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Macroporous Bioglass®-derived scaffolds for bone tissue regeneration

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Abstract

Since it was introduced at the end of the '60s, the 45S5 Bioglass® has played a fundamental role among the materials for orthopedic applications because of its ability to build a stable bond with the surrounding bone. The recent development of bone tissue engineering has led the interest of many scientists in the design of Bioglass®-based scaffolds, i.e. porous systems able to drive and foster the bone tissue regrowth. Among the available techniques to realize scaffolds, the polymer burning out method, which employs organic particles as pore generating agents in a ceramic matrix, combines versatility and low cost. In spite of the advantages of the polymer burning out method, this technique has been rarely applied to 45S5 Bioglass® and a systematic feasibility study has not been carried out on this issue yet. In order to fill this gap, in the present contribution the polymer burning out method was employed to design macroporous scaffolds based on 45S5 Bioglass®. Different amounts of organic phase were used to obtain samples with different porosity. The samples were characterized from a microstructural point of view, in order to evaluate the pore morphology, dimension and degree of interconnectivity. Such findings proved that a proper setting of the processing parameters made it possible to achieve very high porosity values, among the best ones obtained in the literature with the same technique, together with an appreciable mechanical behaviour, according to compression tests. Finally, the scaffolds bioactivity was assessed by means of *in vitro* tests in a simulated body fluid (SBF) solution. Moreover, in the view of a potential application for bone tissue engineering, a preliminary biological evaluation of the obtained scaffolds to sustain cell proliferation was carried out.

Keywords: B. Porosity; D. Glass; D. Glass ceramics; E. Biomedical applications

1. Introduction

Until the '60s, the concept of a material that would not be rejected or surrounded by fibrous tissue after the implantation in the human body seemed unimaginable. It was the 1969 when Hench and coworkers [1], in Florida, not only reached this goal, but also went beyond it. For the first time, in fact, they developed a special soda-lime phosphosilicate glass able to bond to the living bone without forming an interfacial scar tissue. That lucky composition was only the first one discovered in a family of bioactive glasses called Bioglass®. Among them, the so-called 45S5 Bioglass®, whose proportions are 45 wt.% SiO_2, 24.5 wt.% CaO, 24.5 wt.% Na_2O and 6 wt.% P_2O_5, is the most bioactive one, being able to bond well to both hard and soft tissues [2,3].

The development of a stable bond between bioactive glasses and bone tissue was shown to involve a sequence of eleven reaction steps [4,5]. The first 5 steps occur on the material surface and lead to the crystallization of a biologically reactive hydroxyapatite layer (HA). HA is chemically and structurally equivalent to the mineral phase of bone and provides the bonding interface with the surrounding bone tissue. The HA precipitation and crystallization process can be also observed by means of *in vitro* tests, which are based on an acellular solution (simulated body fluid, SBF) with specific ion concentrations mimicking the human extracellular fluid [6,7].

Since it was discovered, the 45S5 Bioglass® has been widely employed for several clinical uses including dental, maxillo-facial and orthopedic applications [8,9]. In the last years the interest of several investigators has been focused on

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the possibility of employing bioactive glasses for the development of scaffolds for bone regeneration [10,11]. In fact, according to the tissue engineering approach [12], bone reconstruction is shown to be a promising alternative to the traditional approach whenever it is mandatory to repair skeletal defects. Current research looks at 45S5 Bioglass® for scaffold applications mainly because of its high bioactivity level. Moreover, 45S5 Bioglass® may be easily resorbed by the body and this is an important property for scaffolds [13]. These structures, in fact, act as temporary templates for natural cell attachment and proliferation [14] and therefore they should be resorbed at the same rate as the tissue is repaired. Moreover they should not release any toxic degradation products. From this point of view, recent studies have demonstrated that the ionic products of bioactive glass dissolution activate and exert a direct control over the genes that regulate the osteoblasts cell cycle [15,16]. The clinical consequence is the enhancement of new bone formation with a rapid filling of the bone defect. Other studies have been devoted to investigate the possible antibacterial effect exerted by the reactions that involve the 45S5 Bioglass® surface [17,18]. However, there are several hurdles dealing with Bioglass®-based scaffolds that need to be overcome. First of all, scaffolds for bone regeneration should be highly porous to allow the cell infiltration and the diffusion of fluids and nutrients [14]. Nevertheless, the required porosity lowers drastically the mechanical behaviour of the 45S5 Bioglass®, whose mechanical properties are intrinsically poor, mainly due to its brittleness. A compressive strength of 0.27–0.42 MPa is reported for a porous 45S5 Bioglass® body with a porosity of about 90% and pore size of 510–720 μm [10]. For comparison, human cancellous bone has a compressive strength of 4–12 MPa and an elastic modulus of 0.1–0.5 GPa [19]. However, a significant decrease in compressive strength of cancellous bone is usually associated with aging [20]. Another open issue regards the effects of sintering techniques on the bioactivity of 45S5 Bioglass®. This material, in fact, fully crystallizes prior to significant densification by sintering, and it is not clear whether the material remains resorbable and able to bond to bone. Recently several authors have pointed out that thermal treatments on 45S5 Bioglass® slightly decrease the kinetics of the HA layer formation without inhibiting the development of such layer [21–23]. For these reasons, in the last few years an increasing interest has been devoted to the use of Bioglass® for scaffolds applications.

Several techniques are available to realize bioceramic scaffolds. Among them, the burning out method combines low cost, simplicity and versatility [24,25]. According to this approach, organic particles are employed as pore generating agents in a ceramic matrix. The organic phase is added to the ceramic powders and subsequently it is thermally removed during sintering. Different organic agents can be incorporated into the ceramic powders, such as polyethylene [26], sucrose [27], gelatine [28], corn, potatoes and rice starches [29].

Surprisingly, this technique is not very common in the production of Bioglass®-based scaffolds, therefore feasibility studies are necessary with the aim to develop an adequate protocol. Probably one of the main difficulties to face is the achievement of highly porous samples with adequate mechanical properties and manageability. Specifically, scaffolds should have an open and interconnected porosity higher than 50 vol.% [10]. A porosity value between 50 and 70 vol.% has been reported for scaffolds based on a SiO_2–CaO–K_2O glass–ceramic system and realized with the burning out method [24]. Elsewhere [25], a 48 vol.% porosity has been obtained for SiO_2–P_2O_5–CaO–MgO–Na_2O–K_2O glass–ceramic scaffolds. However, lower porosity values are often reported [28–30].

