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Preparation of poly(vinyl alcohol)-chondroitin sulfate hydrogel as matrices in tissue engineering

Chih-Ta Lee, Po-Han Kung, Yu-Der Lee*

Department of Chemical Engineering, National Tsing Hua University 101, Section 2, Kuang Fu Road, Hsin Chu, Taiwan 300, ROC

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Abstract

Poly(vinyl alcohol) (PVA)-Chondroitin sulfate(CS) hydrogels are prepared using glutaraldehyde as the crosslinking agent. The obtained hydrogels, which have the advantages of both PVA and CS, can be used as a material for scaffolds in tissue engineering. They promote not only cell adsorption, but also cell growth. The cells that adhere to the CS20 hydrogel are connected in the form of a dense sheet. The number of cells in CS20 hydrogel was 2.56 times that in CS00 hydrogel, which is hydrophilic and does not favor cell adhesion. A cell test also shows that the CS contained in the scaffolds promotes the effective growth of BHK cells on the surface. It promotes interaction between cells and scaffold. Additionally, the strength of PVA provides scaffolds with mechanical properties appropriate for culturing cells in vitro.

Keywords: Hydrogel; Poly(vinyl alcohol); Chondroitin sulfate; Tissue engineering; Crosslinking

1. Introduction

Hydrogels, highly hydrated polymer networks, are formed by the chemical or physical crosslinking of the hydrophilic polymer. The characteristics of hydrogels, including sensitivity to the environment, tissue-like water content and elasticity afford the potential for biomedical application. For instance, hydrogels are used for delivering drugs (Ichikawa & Fukumori, 2000; Matsumoto, Ikeda, Harada, & Kataoka, 2003), artificially dressing burns (Choi, Hong, Lee, Song, Park, & Nam, 1999; Yannas, Lee, Orgill, Skrabut, & Murphy, 1989), cell encapsulation (Lim & Sun, 1980; Uludag, Vos, & Tresco, 2000), and constructing a scaffold for use in tissue engineering (Lee & Mooney, 2001; Tateishi, Chen, & Ushida, 2002).

Tissue engineering is a new biomedical technology. This new area of medical science consists of three parts: cells, growth factors and scaffold (Byung & David, 2003). The goal is to regenerate new organs after damage. Unlike cells and growth factors, the scaffold, which provides cells with growing space have been extensively investigated (Lee & Mooney, 2001). The scaffold used in a cell culture for tissue engineering tells cells how to grow into functional 3D tissues or organs. The absence of a scaffold results in the formation of clustered cells, instead of sound organs. The most popular material for use as a scaffold is hydrogel, which has a high water content. The advantages of hydrogels in a tissue engineering scaffold include good biocompatibility, good permeability toward nutrients and the products of the cell, tissue-like elasticity, ease of chemical modification and the ability to undergo gelation in situ (Griffith, 2000; Hoffman, 2002).

Poly(vinyl alcohol) (PVA), generated via the hydrolysis of poly(vinyl acetate), had been investigated as a long-term or permanent scaffold for artificial cartilage, pancreas, bone and aortic heart valve (Lee & Mooney, 2001). The advantages of PVA as scaffolding used in tissue engineering include good biocompatibility and suitable mechanical strength. However, highly hydrophilic PVA exhibits poorer cell adhesion than the moderately hydrophobic polystyrene (Schmedlen, Kristyn, & Jennifer, 2002) because the poor adsorption of adhesion protein is responsible for cell adhesion. Oligopeptide or cell adhesion proteins are introduced into PVA hydrogels to improve the interaction between PVA hydrogels and cells. For instance, the RGDS sequence response that governs the adhesion of epithelial cells, and fibronectin (FN) that is responsible for NIH3T3

* Corresponding author. Tel.: +886 3 571 3204; fax: +886 3 571 5408.
E-mail address: ydlee@che.nthu.edu.tw (Y.-D. Lee).

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fibroblast attachment have been studied (Nuttleman, Mortisen, Henry, & Anseth, 2001).

Chondroitin sulfate (CS) consists of repeating disaccharide units of D-glucuronic acid and *N*-acetyl galatosamine, sulfated at either 4- or 6-positions. CS can bind with core protein to produce highly absorbent aggregan, which is a major structure inside cartilage and acts as a shock absorber, or it can produce sydecan, which is a cell receptor which can interact with adhesion proteins, cells and the extracellular matrix (ECM) (Bukalo, Schachner, & Dityatev, 2001). Investigations of the FN-CS interaction indicated that CS was involved in regulating cell-ECM interaction, such as the adhesion of lymphocytes and melanoma cells, and neural crest cell migration (Barkalow & Schwarzbauer, 1994). In biomedical applications, CS has shown in vivo anti-inflammatory effect in animal models. It also regulates metabolism in vitro (Bali, Cousse, & Neuzil, 2001). CS can be used for treating autoimmune and wasting joint diseases.VISCOAT® (4% chondroitin sulfate$_{(aq)}$ and 3% sodium hyaluronate$_{(aq)}$) is used as a surgical aid in cataract extraction and lens implantation (Tomita, Sando, Sera, & Aoyama, 2004). CS is also a component of the dermal layer of the FDA-approved skin substitute for treating burns (Philips, 1998).

