Materials Science and Engineering C 35 (2014) 354–362

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Decellularized dermis–polymer complex provides a platform for soft-to-hard tissue interfaces

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ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form 4 October 2013
Accepted 3 November 2013
Available online 14 November 2013

Keywords:
Decellularization
Dermis
Poly(methyl methacrylate)
Interface tissue engineering
Tissue–polymer complex

ABSTRACT

To develop a soft-to-hard tissue interface, we made a decellularized dermis/poly(methyl methacrylate) (PMMA) complex by soaking the decellularized dermis in methyl methacrylate (MMA) and an initiator, and then polymerizing the MMA. The decellularized tissue was chosen because of its good biocompatibility and the easiness of suturing it, and MMA because of its hard tissue compatibility and wide use in the biomedical field. The MMA filled the cavities in the dermis and polymerized within 10 min. No leaking or polymer aggregation was observed, implying that a homogenous tissue–polymer complex had formed. The cell infiltration and the integration between the tissue and the dermis occurred in vivo, whereas the cells could not infiltrate the tissue–polymer complex. This implies that the interface tissue should possess both complex and noncomplex parts, where the cells infiltrate the noncomplex part and stop when they encounter the complex part, integrating the soft and hard tissue or hard polymer.

1. Introduction

Preparing a tissue interface that is biologically compatible with soft tissue and hard tissue has been a hot topic in recent years. The purpose of preparing an interface tissue is to regenerate or repair the damaged zone between different types of tissues [1]. The tendon–bone, ligament–bone, and cartilage–bone interfaces possess certain areas that have a gradually changing gradient structure. The preparation of an interface tissue is important because such a material can function as the intermediate for soft tissue integration to the dental materials, percutaneous device, stent, or any artificial materials that should be implanted into the living body. Materials such as collagen/hydroxyapatite, gelatin/hydroxyapatite, polyethylene glycol hydrogel, poly(2-hydroxyethylmethacrylate), polyacrylamide, polycaprolactone, and poly(lactic acid-co-glycolic acid) are used for the purpose of preparing an interface tissue [2–9].

Our research group has focused on using decellularized dermis as the base material in the tissue interface. This is because decellularized dermis is composed of a high percentage of collagen (97%), which is compatible with soft tissue. Decellularized soft dermis is non-inflammatory, mechanically tough, and has good cell adhesion and infiltration abilities, which made thlis material suitable for clinical applications [10–13]. Furthermore, it shows good integration with the host tissue, which makes it a good candidate for soft tissue compatibility. This implies that the material possesses the crucial components for the soft-to-hard tissue interface, which include a suitable microenvironment for cellular proliferation, ingrowth, and differentiation, and it meets the mechanical requirements of the insertion site [1]. For the polymer, we chose poly(methyl methacrylate) (PMMA) because of its long history of biomedical application, especially in the dental field, where it is used as a hard tissue-compatible material [14–16]. We therefore investigated ways to use decellularized tissue and PMMA to make a decellularized tissue–polymer complex to provide a platform as a tissue interface.

The most convenient method to complex decellularized tissue and polymer is to let the tissue absorb a polymer solution and then interlink the polymers. Although such a method may appear very simple, it is not the case. The problems associated with this process include the anchoring and the blending of the materials. Unless using physical force to let the polymer solution to penetrate into the native dermis, it is difficult for the polymers to be absorbed. Even if the polymer had been absorbed, lack of any sort of physical interaction between the tissue and the polymer allows the polymer to be kept inside the tissue. By contrast, we

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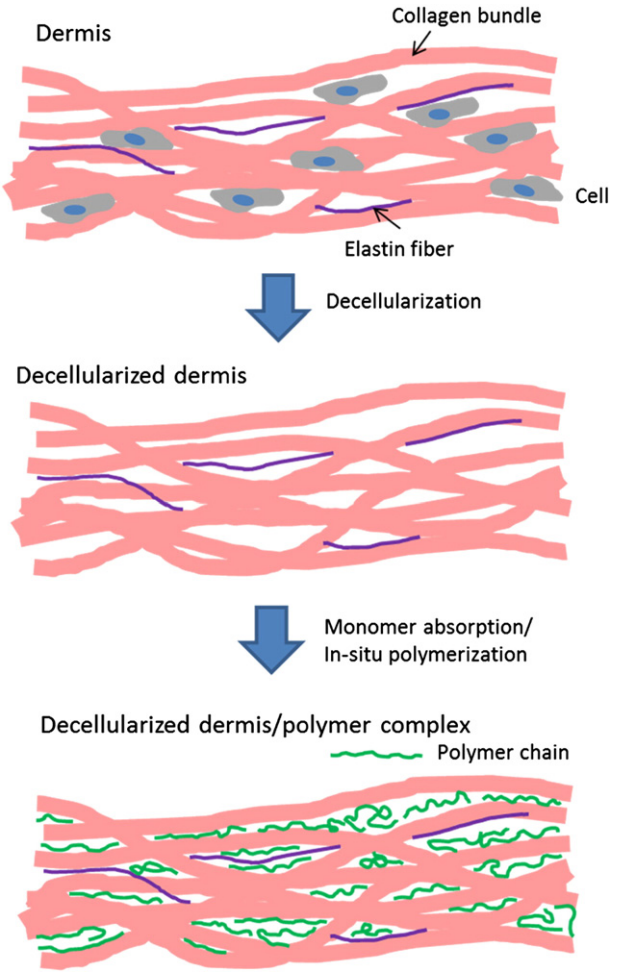
http://dx.doi.org/10.1016/j.msec.2013.11.007