In the present work the burning out method was employed to realize Bioglass® scaffolds. The produced samples were analyzed by a microstructural point of view, paying a particular attention to their porosity (content and morphology). The obtained scaffolds possessed a good manageability coupled with an excellent porous structure. In fact the porosity values were analogous to the highest ones reported in the literature dealing with the burning out approach. The scaffolds bioactivity was assessed by means of *in vitro* tests in a simulated body fluid (SBF) solution. Moreover, a preliminary biological evaluation of the obtained scaffolds to sustain cell proliferation was carried out. As a model the mouse calvaria-derived pre-osteoblastic cell line MC-3T3-E1 was selected. This cell line mimics osteoblast progenitors by expressing markers associated with differentiation into a mineralizing phenotype.

The present contribution offers a comprehensive characterization of the scaffolds obtained via the burning-out method, offering a wide overview on the main properties of the materials, in terms of microstructural features, mechanical behaviour and bioactivity.

2. Materials and methods

2.1. Scaffold fabrication

45S5 Bioglass® powders were used to synthesize scaffolds for bone regeneration and repair according to the burning out method.

45S5 Bioglass® was prepared by melting the raw powder materials (commercial SiO_2, $CaCO_3$, $Na_3PO_4{\cdot}12H_2O$, Na_2CO_3 by Carlo Erba Reagenti, Italy) in a platinum crucible at 1450 °C for 1 h and then by quenching the melt in cold water. The obtained frit was then ball-milled and sieved to a final grain size below 25 μm.

The scaffolds were prepared by mixing the Bioglass® powders with a thermally removable organic phase acting as a pore generating agent. With this aim, two different polyethylene (PE) powders (Goonvean Fibres, UK) mixtures were employed:

- Poly_1: 80 wt.% PE powders with particle size within 90–150 μm and 20 wt.% PE powders with particle size within 300–500 μm;
- Poly_2: 50 wt.% PE powders with particle size within 90–150 μm and 50 wt.% PE powders with particle size within 300–500 μm.

Various amounts of Poly_1 and Poly_2 were added to the Bioglass® powders with the aim of producing samples

characterized by the best compromise between porosity and compactness. As discussed in the following section, based on a preliminary microstructural characterization of the scaffolds, the following glass-to-polyethylene ratios were selected for further investigation:

- Bio1: 40 vol.% of Bioglass® powders and 60 vol.% of Poly_1 mixture;
- Bio2: 50 vol.% of Bioglass® powders and 50 vol.% of Poly_2 mixture.

Both Bio1 and Bio2 were mixed for 30 min. in a plastic bottle using a rolls shaker in order to obtain an effective mixing.

Then Bio1 and Bio2 powders were used to produce green bodies by uniaxial pressing at 140 MPa for 10 s using propanol as a liquid binder. The pressed samples, shaped in form of disks (4 cm of diameter, 0.7 cm of thickness), were heat-treated in a furnace in order to burn out the PE powders and sinter the inorganic phase. The thermal treatment was set at a final temperature of 1050 °C for 3 h. The heating rate was 10 °C/min.

Hereafter the acronyms Bio1 and Bio2 will be also used to name both the PE-Bioglass® powder mixtures and the corresponding prepared scaffolds.

2.2. Microstructural characterization and assessment of in vitro bioactivity

The scaffolds microstructure was investigated by means of a scanning electron microscope, SEM (ESEM Quanta 200, FEI Co., Eindhoven, The Netherland). A particular attention was devoted to the morphology, distribution and interconnection of pores. Moreover, a local chemical analysis was performed by X-ray energy dispersion spectroscopy, EDS (Inca, Oxford Instruments, UK). The SEM was operated in low-vacuum mode with a pressure of 0.5 Torr.

Capillarity tests were performed on the scaffolds to qualitatively assess their permeability, i.e. the presence of a highly interconnected pores network. To this aim, a solution with a viscosity similar to that of the human blood was used. Some drops of blue ink were dispersed into the solution to better observe the fluid infiltration throughout the scaffold.

The total porosity (vol.%) of the scaffolds was evaluated by the following calculation:

$$P\% = \left(1 - \frac{W_f}{W_0}\right) \times 100 \quad (1)$$

where $P\%$ is the total porosity (vol.%), W_f is the measured weight of the scaffold and W_0 is the theoretical one, calculated multiplying the Bioglass® density $\rho = 2.7$ g/cm^3 [31] by the scaffold volume.

The scaffolds were also studied by means of X-ray diffraction (XRD), in order to investigate the crystallinity of the 45S5 Bioglass® after sintering. The samples were analyzed by means of a PANalytical X'pert PRO diffractometer employing a Cu kα radiation. Data were collected in the angular range 10–70° 2θ with steps of 0.02° and 5 s/step.

