Materials Today: Proceedings xxx (xxxx) xxx

Contents lists available at ScienceDirect

Materials Today: Proceedings

journal homepage: www.elsevier.com/locate/matpr

Fabrication and characterisation of poly(vinyl alcohol)/chitosan scaffolds for tissue engineering applications

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Chitosan
Osteoarthritis
Poly(vinyl alcohol)
Scaffold
Tissue engineering

ABSTRACT

In this study, poly(vinyl alcohol)/chitosan (PVA/CS) scaffolds were fabricated by freeze-drying method and the characterisations of obtained scaffolds were carried out to analyse the suitability of the product for biomedical applications such as tissue engineering. The effect of initial PVA concentrations and their hydrolysis degrees, the addition of different molecular weights (MWs) of CS along with the blending ratio of PVA/CS were studied. Fourier transmission infrared (FTIR) spectroscopy showed the presence of chemical bonds in PVA and PAV/CS scaffolds and the energy dispersive X-ray (EDX) spectroscopy confirmed the existence of the chemical element nitrogen in PVA/CS scaffolds. The stability of scaffolds under physiological conditions was studied by swelling and degradation tests, which demonstrated the effect of initial PVA concentration and CS addition on the water resistance of scaffolds. Scanning Electron Microscopy (SEM) images showed highly porous structures which became more uniform in pore size with the addition of CS. The mechanical properties of dry and hydrated scaffolds were also investigated by an unconfined compression test and the compressive modulus and maximum stress at 20% strain were calculated through stress–strain curves. The in-depth characterisation of scaffolds showed that the initial concentration and blending ratio of PVA/CS can be adjusted to tailor the desired properties of scaffolds for different biomedical applications.

1. Introduction

Diseases and injuries can bring degeneration and damage to human tissues that ultimately require a regeneration process. Taking osteoarthritis (OA) for instance, according to World Health Organization (WHO), approximately 10% of men and 18% of women over 60 are suffering from this degenerative joint disease worldwide which results in articular cartilage (AC) degeneration [1]. Furthermore, 80% of people over 75 are suffering from osteoarthritis, becoming one of the most debilitating health problems for the ageing population. At the early stage of this disease, one approach is to replace the damaged areas of AC with donated tissues. However, due to the scarcity of donated tissues and the biocompatibility problems, this alternative does not fulfill the increasing demand. Using artificial materials can be an interesting solution for improving the regeneration of damaged tissues. This potential solution is based on the development of materials such as scaffolds that are transplanted into the human body in order to regenerate the damaged tissues and be degraded after their function.

Tissue engineering aims to regenerate damaged or degenerative tissues by developing biomaterials which could support the growth of new tissues [2]. These biomaterials should, besides not inducing inflammation, toxic reactions and allergenic symptoms in the body, be biocompatible, biodegradable, biofunctional, bioactive, bioresorbable and bioinert. Polymers are ideal candidates for tissue engineering applications due to their acceptable physical properties such as low density, modifiability and controllable degradation [3]. Over the past several decades, a variety of natural and synthetic polymers have been investigated. Natural polymers such as fibrin, chitosan (CS) and collagen have demonstrated good biocompatibility, bio-adhesivity, and low immunogenicity, but their disadvantages such as uncontrollable degradation rate and

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https://doi.org/10.1016/j.matpr.2023.02.303
2214-7853/

weak mechanical properties are not negligible. Synthetic polymers such as poly (lactic acid) (PLA), polypropylene fumarate (PPF), polycaprolactone (PCL), and poly(vinyl alcohol) (PVA) display a controllable degradation rate, the possibility to tune mechanical properties and the tailored architecture. The weakness of synthetic polymers falls in their low biocompatibility which affects the adhesion of cells and causes inflammatory reactions in the host tissue [4].

Using a single material could present some limitations for biomaterials development. In this context, composites which consist of two or more materials with different properties can be considered as an alternative to improve the properties of single-material scaffolds. The combination can be in the form of polymer–polymer blends or polymer-ceramic composites. Polymer-polymer blends are mixtures of two or more polymers such as the combination of synthetic and natural polymers. Polymer-ceramic composites are mixtures of polymer and ceramic such as combining inorganic HA crystals with organic collagen polymer. Composite scaffolds have shown some promising results and become a typical approach used in tissue engineering applications. In this study, PVA and CS polymers were selected in order to develop 3D scaffolds. PVA is prominently used due to its good performance characteristics, such as chemical stability and mechanical properties, controllable biodegradability, biocompatibility, and processability in a wide range of forms [5]. Polysaccharides such as CS which is a natural biopolymer, are extensively used for bone regeneration due to their good bio-adhesivity, antibacterial activity and good similarity with extracellular matrix (ECM) components [6].

Along with the selection of proper materials, the biomaterial fabrication method is as well a key factor for successful tissue regeneration. Biomaterials such as scaffolds should present porous structures with distributed and interconnected pores to ensure cell penetration, adhesion, proliferation, nutrient supply, and waste product elimination. Furthermore, consistent mechanical properties are essential [7]. For non-load bearing tissues, adequate mechanical properties of scaffolds can allow surgical handling during implantation. On the other hand, for load-bearing tissues such as bone or cartilage, sufficient mechanical integrity can ensure the function of the tissue after the completion of implantation [8]. The manufacturing technology has a significant impact on the feature of scaffolds [9]. Previous studies have reported several scaffold fabrication techniques on PVA or CS such as cast-drying, electrospinning, freeze-thawing and freeze-drying [9,10]. Freeze-drying technology is mainly used for porous scaffolds fabrication with the advantage of a lower use of toxic solvents. The scaffolds fabricated through freeze-drying technique have numerous interconnected pores inside the structure.

