Original Article

Fabrication and characterization of biocompatible agarose–chitosan–collagen scaffold for tissue engineering

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Abstract

Introduction: The biocompatible scaffold which promotes cell proliferation is necessary for the development of tissue engineering. The objective of this study was to fabricate and characterize a biocomposite scaffold using natural material, agarose-chitosan-collagen (AG-CH-CL) via freeze-drying method. To avoid cytotoxic effects associated with the chemical cross linker, the freeze-drying process was performed without adding the cross linker reagent.

Methods: The AG-CH-CL scaffold was synthesized using a freeze-drying method. The natural polymers were used in different concentrations to optimize the most desirable scaffold. SEM was used to determine the pore size of the scaffold. Physical functional as well as water uptake ability, degradation rate and chemical group also evaluated. In addition, MTT assay was used to examine biocompatible of the scaffold by growing fibroblast on the scaffold for 4 weeks and the histological structure of the cell-scaffold were determined using H&E staining.

Result: The scaffold made from AG-CH-CL in 3:1:0.5 proportions were generating the most desirable properties, which consisted of three dimensional interconnected porous structures with pore size diameter around 184 μm. The water uptake ability of the scaffold was over 85% and the degradation rate at 4 weeks was only 5%. In addition, the scaffold can apply for supporting human fibroblast culture, showing their low cytotoxicity.

Conclusion: Our findings provide the information for synthetic method of scaffold fabrication that must be accompanied by generating scaffold which contains natural material and avoids the use of toxic reagent. This study indicates potential of biomaterial scaffold for tissue engineering.

Key words: biomaterial, scaffold, tissue engineering

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Introduction

To date, the alternative approach for therapy degenerative disease is tissue engineering using three-dimensional (3D) scaffolds. The ideal scaffold should be elastic, biodegradable and biocompatible to serve as a template to guide cell adhesion and proliferation. Besides these, the scaffold should have an interconnected pore to allow cell penetration and transfer nutrients and metabolic waste products.[1,2] Thus, the material chosen for fabrication of scaffold for tissue engineering is very important. There are varieties of synthetic and natural materials investigated as scaffolds for tissue engineering.[3-5] In this study, we were interested in natural materials such as agarose, chitosan and collagen, because the natural scaffold could mimic a native environment for support cell proliferation. Agarose is a polysaccharide consisting of 1, 3-linked β-D-galactopyranose and 1, 4-linked 3, 6-anhydro-α-L-galactopyranose. The advantage of agarose is good mechanical strength and elasticity.[6,7] However, the scaffold that made from agarose alone is not suitable to mimic the microenvironment for cell proliferation. Chitosan is a linear polysaccharide composed of glucosamine and N-acetyl-glucosamine, obtained by the deacetylation of chitin. Many studies have shown that chitosan scaffold had an excellent biocompatibility, biodegradability, non-toxicity and great adsorption properties.[8-10] However, the biocomposite agarose-chitosan scaffold was an inert polymer, which does not support cell adhesion effectively. Thus, incorporation of extracellular matrix (ECM)-like component could facilitate cell adhesion and proliferation in the porous 3D scaffold. Collagen consisted of three polypeptides chains: glycine, proline and hydroxyproline which arranged as a triple helix. Because of the presence of Arg-Gly-Asp (RGD) sequences, collagen scaffold enhanced cell attachment and proliferation.[11]

At present, the combination of natural polymers have been used to fabrication the tissue engineering scaffold.[12-14] The previous work of Bath *et al*., 2011[6] reported that the scaffold construct from chitosan-agarose-gelatin via cryogelation technology had a high compression modulus and suitable for bioreactor studies which benefit for cartilage tissue engineering. As well as, chitosan-agarose-gelatin cryogels showed a good cell adhesion of primary goat chondrocytes. However, after six weeks of implantation in animal model, the chondrocyte - seeded scaffold has degraded. These results implied that chondrocyte - seeded scaffolds degraded faster than the scaffolds that were not seeded with cells, which might be due to the production of enzymes by the cells along with the other enzymatic factors present in *vivo* conditions. In liver tissue engineering study,[14] the candidate scaffold produced from agarose and chitosan by freeze-drying method. The AG–CH scaffold showed high porosity with interconnected pores, the average pore size diameter around 40–70 μm and degradation rate after 4 weeks of incubation with PBS were 15%. In addition, the primary hepatocytes could proliferation in the AG–CH scaffold as well as the normal hepatic functions like albumin secretion and urea synthesis were higher when culture the primary hepatocytes on the AG–CH scaffold. However, the hepatocyte viability was decreased to 60% after seeded cell on scaffold for 24 hrs. Moreover, the percent cell viability was significantly decreased to 40% after day 10 in AG–CH scaffolds. These results indicated that each type of cell and tissue have a distinct structure and specific function, thus adaption of the ECM scaffolds to improve cell viability and engraftment requires specific design and fabrication of the scaffold templates to creation the microenvironment that accommodate cell growth.