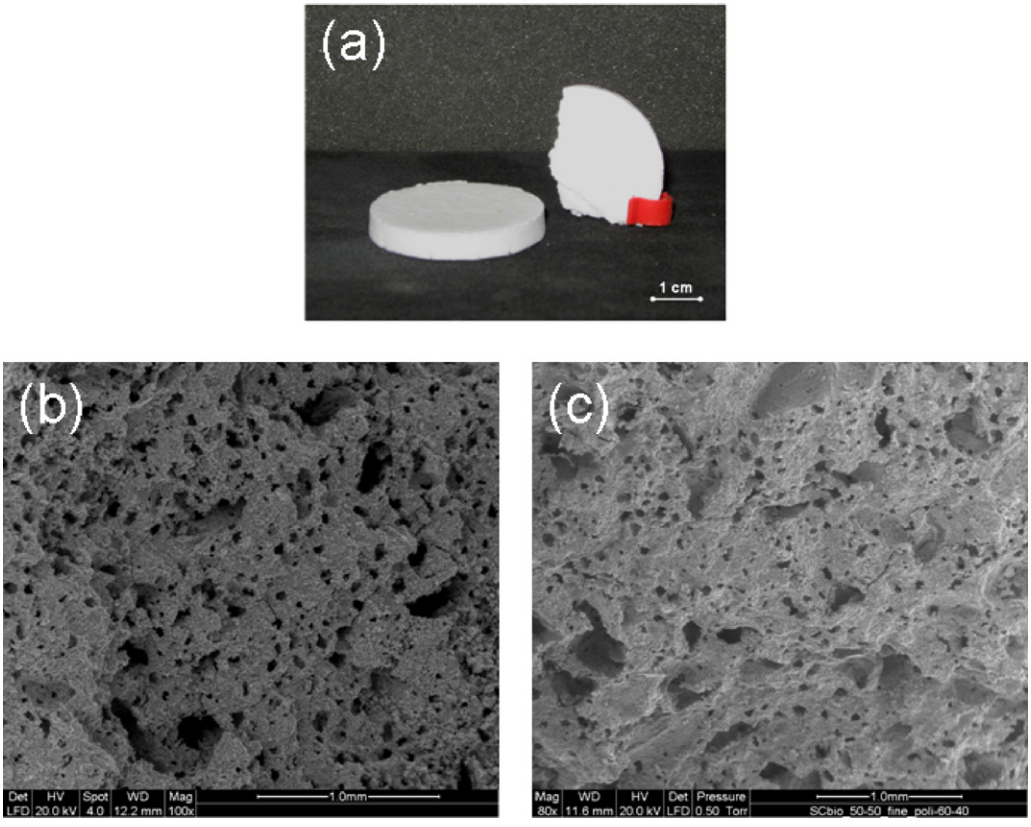

Fig. 1. Bio1 scaffolds (a); micrographs of the Bio1 (b) and Bio2 (c) cross-sections.

The study of the *in vitro* bioactivity was carried out by soaking the scaffolds in a simulated body fluid (SBF) solution, according to the standard protocol developed by Kokubo et al. [6,7]. As already mentioned, SBF has inorganic ion concentrations similar to those of the human extracellular fluid. The samples were soaked in flasks containing 20 ml of SBF and maintained at 37 °C. After given times of 3, 7, 14 and 30 days the samples were extracted from the solution, rinsed with deionized water and then left to dry at ambient temperature. After that, the SEM analysis was repeated in order to evaluate the amount and morphology of the precipitated HA.

2.3. Biological evaluation

Prior to preliminary biological investigations, 45S5 Bioglass® derived samples were cut into pieces of about 1.7 g and placed into a 12 well culture plate. Samples were pre-treated with simulated body fluid (SBF), prepared according to the Kokubo protocol [32] at pH 7.4 for 22 days. Briefly, each sample was soaked in 2 ml of SBF and the solution was refreshed every 24 h to simulate the recirculation of physiological fluid and the consequent formation of HA aggregates on the sample surface [33]. During soaking in SBF, pH evaluations were performed on each sample, to monitor the pH variations due to ion exchange process between the bioactive sample and the surrounding fluids. At the end of the incubation time with SBF, samples were incubated in complete alpha-Minimum Essential Medium (α-MEM) [Sigma] for 48 h prior to cell seeding. To investigate the ability of the prepared 45S5 Bioglass® derived scaffolds to support cell viability for bone tissue regeneration, MC3T3-E1 (CRL 2594) mouse pre-osteoblast cells from American Type Culture Collection [ATCC] were selected. Cells were propagated as indicated by the supplier using α-MEM containing ribonucleosides, deoxyribonucleosides, sodium bicarbonate and supplemented with 2 mM of L-glutamine, 1% of penicillin:streptomycin solution (10,000 U/ml:10 mg/ml), 10% of fetal bovine serum and antimycotic. Cells were seeded onto the scaffolds at a concentration of 3×10^4 per well, in a total volume of 2 ml, and were allowed to proliferate for 11 days. Growth medium was refreshed every 24 h.

Cells viability was investigated by using the alamar-Blue® assay [Invitrogen] a redox indicator that measures quantitatively cell proliferation. As cells grow, there is an increase in metabolic activity giving rise to a reducing environment in the surrounding culture medium, while growth inhibition produces an oxidizing environment. Reduction causes colour change of alamar-Blue® indicator from non-fluorescent (blue) to fluor-

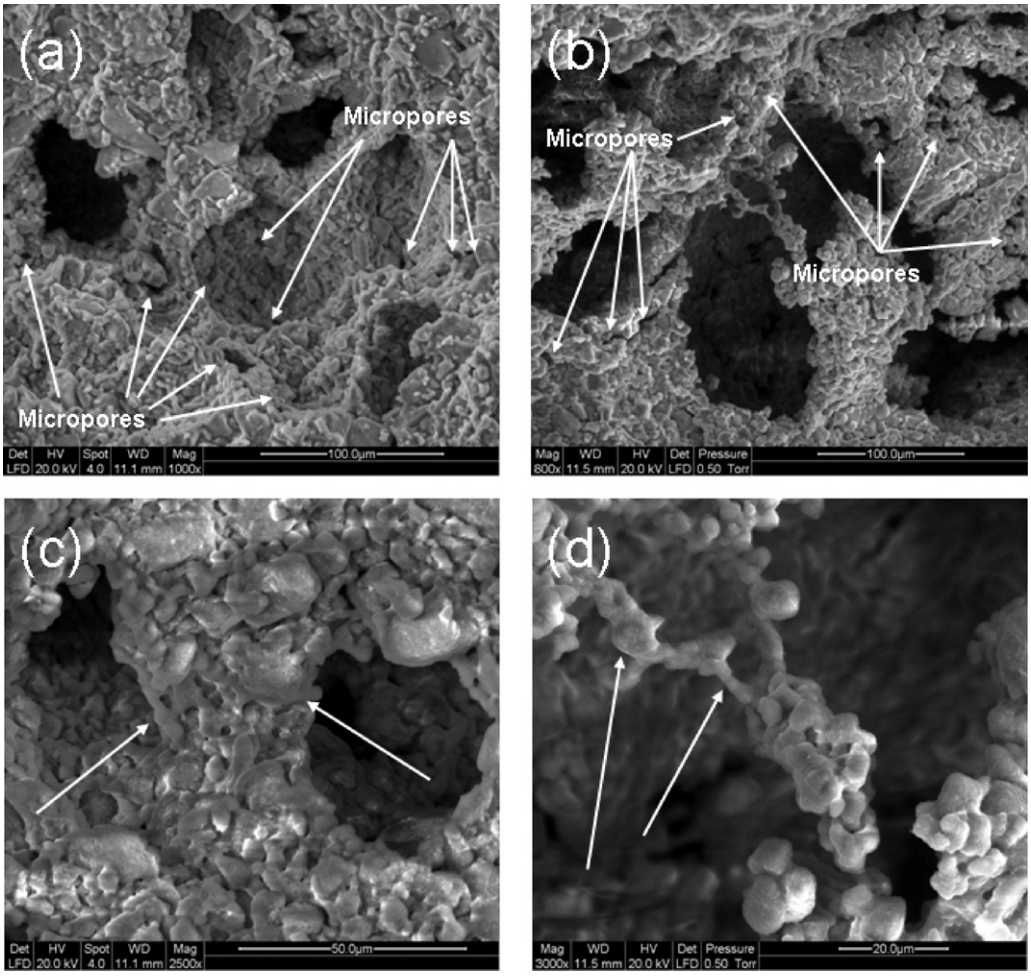

Fig. 2. Micrographs of the Bio1 cross-sections at different magnifications.

