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Proliferation of chondrocytes on porous poly(DL-lactide)/chitosan scaffolds

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Abstract

Porous poly(DL-lactide)(PDLLA)/chitosan scaffolds with well-controlled pore structures and desirable mechanical characteristics were fabricated via a combination of solvent extraction, phase separation and freeze-drying. These scaffolds were further evaluated for the proliferation of isolated rabbit chondrocytes in vitro for various incubation periods up to 4 weeks in order to finally use them for the cartilage tissue engineering. MTT assay data revealed that the number of cells grown on PDLLA/chitosan scaffolds measurably increased with the weight ratio of the chitosan component and was significantly higher than those collected from pure PDLLA scaffolds for the entire incubation period. Scanning electron microscopy examinations, histological observations and proteoglycan measurements indicated that the resulting PDLLA/chitosan scaffolds exhibited increasing ability to promote the attachment and proliferation of chondrocytes, and also helped seeded chondrocytes spread through the scaffolds and distribute homogeneously inside compared to pure PDLLA scaffolds. Immunohistochemical staining verified that these PDLLA/chitosan scaffolds could preserve the phenotype of chondrocyte and effectively support the production of type II collagen.

Keywords: Chitosan; Polylactide; Porous scaffold; Cartilage tissue engineering

1. Introduction

Cartilage is a very important tissue, serving specific functions in human body. Once damaged, cartilage has very little capacity for spontaneous healing because of the avascular nature of the tissue [1]. Although surgical intervention, involving treatments ranging from classical arthroscopic debridement of the injured tissue to recently developed biological approaches such as autologous cell transplantation and orthotopic cartilage transplantation, is relatively effective [2–4], the clinical outcome depends on the patient's symptoms, age, activity level and size of the lesion, and remains essentially unpredictable in a large number of cases [5,6]. In addition, the costs of the surgical procedures are another major factor limiting the widespread use of transplantation [7,8]. As an alternative to transplantation methods, a great number of efforts have been made to develop new substitutes based on porous polymer scaffolds seeded with cartilage cells according to tissue-engineering principles [9,10]. Since these polymer scaffolds will act as a temporary substrate for viable cells and provide the necessary physical support to induce tissue reconstruction, they should be biocompatible with surrounding biological fluids and tissues, biodegradable for easy removal from the body, and favorable for the diffusion of gases and nutrients to cells as well as for the migration of cells themselves. In addition, their mechanical strength needs to be sufficient to maintain the stability of cell-seeded scaffolds during culture in vitro or transplantation in vivo. In certain cases, scaffolds also need to meet some specific requirements to interact strongly with extracellular matrix,

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growth factor and the cell surface receptor for the survival, proliferation, differentiation and maintenance of seeded cells. Therefore, the design and selection of polymeric materials are of great importance for tissue-engineering applications.

Many in vivo and in vitro investigations have been conducted to evaluate biodegradable aliphatic polyesters, mainly involving polyglycolide, polylactide, poly(β-hydroxybutyrate), polycaprolactone and their copolymers [11]. Of these different polyesters, polylactide and its copolymers have been of the subject of interest because of their acceptable physicochemical characteristics. Although polylactides (main commercial products: poly(DL-lactide), PDLLA; poly(L-lactide), PLLA) have been widely used for different biomedical purposes, several significant shortcomings, such as relative brittleness, hydrophobicity, lack of specific cell-recognition sites and acidic degradation products, have been noted [12–15]. In particular, when some scaffolds composed of polylactides are applied to articular cartilage regeneration, solving the problems of prolonged acute inflammatory responses and chronic inflammatory responses caused by the acidic degradation products remains a challenge for the successful and complete repair of cartilage defects [16]. Many efforts have been addressed to overcoming these drawbacks of polylactides. The main strategy applied includes blending, grafting or copolymerizing other synthetic or natural polymers with polylactides to obtain the desired characteristics [17]. However, these efforts in most cases can only improve the properties of polylactides in one or two respects and usually leave the other problems unresolved [18].

Chitosan is a linear polymer and is usually obtained by alkaline deacetylation of chitin, which is the second most abundant biopolymer in nature after cellulose [19]. Chitosan has amino groups and hydroxyl groups in its backbone; this composition renders chitosan itself hydrophilic, but at the same time makes chitosan weakly basic [20–22]. On the basis of the above-mentioned respective characteristics of chitosan and polylactides, it is reasonable to believe that chitosan would provide polylactides with some comprehensive improvements related to hydrophilicity, functional groups, mechanical toughness and buffering of the acidic degradation products of polylactides if these two components could be well blended together. Although blending chitosan with polylactides at a highly miscible level is difficult to achieve due to the fact that chitosan cannot be processed via a melt-processing technique [23], and there are no shared common solvents for both chitosan and polylactides, several efforts have nevertheless been devoted to such blending [24–26]. We have prepared a series of PDLLA/chitosan and PLLA/chitosan membranes using an improved solution-casting and solvent-extracting method [27,28]. The resultant membranes showed acceptably well-blended structures because there was no visual phase separation appearing on the surfaces or inside of the membranes. In addition, these blends have been further fabricated into porous scaffolds [29].

We have found that these porous scaffolds are quite suitable for cartilage tissue engineering in terms of their pore structures, mechanical strength and surface chemical properties as well as integrated degradable characteristics. A number of porous PDLLA/chitosan scaffolds were further investigated for cartilage cell culture in vitro. Histological observations and immunohistochemical staining revealed that these scaffolds exhibited an increasing ability to promote the attachment and proliferation of chondrocytes, and also to help seeded chondrocytes homogeneously spread and distribute. These scaffolds also preserved cell phenotype and effectively supported the production of type II collagen. In the present study, some relevant results are reported.

2. Materials and methods

2.1. Materials

Chitosan was purchased from Fluka. Its degree of deacetylation (DDA) was calculated using first-derivative UV spectra recorded on a spectrophotometer (Varian Cary 300) [30], based on a calibration curve generated from *N*-acetyl-D-glucosamine following our previous method [31]. The viscosity-average molecular weight of chitosan was determined using 0.25 M CH_3COOH/0.25 M CH_3COONa as a solvent system as described in our previous report [32]. The DDA value and viscosity-average molecular weight of chitosan were measured as $83.9 \pm 1.73\%$ and $1.41 \pm 0.19 \times 10^6$, respectively. PDLLA was received from Birmingham Polymers Inc. Its viscosity-average molecular weight was measured using a Ubbelohde-type viscometer in chloroform at 30 °C and calculated via following relationship [33]:

$$[\eta] = 2.21 \times 10^{-4} M_v^{0.77} (dl \quad g^{-1}). \tag{1}$$

The M_v value for PDLLA was obtained as $1.03 \pm 0.12 \times 10^6$ g mol^{-1}.

Phosphate-buffered saline (PBS) packets and some other biochemical reagents were purchased from Sigma and used as received. All common chemicals – acetic acid, *N*-acetyl-D-glucosamine, acetone, ethanol, sodium acetate, sodium hydroxide, chloroform – were obtained from Aldrich and used without further purification.

2.2. Preparation of porous scaffolds

The details of how to prepare porous PDLLA/chitosan scaffolds can be found in our previous reports [27,29]. In the present case, a series of porous PDLLA/chitosan scaffolds was prepared and named as PDLLA/ch10, PDLLA/ch30, PDLLA/ch50. The number following ch denotes the weight ratio of chitosan to PDLLA. By using a similar method and carefully selecting the processing conditions, some pure chitosan porous scaffolds were also built with matched structures and used as controls. In addition, some pure PDLLA porous scaffolds were prepared using a

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