International Journal of Medical, Medicine and Health Sciences
ISSN: 2517-9969
Vol:11, No:5, 2017

Design and Fabrication of a Scaffold with Appropriate Features for Cartilage Tissue Engineering

S. S. Salehi, A. Shamloo

Abstract**—**Poor ability of cartilage tissue when experiencing a damage leads scientists to use tissue engineering as a reliable and effective method for regenerating or replacing damaged tissues. An artificial tissue should have some features such as biocompatibility, biodegradation and, enough mechanical properties like the original tissue. In this work, a composite hydrogel is prepared by using natural and synthetic materials that has high porosity. Mechanical properties of different combinations of polymers such as modulus of elasticity were tested, and a hydrogel with good mechanical properties was selected. Bone marrow derived mesenchymal stem cells were also seeded into the pores of the sponge, and the results showed the adhesion and proliferation of cells within the hydrogel after one month. In comparison with previous works, this study offers a new and efficient procedure for the fabrication of cartilage like tissue and further cartilage repair.

Keywords**—**Cartilage tissue engineering, hydrogel, mechanical strength, mesenchymal stem cell.

I. Introduction

CARTILAGE is an avascular tissue and has a limited capacity for regeneration. Tissue engineering comes to solve these problems with the aid of natural and synthetic materials. There are various attempts for producing scaffolds and using in cartilage tissue engineering. They are made by natural and synthetic materials and with different methods such as wet and dry electrospinning, hydrogel fabrication and other ways. Each method has advantages and suffers from some shortcomings. Hydrogels can be used in a wide range of areas of research and clinical applications such as tissue engineering and drug delivery. Different polysaccharides, proteins, and synthetic polymers are used in cartilage tissue engineering so far including collagen, gelatin, hyaluronic acid, chitosan, polycaprolactone (PCL), polyvinyl alcohol (PVA), etc. Hydrogels should have some properties to be appropriate for cell seeding. Among these features, the hydrogels must have enough porosity for cell penetration and culture. Gelatin is a biopolymer derived from collagen and has two types including type A which is extracted by partial acid or type B from alkaline hydrolysis of animal collagen [1], [2]. Gelatin is the main component of extracellular matrix in animals and contains a chain of amino acids linked together. Chitosan is a natural polysaccharide derived from chitin and insoluble in water [3]. Applications of chitosan in tissue engineering relates to promoting attachment, proliferation and viability of mesenchymal stem cells [4]. PVA is a synthetic polymer with wide range of use in tissue engineering due to high water content as well as elastic and compressive mechanical properties [5]. It can also be applied in the early stages of wound healing and skin tissue engineering [6].

Microstructure of scaffold like porosity and pore size may have influence on cell growth and extracellular matrix secretion. Several parameters such as crosslinking temperature determine the range of pore size. In a study, the crosslinking temperature was changed from 10 °C to 25 °C to prepare gelatin scaffolds and the range of pore size was from 50 to 500 microns [2]. Pore size of hydrogel which can significantly change gel swelling and its drug delivery behavior, increases as concentration of crosslinking agent increases. The results demonstrated that increasing pore size leads to secretion of larger amounts of GAG and respective gene markers for aggrecan, collagen type I, II and collagen type X. Another important result is maintenance of cell phenotype within larger porosity of scaffolds.

In a study, in order to provide compressive strength and cartilage ECM environment, hydroxyapatite (HA) and chondroitin sulfate were poured into collagen sponge [7]. Collagen type I as a scaffold can control the immunological properties of mesenchymal stem cells [8]. As another composite scaffold, chitosan-gelatin is favorable to chondrocyte adhesion, differentiation, and proliferation [7]. Gelatin sponges can be used as engineered cartilage by fertilizing with optimal amount of b-FGF. Otani et al. showed that gelatin 3 wt.% by four weeks after implementation of chondrocytes, and b-FGF and incorporating beta-TCP can be used as an implant for cartilage tissue [9].