Scheme 1. Schematic images of the dermis structure, decellularized dermis structure and dermis/polymer complex structure.

allowed the tissue to absorb a monomer solution, and then polymerized the monomers (Scheme 1). The monomers that fill the cavities that were created when the cells were removed can be polymerized and anchored to the extracellular matrix of the native tissue. We reported the incorporation of the 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer with a cross-linker into a decellularized blood vessel [17]. We could not, however, control the amount of polymer that was absorbed, or regulate the cross-linking rate. This made it difficult to characterize the decellularized blood vessel–polymer complex.

Saturating the decellularized tissue with monomers and then polymerizing them is a much more promising method than incorporating a polymer. Such a method would provide an intermediate phase that possesses compatibility with the soft and hard tissue, or soft tissue and the polymer. That is, the monomers would fill the cavities and polymerize within them, producing a homogenous blend of synthetic material and native tissue. An advantage of using decellularized tissue is the ease of suturing it during implantation. Furthermore, it is expected to result in cell infiltration and integration with the surrounding tissue without serious inflammatory responses [18].

However, we do not know whether it is possible to prepare such a structure or how the host tissue would respond to the decellularized tissue–polymer complex. Here, we discuss the decellularization of skin tissue (dermis) and the preparation and characterization of a decellularized dermis–polymer complex using methyl methacrylate (MMA), including that of its behavior in vivo.

2. Materials and methods

2.1. Materials

Fresh porcine skin was obtained from a local slaughterhouse (Tokyo Shibaura Zouki, Tokyo, Japan). The epidermal tissue and fat were trimmed, and the dermis was washed in phosphate-buffered saline (PBS) (Invitrogen, Tokyo, Japan). MMA (Wako Pure Chemicals, Japan) was purchased and distilled under reduced pressure. Benzyl peroxide (BPO) (Alfa Aesar, MA, USA) was used as the initiator and *N,N′*-dimethyl-*p*-toluidine (DMPT) (Tokyo Chemical Industry, Tokyo, Japan) was used as a co-initiator. DNase I (Roche, Switzerland) and magnesium chloride solutions (Wako Pure Chemicals, Japan) were used for washing away the cell debris.

2.2. Preparation and characterization of the decellularized dermis

2.2.1. Preparation of the decellularized dermis

The decellularized dermis was prepared using the high-pressure methods that have been used to decellularize blood vessels and cornea [19–21]. In short, the dermis was placed in polyethylene bags containing PBS. After sealing, they were hydrostatically pressurized at 980 MPa at 30 °C for 10 min using a cold isotactic pressurization machine (Dr. CHEF, Kobe Steel, Ltd., Hyogo, Japan) to dismantle the cells. Then, the specimens were washed by continuous gentle shaking in saline with 0.2 mg/mL DNase I and 50 mM magnesium chloride at 37 °C for 10 d, treated with 80% ethanol in saline at 4 °C for 3 d, treated with a citric acid buffer at 37 °C for 3 d, and stored in PBS at 4 °C until further use.

2.2.2. Characterization of the decellularized dermis

To observe the structure and cells remaining after decellularization, the specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, and cut into sections that were 4 μm in thickness. These sections were stained with Mayer's hematoxylin and eosin (H–E) stain and elastica van Gieson (EVG) stain. The stained sections were imaged using microscopy (Coolscope, Nikon Co., Ltd., Tokyo, Japan). To collect quantitative data on the residual cells in the tissue, native and decellularized dermis samples were freeze-dried and suspended at 55 °C for 12 h in 0.5 mL of a lysis buffer containing 50 μg/mL protease K, 50 mM Tris–HCl, 1% (w/v) sodium dodecyl sulfate (SDS), 100 mM NaCl, and 20 mM ethylenediaminetetraacetic acid disodium salt (EDTA-2Na). The DNA was extracted with phenol/chloroform and purified by ethanol precipitation. The residual DNA content was measured at 260 nm using an ultraviolet (UV)/visible-light spectrophotometer (V-560, JASCO, Tokyo, Japan).

To determine if the monomer can be absorbed by the decellularized dermis, we immersed the freeze-dried sample in MMA. Then, the sample was removed to measure the change in the weight. Measurements were taken until no further increases in the weight occurred. The same experiment was repeated with polymer/acetone mixture and water to compare the absorptivity of the decellularized dermis. The liquid content was calculated using the equation written below:

$$\text{Liquid content}(\%) = (W_t - W_d)/W_d \times 100$$

where W_t is the weight of a sample at certain time and W_d is the dried weight of that sample.

2.3. Preparation and characterization of PMMA and decellularized dermis/polymer complex

2.3.1. Polymerization of MMA

The polymerization of the MMA was executed by the method that was reported for its application as bone cement [16,22]. After MMA was distilled under reduced pressure to remove inhibitors, the MMA