In the current study, freeze-drying was selected to manufacture PVA and PVA/CS scaffolds. During this study, the effects of PVA hydrolysis degree and CS MW on scaffold properties were investigated in order to select the most appropriate formulation to develop scaffolds for tissue engineering applications. For that, the scaffolds were evaluated to assess the chemical groups and bonds through Fourier-transform infrared spectroscopy (FTIR), swelling capacity, degradation, morphology through scanning electron microscopy (SEM), the presence of chemical elements by energy dispersive X-ray (EDX) spectroscopy and the unconfined compressive mechanical properties.

2. Materials and methods

This research aimed to use freeze-drying method to develop porous PVA/CS scaffolds for tissue engineering applications. As the hydrolysis degree of PVA can affect the final properties of scaffolds, two types of PVA polymers with different hydrolysis degrees were used, i.e. BF-05 grade with 98.5–99.2 mol % hydrolysis and BP-17 grade with 86–89 mol % hydrolysis degree. To investigate the proper concentration of PVA for preparing suitable scaffolds for tissue engineering, the following steps were followed: (1) Fabrication of two types of PVA scaffolds with different concentrations using the freeze-drying technique. (2) Characterisation of scaffolds (FTIR, in vitro degradation, swelling ratio, SEM, and compressive mechanical properties) in order to decide which PVA is the most suitable for tissue engineering applications. (3) Addition of CS into PVA formulation for the improvement of scaffold properties.

Two types of PVA polymers (Chang Chun Petrochemical Co. Ltd., Taiwan) were used which had different hydrolysis degrees. The grade and specification of these PVAs are listed in Table 1 and the chemical structures of PVAs are presented in Fig. 1.

Remarks:

(1) Viscosity (cps) of a 4.00 wt% standard grade poly (vinyl alcohol) solution at 20.0 °C which is determined by Brookfield Viscometer LVF mode with UL adapter at a rotation speed of 60 RPM.
(2) Calculated as Na_2O.
(3) pH is determined by a pH meter (with a sensitivity of 0.001 pH) at 20℃

Low molecular weight (LMW) CS, medium molecular weight (MMW) CS, and high molecular weight (HMW) CS with a degree of deacetylation higher than 75% were provided by Sigma-Aldrich (U.S.A). The chemical structure of CS is shown in Fig. 2a. Acetic acid was used to dissolve CS and was produced by Fisher Scientific (UK).

For freeze-drying method, aqueous solutions of PVAs with concentrations of 2.5, 5, 7.5, 8.5, 10, and 15% (w/v) were prepared by dissolving 1.25 g, 2.50 g, 3.75 g, 4.25 g, 5.00 g, and 7.50 g PVA, respectively, in 50 mL milli-pure water under stirring (300 rpm) at room temperature (22 °C) for 15 mins in order to avoid the formation of lumps. Then the solution was heated up to 90℃ and kept stirring (800 rpm) for 3 h. After that, 1.5 mL of the solution was transferred into each well of a 24 multiwell plate. Then the plate was placed in a freezer at −20 °C for 24 h. Later on, the frozen solutions were freeze-dried (Alpha 2–4 LDplus, Martin Christ Company) under a vacuum (1 mBar) at −80 °C for 24 h in order to evaporate the solvent by sublimation.

A 1% by weight CS solution was prepared by dissolving 0.25 g CS in 25 mL of 1% acetic acid solution, under stirring (250 rpm) at room temperature for 4 h to obtain a homogeneous and transparent solution. As there were three different types of MWs, three different CS solutions were prepared. These solutions were mixed with 8.5% (w/v) PVA solution at different ratios (PVA/CS, 100:0, 75:25, 50:50, 0:100) for 30 mins before pouring 1.5 mL into each well of a 24 multiwell plate. The plate was kept in a freezer for 24 h before freeze-drying for another 24 h.

FTIR spectra were obtained using a Thermo Scientific Smart iTR instrument equipped with a horizontal attenuated total reflectance (ATR). The samples were placed directly onto the ATR crystal and spectra were collected in transmittance mode. Each spectrum was the result of the average of 32 scans at 4 cm^{-1} resolution.

The swelling was calculated gravimetrically according to ASTM D570-98 under physiological conditions. Three specimens of each composition were weighed (W_d) and immersed in 15 mL of phosphate-buffered saline (PBS) solution at pH 7.0 in order to determine the swelling ratio (SR) profile at 37 °C in an incubator (CARBOLITE Gero Furnaces). Then, the samples were removed from the buffer solution at fixed times, wiped with soft tissue paper and reweighed (W_s). The SR was calculated using Eq. (1):

Table 1
Specifications of different PVA polymers used in this study.