HA was mixed with PVA and gelatin, and a composite material of PVA/gelatin/HA was implanted in the dorsal region of rat [10]. The results of this work suggest this mixture as a cartilage scaffold and replacement in cartilage tissue engineering applications. Presence of polyurethane in combination with PVA can also retain the chondrogenic phenotype of the cells [11].

Crosslinking of gelatin is compared between glutaraldehyde (GTA) and genipin (GP) at room temperature [12]. In vitro cell culture within gelatin scaffolds demonstrated that GP crosslinking can promote proliferation of chondrocytes better than GTA and GP is less cytotoxic.

A. Shamloo is with the Sharif University of Technology, Tehran, Islamic Republic of Iran (corresponding author, phone: +98-21-66165691, fax: +98-21-66000021, e-mail: shamloo@ sharif.edu).

S. S. Salehi is with Sharif University of Technology, Tehran, Islamic Republic of Iran.

In this study, a hydrogel is fabricated by using natural and synthetic materials. They are crosslinked with GTA, and different biocompatibility and biodegradation tests are done to examine the properties of different combinations of the ingredients and bone marrow derived mesenchymal stem cells are seeded through the pores of the scaffold for a period of time.

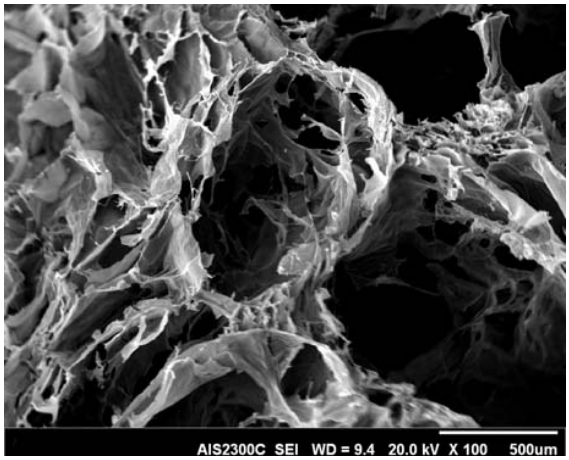
II. Materials and Methods

A. Hydrogel Fabrication

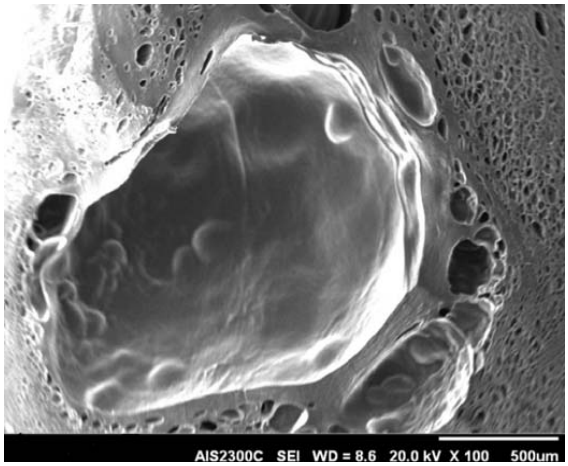
The process of fabricating the hydrogel consists of different stages. Different concentrations of gelatin, chitosan, and PVA were mixed. The hydrogels were prepared by the following procedure. First, gelatin Type A powder was dissolved in DI water and stirred at 50 °C for 1 hour. Then, chitosan with 3, 4 and 5% w/v was dissolved in 2% v/v acetic acid solution with stirring at room temperature for 24 hours. On the other hand, PVA powder was dissolved with 15% and 20% w/v in DI water and heated at 60 °C stirring for 6 hours. Gelatin, chitosan, and PVA solutions were mixed at equal volume ratios. The mixture was placed at room temperature for 1 hour to remove the bubbles. The hydrogels were put in a refrigerator at 4 °C for gelling. After gel formation, the scaffolds were cross-linked using GTA 0.2% w/v at room temperature for 24 hours. Then, the samples were placed in 20 °C refrigerator for 24 hours, -80 °C refrigerator for 24 hours, and transferred to vacuum freeze dryer for 24 hours. After freeze drying, a porous scaffold was formed.