escent (red). Proliferation was measured on day 4, 7 and 11 post seeding as previously described. Briefly, the alamar-Blue reagent diluted 1:10 was added to the culture and incubated for 24 h. Supernatants were then re-plated in 96 well culture plate and analyzed with a Biorad microplate reader. Measurements of resorufin dye absorbance were carried out at 565 nm, with the reference wavelength at 595 nm.

2.4. Mechanical characterization

The mechanical resistance of the scaffolds was determined by means of compression tests (Zwick Roell Z600, equipped with an electronic estensimeter). The specimens were 40 mm large and 7 mm thick disks, obtained with the previously described burning-out procedure. The load was applied along the thickness direction (coincident with the symmetry axis). The cross-head speed was set at 0.01 mm/min and the stress-deformation curve was recorded up to the maximum stress point. In particular, the failure stress σ_f was defined as:

$$\sigma_f = \frac{L}{A} \tag{2}$$

where L (N) was the maximum applied load and A (mm^2) was the nominal area of the cross-section perpendicular to the load axis. For each type of material, five samples were considered to have statistical data.

3. Results and discussion

3.1. Microstructural characterization

Preliminary studies were focused on the feasibility of fabricating porous scaffolds with various PE contents. Several PE-to-Bioglass® ratios were considered and it was found that after adding PE contents higher than 70 vol.% it was not possible to produce samples strong enough to be handled with no damage. On the other hand, PE contents lower than 30 vol.% were insufficient to induce an adequate porosity. For these reasons, the microstructural and *in vitro* characterizations were carried out only on the Bio1 and Bio2 systems.

Bio1 scaffolds are presented in Fig. 1(a). Bio2 samples are macroscopically similar to Bio1 ones, therefore they are not reported. Fig. 1(b and c) shows the Bio1 and Bio2 internal microstructure, revealing a homogeneous pore distribution, as well as a high degree of interconnection between pores. These features are decisive to allow an adequate vascularization and cell penetration *in vivo* [14]. As previously mentioned, both samples, Bio1 and Bio2, were produced with mixtures of large (300–500 μm) and small (90–150 μm) PE particles. This is the reason why the scaffolds exhibit not only a widespread network of micro-pores, but also interconnected macro-pores, which boost the biointegration. The comparison between the Bio1 and Bio2 scaffolds reveals that the Bio1 cross-section has a higher

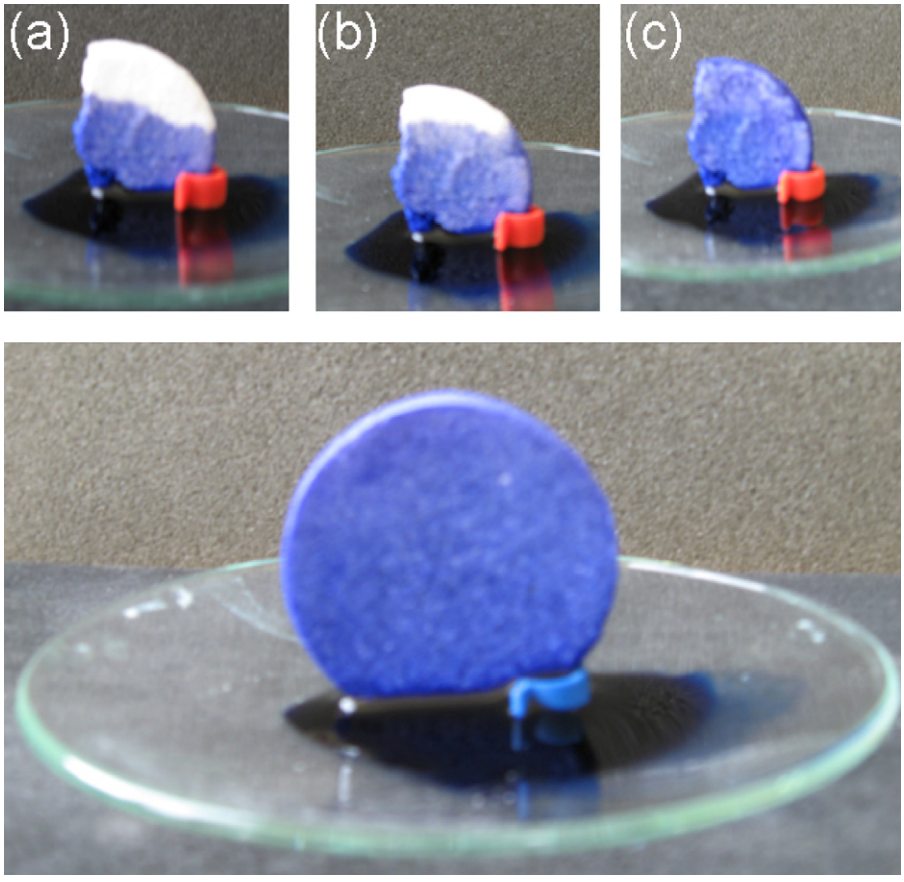

Fig. 3. Capillarity test performed on a Bio1 sample.

porosity compared to the Bio2 one. This is due to the higher content of polyethylene in the former samples.

Fig. 2 shows the Bio1 cross-section at different magnifications. The Bio2 cross-sections are qualitatively analogous to the Bio1 ones, therefore they are not reported. As emphasized in Fig. 2(b), most of the pores are open and widely interconnected, with an average pore size higher than 100 μm. Many authors agree in fixing a minimum pore size of 100 μm to allow a fast cell infiltration inside the whole scaffold [14,34]. The degree of interconnection and the absence of clogged pores are key features for angiogenesis and tissue regrowth. Fig. 2(c) reports a magnification of the samples where two pores can be observed together with the scaffold texture underlying them. It is possible to appreciate the presence of a widespread microporosity, which is due to the gas evacuation during the polyethylene removal. Microporosity, resulting in a large surface area, is a crucial factor to promote diffusion of fluids and nutrients by means of capillarity in-growth [35]. In Fig. 2(c and d) it is also possible to observe the well sintered walls between pores (see arrows), which are thick and dense. A good densification of the pores struts is determinant for maximizing the scaffold mechanical strength. Finally, the rough texture which covers scaffold structures is adequate to foster the absorption of biological metabolites as well as the attachment and proliferation of bone progenitor cells [36,37].