This investigation aims to obtain a composite scaffold by the glutaraldehyde-crosslinking of PVA and CS. CS is expected to promote the interaction between cells and hydrogels and promote the adhesion of cells onto highly hydrophilic hydrogels. The composite PVA-CS hydrogels are not only bioactive as CS, but also tough as PVA hydrogels. The combined advantages are such that the PVA-CS gels have potential in tissue engineering.

2. Experimental methods

2.1. Materials

Chondroitin sulfate (CS) was obtained from TCI and stored in a refrigerator before it was used. Poly(vinyl alcohol) (PVA) (M_w = 16,000 Da) and glutaraldehyde (GA) were purchased from Acros. 1,9-Dimethylmethylene blue (DMB) was purchased from Sigma. Baby-hamster kidney (BHK) cells, culture medium, Trypsin, and trypan blue were supplied by the Protein and Metabolic Engineering Laboratory (Chemical Engineering Department, National Tsing-Hua University). The growth medium was composed of Dulbecco's modified Eagle's medium (Sigma), 10% (v/v) fetal bovine serum (Sigma), 1% (v/v) antibiotic/antimicotic agent and 1% (v/v) sodium bicarbonate solution to control the pH. The phosphate buffer solution (PBS) were gotten by mixing 0.2 g potassium dihydrogen phosphate (Showa), 2.172 g sodium phosphate dibasic anhydrous (Showa), 8 g sodium chloride and 0.2 g potassium chloride within 1000 ml water, then the pH was adjusted to 7.4. The other reagents were analytic grade and used without further purification.

2.2. Analytical instrumentation

A Perkin-Eimer 842 IR spectrometer with a resolution of 2 cm^{-1} was used for the IR analysis of samples in KBr pellets. Solid C^{13} NMR spectra data were obtained using a DSX400 NMR spectrometer. The sample was mechanically tested using a Shimadzu AGS 2000G. The initial distance between the upper and lower clamps (l_o) was 20 mm. The stretching rate was 5 mm/min. The cell morphology of the cultured PVA-CS membranes was observed by SEM (JSM-5600, JEOL) after gold-sputtering. The magnification was limited to ×3000 and the voltage was under 10 kV, to prevent damage by the electron beam.

2.3. Preparation of PVA-CS hydrogels

PVA is a hydrophilic polymer. When it is placed in water, it would swell and then be gradually dissolved into the water. Therefore, when we use PVA as the matrix for cells culture, it needs to be crosslinked to decrease its solubility.

Eighteen weight percentage of PVA$_{(aq)}$ and 20 wt% CS$_{(aq)}$ were prepared, and then both solutions were mixed, as described in Table 1. Six solutions have different CS weight percentages (as shown in Table 1). Five percent of HCl (aq) was added to these solutions, which were stirred for 1 h. After 25 wt% glutaraldehyde was added, reagent solution was poured into PE dishes. PVA-CS hydrogels

Table 1
Feed ratio, composition, and elastic modulus of PVA-CS hydrogels

Code	CS00	CS01	CS05	CS10	CS15	CS20
PVA (18 wt%)(g)	7.051	6.941	6.682	6.371	6.092	5.785
CS (20 wt%)(g)	0	0.075	0.343	0.661	0.989	1.323
HCl (5 wt%) (g)	0.284	0.283	0.278	0.279	0.284	0.285
GA (25 wt%) (g)	0.281	0.288	0.283	0.286	0.281	0.279
Calculated CS wt%[a]	NA	1.55	3.52	5.84	7.98	9.41
Elastic modulus (×10^7 N/m^2)	16.8	15.0	10.8	8.3	7.1	6.1

P.S. CSXX is the sample whose feed percent is XX solute wt% CS.

[a] The amount of CS inside PVA-CS hydrogels are obtained by spectrophotometric method. The formula of calibration curve is $Y = 1624X - 86.597$ and $R^2 = 0.995$. (X, absorbance; Y, concentration; unit, ppm).

were formed after a crosslinking reaction for 2 h. Hydrogels were then immersed into PBS for two days to remove the unreacted residue. The hydrogels were then placed into a vacuum oven to be dried for another 24 h. After drying, the PVA-CS membranes were stored in a desiccator until further testing.