PVA Specifications						
Grade	**Viscosity[1] (cps)**	**Hydrolysis (mole %)**	**Volatile (wt%)**	**Ash[2] (wt%)**	**pH[3]**	**Degree of Hydrolysis (HD, %)**
BF-05	5–6	98.5–99.2	<5	<0.7	5–7	Fully hydrolysed
BP-17	21–26	86–89	<5	<0.5	5–7	Partially hydrolysed

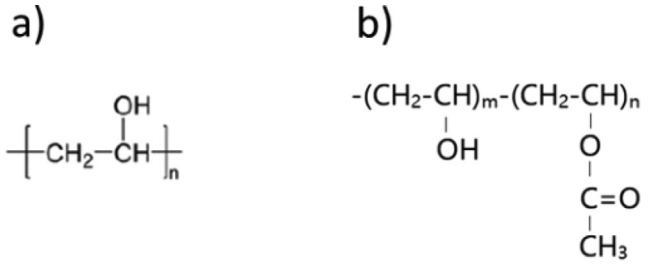

Fig. 1. Chemical structure of (a) fully hydrolysed PVA (98.5–99.2 mol %); and (b) partially hydrolysed PVA (86.0–89.0 mol %).

$$SR\,(\%) = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

A graph depicting SR against time was plotted in order to determine the equilibrium swelling. The degradation degree (DD) was also calculated gravimetrically under the same physiological conditions.

The inner morphology of scaffolds was observed using a HITACHI SU-70 SEM at an accelerating voltage of 5 kV. For pore size evaluation, SEM images were analysed by ImageJ software in order to get pore size distribution and the average of pore size. Before observation, the samples were cryofractured and coated with platinum for 20 s using Hitachi E-1045 sputter coater to be electrically conductive.

EDX analysis was conducted by measuring the energy and intensity distributions of the X-ray signals generated by a focused electron beam on the specimen. With the energy dispersive spectrometer, the chemical elements of the scaffolds were obtained. The elements of scaffolds were observed using a HITACHI SU-70 SEM at an accelerating voltage of 15 kV.

Unconfined compression tests were conducted to evaluate the mechanical properties of both dried and hydrated scaffolds. The hydrated scaffolds were prepared by immersing the scaffolds in PBS solution for 4 h and removing the excess water with soft tissue paper. The unconfined compression test was carried out on each type of scaffold using Texture Analyser (TA. XT plus) with a 500 N load cell for dried scaffolds and a 50 N load cell for hydrated scaffolds at a loading speed of 1 mm/min. The average of diameter and height of the cylindrical samples were measured taking three measurements per scaffold and these values along with the section area were introduced into the analysis program before testing. The force (N) and displacement (mm) were measured and recorded during the compression test. The engineering compressive stress σ and compressive strain ε were then calculated. The samples were subjected to unconfined compression force to 60% strain, and stress–strain curves were plotted during the test. The compressive modulus was calculated as the slope of the curve in the elastic region up to 2% deformation [12], and the maximum stress for both dry scaffolds and hydrated scaffolds was calculated at 20% deformation [13]. Five independent samples were tested for each type of scaffold (n = 5).

3. Results and discussions

In this study, different concentrations of PVA scaffolds and PVA/CS blending ratio were fabricated through freeze-drying technique. Firstly, fully hydrolysed PVA BF-05 scaffolds were studied. Different concentration (2.5%, 5%, 7.5%, 10% and 15% w/v) of PVA BF-05 scaffolds were fabricated. In low PVA BF-05 concentrations (2.5%, 5%, and 7.5% w/v), the scaffolds showed uniform shape, while with higher concentrations (10% and 15% w/v) the scaffolds with irregular shapes were produced. Considering these results, 8.5% (w/v) fully hydrolysed PVA BF-05 scaffolds and partly hydrolysed PVA BP-17 scaffolds were prepared and characterised. As can be seen in Fig. 3, all the scaffolds presented regular shapes. However, scaffolds prepared with PVA BP-17 (Fig. 3b), showed better shape than the PVA BF-05 scaffolds (Fig. 3a). Once the proper PVA concentration was selected, different ratios of PVA/CS were prepared and characterised. The obtained scaffolds kept a regular shape, indicating that the addition of CS did not affect the preparation process of scaffolds.

Fig. 4a shows the FTIR spectra of PVA BP-17 and PVA BF-05. The Large band in the range of 3000–3500 cm^{-1} is ascribed to the stretching of O–H from the intramolecular and intermolecular H-bonds [14]. The band around 1713 cm^{-1} is assigned to C=O stretching. The bands around 1645 cm^{-1} and 1652 cm^{-1} are assigned to the stretching and deformation vibration of O–H [15].

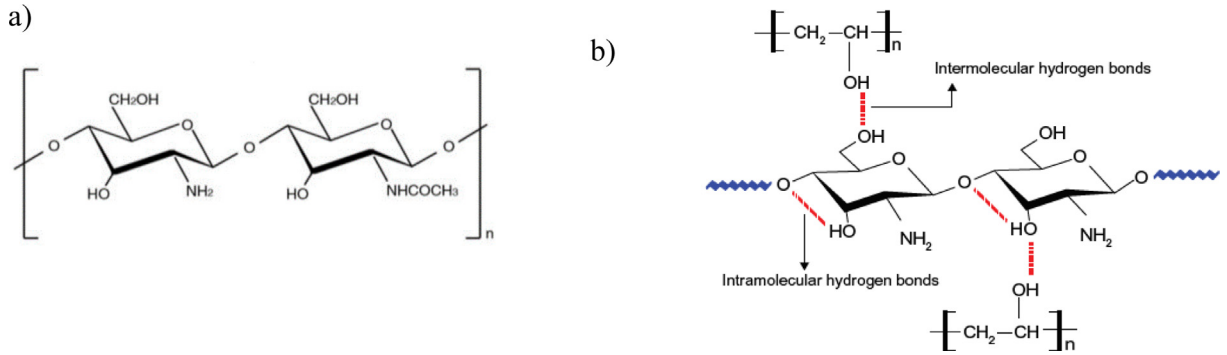

Fig. 2. (a) Chemical structure of chitosan (CS), (b) schematic of intermolecular and intramolecular H-bonds that occurred after blending PVA and CS [11].

