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Effect of polymer concentration in cryogelation of gelatin and poly (vinyl alcohol) scaffolds

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Abstract. The aim of this study was to investigate the effect of total polymer concentration on the chemical structure, morphology of pores, porosity, swelling ratio, degradation of gelatin-poly (vinyl alcohol) (Gel-PVA) cryogel scaffolds. Porous cryogels were prepared with cryogelation technique by using glutaraldehyde as a crosslinker. Functional group composition of cryogels after crosslinking was investigated by Fourier Transform Infrared (FTIR). The morphology of cryogels was characterized via scanning electron microscopy (SEM) and porosity analysis. All of the cryogels had a porous structure with an average pore size between 45.58±14.28 and 50.14±4.26 μm. The cryogels were biodegradable and started to degrade in 14 days. As the polymer concentration increased the swelling ratio, the porosity and the degradation rate decreased. Spongy and mechanically stable Gel-PVA cryogels, with tunable properties, can be potential candidates as scaffolds for tissue engineering applications.

Keywords: gelatin; poly (vinyl alcohol); cryogel; scaffold; tissue engineering

1. Introduction

Tissue or organ failure is one of the important problems in medicine (Gualandi 2011). The aim of tissue engineering is the creation of functional tissues and organs *in vitro* to be used as transplants (Tocchio *et al.* 2015). Both synthetic and natural polymers have been extensively utilized in medicine and tissue engineering applications during the last decades (Ceylan *et al.* 2017). An ideal scaffold should be porous, biodegradable, biocompatible, bioabsorbent, and do not induce an immune reaction or inflammation (Kemençe and Bölgen 2017). The natural polymers are biocompatible and bioabsorbent. On the other hand, synthetic polymers are obviously, including adequate physical and mechanical properties and low-cost (Râpă *et al.* 2014). The aim of blending synthetic and natural polymers is to produce ideal scaffolds for tissue engineering applications.

Among the natural polymers gelatin (Gel) is the primary protein of skin, bone and other tissues. Having low cost and biocompatibility, and biodegradability makes gelatin a biomacromolecule which is widely used in biomedical applications. Polyvinyl alcohol (PVA) is a synthetic polymer and exhibits enormous potential in bone tissue engineering applications because of its mechanical properties, biocompatibility and bioresorbability (Ceylan *et al.* 2016, Rodrigues *et al.* 2013).

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Bioresorbable materials are capable of being absorbed into tissue, starts to dissolve (resorbed) and slowly replaced by advancing tissue (Burg and Orr 2008). Gelatin undergoes hydrolytic cleavage in tissue. On the other hand, PVA cannot be hydrolytically cleaved and degradation mechanism of PVA consists of physical erosion and dissolution (Ceylan *et al.* 2016).

Production techniques affect the porosity and interconnection of pores. To fabricate an ideal 3D scaffold for tissue engineering applications, various techniques have been used such as freeze drying, electrospinning, solution casting, freeze thawing and cryogelation. The cryogelation is a technique producing gels in moderately frozen solutions of monomers, macromers or polymeric precursors (Kemençe and Bölgen 2017, Rodrigues *et al.* 2013). Cryogels have high porosity with interconnected macroporous structure, elasticity and good swelling capability in aqueous media.

The objective of this work was to obtain Gel-PVA composite cryogels for tissue engineering applications. Effect of total polymer concentration on the properties of cryogels was investigated. Physical, chemical, and morphological characterizations were analyzed using different techniques and equipment. Chemical structure and pore morphology were demonstrated by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). Porosity measurement was done by measuring the volume of cryogels (Vs) and volume of the pore (Vp) of the Gel-PVA cryogels. Swelling ratio and degradation profile of the cryogels were also determined.

2. Material and methods

2.1 Materials

Gelatin was obtained from Merck, Germany. PVA with a molecular weight 89.000-98.000 g/mol (%99 hydrolyzed) was purchased from Sigma Aldrich, U.S.A. Glutaraldehyde (25%, aqueous) was obtained from Merck, Germany. Distilled water was used in all experiments.

