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Effect of porogen on the physico-chemical properties and degradation performance of PLGA scaffolds

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ABSTRACT

The aim of the study was to examine the relationship between the structural features of PLGA 3-D scaffolds and their degradation performances. PLGA 3-D scaffolds were fabricated by solvent casting and particulate leaching using salt and sucrose particles as porogen and they were characterized in terms of structure, physico-chemical and mechanical properties. The *in vitro* degradation study was performed at 37 °C in PBS for 28 days and it included the determination of Mw, Mn, PI, water uptake, mass loss and pH changes of the degradation buffer. It was found that the preparation method scaffolds, in particular the choice of the porogen type and was responsible for the three-dimensional structure (pore size, porosity, apparent density and, mechanical properties) of the scaffold and for physico-chemical PLGA polymer modifications (polymer degradation reactions, polymer chain rearrangement, Tg changes). Scaffolds with high porosity (83.8–89.4%) and an interconnected network were obtained.

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1. Introduction

The objective of the regenerative medical therapy is to allow cells to induce tissue regeneration, repairing defective tissue and substituting the biological functions of damaged tissue/organs [1]. Scaffolds for tissue engineering are degradable matrices designed to support cell attachment, proliferation and growth and to finally provide functional living tissue [2]. Since the scaffold is a temporary matrix for cell proliferation and differentiation, the degradation performance of the scaffold must correspond to the regenerative rate of the affected tissue in order to permit concomitant growth and replacement of tissue and matrix clearance [3].

Considering these systems *in vivo*, it is necessary to create an appropriate environment which permits cells to efficiently proliferate and differentiate, inducing regeneration. Indeed, cells need to interact with the extracellular matrix (ECM) to perform their biologically functions in the body. For this reason, the primary function of the biomaterial used in the preparation of scaffolds is to act as an artificial matrix supporting proliferation and differentiation of cells [1]. The materials used to produce scaffolds for regenerative medical therapy range from stiff inorganic materials, such as hydroxyapatite (HA), to synthetic materials. The synthetic materials, such as polymers, can be produced with predictable and reproducible chemical, mechanical and physical features. Nevertheless, when the synthetic material is in contact with the damaged tissue unexpected biological responses may appear owing to the accumulation of polymer degradation by-products [4]. For this reason, there is a great interest in biopolymers which present a well-defined biocompatibility and *in vivo* biodegradability, thus to provide a suitable alternative to produce biocompatible and biodegradable scaffolds with specific physico-chemical, morphological and mechanical properties appropriate for the intended applications [5].

The intrinsic properties of the raw material play a strategic role in the production, structure and morphology and, consequently, in the functional performances of the polymer scaffold [6]. To act as an artificial ECM, the structure and the surface morphology of the scaffolds have to meet general requirements specific for the targeted tissue: i) interconnected pores to ensure cell growth, nutrients and metabolic waste transport flow; ii) three-dimensional architecture; iii) suitable mechanical properties; iv) suitable surface chemistry; v) controllable biodegradation and bio-resorbability [7]. The scaffold shape should also facilitate cell seeding and attachment and promote the cell proliferation and differentiation [8].

The degradation performances of the raw polymer are essential for the biomaterial choice and the scaffold design but also for the long-term success of tissue-engineered construct [9–11]. The scaffold has to be based on resorbable biomaterials exhibiting clinically relevant controlled chemical breakdown and resorption [12]: the degradation of the polymer material has to proceed

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appropriately and has to be in sync with the regeneration of the new tissue/organ [6]. In this approach, the implant is completely replaced by the ready-reconstructed tissue, and no differences between the regenerated and host tissue must be perceptible. Moreover, the bio-resorbable scaffold should present mechanical properties (strength and stiffness) equivalent to the host tissue ones until the bio-resorbable scaffold matrix is substituted by the new tissue [10].

The polymer used in this work was poly D, L-lactic-glycolic-co-polymer (PLGA). PLGA has already been studied for its degradation performance [13], even after sterilization by gamma-irradiation [14,15] and as raw material for the preparation of polymer micro- and nanoparticles containing protein and peptide [16,17]. The polymer has been shown to degrade mainly by the hydrolysis of ester bonds into acidic monomers, which can be removed from the body by physiological metabolic pathways [18,19]. The degradation of copolymer such as PLGA is affected by several factors including the molecular weight, the ratio of hydrophilic polyglycolic acid (PGA) and hydrophobic polylactic acid (PLA) and polymer treatment for the formulation. Poly(orthoester)s biocompatibility has been already demonstrated for biological applications and it has been reported that the polymer degradation rate is a fundamental parameter as it might affect cellular interaction including cell proliferation, tissue regeneration and host tissue response [20].

There are numerous techniques to obtain biodegradable polymeric scaffolds, such as solvent casting/particulate leaching, emulsion and freeze-drying, supercritical CO_2 technology, compression/assembling of polymeric micro-particulate systems [2,5,8,11]. Among these, solvent casting/particulate leaching is one of the most used preparation techniques because it permits good results in terms of scaffold structure (pore size, porosity and interconnected structure). Moreover, this preparation technique is quite simple, and it can be easily scaled up. Its drawback is the use of organic solvent that need to be eliminated through an accurate purification process before achieving the final product.