There are many techniques for fabrication scaffold such as electrospinning, gas foaming, solvent casting, particulate leaching, phase separation, melt molding, solution casting as well as freeze-drying.[15-18] Freeze-drying is one method which is used for synthesizing 3D porous scaffold. The principle of this method is conversion of a solid directly into its gaseous form. Natural biocomposite scaffold by freeze-drying technique for tissue engineering application have been studies.[17, 19-21] Freeze-drying method obtain many advantages for control important properties of the scaffolds such as porosity, size, geometry and the degree of pore in order to mimic the topological and microstructural characteristics of the ECM. Their additional advantage of freeze-drying method is preventing microbial growth because of the absence of water in the scaffold and preserving biological samples that degrade by water. Moreover, the sublimation process can remove any acetic acid which dissolved chitosan due to its volatility, thus low cytotoxicity of the freeze-drying scaffold was observed. These advantages allow their use in biomedical tissue engineering for fabrication the desirable properties of the natural scaffold. Normally, cross linker reagent, glutaraldehyde, were added into polymer during freeze-drying process for creation peptide bond within scaffold. However, the cytotoxic effect of glutaraldehyde has been demonstrated, the mechanism of which is apoptosis.[22, 23]

Therefore, the aim of the study was to fabricate a 3D porous scaffold using agarose, chitosan and collagen by freeze-drying method. In an attempt to avoid glutaraldehyde toxicity, freeze-drying process was performed without the addition of the cross-linker reagent. The biopolymers were prepared using different ratios of agarose, chitosan and collagen to optimize the scaffold characteristics. The synthesized scaffold was further characterized porosity, stability, water uptake ability as well as biocompatible to human fibroblast.

Methods

Materials

Agarose (low EEO, gelling temperature at 38-40°C) was purchased from Sisco Research Laboratories (Mumbai, India). Chitosan (MW: 50000-190000 Da and degree of deacetylation >75%) and collagen peptide were supplied from Himedia (Mumbai, India). MTT reagent (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) were purchased from Sigma-Aldrich (St Louis, MO). Normal human fibroblast was purchased from the American Type Culture Collection (ATCC, CRL-1635, Rockville, MD).

Preparation of agarose–chitosan-collagen (AG-CH-CL) scaffold

For optimizing the scaffold properties, different ratios of agarose, chitosan and collagen were used in this study (Figure 1). Chitosan powder was dissolved in 1% acetic acid solution to make the final concentration; 1% and 2% w/v. Collagen was added in the same tube containing chitosan solution and stirring continued (final concentration; 0.5% and 3% w/v). Agarose (final concentration; 1% and 3% w/v) was dissolved in deionized water by boiling until the solution was clear. Agarose solution was then added to the solution mixture containing chitosan and collagen. The polymer solution was incubated at –80°C for 24 hrs before dried under vacuum at -100°C for 48 hrs (Martin Christ, Germany). The AG-CH-CL scaffold was dried at room temperature for studying their physical and chemical properties and determination of biocompatibility.

Scaffold	Agrose	Chitosan	Collagen	Photographic image
1	1%	1%	0.5%	
2	1%	1%	3%	
3	3%	1%	0.5%	
4	3%	1%	3%	
5	1%	2%	0.5%	
6	1%	2%	3%	
7	3%	2%	0.5%	
8	3%	2%	3%	

Figure 1 The composite AG-CH-CL scaffold could synthesize in 3D structure by freeze-drying condition, which showed sponge-like and elastic property in scaffold 3, 4, 7 and 8. In contrast to scaffold 1, 2, 5 and 6, those have non-flexibility.

Scanning electron microscopy (SEM)

The biopolymer scaffold was placed onto a small piece of double-sided adhesive tape which was placed on a brass stub used as a target. Then, the stub was inserted into the holder in a vacuum system. Gold was heated to produce vapors, which would coat the sample particle on the target. The samples were then recorded at 10 kV, 2500X modification. The pore size was determined by the ImageJ software by selecting the different areas to determine the average pore size of the scaffold.

Water uptake ability

The scaffold was cut into discs (thickness: 0.5 cm and diameter: 1.5 cm) and putted into 24 well plate. After that, scaffold was weighed (Wdry) and immersed in 1XPBS (pH 7.4) for 1 hr. Then the scaffolds were removed from 24 well plate and placed on a gauze pad for 5 minutes to absorb the excess water. Finally, the scaffolds were weighed (W_{wet}) to determine water uptake ability as formula below.

$$\text{Water uptake ability (\%)} = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100$$

Degradation rate

The biomaterial scaffold was cut into disc (thickness: 0.5 cm and diameter: 1.5 cm) and weighed (W_1). Then the scaffold was transferred to 24 well plate and incubated with 1XPBS solution (pH 7.4) at 37°C for 4 weeks. At weekly intervals, triplicated samples were removed and dried at 80°C in a hot air oven and then weighed (W_2). The degradation rate was calculated as:

$$\text{Degradation rate (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Fourier transform infrared (FTIR) spectroscopy

Pure agarose, chitosan, collagen and AG-CH-CL composites scaffold were measured infrared spectrum in Bruker Tensor 27 system FTIR spectrometer. Data were collected from 4000-400 cm^{-1} at a spectral resolution of 2 cm^{-1} with 32 scans co-added. Background spectra were acquired from sample-free-areas before the acquisition of every 10 spectra from the sample.

