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Preparation and endothelialization of decellularised vascular scaffold for tissue-engineered blood vessel

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Abstract The proliferation of cells on the decellularised tissues fixed by chemical crosslinking agent is retarded for cytotoxicity of crosslinked tissues. To overcome this disadvantage, we prepared the decellularised vascular scaffold through fixing the porcine thoracic arteries with 40mL/L ethylene glycol diglycidyl ether (EGDE), and reduced the cytotoxicity of this scaffold by treating it with lysine and coating it with type I collagen, finally endothelialized it in vitro. The EGDE-fixed porcine thoracic arteries were examined morphologically. The fixation index determination and the biomechanics test were also performed. Human umbilical vein endothelial cells (HUVECs) were seeded on the type I collagen-coated surface of different modified vascular tissues (fixed with glutaraldehyde or EGDE or EGDE + lysine), and the growths of HUVECs on the specimens were demonstrated by means of MTT test. Finally, HUVECs were seeded on the luminal surface of the modified porcine vascular scaffolds which were respectively treated in the same manner described above, and then cultured for 7 days. On the seventh day, the HUVECs on the specimens were examined by means of light microscopy, scanning electron microscopy and transmission electron microscopy (TEM). The antigenicity of the vascular tissues can be diminished by EGDE through getting rid of cell in the vascular tissues or reducing the level of free amino groups in the vascular tissues. In this study, it was also found that the EGDE-fixed porcine vascular tissues appeared similar to the native porcine vascular tissues in color and mechanical properties. After treated by 2% lysine and coated with type I collagen, the EGDE-fixed porcine vascular tissues were characterized by low cytotoxicity and good cytocompatible. The HUVECs can proliferate well on the modified vascular tissues, and easily make it endothelialized. The results showed that the modified porcine vascular scaffolds should be a promising material for fabricating scaffold of tissue-engineered blood vessel.

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Introduction

Atherosclerotic vascular diseases such as coronary artery disease and peripheral vascular disease are still the largest cause of mortality in the western societies [1, 2]. Over 600,000 vascular grafts are implanted annually to replace damaged blood vessels [3, 4]; therefore, there is an increasing demand for blood vessel substitute in clinic. However, the traditional blood vessel substitutes such as synthetic grafts, autografts, allografts and xenografts can not meet clinic demand due to mismatch compliance, limited supply and antigenicity etc, especially in small-diameter (<6 mm internal diameter) vascular graft. In an attempt to overcome these limitations vascular tissue engineering is a new multidisciplinary approach to create completely autologous, living blood vessel substitute. The prefabrication of vascular scaffold and its endothelialization will be involved in engineering a blood vessel

substitute. Naturally derived tissues are important biomaterials fabricating vascular scaffold that can offer better constructions for adhesion and growth of cells over synthetic materials and possess mechanical properties similar to those of native vessels [5]. Because of immediate degradation of naturally derived tissues obtained from the abattoir, cadaver or patient and the presence of antigenicity in allogenic or xenogenic tissues, the fresh biological tissues cannot directly be preserved and applied. The use and preservation of these natural biomaterials have typically required pre-treatment aimed at (1) reducing the antigenicity of the materials, (2) enhancing the resistance of the materials to enzymatic degradation, (3) stabilizing the structure of the tissues and maintaining their mechanical properties [6]. Although glutaraldehyde has been widely used in the pre-treatment of natural biomaterials for the purposes mentioned above, its application in clinics was limited by the side-effects of the glutaraldehyde treatment to natural biomaterials such as high cytotoxicity, stiff and calcification. Polyepoxy compound is a hydrophilic crosslinking agent, its treatment to natural biomaterials represents an effort to overcome some of the drawbacks that are typically encountered with glutaraldehyde. However, its use in clinics has also been associated with several problems, including cytotoxicity, mechanical property, and poor cell adhesion [7, 8]. Here, we prepared the biological vascular scaffolds by pre-treatmenting porcine thoracic arteries with ethylene glycol diglycidyl ether (EGDE). The characteristics of the biological vascular scaffolds fixed with EGDE were investigated. The EGDE-fixed vascular scaffolds were also treated with 2% lysine solution to reduce its cytotoxicity. Further, the EGDE-fixed vascular scaffolds were successfully endothelialized in vitro.

