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Nano-yarns Reinforced Silk Fibroin Composites Scaffold for Bone Tissue Engineering

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Abstract

In this paper, novel nano-yarn reinforced scaffolds were fabricated and further biomineralized by submerging in 10×Simulated Body Fluid (SBF). The compressive strength of P(LLA-CL)/Silk Fibroin (SF) nano-yarns reinforced scaffold was 1.72±0.50 MPa and its porosity is 82.8%. Fourier Transform Infrared Spectrum (FTIR) and X-ray Diffraction (XRD) data confirmed the mineral phase was made of Hydroxyapatite (HA). MC3T3-E1 cell proliferation and differentiation on the scaffolds were evaluated. *In vitro* biological evaluation showed that HA coated scaffolds provided higher cell proliferation efficiency (t-test, $P < 0.05$) than uncoated scaffolds.

Keywords: Nanofibres; Wound Dressing; Drug Delivery

1 Introduction

Tissue engineering is considered as a promising alternative therapy to bone defects. The basic strategy of tissue engineering is to fabricate a scaffold to mimic the nature Extracellular Matrix (ECM) [1]. Bone ECM is a kind of hierarchically structured organic–inorganic composite, which consists of collagen fibrils embedded with nanoscopic Hydroxyapatite (HA) crystals [2]. From the bionic aspect, nanofiber is the nearest approach to morphology of bone ECM. Apart from that, a successful scaffold for bone tissue engineering should be i) three dimensional (3D), ii) have interconnected porous network, [3] iii) possess sufficient strength, iv) be biocompatible and osteoconductive [4].

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Liquid-electrospinning first reported by Teo [5] is a new way to fabricate continuous nano-yarns. Nano-yarns are a new type of yarns that consists of nanofibers. On the basis of summarizing forefathers, we added several improvements on its roller to obtain a layer-by-layer 3D nanoyarn structure. We anticipated that modified 3D assembly nanoyarns would mimic the macrostructure of bone with nanofiber components. A certain level of mechanical strength is necessary for the growth of a new bone [6]. However one drawback of nanoyarns is that their cotton-like structure is too soft to counteract the effects of stress in the hard tissue. As we know, fiber reinforce is an effective method to increase the strength of materials. In this study, we introduce nanoyarns into nature protein matrix to give the fiber reinforced scaffolds to solve this problem.

Osteoconduction is an essential element for bone tissue regeneration, an osteoconductive scaffold, such as a scaffold with apatite surface, can promote the formation of a new bone [3, 7]. By soaking the scaffolds in the Simulated Body Fluid (SBF), apatite calcium phosphates would deposit the scaffolds ranging from a few days to 2 weeks [8]. To speed up the process, we adopted a 10×SBF to increase the mineral content deposited on the scaffolds.

Silk Fibroin (SF) has favorable physical characteristics, due to its β-sheet (crystalline)-rich structure and excellent biocompatibility [9]. Poly(l-lactide-co-e-caprolactone) (P(LLA-CL)) has both good biocompatibility and slower degradation rate [1]. In this study, P(LLA-CL) and P(LLA-CL)/SF nanoyarns were fabricated by modified liquid-electrospinning, they were then combined with SF to give the fiber reinforced novel scaffold by freeze-drying. These scaffolds were then biomineralized by submersion in 10×SBF. Scanning Electron Microscope (SEM), Fourier Transforms Infrared Spectrum (FTIR) and X-ray Diffraction (XRD) were used to test the chemical properties of the bioceramic phase. Morphology, proliferation of MC3T3-E1 pre-osteoblasts on these scaffolds were investigated *in vitro*.

2 Experimental

2.1 Materials

A copolymer of P(LLA-CL) (75:25), which has a composition of 75 mol% L-lactide, was supplied by Nara University (Japan). Cocoons of Bombyx mori silkworm were kindly supplied by Jiaxing Silk Co., Ltd. (China). The silk fibroin was prepared as an earlier published procedure [10].

2.2 3D Composite Scaffolds Fabrication

SF (8 wt%) and P(LLA-CL) (8 wt%) were dissolved respectively in 1, 1, 1, 3, 3, 3, -hexafluoro-2 (HFIP, Daikin Industries Ltd, Japan). After SF and P(LLA-CL) completely dissolved, the two solutions were mixed together in different proportions (v/v) of 0:100 and 50:50 whilst being constantly stirred overnight.

These prepared solutions were used for nanoyarn fabrication by the methods showed in Fig. 1. Step 1 – Nanoyarns fabrication with a modified liquid-electrospinning method. In brief, electrospinning was carried out above the water cycling system consisted of two receptacles up and down, a pump used to recirculate the water from the down one to the up one (a hole of diameter 8 mm was drilled in the up one) and a seven-pin plug-like roller rotating in the down flowing water at a lower speed to collect nanoyarns. Irregular nano-yarns were intertwined layer-by-layer