic modulus (Ec) and rational stress and strain were calculated.

### Biocompatibility in vitro

In vitro biocompatibility study was carried out on the neat polymer and the composite by cells culture. Rectangular specimens (10x10x2 mm<sup>3</sup>) were used for the culture cell. Materials were first soaked in cell culture medium for 8 days. MG63 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% foetal calf serum, penicillin and streptomycin. MG 63

cells were seeded onto materials at a density of  $10^5$  cells.ml<sup>-1</sup>. The same number of cells was seeded on 6 control wells, without material. The plates were further incubated at 37°C in humidified air with 5% CO<sub>2</sub>, for a period of 7 and 14 days. The culture medium was changed every two days. In order to evaluate the biocompatibility of the polymer and the composite, MTT test was carried out at 7 and 14 days.

In the cell adhesion study, viability was measured according to MTT-based colorimetric assays. This assay relies on the ability of living cells to reduce a tetrazolium salt into soluble coloured formazan product, thanks to succinate dehydrogenase mitochondrial activity present in living cells. This enzyme converts the yellow MTT in blue formazan crystals. After 7 and 14 days of incubation, MTT test was carried out on the materials. Formazan measurement was realized by spectrophotometry at 570 nm. Results are determined by measuring the optical density, and reported as a percentage of cells on the materials relative to cells without material.

Osteoblast morphology was examined using scanning electron microscopy (SEM) after 14 days of culture on the materials. Two wells, containing polymer and composite specimens without cells, were used for SEM negative control. For the SEM observation, cells were fixed with paraformaldehyde during minimum 24h at 4°C. Cells were then dehydrated through a series of alcohol concentrations (30°, 50°, 70°, 95°, 100°), and by air drying. The day after, cells were gold coated and examined under the SEM at an accelerating voltage of 5KeV.

## RESULTS AND DISCUSSION

### Thermal characterization

Only the second DSC heating run was analyzed, the first one being used to erase the thermal history of the polymer. The P(L,DL)LA copolymer is amorphous, with a glass transition temperature (T<sub>g</sub>) between 52 and 53°C. Addition of bioglass particles on the polymer matrix has no effect on the glass transition temperature.

### Bioactivity study

SEM observation of the composite was realized after different immersion times and compared with observations of the neat polymer (Fig. 1 to 3). Crystals appear on the edges of the composite after 7 days of immersion in SBF (Fig. 1 c and d), and on its surface after 14 days of immersion (Fig. 2b). The XRD analysis of materials after different immersion times in SBF evidenced the existence of the main characteristic diffraction peaks of HA after 14 days soaking in SBF. The peak intensity increased after 28 days soaking in SBF. This was confirmed by the comparison of the XRD spectra with the standard pattern of HA (JCPDS 00 01-1008) (Fig. 4). Others minor peaks (not identified) appear on the diffractograms, and could be attributed to salt crystallization from the SBF solution. XRD and SEM results showed that after 14 days in SBF, an apatite layer appears on the composite surface. This result confirms the bioactive character of the composite. In the literature, a similar result has been observed but with a different kinetic. Y. Shikinami et al.<sup>5</sup> have studied bioactivity of the composite PLLA with HA particles. They have shown HA crystals between 3 and 6 days of immersion in SBF, and a totally covered surface after 7 days.

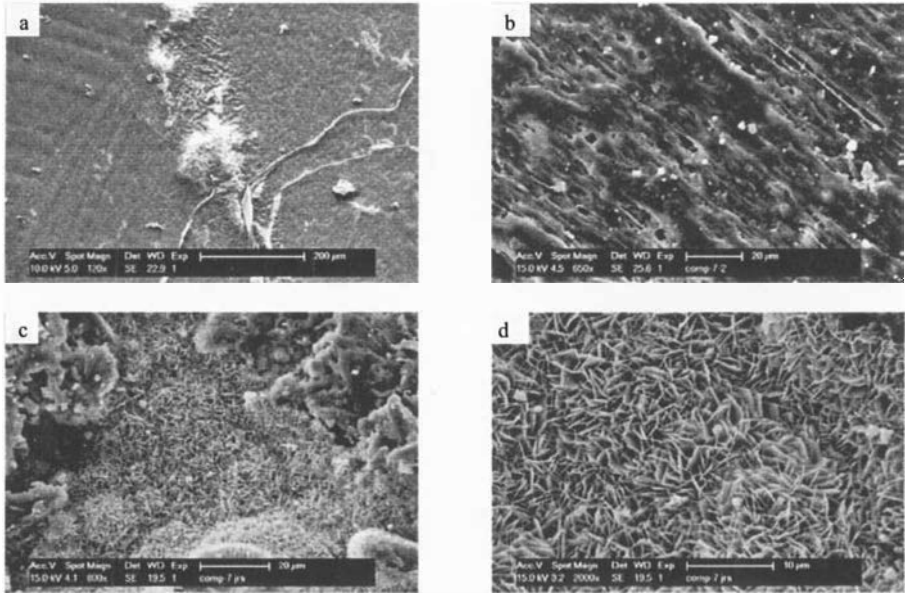


Figure 1. SEM micrographs after 7 days of immersion in SBF, (a) of the neat polymer x 100, (b) of the surface composite x 650, and the edges of the composite (c) x 800, (d) x 2000.

