Preparation and Properties of Decellularized Sheep Kidney Derived Matrix Scaffolds

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Abstract. Scaffolds from tissues or organs have nanoscale microstructures. Derived matrix scaffolds prepared by decellularized method can provide more cell attachment sites, which is conducive to cell adhesion, proliferation, differentiation and other physiological activities on scaffolds. In this study, the sheep kidney decellularized matrix scaffold was prepared by the method of decellularization. Due to the poor mechanical properties of the decellularized matrix, the cross linking method was adopted to enhance its mechanical properties. The decellularization efficiency of sheep renal matrix scaffolds was observed by scanning electron microscopy and histological staining, and the biocompatibility of the scaffolds was investigated by inoculating adipose derived stem cells. It was found that the scaffold had good decellularization effect and good pore structure.

1. Introduction

Biomaterials are widely used in the field of tissue engineering because of their ability to support cell proliferation, migration, and differentiation, allow oxygen and nutrient transport, and mimic natural soft tissue. The biomaterials used to make scaffolds may be natural polymers such as alginate [1], collagen [2], gelatine [3], fibrin [4], and albumin [5], or synthetic polymers such as polyvinyl alcohol [3], polyethylene glycol [6], and polyacrylamide [7]. Scaffolds made from different materials have different properties. For natural polymer materials, although they have good biocompatibility and are conducive to cell adhesion and proliferation, their mechanical properties are poor with a relatively higher degradation rate. Synthetic polymer materials have good mechanical properties and are easy to be processed. Their structure and properties can be modified and regulated as needed which makes
them more suitable for mass production. However, synthetic polymer materials still lack cell action sites with poor cell adhesion.

Bioscaffolds made of extracellular matrix have been widely used in clinical therapy and in vitro and in vivo basic research. Extracellular matrix (ECM) is an acellular three-dimensional polymer network composed of collagen, proteoglycan/glycosaminoglycan, elastin, fibronectin, laminin, and several other glycoproteins [8]. It is highly dynamic because it is continuously deposited, remodeled, and degraded during development until it matures to maintain tissue homeostasis. Therefore, its composition varies with tissue change according to specific requirements [9]. ECM protein can guide the differentiation of embryonic stem cells and is an important factor that affects cell behavior during organogenesis. In particular, if the ECM protein structure is very similar to the ECM structure of the target tissue in vivo, the differentiation to the target tissue can be improved [10, 11].

The successful culture of bioporous scaffolds on cells in vitro provides a potential for further exploration of bioporous scaffolds in vivo, biomedicine and customized applications in animals. In this study, renal derived matrix scaffolds were prepared by decellularized method. Due to the limited mechanical properties of natural matrix materials, chemical cross-linking method was used to improve the mechanical properties of the scaffold. The physical properties and biocompatibility of the scaffolds were tested.

2. Materials and Methods

2.1. Materials

The adipose tissue was provided by the Affiliated Hospital of Dalian Medical University. Human adipose tissue-derived stem cells (ADSCs) were isolated from subcutaneous adipose tissue obtained from liposuction surgery performed on a 32-year-old middle-aged woman. The process strictly adhered to the requirements of the Helsinki Declaration and signed with the patient's consent. ADSCs were cultured in a humidified incubator at 37°C with 5.0% CO2 and DMEM medium contained 10% fetal bovine serum. Sodium dodecyl sulfate (SDS) was purchased from Shenyang WanLei Technology Co., LTD. N-(3-dimethylaminopropyl)-N'-ethyl carbonimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and 2-(N-morpholine) Ethyl sulfonic acid (MES) were purchased from Beijing Balinway Technology Co., LTD. Dulbecco's improved Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA, USA). Calcein-AM, Propyl iodide (PI), and Hoechst 33342 were all from Calbiochem (San Diego, CA).

2.2. Preparation of Decellularized Sheep Kidney Matrix Scaffold

Fresh sheep kidneys were taken from the market, washed with clean water until the water has no obvious blood color, and then put the cleaned sheep kidneys into the refrigerator at -20~30°C and frozen for 10~14 hours. After freezing, the renal pelvis and fibrous capsule were removed, the remaining kidneys were cut into small pieces of 8~12 cm³ in volume. The kidney block was bathed in phosphate (PBS) buffer and washed several times. The treated renal lumps were put into the solution of sodium dodecyl sulfate (SDS) with a mass fraction of 0.5%. Under the condition of low-speed magnetic agitation, the agitation time was 48~72 h (appropriate liquid exchange). The matrix was frozen in a refrigerator at -20~ -30°C for 3~5 h and then freeze-dried for 14~19 h to obtain a porous kidney-derived matrix scaffold. Kidney-derived matrix scaffolds were cross-linked for 36~48h in an ethanol solution containing 50mmol/L 1-(3-dimethylaminopropyl)-3-ethyl carbonimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and ethyl sulfonic acid (MES). Finally, the sheep kidney decellularized scaffolds after cleaning were freeze-dried for 19~24 h to obtain the sheep kidney-derived matrix scaffolds.