Materials and methods

Preparation of decellularised vascular scaffold materials

Crosslink of the porcine thoracic arteries

The porcine thoracic arteries from 6- to 8-month-old Longchang pigs were obtained from a local abattoir. The arteries were 5–10 mm in inside lumen diameters. Warm ischemic time was no more than 6 h from the time of tissue extraction to processing. Excess connective tissue was removed from the ablumenal surface of the arteries with a scalpel. A 4% polyepoxy compound (ethylene glycol diglycidyl ether, EGDE, Sigma Chemical Co., St Louis, MO, USA) solution was employed to fix the porcine arteries, and this polyepoxy compound solution was buffered with sodium carbonate /sodium bicarbonate (0.21 M/0.02 M, pH 10.5). Meanwhile, the samples fixed with 0.625% glutaraldehyde solution that was buffered with phosphate-buffered saline (0.01 M, pH 7.4) were used as a control. The samples of each group were taken out at various elapsed fixation periods (from 7 min to 72 h).

Fixation index determination

It is known that the amount of free amino groups in the tested tissue, after heating with ninhydrin (NHN), is proportional to the optical absorbance of the solution. So the fixation index was determined by NHN assay and defined as [9]:

$$FI(\%) = \frac{\left[(NHN - reactive\_a\min e)_{fresh} - (NHN - reactive\_a\min e)_{fresh}\right]}{(NHN - reactive\_a\min e)_{fresh}} \times 100\%$$

The tested tissue was first lyophilized for 24 h and then weighed. Subsequently, the lyophilized tissue was heated with an NHN solution for 20 min. After heating with NHN, the optical absorbance of the solution was recorded with a spectrophotometer (model UV-150-02, Shimadzu Corp. Kyoto, Japan) using glycine at various known concentrations as standard.

Mechanical examination

The mechanical testing was conducted in a Plexiglas tank containing saline at 37 °C on an Instron material testing machine (Mini 44, Canton, MA, USA). A segment of vessel (nature, EGDE treated or glutaraldehyde treated) was longitudinally opened and cut into 40 mm × 10 mm rectangular slab specimens with the long axis aligned along the circumferential axes of the vessel. The thickness of the sample was obtained using a micrometer. The tested tissue strip was mounted between two screw-tightened brass grips lined with sandpaper to prevent slippage. The upper grip was attached to the fixed load cell, while the lower grip was secured to the bottom of the Plexiglas tank. Prior to each

test, samples were preconditioned with three linear loading cycles to ~33% of the force at failure to remove stress history. The tested tissue strip was then extended at 10 mm/min from 0 g load until the tissue strip ruptured. Fracture was taken to occur when the first decrease in load was detected during extension. The ultimate tensile strength was taken as the force at which fracture occurred divided by the initial cross-sectional area. The linear tensile modulus was defined by the slope of the region extending from 25% to 75% of the peak failure stress.

Histology

The nature samples and the samples cross-linked with EGDE were examined histologically by hematoxylin & eosin (H&E) stain.

The endothelialization of decellularised vascular scaffold materials in vitro

Endothelial cell isolation and expansion

Human umbilical vein endothelial cells (HUVECs) were isolated from human umbilical veins as described [10]. Briefly, endothelial cells were detached from the vein by 20 min incubation at 37 °C with 0.05% trypsin/0.02% EDTA solution in PBS. Subsequently, the cell suspension was centrifuged for 7 min at 200*g*, after which the endothelial cells were seeded in a 75 cm^2 tissue culture polystyrene (TCPS) flask (Corning Costar, Cambridge, MA, USA) coated with 2 mg/mL fibronectin (Sigma). HUVECs were cultured at 37 °C in humidified 95% air/5% CO_2 in culture medium (M199, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 20 mM HEPES, all from Gibco) containing 20% pooled new born calf serum. HUVECs were subcultured in 162 cm^2 TCPS flasks coated with 2 mg/mL fibronectin until passage three. Before testing cellular compatibility, the media was aspirated from TCPS flasks and the cells were washed briefly with Hanks' balanced salt solution. Cells were then dissociated with 0.25% trypsin/0.02% ethylenediaminetetraacetic acid (EDTA) (Sigma) for 1 min, transferred to M199 media containing 20% fetal calf serum, and centrifuged (800 g for 5 min at 4 °C). The resultant pellet was resuspended in the M199 growth medium (containing 15% new born calf serum, 5% foetal calf serum, Endothelial cell growth factor 20 μg/mL, heparin 100 μg/mL, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 20 mM HEPES) to a concentration of 2×10^4 cell/mL. Before seeding, Cell identity was confirmed by in situ von Willebrand/Factor VIII staining (Sigma-Aldrich, Steinheim, Germany) using the EnVision™ AP detection system (Dako, Hamburg, Germany), and by observing the typical cobblestone morphology of endothelial cells in confluent monolayer.