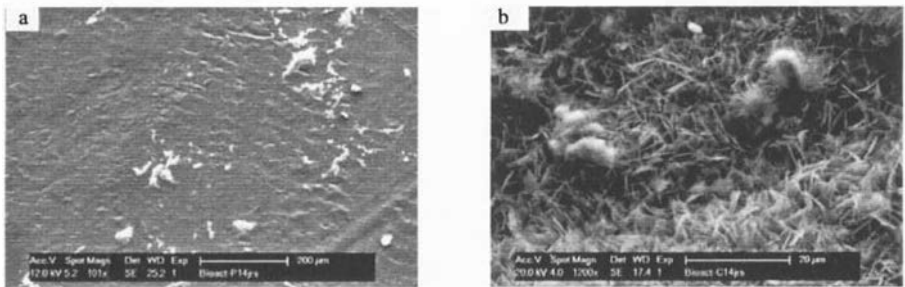


Figure 2. SEM micrographs after 14 days of immersion in SBF, (a) of the neat polymer x 100, (b) of the composite x 1200.

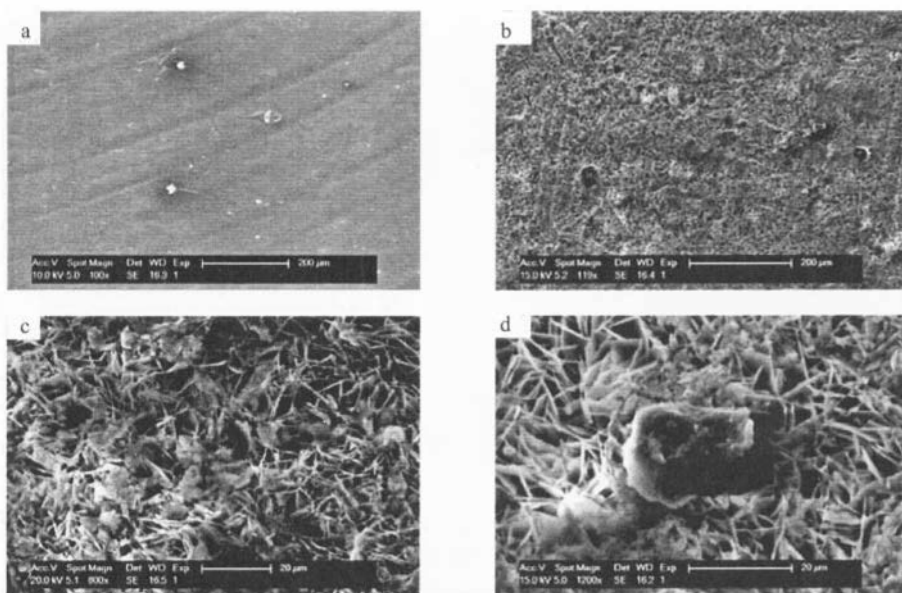


Figure 3. SEM micrographs after 28 days of immersion in SBF, (a) of the neat polymer x 100, (b) of the composite x 100, (c) of the composite x 800, (d) of the composite x 1200.

