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The healing of confined critical size cancellous defects in the presence of silk fibroin hydrogel

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Abstract

In vitro and in vivo behaviour of an injectable silk fibroin (SF) hydrogel was studied through osteoblast cultures and after implantation in critical-size defects of rabbit distal femurs. A commercial synthetic poly(D,L lactide-glycolide) copolymer was used as control material. In vitro biocompatibility was evaluated by measuring LDH release, cell proliferation (WST1), differentiation (ALP, OC), and synthetic activity (collagen I, TGF ß1, IL-6). Bone defect healing rate and quality of the newly formed bone inside the defects were determined in vivo by measuring trabecular bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), mineral apposition rate (MAR) and bone formation rate (BFR/B.Pm). In vitro tests indicated that both materials significantly increased cell proliferation in comparison with the negative control. A significant increase in the TGF-β1 level was found for SF hydrogel in comparison with the control material and negative control. Both materials promoted bone healing when used to fill critical size defects in rabbit femurs. The new-formed bone of the SF hydrogel treated defects showed significantly higher BV/TV, Tb.Th, MAR and BFR/B.Pm and lower Tb.Sp values in comparison with the control gel. At 12 weeks the re-grown bone of the SF hydrogel-treated defects appeared more similar to normal bone than that of the control synthetic polymeric material-treated defects, except for the Tb.N value that differed significantly from that of normal bone ($p<0.05$). MAR and BFR/B.Pm presented significantly ($p<0.05$) higher values for SF hydrogel-treated defects in comparison with controls treated with a synthetic polymeric material, confirming that SF hydrogel accelerated remodelling processes.

Keywords: Silk; Hydrogel; Osteoblast; Biocompatibility; Animal model

1. Introduction

Tissue regeneration has become a great challenge for many illnesses, traumatic lesions or reconstructive needs [1]. Bone has regenerative capabilities that often lead to a spontaneous bone regeneration and growth. The amount of repair, however, depends on the size of the bone defect, lesion site and the patient's health status, age and lifestyle [2]. For critical defects, i.e. defects that would not fully heal spontaneously, or even to accelerate or guide the repair process, the use of bioactive scaffolding material could be of great advantage. Suitable materials can, in fact, fill the cavity or stabilize the defects while exerting beneficial stimuli that promote cell activity and proliferation.

Autogenous and allogenic bone derived materials are usually considered as the first choice by orthopaedic surgeons, because of the high osteopromotive properties of the former and the availability of the latter in bone tissue banks [3,4]. However, the inadequate amount of

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autologous bone utilizable in a patient, the morbidity of the donor site and the need for a second surgical site, on one hand, and the awareness of the risk of unknown infective pathologies when using allografts, have prompted studies on alternative synthetic or biological scaffold materials [1,5,6]. Another disadvantage of donor-derived bone is the variability observed in osteoconductive and osteoinductive properties depending on storing and processing techniques, site of explantation, and donor characteristics, such as age [7].

Many different materials have been used or proposed for bone replacement or repair, such as metals, collagen, glass ceramics, calcium sulphate, natural and synthetic calcium phosphate ceramics, polymers, and cements [8–11].

In recent years silk fibroin (SF), i.e. a protein that, together with sericins, builds up the silkworm filament, has been proposed as implant material and as a scaffold material able to promote tissue regeneration processes [12,13]. Santin et al. studied the inflammatory potential of fibroin films by analyzing in vitro the predisposition of fibroin to interact with proteins and cells involved in the inflammatory response. Fibroin was compared with two model materials having completely different physico-chemical properties: polystyrene and poly(2-hydroxyethylmethacrylate). Results demonstrated that the interaction of fibroin with the humoral components of the inflammatory system was similar to that of the 2 model materials and the degree of activation and adhesion of the immunocompetent cells was more limited [14]. Panilaitis et al. studied the inflammatory potential of silk (unprocessed native fibres, fibroin extracted to remove sericin, extracted fibroin re-coated with sericin, soluble sericin proteins and crystalline domain of silk fibroin) based on the release of the proinflammatory cytokine TNF of macrophages and in comparison with collagen and commercially available silk suture [15]. Results indicated that the suspension of the crystalline particles was the only silk preparation that induced significant TNF release (because of the particulate size range) while macrophages stimulated with other silk preparations failed to respond with elevated levels of TNF in both short and long-term cultures [15]. Meinel et al. demonstrated in vitro that the inflammatory response by bone-marrow derived mesenchymal stem cells grown on silk, silk-RGD, and collagen scaffolds was similar by measuring IL-1β and COX-2 gene expression and IL-1β and PGE2 release [16]. The same authors implanted silk, silk films covalently decorated with cell attachment sequence (silk-RGD), collagen, polylactic acid (PLA) in the muscular tissue of rats and observed that inflammatory tissue reaction was more conspicuous around PLA and collagen films when compared to silk [16]. Moreover, SF films, fibres and gels have been investigated in vitro with different cell types including fibroblasts, epithelial, endothelial, glial, keratinocytes, hepatocyte, osteoblast and marrow stromal cells with promising results that support the use of SF as an implant biomaterial [13,17–27]. The present authors previously investigated the physical and in vitro biological properties of two different SF-based hydrogels and observed that gels did not have any toxic effects and promoted osteoblast proliferation or activity and differentiation depending on the procedures used for their manufacture [27]. However, it is recognized that the ability of a biomaterial to perform a specific function in patients cannot be evaluated only with in vitro tests and that both biocompatibility and bone promoting activity of a candidate implantable orthopaedic material should be confirmed also with in vivo studies [19,28]. As far as using SF for orthopaedic purposes is concerned, to the present authors' knowledge, to date no in vivo bone implantation studies have been carried out.

By considering both the interest in hydrogel materials for orthopaedic and maxillofacial purposes and the absence of in vivo data on SF after implantation in the skeletal system, in the present study a SF hydrogel was inserted into cancellous defects in the rabbit distal femurs and bone response was investigated by means of radiography, histology and histomorphometry. The SF hydrogel was preliminarily studied in osteoblast cultures. A comparison was made between SF hydrogel and a synthetic poly(D,L lactide-glycolide) copolymer with regards to osteoblast behaviour and bone healing rate.

2. Materials and methods

2.1. Materials

A poly(D,L lactide-glycolide) copolymer (ratio 50/50 mol%) powder dispersed in aqueous solution of polyethyleneglycol (PEG) and 15% dextran in order to form a semi-solid slurry-gel form (Sintbone Slurry Gel®; Ghimas S.p.A, Casalecchio di Reno, Bologna, Italy) was used as control material. The material was originally developed and is clinically used for oral surgery [29].

Bombyx mori silk cocoons, bred and selected by Centro Sperimentale di Gelsibachicoltura, in Como, Italy, were degummed by boiling twice, one hour each, in a water solution of Na_2CO_3 (1.1 and 0.4 g/l, respectively), in order to remove the sericins. Degummed silk was then washed several times in distilled water, and then dried at room temperature. The so-obtained fibroin was dissolved in 9.3 M LiBr (Fluka Chemical) water solution (10%, w/v) at 65 °C for 3 h and filtered to eliminate impurities. LiBr was removed by dialysis against distilled water for three days at room temperature using a cellulose membrane with a MWCO: 3500 D (PIERCE). After dialysis the solution was filtered. Citricgel (SF Hydrogel) was obtained by adding