Cellular compatibility of decellularised vascular scaffold materials

A segment of nature vessel with 10 mm in inside diameters was longitudinally opened, and the vessel wall was cut into 0.3 cm × 0.3 cm foursquare slab specimens. These specimens were divided into four groups as follows:

1. fixed with glutaraldehyde for 72 h and coated with type I collagen (Sigma). (The second group, $n = 5$)
2. fixed with EGDE for 72 h and coated with type I collagen. (The third group, $n = 5$)
3. fixed with EGDE for 72 h and treated with 2% lysine solution for 4 days (the solution was changed daily), finally coated with type I collagen. (The fourth group, $n = 5$)
4. As a control experiment for comparison, the polystyrene 96-well flat-bottom culture plate (Corning Costar, Cambridge, MA, USA) was also used as a test specimen. (The first group, $n = 5$)

The M199 growth medium (containing 15% new born calf serum, 5% foetal calf serum, Endothelial cell growth factor 20 μg/mL, heparin 100 μg/mL, 2 mM L-glutamine, 100U/mL penicillin, 100 μg/mL streptomycin, 20 mM HEPES, all from Gibco) was distributed onto each specimen placed in the bottom of well in a 96-well plate. After 24 h culture in a incubator, the M199 growth medium in each well was sucked off, and 200 μL M199 growth medium suspension of HUVECs at 2×10^4 cell/mL was evenly distributed onto each well. The cell culture was maintained at 37 °C in humidified 95% air/5% CO_2. Using the MTT assay, the viable cells cultured on each test specimen were determined at 1, 3, 5, 7 and 9 days after cell seeding. During the cell-culture period, M199 growth medium was changed daily.

In the MTT assay, the cells cultured on each test specimen were washed with PBS twice and viable cell numbers then were determined indirectly by 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, Missouri USA) dye reduction. The MTT assay is based on the reduction of MTT, a yellow soluble dye, by the mitochondrial succinate dehydrogenase to form an insoluble dark blue formazan product. Only viable cells with active mitochondria reduce significant amounts of MTT to formazan [11]. In the test, 200 μL serum free medium and 20 μL MTT solution (5 mg/mL in Ca^{2+} and Mg^{2+} free PBS) were added to culture wells. After incubation for 3 h at 37 °C in a 10% CO_2 atmosphere, the MTT reaction medium was removed and blue formazan was solubilized by 100 μL dimethylsulfoxide

(DMSO). Optical density readings were then performed using a multiwell scanning spectrophotometer (MRX Microplate Reader, Dynatech Laboratories Inc., Chantilly, Virginia, USA) at a wavelength of 570 nm.

Endothelialization experiments

Three 10 cm length of <6 mm internal diameters porcine thoracic arteries were respectively treated in the same manner described in the second group, the third group and the fourth group of chapter ''Cellular compatibility of decellularised vascular scaffold materials'', and then washed with PBS. After culturing with the M199 growth medium (prepared in the same manner described above) in the incubator for 24 h, each vascular construction was clamped at one end, and HUVECs suspension (5×10^5 cell/mL) was injected into the lumen of each conduit several times with the use of a 5-mL disposable syringe with a 26-gauge needle, finally the other end of each conduit was also clamped. The conduits were put into sterile cylinders, and placed on a modified blood roller, and rotated at 16 revolutions per hour for 1 h at 37 °C. After seeding, the conduits were placed in M199 growth media and incubated at 37 °C in a 5% CO_2 atmosphere for 7 days. During the cell-culture period, M199 growth medium was changed daily.