2.2 Preparation of Gel-PVA cryogels

The gelatin and PVA ratios in the Gel-PVA polymer solution was fixed at 90 and 10% (w/w), respectively. Gel-PVA polymer concentration examined were 6, 8 and 10% (w/v). Effect of total polymer concentration on the properties of cryogels was investigated. Polymers were dissolved in deionized water and mixed for 3 h until a homogenous mixture was obtained. After that, glutaraldehyde (5% weight of total polymer amount) was added to the polymer solution. Polymer and glutaraldehyde solution was immediately poured into 2.5 ml syringe and placed into cryostat (Daihan, WCL P12, Korea). The mixture was kept in the cryostat at -12 °C for 2 h. And then kept in the freezer at -16°C for 24 hours. After, the crosslinking reaction completed, the cryogels were thawed at room temperature and washed five times with distilled water to remove unreacted polymers and glutaraldehyde. Before carrying out the characterizations, all cryogels were freeze dried (Free Zone Benchtop Freeze Dry System-7670531, Labconco, U.S.A.). The samples were named 6% Gel-PVA, 8%Gel-PVA and 10% Gel-PVA.

2.3 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was performed (Perkin Elmer, FTIR/FIR/NIR Spectrometer Frontier-ATR, U.S.A.) to investigate the chemical groups in synthesized cryogels, within the range of 400-4000 cm^{-1}.

2.4 Scanning electron microscopy analysis (SEM)

A scanning electronic microscope (SEM, FE-SEM Zeiss/Supra55, Quanta 400F Field Emission, USA) was used to analyze the morphology of synthesized cryogels. The samples were coated with a thin layer of platinum before analysis. SEM was operated at the acceleration of 5 kV and the magnification was 500x.

2.5 Porosity of cryogels

The porosity of cryogels can be calculated by measuring the volume of cryogel (V_c) and the volume of pores (V_p) of the cryogels. Cryogel volume (by using height and diameter) was used to calculate V_c, by using ethanol infiltration method. The weighed cryogels (W_o) were incubated in ethanol and then taken to desiccators for 15 minutes. After that, surface ethanol was removed by using filter paper and then weighed immediately (W_e) (Choudhury *et al*. 2015). V_p was defined as Eq. (1);

$$V_p = (W_e - W_0)/\rho_e \quad (1)$$

where ρ_e (0.805 mg/ml) represents the ethanol density at room temperature.

The porosity of the scaffolds (P%) was calculated with Eq. (2);

$$P\% = (Vp/Vc)x100 \quad (2)$$

2.6 Swelling and degradation studies

In order to examine swelling behavior of the cryogels Eq. (3) was used. The swelling determination was carried out in deionized water at 37 °C in a water bath. Three samples (0.5 cm height and 0.9 cm in diameter) were used to calculate the average swelling ratio. Firstly, the dry weight of the cryogel was recorded. Secondly, the cryogel was immersed in deionized water for a certain time. After that, the scaffold was taken out and filter paper was used to remove excess water from the swollen cryogel. Finally, the weight of wet cryogel was recorded.

$$SR\% = [(M_c - M_o)/M_o]x100 \quad (3)$$

where M_o is the initial dry weight of cryogel, M_c is the swollen weight of cryogel, and SR% is the swelling ratio (Nasri-Nasrabadi *et al.* 2014).

The degradation ratio of cryogels was determined with Eq. (4)

$$DR\% = [(W_o - W_f)/W_o]x100 \quad (4)$$

where W_o is the initial dry weight of cryogel, W_f is the final dry weight of cryogel and DR% is the degradation rate (Choi *et al.* 2013). Three samples (1 cm height and 0.9 cm in diameter) were used to calculate average weight loss. Dry weights of cryogels were recorded. After that, cryogels were transferred to 15 mL tubes filled with deionized water. The temperature of water the bath was adjusted to 37°C. At regular time points, samples were dried and weighed. The degradation profiles of cryogels were followed for 14 days.

