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Mechanical strength and biocompatibility of bredigite ($Ca_7MgSi_4O_{16}$) tissue-engineering scaffolds modified by aliphatic polyester coatings

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Abstract

Bredigite ($Ca_7MgSi_4O_{16}$) is a bioceramic with excellent bioactivity and bioresorbability; nonetheless, its inadequate mechanical strength and biocompatibility limit its tissue-engineering application. In this research, interconnected porous bredigite scaffolds were fabricated by sol-gel, sacrificial sponge replica and sintering processes for bone tissue engineering. In order to improve their strength and cytocompatibility, the scaffolds were coated with poly(lactic-co-glycolic acid) (PLGA) via immersion in acetone-based solutions containing different concentrations (5, 10 and 15% w/v) of the polymer. Based on the results, the PLGA coatings to 10% do not suppress the porosity characteristics of the scaffolds appropriate for tissue engineering. It was also found that the polymeric coatings significantly enhance the compressive strength of the ceramic scaffolds, where this alteration is improved by increasing the PLGA concentration of the coating solution. In addition, the viability of stem cells on the bredigite scaffolds are improved by using the PLGA coatings, with the optimal concentration of 10% PLGA according to MTT and cell attachment studies.

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***Keywords*:** Sol–gel processes (A); Silicate (D); Mechanical properties (C); Biomedical applications (E)

1. Introduction

Osteoporosis, trauma, osteomyelitis, grafting donor are the most common reasons of the damage and loss of bone in the human body. Autografting is a prevalent method to treat bone tissue injuries, albeit it comes with several well-known disadvantages; being a painful procedure and having the increased risk of medical complications like infection and inflammation due to the necessary additional surgery. Also, allograft and xenograft tissues is commonly employed, but they have serious shortcomings, including the risk of diseases being transferred from the tissue donor and a slew of other immunological issues like host tissue rejection and negative responses [1-3]. Bone tissue-engineering scaffolds are synthetic grafting materials offering an alternative to autografts, allografts and xenografts, lacking their aforementioned disadvantages. These scaffolds are typically made of porous resorbable biomaterials, acting as temporary place holders and providing mechanical support during the regeneration of the damages or diseased bone [4]. High interconnected porous structures, high porosity percentage, bioactivity, bioresorbability, biocompatibility and compressive strength are key parameters for ideal bone tissue-engineering scaffolds [5, 6].

Ca-Mg silicate ceramics, such as bredigite ($Ca_7Mg(SiO_4)_4$), akermanite ($Ca_2MgSi_2O_7$), diopside ($CaMgSi_2O_6$) and merwinite ($Ca_3Mg(SiO_4)_2$), are regarded as a new class of bioactive and resorbable inorganic biomaterials, with higher mechanical properties in comparison to other commonly used ceramics such as bioglasses and calcium phosphates

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(apatites) [7, 8]. In addition, these biomaterials can promote the proliferation and differentiation of human cells due to the release of Ca^{2+}, Mg^{2+} and Si^{2+} during bioresorption [9]. Bredigite is a member of calcium-magnesium silicates with an orthorhombic structure. Bredigite exhibits excellent bioresorbability and bioactivity compared to diopside and akermanite, due to the higher content of Ca ions. Wu et al. [8] synthesized bredigite powders by a sol-gel method and thereafter fabricated porous scaffolds via a polymer sponge method. The interconnected porosity size of the obtained bredigite scaffolds was in the range of 300–500 μm. Typically, the bredigite scaffolds exhibited higher compressive strength than tricalcium phosphate with values of 233 and 50 kPa, respectively. Also, this material has showed better biological performance and bone regeneration in comparison to simple calcium silicates, due to the incorporation of magnesium into its crystal structure [7, 10]. Nevertheless, there are two major drawbacks for the application of bredigite scaffolds in bone tissue engineering. One is the high resorption rate of this material which leads to a high concentration of Ca^{2+}, metabolic alkalosis and thereby an undesirable cellular response [11]. The second disadvantage of this material, similar to all bioceramics, is mechanical strength which is not enough for bone tissue engineering [12].

In this work, bredigite scaffolds are fabricated by sol-gel and sacrificial sponge replica methods and coated with poly(lactic-co-glycolic acid) (PLGA). Our hypothesis is that PLGA coating improves the aforementioned drawbacks of bredigite scaffolds, i.e. mechanical strength and physiological alkalosis. This hypothesis arose from the fact that PLGA coating has been frequently reported to increase the compressive strength of some other scaffolds and release acidic products during degradation [13, 14]. Due to its good biodegradability and nontoxicity [15-17], PLGA has been extensively used in biomedical applications. The

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degradation rate of PLGA depends on its lactic/glycolic acid ratio (LA/GA), molecular weight and end-group functionalization [18, 19]. In this study, PLGA 5004A with LA/GA = 50:50, the intrinsic viscosity of 0.4 dl/g, acid-terminated, M_w = 44 kDa, acid number: min 3 mg KOH/g) was selected because:

i) Among the different types of PLGA used commonly, the LA/GA ratio of 50:50 provides the highest degradation rate due to the higher degradation tendency of more hydrophilic glycolic acid component [20].