Selecting the easiest and most common matrix preparation method (solvent/casting particulate leaching method) [21], the authors focused attention on the porogen choice as the critical preparation aspect. Indeed, the porogen is certainly responsible for the three-dimensional structure of the scaffold but it may promote/induce unexpected physico-chemical PLGA modifications. For this reason, scaffold processing variables (organic solvent, porogen and freeze-drying process) were considered to assess their effects on morphological and mechanical features and on the degradation behaviour of PLGA scaffolds. In particular, the goal of this study was to systematically examine the relationship between the structural features of the PLGA 3-D scaffolds and their degradation performances.

2. Materials and methods

2.1. Materials

PLGA polymer (PLGA 8515 DLG 7E, Mw 120 kDa, Mn 97 kDa) was purchased from Lakeshore Biomaterials, Birmingham (USA). Salt (NaCl, Mw 58.443 g/mol, solubility in water 36 g/100 mL at 20 °C), Sugar (Sucrose, Mw 342.296 g/mol, solubility in water 211.5 g/100 mL at 20 °C) and 1,4-dioxan, used for the preparation of PLGA scaffolds, were obtained from Carlo Erba, Milan (Italy). The water used in the preparation of scaffold was distilled and filtered through 0.22 μm Millipore membrane filters (Millipore Corporation, Massachusetts, USA). Unless specified, all other solvents and reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of PLGA scaffolds

PLGA scaffolds were prepared by solvent/casting particulate leaching method [21] using 1,4-dioxan as solvent. Porogen particles (salt and sucrose) were sieved onto a 600–1180 μm diameter group; 700 mg of sieved porogen particles (70% w/w) were added to a Teflon mould (cylindrical vial with a diameter of 10 mm).

700 μL of PLGA solution (15% w/v in 1,4-dioxan) were cast drop by drop into the Teflon mould filled with porogen. The mould containing the porogen and the polymer solution was first maintained at room temperature (RT), overnight to permit the diffusion of the polymer solution through the porogen particles and then it was placed at −25 °C for 24 h. The frozen porogen/polymer mixture was freeze-dried at −50 °C for 12 h to completely remove the solvent. The scaffolds were dialyzed in water (200 mL) at RT for 21 days. The water was changed three times a day for the first week and then once a week. After dialysis the scaffolds were freeze-dried at −50 °C overnight. The prepared scaffolds were stored in a desiccator at −25 °C until characterization. Scaffolds obtained by using salt and sucrose as porogen are indicated in the paper as scaffold 1 and scaffold 2, respectively.

2.2.2. Characterization of PLGA scaffolds

The pore architecture of polymer scaffold was examined by scanning electron microscopy (SEM) (Jeol, Cx, Temcam, Jed, Tokyo; Japan). Samples were sputtered with an Au/Pd coating in argon atmosphere. The coating was repeated two times.

The density and porosity values of the PLGA polymer scaffolds were measured by a modified liquid displacement method [22]: ethanol was chosen as the displacement liquid because it penetrated easily into the pores, it was a PLGA non-solvent and it did not induce shrinkage or swelling of polymer.

Briefly, a weighed polymer scaffold (W) was immersed in a graduated cylinder containing a known volume (V_1) of ethanol. The sample was kept in the non-solvent for 10 min, and then a set of evacuation–repressurisation cycles was conducted to force the ethanol into the pore structure. Cycling was continued until no air bubbles were observed from the surface scaffold. The total volume of the ethanol and ethanol-soaked scaffold was then recorded as V_2. The volume difference ($V_2 - V_1$) was the volume of the scaffold skeleton. The ethanol-soaked scaffold was then removed from the cylinder and the residual ethanol volume was recorded as V_3. The volume ($V_1 - V_3$), that is the ethanol volume retained in the porous scaffold, was defined as the pore volume of the scaffold. The total volume of the scaffold was calculated as follows:

$$V = (V_2 - V_1) + (V_1 - V_3) = V_2 - V_3$$

The density of the scaffold (d) was expressed as:

$$d = W/(V_2 - V_3)$$

And the porosity of the scaffold (ε) expressed as percentage (%) was calculated by:

$$\varepsilon(\%) = (V_1 - V_3)/(V_2 - V_3)100$$

The density and porosity were determined in triplicate ($n = 3$) and expressed as mean ± standard deviation.

2.2.3. Mechanical properties

The compressive mechanical properties of the PLGA scaffolds including compressive strength and compressive modulus were measured with an electromagnetic testing machine (Enduratec Elf 3200, Bose Corporation, Eden Prairie, MN, USA). The machine was

equipped with a load cell of 220N. Different grips were used with the machine depending on the test configuration, i.e. compression or tension. Compression tests were done under displacement control, at a velocity of 0.1 mm/s until the sample was 40% of initial height. Tension tests were done under displacement control, at a velocity of 0.1 mm/s until sample failure. The compressive modulus and compressive strength were calculated as the average of five measurements per scaffold and expressed as mean ± standard deviation (SD).