2.4. Swelling assay

The PVA-CS hydrogels were placed in phosphate buffer solution (PBS) at room temperature until the weight of the hydrated PVA-CS hydrogels did not further increase. The weight of PVA-CS hydrogels, W_h, was recorded at various times. The weight of dried PVA-CS membranes, W_d, was also recorded. The swelling ratio of PVA-CS hydrogels was calculated as $100\times(W_h - W_d)/W_d$.

2.5. Mechanical testing

Dried membranes whose thickness ranged from 0.2 to 0.22 mm were cut into 10×30 mm^2 stripes. The Shimadzu AGS 2000G was used for the mechanical testing of these stripes. The stress vs. strain curves were plotted according to the obtained mechanical data. The elastic modulus was the slope of the line fitted to the initial linear section of these curves.

2.6. Quantitative analysis of CS inside PVA-CS hydrogel

2.6.1. Plotting the calibration curve

The amount of CS inside PVA-CS hydrogels was determined by spectrophotometric method (Farndale, Buttle, & Barrett, 1986). The color reagent was prepared by adding 16 mg 1,9-dimethylmethylene blue, 3.04 g glycine, 2.37 g NaCl and 95 ml 0.1 M $HCl_{(aq)}$ into 1000 ml water. The color reaction was performed by adding 0.1 ml of aqueous CS into 1 ml of color reagent, and A_{525} was read immediately while reagent was used as the blank. Furthermore, cuvettes may be washed with methanol to remove the dye, followed by water to flush out any remaining CS. The calibration curve was obtained by plotting A_{525} of known CS standard solution at concentrations from 25 to 400 ppm. A spectrophotometer (Metertech spectrometer SP 8001) was used.

2.6.2. Estimating the amount of CS inside PVA-CS hydrogels

A 0.1 g sample was hydrolyzed in 3 M $HCl_{(aq)}$ to the boil for 1 h. The solution of the sample was neutralized and its volume was raised to 10 ml by adding water. The procedures were as described above. The amount of CS in PVA-CS hydrogels was determined from the data on absorbance A_{525} and the calibration curve.

2.7. Cells culture

The BHK cell culture on PVA-CS hydrogels was evaluated and observed. The CS-PVA membranes that fit a 12-well culture dish were sterilized with 70% alcohol under UV light overnight, and rinsed several times in PBS. 3×10^5 cells per well were seeded onto the CS-PVA membrane in the culture medium. The culture was maintained at 37 °C and 5% CO_2. After the cells were cultured for three days, they were directly observed under an optical microscope. The number of cells detached by Trypsin and stained by trypan blue, was counted under a microscope.

2.8. Cells morphology

The cultured PVA-CS membrane was rinsed by PBS, immersed in 2.5% glutaraldehyde for 4 h and osmium tetroxide for 1 h at room temperature, to determine the morphology of the BHK cells. After repeated washing by PBS, the membrane was dried by graded ethanol changes (50, 70, 95 and 100%), and CO_2 critical point drying. The gold-sputtered specimens were observed by SEM (JSM-5600, JEOL).

3. Results and discussion

3.1. IR spectra

Fig. 1 shows the IR spectra of pure PVA, pure CS and PVA-CS samples. The hydroxyl groups of PVA exhibit two main absorptions-one is O–H stretching absorption at 3200–3600 cm^{-1}; the other is C–O stretching at 1050–1150 cm^{-1} ($\lambda_{max}=1100$ cm^{-1}). The characteristic peaks of CS not only include O–H and C–O stretching, but also C=O and SO_4^{2-} ($\lambda_{max}=1640$, 1260 cm^{-1}, individually).

The aldehyde groups of GA can react with hydroxyl groups of PVA and CS under acidic conditions, and then form acetal rings (Yeom & Lee, 1996, Fig. 2). The acetal ring exhibits two absorptions whose λ_{max} are at 1001 and

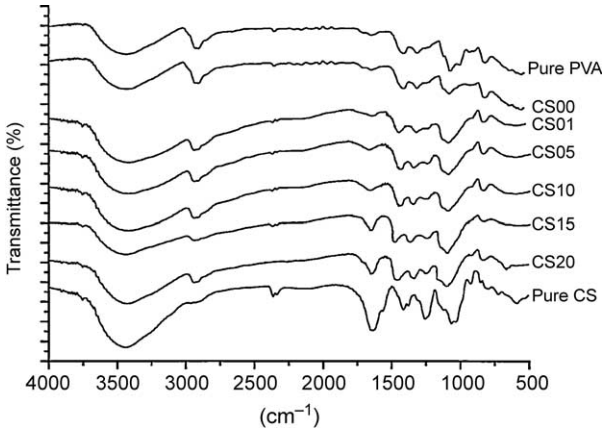

Fig. 1. IR spectra of PVA-CS sample through KBr pellets. Here CSXX is the sample whose feed percent is XX solute wt% CS.