Cell viability assay

To determine the viability and proliferation of normal human fibroblast (ATCC CRL-1635, Rockville, MD) in AG-CH-CL scaffold, MTT assay was carried out. The viable cells contain mitochondrial succinate dehydrogenase. This enzyme can oxidize the MTT solution to an insoluble crystal formazan. Then, the viable cells are solubilized by DMSO resulting in released of solubilized formazan reagent. This blue end product of solubilized formazan quantified by spectrophotometry to measure the optical density (OD). Because the formazan can only occur in viable cells, the level of solubilized formazan is also a measure of the viability of the cells. The scaffold was cut into disc (thickness: 0.5 cm and diameter: 1.5 cm) and immersed in 2% NaOH to neutralize the residual acid in the scaffold. The scaffold was washed 5 times with deionized water. Both sides of the scaffold were sterilized via ultraviolet irradiation in a laminar flow hood. Human fibroblast was plated at a density of 1×105 cells for each scaffold. Cellular scaffolds were incubated at 37°C in a CO_2 incubator and cell culture medium was replaced every three days. The scaffold was harvested at 1, 2, 3 and 4 weeks, washed three times in PBS and then incubated with MTT solution (0.5 mg/ml) for 1 hr. After that, the solution was aspirated and the insoluble intracellular formazan product was solubilized and released from cells by adding 100 µl of iso-propanol and incubated for 10 minutes. The optical density at 570 nm was then measured using a plate reader spectrophotometer.

Hematoxylin and eosin (H&E stain)

The cellular scaffold was harvested at 1, 2, 3 and 4 weeks, washed three times in PBS, and fixed in 4% phosphate-buffered paraformaldehyde at room temperature for 18–24 hrs, dehydrated through

a graded series of ethanol, embedded in paraffin and sectioned to a 10 μm-thick. For histological analysis, sections were then deparaffinized in xylene, rehydrated using a graded series of ethanol mixtures with water and stained with hematoxylin and eosin. The sample was dehydrated again and mounts with coverslip slides using permount.

Results

Three natural polymers, agarose, chitosan and collagen were synthesized as composite scaffold by freeze-dry technology at sub-zero temperature without the addition of the cross-linker reagent. This technique could produce a sponge-like biocomposite AG-CH-CL scaffold as shown in Figure 1. The optimal concentration of polymer ratio was obtained by preparing different concentration of agarose, chitosan and collagen to achieve elasticity, porosity, degradation and water uptake of the scaffold. The elasticity composite scaffold was found to be scaffold 3, 4, 7 and 8. However, scaffold 1, 2, 5 and 6, form a non-stable 3D scaffold and non-elasticity as shown in Figure 1. Thus, scaffold 3, 4, 7 and 8 were examined the other property of the scaffold including pore sized, biodegradation, water uptake as well as cytotoxicity assay.

The pore sized of the scaffolds was determined by SEM. As indicated in Figure 2, SEM images showed interconnected pore with polygonal shape in all scaffold. Large pore size diameter, 184.32 ± 25.12, 209.55 ± 22.44, 155.72 ± 17.68 and 141.13 ± 19.13 μm were observed in scaffold 3, 4, 7 and 8, respectively.

