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Recovery evaluation of rats' damaged tibias: Implantation of core-shell structured bone scaffolds made using hollow braids and a freeze-thawing process

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ABSTRACT

This study prepares biodegradable bone scaffolds helping the recovery of damaged tibias of rats. Polyvinyl alcohol (PVA) plied yarns are fabricated into hollow braids. The braids are combined with hydroxyapatite (HA)/gelatin/PVA mixtures and processed using freeze-thawing and freeze-drying processes in order to form bone scaffolds. These bone scaffolds are observed by scanning electron scope (SEM) and tested for compression strength. Afterwards, recovery of damaged bone, the morphology of the bone, and the histological observation are evaluated. Results indicate a small amount of HA helps in enhancing the compressive strength of bone scaffolds. Results of in vivo assay indicate the damaged tibias of rats recover and function well eight weeks after the implantation, and exhibit a normal morphology. Histological observation confirms the bone scaffolds gradually decompose, allowing tissue infiltration and facilitating ossification. This study successfully produces bone scaffolds with satisfactory mechanical properties helping in the recovery of damaged tibias of rats.

1. Introduction

Human bones are composed of minerals and organic constituents, and the composition ratio is associated with age and health [1,2]. The self-healing ability of bones can cover small-sized or less severe areas of damage caused by an external force or a disease, and bones can fully recover. However, pharmacological aid or surgery is essential for damaged bone when the damage level is too severe for self-healing [3,4]. Common clinical therapies, including repair, replacement, reconstruction, and removal, can provide patients with temporary or permanent support while facilitating the regeneration of the damaged bone tissues [5]. A great number of patients suffer from bone failure, but there are relatively few donors. Therefore, the number of patients who need bone transplantation is increasing. Tissue engineering thus helps to solve the problem of shortage of donors, and eliminates the waiting time usually required for a transplant. Tissue engineering materials have the potential to repair or replace the damaged bones as well as to activate the natural regeneration of bone tissue. Implanting bone scaffolds into the damaged bones allows for the growth and adhesion of osteocytes to the bone scaffolds. After the implantation, the osteocytes grow over the damaged site and eventually fill it, while the bone scaffolds degrade over time [6,7]. The damaged area thus shrinks as a result of these newly and fully generated tissues when the bone scaffold is completely decomposed.

On the other hand, some polymer processing techniques have been commonly used to produce bone scaffolds, such as the freeze-dry method [8–11], the melt deposition method [12–14], and the salting out method [6,15]. The freeze-dry method helps to manipulate the pore

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size and direction, and allows for combination of different polymers to have a three-dimensional and porous sponge structure [8,16,17]. The melt deposition method can form bone scaffolds with high strengths and regular pore size. Moreover, it can also combine materials, such as polylactic acid (PLA), to provide the bone scaffold with high stiffness [12,18,19]. The slating out method can combine PLA solution and polycaprolactone (PCL) solution using NaCl powders. Both solutions use organic solvents, such as chloroform and methylene chloride. Therefore, after the materials are dried and the organic solvents are volatilized, the bone scaffolds can be immersed in water to remove NaCl powders. This convenient and low-cost method thus can manipulate pore size and connectivity using different contents and sizes of NaCl powders [6,20–22].

Incorporating these techniques is advantageous for the production of bone scaffolds, as it can give degradable polymers a three-dimensional structure with porous connectivity, compressive properties, and biocompatibility. However, the product is composed of a mono-structure. For example, products commonly take the form of a sponge, a 3D grid, or a hollow porous tube, which do not resemble the structure of human bones. Hence, there has been an increasing number of studies on bone scaffolds with a bionic structure [23]. Previous studies have developed ways to combine braids or knits with porous polymer materials, indicating that the composites are stably combined [24–27]. Biocompatible and biodegradable fabrics can be combined with bone scaffolds based on different sizes of impaired bones, thereby strengthening the compression resistance. Moreover, different polymers can be combined with bone scaffolds according to the sites of impaired bones. For example, gelatin features a high osteocondution, avirulence, and biodegradation [28–31], while hydroxyl apatite (HA) can induce bone cell growth and accelerate bone tissue recovery. These polymers thus provide the bone scaffolds with interconnected pores, osteoinduction, and osteoconduction [32,33]. In our laboratory, bone scaffolds with a bionic formation were produced by combining knits, braids, and porous polymer materials. Mechanical property tests and an in vitro assay were conducted to evaluate these bone scaffolds, thereby determining that the fabrics and polymers could be stably synthetized [24,34]. However, for in vivo assays, the recovery of damaged bones has so far been examined only by computerized tomography, biomechanical testing, and histological evaluation [25]. There are relatively few studies on comprehensive evaluations in regaining walking ability, including functional recovery, the repaired morphology of damaged bone, and the formation of new bones. Therefore, the in vivo assay is performed on the impaired tibias of rats in this study in order to examine the proposed bionic-structured bone scaffolds in terms of walking ability, recovery of morphology and regeneration of new bones.