2.2.4. Glass transition temperature determination

Glass Transition Temperature (Tg) was determined by means of a 2910 modulated differential scanning calorimeter, MDSC (TA Instruments, Delaware, USA), fitted with a standard DSC cell, and equipped with a liquid nitrogen cooling accessory (LNCA). Samples of about 10 mg were quantitatively transferred to hermetically sealed aluminium pans and subjected to two cooling and heating cycles from −60 °C to +60 °C with cooling and heating rates of 5 °C/min. The DSC cell was purged with dry nitrogen at 40 mL/min. The baseline correction was performed by recording a run with empty pans. The system was calibrated both in temperature and enthalpy with indium as standard material. The data were processed with Thermal Solutions software (TA Instruments, USA) and the results were expressed as the mean of three determinations.

2.2.5. In vitro degradation test

Polymer scaffolds were incubated at 37 °C in 10 mL of phosphate buffer saline (PBS), pH 7.4. The samples were fixed to the vial to ensure the whole scaffold was immersed into the degradation buffer, and then incubated at 37 °C in static conditions. The incubation buffer from each test tube was withdrawn, collected and replaced with fresh PBS buffer at regular intervals (twice a week).

At scheduled times (3, 7, 10, 14, 21 and 28 days) samples were recovered, washed with distilled water and lyophilized (Lio 5P, Cinquepascal s.r.l., Milan, Italy) at about −50 °C, for 24 h. The degradation study was performed on the scaffold samples (scaffolds 1 and 2), and on the PLGA polymer (raw material) used in the preparation of the scaffolds, as control. It included the determination of water uptake, PLGA Mw variations (GPC analysis), weight loss and pH measurement of the degradation buffer.

With the purpose of reducing experimental errors, all of the data presented in the figures of this paper are the average data from 3 parallel samples and expressed as mean ± standard deviation (SD).

2.2.5.1. Water uptake determination. The amount of water absorbed (WA) in the polymer samples incubated at 37 °C in PBS was determined gravimetrically (Mettler Toledo AG 245, Milan, Italy). The samples submitted to the *in vitro* degradation test were weighed immediately after recovering and subsequently rinsed with distilled water. Water content was computed as follows Eq. (1):

$$\text{Water Content}(\%) = \frac{W_t - W_0}{W_t} \times 100 \quad (1)$$

where W_t is the weight of wet sample at time t, and W_0 is the initial weight of the dry sample. Water uptake was determined in triplicate ($n = 3$).

2.2.5.2. Gel permeation chromatography. Gel Permeation Chromatography (GPC) was used to perform the physico-chemical characterization, in terms of average molecular weight (Mw) and polydispersity index (PD) of polymers and scaffolds before and after incubation in PBS (pH 7.4).

The GPC system consisted of three Ultrastyragel columns connected in series (7.7 × 250 mm each, with different pore diameters: 10^4 Å, 10^3 Å and 500 Å), a pump (Varian 9010, Milan, Italy), a Prostar 355 RI detector (Varian Milan, Italy), and software for computing Mw distribution (Cirrus™ GPC software for LC Systems, Varian Milan, Italy). Raw polymer and scaffolds were solubilised in tetrahydrofuran (THF) at a concentration of 20 mg/mL, the sample solutions were filtered through a 0.45 μm filter (Millipore, Massachusetts, USA) before injection into the GPC system, and were eluted with THF at 1 mL/min flow rate. The weight-average molecular weight (Mw) of each sample was calculated using monodisperse polystyrene standards (Mw 1000–150,000 Da). The data were processed as weight-average molecular weight (Mw), average molecular number (Mn) and polydispersity index (PI).

The results reported as average molecular weight (Mw), percentage variation of average molecular weight (ΔMw%), and of polydispersity index (ΔPI%), were calculated by GPC at the fixed time of incubation ($n = 3$). The percentage variation of average molecular weight and polydispersity index were calculated as follows Eqs. (2) and (3):

$$\Delta\text{Mw}(\%) = \frac{\text{Mw}_0 - \text{Mw}_t}{\text{Mw}_0} \times 100 \quad (2)$$

$$\Delta\text{PI}(\%) = \frac{\text{PI}_0 - \text{PI}_t}{\text{PI}_0} \times 100 \quad (3)$$

where Mw_0 is the average molecular weight of sample before incubation, and Mw_t is the average molecular weight of polymer samples at the scheduled time of incubation, PI_0 is the polydispersity index of sample before incubation, and PI_t is the polydispersity index of samples at the scheduled time incubation as calculated by GPC ($n = 3$).