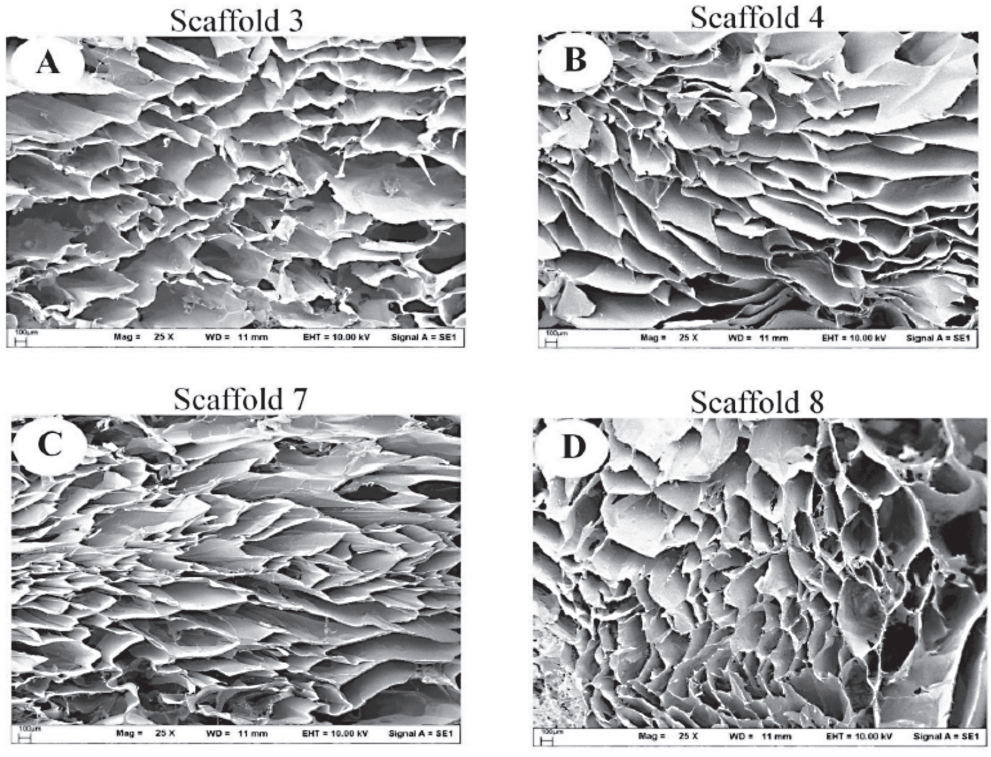

Figure 2 SEM showed an interconnected pore network of all scaffolds, which is having an average pore size diameter around 140–200 μm. SEM micrograph of scaffold 3 (polygonal shape with average pore diameter 184.32 ± 25.12 μm). SEM image of scaffold 4 (long channel shape with average pore diameter 209.55 ± 22.44 μm). SEM image of scaffold 7 (long channel shape with average pore diameter 155.72 ± 17.68 μm). SEM image of scaffold 8 (polygonal shape with an average pore diameter 141.13 ± 19.13 μM). Scale bar = 100 μm.

To further analyses the water absorption properties of the biocomposite AG-CH-CL scaffold, the scaffold was weighted before and after soaking in 1XPBS for 1 hr. All scaffolds showed water uptake ratio in the range of 85.10 - 88.14%. Data were shown as the mean ± SD, n = 3. As demonstrated in Figure 3, the highest water absorption (88.14 ± 0.68%) was observed in scaffold 7. In contrast, scaffold 3 showed the lowest water uptake ability (85.10 ± 0.54%). Because of the water uptake ratio of all scaffolds up to 85%, all type of scaffolds were quickly absorbed water within 5 minutes.

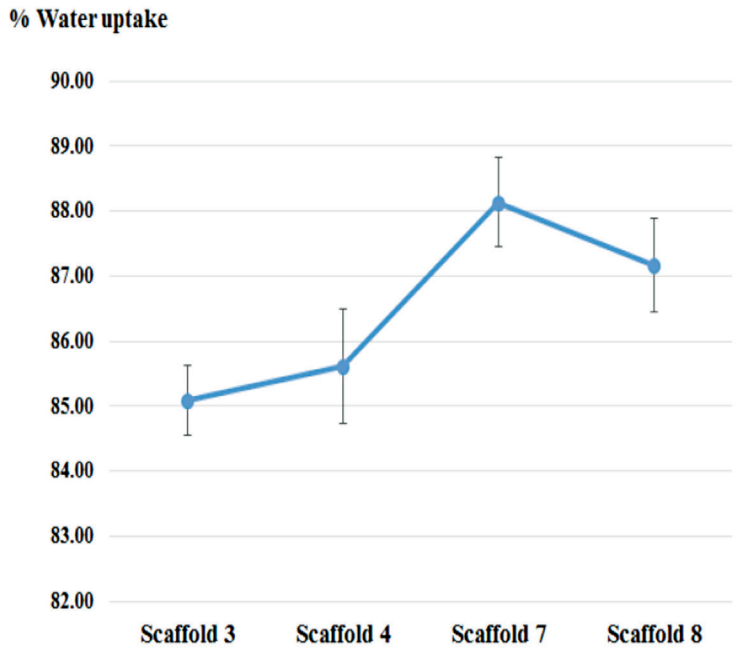

Figure 3 Analysis of water uptake abilities of the scaffold. All scaffolds showed water uptake ratio in the range of 85.10 - 88.14%. Data were shown as the mean ± SD, n = 3.