The proposed bone scaffolds possess a core-shell structure that resembles the morphology of bones, where the shell exhibits high strengths and the core is porous. Employing a braiding technique, PVA plied yarns are fabricated into hollow braids, and then processed using freeze-thawing and freeze-drying. These manufacturing processes ensure the solid bonding of braids and polymers. The resulting bone scaffolds are observed using a scanning electron microscope and evaluated using a compressive strength test and in vivo assay.

2. Materials & methods

2.1. Materials

Polyvinyl alcohol (PVA) fibers (Asiatic Fiber Corporation, R.O.C.) have 75 D/25 f and are dissoluble in water at 36 ± 5 °C. PVA powder (Sigma, US) has a molecular weight of 89,000–98,000. Gelatin (Sigma, US) is type A. Hydroxyapatite (HA, Sigma, USA) has a molecular weight of 502.31 and a particle size ≤250 μm. Glutaraldehyde (Shimakyu's Pure Chemicals, OSAKA, Japan) has a concentration of 25% and is reagent grade.

2.2. Preparation of bone scaffolds

Gelatin powders are added to water at a temperature of 60 °C on a hot plate, and are stirred for 12 h in order to formulate a 15 wt% gelatin solution. PVA powders are added to water at a temperature of 110 °C and stirred for 8 h at this temperature in order to formulate a 10 wt% PVA solution. Afterwards, these two solutions are blended at a temperature of 30 °C and stirred for 4 h in order to formulate a gelatin/PVA mixture. Different amounts of HA powder (0, 0.2, 0.4, 0.6, 0.8, and 1.0 wt%) are added to the gelatin/PVA mixture and stirred for six hours in order to form different HA/gelatin/PVA mixtures.

PVA plied yarns are braided into five-layer hollow braids with an outer diameter of 3 mm and an inner diameter of 2 mm. The hollow braids are cross-linked with glutaraldehyde for 15 min and then thermally treated for 10 min. This process is used to limit the degradation of PVA braids and stabilize their structure, and as such to ensure their high compressive strength. Moreover, the HA/gelatin/PVA mixtures are poured into the hollow PVA braids. The whole set is placed in a freezer at −20 °C for 20 h and then thawed at 25 °C for 4 h. This cycle is repeated three times, and this freeze-thawing method results in a physical cross-linking. Finally, samples are freeze-dried for 48 h, yielding the core-shell structured bone scaffolds as shows in Fig. 1.

2.3. Tests

2.3.1. Scanning Electron Microscopy (SEM) observation

This test is performed in order to observe the morphology of core-shell structured bone scaffolds. Samples are coated with gold for 30 s using an Ion Sputter (E-1010, HITACHI, Japan), and their morphology is observed using an SEM (S3000, HITACHI, Japan) at an accelerating voltage of 15 kV.

2.3.2. Compressive strength test

This test is performed as specified in ASTM D6641M-09. Core-shell structured bone scaffolds are placed on the plane of the compressive clamps, and their compressive strength is measured using an Instron 5566 (Instron, US) at a compressive velocity of 1.3 mm/min in order to examine the effects of HA content.

2.3.3. Degradation evaluation

Samples are weighed and recorded as dry weight (W_0). They are immersed in PBS in a centrifuge tube, which is placed in a shaking water

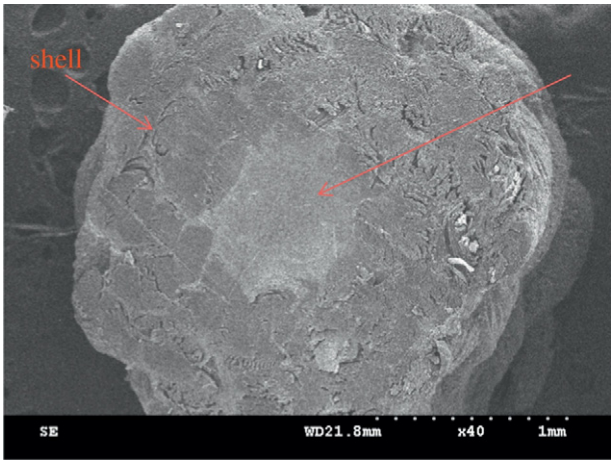

Fig. 1. SEM image of cross-section of the core-shell structured bone scaffold.