ii) The end-group functionalization of PLGA is an important parameter influencing the pH value of the biological environment and the degradation rate of the polymer. Due to the higher acid number of PLGA 5004A (acid-terminated, acid number: min 3 mg KOH/g) in comparison with PLGA 5004 (end-capped: max 1 mg KOH/g), its degradation provides higher acidity, which is hypothesised to buffer the alkalosis of bredigite bioresorption. Also, the degradation rate of acid-terminated PLGA is faster than end-capped one because a free carboxylic group at the end of PLGA leads to higher hydrophilicity [24, 27, 28]; thus, coating with this PLGA variant cannot considerably suppress the excellent bioresorption rate of bredigite scaffolds.

iii) When the intrinsic viscosity of the polymer is high, it does not provide a uniform coating on the pore walls of the scaffold, which leads to the blockage of some pores [13]. Hence, a variant of PLGA with the intrinsic viscosity of 0.4 dl/g was selected in this work.

2. Experimental procedures

2.1. Preparation of powders

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Bredigite powders were prepared by a sol-gel process [8, 21], using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS, Merck, Germany, Purity>98%), magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$, Merck, Germany, Purity>98%) and calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$, Merck, Germany, Purity>98%) as raw materials. TEOS was first mixed with water and 2 M HNO_3, at the molar ratio of TEOS/H_2O/HNO_3 = 1:8:0.16, and hydrolyzed for 30 min under stirring. Then, $Mg(NO_3)_2.6H_2O$ and $Ca(NO_3)_2.4H_2O$ were added into the solution at the molar ratio of TEOS/$Mg(NO_3)_2.6H_2O$/$Ca(NO_3)_2.4H_2O$ = 4:1:7 and stirred for 5 h at room temperature. Afterwards, the solution was maintained at 60 °C for 1 day and dried at 120 °C for 2 days. The obtained xerogel was then calcined at 700 °C for 3 h at a heating rate of 3 °C/min.

2.2. Fabrication of scaffolds

Bredigite scaffolds were fabricated by a sacrificial sponge replica method, based on the procedure of Ref. [8, 22, 23] with a modification of employing sodium alginate instead of polyvinyl alcohol. For this purpose, the calcined powders were suspended in an aqueous solution of sodium alginate (3 wt%) to obtain a slurry with the powder-to-solution mass ratio of 1:3. Polyurethane foam templates (density: 25 ppi, porosity >97%) were cut, immersed in the slurry and compressed to force the slurry to migrate into the pores of the foams. Then, the impregnated foams were dried at 60 °C for 12 h, heated at 300 °C for 1 h to remove the polymeric struts and then sintered at 1350 °C for 3 h at a heating rate of 3 °C/min.

For PLGA coating of the scaffolds, 5, 10 and 15% w/v of PLGA (5004A, Corbion, Netherlands) were dissolved in acetone at room temperature. The sintered scaffolds were dipped into the PLGA solutions under air and vacuum, and thereafter rotated for 5 sec under

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centrifugation at 300 rpm to remove the excess solution. Finally, the PLGA-coated samples were dried at 60 °C for 2 h to remove the solvent.

2.3. Structural characterization

X-ray diffraction (XRD, PANalytical Company X'Pert PRO MPD, CuKα radiation at 40 kV and 40 mA) was used to characterize the phase composition of the samples. The average crystallite size of the sintered bredigite scaffold was also determined from the broadening of XRD peaks using the Scherrer equation [24]:

$$\tau = \frac{K\,\lambda}{\beta\,cos\theta} \quad (1)$$

where τ is crystallite size in nm, K is the shape factor (around 0.9), λ is the wavelength of radiated X-ray (0.154 nm), β is the full width at half maximum of diffraction peak (FWHM) in radians, and θ is the angle of diffraction. In this study, the highest diffraction peak of bredigite was selected to determine the crystallite size. Fourier transform infrared spectroscopy (FTIR, Thermo Nicolet, Avatar, USA) was also performed in a spectral range of 2000-400 cm^{-1}. In addition, the morphology of the prepared powders and scaffolds was investigated by field emission scanning electron microscopy (FESEM, MIRA3 TESCAN, Czech Republic).

2.4. Porosity measurements

The porosity level of the scaffolds was determined according to the following formula by the water Archimedes method [25]:

$$\text{Porosity Percentage} = [(W_w - W_d)/(W_w - W_s)] \times 100 \quad (2)$$

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where W_w is the weight of the scaffold after removing from water, W_d is the weight of the scaffold in air, and W_s is the weight of the suspended scaffold.

2.5. Mechanical testing

The compressive strength of the scaffolds (~15×7.5×7.5 mm^3) was measured by using a universal mechanical machine (SANTAM STM-1) at a crosshead speed of 0.5 mm/min. At least three samples were tested, and the mean value of strength was determined.