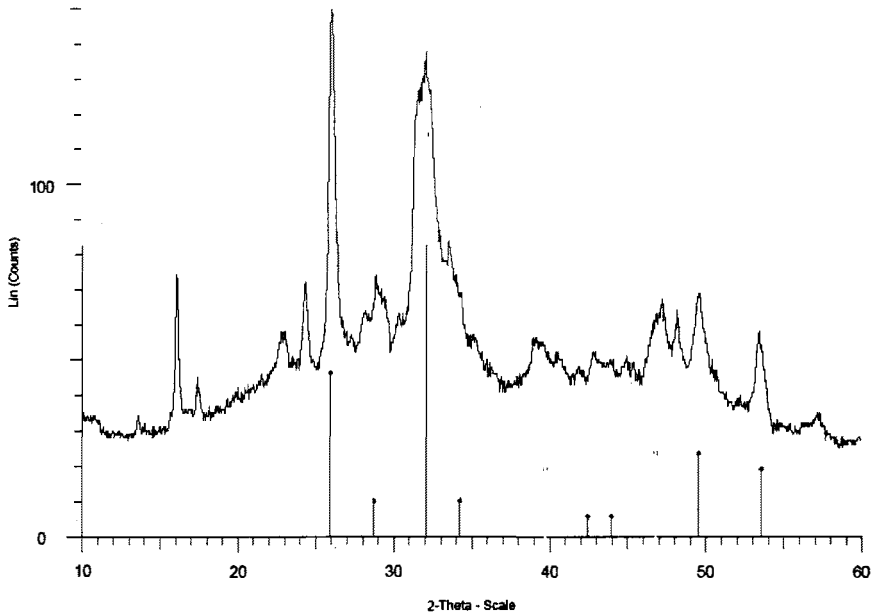


Figure 4. DRX pattern of the composite after 28 days of immersion in SBF at 37°C, comparison with HA standard pattern (JCPDS 00-001-1008).

#### Mechanical characterization

The results of tensile tests for the polymer matrix and the composite are presented in Table I. The addition of ceramic particles decreases significantly the tensile strength, but as expected increases the Young modulus. Stress – strain curves for the polymer and the composite show a first linear part followed by a plastic domain (the strength decreases and reaches a plateau). Extensive cavitation was observed on the polymer specimens at the fracture section. The composite shows a more brittle fracture. Maximal tensile stress obtained with the materials are lower than human cortical bone one (between 130 and 150 MPa). Few materials of this kind have been tested in previous works. Compared with those works, the tensile stress is similar to that obtained by Verheyen<sup>6</sup>, but lower compared to that obtained by Shikinami<sup>5</sup>. The Young modulus obtained here is higher than those observed in the literature<sup>5</sup>. It is to note however that the materials studied by both authors (PLLA/HA composites) are different from the current composite (P(L,DL)LA/BG), and the comparison is difficult.

Table I. Tensile strength and Young modulus of the polymer matrix and the composite.

	Tensile strength (MPa)	Young modulus (GPa)
Polymer	$46 \pm 11$	$3.2 \pm 0.2$
Composite	$31 \pm 2$	$4.4 \pm 0.1$

The compressive tests results for the polymer and the composite are presented in Table II. As expected, and as already reported in previous works<sup>5,6</sup>, the addition of ceramic particles to the polymer matrix improves the Young modulus. The polymer presents slightly higher values of yield stress and maximal strength. Our results are low compared to the literature, but quite close to those obtained by Shikinami et al.<sup>5</sup>, who worked on a PLLA/HA composite. Composite compressive strength is very low compared to cortical human one (130-200 MPa), but remains acceptable.

Table II. Compressive strength and Young modulus of the polymer matrix and the composite.

Materials	Compressive maximal strength (MPa)	Yield stress (MPa)	Young modulus (GPa)
Polymer	101 ± 7	74 ± 1	3.5 ± 0.1
Composite	96 ± 2	62 ± 2	4.1 ± 0.3

#### Biocompatibility in vitro

Figure 5 shows the percentage of cell viability on polymer and composite materials, for MG63 cells. After 7 days of cell incubation, 88% of cell viability was obtained on the neat P(L,DL)LA, while only 56% was obtained on the composite. But after 14 days of cell incubation, 93% and 88% were obtained on the polymer and the composite respectively. These results show that materials are not toxic. After 14 days of culture, cell viability on the composite returns to normality. The apparent decrease in cell viability observed at 7 days, can be due to ions leaching from the bioglass. It might be limited or suppressed if pre-soaking duration was increased. SEM micrographs realized on the materials with cells, show that MG63 cells adhere on both materials after 14 days (Fig. 6).

