Bioglass® coated poly(DL-lactide) foams for tissue engineering scaffolds

Bioglass®-beschichtete Poly(DL-laktid)-Schaummaterialien als Gerüste in der Geweberekonstruktion

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The purpose of this study was to prepare poly(DL-lactic acid) (PDLLA)/Bioglass® composites of foam-like structure, to measure the degree of bioactivity of the composites by studying the formation of hydroxyapatite (HA) after immersion in simulated body fluid (SBF) and to test the initial attachment of human osteoblasts within the porous network. It was found that crystalline HA formed on the Bioglass® coated PDLLA foams after 7 days of immersion in SBF. HA formed also on the surfaces of non-coated PDLLA foams, however the rate and amount of HA formation were much lower than in the composites. The rapid formation of HA on the Bioglass®/PDLLA foam surfaces confirmed the high bioactivity of these materials. Osteoblasts attached within the porous network throughout the depth of the foams. Cell density was found to be higher in the PDLLA/Bioglass® composites compared to the pure PDLLA foams. The composite foams developed here exhibit the required bioactivity to be used as scaffolds for bone tissue engineering.

Key words: Tissue engineering, Bioresorbable polymers, Porous Scaffolds, Bioactive Composites, Bioactive glass

Die vorliegende Arbeit befasst sich mit der Herstellung von porösen Verbundwerkstoffen bestehend aus Poly(DL-Laktidsäure) (PDLLA) und Bioglass® und der anschliessenden Untersuchung der Bioaktivität. Die Bioaktivität wurde anhand von In-vitro-Methoden untersucht: Durch Ermittlung der Bildungsrate von Hydroxylapatit (HA) auf der Oberfläche nach Eintauchen in simulierter Körperflüssigkeit (SBF) und mittels Zellkulturstudien mit menschlichen Osteoblasten. Nach 7 Tagen in SBF hatte die Bildung von kristallinem HA auf der Oberfläche von mit Bioglass®-beschichteten PDLLA Schäumen stattgefunden. Auf der Oberfläche von unbeschichtetem PDLLA konnte ebenfalls die Bildung von HA gezeigt werden, jedoch war die Bildungsrate hier bedeutend langsamer verglichen mit den Verbundwerkstoffen. Die rasche Formung von HA auf der Bioglass®/PDLLA-Schaumoberfläche bestätigt die hohe Bioaktivität dieser Materialien. Die Kolonisierung von Osteoblasten fand innerhalb des gesamten porösen Netzwerkes des Schaumes statt. Die Zelldichte war höher bei Bioglass®/PDLLA-Verbundwerkstoffen verglichen mit unbeschichtetem PDLLA. Die Bioglass®/PDLLA-Verbundwerkstoffe weisen angemessene Bioaktivität für die Anwendung als Gerüste in der Geweberekonstruktion von Hartgewebe auf.

Schlagworte: Geweberekonstruktion, Biolösliche Polymere, poröse Konstrukte, Bioaktive Verbundwerkstoffe, Bioaktives Glas

1 Introduction

The ability to generate new bone for skeletal use is a major clinical need [1]. While bone is capable of self-regeneration after injury, if the injury is particularly severe, the bone may not heal correctly. In these cases, a fibrous non-union is formed requiring additional treatment to resume healing in order to restore mechanical function. Much research has been carried out to find materials that aid this restoration (see for example refs. [2-5]). Autologous bone and allograft bone have been found to be quite successful [6]. However, there are certain problems associated with such materials including limited availability and donor site morbidity in the case of autogenous bone or tissue rejection in the case of allogenic bone [7].

Various inorganic biomaterials such as bioactive ceramics including hydroxyapatite (HA), tricalcium phosphate and selected compositions of phosphate and silicate glasses are already used widely for bone regeneration applications, their biocompatibility being due to their structural similarity to the mineral phase of bone [8]. Bioglass®, a Class A bioactive material, promotes osteoconduction and osteoproduction as a consequence of rapid reactions on the bioactive glass surface involving the dissolution of Si, Ca, P and Na ions that give rise to both intracellular and extracellular responses at the interface of the glass [8,9]. Bioactive glasses as well as hydroxyapatite are being therefore considered for the fabrication of scaffolds for bone regeneration and bone tissue engineering [7-11].

Similarly, porous structures made of synthetic biodegradable polymers, for example poly(*α*-hydroxy acids) such as poly(lactid acid) and poly(glycolic acid), are of interest for tissue engineering scaffolds [3,12,13]. They are frequently combined with bioactive inorganic phases, e.g. hydroxyapatite or bioactive glasses, to form composite scaffolds [13, 14]. In these composite systems several favourable properties can be achieved; bioactivity is enhanced, the scaffold degradation rate can be controlled and the mechanical properties