2.6. Physiological pH analyses

To investigate the influence of the degradation of the scaffolds on physiological pH, the scaffolds were immersed in the simulated body fluid (SBF) at the scaffolds-to-SBF ratio of 1/200 g/ml. The pH values of the SBF were monitored every day using an electrolyte type pH meter (PHS-2C, Jingke Leici Co., Shanghai, China) for one week. Three samples from each group were tested to obtain average pH values.

2.7. Biocompatibility assessments

The fabricated scaffolds of 3×3×3 mm^3 in size were immersed in ethanol, rinsed with phosphate buffered saline (PBS) and then exposed to UV radiation for sterilization. 3,500 dental pulp stem cells were seeded onto the sterilized scaffolds with three repetitions. The culture medium containing cells without any scaffolds was also considered as the control. The plate was then incubated for 1, 3 and 7 days under a humidified atmosphere of 5% CO_2 in air. The MTT assay was performed to determine the cell viability on the scaffolds, in accordance with the protocol described previously [23]. To do so, the optical density of viable cells at the

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wavelength of 545 nm was measured by a microplate reader (ChroMate-4300, FL, USA). The one-way analysis of variance with a statistically significance level less than 5% ($p < 0.05$) was used to compare the cytocompatibility data. The cell attachment on the optimal sample was also observed after fixing in a glutaraldehyde solution and washing by PBS and ethanol.

3. Results and Discussion

3.1. Structural characterization

The XRD pattern of the sintered bredigite scaffold is shown in Fig 1. The detection of bredigite peaks without any impurity phases indicates the process merit for the synthesis of this bioceramic. The mean crystallite size of bredigite was also measured to be almost 40 nm, based on the Scherrer equation (Eq. 1).

Fig. 2 presents the FESEM micrographs of the powder calcined at 700 °C, i.e. the substance which was used to deposit on the sacrificial urethane foams, and a strut of the bredigite scaffold sintered at 1350 °C. According to the micrographs, calcination at 700 °C develops nanoparticles of about 25 nm in diameter with a high level of agglomeration due to the high surface energy of the nanospecies. In contrast, microparticles of irregular shapes with considerable sintering necks are obtained after sintering at 1350 °C, suggesting the proper sinterability of the nanoparticles during the used densification process.

The FTIR spectra of the bare and 15% PLGA-coated bredigite scaffolds in the spectral range of 2000-400 cm^{-1} are depicted in Fig. 3. For the bare sample, the peaks of 516 and 553 cm^{-1} are attributed to the bending and stretching vibrational modes of O–Mg–O and Ca–O bonds, respectively. The bending Si–O–Si band of the SiO_4 tetrahedron is clearly recognizable at 616 cm^{-1}, and vibrational peaks of 1000-1100, 960 and 873 cm^{-1} are also

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assigned to the stretching mode of Si–O. It would be worth mentioning that all of the detected FTIR peaks are in good agreement with the functional groups of bredigite [26-28], confirming the formation of bredigite which was realized by the XRD phase analysis. Regarding the PLGA-coated sample, two peaks of 1089 and 1130 cm^{-1} correspond to the stretching vibration of the C-O bonding. The stretching mode of C-O-C is also detected at 1168 cm^{-1}, which is attributed to the end groups of the polymer chains. The broad peak of 1618 cm^{-1} and the strong peak of 1752 cm^{-1} are also related to the stretching mode of C=O in the ester bond. Additionally, two sharp peaks of 1393 and 1427 cm^{-1} are assigned to the bending mode of C-H, whereas the peak at 1454 cm^{-1} corresponds to the stretching mode of C-H. Apart from the bredigite vibrations, the other peaks assigned for the coated scaffold are compatible with the functional groups of PLGA [29-32], verifying the successful deposition of PLGA on the bredigite scaffold.

Fig. 4 represents the FESEM micrographs of the bredigite and bredigite-PLGA scaffolds. As can be seen, the bredigite scaffold displays an interconnected porous structure which is a desired feature for bone tissue engineering. After coating with 5 and 10% PLGA, the scaffolds still exhibit highly interconnected porous networks. Nevertheless, when the concentration of PLGA in acetone increases to 15%, the deposited PLGA coating blocks some of the pores of the bredigite scaffold. Disadvantageously, this feature of scaffolds can disturb the cellular penetration and the adequate diffusion of nutrients to cells required for tissue engineering by porous scaffolds [3]. In addition, as can be seen in the high-magnification micrographs, the smoothness of the strut surfaces increases by PLGA coating and increasing the PLGA concentration. Typically, 5% PLGA-acetone solution is not able to completely fill micropores, thereby creating a roughness coating on the surface. However, 10

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and 15% PLGA-acetone solutions are able to fill the micro-pores entirely. Also, the increase of the coating and struts thickness by increasing the PLGA concentration reduces the size and volume percentage of pores (Table 1). Typically, the deposition of the 10% PLGA solution on the bredigite scaffold develops a coating thickness of about 16 μm with a uniform coverage and dense feature without any gap with the bredigite substrate (Fig. 5).

