Fabrication of Cellulose-Gelatin Based Endothelialed Vascular Graft with SMCs/ADSCs Seeding in Bioreactor

Ts-Ching Yu and Ming-Long Yeh*

Medical Engineering Department, National Chen Kung University, Taiwan

Abstract—**Cardiovascular disease (CVD) is a major health issue over the worldwide. The application of tissue engineering technology holds great promise for improving outcomes in CVD patients. Currently, the use of synthetic vascular grafts has several limitations, including thrombogenicity, increased risk of infection, and lack of growth potential. Natural vascular grafts have many advantages including excellent degradation, biocompatibility, but low mechanical property. Therefore, to form a biodegradable and antithrombotic vessel graft as temporary substitute is a problem to overcome in recent researches. The purpose of our study is using natural materials to rapidly form an endothelialized vascular graft in vitro. We utilized cellulose and gelatin to fabricate a composite scaffold. In order to enhance the mechanical property and decrease the degradation rate of scaffold, we added genipin to crosslink cellulose and gelatin. We mixed the cellulose, gelatin, and genipin solution, and injected solution into self-made mold to form tubular hydrogel. At last, we could obtain the tubular scaffold by freeze-drying. With regard to fabrication of vascular graft, the smooth muscle cells (SMCs) extracted from rabbit's carotid arteries would be seeded into scaffold to secret the collagen and elastic fiber, while adipose derived stem cell (ADSCs) would be seeded onto the lumen of scaffold as the source of endothelial cells. The material property of scaffold were analyzed by swelling test, biocompability, and FTIR. Moreover, we observe the SMCs distribution and extracellular matrix (ECM) deposition of vascular graft by histology.**

Keywords— **natural polymer, genipin, SMCs, endothelia cell.**

I. Introduction

Natural vascular grafts have many advantages including excellent degradation, biocompatibility, but low mechanical property. Therefore, to form a biodegradable and antithrombotic vessel graft as temporary substitute is a problem to overcome in recent researches. The purpose of our study is using natural materials to rapidly form an endothelialized vascular graft in vitro.

Cellulose can be considered for biomedical application because of owning biocompatibility and available[1]. Gelatin was chosen in this study because it is a derivative of collagen. Gelatin exhibit anti-antigenicity, high biodegradable rate, and high cell affinity properties[2]. In order to afford cells enough time to proliferate within the scaffold in vivo, we have to reduce the degradable rate of the scaffold through crosslinking. Genipin was used as an ideal crosslinking agent, which has less cytotoxicity compared to traditional crosslinking agent[3]. The cellulose/ gelatin hydrogel are nontoxic, inexpensive, and own high surface-to-volume ratio of pores, which is good for cell to adhesion and proliferation.

Studies have shown that smooth muscle cells (SMCs) could secrete the type I collagen that mimicking the extracellular matrix. Rabbit SMCs are seeded by perfusion of a cell suspension from the lumen (inner diameter 2 mm) through the wall of a tubular scaffold.

For endothelialization, it's reported the ability of adipose-derived stem cells(ADSCs) to differentiate into cells functional features of endothelial cells when treated with the shear stress[4]. Mechanical stimulation of ADSCs seeded in vascular tissue engineering scaffolds promotes the endothelial layer formation. Moreover, various growth factors were used to promote biomaterial endothelialization.

In our study, cellulose and gelatin were fabricated as a tubular scaffold, and seeded with SMCs to mimic the natural vessel environment.

II. Materials and Methods

Type B gelatin (pharmaceutical grade, pH 5.64, pI 4.9) were used. Cellulose(Degree of polymerization < 350, Particle Size: < 1% +60 mesh, Alfa Aesar). Genipin (Molecular Weight 226.23, ≥98% (HPLC), powder, Sigma) All other chemicals used in this work are of analytical grade.

Scaffold fabrication

Type B gelatin were swollen in deionized water at room temperature and then dissolved at 37°C under agitation to obtain 50 wt%(w/w) solutions. Add 1g cellulose into 3.5ml deionized water, then, add 1ml gelatin solution under agitation. The gelatin/cellulose solution was cross-linked by genipin, and injected into designed mold. A tubular rod is as the mold(outer diameter: 4mm, internal diameter: 2mm; length: 5cm), After injecting, the mold was rotated at room temperature for 10 hours.

Swelling property

* Corresponding author.

