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In vitro enzymatic degradation of a biological tissue fixed by alginate dialdehyde

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ABSTRACT

Biological tissues must be chemically fixed before they can be implanted in humans as tissue engineering scaffolds. To provide an ideal tissue engineering scaffold material, which is biodegradable and cytocompatible, a novel crosslinking agent, alginate dialdehyde (ADA), was employed to fix biological tissues by our group. The study mainly investigated the enzymatic degradation of ADA fixed biological tissues in vitro. Glutaraldehyde, the most commonly used crosslinking agent for biological tissue fixation, was employed as a control. The results suggested that, the ADA fixation could enhance the resistance against enzymatic degradation of biological tissues effectively. Meanwhile, compared to glutaraldehyde-fixed tissues, the ADA-fixed tissues could also degrade gradually over time. Moreover, the ADA crosslinking reagent itself had a stimulatory effect on cell proliferation when at an appropriate concentration. The results obtained in this study demonstrate that ADA fixation might provide a successful example of the biodegradable scaffold materials in tissue engineering.

1. Introduction

Tissue loss or end-stage organ failure resulting from an injury or a disease is a major concern in healthcare as they are costly and devastating problems (Langer & Vacanti, 1993). Tissue engineering has now emerged as a potential alternative to tissue or organ transplantation (Chapekar, 2000). Tissue engineering is an interdisciplinary field that involves the knowledge and technology of cells, engineering scaffold materials, and suitable biochemical factors to create artificial organs and tissues, or to regenerate damaged tissues (Langer & Vacanti, 1993). Recently, the development of scaffold materials responsible for cells growing has been a major concern in tissue engineering. A major objective of the development of scaffold materials is to mimic the structure and function of extracellular matrix in a living system (Ishii, Ying, Yamaoka, & Iwata, 2004). Therefore, compared to the synthetic materials, naturally derived biological tissues show great promise in tissue engineering applications (Schmidt & Baier, 2000). Naturally derived biological tissues are composed primarily of extracellular matrix components, which can provide a natural substrate for cells attachment, proliferation, and differentiation in its native state (Chen, Harding, Ali, Lyon, & Boccaccini, 2008). Furthermore, these natural materials may also offer improved mechanical and shape compatibility compared to synthetic scaffolds. For the above mentioned reasons, when repopulated with autologous or genetically engineered cells, naturally derived biological tissues can serve as the ideal scaffold materials for tissue engineering. However, due to the immediate degradation and presence of antigenicity after implantation, these naturally derived tissues must be chemically fixed before they can be used in the clinical applications (Schmidt & Baier, 2000).

In recent years, various synthetic crosslinking reagents, such as formaldehyde, glutaraldehyde (Cheung, Perelman, Ko, & Nimni, 1985) and polyepoxy compound (Sung, Shih, & Hsu, 1996) have been widely used in the fixation of natural biological tissues, however, these synthetic crosslinking reagents are all highly (or relatively highly) cytotoxic (Nishi, Nakajima, & Ikada, 1995; Yu, Wan, & Chen, 2008). In an attempt to overcome the aforementioned cytotoxic effect of synthetic crosslinking reagents, a naturally occurring crosslinking agent, alginate dialdehyde (ADA), was developed to fix biological tissues by our group.

Alginate (ALG), an important naturally occurring carbohydrate polymer extracted from brown algae, has been widely used in a number of biomedical applications, mainly due to its high biocompatibility (Goh, Heng, & Chan, 2011; Knill et al., 2004; Isiklan, Inal, Kursun, & Ercan, 2010; Wang, Fu, Zhang, Yu, Li, & Wan, 2010). In recent years, It has been reported that alginate can be oxidized

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with periodate and form multiple functional aldehyde groups (alginate dialdehyde, ADA) (Bouhadir et al., 2001). Therefore, ADA may react with the free amino groups using its multiple functional aldehyde groups in the same way as glutaraldehyde (Xu, Li, Yu, Gu, & Zhang, 2012). On the other hand, ADA is also highly susceptible to biodegradation, therefore, has potential to be used in a variety of biomedical applications wherein biocompatibility and biodegradability are important criteria. (Balakrishnan, Lesieur, Labarre, & Jayakrishnan, 2005). These advantages of ADA in biomedical applications prompted us to use this naturally occurring crosslinking agent to fix biological tissues. In our previous study, it was found that ADA not only could crosslink the biological tissues effectively, but also had an excellent cytocompatibility for biological tissue fixation (Xu et al., 2013, 2012).

Except for the biocompatibility, degradability is generally a desired characteristic for tissue engineering scaffold materials because the second surgery to remove them (such as a heart patch) would be averted if the substrate could be removed by the physiological system of the host body (Chen et al., 2008). Theoretically, even all scaffolds used in tissue engineering are intended to degrade slowly after implantation in the patient and be replaced gradually by new tissue (Griffith & Naughton, 2002). Complete degradation of the tissue engineering scaffolds could alleviate many concerns about the long-term implant biocompatibility (Slaughter, Khurshid, Fisher, Khademhosseini, & Peppas, 2009).

