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Development of bioactive glass based scaffolds for controlled antibiotic release in bone tissue engineering via biodegradable polymer layered coating

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Highly porous 45S5 Bioglass®-based scaffolds coated with two polymer layers were fabricated to serve as a multifunctional device with controlled drug release capability for bone regeneration applications. An interior poly(D,L-lactide)/poly(ethylene glycol)-(polypropylene glycol)-poly(ethylene glycol) triblock copolymer (Pluronic P123) coating improved the mechanical stability of Bioglass-based scaffolds, while an exterior natural polymer (alginate or gelatin) coating served as an antibiotic drug carrier. The results showed improved mechanical properties of Bioglass-based scaffolds by the bilayer polymer coating. In addition, hydrochloride tetracycline loaded in either alginate or gelatin coatings was released rapidly at the initial stage (~1 h), while the released rate subsequently decreased and was sustained for 14 days in phosphate buffered saline. Therefore, these layered polymer coated scaffolds exhibit attractive characteristics in terms of improved mechanical properties and controlled drug release, simultaneously with the added advantage that the drug release rate is decoupled from the intrinsic scaffold Bioglass degradation mechanism. The layered polymer coated scaffolds are of interest for drug-delivery enhanced bone regeneration applications. © *2014 American Vacuum Society*. [http://dx.doi.org/10.1116/1.4897217]

I. INTRODUCTION

Recent developments in bone tissue engineering provide alternative approaches for the repair of bone defects caused by trauma and infection.[1] Bone repair scaffolds loaded with drugs (i.e., antibiotics and antitumoral medicaments) and/or growth factors attract increasing attention since they can protect against infections but also regulate cell growth and they can also enhance bone regeneration.[2–9] Basically, bone scaffolds should be biocompatible, biodegradable, osteoconductive and, in improved scaffold designs, they should be able to act as a local drug carrier.[2,9–14] Scaffolds are usually made from tailored combination of inorganic and organic phases, forming composite structures aiming to replicate the structure and composition of bone tissue.[2,3,13,15] Several bioactive glasses and bioceramics have been used as the inorganic phase in drug eluting composite scaffolds, including hydroxyapatite (HA),[6,7,16] calcium phosphate (CaP),[17–19] and Bioglass®.[20–23] Scaffolds composed of a single inorganic component usually have low drug binding affinity, and thus, they do not allow a controlled drug release.[13] This is particularly the case for bioactive glass scaffolds derived from molten glasses,[20–23] which do not have a suitable intrinsic mesoporosity to be used as drug reservoirs.[3,10] Therefore, several natural- and synthetic-derived biodegradable polymers have been explored as the organic component for development of composite scaffolds, such as collagen,[24–26] gelatin,[27–29] chitosan,[2,18,19,30] alginate,[29–31] and polyesters.[16,32–35] As early reported by Yaylaoğlu *et al.*,[17] CaP/gelatin composite scaffolds have been loaded with gentamicin for *in-situ* drug delivery enhanced bone tissue engineering. Continuous release of the drug upon 4 weeks *in vivo* was observed with the release rate depending on the

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degradation rate of the gelatin component. Kim *et al.*[6] developed HA-based scaffolds with controlled tetracycline release function by using of the polycaprolactone (PCL)/HA hybrid coating. The scaffolds exhibited improved mechanical properties due to the presence of the PCL hybrid coating, while the drug entrapped in the polymeric coating was shown to be released in a sustained manner. Indeed biodegradable polymers can be conveniently used as coatings of inorganic scaffolds in order to achieve better mechanical properties, and at the same time, such coatings can function as a drug carrier.[3,32] Usually, coatings in the form of a single polymer layer or formed by microspheres have been investigated.[14] For example, vancomycin loaded poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) coated 45S5 Bioglass-based scaffolds presented improved mechanical strength (around five times higher compressive strength than that of uncoated scaffolds) and provided sustained drug release, as reported by Li *et al.*[21] The coated scaffolds provided a lower initial burst release when compared to the uncoated scaffolds, followed with a controlled release over 6 days in phosphate buffered saline (PBS). Francis *et al.*[20] have reported that gentamicin loaded poly(hydroxybutyrate) microspheres coated onto 45S5 Bioglass-based scaffolds presented slow drug release (in comparison to free microspheres) while the bioactivity of the scaffolds was not impaired. Other studies involving microsphere coating of scaffolds were reported by Meng *et al.*[36] and Li *et al.*,[37] who considered tetracycline and vancomycin as the encapsulated drugs, respectively. On the other hand, the use of dual coating layers to enhance the functionality of the scaffolds has been explored only to a limited extent. Multifunctional scaffolds based on PCL and vancomycin-loaded chitosan coated Bioglass-based scaffolds were studied recently by Yao *et al.*[22] PCL coating improved the mechanical strength of the scaffolds about three times, while the vancomycin-loaded chitosan coating exhibited a controlled drug release upon 11 days of immersion in PBS. In another recent study, vancomycin-loaded poly(*n*-isopropylacryliamide-*c*-acrylic acid) microgels dispersed in poly (lactic-co-glycolic acid) coated 45S5 Bioglass-based scaffolds[23] showed improved mechanical properties and suitable bioactivity, as well as exhibiting controlled release rate of vancomycin from the drug-loaded microgels. In addition, multilayered poly(β-amino ester) films containing vancomycin have been coated onto gelatin sponges by using spray layer-by-layer assembly.[38] Both 60- and 120-coating layers on the sponges exhibited controlled vancomycin release over 6 days. It was reported that the ability of drug loading and the capability of drug release could be controlled by adjusting the number of coating layers.[38]

In the present study, a new strategy to develop multilayered polymer coatings on scaffolds was developed. Multifunctional scaffolds were fabricated by coating Bioglass-based foams with two different biodegradable synthetic and natural polymers forming layered coatings. Poly(D,L-lactide) (PDLLA) was chosen as the first coating layer, aiming at improving the mechanical properties and structural stability of Bioglass-based scaffolds, while either alginate or gelatin were loaded with tetracycline hydrochloride (TCH) and applied on the PDLLA coated scaffolds as a second coating layer. Natural polymers were used as the drug carrier component because such polymers are compatible with water soluble drugs like TCH. In addition, natural polymers exhibit superior biocompatibility and therefore are preferred as the outer coating layer to facilitate the adhesion and proliferation of cells (i.e., osteoblasts).[13] Given the chemical incompatibility between PDLLA and alginate or gelatin, a new strategy was investigated involving the modification of the surface chemistry of the PDLLA coating by blending with an amphiphilic polymer (i.e., P123 copolymer). The mechanical properties and drug release behavior of the multifunctional scaffolds (both with alginate- and gelatin-drug carriers) were investigated.