2.2.5.3. Weight loss determination. The weight loss (WL) of samples was determined gravimetrically (Mettler Toledo AG 245, Milan, Italy) at scheduled times, the samples subjected to the incubation in PBS were freeze-dried before gravimetric analysis. Weight loss was determined in triplicate, and computed as follows (Eq. (4)):

$$\text{Weight Loss}(\%) = \frac{W_0' - W_t'}{W_0'} \times 100 \quad (4)$$

where W_0' is the weight of polymer samples before incubation, and W_0' the weight of the samples after incubation and freeze-drying ($n = 3$).

2.2.5.4. pH of degradation buffer. The pH of the degradation buffer in every test tube was measured with a pH meter (pH meter 827 phyla; Metro, Switzerland) twice a week.

3. Results and discussion

3.1. Scaffold characterization

PLGA scaffolds were prepared by solvent/casting particulate leaching method using 1,4-dioxan as solvent to dissolve the PLGA polymer. 1,4-dioxan was selected among a few solvents (THF, methylene chloride, pyridine and dimethyl carbonate) considering the evaporation point (101.3 °C) and the melting temperature (12 °C). The higher evaporation temperature value reduces the solvent evaporation during the preparation process allowing the complete diffusion of dropped polymer solution through the porogen particles. Moreover, 1,4-dioxan presents a melting point around 12 °C that makes it suitable for sublimation during the following lyophilisation process. As observed in a preliminary study (not reported data) scaffolds prepared using a solvent with a freezing point lower than −25 °C as methylene chloride (−95 °C) exhibited

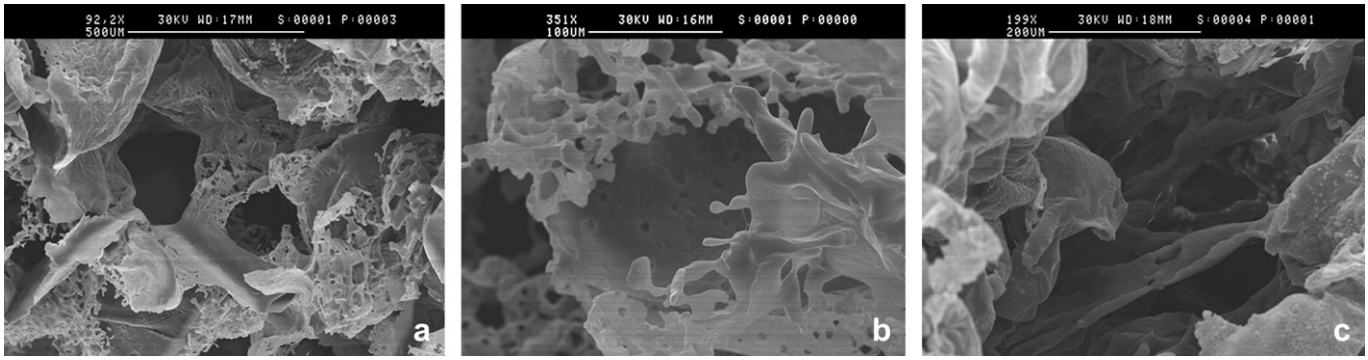

Fig. 1. Scanning electron photomicrographs of PLGA scaffolds prepared using salt as porogen: (a) view transverse section at low magnification (92.2×), (b) wall pore structure (magnification 351×), (c) interconnected scaffold network (magnification 199×).

a rubbery texture, due to the residual solvent in the polymeric matrix even after the lyophilisation step.

During the preparation process scaffolds were submitted to two freeze-drying cycles: the first one before dialysis to remove residual solvent (1,4-dioxan) that could compromise the safety of the construct, the second freeze-drying cycle after the leaching step to eliminate remaining of water which may be responsible of hydrolytic degradation reactions. Residual amount of 1,4-dioxan (data not reported) was opportunely determined to be below the concentration limit reported in ICH Q3 C (R3) (380 ppm) [24].

Figs. 1 and 2 show the morphology of PLGA scaffolds prepared using salt and sucrose porogen particles, respectively. Scanning electron photomicrographs illustrate polymer scaffolds with high porosity and well interconnected network (Figs. 1c and 2a). Highly porous structures are necessary to create suitable interaction of cells with scaffold. The pore architecture is characterized by: i) pore size, ii) pore shape, iii) pore interconnectivity, iv) degree of porosity and v) surface area. The microstructure determines cell interactions with the scaffold, as well as molecular transport (nutrients, wastes and biological chemicals *e.g.* growth factors) within the scaffold. Specifically, pore size determines the cell seeding efficiency into the scaffold [25]; very small pores prevent the cells from penetrating into the scaffold, while very large pores prevent cell attachment due to a reduced area to be colonized by cells. Subsequently, cell migration within a scaffold is determined by degree of porosity and pore interconnectivity. A scaffold with an open and interconnected pore network and a high degree of porosity (~90%) is described as ideal to interact and integrate with the host tissue [26].