The average porosity, resulting from density measurements, is about 70 vol.% for Bio1 samples and 60 vol.% for Bio2 samples. These values are rather satisfactory, especially in comparison to the values commonly reported in the literature, which often describe 50 vol.% porosity or even less for a burning out approach [27–29], as mentioned in Section 1. It is worth noting that these values, calculated according to Eq. (1), are higher than the respective PE amounts used to produce the Bio1 and Bio2 samples. This fact should be ascribed to the removal of the decomposition gases, which results in an increased widespread microporosity, as already observed in the literature [24].

An adequate permeability to fluids is one of the most important consequences of microporosity. The results of capillarity tests used to qualitatively evaluate the scaffolds permeability are reported in Fig. 3. A face of the scaffold (Bio1) was put into contact with the fluid and the capillary up-take of the fluid within the scaffold porosity was immediately observed. Despite the Bio1 porosity is slightly higher than the Bio2 one, however the capillarity test results were similar.

The presence of one or more crystalline phases in the Bioglass®-derived glass–ceramic scaffolds was investigated by means of XRD analysis (Fig. 4). In fact, the thermal treatment necessary to remove the PE particles and to sinter the glass powders exceeded the 45S5 Bioglass® crystallization temperature, therefore the nucleation and growth of crystalline phases within the amorphous matrix were expected. According to the XRD spectra reported in Fig. 4, it is possible to note the amorphous nature of the initial glass powder and the

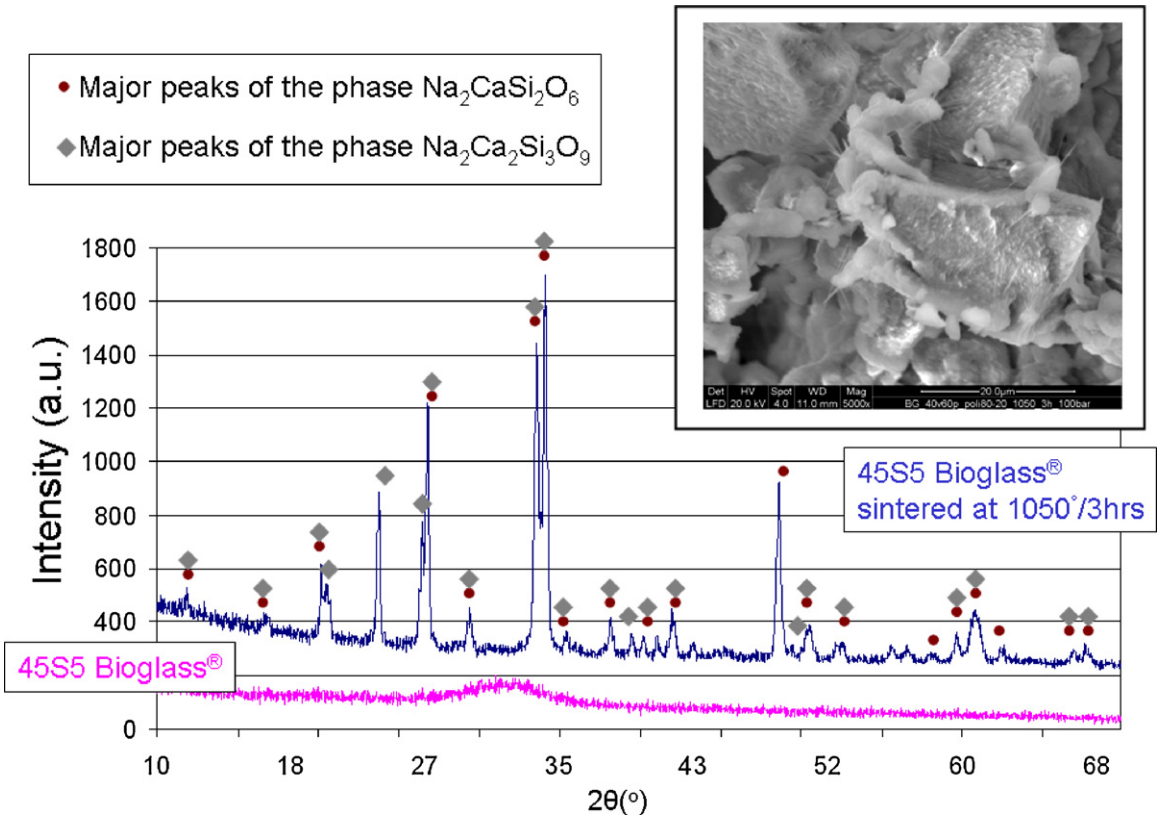

Fig. 4. XRD spectra of 45S5 Bioglass® powder unsintered and of the Bio1 scaffold, i.e. subjected to a sintering process at 1050 °C for 3 h. Inset: needle-like crystals on the surface of the scaffold.