II. MATERIALS AND METHODS

A. Fabrication of TCH-loaded layered polymer coated scaffolds

45S5 Bioglass-based scaffolds were prepared by using the foam replication method originally reported by Chen *et al.*[39] Briefly, poly(vinyl alcohol) (PVA), purchased from Merck KGaA, Germany, was dissolved in deionized (DI) H_2O with concentration of 3.5 wt./vol. %. Afterward, 40 wt./vol. % of 45S5 bioactive glass powder (45% SiO_2, 24.5% CaO, 24.5% Na_2O, and 6% P_2O_5 by weight) was added to the PVA solution. The whole procedure was carried out at 80 °C under vigorous magnetic stirring for 2 h. Polyurethane (PU) foam "Eurofoam" with 45 ppi (pore per inch) served as sacrificial template. PU foams (of dimensions $10 \times 10 \times 10\,mm^3$) were immersed in the prepared slurry for 1 min. The foams were then removed and the extra slurry was squeezed out manually. The samples (green bodies) were then dried in an oven at 60 °C for 12 h. The coating thickness of the green bodies was increased by repeating the slurry coating procedure for three times. The green bodies were first heated up at 450 °C for 1 h to burn out the PU template and then at 1100 °C for 2 h in order to sinter the 45S5 Bioglass scaffolds (heating rate was 2 °C/min and cooling rate was 5 °C/min). For the preparation of polymer coatings, PDLLA (Purac Biomaterials, Gorinchem, Netherland) was dissolved in dimethylcarbonate (DMC) with a concentration of 5 wt./vol. % at room temperature while stirring for 2 h. Then poly(ethylene glycol)-(polypropylene glycol)-poly (ethylene glycol) triblock copolymer (Pluronic P123, $M_n \sim 5800$ Da; Sigma) was added into the PDLLA solution with a PDLLA to P123 weight ratio of 9/1. The mixture was continuously stirred until P123 was completely dissolved. For production of coatings, 45S5 Bioglass scaffolds were immersed in 5 ml of the polymer solution for 5 min. Subsequently, scaffolds were removed from the solution and dried at room temperature for 24 h. These coated scaffolds were labeled as PL/P123–*c*-BG.

TCH-loaded alginate and gelatin solutions were prepared as follows. Sodium alginate (Mw ~ 200 000 Da; Sigma) was dissolved in DI H_2O with a concentration of 1.5 wt./vol. % at room temperature and stirred for 2 h. For gelatin, a

concentration of 1.5 wt./vol. % gelatin (type A from porcine skin with 300 g bloom; Sigma) was dissolved in DI H_2O at 50 °C while stirring for 1 h. Then, 375 μg/ml TCH ($C_{22}H_{24}N_2O_8 \cdot HCl$; Appli Chem GmbH, Darmstadt, Germany) was added into both alginate and gelatin solutions. Finally, the PL/P123–*c*-BG scaffolds were immersed in the TCH-loaded alginate and gelatin solutions (5 ml of solution/scaffold) for 5 min and dried at room temperature for 24 h. The drug-loaded scaffolds were labeled as T-Alg-*c*–(PL/P123–*c*-BG) and T-Gel-*c*–(PL/P123–*c*-BG) for alginate and gelatin as the drug carriers, respectively. TCH loaded uncoated scaffolds were prepared as control, by simply dipping uncoated scaffolds in TCH/DI H_2O with a TCH concentration of 375 μg/ml for 5 min. Then, the scaffold was taken out and dried at room temperature for 24 h. These samples were labeled as T-BG. The initial assessment of the presence of the drug was carried out by simple visual inspection given the expected change of color of the scaffold surface when incorporating the drug.

B. Characterization techniques

1. Capillarity test

In order to evaluate surface characteristics of the polymeric coatings, a qualitative capillarity test was performed according to Ref. 40. Briefly, a TCH-loaded polymeric coating solution, which served as a testing fluid, was prepared following the same procedure as the coating solution (described above). In this case, TCH-loaded gelatin solution, which exhibits a yellow color, was added in a glass vial. Then, a coated scaffold was slowly placed on the surface of the solution, while the testing time was recorded until the scaffold was completely wet (the fluid ascended through the entire porous network of the scaffold). PDLLA-*c*-BG and PL/P123–*c*-BG scaffolds were tested in order to compare the surface property of the different polymeric coatings and how they would affect scaffold capillarity.

2. Contact angle measurement

In order to evaluate the hydrophilicity of each polymeric coating, the wettability of pellets prepared following the same conditions as for 3D scaffolds was measured using a water contact angle instrument (DSA30, Kruess, Germany). The pellets were prepared as follows: 0.3 g of Bioglass powder were added in a stainless steel die (diameter: 10 mm) and pellets were obtained by cold uniaxial pressing using an electrohydraulic press (MAUTHE MASCHINENBAU PE-010; Wesel, Germany) working at a load of 4×10^4 N. The obtained pellets were sintered using the same conditions used for porous Bioglass scaffolds. As-sintered Bioglass pellets were then coated with TCH, PL/P123, and TCH loaded alginate and gelatin following the same procedures described above.

3. Microscopy

The microstructure of the scaffolds was characterized by scanning electron microscopy (SEM; LEO 435VP, Zeiss Leica). The scaffolds were cross-sectioned by using a razor blade. The samples were then sputter-coated with carbon and observed at an accelerating voltage of 10 kV.

4. Chemical analysis

The chemical structure of the scaffolds was investigated by using Fourier-transform infrared spectroscopy (FTIR) (Nicolet 6700). Bioglass-based scaffolds were grinded and the obtained powder was mixed with potassium bromide (KBr) powder in a weight ratio of 1/300 (scaffold/KBr). The mixture was pressed into a pellet by using an electrohydraulic press at a load of 10^5 N. Pellets were measured by using FTIR in transmission mode with a resolution of 4 cm^{-1} in the wavenumber range of 4000–400 cm^{-1}.

5. Mechanical testing

Polymer coated cubic Bioglass scaffolds of nominal dimensions $8 \times 8 \times 8$ mm^3 were tested in compression using a universal testing machine (Zwick Z050). The cross-head speed used was 2 mm/min, the preload was 0.1 N, and the maximum load was 50 N. Stress–strain curves were recorded to determine the mechanical properties. Eight specimens were tested for each scaffold type and the results are presented as average ± standard deviation (SD).