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and structural integrity of the scaffolds can also be improved [13]. Other potential advantage of combining a bioactive inorganic phase with degradable polymers, in particular poly(α-hydroxy acids), is that the ion dissolution of the inorganic phase can counteract the acidic degradation of the polymer, which has been reported to lead to inflammatory responses [15]. Because of the mentioned advantages, composite materials based on biodegradable polymers and bioactive ceramics (e.g. hydroxyapatite, Bioglass®), are being increasingly considered for bone regeneration and tissue engineering scaffolds [13-25]. In recent studies, a particular commercially available bioactive glass composition, 45S5 Bioglass®, has started to be used as particulate filler and coating in a variety of biodegradable polymer structures, including fibres [26], meshes [27] and foams [28,29]

In this study, the previous research is expanded focussing on the development of composite materials based on poly(D,L-lactic acid) (PDLLA) foams and 45S5 Bioglass® coatings as candidate materials for scaffolds for bone regeneration. The degree of bioactivity was measured by studying the formation of hydroxyapatite on the surfaces of the samples after immersion in simulated body fluid (SBF). Initial osteoblast attachment and infiltration into the porous architecture of the composite foams were also analysed.

2 Experimental

2.1 Materials

PDLLA foams of cylindrical shape (8 mm diameter, 3-5 mm height) were supplied by CERM, University of Liege, Belgium. The foams were fabricated from Purasorb® (Purac Biochem, Holland) by a thermally induced phase separation process (TIPS), which has been described in the literature [30]. Macroporous foams with high porosity and tailored pore structure were used in this study. The foam porous structure comprised macropores of approximately 100μm in diameter and oriented micropores with an average pore diameter of 10μm, forming an interconnecting network (*Fig. 1*). The structure of the porosity of similar foams to those used here has been characterised in detail in previous studies [31] and general properties of the foams are summarised in *Table 1* [28].

The Bioglass® used was a melt-derived 45S5 grade powder provided by US Biomaterials Corporation (Alachua, Florida, USA). The powder had a mean particle size <5μm and a density of 2.66 g cm^{-3}. The composition of this glass (in wt.%) is 45 % SiO_2, 24.5 % Na_2O, 24.5 %CaO and 6 % P_2O_5 , which is the first bioactive glass composition developed by Hench et al. [32].

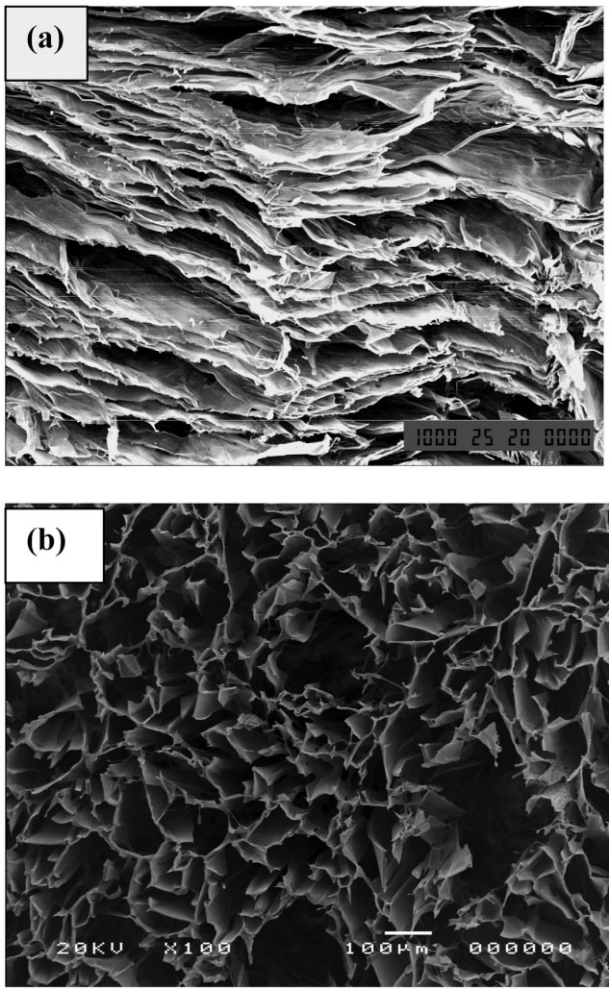

Figure 1. SEM micrographs of an as-received PDLLA foam sample showing the typical porosity structure in (a) longitudinal and (b) transversal sections. The pore size was between 10 and 100μm in diameter.

Abb. 1. REM-Aufnahmen eines PDLLA-Schaumes im Anlieferungszustand. Die Porositätsstruktur ist im (a) Längsschnitt und (b) im Querschnitt zu sehen. Der Porendurchmesser ist im Bereich von 10-100 μm.