B. Scanning Electron Microscopy

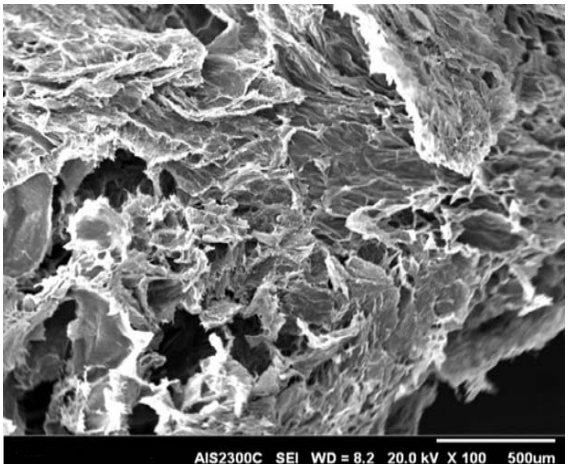
Gelatin-Chitosan-PVA hydrogels with different concentrations were blended and sputter coated with a thin gold layer for scanning electron microscopy. The average pore size was estimated by using at least 15 pores of hydrogels. Morphology of the scaffolds can be seen in Fig. 1.



(a)



(b)



(c)

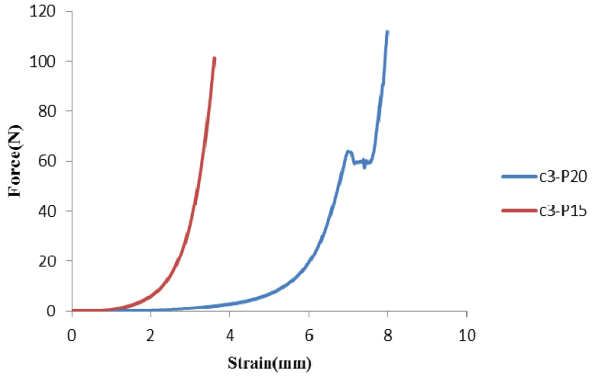
Fig. 1 Scanning electron microscopy of the porosity of the hydrogel for (a) 3 wt.% chitosan, (b) 4 wt.% chitosan and (c) 5 wt.% chitosan

Fig. 1 shows that increasing chitosan concentration would result in decreasing porosity of the scaffold. On the other hand, as the concentration of chitosan increases, its viscosity also would be greater which is not suitable for cell seeding. So, the concentration of 3 wt.% has the largest porosity. Other properties of the hydrogel should be checked to choose a combination of gelatin, chitosan and PVA.

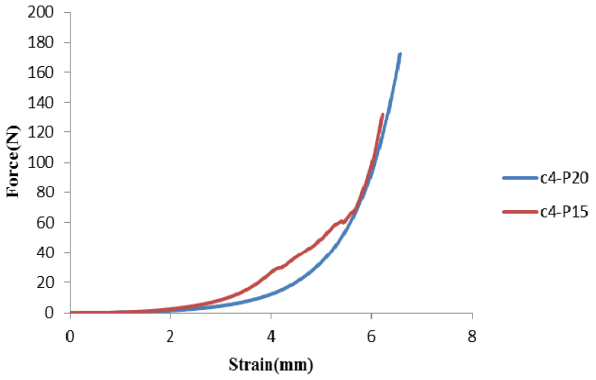
C. Mechanical Strength

Compressive strength of hydrogels in wet state were determined using a tabletop uniaxial material testing machine (H10K-S) equipped with a 10 N load cell with a rate of 1 mm per minute [13], [15], [16]. Specimens had cylindrical shape (14mm, 8mm) and three samples were tested for each combination of natural and synthetic polymers. Young's modulus of elasticity was calculated according to the slope of linear region of force-strain curve for each type of hydrogels. Force-displacement diagram is drawn for three concentrations of chitosan, i.e. 3, 4, and 5% (w/v). The content of gelatin is

