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Development of silk-based scaffolds for tissue engineering of bone from human adipose-derived stem cells

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ABSTRACT

Silk fibroin is a potent alternative to other biodegradable biopolymers for bone tissue engineering (TE), because of its tunable architecture and mechanical properties, and its demonstrated ability to support bone formation both in vitro and in vivo. In this study, we investigated a range of silk scaffolds for bone TE using human adipose-derived stem cells (hASCs), an attractive cell source for engineering autologous bone grafts. Our goal was to understand the effects of scaffold architecture and biomechanics and use this information to optimize silk scaffolds for bone TE applications. Silk scaffolds were fabricated using different solvents (aqueous vs. hexafluoro-2-propanol (HFIP)), pore sizes (250–500 μm vs. 500–1000 μm) and structures (lamellar vs. spherical pores). Four types of silk scaffolds combining the properties of interest were systematically compared with respect to bone tissue outcomes, with decellularized trabecular bone (DCB) included as a "gold standard". The scaffolds were seeded with hASCs and cultured for 7 weeks in osteogenic medium. Bone formation was evaluated by cell proliferation and differentiation, matrix production, calcification and mechanical properties. We observed that 400–600 μm porous HFIP-derived silk fibroin scaffold demonstrated the best bone tissue formation outcomes, as evidenced by increased bone protein production (osteopontin, collagen type I, bone sialoprotein), enhanced calcium deposition and total bone volume. On a direct comparison basis, alkaline phosphatase activity (AP) at week 2 and new calcium deposition at week 7 were comparable to the cells cultured in DCB. Yet, among the aqueous-based structures, the lamellar architecture induced increased AP activity and demonstrated higher equilibrium modulus than the spherical-pore scaffolds. Based on the collected data, we propose a conceptual model describing the effects of silk scaffold design on bone tissue formation.

1. Introduction

Numerous approaches have been made towards development of an "ideal" scaffold for bone tissue engineering [1,2]. Silk fibroin, obtained from silkworms, demonstrates great biocompatibility along with outstanding mechanical properties [3] and proteolytic degradation [4]. In tissue engineering, silk fibroin has been extensively used for multiple types of scaffolds [5–8]. Various modifications of silk scaffolds have been fabricated with a wide range of chemical, structural and biomechanical modifications [6,9,10]. Silk sponges have been used for cartilage [11–13] and fat [14,15], silk tubes for blood vessels [16] and silk fibers for ligaments [17,18]. Porous sponge scaffolds are suitable for bone tissue formation, as they enhance cell attachment, proliferation and migration. In addition, the high porosity (92–98%) [19–21] facilitates nutrient and waste transport into and out of the scaffolds.

Porous silk sponges can be fabricated using porogens, gas foaming or lyophilization methods [22,23]. Among these, NaCl salt leaching is one of the simplest and most effective fabrication methods, resulting in scaffolds with spherical pores and different morphologies. Silk scaffolds are generally fabricated using two different silk preparation methods: aqueous and solvent (hexafluoro-2-propanol; HFIP) based. HFIP does not solubilize salt particles, therefore pore sizes in these sponges reflect the size of the porogen used in the process [22,23]. On the other hand, aqueous-based silk sponges demonstrate pore sizes 10–20% smaller than the size of salt crystals. This is due to partial solubilization of the

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