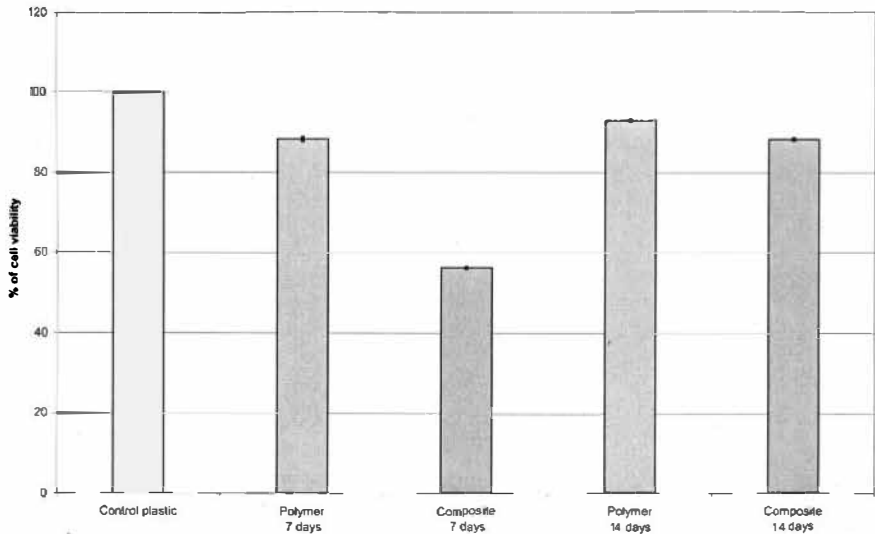


Figure 5. Cell viability on the polymer and the composite after 7 and 14 days of incubation.

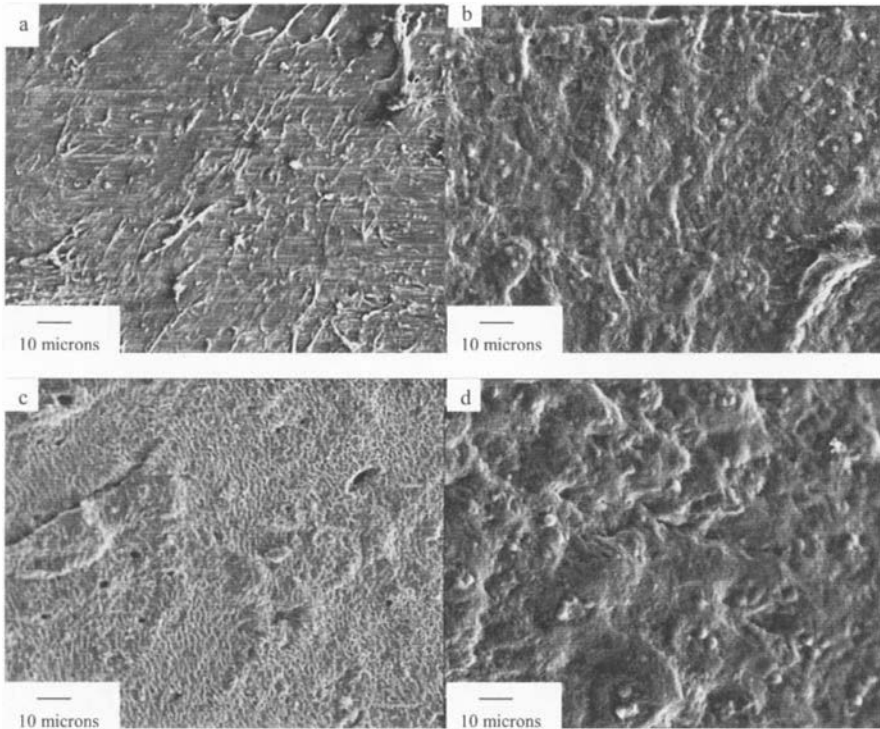


Figure 6. (a) Electron micrograph of the polymer without cell, 1000 x, (b) of the polymer with cells, 1000 x, (c) of the composite without cell, 1000 x, (d) of the composite with cells, 1000 x.

## CONCLUSION

The addition of 30% in weight of BG particles in the P(L,DL)LA matrix causes a decrease in tensile strength and a slight decrease in compressive strength. The Young modulus of the composite is however increased. Further mechanical tests have to be carried out to better understand the mechanical behaviour of the composite: precise elastic modulus values can be obtained by ultrasonic waves propagation. Bioactivity study has confirmed bioactivity ability of the composite. A complementary study has been carried out on the polymer, the composite and the bioglass with a more complete kinetic. First results tend to show the apparition of HA crystals even after 3 days by DRX. Complementary studies will be conducted on cell cultures, in order to better characterize the extracellular matrix, in particular collagen I and osteocalcin assays. Moreover, osteoblast differentiation will be determined by alkaline phosphatase assay. An in vitro degradation study will be realized, to simulate the degradation of the composite and the evolution of its properties. Biocompatibility in vivo study is planned, in order to check the biocompatibility of the composite. Composites with amounts of bioglass fillers (20 and 50% wt) will also be studied. The final aim is to choose the best composition that presents optimal mechanical, chemical and biological properties for

bone fixation devices. The first study seems to prove that this material is a good target for medical devices conception, and in particular bone fixation devices, like plates, screws, pins ... for orthopaedic, oral-maxillo facial surgeries. This composite is a dense material, easy to shape, biocompatible and bioresorbable.

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