The degradation rate of AG-CH-CL scaffold was determined after 4 weeks of incubation with 1XPBS at 37°C. Data were shown as the mean ± SD, n = 3. The degradation rate after 4 weeks of scaffold 3, 4, 7 and 8 were 5.40 ± 1.24%, 11.05 ± 0.69%, 16.45 ± 0.73% and 11.61 ± 1.34%, respectively. All types of the scaffolds gradually degraded throughout the entire time period as shown in Figure 4. Scaffold 3 which comprises of 3% agarose, 1% chitosan and 0.5% collagen showed the lowest degradation rate, while scaffold 7 which made from 3% agarose, 2% chitosan and 0.5% collagen exhibited the highest degradation rate.

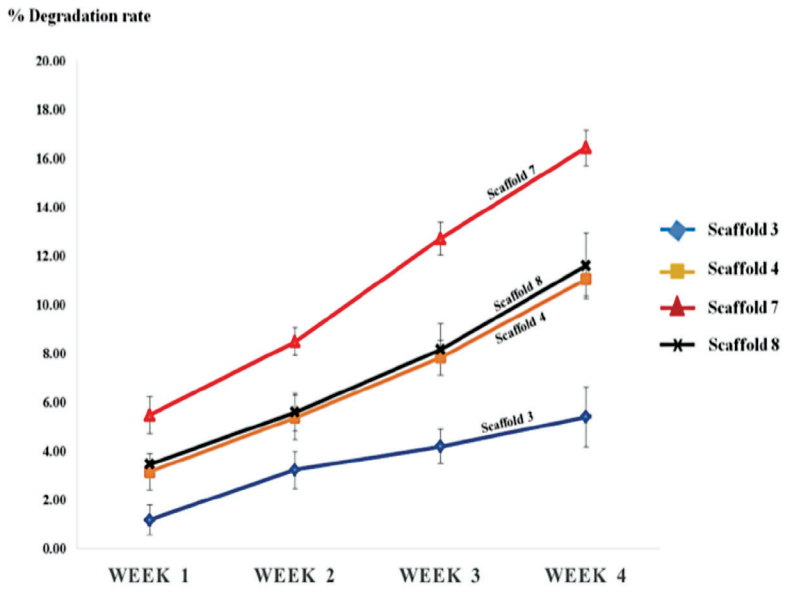

Figure 4 Degree of degradation of AG-CH-CL scaffold after 4 weeks of incubation with 1XPBS under room temperature.

Among the biocomposite AG-CH-CL scaffold, we found that scaffold 3 was the most preferable property for tissue engineering such as good elasticity, high porosity, water uptake abilities as well as the stability in 1XPBS solution. Therefore, scaffold 3 was selected for chemical group analysis via FTIR and cytotoxicity test by MTT assay.

Figure 5 shows representative 2nd derivative IR spectra from 4000 to 900 cm^{-1}. The average spectra of agarose[9] (Figure 5A) showed peak near 3370, 1633, 1370, 1041 and 928 cm^{-1}, as well as IR absorbance bands at 3349, 1623, 1536, 1394, 1238 and 1020 cm^{-1} that correlated with collagen[24] (Figure 5B). The IR absorbance of chitosan[25] (Figure 5C) showed peak at 3388, 1652, 1587, 1391, 1151 and 1035 cm^{-1}. The functional group related to FTIR bands as listed in Table 1. The absorbance of agarose, collagen and chitosan were observed in scaffold 3.

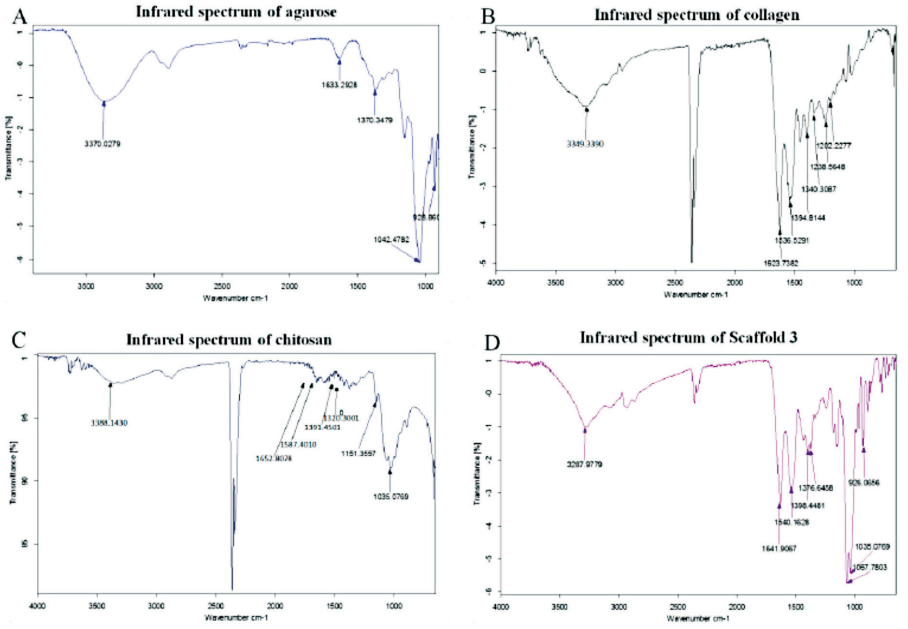

Figure 5 The representative 2nd derivative IR spectra from (A) agarose, (B) collagen, (C) chitosan and (D) scaffold 3.