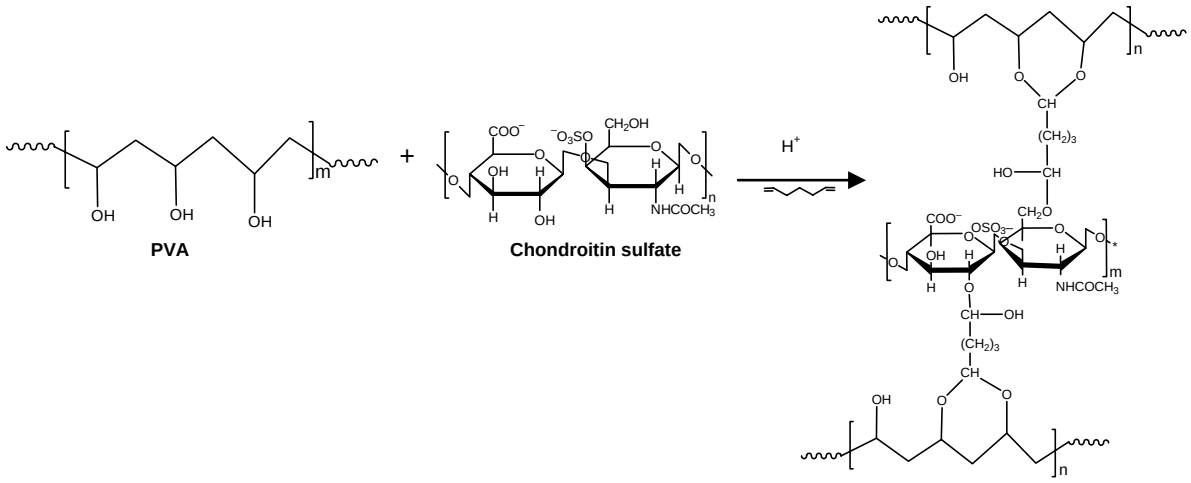

Fig. 2. The glutaraldehyde-crosslinking reaction of PVA-CS hydrogels.

1134 cm^{-1}, so the formation of the acetal ring broadens the C–O stretching peak (Fig. 1, pure PVAand CS00) (Kim, Mia, Lee, Won, & Kang, 2002). The spectra of the other PVA-CS samples also exhibit a broadened C–O stretching zone. The spectra of the PVA-CS samples show the characteristic peaks of not only C–O and O–H, but also of CS at 1640 and 1260 cm^{-1}. The intensities of the C=O and SO_4^{2-} absorptions increase with the percentage of fed CS (Fig. 1). The IR spectra demonstrate that PVA-CS hydrogels had been successfully prepared.

3.2. Solid C^{13} NMR spectra

PVA-CS hydrogels are insoluble polymer networks that cannot easily undergo common liquid H^1 or C^{13} NMR, but can undergo solid C^{13} NMR measurement. Fig. 3(a) reveals that the peaks of the CS00 (pure PVA) spectra are attributed to CH_2 (45.3 ppm) and CHOH (70.8 ppm); where the characteristic NMR peaks of CS (Fig. 3(f)) include (1) $NHCOCH_3$ (24.1 ppm) in *N*-acetyl galatosamine's moiety, (2) C2 of the carbon ring in *N*-acetyl galatosamine's moiety (51.9 ppm), (3) C–O (60–80 ppm), (4) C1 in the carbon ring (104.1 ppm), (5) C=O (175.5 ppm). Fig. 3(c)–(e) show the characteristic peaks of CS at 24, 104 and 175 ppm, respectively. As the CS content increase in the hydrogels, the relative intensity of the peaks also increased. The results of the solid state NMR and the IR spectra can make sure that we have successfully synthesized the hydrogels.

3.3. Estimating the amount of CS

Dimethylmethylene blue (DMB) is a strongly metachromatic dye for the histochemical detection of sulfated glycosaminoglycans (GAG) and was used in an analytical procedure at pH 3.0. The DMB assay relies on spectrophotometric detection of metachromatic changes in DMB which occur when the cationic dye binds to sulfate present in GAG chain.

