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Preparation, characterization and *in vitro* analysis of novel structured nanofibrous scaffolds for bone tissue engineering

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ABSTRACT

In a previous study, a three-dimensional nanofibrous spiral scaffold for bone tissue engineering was developed, which showed enhanced human osteoblast cell attachment, proliferation and differentiation compared with traditional cylinder scaffolds, owing to the incorporation of spiral structures and nanofiber. However, the application of these scaffolds to bone tissue engineering was limited by their weak mechanical strength. This limitation triggered the design for novel structured scaffolds with reinforced physical characteristics. In this study, spiral polycaprolactone (PCL) nanofibrous scaffolds were inserted into poly(lactide-*co*-glycolide) (PLGA) microsphere sintered tubular scaffolds to form integrated scaffolds to provide mechanical properties and bioactivity appropriate for bone tissue engineering. Four experiment groups were designed: PLGA cylinder scaffold; PLGA tubular scaffold; PLGA tubular scaffold with PCL spiral structured inner core; PLGA tubular scaffold with PCL nanofiber containing spiral structured inner core. The morphology, porosity and mechanical properties of the scaffolds were characterized. Furthermore, human osteoblastic cells were seeded on these scaffolds, and the cell attachment, proliferation, differentiation and mineralized matrix deposition on the scaffolds were evaluated. The integrated scaffolds had Young's modulus 250–300 MPa, and compressive strength 8–11 MPa under uniaxial compression. With the addition of an inner highly porous insert to the tubular shell, human osteoblast cells seeded on the integrated scaffolds showed slightly higher cell proliferation, 20–25% more alkaline phosphatase expression and twofold higher calcium deposition than those on the cylinder and tubular scaffolds. Furthermore, compared with sintered PLGA cylinder scaffolds, the integrated scaffolds allowed better cellular infiltration Therefore, this design demonstrates great potential for integrated scaffolds in bone tissue engineering applications.

1. Introduction

Bone tissue engineering using cells, scaffolds and bioactive molecules has revolutionized treatment for large bone defects caused by trauma or disease. Tissue-engineered bone product not only provides a bone implant to support the host structure, but also allows new tissue regeneration and integration with the host tissue [1]. Ideal bone tissue engineered scaffolds should provide a three-dimensional (3D) matrix with high mechanical strength adequate to support the newly formed tissue, high porosity allowing new tissue formation and growth within the scaffolds, a biomimetic structure for nutrient transport and waste removal, good biocompatibility and an appropriate biodegradation rate [2]. For the design of scaffolds, high porosity usually leads to a decrease in biomechanical strength, which is also of great importance for bone implants [3]. In order to solve the conflict between optimizing porosity and maximizing mechanical properties, many strategies have been used to reinforce porous scaffolds.

Several studies have focused on the design of scaffolds with optimized architecture to fulfill both mechanical and regeneration requirements [4,5]. For example, Kong et al. [6] developed a multilayer scaffold with different pore size and porosity from the center to the exterior tube. The exterior tube with a small pore size provided mechanical strength, while the core of the multilayer scaffold had a large pore size facilitating nutrient supply and bone regeneration. Heilmann et al. [7] developed a graded hydroxyapatite/calcium carbonate composite with porosities graded from 5% to 90% using a combined slip infiltration and dip-coating technique. Ramay and Zhang [8] integrated the gel-casting technique with the polymer sponge method to prepare macroporous hydroxyapatite scaffolds, which combined the advantages of both approaches with improved porous structure and enhanced mechanical strength. However, none of these designs completely solved the problem of the conflict between porosity and mechanical strength, wherein compromise of the two parameters is still needed to fulfill the requirements of a bone substitute.

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In addition to the structure, the fabrication technique has also been reported to affect the mechanical property of the scaffolds, given the same material and specific porosity level. Scaffolds prepared by conventional methods, including solvent casting, phase separation and gas foaming techniques, were usually restricted by insufficient mechanical strength due to the low polymer content, which caused high porosity [9]. A microsphere-based sintering technique was then invented for scaffold fabrication and has been intensively investigated in recent years, as the unique structure can achieve 100% pore interconnectivity and higher mechanical properties than conventional techniques [10]. Microsphere-based poly(lactide-*co*-glycolide) (PLGA) sintered scaffolds have been reported with significantly larger compressive modulus than PLGA scaffolds from conventional foaming techniques [10]. However, after 21 days' culture of osteoblast cells on the microsphere-sintered scaffolds, the cells can only penetrate ~700 μm through the thickness of a 2000 μm disk, owing to the limited porosity of the scaffolds [11]. In order to solve this problem, Kofron et al. [12] developed PLGA tubular scaffolds with lumen inside, allowing nutrient transport and waste removal. The limitation associated with these tubular scaffolds is that the empty lumen could not provide the necessary surface for the attachment of the osteoblast cells.