6. In vitro *drug release profile*

The *in vitro* drug release behavior of the scaffolds, including T-BG, T-Alg-*c*–(PL/P123–*c*-BG), and T-Gel-*c*–(PL/P123–*c*-BG) scaffolds, with dimensions $8 \times 8 \times 8$ mm^3 were evaluated. Each scaffold was immersed for up to 14 days in a glass vial containing 5 ml of PBS (0.1 M; Sigma) solution at 37 °C and *p*H 7.4. At given interval times, 2 ml of PBS solution was taken and replaced with fresh PBS. The absorbance of the drug containing PBS solution at the wavelength of 362 nm was measured by using a UV spectrophotometer (Specord 40; Analytikjena, Germany). Then, the amount of drug released was determined by using a linear relationship between absorbance and known concentrations of TCH (2.5–100 μg/ml), as given

Absorbance = [0.0268 × concentration (μg/ml)] − 0.1206, $R^2 = 0.99$.

The amount of drug release was reported as a percentage of cumulative drug release ± SD with respect to the immersion time.

7. In vitro *bioactivity*

In order to confirm the *in-vitro*, acellular bioactivity of scaffolds after coating with synthetic PDLLA, PDLLA-*c*-BG scaffolds of dimensions $8 \times 8 \times 8$ mm^3 were investigated using the Kokubo simulated body fluid (SBF) protocol.[41] Each scaffold was placed in a polystyrene bottle containing 50 ml of SBF solution at 37 °C and *p*H 7.4. After 3 days of immersion, the scaffold was extracted, washed twice with DI water, and dried at room temperature. Afterward, possible HA formation on scaffold surfaces and also morphological changes of the surface were analyzed by using SEM.

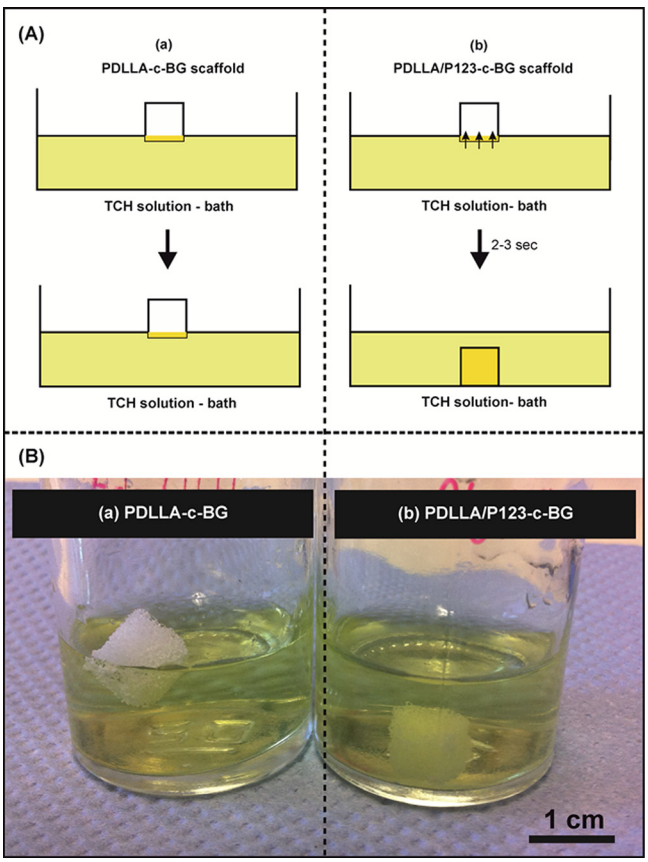

FIG. 1. Scheme of the capillarity test of Bioglass-based scaffolds, showing the effect of surface chemistry on the permeability of the porous scaffolds with (left): PDLLA-only and (right) PDLLA/P123 coating.

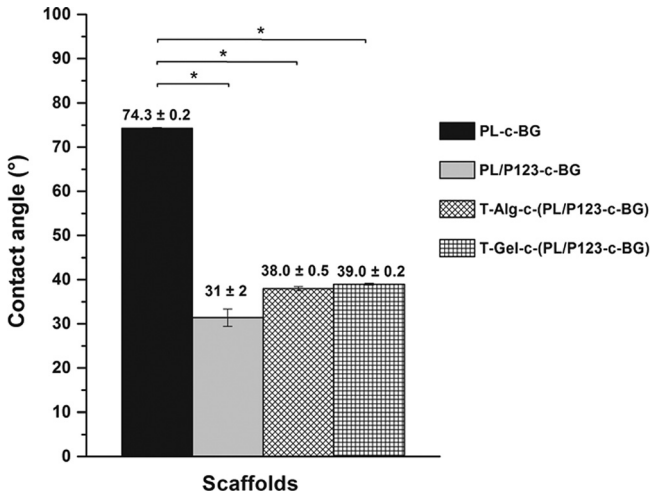

FIG. 2. Contact angles of Bioglass-based scaffolds, showing the surface wettability of different coatings. * indicates the significant difference ($p < 0.05$) of the modified coatings on the Bioglass scaffolds in comparison with PL-*c*-BG scaffolds.

8. Statistical analysis

The data were analyzed by using one-way ANOVA analysis and Turkey's multiple-comparison test to determine statistical differences. A confidence interval of 95% ($p = 0.05$) was used for all analyses.

III. RESULTS AND DISCUSSION

A. Surface property of polymeric coatings

A double layered coating based on PDLLA and alginate or gelatin was designed to be applied on 45S5 Bioglass-