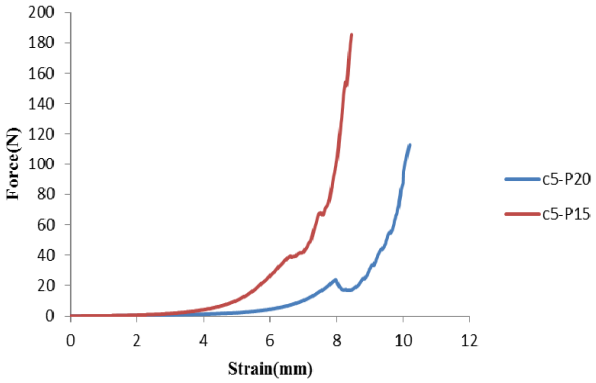
remained constant in all of the samples, and the concentration of PVA is changed between 15 and 20% (w/v).



(a)



(b)



(c)

Fig. 2 Force- Tension graph for hydrogels with 5 wt.% gelatin, and (a) 3 wt.% chitosan(c-3), (b) 4 wt.% chitosan (c-4) and (c) 5 wt.% chitosan (c-5). Red lines indicate using 15 wt.% PVA (p-15) and blue ones are hydrogels with 20 wt.% (P-20)

Mechanical strength test in each figure shows that increasing PVA content would result in decreasing compressive mechanical strength and increasing chitosan content leads to increasing the Young's modulus of elasticity. So, a hydrogel consisting of chitosan (5% w/v), gelatin (5% w/v), and PVA (15% w/v) was selected having good mechanical strength with respect to other compositions. In order to test other properties of the hydrogel, this composite hydrogel was chosen.

D.In vitro Cell Culture

The scaffolds were immersed in PBS for 15 min and then in DI water for 15 min three times. They were soaked in 70% ethanol for three hours and were put in a humidified incubator at 37 °C and 5% CO_2 with culture medium for 24 hours. All the above steps were done under a laminar flow hood.

Bone marrow derived mesenchymal stem cells were seeded in cell culture flasks and supplied with DMEM containing 10% fetal bovine serum, 100 U ml^{-1} penicillin and 100 µg ml^{-1} streptomycin. They were cultured until passage five, and the culture medium was changed every two days. Hydrogels were seeded with density of 16 million cells per milliliter in 24-well culture plates. The culture medium was changed every two days after seeding cells in the scaffolds.

E. MTT Assay

3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to measure the viability of cells seeded within porosity of scaffolds and mitochondrial activity of bone marrow derived mesenchymal stem cells after 3, 5, 14, and 30 days after culturing. Briefly, half of culture medium was removed and replaced with MTT (5 mg/ml) [13], [14]. Three sponges were used for each MTT test. Hydrogels without cells and only with culture medium were used as standard samples, and treated culture plates were control groups. The hydrogels were incubated at 37 °C and 5% CO_2 for four hours. After removing the whole medium, the converted dye was dissolved in DMSO and was shacked for five minutes at 37 °C. The absorbance was measured using ELIZA reader.

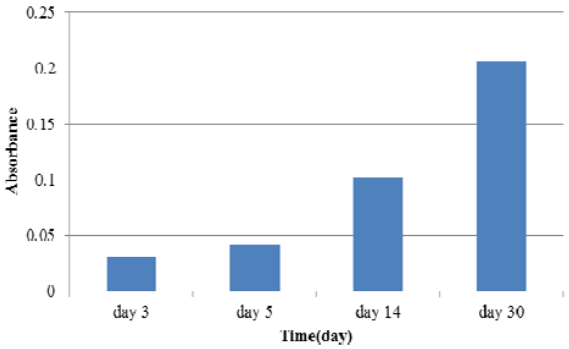


Fig. 3 MTT assay of cell seeded scaffolds after one month

Standard and control samples were also considered, and the results of MTT assay were calibrated with respect to these limits between zero and one. Fig. 1 shows the viability and proliferation of cells after one month. This increasing trend shows that porosity of the sponge is enough, and nutrients can penetrate to the bottom of the hydrogel. On the other hand, the hydrogel is non-toxic and compatible with cells and their growth and proliferation.