Table 1. Properties of the PDDLA foams [28]
Tabelle 1. Eigenschaften der PDLLA-Schaummaterialien [28]

Density (g cm^{-3})	0.06
Pore Size (μm)	10-100
Crystallinity (%)	~ 0
Glass Transition Temperature Tg (°C)	65
Melting Temperature (°C)	171
Porosity (%)	93
Total Porous Volume (cm^3 g^{-1})	11.08

2.2 Composite Fabrication

An aqueous slurry-dipping technique was used to coat the PDLLA foams with Bioglass® particles, as firstly used and described by Roether et al. [28]. Briefly, a slurry was made using 3 g Bioglass® powder and 5.33ml of distilled water. This ratio was determined to give the ideal composition required to achieve a uniform and stable coating of Bioglass® particles around and within the PDLLA foams, as reported earlier [28]. This mixture was then stirred with a glass rod followed by magnetic stirring for 30 minutes to ensure adequate mixing producing a slurry with uniform dispersion of solid (glass) particles. The foam samples were initially soaked in ethanol for 30 minutes to reduce their hydrophobicity so that they

would not float to the surface of the slurry during the coating procedure. The foam samples where then placed in the slurry. The mixture was stirred with a glass rod to ensure the adequate mixing of Bioglass® particles in a clear, well-dispersed suspension. The foams were also carefully compressed with the glass rod to ensure that Bioglass® particles could infiltrate the central structure but the overall shape of the foam was maintained. Each foam was coated for 3 minutes. The coated samples were then carefully removed from the slurry and left to dry in a dessicator at room temperature.

2.3 Immersion in Simulated Body Fluid (SBF)

SBF is designed to contain similar ions in concentrations comparable to those present in blood plasma and it is very frequently used to assess the bioactivity of materials, which is characterised by the rapid formation of hydroxyapatite upon exposure of the material surfaces to the fluid [33]. The exact composition and method used to formulate the SBF used in this study were based in the work of Kokubo et al. [33].

Uncoated and Bioglass® coated foams were incubated in SBF at 175 rpm on an orbital shaker and maintained at 37 °C for various time points. Samples were removed from the SBF and washed in acetone before leaving to dry in a dessicator. The dried samples were then characterised using scanning electron microscopy (SEM), X-ray diffraction (XRD) analysis and Raman spectroscopy to investigate the formation of HA. The SBF solution was changed every 3 days. Regular measurements of the changes in pH were also carried out when the solution was changed.

2.4 Cell Culture

Human primary osteoblasts (HOBs) were isolated from the femoral head of patients undergoing total hip replacement. Bone samples were cut into fragments of approximately 3 mm x 3 mm. Fragments were washed several times in phosphate buffered saline (PBS) to remove blood cells and debris with a final wash in culture medium. Fragments were then placed into culture flasks in complete Dulbecco's Modified Eagles Medium (DMEM) containing 10 % Foetal Bovine Serum (FBS), 1 % glutamine, 2 % penicillin/streptomycin and 0.85 mM ascorbic acid. The fragments were incubated at 37 °C in a humidified incubator with 5 % CO_2. After culture for 7-10 days, fragments were subjected to trypsin (0.02 %) and collagenase (0.162U/ml) digestion for 20 minutes at 37 °C on a roller mixer. The resulting cell suspension was then centrifuged at 1000rpm and enzyme digestion of the fragments repeated. The whole process was performed a total of 5 times and cells pooled. HOBs were cultured using the procedure described above on PDLLA foams, PDLLA foams coated with Bioglass® particles and Thermanox discs as positive controls, at a density of 40,000 cells/cm^2. A commercially available (ECACC) human osteosarcoma cell line (MG-63) was also used and maintained as above.

2.5 Staining of cell nuclei

Samples were washed in PBS and fixed with 4 % paraformaldehyde for 10 minutes at room temperature. Foams were then carefully cut in half with a scalpel and stained with 10μg/ml propidium iodide at room temperature, washed in PBS and mounted under coverslips using Vectashield. Samples were then viewed under a Bio-Rad confocal microscope.

Cells were counted in random fields of view through the entire depth of the foams.

3 Characterization of foams

3.1 Scanning electron microscopy (SEM)

As-received foam samples, Bioglass® coated foams (before immersion in SBF) as well as coated and uncoated foams after immersion in SBF for 1 to 3 weeks were characterized using SEM. Samples were cut in the transverse direction with a sharp razor. The foams were mounted to show the surface and the transverse sections of each set of samples. The samples were then gold coated and observed using a JEOL (JSM 220) scanning electron microscope.

3.2 X-Ray Diffraction (XRD)

X-Ray diffraction (XRD) was used to detect the formation of crystalline HA on the surface of the samples. After immersion in SBF, a small section was cut from each sample using a sharp razor blade. The samples were then mounted on the XRD stage using blu-tack. A Phillips PW 1700 Series Automated Powder Diffractometer using K_a radiation at 40V, 40 mA, with a secondary crystal monochromator was used.

3.3 Raman Spectroscopy

Raman spectroscopy measurements (Renishaw 2000 system) were carried out to detect the formation of HA on the foam surfaces after immersion in SBF. This technique was used here to corroborate results from XRD and SEM.

4 Results

4.1 Foams structure

The typical porosity structure of the PDLLA foams can be seen in Figure 1(a,b), showing the pores in longitudinal and transversal sections of an as-received sample. The pore sizes were found to be between 10 and 100μm in diameter. The pores appear oval shaped because they have been slightly distorted during sample preparation for SEM.

A typical Bioglass® coated sample is shown in *Figure 2(a,b)*. In Figure 2a, the homogeneous distribution of Bioglass® particles throughout the cross section of the foam can be seen, while Figure 2b shows the inner structure of the coated foam at high magnification confirming the presence of Bioglass® particles within the porous network. These observations confirm the suitability of the slurry-dipping method employed here for efficient coating of PDLLA foams with Bioglass® microparticles.