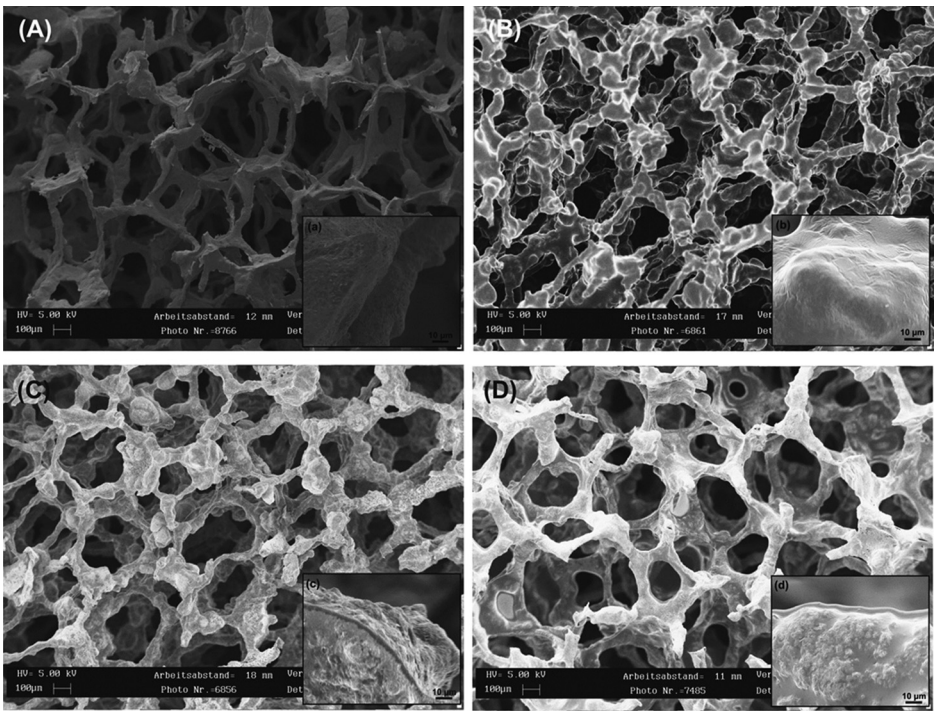

FIG. 3. SEM images of the scaffolds showing the pore structure and morphology of coating surfaces of: (A, a) T-BG scaffolds, (B, b) PL/P123-*c*-BG scaffolds, (C, c) T-Alg-*c*-(PL/P123-*c*-BG) scaffolds, and (D, d) T-Gel-*c*-(PL/P123-*c*-BG) scaffolds.

based scaffolds to impart drug delivery capability. The challenge in developing such synthetic-natural polymer layered coatings is the difference of surface chemistry between the hydrophobic PDLLA and hydrophilic alginate or gelatin. As a result of this polymer incompatibility, the TCH-loaded alginate and gelatin solutions could not infiltrate in the preliminary experiment the porous structure of the unmodified PDLLA-*c*-BG scaffold. This observation was qualitatively confirmed by the capillarity test (Fig. 1) carried out using the TCH-loaded gelatin solution (yellow color) as a test solution. The test involves the determination of the capillarity of a structure,[40] and it revealed the lack of suitable capillarity (related to the wettability) in the case of PDLLA-*c*-BG scaffolds. The scaffolds were seen to remain on the surface of the coating solution, as illustrated in Fig. 1(A). Consequently, the TCH-loaded gelatin solution could not infiltrate the pore structure and coating of the struts was not successful. In order to overcome this problem, a modification of the surface chemistry of PDLLA-*c*-BG scaffold was necessary. The approach developed in this study involved the addition of P123 copolymer in order to increase the hydrophilicity of the PDLLA-*c*-BG scaffold, leading to a degree of hydrophilicity matching that of alginate and gelatin. Indeed blending Pluronic polymers (i.e., F127 and P123) with synthetic polymers (i.e., polyethersulfone and poly (lactic acid)) has been reported to increase the wettability of such synthetic polymers without affecting their degradation rate.[42,43] The amphiphilic P123 copolymer was used because it contains both hydrophilic and hydrophobic groups, which can be homogeneously blended with PDLLA in DMC solution. By using this approach, the capillarity effect was obvious in the case of PDLLA/P123–*c*-BG scaffolds, as the surface wettability increased and the TCH-loaded gelatin solution ascended through the whole pore network of the scaffold in few seconds, as shown in Fig. 1(B).

The increase in the hydrophilicity of PDLLA-*c*-BG scaffolds was also confirmed by the water contact angle values, as shown in Fig. 2. The applied drops had a small volume such that influence due to gravity is negligible as shown by Härth and Schubert.[44] After coating with PDLLA/P123 blend, the contact angle value of the scaffolds was significantly decreased (from $74.3° \pm 0.2°$ for pure PDLLA-*c*-BG to $31° \pm 2°$ for PDLLA/P123–*c*-BG) to nearly the values of T-Alg and T-Gel coatings ($38.0° \pm 0.5°$ and $39° \pm 0°$, respectively). These results confirmed that the blend of PDLLA and P123 copolymer can drastically modify the surface chemistry of pure PDLLA and thus the TCH-loaded alginate and gelatin solutions could efficiently infiltrate the porous structure of the scaffolds, forming layered polymer coatings, as desired in this study.

B. Microstructure

Figures 3(A)–3(D) show SEM micrographs of different coated scaffolds at different magnifications. As it is well known from the literature,[20–23,39] this type of scaffolds fabricated by the foam replica method exhibits highly interconnected porosity and the pore volume fraction of scaffolds before polymer coating is >90%. The morphology of the scaffolds after coating with PDLLA/P123 blend is shown in Fig. 3(B). The surface of the coated scaffold was homogeneous and smooth compared to the surface of T-BG scaffolds [Fig. 3(A) and inset], which might be the result of the used P123 copolymer, considering that P123 copolymer shows an ability to enhance the rheological property of polymer blends.[45] It is thus obvious that the polymer homogeneously covered the entire strut even though some uneven areas could be observed, as shown in the inset in Fig. 3(B). After coating with TCH-loaded alginate and gelatin as second coating layers, as shown in Figs. 3(C) and 3(D), even though the color of the scaffolds became yellow, no morphological changes of the struts were observed by SEM [both T-Alg- and T-Gel-*c*–(PL/P123–*c*-BG) scaffolds] compared to the PDLLA/P123–*c*-BG scaffolds [Fig. 3(b)]. In detail, a fairly homogeneous coating not showing blocking of pores was observed [see Figs. 3(c) and 3(d)]. However, at higher magnification [inset in Figs. 3(c) and 3(d)], a rougher surface