Table 1 presents the amount of CS assayed by the spectrophotometric method. The calculated amounts of CS in PVA-CS hydrogels are lower than those in the feed, perhaps because the unreacted CS is washed out. However, the calculated amount of CS01 isn't reasonable and more than 1%. The deviation could arise from that the calibration curve didn't pass through the zero point (Groves, Shellis, & Dieppe, 1997). It may be explained by the tiny absorbance of DMB in the absence of CS (Chandrasekhar, Esterman, & Hoffman, 1987; Farndale et al., 1986). In this case, as the feed concentration lower than 1% may be not suitable for the line of calibration curve. The linear response of the standard curve was obtained for the various CS concentrations (up to 400 ppm, $r^2=0.995$). Above 400 ppm, the absorbance was less than that predicted from the linear part of the curve. This was mainly attributable to precipitation of the CS/DMB complex, which was visible as turbidity at high CS concentration. The CS concentrations were stable for at least 1 h as the concentration less than 200 ppm. Therefore, this method may also be used for quantification of sulfated GAG in cartilage cell culture.

3.4. Elastic modulus

CS has much poorer mechanical properties than PVA. The addition of CS into PVA hydrogel should weaken the hydrogel and lower its elastic modulus below that of pure PVA hydrogel. The results of the mechanical tests in Table 1 prove this prediction. A hydrogel of pure PVA also crosslinked by glutaraldehyde has higher elastic modulus (CS00) than other hydrogels. A higher CS content in the hydrogel corresponds to a lower modulus. However, CS20 with the lowest elastic modulus (6.1×10^7 N/m^2) can still be used to form the scaffold. Besides, the elastic modulus for PVA without glutaraldehyde was also measured (2.19×10^7 N/m^2), but it is brittleness and it would dissolve in water, so it is not suitable for using as cells culture matrix.

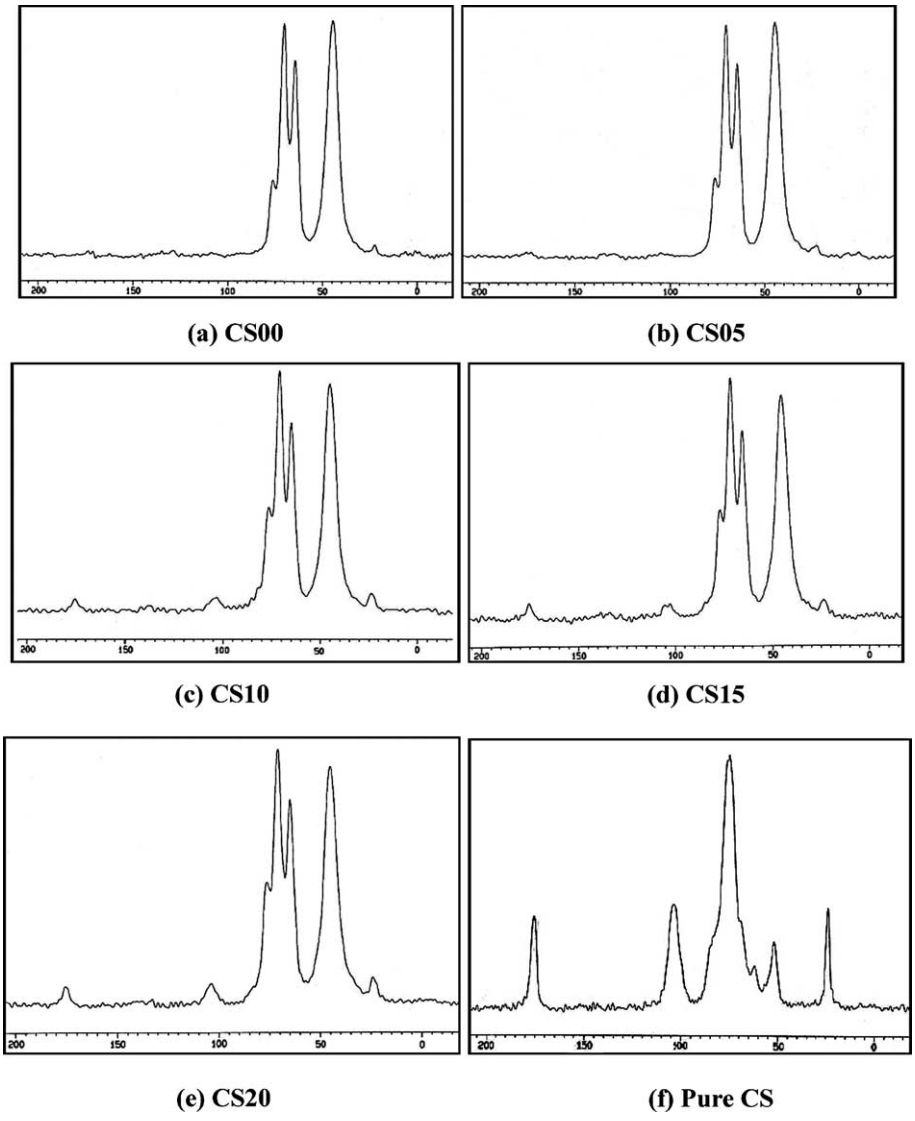

Fig. 3. Solid C^{13} NMR spectra of PVA-CS hydrogels.