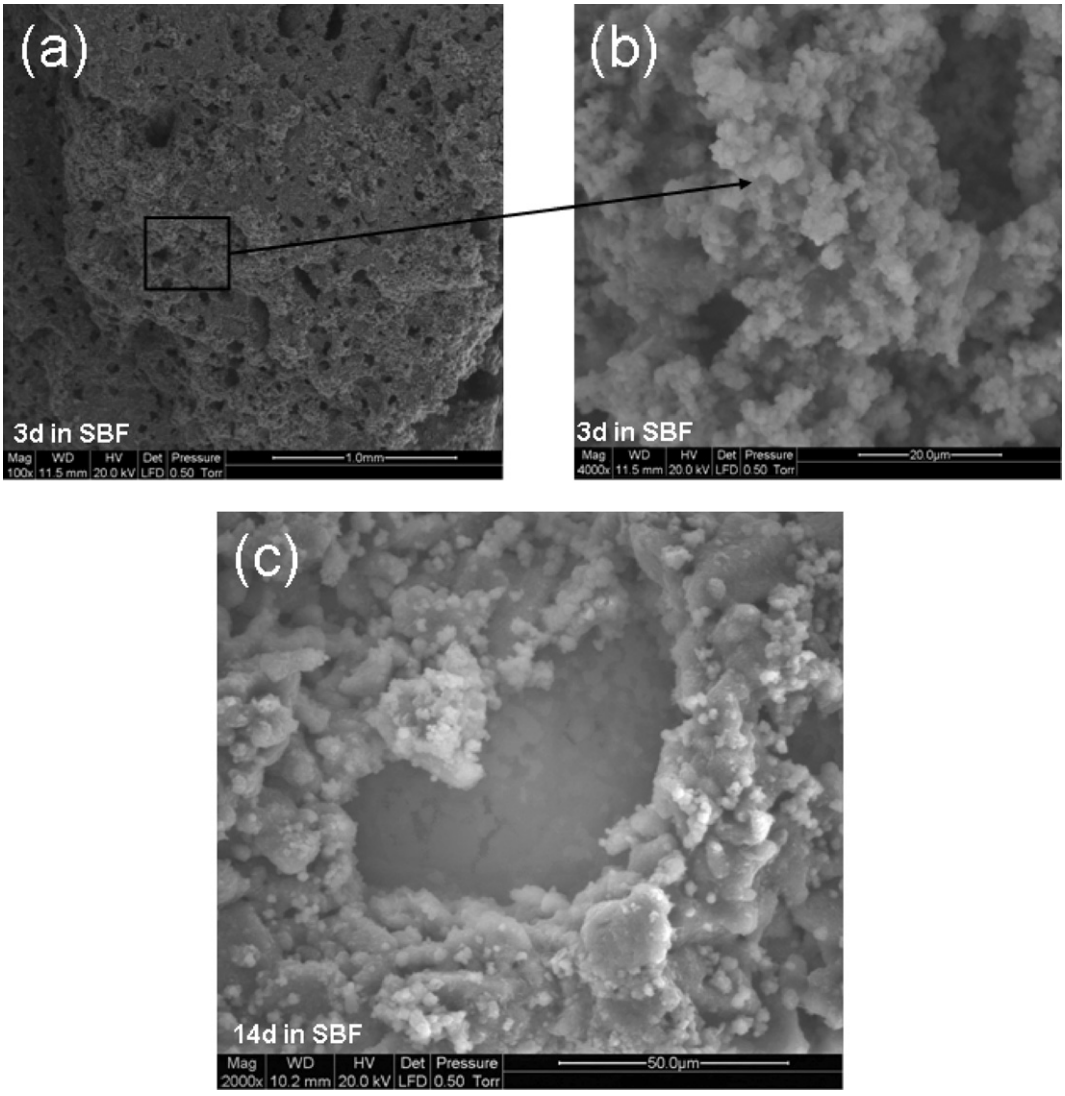

Fig. 5. Micrographs of the Bio2 scaffold after 3 days in SBF (a and b); micrograph of a pore on the Bio1 cross-section after 14 days in SBF (c).

development of crystalline phases induced by the thermal treatment. As regards the diffractogram of the scaffold, the angular location and the intensity of the peaks can be ascribed to the $Na_2CaSi_2O_6$ and $Na_2Ca_2Si_3O_9$ crystalline phases, which coexist within the residual glass matrix. Both the $Na_2CaSi_2O_6$ and $Na_2Ca_2Si_3O_9$ crystalline phases have been identified in previous analogous works, and there is no unanimity on which phase is the most likely. For example, the $Na_2CaSi_2O_6$ phase was reported by Lin and coworkers [38] and by Lefebvre and coworkers [39], while Boccaccini et al. [11,22] or Hench and coworkers [21,38] agree on the $Na_2Ca_2Si_3O_9$ phase. In the inset of Fig. 4 a micrograph of the Bio1 scaffold surface is reported, in which it is possible to note the formation of needle-like fine crystals, produced as a result of the crystallization phenomena.

3.2. Assessment of in vitro bioactivity

The investigation of the sample bioactivity was addressed via *in vitro* tests in SBF. These tests are particularly relevant, since the partial crystallization occurred during the thermal treatment may have modified the intrinsic bioactivity of the Bioglass®, as mentioned in Section 1, as reported in the literature [21–23], the newly formed crystal phases may be much less bioactive than the starting amorphous Bioglass®. In addition, the residual amorphous phase differs from the original glass composition [22].

Micrographs of the Bio1 surface after 3 days in SBF are reported in Fig. 5(a and b). A silica gel covers the surface. It is possible to observe white spherical agglomerates, with the typical morphology of the HA precipitated in SBF. In particular, the HA precipitate covers also the internal pore surface. This fact can be appreciated even better in Fig. 5(c), which refers to a sample immersed in SBF for 14 days. An EDS analysis was also performed to assess the composition of the precipitate. The corresponding results are shown in Fig. 6 for a Bio1 sample after 14 days in SBF. In particular, Fig. 6(c) proposes the EDS spectrum of the surface depicted in Fig. 6(a); moreover, as a term of comparison, the EDS spectrum of the same scaffold before immersion in SBF is reported in Fig. 6(b). Some considerations may be made. First of all, after soaking in