3.2. Mechanical analyses

Fig. 6 shows the effect of the vacuuming process during the PLGA deposition on the compressive strength of the scaffolds coated with the 10% PLGA-acetone solution. It was measured from the stress-strain curves that the strength of the sample prepared under vacuum is 220% higher than that of the sample prepared under air. Indeed, the surface tension of the interfaces does not allow the complete penetration of the PLGA-acetone solution into the air-entrapped pores of the bredigite struts. This phenomenon is more drastic for the higher concentration of the polymers due to the increase in the solution viscosity. In this regard, the vacuuming process removes air from the pores and alters the surface tension of the interfaces, thereby overcoming this drawback somewhat. Thus, this process was used for all of the PLGA-coated samples to benefit from the optimal polymer penetration and scaffold strength.

The compressive stress-strain curves of the bare and PLGA-coated bredigite scaffolds are revealed in Fig. 7. The extracted mean value of the strength of the samples is also listed in Table 1. The compressive strength of the bredigite scaffold is around 0.31 MPa. After coating with 5, 10 and 15% PLGA, the strength increases by almost 290, 461 and 780%, respectively. Similar results have been also found by Lang Zhao et al. [13] on calcium silicate scaffolds with the porosity level of 67.8% and compressive strength of 1 MPa. After coating by PLGA,

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the compressive strength increased to about 320%, and the porosity decreased to around 5.5%. Nonetheless, O'Shea et al. [33] prepared PLGA-coated bioactive glass-ceramic scaffolds. After coating, the porosity level decreased to only 2% and the compressive strength increased from 0.12 MPa to 0.25 MPa, which are very lower values than the PLGA-coated bredigite scaffolds fabricated in this work. This difference is attributed to the higher inherent strength of silicates like bredigite in comparison to other conventional ceramics like apatites, bioglasses and glass-ceramics [26, 34, 35]. There are three reasons responsible for the enhancement of the compressive strength of the scaffolds with the polymer coatings:

1) The struts of the bredigite scaffold, according to the high-magnification FESEM micrograph (Fig. 4b), are characterized by a high level of pores which act as preferred locations for stress concentration and crack initiation during mechanical loading. According to Figs. 4d, 4f and 4h, PLGA fills the pores and therefore modifies the brittleness of the bredigite struts.
2) According to the Archimedes densitometry (Table 1), the employment of the polymer coatings enhances the density of the bredigite-based scaffolds. The direct relation of the density and mechanical strength of porous structures is evident.
3) PLGA inherently has a certain amount of strength and toughness and can create an intertexture inside the bredigite scaffold. This leads to a linkage in the inorganic phase and subsequently to the increase of the scaffold strength.

It is noticeable that the compressive strength of cancellous bone is in the range of 1-12 MPa [36]. That is, the PLGA-coated bredigite scaffolds fabricated in this work have satisfactory strength levels for bone tissue-engineering applications. It would be worth mentioning that bone ingrowth into porous scaffolds further enhances the compressive

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strength of the scaffolds during implantation. In this regard, Tamai et al. [37] reported that the compressive strength of interconnected porous hydroxyapatite scaffolds increases with time by about 200% after 9 weeks of implantation.

3.3. Physiological pH variations

The pH value of the SBF in contact with the scaffolds is demonstrated in Fig. 8. As can be observed, pH for the bredigite scaffold rapidly increases with soaking time. The low concentration of silicon and bridging oxygens in bredigite ($Ca_7MgSi_4O_{16}$) is responsible for its high bioresorption rate. Accordingly, the local concentration of cations, particularly Ca^{2+}, in the surrounding biological environment is enhanced; instead, the adsorption of hydrogen ion (H^+) increases pH (ion exchange mechanism). On the contrary, the PLGA coating limits the increase of pH caused by the bioceramic resorption, where pH is reduced by increasing the PLGA concentration of the coating solution. This is due to, on the one hand, the reduction of the release rate of the cations from the bredigite substrate and, on the other hand, the acidic character of PLGA hydrolysis products, i.e. glycolic and lactic acids. This confirms our hypothesis that the PLGA coating can effectively buffer the physiological pH of the bredigite scaffolds, which is a critical parameter for biocompatibility.