J. Goh and C.T. Lim (eds.), *7th World Congress on Bioengineering 2015*,
IFMBE Proceedings 52, DOI: 10.1007/978-3-319-19452-3_10

The water absorption capacities of the scaffolds were determined by swelling them in phosphate buffered saline (PBS) at room temperature. The freeze-dried scaffolds were placed in the PBS solution for 5 h. The swelling ratio of the scaffold was calculated from the equation:

$$\text{Swelling Ratio (\%)} = \frac{Wt - W0}{W0} \times 100\% \quad (1)$$

Smooth muscle cell extraction

SMCs were isolated from rabbit aortas. After removing endothelium, adventitia, fat and connective tissue, were dissected and incubated in a sterile conical tube containing an enzymatic dissociation buffer under agitation on an orbital shaker (60 rpm) for 90 min at 37◦C. This buffer contains 1.0 mg collagenase in 10 ml DMEM medium. Following the complete dissolution of the matrix, centrifuged at 1000 × g for 5 min.

Cell seeding

The novel cell seeding technique is named swell-rotating method, which utilizes the water absorption property of gelatin/cellulose hydrogel. The cell would be penetrated into scaffold wall during the scaffold absorbed the medium. Prepare the medium containing ($1.5x10^5$) cells. The freeze-dried scaffolds were immersed in medium and shaked for 1 hour at 37◦C. Then, the scaffold was maintained in the medium for 4 hour in order to assist cells to adhesion. The scaffold was put into 4% formalin a half hour and underwent frozen cut. For observing the cell distribution, the cells would be stained DAPI before seeding.

Cell viability

ADSCs were seeded onto gelatin/cellulose composite sheet scaffold for 1, 3, 5, 6 days. Prepare the culture medium of the cells and composite sheet in 96-well plates containing a final volume of 100 µl/well. An optional set of wells can be prepared with medium only for background subtraction. Add 20 µl MTS solution to each well. Incubate 1 hour at 37°C. Record absorbance at 490 nm.

FTIR

The FTIR spectra of freeze-dried gelatin, and composite scaffold in absorption mode in the range of 4000~650 cm^{-1}. 32 scans were performed to establish accuracy.

III. Results and Discussions

Scaffold fabrication

The gelatin/cellulose composite scaffold contains elasticity, and appropriate mechanical strength properties. The scaffold exhibit deep blue color because of the crosslink between –NH group on the gelatin and genipin(Figure 1).

Cell viability

All samples biocompatibility was determined by MTS assay. By 5th culture of ADSCs and cross-linked scaffold shows good cell compatibility. Day 1 cell viability is up to 92%, and day 6 reaches about nearly 80%. It shows that gelatin/cellulose cross-linked scaffold afford a nontoxic environment for cell to live(Figure 2).

Swelling test

The swelling ratios at 5th hour were investigated since it was the least time for the initially cell attachment[5]. The results showed that the weight of swollen scaffolds were two times than the dried scaffolds, as presented in Table 1. Gelatin is widely known for its hydrophibic, which allows gelatin to absorb water up to 5 times of its dry weight. It shows that crosslinking may influence the absorption ability of the material.

Cell seeding

It's obviously to see that seeding ADSCs into the three-dimensional composite scaffold by swell-rotating method is an efficient way. Histologic analysis showed that many cells adhered to the scaffold within short incubation time, and adhered more uniformly around the scaffold lumen. Figure 3a and 3b showed the whole cell distribution of the scaffold, figure 3c and 3d showed the cell accumulation within the pore. Because of auto-fluorescence, cellulose exhibits red color. The swell-rotating method increases cell seeding efficiency. Compared to other cell seeding methods, it has higher cell attachment ratio, and more uniform cell distribution in the scaffold.

FTIR

The figure 4a shows the FTIR spectra of the dried gelatin films in the 900-1800 cm^{-1} finger-point region of wave numbers. According to previous studies [6.7], the absorption peaks at 1690-1760, 1500-1600, 1340-1470, 1180-1360, 1050-1300 cm^{-1} were attributed to C=O, C=C, C-H, C-N, and C-O, respectively. The results demonstrated that the FTIR spectra of dried composite films corresponded to gelatin cross-linked with genipin. Conformation of the cellulose chains and their strong packing depends on intermolecular and intramolecular H-bond. As the free –OH group peak appears at 3650-3590 cm^{-1}. Figure 4b shows that the broader peak in the 3600-3200 cm^{-1}. It's implies that the H-bond of scaffold decreases, and cellulose indeed cross-linked with genipin.

IV. Conclusions

The gelatin/cellulose composite scaffold was stabilized by crosslinking with genipin. The scaffold exhibits good swelling ability enough to be utilized for cell seeding, and excellent biological environment for cell proliferation. The swell-rotating method causes to uniform and dense cell attachment. Implanting the scaffold into rabbit artery is the next step, and I will observe thrombogenicity by angiography.

V. Figures and Table

Figure 1. The genipin cross-linked gelatin/cellulose composite scaffold.