3.5. Swelling ratio of PVA-CS hydrogels

The high water content of the hydrogels provide them important characteristics that support tissue engineering, such as tissue-like elasticity, good permeability toward nutrients and the easy growth of cells (Li, Williams, Sun, Wang, Leong, & Elisseeff, 2004; Nuttelman et al., 2001).

The strong repulsion of negative charges and polar groups cause CS to be highly hydrophilic and absorbent. PVA-CS hydrogels are expected to show more capacity for water than pure PVA hydrogels. Fig. 4 shows that the swelling ratio of the PVA-CS hydrogels increases with the amount of CS. Only 240 min is required to reach the equilibrium swelling ratio for all PVA-CS hydrogels. The higher water content and faster swelling can be attributed to the existence of CS. The CS possesses negative

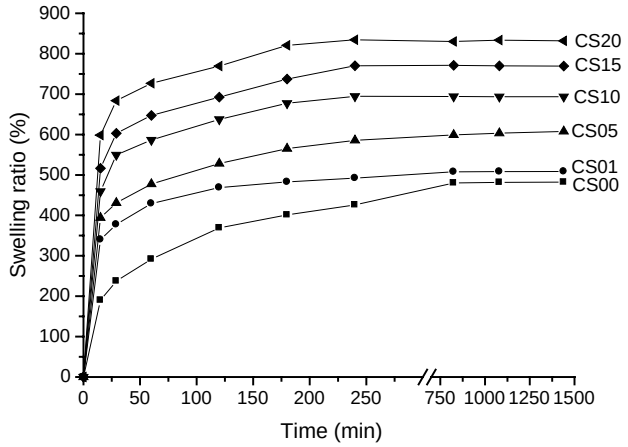

Fig. 4. Swelling ratio of PVA-CS hydrogels content different composition of Chondroitin sulfate.

charges and helps the gels to swell highly. The presence of ionization groups on polymer chains results in swelling of the hydrogels much beyond that can be achievable by nonelectrolyte hydrogels. Swelling ratio of the ionic gel is determined by two main factors, one is the electrostatic repulsion between the polymer chain and the other is ions present in the buffer solution (Donnan equilibrium). In the way, the swelling of the polyelectrolyte hydrogel is mainly due to the electrostatic repulsion among charges present on the polymer chain. The extent of the swelling ratio is influenced by pH, ionic strength, etc. which reduce electrostatic repulsion. Therefore, these hydrogels may also apply to drug delivery system.

3.6. BHK cell culture on PVA-CS membrane

Comparisons between PVA and PVA-CS hydrogels as tissue engineering matrices were initially made by assaying with a BHK cell culture for three days. Fig. 5(a) shows that few BHK cells adhere to the surface of CS00, indicating that CS00 is biocompatible with BHK cells, but the number of adherent cells is lower than that on the PVA-CS hydrogels. The highly hydrophilic CS00 exhibits poor cell adhesion, unlike PVA-CS hydrogels. The bioactive CS promotes BHK cell's attachment and growth onto PVA-CS hydrogels (Fig. 5). The number of cells for CS20 ($=1.23\times10^6$ cells/ml) is slightly lower than the control number, but 2.56 times the number for CS00.