As previously mentioned, ADA was a promising crosslinking agent for biological tissue fixation, due to its excellent cytocompatibility. The present study was undertaken to further investigate the enzymatic degradation of a biological tissue fixed by ADA. In the study, fresh porcine aortas procured from a slaughterhouse were used as raw materials. Glutaraldehyde, the most commonly used crosslinking agent for biological tissue fixation (Jayakrishnan & Jameela, 1996), was employed as a control.

2. Materials and methods

2.1. Materials

Sodium alginate (viscosity: 495 cps at 25 °C) was obtained from Qingdao Jingyan Biotechnology Co. Ltd. (China). Glutaraldehyde and Diphenyl tetrazolium bromide (MTT) were obtained from Sigma–Aldrich (St. Louis, MO, USA). EDTA and Triton X-100 were obtained from Amresco Co. (USA). DNaseI and RNseA were obtained from Aladdin Co (Shanghai, China). Fetal bovine serum was purchased from Hyclone Laboratories (Logan, UT, USA). Collagenase type I, DMEM, trypsin, penicillin and streptomycin were purchased from Gibcobrl (Grand Island, NY, USA). Sodium periodate and all other chemicals of the analytical reagent were purchased from Kelong Co. (Chengdu, China).

2.2. Preparation of ADA

ADA was prepared according to our previously reported method (Vieira, Cestari, Airoldi, & Loh, 2008; Wang et al., 2010a,b). The sodium alginate (5 g) was dissolved in 200 ml distilled water and 50 ml pure ethanol by prolonged magnetic stirring in a beaker. And then 5.7 g of sodium periodate were added to the solution in the dark at room temperature to prepare the product. After 24 h, the reaction was neutralized by the addition of 10 ml ethylene glycol to reduce the excess periodate. The reaction mixture was continuously stirred under dark for 2 h. 5 g of sodium chloride was then added to the solution, followed by precipitation with 800 ml of ethanol. The precipitates were then dissolved in about 100 ml distilled water again and reprecipitated by the addition of 600 ml ethanol. This process was repeated three times. Furthermore, the product solution was dialyzed using dialysis tube (MWCO, 3500) against distilled water with several changes of water until it was free from periodate (Balakrishnan et al., 2005). The dialyzate was then lyophilized to obtain the product.

2.3. Decellularization and crosslinking process

Decellularized porcine aortas tissues were obtained according to previously described methods (Yu, Liu, Xu, & Wan, 2010). Briefly, fresh porcine aortas were procured from a local slaughterhouse and treated with 0.1% trypsin and 0.02% EDTA in PBS solution for 4 h at 37 °C. Then, the aortas tissues were washed with sterile PBS, followed by treatment in a hypotonic tris solution with 1% Triton X-100 for 48 h. Finally, the tissues were further incubated with RNaseA (0.02 mg/ml) and DNaseI (0.2 mg/ml) for 4 h at 37 °C.

After washing with sterile PBS solution, the decellularized aortas were fixed in a 15% ADA solution, which was buffered with phosphate-buffered saline (pH 7.4). Meanwhile, the samples fixed with 0.625% glutaraldehyde solution (buffered with phosphate-buffered saline, pH 7.4) were used as controls. The samples of each group were all fixed at 37 °C for 72 h under continuous shaking (Xu et al., 2013).

2.4. In vitro enzymatic degradation

To assess the resistant of all the samples to enzymatic degradation, in vitro enzymatic degradation of porcine aortas tissues was evaluated according to a modified method used by Sung, Chang, Liang, Chang, and Chen (2000) and Yao et al. (2004). The degradation was performed using collagenase type I digestion (with an activity of 125 U/mg solid). Fresh tissues, GA-fixed tissues and ADA-fixed tissues were immersed in the 250 U/ml collagenase/PBS solution, incubated at 37 °C under continuous shaking. The degradation was discontinued at predetermined intervals (30 min, 1 h, 3 h, 6 h, 12 h and 24 h) by an addition of 10 mM EDTA solution. In addition, all the samples before and after degradation were photographed using a digital camera.

2.5. The weight loss during degradation process

In the weight loss study, all the tested samples were first lyophilized and then weighed before and after enzymatic degradation. The degradation rate or weight loss percentage ($\Delta W\%$) was then calculated according to the formula:

$$\Delta W\% = \frac{W_0 - W_t}{W_0} \times 100\%,$$

where W_0 represents the initial weight of each sample and W_t represents the weight of the corresponding sample after enzymatic degradation.

2.6. Mechanical testing

The mechanical properties of samples before and after degradation were all examined based on our previous described methods (Yu et al., 2010). Briefly, to prepare specimens for tensile strength testing, each sample from individual group was trimmed as a test strap of 4 mm × 40 mm along collagen fiber, then the thickness and width of the sample was obtained using a micrometer. Five tissue straps in each group were extended on an Instron material testing machine (Instron Co., USA) from 0 g load until the tissue strip ruptured at a constant speed of 10 mm/min. After measurement, the ultimate tensile stress and the ultimate tensile strain were recorded before failure. During testing, the tissue strips were kept in air.