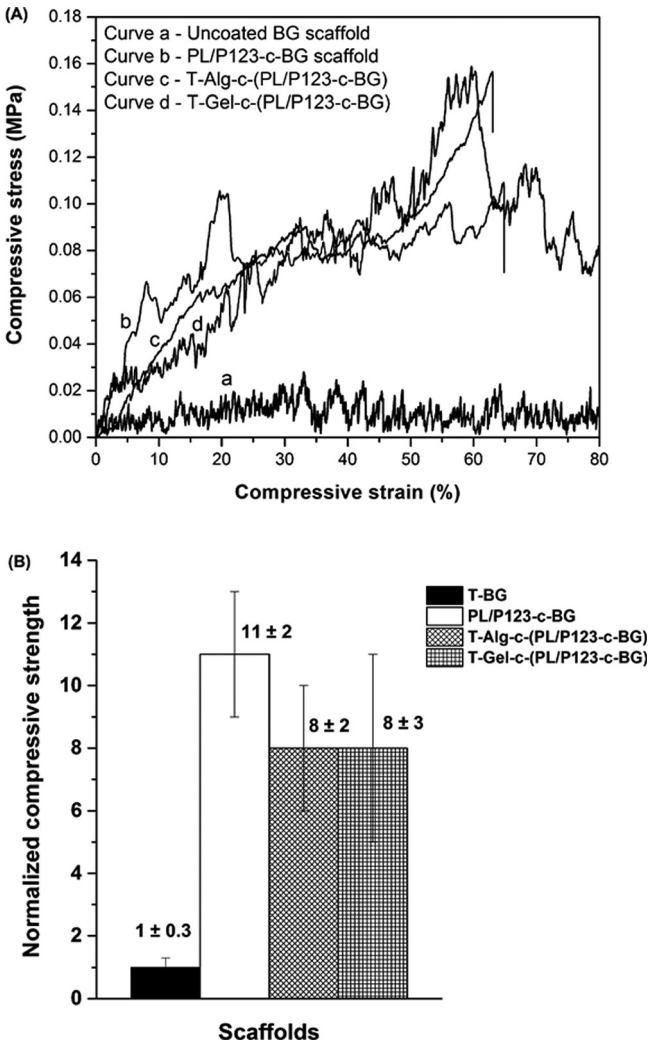

FIG. 4. Mechanical properties of polymer coated Bioglass scaffolds: (a) representative compressive stress–strain curves and (b) normalized compressive strength of the scaffolds.

of the TCH-loaded polymer coatings could be observed in comparison with PDLLA/P123–*c*-BG scaffolds [inset in Fig. 3(A)]. This observation was the same reported in the previous study of Mouriño *et al.*,[46] who found that the surface of Bioglass-based scaffolds similar to the ones used in this study became rougher after coating with a second layer of alginate.[46] This effect is probably caused by polymer agglomeration during drying. In detail, the alginate coating, for example, took longer time to be dried compared to the PDLLA/P123 coating and thus this differential shrinkage is likely responsible for the generation of a marked surface rugosity. This rough surface is believed to be a positive result for improved cell adhesion and proliferation.

C. Mechanical properties

Typical stress–strain curves shown in Fig. 4(a) as well as the normalized compressive strength value in Fig. 4(b) illustrate the improvement of the mechanical properties of Bioglass-based scaffolds by the layered polymer coatings. The stress–strain curves show a jagged shape typical of this type of brittle foams, which has been discussed in previous studies[47] and is related to buckling and localized fracture of the struts with increasing load. It can be noted that the jagged character of the stress–strain curves is reduced for the coated scaffolds. It is also observed that scaffolds coated with PDLLA/P123 exhibited improved compressive strength up to ten times in comparison with the uncoated scaffolds. This result can be ascribed to the formation of a uniform PDLLA/P123 coating on the struts, as well as to the effect of polymer filling of cracks present on the surface of the struts, which will impede catastrophic crack propagation, as discussed in the literature,[32] for example, by a crack bridging mechanism.[48] The mechanical strength of the polymer coated Bioglass-based scaffolds in the present study was higher than that of similar scaffolds reported in previous studies.[21,22] This result can be due to the fact that the Bioglass-based scaffolds in the study of Li *et al.*,[21] for example, were partially coated with polymer, while in the present work, the polymer fully covered the struts. Moreover, the second coating layer, either TCH-loaded alginate or TCH-loaded gelatin, did not further enhance the mechanical strength of the scaffold. As represented in the compressive stress–strain curves of both T-Alg-*c*- and T-Gel-*c*–(PL/P123–*c*-BG) scaffolds [Fig. 4(a)], the curves show similar trend to that of PL/P123–*c*-BG scaffolds. The reason for this result is likely the fact that only a thin layer of alginate or gelatin is formed due to the low polymer concentration used. According to these results, the mechanical properties of the layered polymer coated Bioglass scaffolds are dominated by the first synthetic polymer layer (PDLLA/P123), and this layer led to a significant increase of the compressive strength and of the area under the stress–strain curve in comparison with uncoated scaffolds.

D. Chemical structure

FTIR analysis was performed on coated scaffolds to confirm the presence of the polymer coating and the drug entrapment. First, the spectra of the Bioglass-based scaffolds before (BG) and after drug loading without polymer carrier (T-BG) were considered (Fig. 5). In detail, the spectrum of the T-BG scaffold presents the characteristic peaks of Bioglass, including a double peak at the wavenumber 1100–1040 cm^{-1} attributed to Si-O-Si stretching mode and the peak at the wavenumber 458 cm^{-1} attributed to the Si-O-Si bending mode.[49,50] The characteristic peaks of Bioglass were not changed after loading with TCH, indicating that loaded TCH molecules did not initiate a chemical reaction with Bioglass. This result suggests that the loaded TCH molecules on the Bioglass scaffolds did not loose their activity.

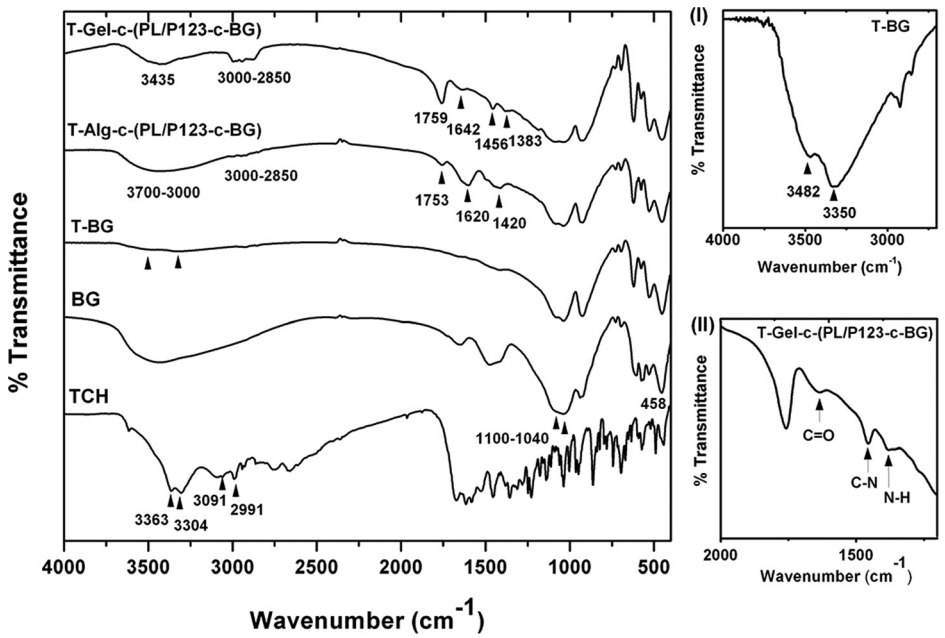

FIG. 5. FTIR spectra of TCH, BG, TCH-loaded Bioglass scaffolds, and TCH-loaded polymer coated Bioglass scaffolds.