3.4. Biocompatibility

Fig. 9 illustrates the MTT results of the cell culture on the scaffolds with the significance level of less than 5% ($p < 0.05$). The increase of the optical density values, equivalent to the number of viable cells, with the culture time is a demonstration of cell proliferation on the bare and coated scaffolds. For all of the culture periods, the cell viability

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of the bare sample is lower than that of the control. The comparatively low cell viability on the bare sample is attributed to the detrimental effects of high calcium release [38, 39] and metabolic alkalosis (Fig. 8), both due to the high bioresorption rate of bredigite. The application of the PLGA coatings and the increase of the PLGA concentration considerably improve the cytocompatibility of the scaffolds for the first and third days of culture, so that the scaffolds coated with the high concentrations of PLGA exhibit higher cell viability levels even than the control. The improvement in cytocompatibility with the employment of the PLGA coatings is explained by the limited resorption rate of bredigite and the buffering effect of the polymer degradation (Fig. 8). Nevertheless, at the 7^{th} day of culture, the highest cell viability is obtained for the bredigite-10% PLGA scaffold, and in contrast to the other culture periods, the cytocompatibility level of the bredigite-15% PLGA scaffold is reduced to that of the control and bredigite-5% PLGA scaffold. The relatively low pore interconnectivity (Fig. 4g) and percentage (Table 1) of the bredigite-15% PLGA scaffold impede the transport of nutrients to cells and thus deteriorate the cell viability, which is in accordance with the literature [40, 41].

It is eventually concluded that the bredigite-10% PLGA scaffold is the optimal sample of this research for bone tissue-engineering applications, benefiting simultaneously from suitable pore interconnectivity, mechanical strength and cytocompatibility. This is in good agreement with the broad morphology of cells with the high level of cytoplasmic extensions on the bredigite-10% PLGA scaffold (Fig. 10).

4. Conclusions

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In this work, porous sol-gel derived bredigite scaffolds with the porosity amount of about 90% and the mean pore size of almost 700-900 μm were prepared using the polymer sponge process. After coating the bredigite scaffolds by the different concentrations (5, 10 and 15% w/v) of PLGA-acetone, the porosity of the samples decreased for almost 8.0, 12.8 and 18.4%, and their mechanical strength increased for about 290, 461 and 780%, respectively. In contrast to the 5 and 10% PLGA coatings, some micropores of the scaffolds were disadvantageously blocked with the 15% PLGA coating. It was also realized that by the PLGA coating and increasing the PLGA concentration, the detrimental enhancement of physiological pH and extreme calcium release caused by the fast resorption of bredigite is restricted. This led to the improvement of cytocompatibility with respect to dental pulp stem cells. Typically, the bredigite-10% PLGA scaffold was concluded as the optimal candidate for bone tissue-engineering applications from the structural, mechanical and biocompatibility viewpoints.

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Figures

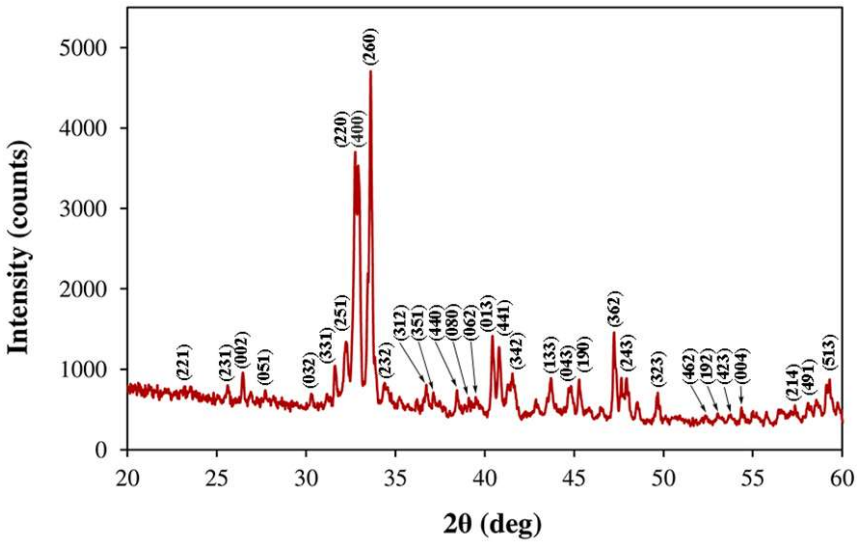

Fig. 1. XRD pattern of the scaffold sintered at 1350 °C. All peaks belong to the bredigite phase.