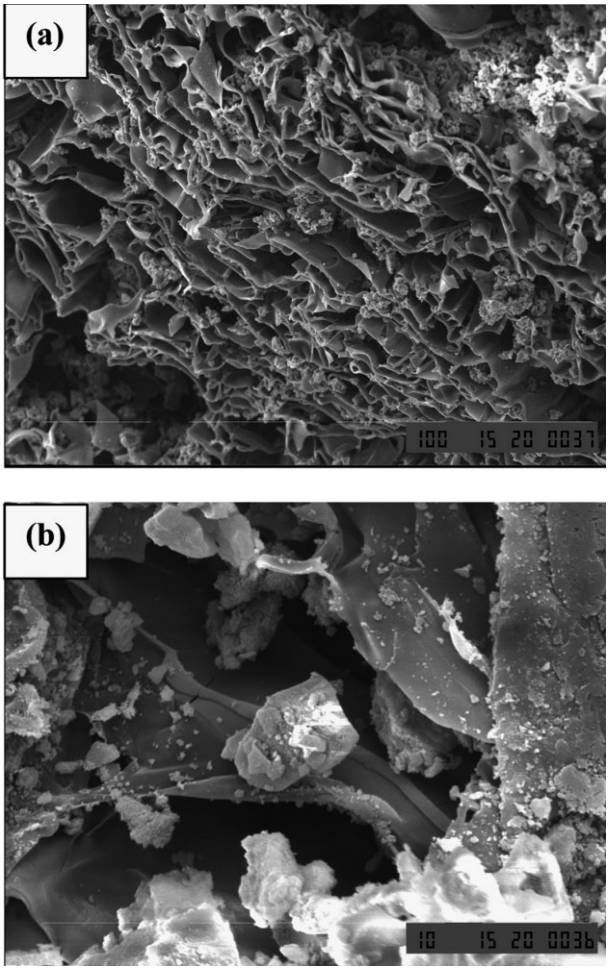

Figure 2. SEM micrographs of a Bioglass® coated PDLLA foam showing (a) the homogeneous distribution of Bioglass® particles throughout the cross section of the foam and (b) the inner structure of the foam confirming the presence of Bioglass® particles within the porous network.

Abb. 2. REM-Aufnahmen eines mit Bioglass® beschichteten PDLLA-Schaums. Die homogene Verteilung von Bioglass®-Teilchen über den gesamten Querschnitt wird in Abbildung (a) deutlich, Abbildung (b) illustriert das innere Gefüge des Schaumes, in welchem Bioglass®-Teilchen innerhalb der gesamten porösen Struktur vorhanden sind.

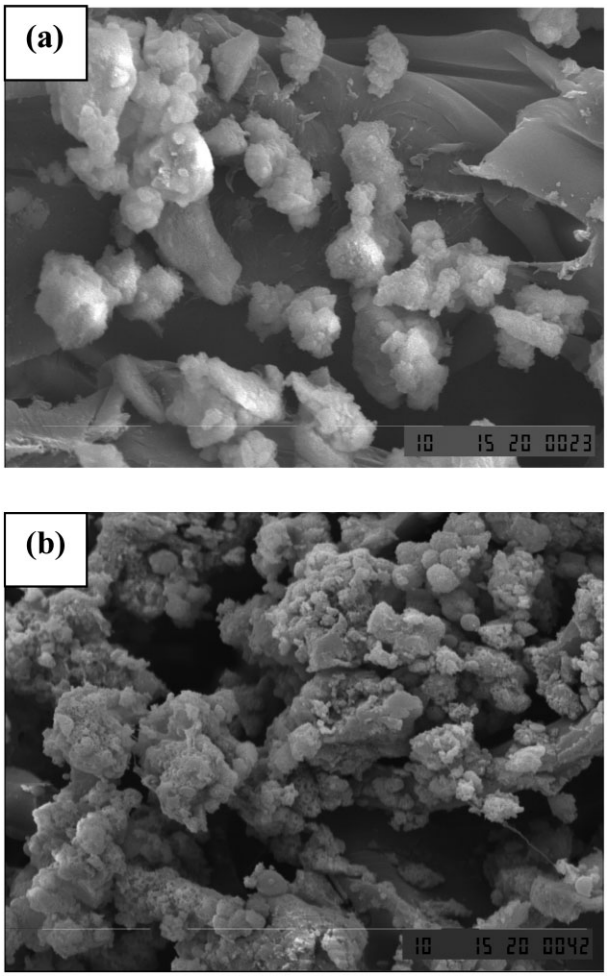

Figure 3. SEM micrographs of cross sections of Bioglass® coated foams after (a) 1 week and (b) two weeks immersion in SBF. In (a) both Bioglass® particles (jagged shape) as well as apatite crystals (rounded particles) can be seen, while in (b) only HA particles are observed.