Moreover, a double peak at 3482 and 3350 cm^{-1} in the spectrum of T-BG scaffold [see the inset (I) in Fig. 5] can be understood as an overlapping effect between the –OH stretching broad peak of Bioglass (3700–3000 cm^{-1}) and a double peak of TCH (3363 and 3304 cm^{-1}) and –CH stretching of phenol framework in TCH.[11] In contrast, the spectrum of T-Alg-*c*–(PL/P123–*c*-BG) scaffold presents the peaks at 1620 and 1420 cm^{-1} assigned to $–COO^-$ asymmetric and symmetric stretching modes, respectively, confirming the presence of alginate in the coated scaffold. Moreover, the peak at 1753 cm^{-1} observed in the spectrum of T-Alg-*c*–(PL/P123–*c*-BG) scaffold is attributed to –C=O stretching and it is ascribed to the PDLLA coating. As observed also in the spectrum of T-Gel-*c*–(PL/P123–*c*-BG) scaffold, the –C=O stretching (related to PDLLA coating) appears at the wavenumber 1759 cm^{-1}. In addition, the peak at 3435 cm^{-1}, assigned to –NH stretching, confirms the presence of gelatin. Other detectable peaks at 1642 and 1456 cm^{-1}, assigned to –C=O and –C–N stretching, and the peak at 1383 cm^{-1}, assigned to –NH bending, as shown in the inset (II) in Fig. 5, further confirm the existence of gelatin coating. However, the characteristic peaks of TCH in the "finger print" region (1500–500 cm^{-1}) were not obvious in the spectra of the coated scaffolds. Also, changes of the peak position, in either alginate or gelatin, were not observed in any spectra of the coated scaffolds. Therefore, possible molecular interaction between the drug and the polymer coating could not be confirmed based on FTIR results.

E. *In vitro* drug release

1. Release profile

Figure 6(A) shows the cumulative percentage of TCH release from the Bioglass scaffolds for up to 14 days of immersion in PBS. T-BG scaffolds showed an initial burst release of 53% at 1 h, which increased to 99% in 4 h. Even if the absolute amount of drug incorporated was not determined in this study, this result confirms the low drug binding affinity of the uncoated Bioglass scaffolds. In contrast, in polymer coated scaffolds, lower initial burst release values (1 h) at 27% and 22% for alginate and gelatin coatings, respectively, were measured. At longer release periods, both TCH-loaded polymer coated scaffolds provided almost complete drug release, i.e., ~99% over 14 days, in a sustained manner. This drug release kinetic is favorable as it should not only facilitate an effective initial antibacterial effect but also promote long term protection against infection. Both alginate and gelatin carriers provided a similar release profile, including (1) an initial burst release as a result of the release of the free drug molecules present on the surface and (2) a further relatively slow release induced by the drug molecules "protected" by the polymer coating. As described in the literature,[6,7,51] drug molecules embedded in polymers can diffuse through available pathways, i.e., pores and channels, into the medium. Diffusion pathways can be influenced by the presence of an inhomogeneous coating accompanied by the intrinsic degradation of the coating. Considering the result of the degradation study [Fig. 6(b)], gelatin coated scaffolds were seen to exhibit slightly faster degradation rate compared to alginate coated scaffolds. This result can be explained by the fact that the alginate coating might partly crosslink with calcium ions in PBS,[52] which should lead to slower degradation of the alginate coating. Also, the initial burst release measured on T-Alg-*c*–(PL/P123–*c*-BG) scaffolds was slightly lower than that of T-Gel-*c*–(PL/P123–*c*-BG) scaffolds. In addition, the possible interaction of the negatively charged drug (TCH) and the positively charged polymer (gelatin) was not observed in this study, and a superior binding affinity of TCH and gelatin cannot be confirmed by the results of drug release. Therefore, it seems that the key factors influencing the release of the drug from the natural polymer coated Bioglass-based scaffolds are mainly related to coating homogeneity and dissolution/degradation kinetics of the coating. Compared to previous recent studies,[21,22] the initial burst release of the TCH-loaded alginate coating (22%) in the present study was significantly lower,

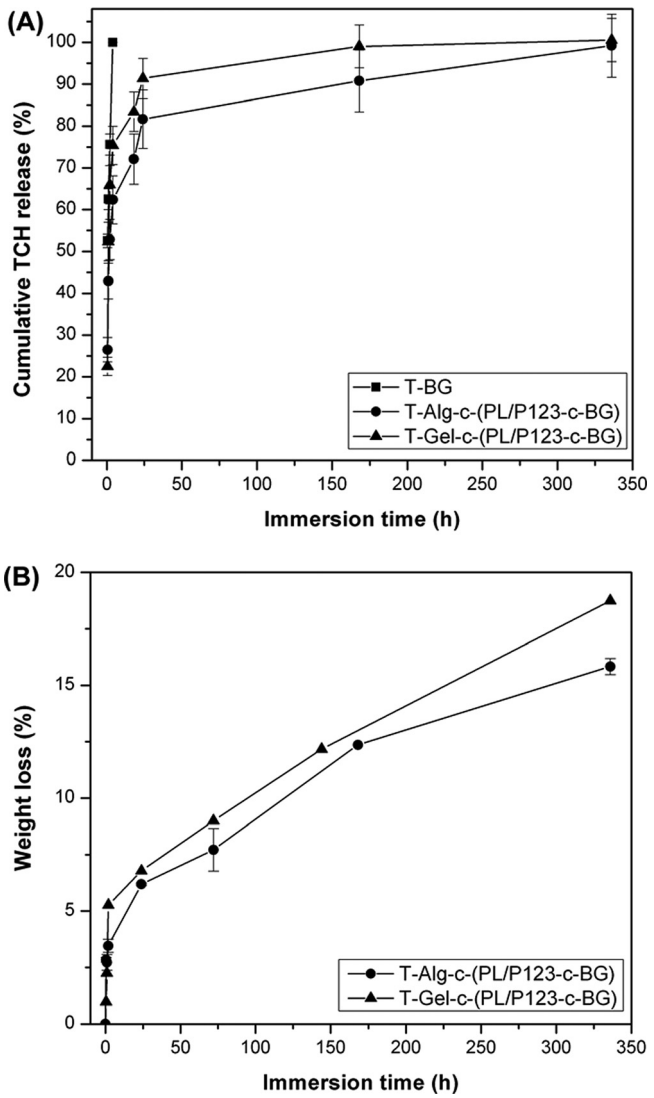

FIG. 6. (a) Drug release profile and (b) degradation behavior of TCH-loaded polymer (alginate and gelatin) coated Bioglass scaffolds.