F. Swelling Analysis

Phosphate buffered saline (PBS, pH=7.4) is a commonly swelling medium for testing the swelling property of hydrogels. Three samples of hydrogels weighed (W_0) and immersed in PBS for 1, 2, 4, 8, 24, and 48 hours at room temperature to estimate the crosslink density which is important for cell adhesion and growth [16]. After soaking time, the sponges were wiped with filter paper to remove excess water and then weighed (W_t). The swelling ratio can be calculated based on below expression,

Swelling ratio (%) = $((W_t - W_0)/W_0) * 100$

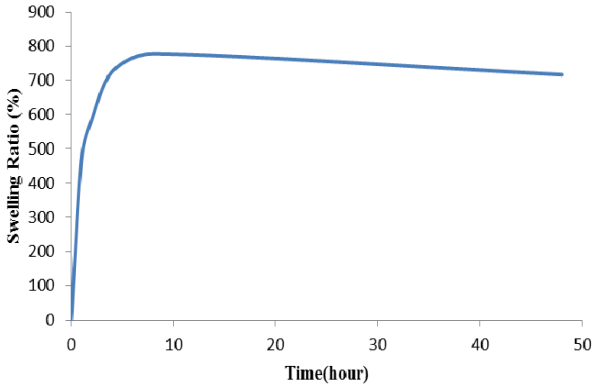


Fig. 4 Swelling ratio of sponges after two days

As it can be seen, the water content of the hydrogel reaches saturation after 24 hours and can absorb water to seven times its volume due to a structure with high porosity. This means that after cell seeding, culture media along with nutrients can be saved within the sponge.

III. Conclusion

The results of this study demonstrated that composite hydrogel containing gelatin, chitosan, and PVA fabricated by freeze drying has very good properties such as biocompatibility, biodegradability, and mechanical strength similar to cartilage tissue. Different concentrations of each of the ingredients were tested, and the best composition was selected and crosslinked to each other. Then, the hydrogels were freeze dried and sterilized to be ready for seeding bone marrow derived mesenchymal stem cells. MTT assay after one month of culturing cells within the porosity of the scaffold showed adhesion and proliferation of cells within the sponge. SEM images from the cross section of the sponge proved that this hydrogel has enough porosity for seeding cells and enough culture media can penetrate to the bottom of it. The scaffold developed in this manner can also accommodate drug delivery for cartilage tissue to empower with more functions. Further studies are necessary to investigate the tensile and cyclic modulus of elasticity and viscoelastic properties of the hydrogel. By proving these features and considering ethics in this realm, this hydrogel can be replaced in injured tissue to mimic the functions of the cartilage.

References

[1] S.-M. Lien, L.-Y. Ko, and T.-J. Huang, "Effect of pore size on ECM secretion and cell growth in gelatin scaffold for articular cartilage tissue engineering," *Acta Biomaterialia,* vol. 5, pp. 670-679, 2009.

[2] M. E. Hoque, T. Nuge, T. K. Yeow, N. Nordin, and R. Prasad, "Gelatin Based Scaffolds for Tissue Engineering-A Review," *Polymers Research Journal,* vol. 9, p. 15, 2015.

[3] E. Mirzaei B, A. Ramazani SA, M. Shafiee, and M. Danaei, "Studies on glutaraldehyde crosslinked chitosan hydrogel properties for drug delivery systems," *International Journal of Polymeric Materials and Polymeric Biomaterials,* vol. 62, pp. 605-611, 2013.

