

Silk fibroin as an adaptable 3-D scaffold for defect repair in subchondral bone

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Background: Fractures and bone defects in the subchondral bone have a common problem regarding bone healing: the balance of bone homeostasis is disturbed and even in the presence of autogenous bone grafts or synthetic bone replacements, newly formed bone may be resorbed, cyst formation may develop and on the long run collapse of the overlying articular surface and/or degeneration of the articular cartilage may occur^{1, 2}. There is evidence that in subchondral bone the stiffness geometry and structure of the applied synthetic material as well as the local inflammatory response of the tissue to the surgical trauma play a major role in the failure of bone healing and subsequent degeneration of the articular cartilage. The aim of the first part of this study was to demonstrate that through adaptations of geometrical, mechanical and structural properties of a 3-D silk scaffold to the local requirements of the subchondral bone in the proximal tibia in sheep *i)* bone healing could be achieved, and *ii)* degeneration of overlying cartilage could be avoided. In the second part of the study biocompatibility issues of the silk scaffold were addressed by comparing different preparation methods for the 3-D silk matrix in a drill hole model in sheep.

Materials&Methods: For both parts of the study adult, Swiss Alpine sheep between 2 to 3 years of age were used. Surgeries were performed with the sheep under general anesthesia and appropriate postoperative analgesia. After recovery, the sheep were kept in stalls in small groups until they were allowed to roam free on pasture at 4 weeks after surgery.

Part I: Three groups were made, where as bone replacement one group received the 3-D test implants (UPW) sterilized by ethylene oxide. Controls were hydroxyapatite (HA) granules and autogenous bone grafts. Implants were followed for 2 weeks (silk, autografts) and 2 months (silk, HA, autografts). A total of 30 sheep were operated.

A rectangular defect of 15mm width, 10mm heights and 18mm depth was made at the medial

aspect of the proximal tibia, just cranial of the collateral ligament and 4mm distally from the proximal rim of the tibia shaft. The overlying articular cartilage was never touched and neither was the joint capsule opened.

Part II: Four different silk preparations were tested, which differed in the source (2 silk distributors) and in the silk scaffolding process, namely silk dissolved in an organic solvent using NaCl salt crystals as porogen vs. a water based scaffolding process using paraffin spheres as a porogen (UPW) (n=4 groups à 6 samples). Leaching of the porogen out of the scaffolds was either by aqueous buffer (hexafluoroisopropanol; HFIP-scaffolds) or hexane (UWP-scaffolds)^{3, 4}. For sterilization all scaffolds in part II of the study were steam autoclaved. A previously established drill hole model in sheep was used to test biocompatibility. Briefly, drill holes of 8mm diameter and 13mm depth were created bilaterally in the proximal and distal metaphyses and epiphyses of the humerus and femur. The holes were filled with the 4 different silk scaffolds and followed for 2 months, when sheep were sacrificed.

For both, part I and II, bone samples containing the silk scaffolds were harvested, radiographed (Faxitron), and processed for histology of non-decalcified bone samples embedded in plastic resin. Ground (30-40µm) and thin (5µm) sections were prepared and either (surface-) stained with toluidine blue or von Kossa/McNeal. (Semi-) quantitative and qualitative evaluation for both studies was focused on cellular reactions and new bone formation. For part 1 degradation of hyaline cartilage was assessed biochemically and histologically, with the latter in relation to the measured distance from the subchondral defect to the overlying cartilage surface. Statistical evaluation of the measured variables was performed using factorial analysis of variance followed by a posthoc test according to Scheffe.

Results: After 2 months silk scaffolds were infiltrated with cells even in the most central parts in both studies. Disparate biocompatibilities were

obtained for part I and part II of the study. Whereas the (foreign body) reaction in response to UPW scaffolds was strong in part I, a substantially better biocompatibility was demonstrated in part II. Overall, the UPW scaffolds were equal or slightly better as compared to the HFIP scaffolds regarding infiltration with giant foreign body or mononuclear cells, but there were slight differences between combinations of scaffolding protocols and silk sources regarding new isles of bone formation at 2 months after implantation. The source of the silks did not have an effect on the experimental outcome.

Degeneration of the hyaline cartilage overlying the rectangular defect occurred in 100% of the sheep in part I, although the cartilage itself was never touched. Proteoglycan content was lowest in the group with the hydroxyapatite bone substitute and equal in the autograft or silk scaffold group. The severity grade of cartilage degeneration was related to the distance measured between the proximal rim of the tibia and the defect.

Discussion & Conclusion: It could be demonstrated in this study, that silk scaffolds can be used as bone substitutes. At this point there is no clear indication about the cause(s) for the difference in biocompatibility between the two parts of the study. Potential causes could be differences in sterilization protocols (ethylene gas vs. autoclave) resulting in ethoxylated silk and/or the final residual solvent concentrations in the scaffold after manufacturing and sterilization, rather than the biocompatibility of silk fibroin per se derived from different sources.

Furthermore, it was shown that degeneration of hyaline cartilage overlying a rectangular defect was mostly related to the distance between the defect and the calcified cartilage zone as well as the type of the implant used as bone replacement. The use of stiff hydroxyapatite granules resulted in more proteoglycan depletion compared to the more elastic silk scaffold or autogenous grafts. Local inflammation seemed to play a minor role in relation to cartilage degradation.

It can be concluded that *i)* subchondral defects in close proximity to the calcified cartilage zone result in degeneration of the overlying hyaline cartilage already after 2 weeks, *ii)* silk scaffolds may have some advantage from a mechanical (stiffness) point of view when used as bone replacement in subchondral bone and, *iii)* if scaffolding, leaching and sterilization protocols are validated local (foreign body) reaction may be well controlled using silk fibroin scaffolds. The possibility of the new scaffolding protocol (UPW) to accommodate growth factors⁵ that enhance bone formation may even further ameliorate the situation of the overlying hyaline cartilage and slow down its degradation as a response to injury to subchondral bone.

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