The PLGA scaffold prepared with salt particles as porogen has irregular pore range from few microns up to about 300 μm (Fig. 1a). The micro-porosity of scaffold 1 (Fig. 1a and b) may be due to a phenomenon known as *solid–liquid phase separation* which is ascribed to solvent crystallization. When the temperature of the polymer solution is lower than the solvent freezing point (crystallization temperature) 1,4-dioxan crystallizes, and the polymer phase is expelled from crystallization front 'as impurity'. A continuous polymer-rich phase is formed by aggregation of polymer fractions excluded from solvent crystals. After solvent crystals have been sublimated, the scaffold is produced with a microporosity similar to the geometry of solvent crystals. The crystallization of solvent is perturbed by the presence of solid porogen particles. The salt or sucrose particles randomly dispersed in the PLGA/1,4 solution in dioxan modify the solvent crystallization front by reducing and limiting the crystal growth, making crystals of solvent smaller and asymmetrical. After solvent sublimation the polymer-rich phase becomes the skeleton of the scaffold and the spaces taken by solvent crystals develop into pores [27,28].

When sucrose is used as porogen in the preparation of PLGA scaffold the structure of the polymer matrix is revealed much different with respect to the structure of the scaffold prepared using salt particles as porogen. In contrast to the irregular pore structure observed with salt (Fig. 1), the scaffold 2 presents cubical pores with the geometry of sucrose crystals (Fig. 2b, c). Figs. 1c and 2a are interesting in order to show that most of the pores are fully interconnected for both types of scaffolds.

As reported in Table 1, the apparent density value of the scaffold 1 is higher (0.12 g/L) than the density of corresponding scaffold 2 (0.088 g/L): the more dense structure of scaffold 1 may be due to the presence of salt during the leaching step. Chen [29] and Sibambo [30] reported how the presence of soluble salt (as NaCl) can affect the inter- and intra-molecular hydrogen bonding, polar interactions and hydrophobic interactions between polymer chains

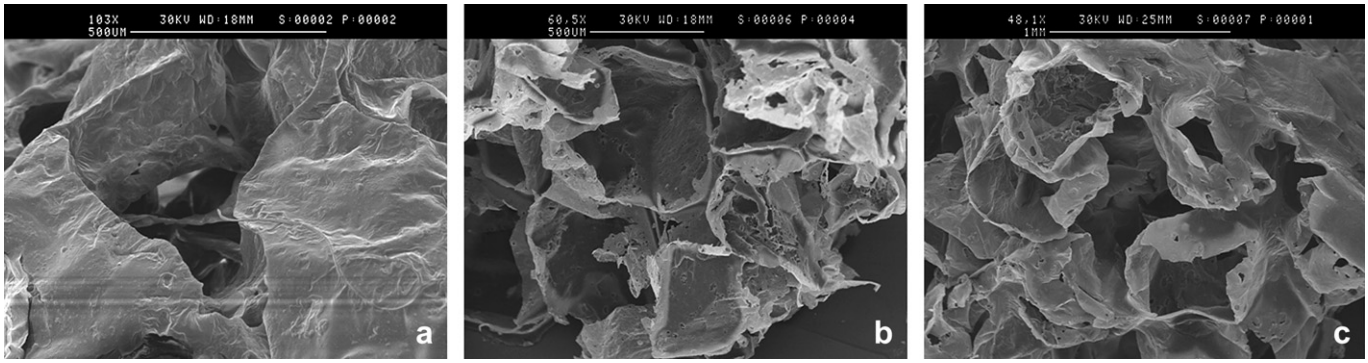

Fig. 2. Scanning electron photomicrographs of PLGA scaffolds prepared using sucrose as porogen: (a) interconnected scaffold network (magnification 103×), (b) view transverse section at low magnification (60.5×), (c) porous structure details (magnification 48.1×).

Table 1
Apparent density, porosity and pore size of PLGA scaffolds prepared using different porogens.

Scaffold #	Porogen	Apparent density (g/L)[a]	Porosity (%)[a]	Pore size (μm)[b]	Compressive modulus (MPa)	Yield strength (MPa)
1	NaCl	0.12 ± 0.03	83.77 ± 0.62	200–300	3.66	0.4
2	Sucrose	0.088 ± 0.01	89.44 ± 0.4	200–600	2.43	0.22

[a] Determined by displacement method (solvent − ethanol).
[b] Determined by SEM.

within polymer matrices during the hydration. As the polymeric matrix hydrates, the salt ionizes and the solvated ions react with C–O and H-groups forming hydrogen and salt-oxygen bonds between PLGA chains and finally resulting in the cross-linking of lactide–glycolide units in the PLGA molecular structure [31].