[4] B. Balakrishnan and R. Banerjee, "Biopolymer-based hydrogels for cartilage tissue engineering," *Chemical reviews,* vol. 111, pp. 4453-4474, 2011.

[5] M. I. Baker, S. P. Walsh, Z. Schwartz, and B. D. Boyan, "A review of polyvinyl alcohol and its uses in cartilage and orthopedic applications," *Journal of Biomedical Materials Research Part B: Applied Biomaterials,* vol. 100, pp. 1451-1457, 2012.

[6] A. Karimi and M. Navidbakhsh, "Mechanical properties of PVA material for tissue engineering applications," *Materials Technology,* vol. 29, pp. 90-100, 2014.

[7] W. Zhao, X. Jin, Y. Cong, Y. Liu, and J. Fu, "Degradable natural polymer hydrogels for articular cartilage tissue engineering," *Journal of Chemical Technology and Biotechnology,* vol. 88, pp. 327-339, 2013.

[8] T. Yuan, L. Zhang, K. Li, H. Fan, Y. Fan, J. Liang, *et al.*, "Collagen hydrogel as an immunomodulatory scaffold in cartilage tissue engineering," *Journal of Biomedical Materials Research Part B: Applied Biomaterials,* vol. 102, pp. 337-344, 2014.

[9] Y. Otani, M. Komura, H. Komura, T. Ishimaru, K. Konishi, H. Komuro, *et al.*, "Optimal amount of basic fibroblast growth factor in gelatin sponges incorporating β-tricalcium phosphate with chondrocytes," *Tissue Engineering Part A,* vol. 21, pp. 627-636, 2015.

[10] M. Wang, Y. Li, J. Wu, F. Xu, Y. Zuo, and J. Jansen, "In vitro and invivo study to the biocompatibility and biodegradation of hydroxyapatite/poly(vinyl alcohol)/gelatin composite," *Journal of Biomedical Materials ResearchPart A,* vol. 85, pp. 418-426, 2008.

[11] M. A. Shokrgozar, S. Bonakdar, M. M. Dehghan, S. H. Emami, L. Montazeri, S. Azari, *et al.*, "Biological evaluation of polyvinyl alcohol hydrogel crosslinked by polyurethane chain for cartilage tissue engineering in rabbit model," *Journal of Materials Science: Materials in Medicine,* vol. 24, pp. 2449-2460, 2013.

[12] S.-M. Lien, W.-T. Li, and T.-J. Huang, "Genipin-crosslinked gelatin scaffolds for articular cartilage tissue engineering with a novel crosslinking method," *Materials Science and Engineering: C,* vol. 28, pp. 36-43, 2008.

[13] T. Miao, E. J. Miller, C. McKenzie, and R. A. Oldinski, "Physically crosslinked polyvinyl alcohol and gelatin interpenetrating polymer network theta-gels for cartilage regeneration," *Journal of Materials Chemistry B,* vol. 3, pp. 9242-9249, 2015.

[14] X. Li, J. Xie, X. Yuan, and Y. Xia, "Coating electrospun poly (ε-caprolactone) fibers with gelatin and calcium phosphate and their use as biomimetic scaffolds for bone tissue engineering," *Langmuir,* vol. 24, pp. 14145-14150, 2008.

[15] R. Zheng, H. Duan, J. Xue, Y. Liu, B. Feng, S. Zhao, *et al.*, "The influence of Gelatin/PCL ratio and 3-D construct shape of electrospun membranes on cartilage regeneration," *Biomaterials,* vol. 35, pp. 152-164, 2014.

[16] Z.-S. Shen, X. Cui, R.-X. Hou, Q. Li, H.-X. Deng, and J. Fu, "Tough biodegradable chitosan-gelatin hydrogels via in situ precipitation for potential cartilage tissue engineering," *RSC Advances,* vol. 5, pp. 55640-55647, 2015.