Abb. 3. REM-Abbildungen der Querschnitte von Proben, die mit Bioglass® beschichtet wurden, nach 7 (a) bzw. 14 Tagen (b) in SBF. Nach 7 Tagen, Bild (a), können Bioglass®-Teilchen (unreguläre, schartige Form) sowie Apatitkristalle (gerundete Teilchen) identifiziert werden, wogegen auf Bild (b) nur HA-Kristalle erkennbar sind.

4.2 SBF studies

Figure 3a shows the transverse section of a Bioglass® coated foam after 1 week immersion in SBF. On this image, Bioglass® particles as well as apatite crystals can be seen. Bioglass® particles and apatite crystals may be differentiated by their appearances; while Bioglass® particles have jagged edges, apatite crystals have a more rounded structure. The image in Figure 3a confirms, thus, that apatite crystals have formed within the central structure of the foam after just one week immersion in SBF. After 2 weeks immersion in SBF (*Figure 3b*) an increasing concentration of HA crystals are detected, which are uniformly distributed on the surface of the foams. SEM examination revealed that both the amount and morphology of HA formed on the foams change with immersion time in SBF. *Figure 4*, which is a SEM image of the longitudinal section of a sample that had been soaked for 3 weeks in SBF, shows the large amount of apatite crystals formed on the sample, which have led to formation of a continuous layer covering the sample surface.

Figure 5a shows the longitudinal section of an uncoated foam sample after immersion in SBF for 2 weeks. Apatite formation does not appear to have occurred in the interior of the foams. The surfaces of the foams after 3 weeks immersion in SBF, however, indicated the presence of small crystals, as shown in *Figure 5b*. However, the apatite formation is limited in comparison to the amount formed on the composite samples after the same immersion time (3 weeks) (Figure 4).

The crystallinity of the HA formed on the coated and uncoated samples was confirmed by XRD analysis, as shown in *Figure 6*. *Figure 7* shows the Raman spectra for the Bioglass® coated foams before and after immersion in SBF for 1 and 2 weeks. It is seen from these spectra that HA has formed after 1 week in SBF with an increasing amount of HA formed after 2

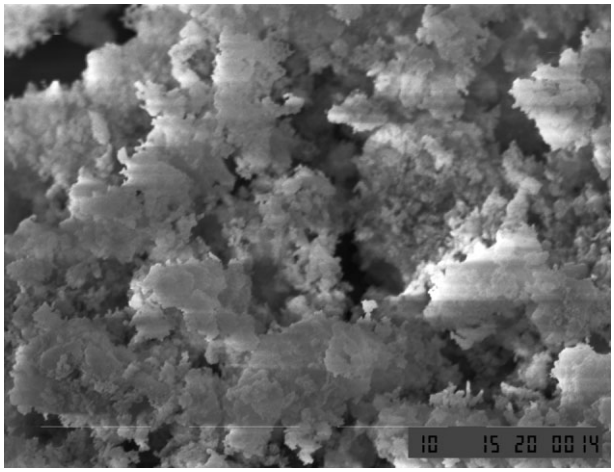

Figure 4. SEM image of the longitudinal section of a Bioglass® coated foam after immersion for 3 weeks in SBF, showing the formation of a continuous HA layer covering the sample surface.

Abb. 4. REM-Aufnahme des Längsschnitts eines mit Bioglass®-bschichteten Schaums nach 21-tägigem Eintauchen in SBF. Eine durchgängige HA-Beschichtung auf der Oberfläche der Probe kann beobachtet werden.

weeks in SBF. As the peaks for HA intensify, the peak for PDLLA appears to decrease. This may be representative of the degradation of the polymer as well as the increasing quantity of apatite formed over time, as also confirmed by SEM (Fig. 3).

4.3 Cell Culture

Cells were counted in the upper section, middle section and lower section of the foams, as shown in *Figure 8*. More cells were observed attached within pores of the upper section compared to the middle and lower sections after 90 minutes on both uncoated and Bioglass® coated PDLLA foams. However, at this initial timepoint, more cells were observed in the pure PDLLA foam compared to the coated sample, although these values were not found to be significantly different as determined by a one way ANOVA with Tukey-Kramer post-test.