At the seventh day postseeding, segments (2.5 cm) were cut from the end of each conduit. These segments were processed through a series of dehydrating EtOH–H_2O rinses and embedded in paraffin. Processed segments were mounted onto a microtome and sectioned into 6 mm slices in the longitudinal orientations. Following removal of the paraffin through a series of rehydrating rinses with xylene substitute and EtOH–H_2O; the sections were stained with Hematoxylin and Eosin Y (H&E) to color the cell nuclei blue, and observed with a light microscope.

Samples for SEM examination were fixed in 2% glutaraldehyde solution, and gradient dehydrated at critical point followed by AuPd sputtering. Samples were observed in JSM-255 scanning microscope (JEOL, Japan).

For transmission electron microscopy (TEM), after prefixation with glutaraldehyde, cell samples were rinsed with cacodylate buffer and postfixed in O_sO_4 with 1.5% $K_3Fe(CN)_6$ for 2 h at room temperature. The samples were dehydrated by graded series of ethanol and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (Hitachi, H-7500, Japan), operated at 60 kV.

Statistics

Result data were expressed as mean ± standard error of the mean. Comparisons between groups were performed by ANOVA test. Statistical significance was set at $P < 0.05$.

Results

Crosslinking characteristics

After fixation, the color of the vascular tissues fixed with EGDE remained natural, while that fixed with glutaraldehyde turned yellowish. It was noted that all the fixed vascular tissues tended to be stiffer than the fresh one. The EGDE-fixed vascular tissues were more pliable than the glutaraldehyde-fixed counterpart. Fixation index of the vascular tissues fixed with EGDF or glutaraldehyde are showed in Fig. 1:

As shown in the figure, the fixation index of the glutaraldehyde-fixed vascular tissues increased more rapidly than its EGDE-fixed counterpart at the beginning of fixation. This indicated that the initial fixation rate of glutaraldehyde was faster than that of the EGDE. However, the fixation indices of both studied groups were comparable after 5 h of fixation. After 72 h of fixation, the fixation index of both the glutaraldehyde-fixed and EGDE-fixed vascular tissues reached maximum (>90%).

Mechanical properties

Biomechanical analysis was performed on the fresh, the EGDE-fixed and the glutaraldehyde-fixed vascular tissues. The results are showed in Table 1:

As shown in the table, the values of ''maximum load'', ''energy at break'' and ''tensile stress at maximum load'' for the glutaraldehyde-fixed vascular tissues were greater than that of the fresh and the EGDE-fixed vascular tissues ($P < 0.05$). But there is no significant difference in ''tensile strain at maximum load'' and ''E-Modulus'' among the fresh, glutaraldehyde-fixed and EGDE-fixed vascular tissues. In contrast, there were no statistical differences in all test items for those EGDE-fixed vascular tissues with respect to fresh vascular tissues.

Ultrastructures

Histological examination of the vascular tissues after the fixation process showed intact total framework. H&E staining did not show any signs of remaining nuclear material in the vessel walls (data not shown), indicating successful decellularization through the thickness of the vessels. The H&E staining also showed that the microcosmic structure of collagen preserved well after fixation with EGDE.

Cellular compatibility

Figure 2 illustrates the time curves of the optical density readings obtained in the MTT assay for the HUVECs

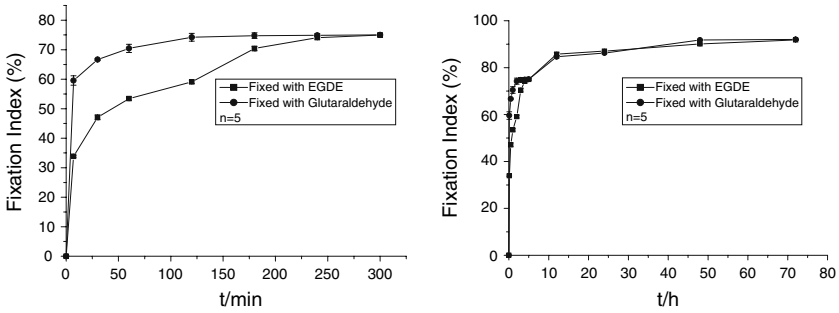

Fig. 1 Fixation indices of the porcine vascular tissues fixed with EGDE or GA (left, fixed within 5 h; right, fixed within 72 h)