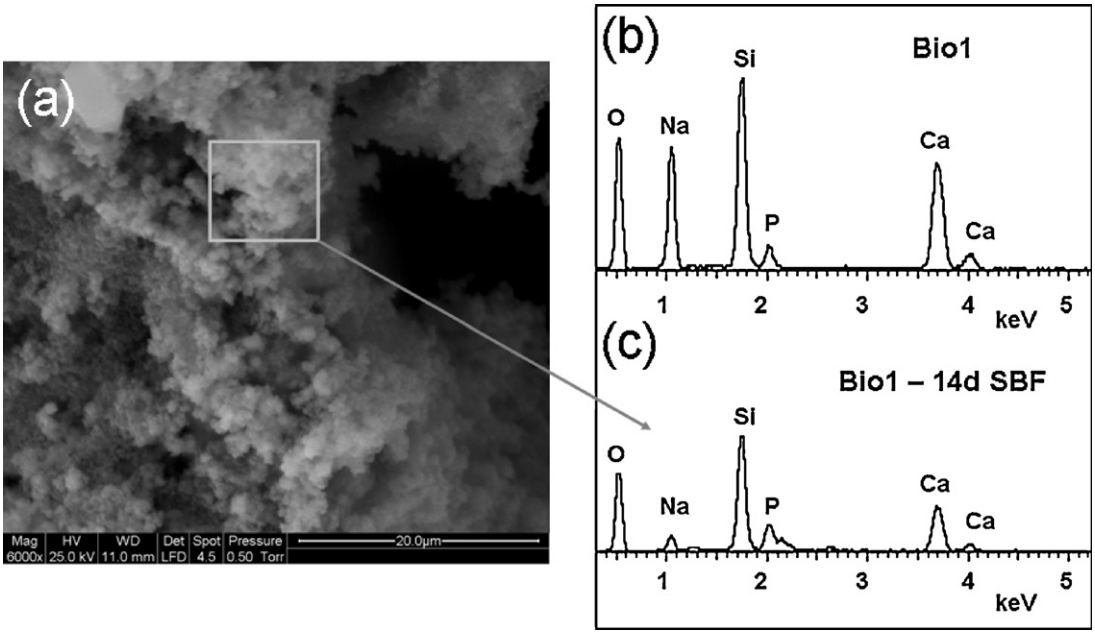

Fig. 6. Micrograph of the Bio1 scaffold after 14 days in SBF (a). EDS analysis of the Bio1 before soaking in SBF (b) and after 14 days of immersion (c). The analysis in (c) is carried out on the whole area reported in (a).

SBF, the sodium content of the sample surface is reduced. This fact agrees well with the model proposed by Hench [2,40,41] to describe the HA precipitation and crystallization on bioactive glasses in a biological environment. In fact, this process can be roughly described as a sequence of 11 reactions steps; the first 5 steps, i.e. ion leaching, dissolution of the glass network and silica-gel polymerization, precipitation and crystallization of HA, can be observed also *in vitro*, since the presence of the osteoblasts is not necessary. According to Hench, the first chemical step is *ion leaching*, where Bioglass® starts its dissolution by exchanging Na^+ ions with H^+ ions from the physiological solution. Therefore, the sodium content of the Bio1 surface decreases as a consequence of the dissolution process. After soaking in SBF, the Si is apparently lower in amount because the silica gel layer is covered by the precipitated HA and, in fact, the ratio between Ca and P, i.e. 1.67, is similar to that in stoichiometric HA [42].

Apart from local fluctuations, the Ca/P ratio in the globular precipitates approaches 1.67 already after 14 days in SBF. Therefore, it is possible to identify them as part of a growing apatite layer. Increasing the soaking time, the HA layer become more uniform all over the scaffold surface. The results dealing with the Bio2 samples are very similar the Bio1 ones, so, in this

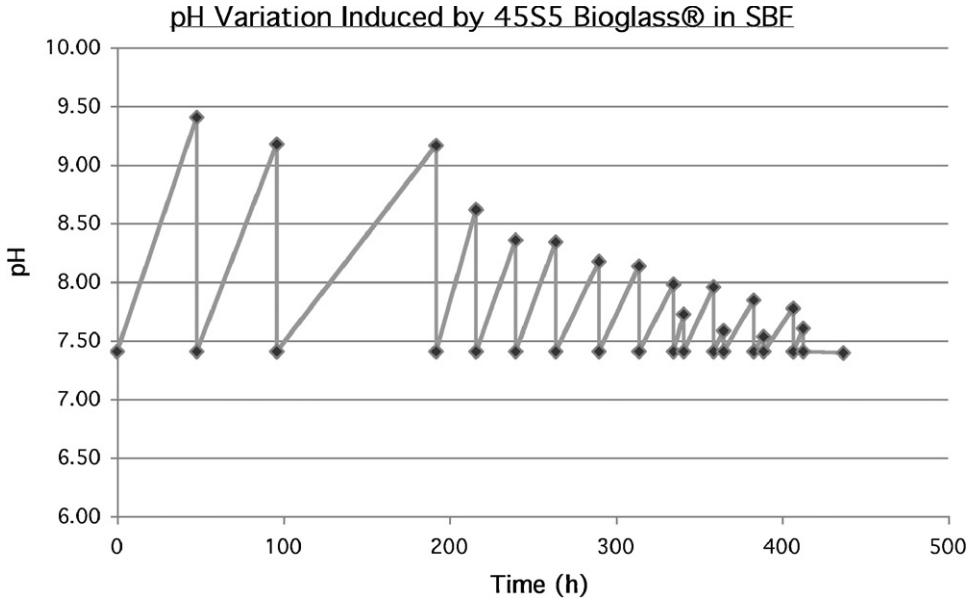

Fig. 7. pH variation induced by a 45S5 Bioglass® derived scaffold in SBF, solution every 24 h.

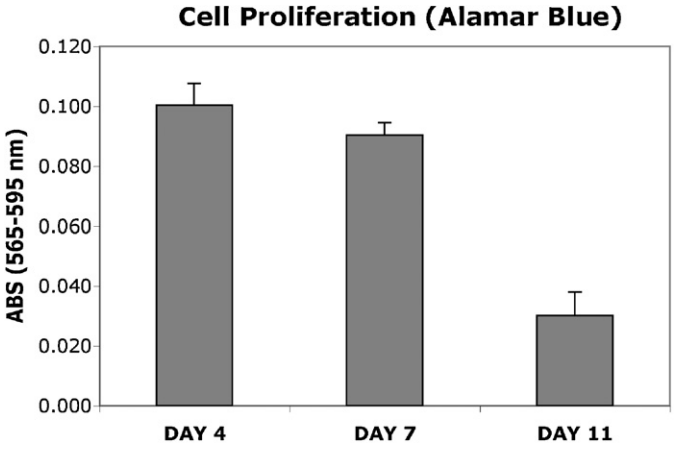

Fig. 8. Cell proliferation onto the scaffolds evaluated by alamar Blue assay.

case, the porosity difference between them does not influence the degree of bioactivity.

As a result of this analysis, it is possible to observe that, despite its crystallization, the 45S5 Bioglass® remains bioactive.

3.3. Biological evaluation

The rate and the amount of ion release and the related pH variation, when a glass–ceramic is placed in contact with physiological fluids, are extremely important for its biocompatibility. It is known that osteoblasts prefer moderately alkaline conditions, i.e. pH values close to 7.8, while changes in pH cause severe damage to cell viability [43]. Hence, pH variations were studied by soaking the scaffolds in SBF for 22 days and refreshing the solution every 24 h. Fig. 7 reports the pH trend for 45S5 Bioglass®-derived scaffold during its soaking in SBF.