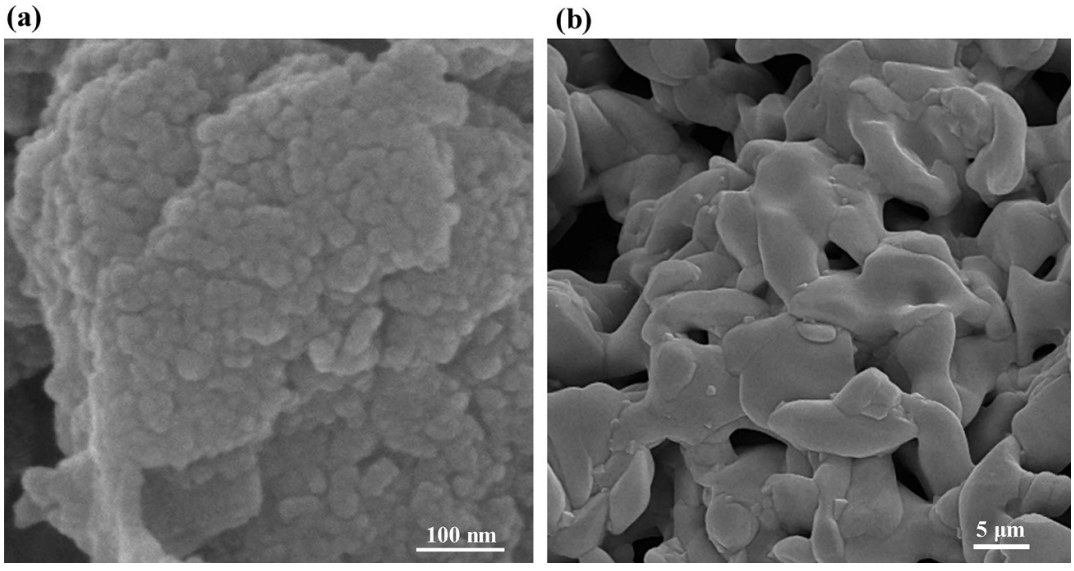

Fig. 2. FESEM micrograph of the sol-gel derived powder calcined at 700 °C (a) and bredigite scaffold sintered at 1350 °C (b).

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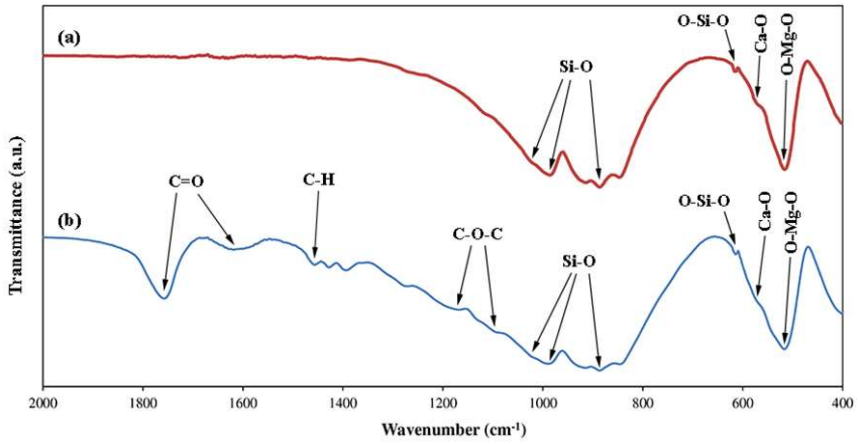

Fig. 3. FTIR spectra of the bare (a) and PLGA-coated (b) bredigite scaffolds.

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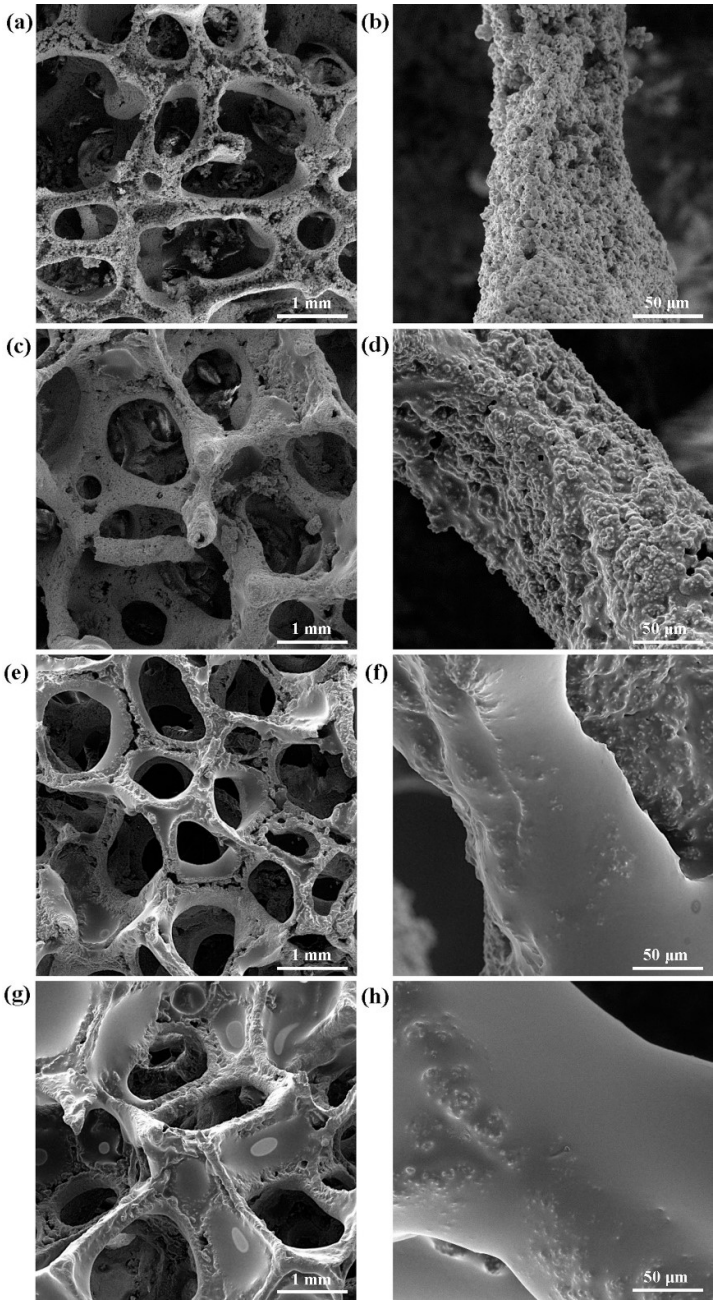