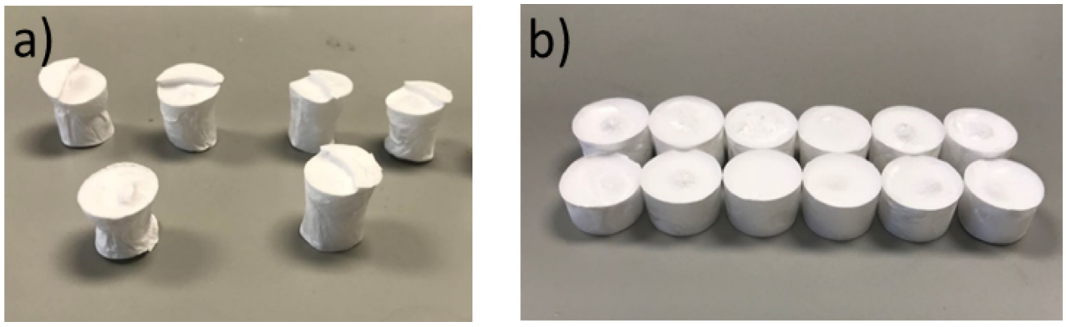

Fig. 3. (a) PVA BF-05 and (b) PVA BP-17 scaffolds with 8.5% (w/v) concentration.

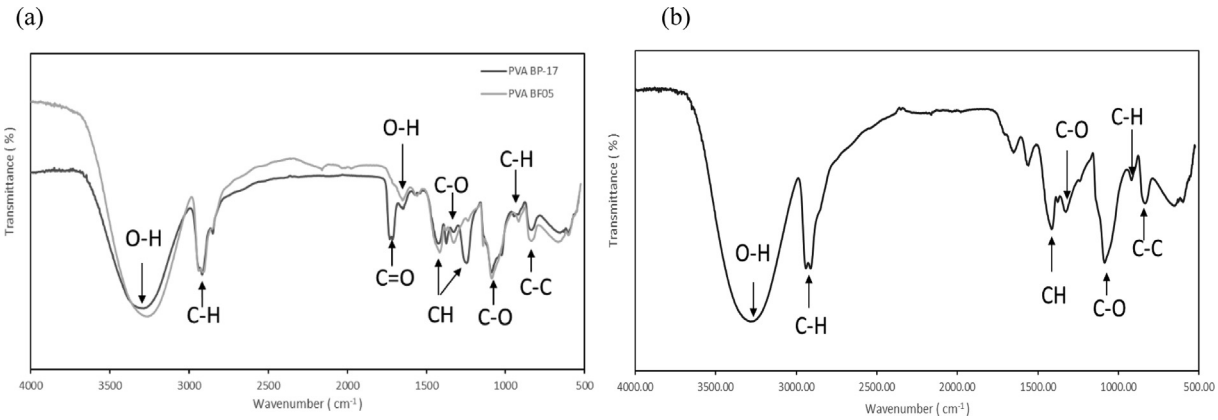

Fig. 4. FTIR spectra of (a) PVA BP-17 and PVA BF-05, (b) chitosan (CS), and (c) PVA BF-05/CS.

The bands around 2911, 2917, 2849, 1417, 1423, 1266 and 917 cm^{-1} are attributed respectively to the stretching and bending vibration of C–H bonds from methyl or methylene groups [15]. The bands around 1329, 1087, 1374 and 1088 cm^{-1} are assigned to the deformation vibration of C-O and bands around 834 and 837 cm^{-1} are attributed to the stretching vibration of C–C [15]. From the results, it is clear that the intensity of the band assigned to C=O stretching decreased when the hydrolysis degree rose. This band became important with the increase of the acetate amount, compatibly decreases when the hydrolysis degree increases [14,16]. The FTIR spectrum of the PVA BF-05/CS is shown in Fig. 4b. As the FTIR spectra of scaffolds at different PVA:CS ratio showed similar bands, the spectrum of PVA:CS blended at 75:25 was shown in order to analyse the interactions between the polymers. It is worth mentioning that the band corresponding to the stretching vibration of O–H located at 3266 cm^{-1} of PVA was shifted to a higher wavenumber (3278 cm^{-1}), illustrated in Fig. 2b, indicating physical interactions such as H-bonding between PVA and CS.

The swelling property of scaffolds is an important parameter in tissue engineering since it is essential for cell growth and proliferation. A low degree of swelling could decrease the absorption of nutrition from the fluid taken up by the scaffold which could affect cell proliferation, while a high degree of swelling could increase the pore size eventually affecting cell attachment [17].

Firstly, PVA BF-05 scaffolds with concentrations of 2.5, 5.0, 7.5, and 10% (w/v) were analysed. Only 10% (w/v) PVA scaffolds showed an adequate swelling ability (Fig. 5a), since the other scaffolds (2.5–7.5% (w/v)) were broken into pieces, making the swelling measurement complicated. Therefore, it was decided that these low PVA concentrations were not suitable for tissue engineering application. 10% (w/v) PVA BF-05 scaffolds reached a swelling equilibrium within 4 h, showing a maximum swelling ratio of 360%. However, as mentioned above, these scaffolds did not present regular shape and so, 8.5% (w/v) PVA BF-05 scaffolds were investigated by swelling test. The maximum swelling ratio of 8.5% (w/v) PVA BF-05 scaffold was 406%, and it reached a swelling equilibrium within 24 h. Therefore, the initial PVA concentration not only affected the shape of scaffolds but also affected the swelling behaviour of samples, since 10% (w/v) PVA BF-05 scaffolds showed lower values (360%) than 8.5% (w/v) scaffolds (406%).