3. Results and Discussions

3.1 FTIR analysis

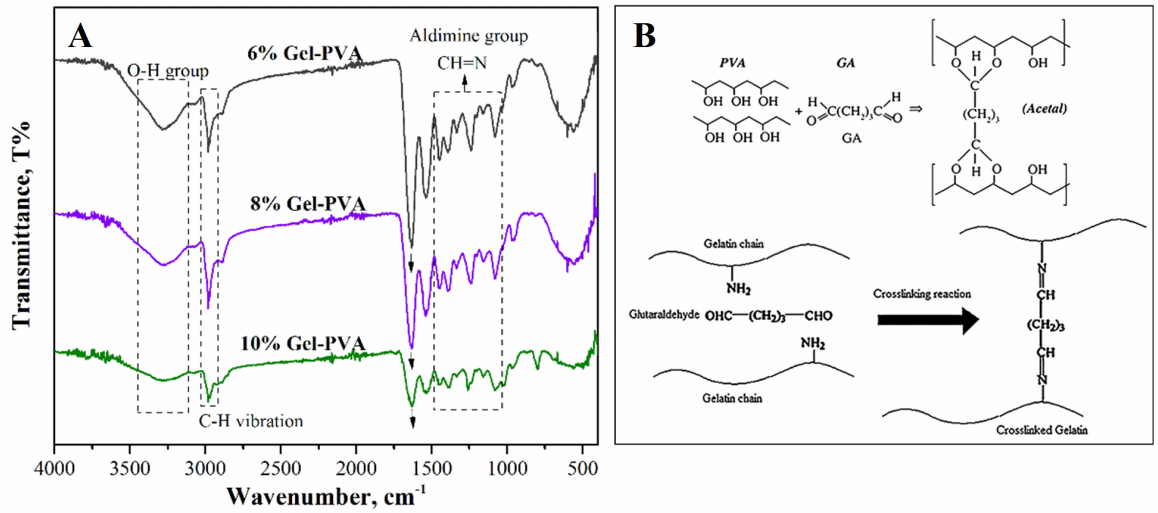

Fig. 1 A) FTIR spectra of cryogels B) Reaction mechanism of PVA-Glutaraldehyde and Gelatin-Glutaraldehyde

FTIR analysis was employed for chemical characterization of cryogels obtained by crosslinking different amounts of Gel-PVA (6% Gel-PVA, 8% Gel-PVA and 10% Gel-PVA) with constant amount of glutaraldehyde. Glutaraldehyde, as a crosslinking agent, links the gelatin and PVA chains with its functional aldehyde groups. The FTIR spectrum of cryogels is demonstrated in Fig. 1 The broad band around 3250 cm^{-1} corresponds to the intermolecular and intramolecular hydrogen bonds (O-H) of PVA (Mansur *et al.* 2008). The vibrational peak observed at 2900 cm^{-1} is the result of the C-H stretch from alkyl groups and the peak started at 1740 cm^{-1} is due to the C=O and C–O stretches of the acetate groups in PVA (Alhosseini *et al.* 2012). The crosslinking reaction between the aldehyde groups of glutaraldehyde and ε-NH_2 functional group of adjacent lysine residues of gelatin and two adjacent hydroxyl groups of PVA was proven by the existence of the peak at 1645 cm^{-1} in Fig.1 B (Ceylan *et al.* 2016). Also, the characteristic absorption of the aldimine linkage at 1450 cm^{-1} and additional peaks of CH=N groups from 1100 to 1740 cm^{-1} show the crosslinking reaction between glutaraldehyde and gelatin (Imani *et al.* 2013, Nguyen and Lee 2010).

3.2 Morphological analysis

The total polymer concentration was altered and the morphology of the cryogels was analyzed by SEM (Fig. 2). An interconnected porous structure was observed for all cryogels. As increasing the polymer concentration to 10% irregular pores in the structure was observed. As decreasing the polymer concentration to 6%, more homogenous porous structure was obtained. The average pore diameter was found to be between 45.58±14.28 and 50.14±4.26 μm. Pore size greatly affects the growth and penetration of cells in the scaffolds. In addition to this, the interconnected porosity plays a significant role in cell survival (cell ingrowth, vascularization and nutrient diffusion), proliferation and migration (Annabi *et al.* 2010). The average pore size and porosity values of spongy cryogels were demonstrated in Table 1. It was observed that the porosity decreased with increasing the total polymer concentration.

