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Bone tissue induction, using a COLLOSS®-filled titanium fibre mesh-scaffolding material

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Abstract

Scaffold materials for bone tissue engineering often are supplemented with bone morphogenetic proteins (BMPs). In the current study we aimed to investigate COLLOSS®, a bovine extracellular matrix product containing native BMPs. Hollow cylindrical implants were made, with a length of 10 mm, a 3 mm inner diameter, and a 5 mm outer diameter, from titanium fibre mesh. The central space of the tube was filled with 20 mg COLLOSS®. Subsequently, these implants, as well as non-loaded controls, were implanted subcutaneously into the back of Wistar rats, with $n = 6$ for all study groups. After implantation periods of 2, 8, and 12 weeks, tissue-covered implants were retrieved, and sections were made, perpendicular to the long axis of the tube. Histology showed, that all implants were surrounded by a thin fibrous tissue capsule. After 2 weeks of implantation, the COLLOSS® material was reduced in size inside the loaded implants, but no bone-like tissue formation was evident. After 8 weeks, in two out of six loaded specimens, new-formed bone- and bone marrow-like tissues could be observed. After 12 weeks, this had increased to five out of six COLLOSS®-loaded samples. The amount of bone-like tissue did not differ between 8 and 12 weeks, and on average occupied 15% of the central space of the tube. In the non-loaded control samples, only connective tissue ingrowth was observed. In conclusion, we can say that COLLOSS® material loaded in a titanium fibre mesh tube, showed bone-inducing properties. The final efficacy of these osteo-inductive properties has to be confirmed in future large animal studies.

Keywords: Bone tissue; Tissue engineering; Titanium fibre mesh; COLLOSS®; Animal study

1. Introduction

In the field of reconstructive surgery there is an enormous demand for bone transplants. In tissue engineering (TE) scientists try to meet with these demands by producing biologically active (i.e. cell and/or growth factor loaded) and innovative scaffold materials [1]. This is done to regenerate and close large bone defects. The 'ideal' scaffolding material for TE has to satisfy many, often irreconcilable, demands: the material should provide adequate strength, be biocompatible, be moldable into a desired shape, and be easy to sterilize. In previous studies we have already evaluated the efficacy of various types of scaffold materials. Among these, titanium (Ti) fibre mesh has been proven to be a very suitable scaffold material for bone regeneration because it meets with most of the above-mentioned demands. Studies have shown that loading osteo-inductive factors, or culturing bone-marrow cells within the porosity of the mesh results in bone tissue formation in both ectopic and orthotopic locations [2–5].

When growth factors are administered to enhance bone formation, great emphasis lies on the transforming growth factor (TGF)-β superfamily. Well-known members of this superfamily are the bone morphogenetic proteins (BMPs). However, high concentrations of BMP are required to regenerate bone tissue in large animals and humans. Unfortunately, BMPs are very expensive which hampers their wide clinical use, and justifies the search for equally potent and highly cost-effective

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alternatives. An interesting candidate material in this respect seems to be COLLOSS®, a lyophilized complex of the extracellular matrix proteins extracted from diaphyseal bovine bone. This COLLOSS® material is currently employed in clinical applications as filler for bone fractures, empty spaces in bone, gaps around dental implants, etc. In these clinical applications the material has been shown to give rise to adequate amounts of new bone formation. However, these findings all deal with orthotopic (bone) implant sites [6,7]. Little information is available on the bone inductive potency of COLLOSS®.

Consequently, in this study we loaded a titanium wire mesh scaffold with a tube-like shape with COLLOSS® material, and evaluated these constructs in an ectopic rat model. We hypothesized that the material was osteoinductive, i.e. that bone would be formed inside the titanium mesh tube.

2. Materials and methods

2.1. Implants

From a sheet of titanium fibre mesh (Bekaert, Zwevegem, Belgium) tube-shaped implants were made with a length of 10 mm, a 3 mm inner diameter, and a 5 mm outer diameter. The used titanium fibre mesh had a fibre diameter of 45 μm, and a volumetric porosity of 86%. The central space of the tube was filled with 20 mg bovine collagen lyophilisate (COLLOSS®, OSSACUR AG, Oberstenfeld, Germany). The implant design is shown in Fig. 1. Empty implants (i.e. without COLLOSS® material) were used as controls. Similar implant models were used in earlier studies [8].

Fig. 1. The implant as used for this study. Titanium fibre mesh material was used to make a cylinder of 1 cm in length, 3 mm inner diameter, and 5 mm outer diameter. The central space of the cylinder was filled with 20 mg of dry COLLOSS® material.

2.2. Animal model and implantation procedure

Eighteen healthy young Wistar rats (40–43 days) were used for the implantation study. Approval was obtained from the University Animal Ethics Committee, and all national guidelines for the care and use of laboratory animals were observed. Surgery was performed under general anesthesia. The back of the animals was shaved; the skin was washed, and disinfected with povidone–iodine. On each flank, parallel to the spinal column, a small incision of about 10 mm was made through the skin. Using blunt dissection subcutaneous pockets were created. Thereafter, one of each implant type was positioned in the pockets (i.e. two implants per animal, one loaded and one control). Finally the incisions were closed (Fig. 2).

2.3. Histological procedures

After implantation periods of 2, 8, and 12 weeks, animals were sacrificed and tissue-covered implants were retrieved ($n = 6$ for each implantation time). These were fixed in 4% buffered formaldehyde for 1 week, dehydrated in a series of ethanol–water solutions, and embedded in MMA. After polymerization, 10 μm sections of all implants were cut using a modified diamond blade sawing technique [9]. Sections were made perpendicular to the longitudinal axis of the tube-shaped implants, and stained with basic fuchsin/methylene blue. As it might be expected that the amount of new-formed bone-like tissue might change with the position of each sample, three sections were used for evaluation. These three sections were obtained at different positions through the implant.

2.4. Histomorphometry

All histological sections were digitally photographed and histomorphometrically evaluated. First the number of positive implants (i.e. the implants showing the presence of bone-like tissue) was counted. When a positive implant was found in all three sections of that implant, the area of new-formed bone tissue was selected, and measured in mm^2, using image analysis software (Scion Image Beta 4.02 Win, Scioncorp, Frederick, MD, USA). Statistical analysis was performed using GraphPad Instat (v3.05, GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Macroscopic clinical findings

All the rats appeared to be in good health throughout the test period. There were no signs of haematoma, severe inflammation, or other complications around the implantation site. At the end of the various study