Effect of washing process after decellularization process by SDS

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(2010年2月24日　受理)

Abstract

One of the major decellularization is with surfactants. Especially, SDS has been used because of the toxicity. Remaining SDS after washing process has not been discussed although the remaining SDS affects cell migration/growth. In this paper, we will discuss residual SDS after different washing hours. The effect of SDS decellularization was observed by dynamic contact angle and AFM analyses. The residual SDS over washing time was observed by Laser Raman spectroscopy. Contact angle analysis showed that SDS bounds to the proteins to make the surface rough. AFM data showed that SDS affects the collagen 3-dimentional matrix. Washing results showed that drastic removal of SDS remaining after 30 minutes of washing with 75% Ethanol.

1. Introduction

Medical treatment of using biologically derived material has been studied in many fields. However, there are many further research required to be a reliable treatment. Major requirements for bio-derived materials were stated as follows: 1) cells can proliferate on the scaffold, 2) cells can migrate through the scaffold, 3)would not inhibit regeneration, 4)would degrade as regeneration, and 5) scaffold is non toxic. In this study, materials (Veins) which derived from Human Umbilical cord are used (HUV) as a scaffold for tissue engineering applications. Preliminarily studies have shown that HUV materials can be prepared in tubular format with constant wall thickness[1)], or flat sheet with constant thickness. In order to use HUV material for medical implantation, the material needs to be decellularized followed by seeding cells for implantation[2)]. One of the major techniques is to use surfactants for decellularization[3),4)]. We have then compared the Sodium Dodecyl Sulfate (SDS) and ethanol treatments with Ethanol, SDS decellularization. Decellularized materials were measured contact angles.

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2. Experimantal

2.1 Materials

Scaffolds used in this research were Human Umbilical Cord veins dissected from Human Umbilical Cord, which were donated from University of Oklahoma. Human umbilical cords were washed with water to remove blood, and donated by Norman regional hospital. Human umbilical cords were loaded on stainless steel rod for the length of 10cm, and stored in refrigerator at -80 °C for 6 to 12 hours. Then, human umbilical cords were dissected by machine lathe at 3600rpm, with travel speed for 10cm per minutes. After dissection, HUVs were stored in -80 °C until use.

2.2 Dissection Method

Figure 1 shows the picture of HUV dissection processes. Insert precision SS tube through vein. Then, 'zip-tie' free ends and tension.

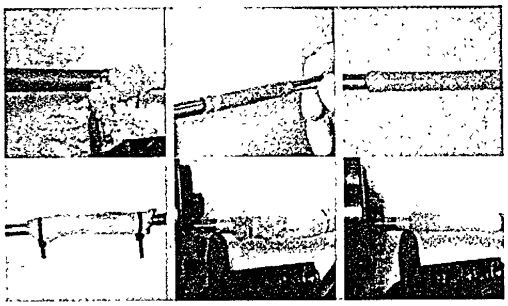

Fig. 1 Pictures of HUV dissection processes

Freeze to -20 °C for at least 6 hours, to pre-cool the material. After 6 pre-cool process, the materials were cooled down in -80 °C freezer for minimum 8 h. Frozen materials were then inserted into Lathe. The lathe was set rotation at 3900 rpm. Horizontal movement was assisted by the lathe for cutting rate 5 mm/sec. HUVs were thawed in room temperature for decellularizing process.

2.3 Decellularization

HUV were cut in approximately 1 cm for processing. HUVs were decellularised with 1% SDS solution for 24 hours. Then, HUVs were stirred in 75% Ethanol with 60 rpm for 0, 1, 5, 30, 60 minutes, and 12, 24, 36, 96, and 120 hours. After washing, HUVs were cut open on slide glass and pressed another slide glass from top to dehydration as shown in Fig. 2.

2.4. Analysis

Dehydrated SUVs were analyzed with Laser Raman Spectrometer. Wavelength was 532nm, x100, Data was taken from top to change focus to see depth wise (Z axle). Also the scaffold was cut to see the SDS (x-y axle). Also, SEM image was taken after SDS treatment.