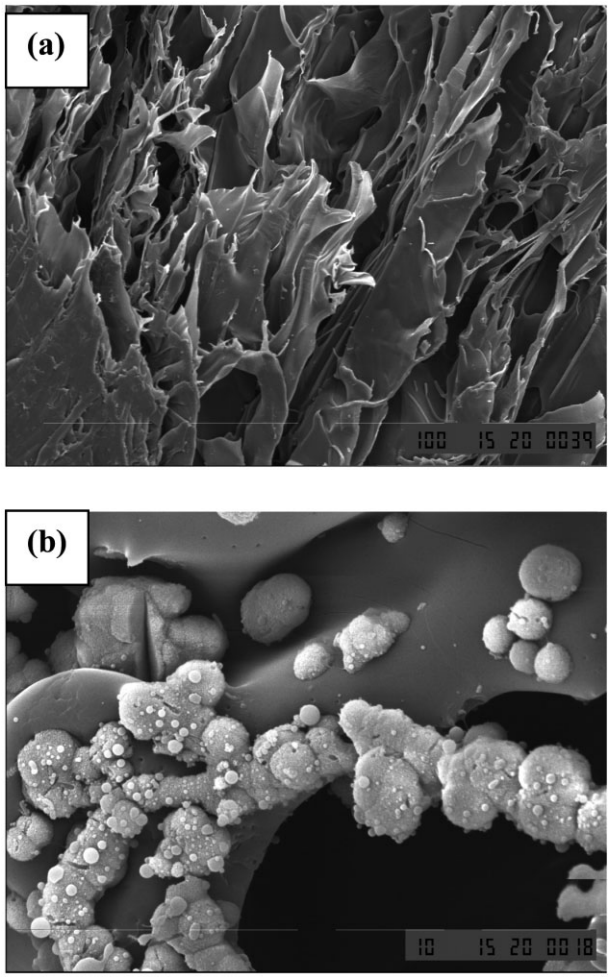

Figure 5. (a) SEM micrograph of the longitudinal section of an uncoated PDLLA foam sample after immersion in SBF for 2 weeks showing no apatite formation. (b) SEM micrograph of the surface of an uncoated PDLLA foam sample after 3 weeks immersion in SBF indicating the presence of small HA crystals.

Abb. 5. (a) REM-Aufnahme des Längsschnittes eines unbeschichteten PDLLA-Schaums nach Eintauchung in SBF für 14 Tage, auf dem kein HA gebildet wurde. (b) REM-Aufnahme der Oberfläche eines unbeschichteten PDLLA-Schaumes nach 21 Tagen in SBF. Hier ist die Bildung von winzigen HA-Kristallen erkennbar.

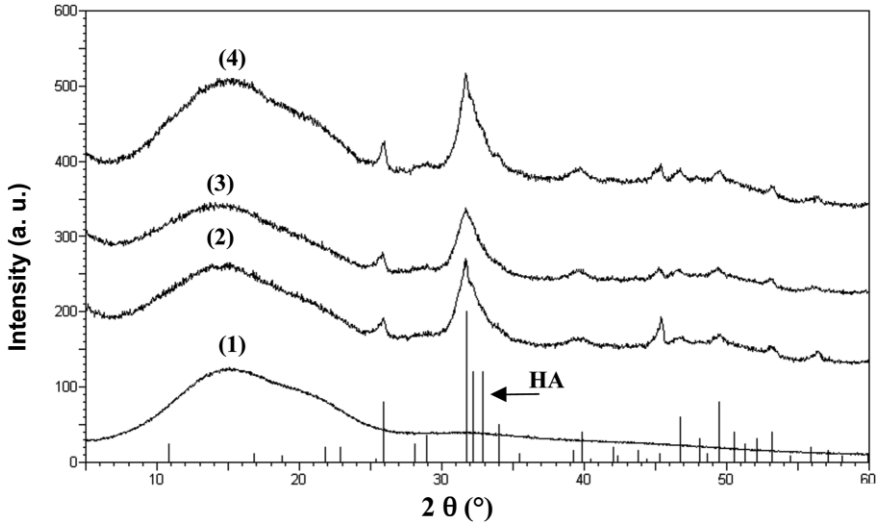

Figure 6. XRD patterns of Bioglass® coated and uncoated PDLLA foams demonstrating crystallinity of HA formed after immersion in SBF: (1) coated sample, before immersion, (2) coated sample, immersion time: 1 week, (3) coated sample, immersion time: 3 weeks, (4) uncoated sample, immersion time: 3 weeks.

Abb. 6. XRD-Bild von mit Bioglass®-beschichteten und unbeschichteten PDLLA Schäumen, in dem die Kristallität von gebildetem HA nach Eintauchen in SBF illustriert wird: (1) beschichtete Probe vor dem Eintauchen, (2) beschichtete Probe nach 7 Tagen, (3) beschichtete Probe nach 21 Tagen und (4) unbeschichtete Probe nach 21 Tagen.

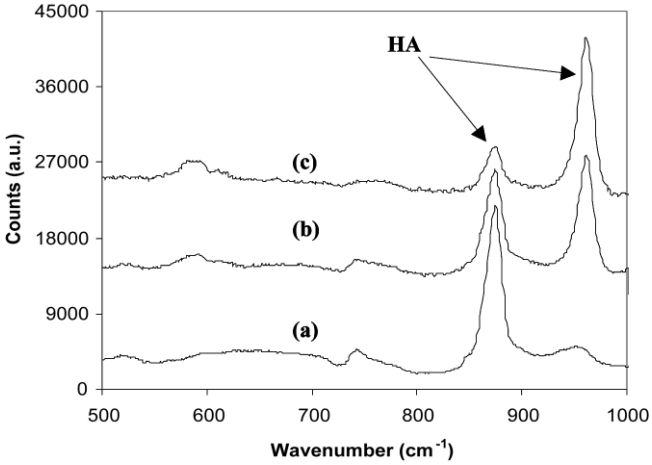

Figure 7. Raman spectra for the Bioglass® coated foams before (a) and after immersion in SBF for 1 (b) and 2 (c) weeks. The peaks marked by the arrows indicate that HA has formed after 1 week in SBF with an increasing amount of HA formed after 2 weeks in SBF.