Table 1 The average pore size and porosity of cryogels

Cryogels	Pore size, µm	Porosity, %
6% Gel-PVA	50.14±4.26	34.25±0.51
8% Gel-PVA	45.64±10.17	30.02±0.81
10% Gel-PVA	45.58±14.28	24.27±1.37

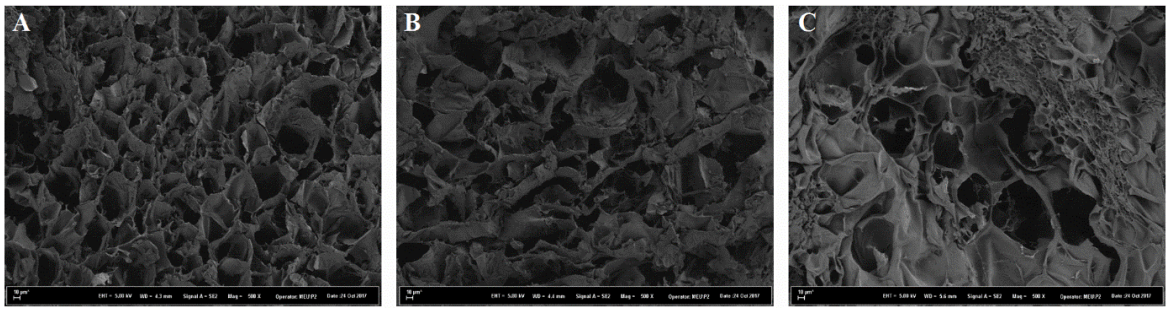

Fig. 2 SEM images of cryogels (A: 6% Gel-PVA, B: 8% Gel-PVA, C: 10% Gel-PVA at magnification of 500x)

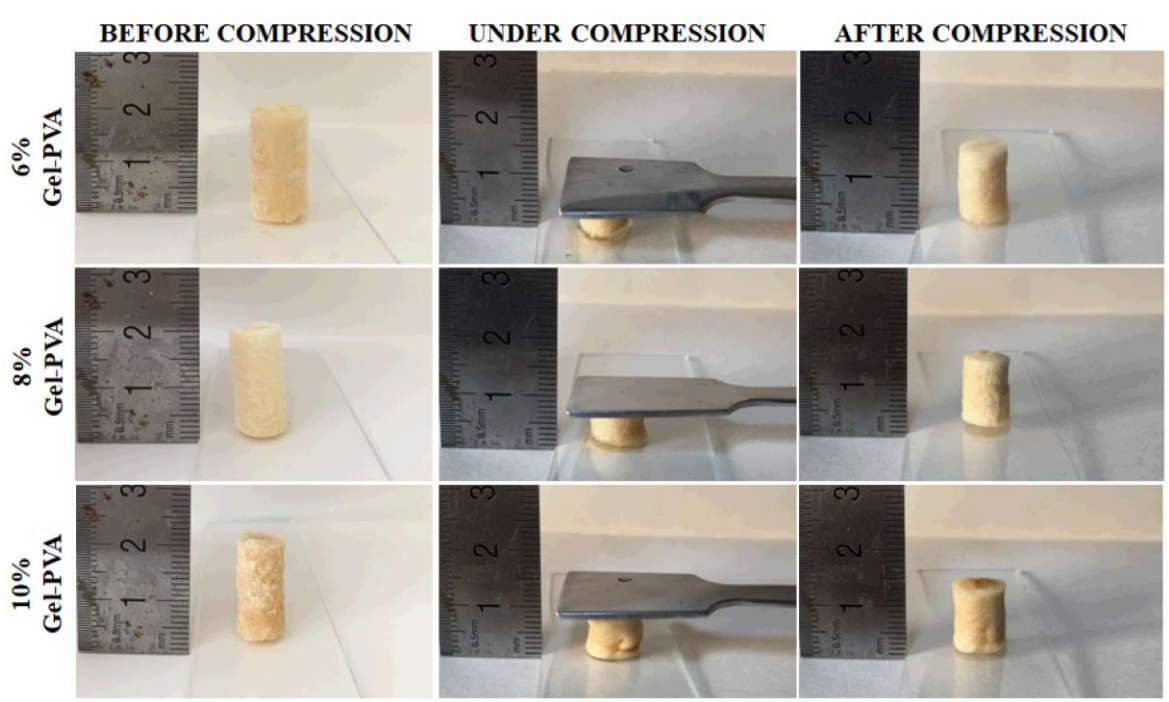

Fig. 3 Images of Gel-PVA cryogels (before, under and after compression)

Compression analysis performed on the cryogels showed that they were highly elastic. Fig. 3 shows the image of cryogels before, under and after compression. The Gel-PVA cryogels could be compressed and did not show significant changes in their dimensions after applying force.