On the other hand, swelling behaviour of PVA BP-17 scaffolds was analysed and for that, 8.5% (w/v) PVA BP-17 scaffolds were prepared. Scaffolds prepared with lower hydrolysis degrees presented the maximum swelling of 160% after 15 min of immersion in PBS, showing lower swelling values in following time points, becoming fully dissolved within 24 h. In order to check the effect of initial PVA concentration on swelling behaviour, 10% (w/v) PVA BP-17 scaffolds were also prepared, and the same results were obtained. So, it was decided that PVA BP-17 was not a good polymer to prepare scaffolds for tissue engineering applications. These results were related to the hydrolysis degree (HD) of the PVA BP-17 used in this study. Water resistance increases with the HD of the PVA polymer [16]. As previously shown, PVA BP-17 presented a lower HD degree (86–89%) than PVA BF-05 (98.5–99.2%).

Taking 8.5% (w/v) PVA BF-05 scaffolds as the most appropriate type of PVA and concentration regarding shape and water stability, different ratios (50:50 and 75:25) and MWs (LMW, MMW and HMW) of 1% (w/v) CS were added into PVA formulation and the swelling behaviour was analysed. The PVA/CS scaffold at the ratio of 50:50 did not present appropriate swelling behaviour since they rapidly got broken into pieces within 4 h in PBS at 37 °C, making the swelling analysis difficult. Fig. 5b shows the swelling behaviour of 8.5% (w/v) PVA BF-05 scaffolds without and with LMW and MMW CS at the ratio 75:25. It was observed that the addition of

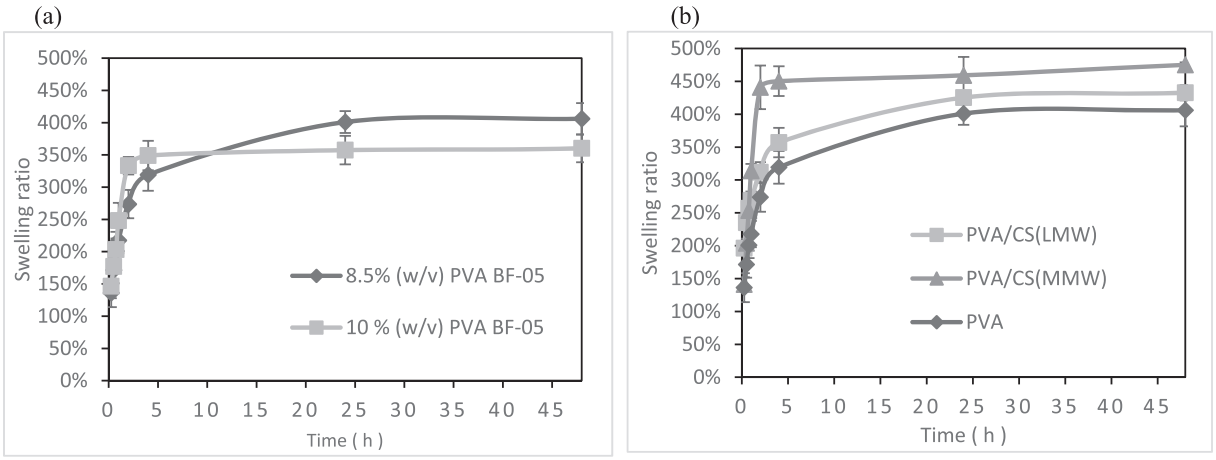

Fig. 5. Swelling behaviour of (a) 8.5 % (w/v) PVA BF-05 scaffolds and of 10 % (w/v) PVA BF-05 scaffolds, and (b) 8.5% (w/v) PVA BF-05/1% (w/v) CS with low molecular weight (LMW) (coded as PVA/CS(LMW)), 8.5% (w/v) PVA BF-05/1% (w/v) CS with medium MW (MMW) (coded as PVA/CS(MMW)) and 8.5% (w/v) PVA BF-05.

CS notably increased the water absorption of PVA scaffolds. This could be related to a decrease in PVA polymeric chain physical interactions when CS was added, as well as the presence of weaker H-bonds between PVA and CS polymers as showed in FTIR analysis. In addition to that, the increase in swelling could be due to the hydrophilic nature of CS which could favour the transport of the water molecules through the network [18,19] increasing the water absorption behaviour of PVA/CS scaffolds. A previous study reported that PVA/CS scaffolds possess a great swelling capacity because of their highly porous structure and the presence of hydrophilic groups (such as hydroxyl groups and amino groups), capable of establishing H-bonds with water molecules [20].The samples with HMW CS disintegrated into small pieces within 1 h. However, the other PVA/CS scaffolds (LMW and MMW) reached the swelling equilibrium in 24 h, showing maximum swelling ratios of 433% and 475% for LMW and MMW CS, respectively. These results showed that the swelling behaviour of PVA/CS scaffolds increased with CS MW. This can be related to a higher chain-relaxation ability in a higher MW of CS as a result of increasing the entanglement of the polymeric chain [18].