Abb. 7. Raman-Spektra für Bioglass®-beschichtete Schäume vor (a) und nach Eintauchen in SBF für 7 (b) und 14 Tage (c). Die Höchstwerte, die durch Pfeile gekennzeichnet sind, weisen auf die Bildung von HA nach sieben Tagen (a) und die vermehrte Bildung von HA nach 14 Tagen in SBF hin.

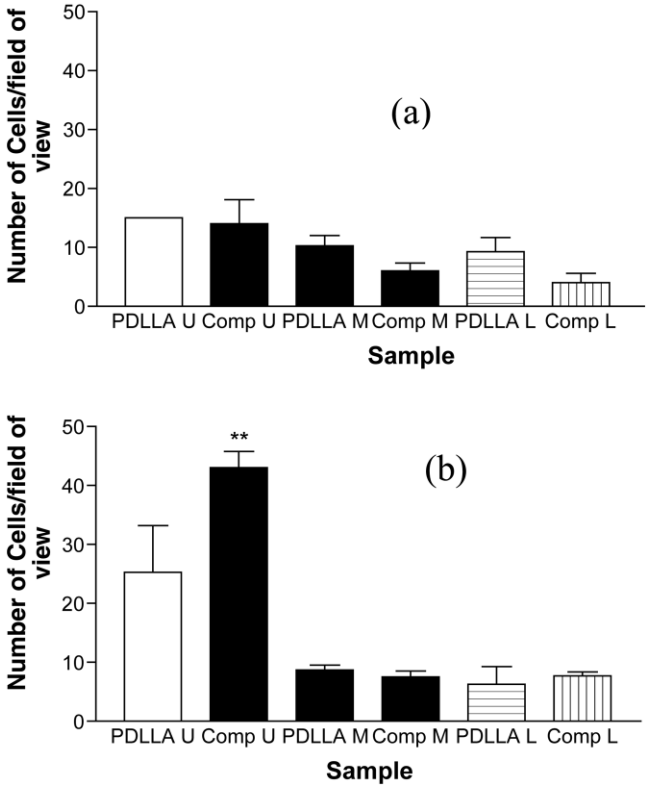

Figure 8. Graph showing cell attachment/colonisation throughout the porous network of foams after 90 minutes (a) and 24 hours (b) culture. Labels "U", "M" and "L" refer to upper, middle and lower regions of the porous structures. Mean values +/– standard error of the mean are shown where n=4. A significantly higher number of cells were observed in the upper region of the Bioglass® coated (composite) foam compared to the PDLLA alone as shown by a one way ANOVA with Tukey Kramer post-test.

Abb. 8. Der Graph zeigt die Fixierung bzw. Kolonisierung der Zellen innerhalb des porösen Gefüges der Schäume nach 90 Minuten (a) und nach 24 Stunden (b) in Zellkultur. Die Kennzeichnung „U", „M" und „L" bezieht sich auf die obere, mittlere bzw. untere Region des porösen Gerüsts. Die Mittelwerte ± Standardabweichung waren für n = 4. Eine wesentlich höhere Anzahl von Zellen wurde in der höheren Region der PDLLA/Bioglass®-Verbundwerkstoffe beobachtet verglichen mit unbeschichteten PDLLA-Schäumen ermittelt mit „ANOVA" mit „Tukey Kramer"-Test.

After 24 hours cell numbers had increased and more cells were observed attaching within the porous network of the coated foam. The number of cells attached within the upper portion of the coated foam was significantly higher than in the same region of the PDLLA alone.

5 Discussion

Various bioactive ceramics such as hydroxyapatite, tri-calcium phosphates and Bioglass® have been developed to be used clinically in bone repair [8]. These have been found to bond with bone through a layer of bone-like apatite formed on the surface of the ceramics when implanted into the body [8-11,32]. This apatite has been characterised as carbonate-containing HA and was not observed at the interface between non-bioactive (or bio-inert) materials and bone [34,35]. The approach followed in the present study was to combine bioactive glass (45S5 Bioglass®) and a resorbable polymer (PDLLA) to form bioactive and biodegradable porous scaffolds for bone tissue engineering. The cumulative results from SEM, XRD and Raman spectroscopy obtained on the foams developed here after immersion in SBF lead to the conclusion that the materials exhibit a high level of bioactivity: crystalline hydroxyapatite is formed on the Bioglass® coated foams after only 1 week immersion in SBF. The amount of HA formed increases with increasing time in SBF. These findings are in broad agreement with previous results on similar polymer/HA and polymer/Bioglass® composites [13, 16-29].