3. Results and discussion

3.1 Dynamic contact angle

Dynamic contact angle was measured

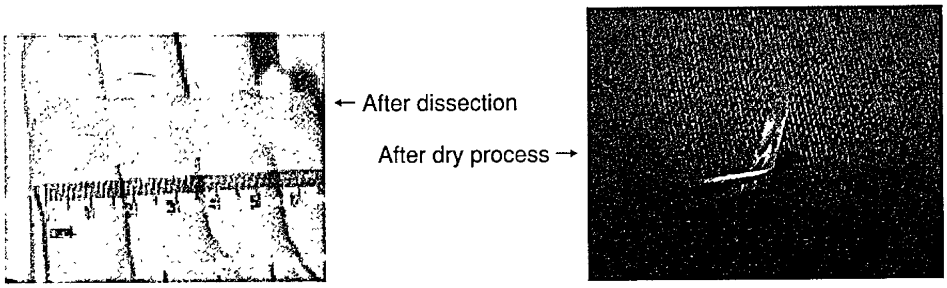

Fig. 2 Before and after drying process

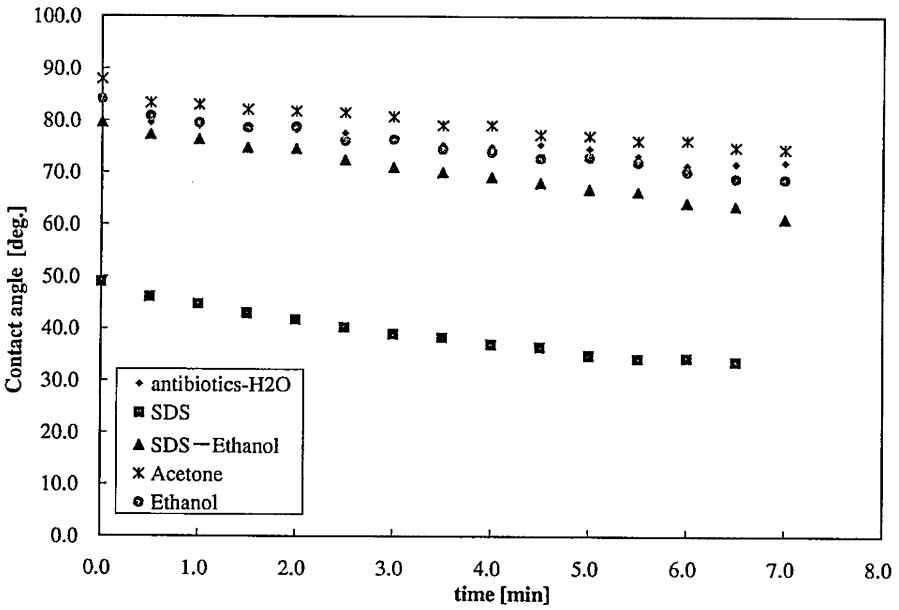

Fig. 3 Changes in dynamic contact angle at 23℃ as a function of time

overtime. Figure 3 shows the changes in dynamic contact angle at 23 °C as a function of time. The result showed that only SDS treated material showed lower contact angles. The difference may come from the roughness of the treated scaffold, or the residual SDS interferes with water droplet.

3.2 AFM

AFM data of SDS-Ethanol, SDS, and Ethanol treated material are shown in Fig.4. Non-treated material was taken for control. The result show that the SDS treated material has rough surface than other samples. Increase of the roughness means that SDS bound with lipids and break the collagen net work, which resulted roughen the surface.

3.3 LASER Raman Spectroscopy

LASER Raman spectroscopy was observed to determine if there ware any SDS remaining at the surface of the material after SDS (24

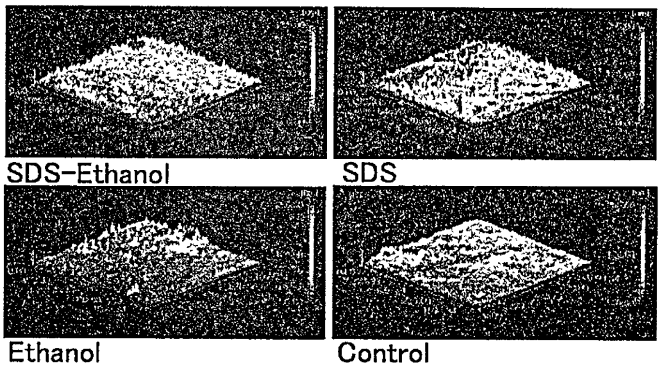

Fig. 4 AFM analysis

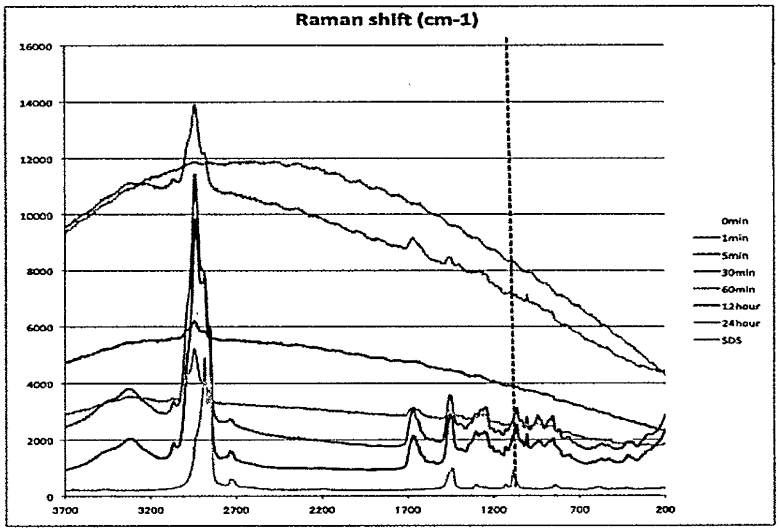

Fig. 5 Laser Raman spectroscopy data

hours) - ethanol treatment (from 1minutes to 24 hours) as shown in Fig.5. SDS has peak at 1080 cm^{-1}. Result shows that the 30 minutes of ethanol treatment would greatly reduce the SDS contents from the material

4. Conclusions

Dynamic contact angle measurement, and AFM, SEM observation showed that the SDS treated material had a very rough surface. However, presence of SDS may greatly affect cell growth. LASER Raman spectroscopy data showed that the 30 minutes of ethanol treatment to wash off residual SDS from the material was effective. LASER Raman spectroscopy observation in depth wise was needed to check if there is any SDS remaining in the material. In addition, the cell seeding observation was also needed to see the cell growth between SDS and cell growth.

References

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