Table 1 Functional group related to FTIR bands[9, 24, 25]

Natural polymer	Wavenumber (cm^{-1})	Functional group
Agarose	3370	OH stretching from hydroxyl group
	1633	Amide I
	1370	Amide III
	1042	Glycosidic bond
	928	C-O-C vibration of 3,6-anhydro-L-galactopyranose
Chitosan	3388	OH stretching from hydroxyl group
	1652	Amide I
	1587	Amide II
	1391	Amide III
	1151	C–O–C stretching
	1035	Glycosidic bond
Collagen	3349	OH stretching from hydroxyl group
	1623	Amide I
	1536	Amide II
	1394	Amide III
	1238	PO2 stretching
	1202	C–O–C stretching
AG-CH-CL scaffold	3287	OH stretching from hydroxyl group
	1641	Amide I
	1540	Amide II
	1398-1376	Amide III
	1067-1035	Glycosidic bond
	926	C-O-C vibration of 3,6-anhydro-L-galactopyranose

MTT assay was performed as an indicator for cytotoxicity of scaffold 3. The numbers of fibroblast cells on scaffold 3 were increased at indicated time point as shown in Figure 6.

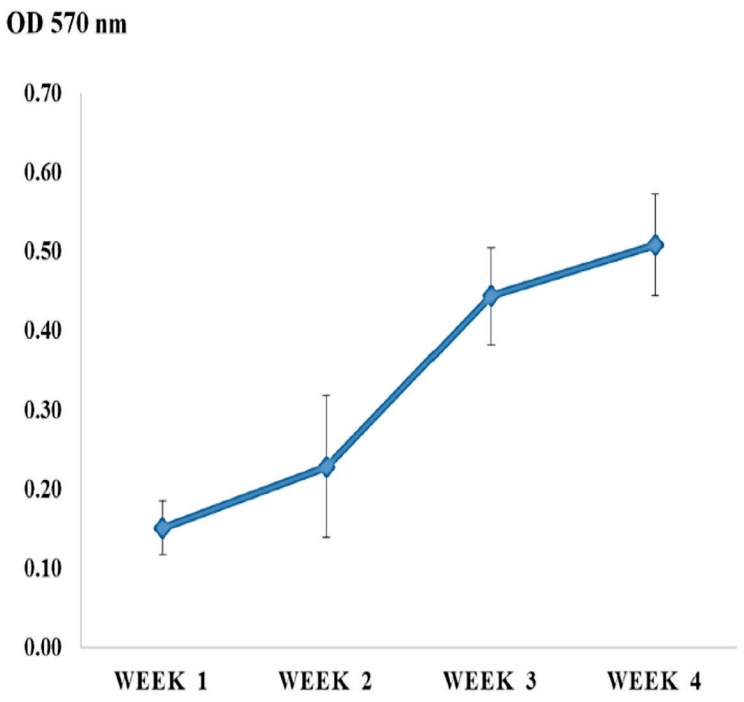

Figure 6 The cell viability and proliferation by MTT assay after 4 weeks on scaffolds 3 of fibroblast culture. The viable cell at each week was increased. Data were shown as the mean ± SD, n = 3.

Figure 7 demonstrates the H&E staining of fibroblast cells cultured on scaffold 3 for 4 weeks. Nucleus of fibroblast cell was stained deep blue, whereas the cytoplasm and extracellular matrix was pink staining. Histological image exhibited the fibroblast cells could attach to scaffold on the first week after culture. At 2, 3 and 4 week, the attached cell on the scaffold were increased. These finding suggested that the scaffold 3 was efficient for support cell adhesion and cell proliferation.