e.g., it was 63% in vancomycin-loaded chitosan coating[22] and 33% in vancomycin-loaded PHBV coating.[21] However, the different drug used in the present study should be taken into consideration. On the other hand, TCH-loaded PCL/HA coated HA scaffolds have released, at the initial stage (1 h), 44% of the load,[16] indicating that the coating developed in the present study enables better release control reducing the initial burst release. In addition, more reduced initial burst release and slower release rate were presented by Meng *et al.*[36] who investigated TCH-loaded P(3HB) microspheres coated on Bioglass-based scaffolds. This effective controlled release was suggested to be the consequence of an efficient drug encapsulation in microspheres compared to polymer coatings as presented in this study. In addition, water-insoluble polymers like P(3HB) used as a drug carrier[36] exhibit slower degradation rate in aqueous solutions compared to water-soluble polymer-based carriers, i.e., alginate and gelatin. Also, investigation by immersion in SBF solution has shown HA formation onto the surface of scaffolds, and it was reported that HA formation inhibited the diffusion of the drug consequently decreasing drug release.[36] In fact, the drug-loaded bilayer polymer coated scaffolds developed in this study by using a dipping method are more simple to fabricate compared to drug-loaded microsphere or microgel coated scaffolds, while they similarly exhibited a controlled drug release capacity.

The present approach using natural polymers such as alginate and gelatin as drug carrier seems to lead to convenient performance of the scaffold as drug delivery device in terms of water soluble drug entrapment and protection of the drug. Another important feature of the present approach is that it is possible to decouple the mechanical stability function of the coating (provided by the synthetic polymer) from the drug release function of the coating (provided by the natural polymer).

2. Morphology after drug release

SEM analysis was used to observe the morphological change of the scaffolds after 14 days of drug release (Fig. 7). The polymer coating was partly maintained on both scaffolds. In Fig. 7(a), it can be observed that the surface of the T-Alg-*c*–(PL/P123–*c*-BG) scaffold became rougher, while pores and channels were generated, indicating the dissolution of the polymer coating. The appearance of a smoother surface underneath can also be observed, which is probably the PDLLA/P123 coating, as depicted by the solid arrows in Fig. 7(a). Similarly, the SEM image of the T-Gel-*c*–(PL/P123–*c*-BG) scaffold in Fig. 7(b) shows the generation of cavities on the coating surface. In contrary to the T-Alg-*c*–(PL/P123–*c*-BG) scaffold, the residual polymeric coating (depicted by a solid arrow) was the PDLLA/P123 layer, while the outer drug-loaded gelatin coating could not be distinguished. It is likely that the gelatin coating fully decomposed after 14 days of immersion in PBS. In addition, the PDLLA/P123 coating partly degraded and the surface of the Bioglass strut can also be observed [dashed arrow in Fig. 7(b)]. The release of TCH-loaded alginate and gelatin carriers, which is predominantly influenced by the degradation of the polymer coating, is thus confirmed. Moreover, it

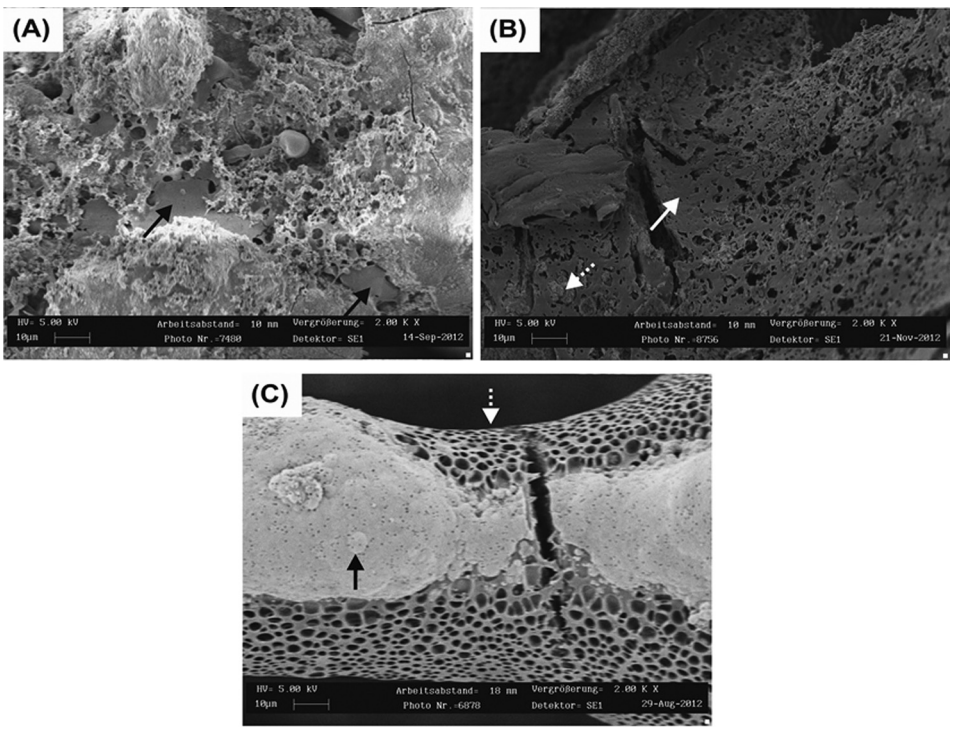

FIG. 7. SEM images of TCH loaded Bioglass scaffolds after *in vitro* release in PBS for 14 days: (a) T-Alg-*c*-(PL/P123-*c*-BG); the arrows predicting the PL/P123 coating and (b) T-Gel-*c*-(PL/P123-*c*-BG) scaffolds; dashed arrow depicting the Bioglass struts and solid arrow line predicting PL/P123 coating, and SEM image of (c) pure PDLLA-*c*-BG scaffolds after immersion in SBF for 3 days.