The degradation of PVA and PVA/CS scaffolds under physiological conditions was evaluated. The scaffolds maintained their shape and integrity after 7 days of immersion. PVA scaffolds presented the lowest degradation rate (11.15% w/v), compared to PVA/CS scaffolds (Fig. 6). The addition of LMW CS showed a slight increase in scaffolds' degradation percentage (11.59% w/v), which was more notable when MMW CS was used (13.28% w/v). As was expected from swelling results, PVA and PVA/CS degradation results showed a similar trend as a function of MW. PVA/CS(MMW) scaffolds presented higher swelling and degradation values than PVA/CS (LMW) scaffolds.

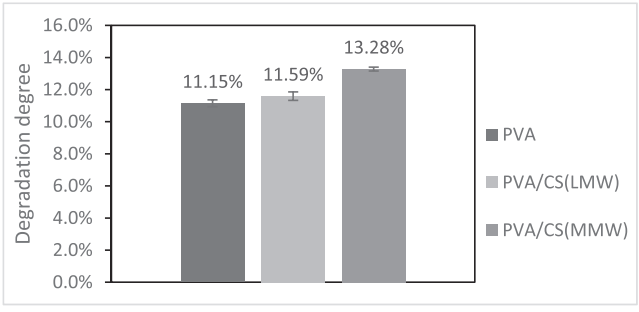

Fig. 6. The degradation ratio of PVA scaffolds, PVA/CS (LMW) scaffolds and PVA/CS (MMW) scaffold.

The degradation results showed a complete dissolution in PBS of PVA BP-17 scaffolds within 24 h. The degradation test showed lower degradation values (11.15 % w/v) of PVA BF-05 scaffolds after 7 days in PBS. These differences in degradation behaviours can be attributed to the HD of PVA polymers that determines the chemical structure of the polymer and so, the presence of physical bonds such as H-bonds, as stated in swelling results. According to Limpan, et al. [16], fully hydrolysed PVA polymers are more water resistant than partially hydrolysed PVA polymers, due to the presence of physical interaction between PVA molecules such as H- bonds. The presence of higher H-bonds amount into PVA BF-05 scaffolds could slow down the absorption of PBS within the scaffold's network and increase their physical integrity, showing better water stability than PVA BP-17.

Regardless of the CS MW, PVA/CS scaffolds showed an increase in the degradation which would be attributed to the loss of polymer mass, mainly CS. PVA and CS both are biodegradable and non-toxic polymers and CS is a natural polymer with better biodegradable character than PVA. CS can be degraded in vivo by several enzymes, mainly by lysozyme (a nonspecific protease in all mammalian tissues) [21]. In the application of tissue engineering, scaffolds should degrade at a rate which could match the regeneration of new tissues [22] and therefore, scaffolds employ for different tissues require varying degradation rates. Considering this and bearing in mind that CS addition modified the degradation percentage of PVA scaffolds, these results suggested the potential of controlling the degradation of PVA/CS scaffolds in order to design biomaterials with tailored properties such as degradation and swelling behaviour for biomedical applications. Furthermore, by controlling scaffolds' degradation and water absorption capacity, the release of additives such as bioactive or drugs can be controlled for drug delivery applications. Drug delivery systems have emerged as an alternative method for disease and after-surgery treatments. The active substance is loaded on a carrier such as a scaffold and is released at a specific rate avoiding over dosage and side effects [21].

In previous sections, 8.5% PVA BF-05 scaffolds showed the appropriate shape and swelling behaviour and therefore, this formulation and PVA type were used in order to keep characterising PVA and PVA/CS scaffolds. In this case, although regular shapes were obtained with all CS MWs, all scaffolds prepared in a 50:50 ratio and with HMW CS at 75:25 ratio did not present appropriate swelling behaviour and so, the inner morphology of 8.5% (w/v) PVA

BF-05 and 8.5% (w/v) PVA BF-05/LMW-MMW CS scaffolds at ratio 75:25 were analysed.

As freeze-drying is one of the most effective methods to create numerous cavities within the bulk material, SEM analysis was performed on the fractured sections of the scaffolds to evaluate the effect of CS addition and PVA:CS ratio on the morphology and pores sizes of the scaffolds. Fig. 7 showed the cross-sectional micromorphologies of PVA, PVA/CS (LMW) scaffolds and PVA/CS (MMW) scaffolds. All samples presented a porous structure with open pores and particularly, the inner PVA scaffolds' structures (Fig. 7a) showed more irregular pore sizes than PVA/CS scaffolds. It is worth mentioning that the addition of CS into PVA scaffolds (Fig. 7b and 7c) notably changed the inner structure of scaffolds. More regular pore sizes were observed in PVA/CS scaffolds irrespective of CS MW. Along with structure information, these images showed a homogeneous mixture of PVA and CS since there was no presence of phase separation. This may be attributed that the good miscibility of PVA and CS at all the compositions due to the intermolecular H-bonding interactions between PVA and CS [23].

As the distribution of the pore size is influenced by the composition, ImageJ computer software was used to analyse SEM images and measure the pore sizes (Fig. 8). PVA scaffolds presented a wide range of pore size distribution:20% of pores in the size lower than 5 μm, 44% of pores in the size range of 5–10 μm, 16% of pores in the size range of 10–20 μm and 20% of pores in the size above 20 μm. When CS was added, the pore size distribution of scaffolds notably changed. Above 90% of pores were in the size lower than 5 μm and less than 10% of pores were in the size range of 5–10 μm. PVA with LMW CS and MMW CS showed similar pore size distributions.