Table 1 Mechanical properties of porcine vascular tissues ($\overline{X}$ ± S, n = 5)

Treated arteries	Maximum load (N)	Energy at Break (J)	Tensile strain at maximum load (mm/mm)	Tensile stress at maximum load (Mpa)	E-Modulus (Mpa)
Fresh vessel	16.64 ± 3.99	0.15 ± 0.04	1.06 ± 0.22	1.19 ± 0.29	2.21 ± 0.66
EGDE-fixed-vessel	15.10 ± 3.78	0.11 ± 0.02	0.98 ± 0.04	0.95 ± 0.22	2.06 ± 0.70
GA-vessel	32.42 ± 7.6*	0.39 ± 0.15**	1.08 ± 0.20	1.99 ± 0.47*	2.93 ± 0.90

* $P < 0.05$ compared with Fresh vessel and EGDE-fixed vessel tissues

** $P < 0.05$ compared with EGDE-fixed vessel tissues

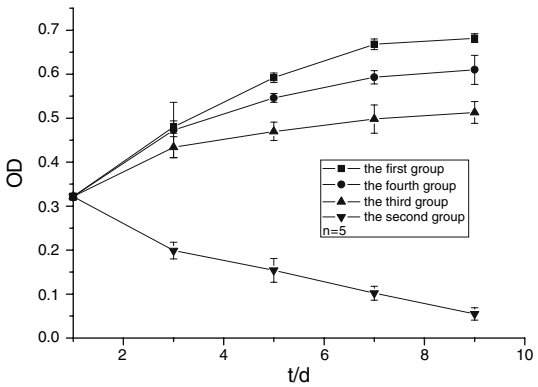

Fig. 2 Curves of growth rate of the HUVEC cultured on scaffold biomaterials

cultured on the surfaces of polystyrene culture plate and each group of the tissues treated in the same manner described in chapter ''Cellular compatibility of decellularised vascular scaffold materials''.

As shown in the figure, the optical density reading for the cells cultured on the surface of the tissues of the second group declined continuously. On the other hand, the optical density readings for the cells cultured on the surfaces of the tissues of the first group, the third group and the fourth group increased with increasing the culture duration. The results suggest that the cellular compatibilities of the EGDE-fixed tissues and the EGDE-fixed + lysine treated tissues were significantly superior to their glutaraldehyde-fixed counterpart. Compared with the EGDE-fixed tissues, the cellular compatibilities of the EGDE-fixed + lysine treated tissues were better.

Endothelial cell culture on decellularised vascular scaffold in endothelialization experiments

After coating with type I collagen, several decellularised vascular scaffolds were seeded with HUVECs and examined histologically after 7 days. As shown in Fig. 3, cells seeded and cultured on the luminal surface of the vascular scaffolds treated with EGDE and lysine were unable to invade the scaffold and formed confluent monolayer, while no cell was observed on the luminal surface of the vascular scaffold fixed with glutaraldehyde. Compared with cells cultured on the surface of the vascular scaffolds treated with EGDE and lysine, the counterpart of the vascular scaffold treated solely with EGDE did not reach a confluent monolayer.

As shown in Fig. 4, scanning electron microscopy showed the results similar to the light microscopy.

TEM revealed a monolayer of endothelial cells with fully spread shape on the luminal surface of the vascular scaffolds treated with EGDE and lysine after 7 days of proliferation (Fig. 5). Cross-sections of HUVECs showed a close, continuous contact with the underlying vascular scaffold. In the decellularised vascular scaffold materials, the distinct periodicity of longitudinally cut, intact collagen fibers could clearly be observed.