The presence of salt would change the structure of the surrounding water layer and cause changes in polymer conformations. These changes are supposed to amplify the Brownian fluctuations and frequency of contacts between polymer segments resulting in the matrix dehydration, the reduction of the average distance between the polymer chains and the increase of the hydrophobic interactions. Moreover, solvated ion pairs are able to attract the adjacent cations within the polymeric matrix: this to contribute to the cross-linking of lactide and glycolide chains in the PLGA molecular structure. The cross-linking reactions depends on several factors: i) the ionization energies of the salt ion, ii) hydration enthalpies in solution, iii) the dissociation constant (pKa) of the salt, and iv) the thermodynamic stability of the monomeric PLGA units. The attraction of adjacent cations during the cross-linking is controlled by the coordination number of salt and the atomic size of ions [30]. As previously observed, the ions and/or salt that possess a high coordination number as NaCl (coordination number: 6) and high atomic size have the most influence on the cross-linking reaction. Moreover, the cross-linking degree is dependent not only to the type of ion/salt but also to the salt concentration: at lower concentration (0–5% w/v) NaCl significantly increases the resilience of PLGA scaffold: any further increments in the amount of NaCl (>5% w/v) leads to a reduction of flexibility [30]. These changes of the native PLGA polymeric structure create a microenvironment which enhances the viscoelastic behaviour of PLGA resulting in a more dense and robust PLGA scaffold which increases the resistance of the scaffold to deform under stress during compressive analysis [30].

As observed by the results of the compressive mechanical properties study (Table 1) the compressive modulus and the compressive yield strength of the scaffold 1 are significantly higher than those of scaffold 2. These data demonstrates the positive effect of salt particles in enhancing the mechanical performance of the polymeric scaffold.

DSC analysis shows an increase of Tg for scaffold 1 (46.6 °C) compared with the PLGA polymer (44.4 °C). This finding could be associated with structural changes induced by the presence of salt, as mentioned above: hydrophobic interaction between PLGA segments reduces the mobility of the polymer chains within the matrix and consequently the transition from the glassy to the rubbery state is observed at a higher temperature value with respect to the native polymer. The Tg reduction detected for scaffold 2 (42 °C) versus 44.4 °C for PLGA polymer may be due the polymer chains degradation during the production of the scaffold which led to a decrease of polymer Mw and Mn and consequently to an increase of free volume and chain mobility.

Scaffolds 1 and 2 display a reduction of average molecular weight (ΔMw%) of about 21% and 17%, respectively (Table 2). Since the number average molecular weight (Mn) reduction was significant for both PLGA scaffolds (26 and 20%, respectively), it is possible to hypothesize that the degradation reactions evaluated throughout the scaffolds preparation have a crucial effect on the terminal end groups of polymer chains causing a more rapidly decay of Mn compared to Mw.

The smaller pore size observed in the scaffold structure (200–300 μm scaffold 1 and 200–600 μm scaffold 2) compared with the original size of the porogen particles (600–1180 μm) can be a consequence of the shrinkage of PLGA chains during freeze-drying process. Indeed, the shrinkage can be ascribed to the molecular rearrangement of the polymer chains during the freeze-drying process. The freezing temperature would definitely regulate the crystallization speed of 1,4-dioxan and water inside the polymer matrix and in turn, the size of solvent/water crystals would eventually determine the inner porous structure [27]. The higher pore size revealed from SEM analysis for Scaffold 2 may also be ascribed to residual fractions of sugar remained in the porous polymer matrix from the leaching step which may protect the porous structure against mechanical stresses induced by freeze-drying treatment. As reported in several studies freeze-drying step may produce many stresses that can have an effect on the polymer system: namely, the stress of freezing and dehydration. The crystallization of ice may exercise a mechanical stress on porous structure leading to its destabilization. For these reasons, special excipients could be added to the pharmaceutical formulation before freezing to protect this brittle system (such as lactose, sucrose, trehalose, glucose, glycerol, mannitol, sorbitol, PVP and PEG). These excipients are frequently added in order to protect the product from freezing stress (cryoprotectant) or drying stress (lyoprotectant) and also to increase its stability upon storage. The sugars are known to vitrify at a specific temperature, known as 'Tg'. The immobilization of the porous network within a glassy matrix of cryoprotectant may prevent the collapse of the pores or eventually their fusion and protect the porous structure against the mechanical stress of ice crystals [32].

3.2. In vitro degradation

Ideally, scaffolds are designed to be completely replaced by the regenerated extracellular matrix by integrating with the surrounding tissue, eliminating the need for further surgery to remove it [20]. Scaffolds should degrade with a controllable degradation rate, as well as with the controllable production of degradation products. Sung et al. studied the effect of the degradation rate on cellular interaction including cell proliferation, tissue regeneration and host response. The comparative study of fast- versus slow-degrading three-dimensional scaffolds indicated that

Table 2
Physico-chemical characteristics of PLGA scaffolds and raw polymer.

	Scaffold 1	Scaffold 2	Raw polymer
Mw (kDa)[a]	93	97	120
Mn (kDa)[a]	72	77	97
PI[a]	1.29	1.27	1.22
Tg (°C)[b]	46.60	42	44.4

[a] Determined by GPC.
[b] Determined by DSC (maximum standard error value 0.5).

fast degradation negatively affects cell viability and migration into the scaffold *in vitro* and *in vivo* [20].

The *in vitro* degradation of PLGA scaffolds was studied in PBS at 37 °C, for 28 days in static condition. The *in vitro* degradation of PLGA polymer (raw material) was also performed and considered as a control. Polymer degradation was monitored by measuring the Mw and Mn changes, weight loss, water adsorption, and changes of incubation buffer pH.