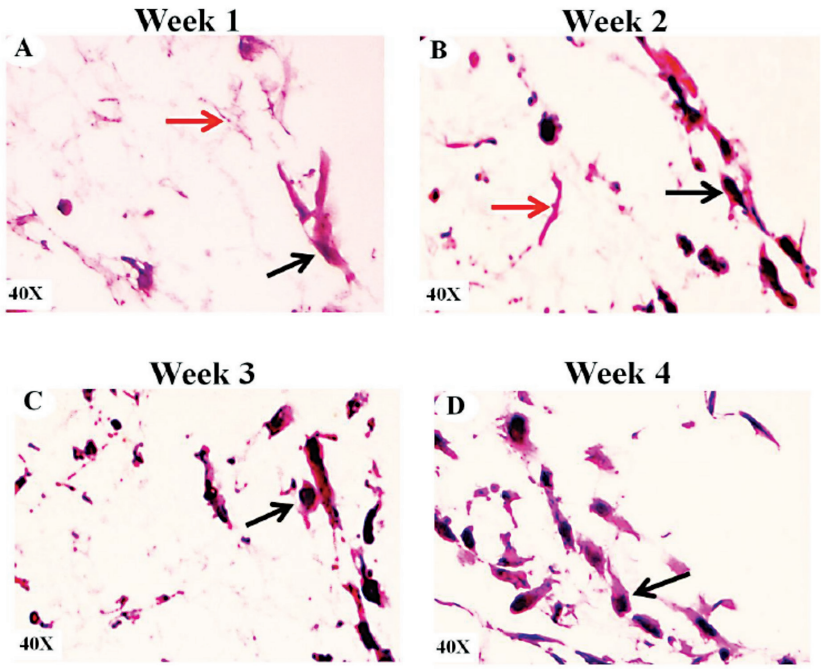

Figure 7 Histological of fibroblast cell culture on scaffolds after (A) 1 week, (B) 2 weeks, (C) 3 weeks, (D) and 4 weeks with 40X magnification. Red and black arrows indicate scaffolds and fibroblast, respectively

Discussion

The 3D scaffold was synthesized using a mixture of agarose, chitosan and collagen as a scaffold to guide cell adhesion, proliferation and tissue development to repair the damage organ. The optimum ratio of agarose, chitosan and collagen was found to be 3:1:0.5 by freeze-drying method for synthesis of AG-CH-CL biocomposite scaffold. Scaffold 3, 4, 7 and 8 which contained 3% agarose had a good elastic property more than other scaffolds that contained only 1% of agarose. These results indicated that, agarose was involved in the elastic strength of the scaffold.[8] The pore size of the scaffold could enhance the cell proliferation, as the waste product and nutrient can transport efficiently throughout the scaffold. In this case, AG-CH-CL scaffold contained interconnected pore with an average pore size diameter around 140 – 200 μm. The smaller pore size diameter was found with higher chitosan concentration in composite scaffold 7 and 8. In addition, scaffold 7 and 8 had absorption capacity better than scaffold 3 and 4. This might be due to that chitosan contains several functional groups, such as hydroxyl (-OH), ether (-COC) and amide (-NH), that are hydrophilic.[26, 27] Thus, the water uptake ability increase along amount of chitosan. As well as, scaffold 3 which comprise of 1% chitosan showed the lowest degradation rate, while scaffold 7 which made from 2% chitosan exhibited the highest degradation rate. These results indicated that, the degradation properties were directly related to the water uptake of the scaffold; the higher the water uptake, the higher the degradation rate.

The infrared spectrum showed that the absorbance of agarose, chitosan and collagen were also observed in scaffold 3. This finding suggested that the spectrum of composite AG–CH-CL scaffold represents a combination of functional groups present in the native polymer chains of agarose, chitosan and collagen. Remarkably, the absorption peak of the CO_2 (peak around 2400 and 600 cm^{-1}) in the environment28 also appeared in the

spectrum of chitosan and collagen. However, the unique molecular fingerprint of collagen and chitosan were peak near 3300 and 1700-900 cm^{-1}, which does not interfere by the absorption peak of the CO_2. To eliminate this effect from the environment, the spectrum will be collected by minimizing the time between the background measurement and the sample measurement, as well as purging the light path with high purity nitrogen to obtain more stable environment[28] for further studies.

Although, AG-CH-CL scaffold could support human fibroblast adhesion and proliferation, low cell growth on the scaffold at the first week of cell culture were observed. These might be due to cell proliferation in the early stages required adaptation to new environment as well as production an adhesion molecules such as integrin to bind to integrin binding site on the surface of the scaffold before starting to proliferation. In addition, some of the cells will attach to the scaffold fibers and some will move through gravity and migration directly to the bottom surface of the tissue culture plate.[29] Although low cell numbers at the first week observe by MTT, fibroblast could increase proliferation over time until 4 weeks. This might suggest that the 3D interconnected porous structure of the scaffold produces a preferable microenvironment for cells to growth. One possible interpretation is that cells penetrate inside the porous scaffold, leading to an increase in cell proliferation within the scaffold at 4 week of cell culture. The 3D cell culture scaffold has an advantage over 2D scaffold because the 3D scaffold allows cells to grow in all directions in *vitro*, similar to how they would in *vivo*. This indicates that the larger surface area of the 3D scaffolds provides a preferable environment for cell expansion than the 2D scaffold.[6]