Fig. 4. FESEM micrographs of the bredigite (a, b), bredigite-5% PLGA (c, d), bredigite-10% PLGA (e, f) and bredigite-15% PLGA (g, h) scaffolds in two magnifications.

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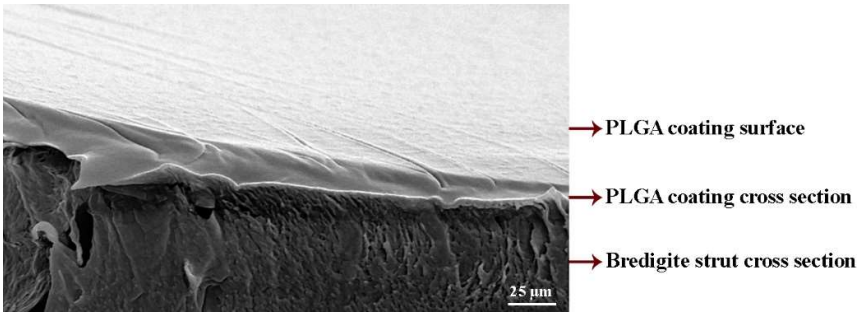

Fig. 5. Cross-sectional FESEM micrograph of the 15% PLGA layer on the bredigite scaffold.

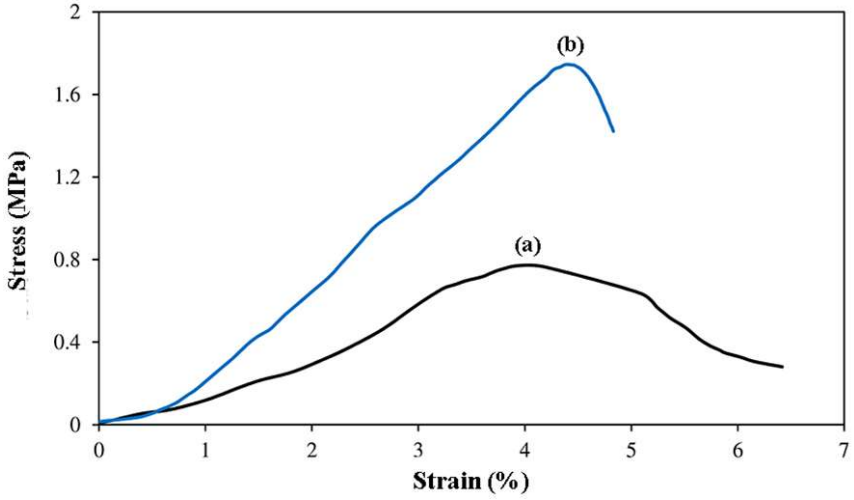

Fig. 6. Compressive stress-strain curves of the bredigite-10% PLGA scaffolds prepared under air (a) and vacuum (b).

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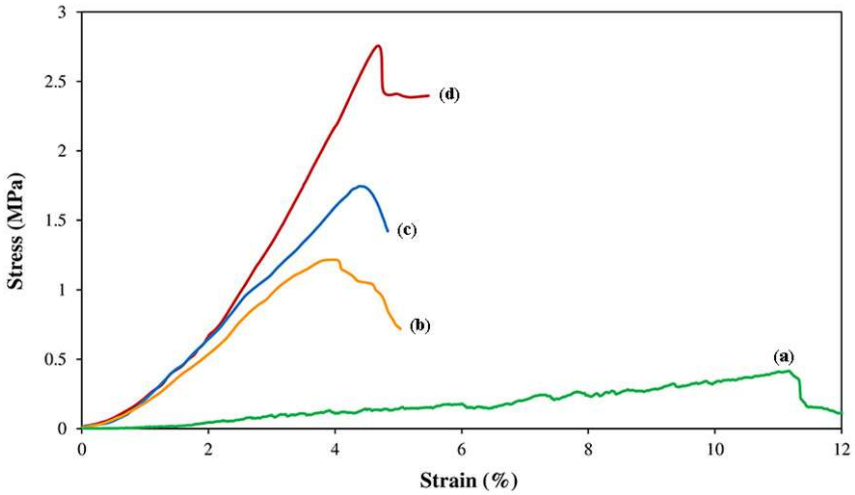

Fig. 7. Compressive stress-strain curves of the bredigite (a), bredigite-5% PLGA (b), bredigite-10% PLGA (c) and bredigite-15% PLGA (d) scaffolds.