HA was also seen to form on the pure PDLLA foams, however only after immersion in SBF for 3 weeks and only on the outer surfaces of the foams. The HA formation on pure PDLLA foams is somewhat surprising but it may be explained in the light of findings reported in the literature, as discussed next. For example, Zhang et al. [36] conducted experiments involving composites prepared from highly porous poly(L-lactic acid) (PLLA) foams and apatite. Bonelike apatite was 'grown' on the surfaces of pore walls throughout the PLLA foams during immersion in SBF at 37 °C. It was found that after 30 days, a large number of HA microparticles with a diameter of up to 2μm was formed on the surfaces of the PLLA pore walls. The porous polymer-apatite composites formed were created as a new type of composite scaffold for bone tissue engineering [36]. This method of preparing composites by growing apatite on PLLA substrates by immersion in SBF for prolonged periods of time supports the evidence produced in this study of the formation of HA on the PDLLA foams (e.g. as presented in Figure 5b).

Extensive research has been carried out investigating the mechanism of apatite formation on CaO-SiO_2 based glasses and glass-ceramics during immersion in SBF [8]. Kokubo et al [34, 35] proposed that calcium ions dissolved from the ceramics increase the ionic activity product of the apatite in the surrounding body fluid, which is already supersaturated

in respect to apatite. Moreover the hydrated silicon oxide surfaces of the bioactive glasses provide favourable sites for apatite nucleation [8, 34,35]. The same principle is exploited in the present study where 45S5 Bioglass® particles are added to the polymer foams as a coating. It has been shown extensively in the literature that the dissolution of Bioglass® particles aid the formation of HA [7,8,27-29]. The same effect can occur if the SBF in which the materials are immersed is supersaturated, i.e. if it contains a high concentration of calcium-phosphate ions by itself without the need for addition of a bioactive material [16]. The modification of the surface of materials by inducing the formation of bonelike mineral layers, such as calcium phosphate or HA layers, in contact with supersaturated simulated body fluid has been termed "biomimetic approach" [37, 38]. Besides the study of Zhang et al. [36] on PLLA scaffolds mentioned above, the biomimetic approach has been applied to a variety of synthetic degradable [16, 39] and non-degradable [38] polymers, natural polymers (e.g. gelatin) [40], metals [41,42] and bioinert ceramics [43]. In the procedure developed by Habibovic et al. to coat titanium implants with a bioactive layer [41], the procedure involved two steps; first the implants were soaked in a solution that was 5 times more concentrated than regular simulated body fluid. A thin layer of calcium phosphate was deposited on the implants. The implants were then immersed in a second SBF solution, which had decreased amounts of crystal growth inhibitors. This resulted in a thick layer of crystalline calcium-phosphate on the titanium implants [41]. This effect was also studied by Du et al [37] where a solution of concentrated SBF was used to produce a potential osteoconductive calcium phosphate coating on a commercial Polyactive® polymer scaffold. Despite these previous experiences, the mechanism of HA formation in the present PDLLA foams remains unclear since in the present study a normal (i.e. non-concentrated) SBF suspension was used. In case of SBF supersaturated with calcium and phosphate ions, precipitation and growth of the crystals is favoured, as discussed by Laurencin and Lu [16]. It is however unknown if or how the polymer surface plays a role in the nucleation and growth of HA crystals, as found in the present experiment after 3 weeks immersion in SBF. It is likely that in solution and during degradation, the polymer surface contains a high concentration of hydroxyl groups, which may attract calcium ions from the solution and initiate the formation of the calcium phosphate or HA layers [16,39].

The osteoconductivity of bone tissue engineering scaffolds can be enhanced by the formation of a HA layer on the surface of the constructs prior to cell seeding, as reported in the literature [8,9,39]. The osteoblast cell infiltration study conducted here demonstrated that cells were able to migrate through the porous network and colonise the lower section of the foams. Also, after 24 hours a higher cell density was observed in the Bioglass® coated foams compared to the pure PDLLA foams. There were however, more cells observed in the upper regions of the foams. This is not unexpected as the culture technique used was that of placing a cell suspension on top of the samples. The fact that some cells were observed in the middle and lower regions is encouraging. Current studies focus on introducing relevant changes in the culture technique to allow greater infiltration and migration of cells into the porous network and further osteoblast responses will be analysed over longer time periods.

The results presented in this paper combining bioactivity assessment and osteoblast cell attachment and infiltration allow to conclude that the developed PDLLA/Bioglass® composite foams are promising materials for the tissue engineering of bone. Studies of the mechanical properties of the foams should determine if load bearing applications of these materials will be also possible, this being the focus of on-going research.

6 Conclusions

Poly(DL-lactic acid) acid (PDLLA)/Bioglass® composite foams were fabricated using a slurry-dipping technique. Two types of in-vitro studies were carried out; SBF immersion tests and osteoblast cell culturing on pure PDLLA foams and PDLLA/Bioglass® composites.

The following conclusions can be drawn:

1. With increasing periods in SBF increasing amount of crystalline hydroxyapatite was formed on the composite samples.
2. Immersion in SBF also formed HA on the pure PDLLA foams (although only after 3 weeks in SBF and in lower quantities than on the composites). This was attributed to a biomimetic effect, however further experiments should confirm this hypothesis.
3. Osteoblast cell infiltration was observed throughout the porous network of the foams indicating the osteoconductive character of the materials.

The porous degradable composites developed in this study are therefore candidate materials for bone tissue engineering scaffolds.

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