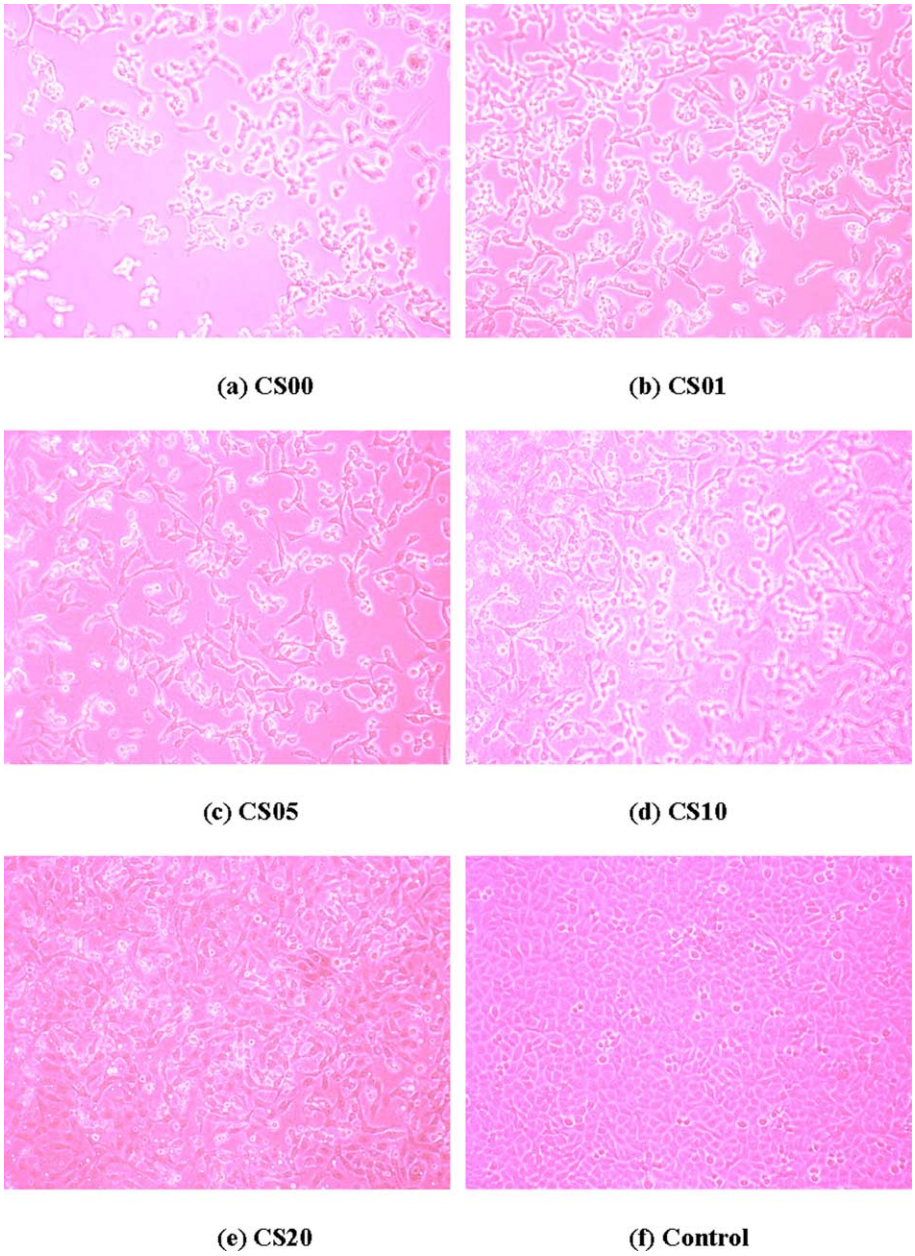

Fig. 5. BHK Cells morphology on PVA-CS membrane after 3 days. Control is the polystyrene plate coated with collagen.

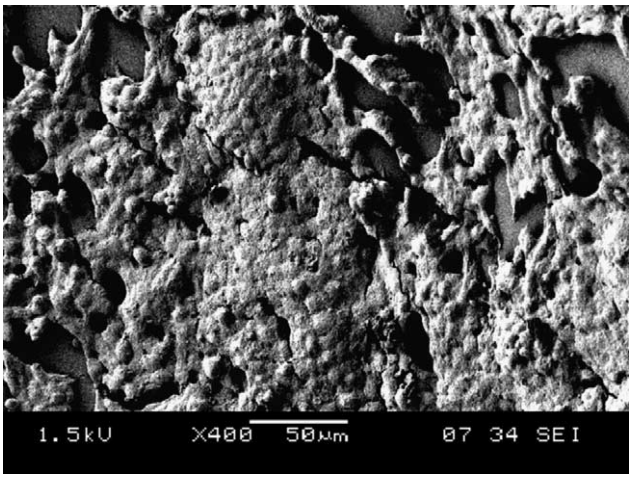

Fig. 6. The cell morphology of BHK cells onto CS20 observed by SEM after 1 week's culture.

Increasing the culture period of CS20 from three days to one week yields the cell growth behavior observed by SEM (Fig. 6). The BHK cells not only become attached to the CS20, but also connect with each other to form a dense sheet of BHK cells thereon.

4. Conclusion

The PVA-CS hydrogels, crosslinked by glutaraldehyde, provide the advantages of both PVA and CS. The mechanical properties of composite hydrogels facilitate the culturing of cells and make them bioactive toward the cells. The PVA-CS hydrogels have potential use in tissue engineering. In the following paper, the biological effect of the CS to the cells growth in the hydrogels will be discussed.

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References

Bali, J. P., Cousse, H., & Neuzil, E. S. (2001). Biochemical basis of the Pharmacologic action of chondroitin sulfates on the osteoarticular system. *Seminars in Arthritis and Rheumatism*, *31*, 58–68.

Barkalow, F. J., & Schwarzbauer, J. E. (1994). Interactions between fibronectin and chondroitin sulfate are modulated by molecular context. *Journal of Biological Chemistry*, *269*, 3957–3962.