The 22 days of conditioning in SBF stabilized the *in vitro* pH of the prepared scaffolds near to the physiological values (7.4). In addition, the extensive washing reduces the presence of contaminants that may derive from the fabrication process and induces the formation of a HA surface layer for mineralized tissue attachment.

Anyhow, prior to cell seeding, samples incubated in complete culture medium for 48 h showed a mild pH increase with an average value of 8.19, probably due to persistent ions exchange in the supernatant. Quantitative evaluation of cell proliferation performed by alamar blue assay at days 4, 7 and 11 highlighted the presence of proliferating cells onto the scaffolds particularly at day 4, with a decrease in viable cells at days 7 and 11 (Fig. 8).

These data could be explained by taking into account the preliminary results obtained from the pH monitoring in culture medium that showed an induction of medium alkalinization. Alkalinization of the culture medium caused by 45S5 Bioglass® was not unexpected since, as reported by the literature [43], reactions occurring to the material in physiological solution involve a mandatory disappearance of the protons. At the beginning a weak increase of the pH facilitated an initial cell proliferation of MC3T3 that prefer a weakly alkaline environment, which stimulates glycolytic activity and calcium release [43]. Nevertheless a continuous ionic exchange between bioactive glass–ceramics and the surrounding fluids could increase pH values causing a cell proliferation decrease. Considering the rising of the pH, changes in $[H^+]$ can have multiple effects on cell metabolism and function, as well as the influence on cell respiration causing enzyme alterations and affecting the diffusion of physiological nutrients and gases to cells.

Conditions for the obtainment of physiological buffering of the prepared Bioglass® scaffolds still needs to be optimized. Nevertheless, the preliminary biological evaluation suggests a possible promising role of the macroporous 45S5 Bioglass® derived scaffolds for applications in bone tissue regeneration.

3.4. Mechanical characterization

Typical examples of the stress-deformation curves for the scaffolds Bio1 and Bio2 are reported in Fig. 9(a) and (b),

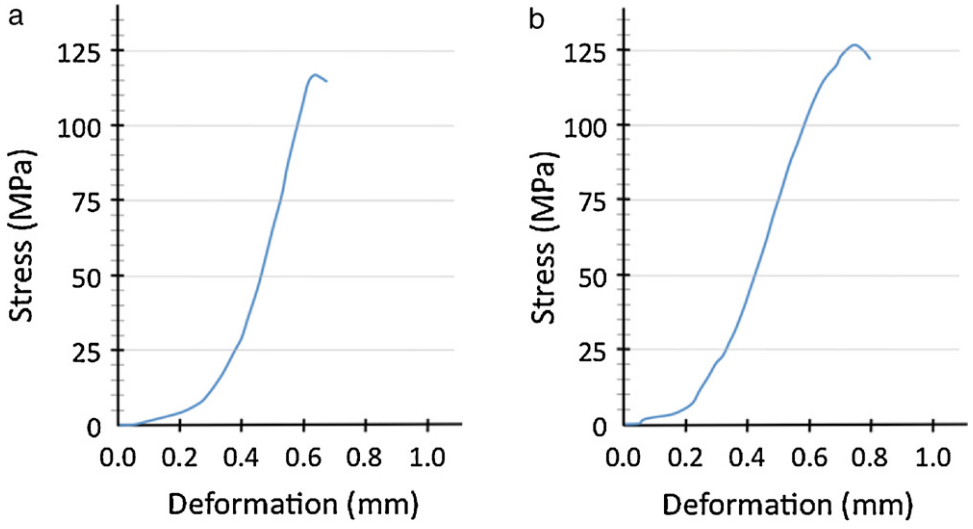

Fig. 9. Stress-deformation curves for Bio1 (a) and Bio2 (b) samples.

respectively. In both cases, a positive slope can be observed, up to a peak point that identifies the failure stress σ_f. After that, if the applied load is further increased, an apparent stress drop occurs, due to the collapse of the first struts. However it is worth noting that the structure is still able to withstand higher loads, since the cracking phenomena at σ_f only involve the weakest trabeculae. From this point of view, this definition of the failure stress σ_f is conservative, since it represents the limit of the Hookean-like region and not the complete breakdown of the scaffolds. The failure stress is 117.7 ± 1.8 MPa for the samples Bio1 and 123.2 ± 2.9 MPa for the samples Bio2. The improved resistance of the Bio2 materials is predictable, since the introduction of a higher PE volume fraction increases the porosity of the Bio1 scaffolds and hence reduces their mechanical strength. Nevertheless, independently of the scaffolds porosity, the failure stress values are very high with respect to those usually reported in the literature [44]. A similar behaviour was noticed by Baino et al. for high-resistance fluoroapatite-containing glass–ceramic scaffolds obtained via powder pressing and burning out [45]. In particular, they found that the scaffold resistance was superior if the load was applied along the compaction direction (as it is the case with the tests of the present contribution) or along a transversal direction. The anisotropic mechanical response of the scaffolds was due to the elongated shape of the pores, which resulted from the deformation of the polymer particles caused by the pressure applied to compact the powders. Analogously, the high resistance observed during the mechanical tests of the Bio1 and Bio2 scaffolds could be attributed to a similar preferential orientation of pores. Unfortunately it is not straightforward to appreciate such structural anisotropy in SEM images, since pores are very large and abundant.

4. Conclusions

The polymer burning out method was applied to realize Bioglass®-based bioceramic scaffolds for bone tissue regeneration. The proposed method, in which polyethylene powders are employed as pore formers in a glass–ceramic matrix, combines simplicity, versatility and low cost.

With the aim of assessing its applicability to the 45S5 Bioglass®, two series of samples with different porosity values were realized and characterized. The porosity, resulting from density measurements, was about 70 vol.% for Bio1 samples and 60 vol.% for Bio2 samples. These values are among the highest ones proposed by the literature regarding the burning out method, therefore the use of polyethylene powders in various amounts showed to be an effective method to control porosity and pore size. The pores were open and well interconnected, with an average pore size higher than 100 μm. All the pores showed dense sintered struts, which results in an appreciable mechanical behaviour for the obtained scaffolds, according to compression tests. The samples proved to possess a high *in vitro* bioactivity, regardless of their different porosity values. Moreover, the preliminary biological evaluation suggests a possible promising role of the macroporous 45S5 Bioglass® derived scaffolds for applications in bone tissue repair and regeneration.

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