2.3. Decellularization Efficiency Test

Scanning electron microscopy (SEM, SU1510, Hitachi High Technologies, Japan) was used to observe the microstructure, pore size, and vascular structure of the sheep kidney matrix after decellularization and cross-link. In addition, histological staining analysis was performed on the sheep kidney matrix before and after decellularization. Sample treatment: The samples were fixed by 2.5%
glutaraldehyde, dehydration through different concentrations of ethanol (50%, 70%, 80%, 90%, 100%), embedded by paraffin, and sliced by 3 mm for hematoxylin, heosin staining (H&E), and Masson trichromatic staining.

2.4. Biocompatibility
Adipose stem cells (ADSCs) are cultured in DMEM medium with 10% fetal bovine serum in a humidified incubator at 37°C and 5% CO₂. The medium is changed every two days. 80% of the cells fill the culture flask for passage. Using ADSCs to investigate the biocompatibility of kidney-derived decellularized matrix scaffolds. The kidney-derived decellularized matrix scaffold was cut into small pieces with a size of 5mm x 5mm x 2mm, soaked in 75% alcohol and placed in an ultra-clean table for UV sterilization overnight. Soak in PBS for two hours, changing the liquid every hour. Place the scaffold in a 24-well plate, adopt a double-sided inoculation method, and draw 20μL of ADSC cell suspension with a cell density of 2x10⁵ cells/ml to inoculate on the scaffold. After the cells are inoculated with the scaffold, when they are cultured for 10 days, add 2μmol/L Calcein-AM, 4μmol/L PI and 5μg/L Hoechst 33342 staining solution and incubate in an incubator for 30 minutes. Observe and take pictures under a focal laser microscope.

3. Results and Discussion

3.1. Decellularization Efficiency Detection
Figure 1 shows the whole process of the sheep kidney decellularized matrix scaffold. Sheep kidney matrix is elastic, it is light red before decellularization, and becomes transparent after decellularization. From the change of the appearance, it can be preliminarily judged that the decellularization effect is good. When a certain pressure is applied to the matrix, the sheep kidney matrix will undergo certain deformation.

![Figure 1. Preparation and physical image of decellularized sheep kidney matrix scaffold](image-url)
Figure 2. Detection of efficiency of decellularized sheep kidney scaffold.
(a) Masson staining of non-decellularized sheep kidney matrix; scale: 40μm
(b) HE staining of non-decellularized sheep kidney matrix; scale: 40μm
(c) Masson staining of sheep kidney after decellularization; scale: 40μm
(d) HE staining of sheep kidney after decellularization; scale: 40μm
(e) SEM examination of non-decellularized sheep kidney matrix;
(f) SEM examination of uncrosslinked sheep kidney matrix scaffold;
(g) SEM examination of crosslinked sheep kidney matrix scaffold.

The histological staining of HE and Masson before and after decellularization of sheep kidney matrix is shown in Figure 2 (a) (b) (c) (d). HE staining indicates the integrity of the pore structure of the kidney-derived matrix, and Masson staining indicates the integrity of the collagen structure. The arrow in the figure indicates an important factor that characterizes the effect of decellularization: glomerular structure.

The micro morphology of renal matrix, decellularization and cross-linked scaffolds is shown in Figure 2 (e) (f) (g). In tissue engineering, the key factor affecting the performance of the scaffold is the pore structure of the scaffold. Therefore, in this experiment, the microstructure of the prepared matrix scaffold was further observed by scanning electron microscope. It can be seen from the figure that the scaffold has a rich multi-structure of interconnected holes, which is conducive to the contact and mass transfer between cells. The surface of the microstructure of the solid sheep kidney is very dense. Although there is no significant difference between the uncrosslinked and crosslinked derivatized matrix scaffolds, further observation revealed that the two have differences in microscopic
morphology. Part of the structure of the uncrosslinked sheep kidney matrix scaffold was damaged, and the pore size was not obvious. After cross-linking, the decellularized matrix scaffold has a uniform pore size distribution and a complete structure. This may be due to the repair of the scaffold structure during the cross-linking process and the reconnection of the damaged amide bond during the decellularization process, the pore size ranges from 268 to 379μm.

3.2. Biocompatibility of Sheep Kidney Matrix Scaffolds

The biocompatibility test of the sheep kidney matrix scaffolds is shown in Figure 3. Good biocompatibility will be more conducive to clinical treatment and in vitro and in vivo basic research. ADSCs were used to investigate the biocompatibility of scaffolds. Calcein-Am is a cell staining reagent that can fluorescently label living cells. The Hochest marks all the nuclei, and PI is often used for apoptosis. After 10 days of culture, the area of the colony became larger and larger as the number of cells increased (red arrow). Hochest staining also revealed a gradual increase in the number of nuclei. No red staining cells (dead cells) were found by PI staining, indicating that the renal matrix scaffold has good biocompatibility.

Figure 3. Growth of adipose tissue stem cells (ADSCs) on sheep kidney derived matrix scaffolds. The cells were dyed alive with Calcein-Am, PI and Hoechst 33342, respectively. Scale: 100μm. The red arrows indicate cell colonies.

4. Conclusion

In this study, the sheep kidney cells were removed by decellularization reagent, and the renal matrix scaffold was prepared by freeze-drying technology. Due to the limited mechanical properties of the decellularized matrix, chemical crosslinking was used to increase the mechanical properties of the scaffold. By histological staining and scanning electron microscopy, it was found that the scaffold had good decellularization effect and good pore structure. Finally, ADSCs were implanted into the scaffold, which proved that the crosslinked matrix scaffold had good biocompatibility.

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