seems that the bioactivity of Bioglass was not significantly inhibited by the presence of the layered polymer coating, since the amorphous PDLLA coating exhibited partial degradation during immersion in PBS, as confirmed by the cavities observed in Figs. 7(a) and 7(b), which enables direct contact of the Bioglass surface with the medium. This phenomenon is expected to lead to formation of HA on the strut surfaces when the scaffold is immersed in SBF. As shown in Fig. 7(c), for example, formation of HA was observed in the case of pure PDLLA-*c*-BG scaffolds after 3 days of immersion in SBF (as depicted by a solid arrow), while the degradation of the PDLLA coating took place (as depicted by a dashed arrow). It can be suggested that in the present drug-loaded polymer coated scaffolds, HA formation is possible by the degradation of the polymer coating during immersion in SBF. However, this behavior should be confirmed further by changing the medium from PBS to SBF in order to study the kinetics of HA formation on TCH-loaded layered polymer coated scaffolds. It should be highlighted that the complex degradation mechanism of the scaffold, composed of three phases degrading at different rates, and the formation of HA on the surfaces, make it difficult to establish a quantitative correlation between weight loss of the scaffold and drug release kinetics.

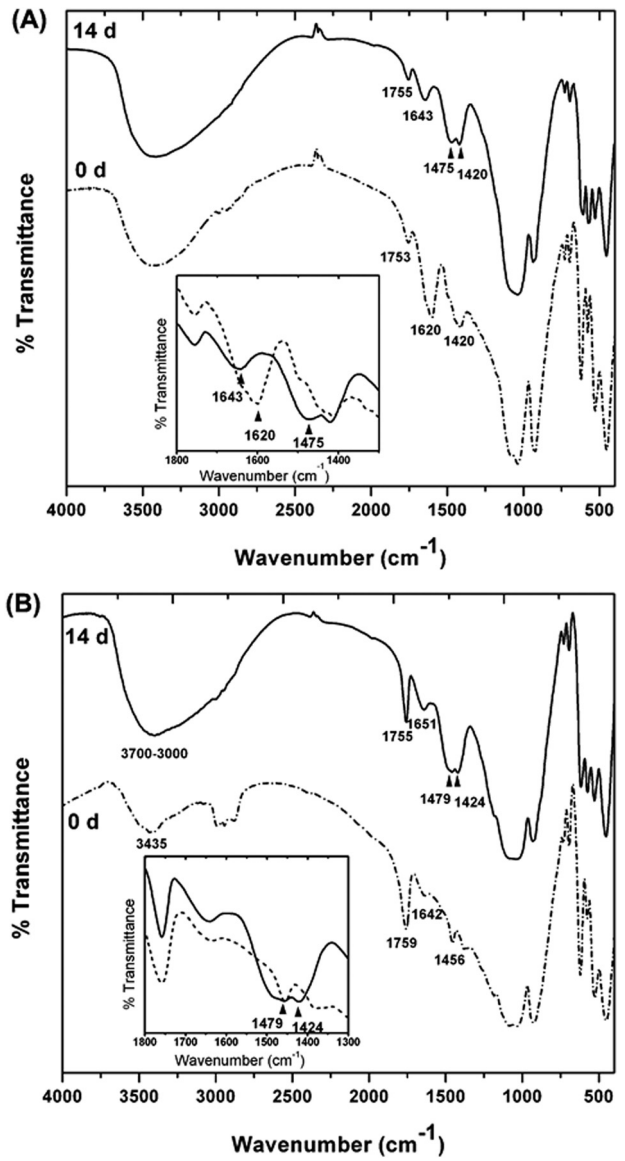

FIG. 8. FTIR spectra of T-Alg- and T-Gel-*c*-(PL/P123-*c*-BG) scaffolds after 14 days of immersion in PBS.

3. Chemical structure after drug release

The FTIR spectra shown in Fig. 8 enable to detect the chemical changes of the scaffold surfaces after 14 days of immersion in PBS. First, a new peak at wavenumber 1475 cm^{-1} was observed in the spectra of T-Alg-*c*–(PL/P123–*c*-BG) scaffold after immersion in PBS [see Fig. 8(a)]. The broad double peak can be assigned to the overlapping of $–COO^-$ stretching band of alginate with $–CH_2–$ bending band of PDLLA.[53] Moreover, the peak at 1620 cm^{-1}, assigned to the stretching vibration of $–COO^-$ in alginate, was lower in intensity suggesting that the alginate content is reduced after immersion. Moreover, this peak was shifted to 1643 cm^{-1}, which is possibly due to protonation of carboxylate groups.[54] Therefore, these results confirm that the alginate coating remains on the scaffold after 14 days of immersion in PBS. The FTIR spectrum of the T-Gel-*c*–(PL/P123–*c*-BG) scaffold is reported in Fig. 8(b). The double peak at wavenumber 1479 and 1424 cm^{-1} appeared after immersion. Similarly to the T-Alg-*c*–(PL/P123–*c*-BG) coated scaffold discussed above, the absorption bands corresponding to PDLLA seem stronger in the spectrum of the scaffold after immersion, which is the result of degradation of the gelatin coating. The degradation of the gelatin coating is evidenced by the broadening of the peak of –NH stretching (3700–3000 cm^{-1}).

IV. CONCLUSIONS

Multifunctional layered polymer coated Bioglass-based scaffolds with drug delivery capability were fabricated by coating Bioglass foams with two different polymer coatings, namely, PDLLA/P123 blend and alginate or gelatin. The scaffolds exhibited improved mechanical properties and superior drug delivery function in PBS characterized by a relatively low initial burst release and subsequent controlled drug release. Even if the absolute concentration of drug contained in the scaffolds was not measured, both alginate and gelatin were confirmed as suitable drug carrier, and they did not show significantly different performances in their degradation and release behaviors. The multifunctional scaffolds fabricated, exhibiting improved mechanical properties and controlled drug release, coupled with the high bioactivity characteristic of Bioglass, belong to a growing family of advanced composite scaffolds for bone tissue engineering. Further studies should consider the drug release kinetics of the scaffolds in realistic biological conditions in which the surface reactivity of the bioactive glass surface, in particular, the formation of a HA crystalline surface layer, may further affect the drug release kinetics.

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