The changes in weight-average molecular weight (Mw) of PLGA scaffolds and polymer raw material are presented in Fig. 3. The Mw of the tested samples decreases throughout the whole incubation time. The PLGA polymer (raw material) shows the fastest reduction of molecular weight over the incubation time. At day 28, the reduction percentage of molecular weight (ΔMw%) is about 66%, versus 11% and 16% determined for scaffolds 1 and 2, respectively. As polyorthoester, PLGA degrades through a hydrolytic reaction in which a molecule of water hydrolyses the polyester bond between two linked up acid molecules breaking apart the polymer chains [23]. The hydrolysis reaction is the rate-limiting step in the bulk-erosion mechanism, whereas the water uptake of the polymer complex may be considered relatively fast.

The first-order polymer degradation may be expressed in terms of degradation rate constant (k). The ks of the tested samples were determined using the linearised form of the exponential trend line fitted to the Mw degradation data as follows:

$$\ln(\mathrm{Mw}) = \ln(\mathrm{Mw}_0) - kt$$

The k values of PLGA polymer (raw material), scaffolds 1 and 2 are calculated to be 0.0315, 0.0044, and 0.0072 day^{-1}, respectively. This trend may seem conflicting, considering that the polymer scaffolds display a higher macro-porosity which may therefore enhance the water uptake.

In this instance, greater macro-porosity may have increased the rate of scaffold wetting and localized more water molecules nearer to the polyesters bonds within the polymer chains, making them more susceptible to hydrolysis. Instead, the slower rate of degradation that was underlined in the PLGA scaffolds is likely due to the autocatalytic reactions which can take place inside the polymeric matrix. The rod shape and the porosity of few microns typical of the polymer raw material may block the diffusion of degradation products through the matrix into the surrounding incubation buffer and cause the accumulation of polymer degradation products in the polymer matrix responsible for the autocatalytic reactions. The macro-porosity of PLGA scaffolds (Figs. 1 and 2) may have contributed to reduce their degradation rate as the lactic and glycolic acids are able to diffuse through the remaining polymer chains and decrease the possibility for catalytic reactions.

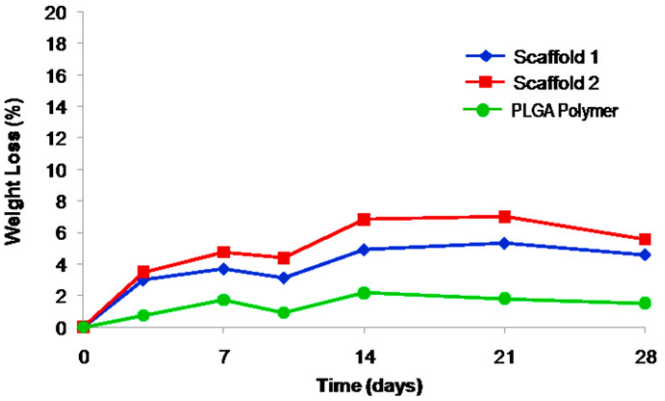

Fig. 4. Weight Loss of PLGA polymer scaffolds (scaffolds 1 and 2) and PLGA polymer (raw material) during *in vitro* degradation study.

As previously reported a significant Mw reduction is observed during the preparation of scaffolds: no further Mw decrease is highlighted for scaffolds 1 and 2, which means that the degradation process is still in its early stage (Fig. 3). The lower k value calculated for scaffold 1 (0.0044 day^{-1}) compared with scaffold 2 (0.0072 day^{-1}) may be likely ascribed to the cross-linking reactions induced by salt particles in the hydrated PLGA matrix.

Weight loss (WL) of PLGA scaffolds is presented in Fig. 4. The PLGA polymer (raw material) shows a slower WL during the whole incubation time. At day 10, an increment of WL is identified for all tested samples, which is consistent with the polymer average molecular weight decrease, quantified by GPC (Fig. 3). The faster WL detected for PLGA scaffold samples could be associated to the higher facility of the scaffolds construction to absorb water when soaked in PBS. Following the implantation, the biopolymer structure interacts with the surrounding fluids: firstly, it imbibes the fluids then it starts the degradation reactions. The uptake of water, which is identified as a plasticizing compound [33], makes the polymer chains more plastic [14] and encourages the reorganization of polymer segments and, as a consequence, leads to adjustments of the porosity, density and dimension of the construction. Concurrently, a higher water uptake enhances the hydrolysis reactions. Indeed, it is very essential to estimate the water uptake of biopolymers, since it indicates the affinity of polymer system with water and, accordingly, its susceptibility to hydrolysis [34].