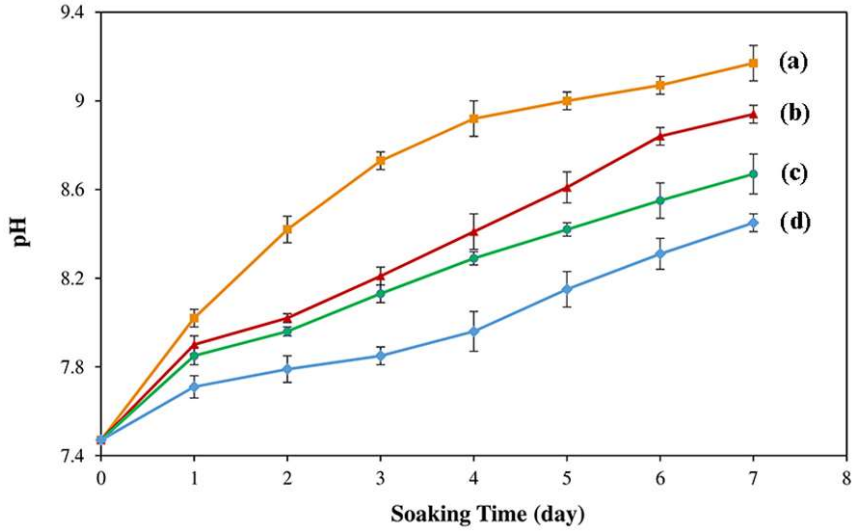

Fig. 8. pH variations of the SBF in contact with the bredigite (a), bredigite-5% PLGA (b), bredigite-10% PLGA (c) and bredigite-15% PLGA (d) scaffolds.

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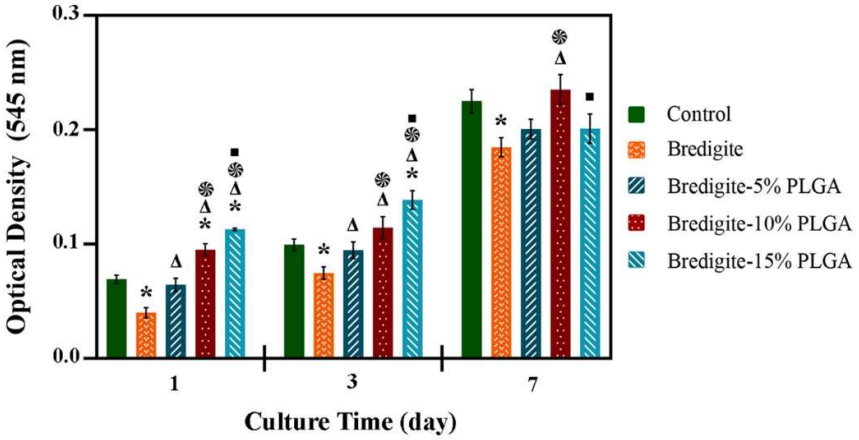

Fig. 9. MTT results of the cell culture on the scaffolds. *, Δ , ֍ and ■ indicate significance differences ($p < 0.05$) with respect to the control, bredigite, bredigite-5% PLGA and bredigite 10% PLGA scaffolds, respectively.

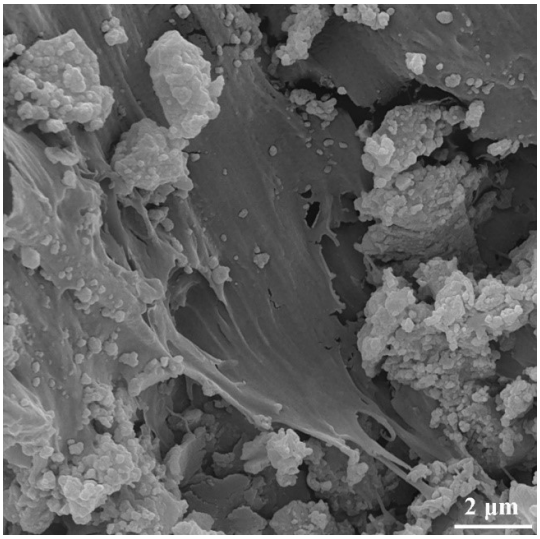

Fig. 10. SEM micrograph of a cell cultured on the bredigite-10% PLGA scaffold for 24 h.

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Table

Table 1. Characteristics of the prepared scaffolds.

Scaffold type	Porosity (%)	Compressive strength (MPa)
Bredigite	90.7 ±0.7	0.31 ±0.09
Bredigite-5% PLGA	82.7±2.1	1.21 ±0.08
Bredigite-10% PLGA	77.9±2.4	1.74 ±0.14
Bredigite-15% PLGA	72.3±2.2	2.73 ±0.12