Some studies showed that the addition of CS in PVA changed the organization of scaffolds' internal structure, becoming more porous [20,24]. The morphology and porosity of scaffolds can be affected by the preparation conditions. In the freeze-drying process, the aqueous PVA and PVA/CS solutions were frozen where the ice crystals were growing until they met the faces of other crystals. Having different solutions with different viscosities and MWs, could affect the growth of ice crystals, which were later on sublimated during the freeze-drying process leaving pores with different sizes [20]. These morphology changes could be related not only due to an increase of PVA solution viscosity when CS was added into the formulation [11], but also because some physical interactions between PVA and CS occurred as shown in FTIR results. These results indicated that CS addition plays an important role in the tuning of pores size and pore distribution of PVA scaffolds. Furthermore, these changes in porosity could affect the swelling behaviour of PVA scaffolds, promoting the water absorption of PVA scaffolds when CS was added [25,26].

The PVA and PVA/CS scaffolds were analysed by EDX spectroscopy in order to observe the effect of CS addition on scaffolds' chemical elements. Considering the chemical elements presented in PVA (C, H, O) and CS (C, H, O, N), special attention was given to Nitrogen (N) element. As can be seen in Table 2, there was no presence of the N element in PVA scaffolds while PVA/CS scaffolds showed the presence of this element.

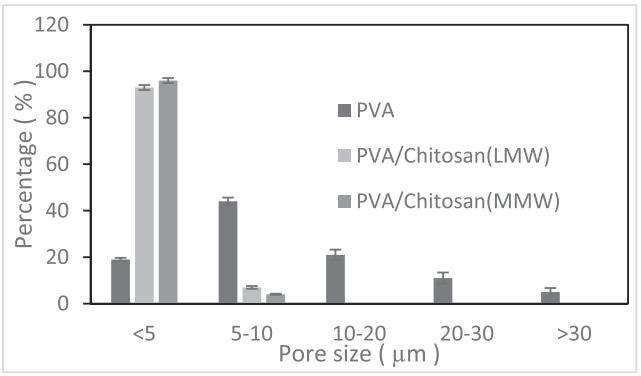

Fig. 8. Pore size distribution of PVA and PVA/CS of scaffolds.

Table 2
Atom percentages of chemical elements presented in PVA and PVA/CS scaffolds.

Atom (%)	C	O	N
PVA	57.75	42.25	–
PVA/CS (LMW)	57.80	41.30	0.90
PVA/CS (MMW)	56.93	42.11	0.96

EDX spectroscopy results showed that CS has been successfully blended with PVA because the element of Nitrogen (N) which typically exists in CS presented in PVA/CS scaffolds. This was consistent with the literature where it has been reported that PVA/CS scaffolds have good chemical properties which was mainly attributed to the interactions between CS and PVA in the blend through hydrophobic side-chain aggregation and intermolecular and intramolecular H-bonds [11], as shown in Fig. 2b.

The mechanical properties of scaffolds are important factors that affect their application in tissue engineering since the scaffold structure not only supports the cell/tissue construction but also in the case of load-bearing tissues such as AC, the scaffolds should provide mechanical support to the applied loading. In order to assess the effect of the CS addition and CS MW on the mechanical properties of the scaffolds, the stress–strain curves, obtained by unconfined compression tests at 0–60% strain were analysed. The unconfined compression tests were conducted on the scaffolds both in dry and hydrated conditions. Within 2% strain, the stress–strain curve showed a linear region and so, the compressive modulus (elastic modulus) at the 2% strain was calculated (Fig. 9a).

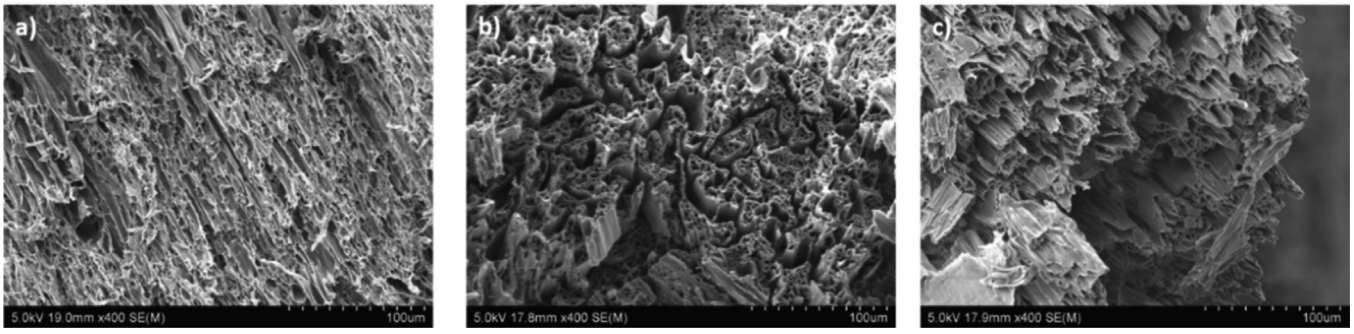

Fig. 7. SEM image of (a) 8.5% (w/v) PVA-BF05; and 8.5% (w/v) PVA-BF05/1% (w/v) (CS) with (b) low molecular weight (LMW), and (c) medium MW (MMW) CS. Magnification x400.