Genipin is extracted from the fruit of the Gardenia Jasminoides Ellis plant and is regarded as a kind of natural cross-linking agent. The previously study established a genipin-cross-linked type II collagen scaffold and determine the biological effects of the scaffold on ASCs differentiating into a nucleus pulposus-like phenotype.[30] They reported that the pores of the genipin-cross-linked scaffold became irregular and the skeleton of the scaffold became twisted and folded. Moreover, the porous structure of the genipin-cross-linked scaffold gradually disappeared as the concentration of genipin increased. However, the stability of the genipin-cross-linked scaffold were increased up to 21 day when compared to non-cross-linked scaffold. These results indicated that the scaffold cross-linking by genipin increased the stability of the type II collagen scaffolds, but deformed the configuration of scaffolds and changed the intrinsic properties of type II collagen. However, this did not affect the cell proliferation on the scaffold because the scaffolds cross-linked with genipin also increased ASCs cell proliferation for 21 day when compared with the non-cross-linked scaffold. Our study showed the similar result of fibroblast cell proliferation on the AG-CH-CL scaffold for 4 weeks. The stability of the scaffold after 4 weeks of incubation with 1XPBS was only 5%, which could also support cell proliferation and differentiation as the genipin-cross-linked scaffold. However, both cross-linked and non-cross-linked scaffold could also design as the most preferable property to promote cell proliferation and differentiation, depending on the other factors such as the type of polymer, the specific characteristic of the polymer as well as technique for fabricate the scaffold. All these results suggested that the genipin-cross-linked scaffold as well as non-cross-linked scaffold should optimize the optimal condition and requires specific design to establish a suitable 3D porous scaffold for promotes cell proliferation and differentiation for individual cell types.

Conclusion

In the present work, 3D porous scaffold composed of 3% agarose, 1% chitosan and 0.5% collagen were successfully fabricated using freeze-drying method without cross linker reagent. Moreover, the composite scaffolds could apply as scaffolds for human fibroblast, showing their good biocompatibility and low cytotoxicity. Our data indicated that, this biocomposite scaffolds can be used as a potential scaffold for tissue engineering. Further confirmation of pore size stability after immerse scaffold in culture media by SEM is required to check whether degradable polymeric obstruct interconnected pore inside the scaffold. These results will lead to the effective technique for production scaffold as well as culture process for promote cell proliferation and differentiation for therapeutic regenerative disorders.

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Conflict of Interest Disclosure

The authors declare no competing financial interest.

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บทคัดย่อ

การผลิตและการศึกษาคุณสมบัติของโครงเลี้ยงเซลล์ชีวภาพจากอะกาโรส - ไคโตซาน – คอลลาเจนสำหรับงานวิศวกรรมเนื้อเยื่อ

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บทนำ: การผลิตโครงเลี้ยงเซลล์แบบแช่เยือกแข็งมีขั้นตอนการเติมสาร cross link ซึ่งมีรายงานว่ามีความเป็นพิษต่อเซลล์ งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อผลิตและศึกษาคุณสมบัติของโครงเลี้ยงเซลล์จากอะกาโรส - ไคโตซาน – คอลลาเจน ด้วยเทคนิคแช่เยือกแข็งโดยไม่ใช้สาร cross link

วิธีการศึกษา: ผลิตโครงเลี้ยงเซลล์ด้วยอะกาโรส - ไคโตซาน – คอลลาเจน ขึ้นรูปโดยเทคนิคแช่เยือกแข็ง ศึกษาขนาดรูพรุน การอุ้มน้ำ อัตราการสลายตัว ทดสอบความเป็นพิษของโครงเลี้ยงเซลล์ต่อเซลล์ไฟโบรบลาสต์ด้วยเทคนิค MTT รวมทั้งศึกษาการเจริญเติบโตของเซลล์โดยการย้อม H&E

ผลการศึกษา: โครงเลี้ยงเซลล์ที่ใช้ อะกาโรส - ไคโตซาน – คอลลาเจนในสัดส่วน 3:1:0.5 มีคุณสมบัติเหมาะสมในการเลี้ยงเซลล์มากที่สุด เนื่องจากมีรูพรุนเชื่อมต่อกัน ขนาดประมาณ 184 ไมครอน การอุ้มน้ำสูงถึง 85% และมีอัตราการสลายตัวที่ 4 สัปดาห์เพียง 5% นอกจากนี้เซลล์ไฟโบรบลาสต์ยังสามารถเจริญบนโครงเลี้ยงเซลล์ได้ดี

สรุปผลการศึกษา: งานวิจัยนี้ชี้ให้เห็นถึงวิธีในการผลิตโครงเลี้ยงเซลล์ที่เหมาะสมต่อการนำไปใช้ ซึ่งควรผลิตจากสารธรรมชาติและใช้สารเคมีให้น้อยที่สุด การศึกษาครั้งนี้แสดงให้เห็นว่าโครงเลี้ยงเซลล์ที่ผลิตได้มีศักยภาพในการนำไปใช้ประโยชน์ด้านวิศวกรรมเนื้อเยื่อ

คำสำคัญ: วัสดุชีวภาพ, โครงเลี้ยงเซลล์, วิศวกรรมเนื้อเยื่อ