Therefore, a new design of scaffold is proposed, which mimics natural bone's structure, as shown in Fig. 1, where the scaffolds are composed of two parts: a highly porous inner spiral part integrated with a rigid outer tubular part. The two parts were fabricated separately using different techniques for different aims: the outer part, with a rigid scaffolding structure, was intended to provide support for the host tissue, while the inner part was intended to promote bone regeneration. Specifically, the outer tubular part was made of PLGA sintered microparticles, and the inner spiral structured scaffolds consisted of nanofiber-coated highly porous thin polycaprolactone (PCL) sheets made by the conventional solvent casting/porogen leaching and electrospinning technique. The insert was designed in a spiral 3D shape with thin scaffold walls to allow cells to grow completely across, and with open gaps to provide sufficient space for nutrient supply and waste removal, as shown in Fig. 1. Nanofibers were deposited on the surface of this spiral structured scaffold. These coated nanofibers serve as extracellular matrix (ECM) mimics for cell proliferation, as the nano size of the nanofibers resembled the structure of ECM. Previous studies [13] found that spiral structured nanofibrous scaffolds promote tissue regeneration by providing a superior mass transport system through the gaps between the spiral walls compared with conventional cylindrical scaffolds. The incorporation of nanofibers on the spiral wall was also shown to facilitate cell attachment, proliferation and differentiation. In the current study, these spiral scaffolds would then be inserted into tubular PLGA sintered scaffolds to form a complete integrated structure with sufficient mechanical properties. It was hypothesized that, by combining these two parts, the integrated scaffolds would fulfill the requirements for both sufficient mechanical properties and enhanced regeneration with the inner nanofibrous spiral scaffolds.

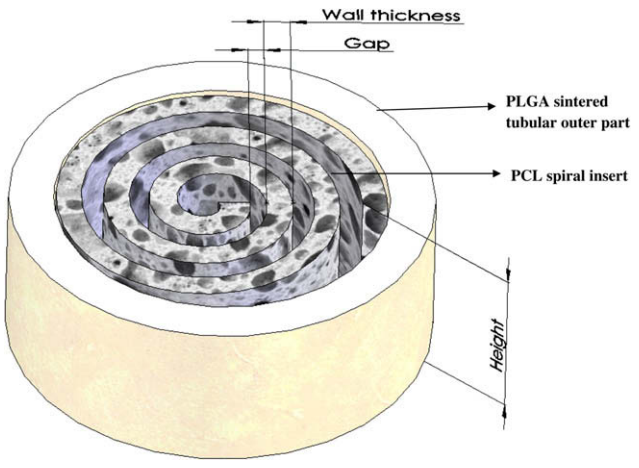

Fig. 1. Schematic diagram showing the design of integrated scaffold.

In this study, four experimental groups were designed: cylinder scaffold; tubular scaffold; tubular scaffold with a spiral structured inner core; and tubular scaffold with nanofiber containing a spiral structured inner core. The morphology, porosity and mechanical properties of the scaffolds were characterized. Furthermore, human osteoblast cells were seeded on these scaffolds, and cell attachment, proliferation, differentiation and mineralized matrix deposition on the scaffolds at days 4, 8, 14 and 21were evaluated.

2. Materials and methods

2.1. Fabrication of integrated scaffolds

Four groups of scaffolds were fabricated: PLGA sintered cylinder scaffolds (denoted cylinder scaffolds); PLGA sintered tubular scaffolds (denoted tubular scaffolds); integrated scaffolds with a PCL spiral insert without fiber coating (denoted porous scaffolds); and integrated scaffolds with a PCL spiral insert with fiber coating (denoted fibrous scaffolds).

All chemicals were from Sigma–Aldrich unless specified otherwise.

2.1.1. Spiral nanofibrous insert

PCL (MW 80,000) sheets were fabricated with an average thickness of 0.2 mm, using the solvent evaporation method. Briefly, PCL in dichloromethane (DCM) (20%, w/v) was spread on the surface of a glass Petri dish. Then DCM was evaporated under reduced pressure to form a dry PCL thin layer ~0.2 mm thick. For PCL fibrous group, PCL in hexafluoroisopropanol (10% w/v) (Oakwood Products, West Columbia, SC) were then electrospun into nanofibers with a constant flow rate (Q = 0.08 ml min^{-1}; KD Scientific syringe pump, Holliston, MA) to a 20-gauge needle connected to a high-voltage power supply (Gamma High Voltage Research ES-30P, Ormond Beach, FL) and deposited on the surface of the PCL porous sheet. Both sides were coated with electrospun nanofibers in the same manner. The sheet was then rolled with a piece of copper sheet 0.15 mm thick, which acts as the mold to form a spiral structure. After being incubated at 45 °C in an oven for 30 min, the scaffold was immediately transferred to −80 °C and was kept there for 24 h to immobilize the shape. The copper mold was finally removed. The dimensions of spiral were designed as 2 mm in diameter and 10 mm long, which could be measured by a digital caliper (TedPella, Redding, CA). PCL spiral inserts with fiber coating (denoted fibrous inserts) and without fiber coating (denoted porous inserts) were fabricated to be part of the integrated porous or fibrous scaffolds, as explained below.

2.1.2. PLGA microsphere sintered outer scaffolds

The preparation followed the methods described previously [11,12]. Briefly, the biodegradable polymeric microspheres were fabricated using PLGA copolymer (85:15) (Lakeshore Biomaterials, Birmingham, AL) through an emulsion–solvent evaporation technique as the result of phase separation and polymer precipitation. The PLGA was dissolved in methylene chloride at 10% (w/v). The solution was slowly poured into a 1% (w/v) polyvinyl alcohol (molecular mass 30,000–70,000 Da) solution, stirred at 325–330 rpm, and the solvent was allowed to evaporate overnight at

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