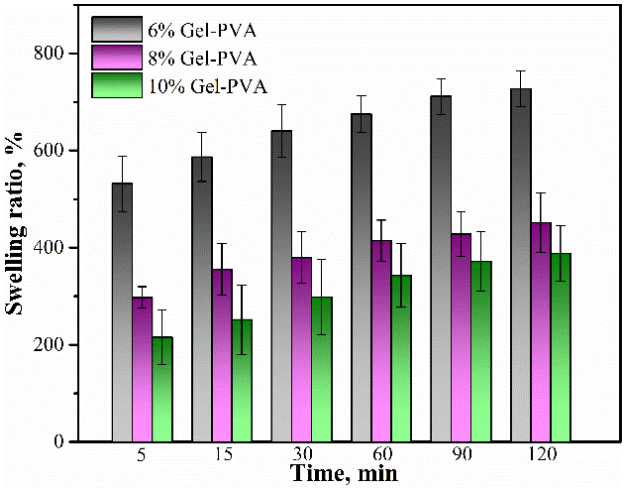

Fig. 4 Swelling profile of Gel-PVA cryogels

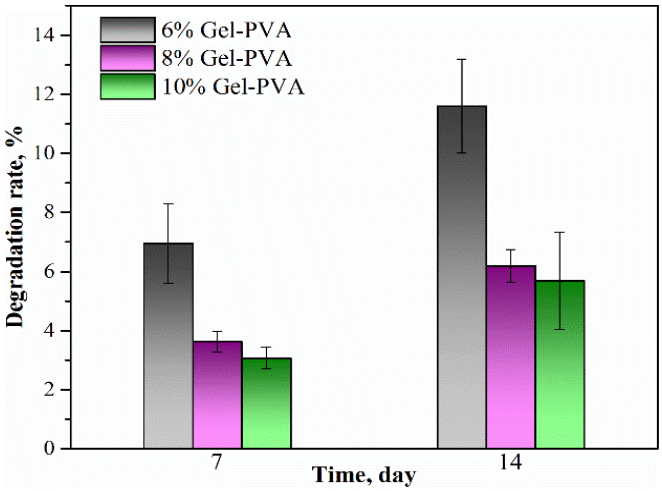

Fig. 5 Degradation profile of Gel-PVA cryogels

3.3 Swelling and degradation studies

The swelling profiles of cryogels are presented in Fig. 4. The interconnected pore structure of the cryogels provided a fast swelling kinetic (Dispinar *et al.* 2012). Therefore, all cryogels have the ability to retain more water than their own dry weight. It was observed that when the polymer concentration decreased swelling ratio of cryogels increased. After 120 minutes, the swelling ratio of 6, 8 and 10% Gel-PVA cryogels were 726.95±37.01, 450.87±60.91 and 387.52±56.87%, respectively. A more spongy structure was formed as total polymer concentration decreased. Higher porosity caused more water uptake, therefore the swelling ratio was increased.

Another important point is degradation behavior of cryogels to determine how far cryogel can withstand to support suitable tissue formation or restoration (Odabas 2016). Degradation profile of cryogels for 14 days is shown in Fig. 5 For all cryogels, as the total polymer concentration increased, the degradation rate decreased.

4. Conclusion

Gel-PVA cryogels with interconnected pore structures were fabricated by cryogelation method. Spongy and mechanically stable cryogels were obtained. The effect of total polymer concentration was investigated. According to the results, as the polymer concentration increased the swelling ratio, the porosity and the degradation rate decreased. The average pore diameter was found to be between 45.58±14.28 and 50.14±4.26 μm. Gel-PVA cryogels with tunable properties can be potential candidates as scaffolds for tissue engineering applications.

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