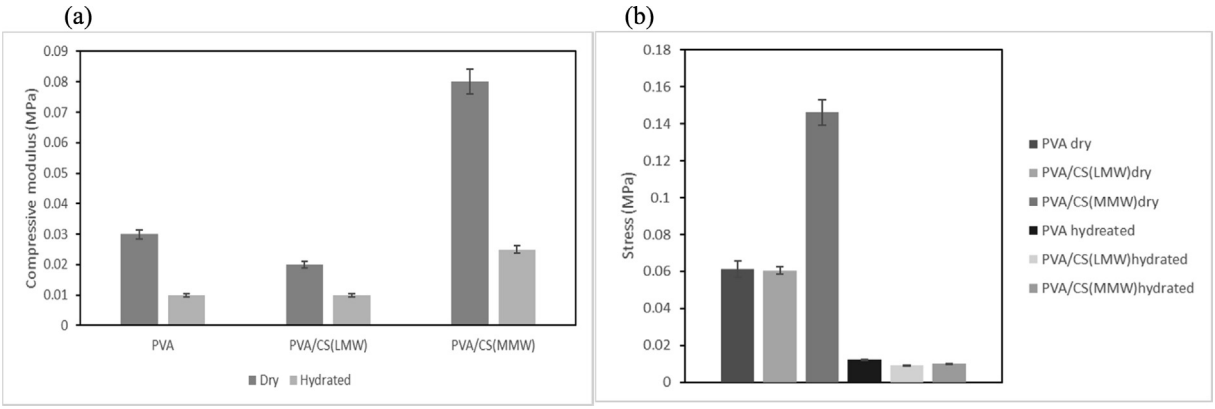

Fig. 9. (a) Compressive modulus of PVA, PVA/CS (LMW) and PVA/CS (MMW) scaffolds in dry and hydrated condition within 2% strain, (b) Stress values at 20% strain of PVA, PVA/CS (LMW) and PVA/CS(MMW) both in dry and hydrated condition.

The mechanical behaviour of dry PVA scaffolds was notably changed with the addition of CS. The compressive modulus of PVA (30 kPa) decreased when LMW CS was added (20 kPa), but it increased when MMW CS (80 kPa) was added into the formulation. These behaviours could be related to changes in morphology and physical interactions as shown by SEM and FTIR, respectively. The morphological features such as pore size and porosity highly influence the mechanical properties of scaffolds [27]. The differences in pore arrangement, pore size and porosity are the main factors which contribute to the variation of mechanical properties of the scaffold [27]. This is consistent with SEM analysis results in the present study, PVA/CS(MMW) presented higher porosity and smaller interconnected pores which made the scaffolds have higher compressive modulus.

On the other hand, hydrated samples showed lower compression strength than the dry samples since water could act as a plasticiser, making the scaffolds less rigid (Fig. 9a). The compressive modulus of hydrated scaffolds was reduced by more than 50%, resulting in 10 kPa, 10 kPa and 25 kPa, for PVA, PVA/CS(LMW) and PVA/CS(MMW) scaffolds, respectively. Stresses at 20% strain of scaffolds are depicted in Fig. 9b. As can be observed, the dry PVA/CS (MMW) scaffolds showed the highest stress value (146 kPa), which was more than two times the value of PVA and PVA/CS(LMW) scaffolds (60 kPa). However, when the scaffolds were hydrated, the stresses at 20% strain of all three types of scaffolds decreased dramatically, showing the same values. Hydroxyl group in PVA and amide group in CS interacted with water molecules by H-bonds which led to hydrating effect and led to a decrease in mechanical properties of scaffolds [28].

Considering all these results, it could be said that the addition of CS LMW notably decreased the rigidity of PVA scaffolds while the presence of CS MMW in the scaffolds significantly increased the stiffness of the material. This could be related to the interactions between PVA and CS through hydrophobic side-chain aggregations and intermolecular and intramolecular H-bonds [11]. In addition to physical bonds, the MW could have an impact on the mechanical properties of scaffolds. When CS MW increased, the mechanical properties increased. The morphology changes could also improve the mechanical properties of PVA/CS scaffolds. When the scaffolds were hydrated, a decrease of over 50% in compressive modulus was observed. This was related to the plasticising effect of water [29]. Regarding CS incorporation, the addition of different MWs of CS in PVA scaffolds increased the stiffness of hydrated samples. However, in order to further enhance the mechanical properties of these scaffolds, the addition of new compounds into the formulation could be a promising approach to improve the mechanical properties of PVA/CS scaffolds. Previous studies showed that hydroxyapatite or calcium phosphate biomaterial could be added to CS scaffolds to improve the mechanical strength for cartilage tissue engineering applications [30].

4. Conclusions

PVA/CS scaffolds were fabricated by freeze-drying method and their characterisation was carried out according to their biomedical application. The effect of hydrolysis degree (HD) of PVA in polymers' chemical structures and water resistance were investigated through FTIR and swelling tests. High HD PVA possess more –OH group for promoting physical bonds that provided better water stability of scaffolds. Therefore, PVA-BF05 polymer was more suitable for tissue engineering applications than PVA BF-17. The addition of CS (LMW and MMW) into PVA scaffolds at a ratio of 75:25 (PVA:CS) can increase the swelling ratio and degradation rate, tailor the porous structure with more uniform porosity and pore size and increase mechanical properties of PVA scaffolds in the dry state. These effects were more notable for MMW than LMW CS. Taking all the results into account and considering the controllability of properties such as swelling, degradation and porosity of scaffolds, these PVA/CS materials can be used for different biomedical applications such as wound healing, bone tissue engineering, cartilage regeneration and drug release applications. In order to promote the use of these scaffolds in cartilage regeneration applications, the mechanical properties of PVA/CS scaffolds have to be improved.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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