On the other hand, after incubating in incubators for 7 days, Factor VIII positive cells that formed confluent

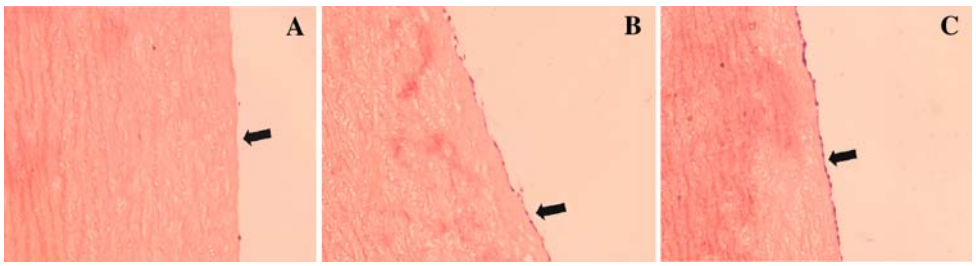

Fig. 3 HUVECs grown (7 days) on the luminal surface tubular vascular scaffold after decellularising; note the acellularity of the decellularised material. The cells (C) formed a monolayer (arrowheads) that was unable to invade below the tubular vascular scaffold luminal surface (150 × magnification, H&E stain). (**A**, glutaraldehyde-fixed; **B**, EGDE-fixed; **C**, EGDE-fixed + lysine treated)

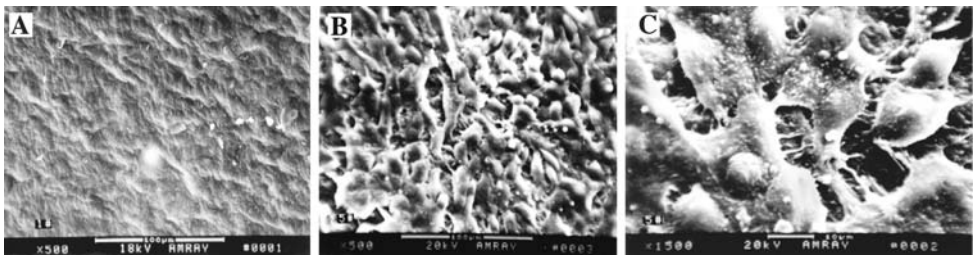

Fig. 4 Representative SEM-images of HUVECs on the luminal surface of vascular scaffold treated with glutaraldehyde (**A**) and treated with EGDE and lysine (**B**, **C**), cultured for 7 days

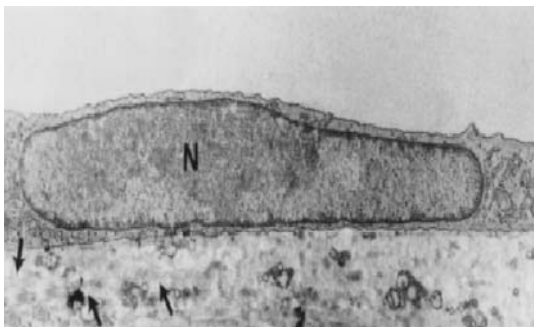

Fig. 5 Representative TEM-images of HUVECs on the luminal surface of vascular scaffold treated with treated with EGDE and lysine (10,750 × original magnifications). N: nucleus, arrow: collagen periodicity

monolayer were detectable on the luminal surfaces of the vascular scaffolds treated with EGDE and lysine (data not shown). This result indicated that the cells cultured on the luminal surfaces of the vascular scaffolds treated with EGDE and lysine preserved the activities of endothelial cells.

Discussion

The choice of vascular scaffold is very important in engineering a blood vessel substitute. Because biological tissues are derived from living body and can provide an environment similar to human body for cells proliferation, so they are perfect biomaterials fabricating vascular scaffold that can offer better constructs for adhesion and growth of cells over synthetic materials. Because of the immediate degradation of biological tissues in vitro, crosslinking (pre-treatmenting) is necessary to reduce biodegradation and antigenicity, stabilize the structure of the tissues and maintain their mechanical properties for practical application of biological tissues.

In this study, the epoxy compound (EGDE) was employed to pre-treatment (crosslink) the biological vascular scaffold. This epoxy compound contains two epoxide groups, and can react with amino, carboxyl and hydroxyl groups. Some researchers reveal that the most reactive functional group of the amino acid residues with epoxide within the biological tissues is the lysyl ε-amino groups [12]. The biological tissues are crossklinked by EGDE through this fixative mechanism. After fixation, it was noted that the EGDE-fixed tissues were more pliable than the glutaraldehyde-fixed tissues. This may be due to that there are a few ether bonds (–O–) in the EGDE, which may serve as flexible joints in the cross-linking bridge. In contrast, in glutaraldehyde, there are only carbon–carbon bonds (C–C), which are known to be relatively inflexible. Therefore, the glutaraldehyde-fixed tissues are usually stiffer than the EGDE-fixed tissues.

