博 士 論 文 の 内 容 の 要 旨

氏名	Burger, Dennis Eugen
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論文題目	Study of silk and cellulose materials for bone tissue engineering and environment friendly production of functional cellulose nanofibers (骨組織再生のためのシルク/セルロース複合材料開発と環境に優しい機能性セルロースナノファイバー製造に関する研究)

Current methods employed for healing problematic bone fractures strongly rely on the use of autologous bone grafts, whereas bone matrix is harvested from the patient and implanted at the critical bone fracture site. As the harvest itself can lead to chronic pain at the donor site, other materials are investigated for application as bone grafts; this is done in the field of bone tissue engineering. Scaffolds used for bone tissue engineering need to have a variety of features to accommodate bone cells. The scaffold should mimic natural bone, it should have appropriate mechanical strength, support cell differentiation to the osteogenic lineage and offer adequate porosity to allow vascularization and bone in-growth. Silk fibroin is a desirable material for many tissue engineering applications due to its superior moldability and beneficial effect on cells. As silk fibroin is obtained from the silkworm, it has also great possibilities for improvement by genetic engineering of the silkworm. Cellulose is also of interest for tissue engineering application, as the strong mechanical properties and biocompatibility are desirable for use of cellulose as structural bone tissue engineering scaffold component.

In the first part of this dissertation the aim was to develop a new process to fabricate a bone tissue engineering scaffold by creating a porous composite material made of silk fibroin and cellulose. Silk fibroin and cellulose were both dissolved together in *N*,*N*-dimethylacetamide/LiCl and molded to a porous structure using NaCl powder. The hydrogels were prepared by a sequential regeneration process: Cellulose was solidified by water vapor treatment, while remaining silk fibroin in the hydrogel was insolubilized by methanol, which lead to a cellulose framework structure embedded in a silk fibroin matrix. Finally the hydrogels were soaked in water to dissolve the NaCl for making a porous structure. The cellulose composition resulted in improving mechanical properties for the hydrogels in comparison to the silk fibroin control material. The pore size and porosity were estimated at around 350 µm and 70 % respectively. The hydrogels supported differentiation of MC3T3 cells to osteoblasts and are expected to be a good scaffold for bone tissue engineering.

The second part of the dissertation addresses the project of developing basic fibroblast growth factor-binding recombinant silk fibroin by expression with transgenic silkworms, *Bombyx mori*. Basic fibroblast growth factor application leads to enhanced osteoblast adhesion and promotion of angiogenesis, which is vital for malnourished bone defects. In preparation, a transgenic SF L-chain sequence which codes for a basic fibroblast growth factor-binding peptide, called P7, in the C-terminal region was prepared and subsequently introduced into the genome of silkworms via the *piggyBac* Transposon system. This led to 22 % of all expressed SF L-chains to be recombinant. Enhancement of basic fibroblast growth factor binding affinity of recombinant silk fibroin compared to that of the control, non-transgenic silkworm, was also confirmed.

The final part of the dissertation initially aimed at making silk fibroin/cellulose composites for bone tissue engineering via dissolution in $ZnCl_2$ hydrate solution. While this approach was unfortunately difficult due to a lack of an appropriate coprecipitation mechanism, a very

small fiber size cellulose was found in the precipitation yielded by the process. From this new finding, the project goal was changed to the development of an energy-saving and straightforward treatment of cellulosic pulp to obtain functional cellulose nanofibers with catalytic activity and flame-retardant properties. For this purpose, dried cellulose pulp was mixed with the recyclable swelling agent, $ZnCl_2$ hydrate, at room temperature. The mild treatment affected the crystal structure through a partial amorphization, yielding a mix of native cellulose I and regenerated cellulose II. This treatment tremendously facilitated the fibrillation into a cellulose nanofiber (CNF) network. In comparison to fibrillated cellulose from non-treated pulp, the $ZnCl_2$-treated counterpart featured higher viscosity, film transparency, better mechanical properties and higher heat stability. Films produced from these nanofibers showed flame-retardant properties without any further modification. The $ZnCl_2$-CNF showed high reactivity in fiber surface acetylation and allowed a fast and efficient reaction while using very mild conditions. The cellulose nanofibers obtained by this process will also be useful for application as structural compound in BTE composite scaffolds.