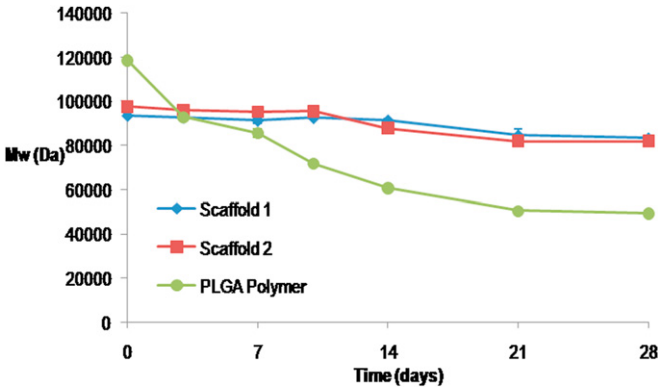

Fig. 3. Molecular weight changes of scaffold 1, scaffold 2 and PLGA polymer (raw material) during *in vitro* degradation study.

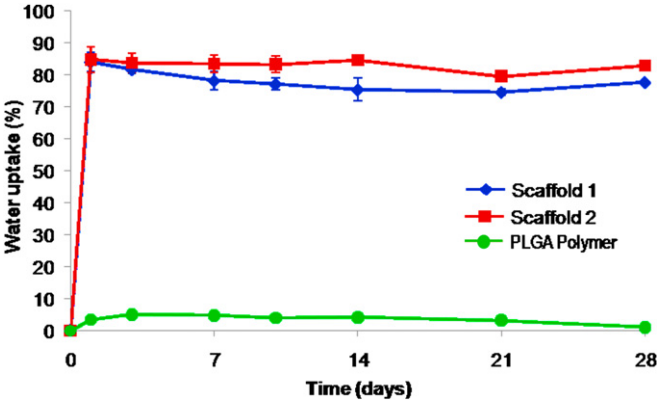

Fig. 5. Water uptake of PLGA polymer scaffolds (scaffolds 1 and 2) and PLGA polymer (raw material) during *in vitro* degradation study.

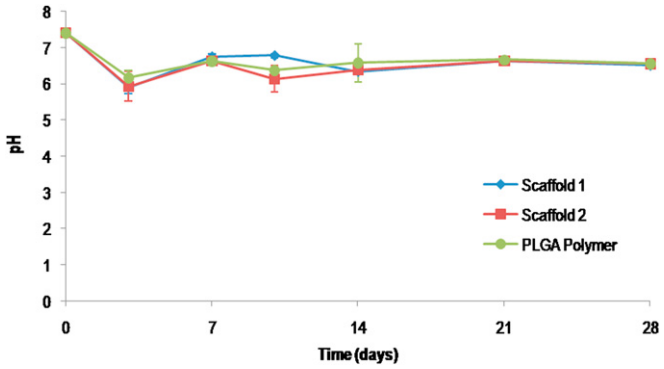

Fig. 6. pH variations of incubation buffer versus time for PLGA polymer scaffolds (scaffolds 1 and 2) and PLGA polymer (raw material) during *in vitro* degradation study.

Moreover, the lowest weight loss observed for PLGA polymer may be justified considering the micro-porosity of the polymer raw material that reduces the water adsorption rate inside the matrix and at the same time represents a barrier for the flow of the degradation products to the surrounding buffer. At day 28 the WL recorded is <10% for all the samples tested: this evidence denotes that at the end of incubation time, the scaffolds are still far from the total degradation and they preserve their original structure.

Scaffolds WA increase much more rapidly and a larger amount of water diffuses through the porous structure than in the PLGA polymer raw material (Fig. 5). After 3 days of incubation in PBS, WA of PLGA scaffolds was 80% versus a WA < 10% for PLGA raw material. After this time, WA plateau point is reached in PLGA raw material sample, while WA continues slightly to increase for scaffolds samples. At the end of the incubation time (28 days), the WA of scaffolds samples ranged between 77.6 and 83% in opposition to a WA < 10% for PLGA raw material. The capability of a scaffold to uptake and keep water within the construct is an important feature for developing a suitable scaffold for tissue regeneration because the medium flow is essential for the nutrient and metabolic exchanges [35].

pH changes of the aqueous buffer (Fig. 6) during the incubation time were determined for all the tested samples in order to verify the release of the acidic oligomers from the polymeric matrix and, eventually, to support the data obtained by GPC. The pH of the incubation buffer for PLGA scaffolds and PLGA raw material decreased greatly at the early of time incubation (at day 3) and, then, stabilized around 6, a value lower than the initial pH (7.4) during all the incubation time (28 days). The pH reduction observed at day 3 may be due to the dissolution of acidic oligomers compounds, formed during the preparation procedure, in the aqueous system.

4. Conclusions

PLGA scaffolds prepared by solvent casting/particulate leaching by salt and sucrose as porogens maintained their morphology and structure for all time of incubation (28 days) and no significant degradation process of polymer was revealed. The results i) highlighted that the scaffold preparation induces significant polymer degradation and ii) confirmed that the degradation manner of polymeric scaffolds depended on the original preparation method and physico-chemical properties of scaffold. It is essential to note that this study does not account for the degradation associated with cell activities. In any case, these biological phenomena may significantly change the reported degradation performance.

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