Although the fixation index of the EGDE-fixed tissues was lower than that of the glutaraldehyde-fixed tissues at the beginning of fixation due to slower rate of EGDE fixation compared with that of glutaraldehyde fixation, the fixation indices of both chemical agents were comparable

after 5 h of fixation (Fig. 1). This suggested that EGDE is as effective a crosslinking agent as glutaraldehyde.

In this study, the biological vascular scaffolds were completely decellularized by EGDE. The complete removal of original resident cells in biological vascular scaffolds reduced the antigenicity derived from cells, and thus markedly diminished the immune response elicited to these materials in vivo [13]. The maximum (>90%) of fixation index was achieved at the end of fixation (Fig. 1). A higher fixation index often implies a lower level of free amino groups left in the fixed tissues. The reduction of free amino groups in the biological tissue also diminished its antigenicity [14]. In addition, the polypeptide, glycolipid and lipopolysaccharide in the biological vascular tissues were crosslinked to form insolubility macromolecule by EGDE. This can mask the epitope of the biological tissues, and thus reduced its antigenicity. In a word, the antigenicity of biological tissues was very low after complete fixation with EGDE.

After fixing with EGDE, the total structure of the vascular tissues remained integrity, and the microcosmic structure of collagen was largely preserved. This structure similar to that of fresh tissues was suitable for the adhesion and proliferation of cells. As showed in Table 1, the EGDE-fixed vascular tissues were in possession of the mechanical properties similar to the natural vascular tissues. This indicated that the EGDE-fixed vascular tissues were suitable to serve as the scaffold of tissue-engineered blood vessel.

With the advantages mentioned above, there are also some drawbacks existing in the EGDE-fixed vascular tissues, such as cytotoxicity. It is reported that the cytotoxicity of the EGDE-fixed vascular tissues is attributed to the continuous release of unreacted EGDE remained within tissues. Proteins or polysaccharides present on and inside the cells may undergo irreversible chemical modification by the EGDE, and thus result in the death of cells [15]. Therefore, the 2% lysine solution was used to treat the EGDE-fixed vascular tissues in order to reduce its cytotoxicity in this study. The epoxide groups of unreacted EGDE within tissues were masked by the ε-amino groups of lysine through reacting with the residual EGDE within tissues, and the cellular compatibilities of the EGDE-fixed vascular tissues were markedly increased. The type I collagen was also coated on the surface of the EGDE-fixed vascular tissues in order to further facilitate the adhesion of cells. The MTT assay results of cellular compatibility for decellularised vascular scaffold materials showed that the methods applied in this study were practicable (Fig. 2).

In the endothelialization experiments, three 10 cm length of <6 mm internal diameters porcine thoracic arteries were respectively treated in three different manners (refer to chapter ''Cellular compatibility of decellularised vascular scaffold materials''), then HUVECs were seeded and cultured on the luminal surfaces of these decellularised vascular scaffolds for 7 days. The results showed that the luminal surface of the decellularised vascular scaffolds treated with EGDE and lysine was successfully repopulated with HUVECs, and resulted in a complete coverage of HUVECs (Figs. 3 and 4). After culturing for 7 days, the HUVECs on the luminal surface of the decellularised vascular scaffolds treated with EGDE and lysine still preserved the activities of endothelial cells (Fig. 5). These indicated that the innovative approach presented in this study could significantly increase the cellular compatibilities of decellularised vascular scaffold materials and be in favour of its endothelialization.

Conclusions

In summary, we have developed a decellularised vascular scaffold by fixing porcine thoracic artery with EGDE and made it endothelialized in vitro. The scaffolds were characterized by low cytotoxicity, low antigenicity and availability to preserve. Meanwhile, it showed the favorable characteristic similar to the nature vessel. This scaffold should be a promising material for fabricating scaffold of tissue-engineered blood vessel. The cellular compatibilities of the EGDE-fixed vascular scaffolds were markedly increased through treating it with lysine and coating type I collagen on its luminal surface. The HUVECs seeded on the luminal surface of the EGDE-fixed vascular scaffolds proliferated very well and reached a confluent monolayer, finally endothelialized the scaffolds.

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