Bukalo, O., Schachner, M., & Dityatev, A. (2001). Modification of extracellular matrix by enzymatic removal of chondroitin sulfate and by lack of tenascin-R differentially affects several forms of synaptic plasticity in the hippocampus. *Neuroscience*, *104*, 359–369.

Byung, S. K., & David, J. M. (2003). Development of biocompatible synthetic extracellular matrices for tissue engineering. *Trends in Biotechnology*, *16*, 224–230.

Choi, Y. S., Hong, S. R., Lee, Y. M., Song, K. W., Park, M. H., & Nam, Y. S. (1999). Study on gelatin-containing artificial skin: I. Preparation and characteristics of novel gelatin-alginate sponge. *Biomaterials*, *20*, 409–417.

Chandrasekhar, S., Esterman, M. A., & Hoffman, H. A. (1987). Microdetermination of proteoglycans and glycrosaminoglycans in the presence of guanidine hydrochloride. *Analytical Biochemistry*, *161*, 103–108.

Farndale, R. W., Buttle, D. J., & Barrett, A. (1986). Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethyl-methylene blue. *Biochimica Biophysica Acta*, *883*, 173–177.

Griffith, L. G. (2000). Polymeric biomaterials. *Acta materialia*, *48*, 263–277.

Groves, P. J., Shellis, R. P., & Dieppe, P. A. (1997). Use of polyvinyl alcohol to stabilize binding of sulfated glycosaminoglycans to dimethylene blue. *Analytical Biochemistry*, *245*, 247–248.

Hoffman, A. S. (2002). Hydrogels for biomedical applications. *Advanced Drug Delivery Reviews*, *54*, 3–12.

Ichikawa, H., & Fukumori, Y. (2000). A novel positively thermosensitive controlled-release microcapsule with membrane of nano-sized poly (*N*-isopropylacrylamide) gel dispersed in ethylcellulose matrix. *Journal of Controlled Release*, *63*, 107–119.

Kim, J. H., Mia, B. R., Lee, K. B., Won, J., & Kang, Y. S. (2002). Coordination structure of various ligands in crosslinked PVA to silver ions for facilitated olefin transport. *Chemical Communications*, *22*, 2732–2733.

Lee, K. Y., & Mooney, D. J. (2001). Hydrogels for tissue engineering. *Chemical Reviews*, *101*, 1869–1880.

Li, Q., Williams, C. G., Sun, D. D. N., Wang, J., Leong, K., & Elisseeff, J. H. (2004). Photocrosslinkable polysaccharides based on chondroitin sulfate. *Journal of Biomedical Materials Research*, *68*, 28–33.

Lim, F., & Sun, A. M. (1980). Microencapsulated islets as bioartificial pancreas. *Science*, *210*, 908–910.

Matsumoto, A., Ikeda, S., Harada, A., & Kataoka, K. (2003). Glucose-responsive polymer bearing a novel phenylborate derivative as a glucose-sensing moiety operating at physiological pH conditions. *Biomacromolecules*, *4*, 1410–1416.

Nuttelman, C. R., Mortisen, D. J., Henry, S. M., & Anseth, K. S. (2001). Attachment of fibronectin to poly(vinyl alcohol) hydrogels promotes NIH3T3 cell adhesion, proliferation, and migration. *Journal of Biomedical Materials Research*, *57*, 217–223.

Phillips, T. (1998). New skin for old developments in biological skin substitutes. *Archives of Dermatology*, *134*, 344–348.

Schmedlen, R. H., Kristyn, K. S., & Jennifer, J. L. (2002). Photocrosslinkable polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering. *Biomaterials*, *23*, 4325–4332.

Tateishi, T., Chen, G., & Ushida, T. (2002). Biodegradable porous scaffolds for tissue engineering. *Journal of Artificial Organs*, *5*, 77–83.

Tomita, N., Sando, S., Sera, T., & Aoyama, Y. (2004). Macrocyclic proteoglycan mimics. *Potent inhibition of cell adhesion by a bundle of chondroitin sulfate chains assembled on the calyx[4]resorcarene platform. Bioorganic and Medicinal Chemistry letters*, *14*, 2087.

Uludag, H., Vos, P. D., & Tresco, P. A. (2000). Technology of mammalian cell encapsulation. *Advanced Drug Delivery Reviews*, *42*, 29–64.

Yannas, I. V., Lee, E., Orgill, D. P., Skrabut, E. M., & Murphy, G. F. (1989). Synthesis and characterization of a model extracellular matrix that induces partial regeneration of adult mammalian skin. *Proceedings of the National Academy of Sciences of the United States of America*, *86*, 933–937.

Yeom, C. K., & Lee, K. H. (1996). Pervaporation separation of water-acetic acid mixtures through poly(vinyl alcohol) membrancs crosslinked with glutaraldehyde. *Journal of membrane science*, *109*, 257–265.