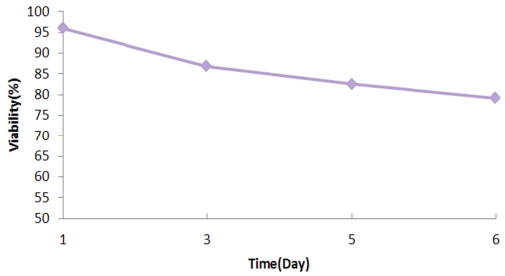

Figure 2. MTS assay cell viability that cultured for 1, 3, 5, 6 days.

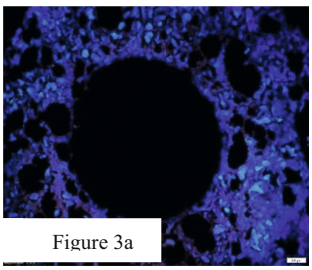

Figure 3a

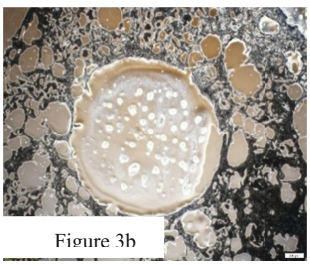

Figure 3b

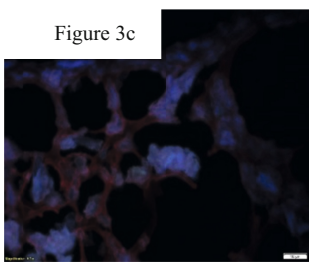

Figure 3c

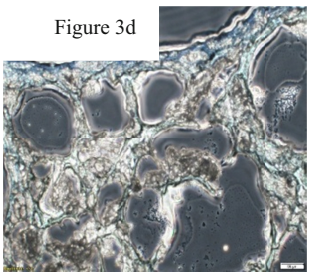

Figure 3d

Figure 3. Using swell-rotating cell seeding method. (a) The whole section of scaffold in fluorescency. (b) The whole section of scaffold in white light. (c) The cells accumulation in the pores of scaffold in fluorescency under 200X. (d) The cells accumulation in the pores of scaffold in white light under 200X.

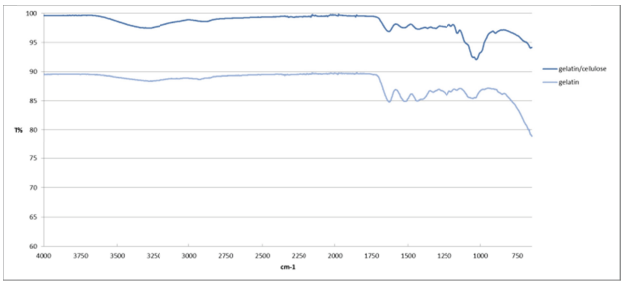

Figure 4. FTIR spectrum of dried gelatin powder and genipin cross-linked gelatin/cellulose powder with a scanning range of 4000-650 cm^{-1}.

	dried weight(g)	*wet weight(g)*	*swelling ratio(%)*
gelatin	0.52	2.95	467.3
gelatin/ cellulose	0.28	0.86	211.4

Table 1. The swelling ratio of gelatin and gelatin/cellulose composite.

Acknowledgment

This work was supported in part by the National Chen Kung University, Biomedical Engineering Department.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. John Rojas, Eduardo Azevedo.(2011) Functionalization And Crosslinking of Microcrystalline Cellulose in Aqueous Media: A Safe And Economic Approach. IJPSR. Volume 8, Issue 1, Article-006
2. Hutson, Che B. et al.(2011) Synthesis and Characterization of Tunable Poly(Ethylene Glycol): Gelatin Methacrylate Composite Hydrogels. Tissue Engineering Part A 17.13-14 : 1713-1723.
3. Balamurugan Manickam, Rajesh Sreedharan et al.(2014) Genipin – The Natural Water Soluble Cross-linking Agent and Its Importance in the Modified Drug Delivery Systems: An Overview. Current Drug Delivery, 139-145
4. Francesca Colazzo, Fahad Alrashed et al.(2014) Shear stress and VEGF enhance endothelial differentiation of human adipose-derived stem cells. Growth Factors; 32(5): 139–149

5. Juthamas Ratanavarporn, Siriporn Damrongsakkul. et al.(2006) Comparison of Gelatin and Collagen Scaffolds for Fibroblast Cell Culture. JOM, Vol.16 No.1 pp31-36
6. Bai-Shuan Liu, Chun-Hsu Yao et al.(2008) Effect of Drying Method on the Characteristics of Genipin Cross-linked Gelatin Films. JMBE. 29(1): 29-38
7. Shih-Ta Chang, Li-Chen Chen et al.(2012) Nano-biomaterials application: Morphology and physical properties of bacterial cellulose/gelatin composites via crosslinking. Food Hydrocolloids; 27 ,137-144