7. Summary

Histological evaluation of a bioactive implant for spondylodesis in the cervical spine (an experimental study in a sheep model)

The fusion of vertebral segments is an established treatment for spinal disorders. Harms-Cages were used as intervertebral implants for spondylodesis since 1986. This cage is originally filled with autologous cancellous bone graft which promotes bone remodeling with its osteoinductive and osteoconductive properties.

The purpose of this *in vivo* study was the histomorphological and histomorphometrical comparison of a sheep cervical spine fusion-model by using the Harms-Cage. Thirty-one sheep underwent C3 / C4 discectomy and fusion. They were divided into four groups, according to their treatment: group 1: Harms-Cage filled with mineralized collagen scaffold (n = 8); group 2: Harms-Cage filled with mineralized collagen scaffold combined with PRP (n = 8); group 3: Harms-Cage filled with mineralized collagen scaffold combined with 150 µg rh BMP-2 (n = 8); group 4: Harms-Cage filled with mineralized collagen scaffold coated with cyclic RGD-Peptide (n = 7); group 5: untreated control segment (n = 8). Group 5 acted as referee. After 12 weeks, sheep were sacrificed and specimen were taken. Histological handling followed. All specimen were stained with masson – goldner - trichrom, safranin - orange / lightgreen, safranin – orange / von Kossa and astrablue. The quantitative analysis showed the intervertebral tissue, vascularisation and foreign body reaction in the former intervertebral disc-space. The following histomorphometrical analysis calculated the percentage rate of bone, cartilage and mineralized cartilage in the defined “Region of Interest” (ROI).

The histological analysis revealed the following results: The vertebral disc-space was filled with fibrous tissue and cartilage in group 1. Partially residual tissue of mineralized collagen was demonstrated. In one specimen a bony fusion could be detected. The specimens of group 2 showed no bony fusion at all. Vertebral bodies were separated by a layer of fibrous tissue and cartilage in contact to new built bone. Group 3 was characterized by advanced bone modeling and remodeling even ventral of the vertebral disc space. Three specimens showed bony fusion. The specimens of group 4 showed advanced bone regeneration. Two bony fusions were verifiable. In group 5, the intervertebral disc was present.

All specimens of the groups 1, 3 and 4 presented significantly more bone tissue in the examined area than the native specimens. There was no significant difference in mineralized cartilage between all examined groups. The results showed that all tested treatments for spinal fusion have the ability for equally replacing the bone graft - “Golden Standard” at the 12-week examination time point. The cage augmentation with mineralized collagen scaffold (group 1) and mineralized collagen scaffold combined with PRP (group 2) did not show a significant effect on the interbody fusion and bone growth. Foreign body reactions were associated with cage sintering.

The additional treatment of the interbody fusion device with rh BMP-2 (group 3) and the cyclic RGD-Peptide (group 4) was a successful combination for the application in the cervical spine. Both methods showed better results than the Harms-Cage filled with autologous bone graft. Nevertheless the usage of rh BMP-2 for spinal fusion is associated with some risks, specified in literature. In this study, no complications were found. It can be suggested that the ventral bony callus is caused by the method of application of the growth factor at the mineralized collagen scaffold and not by rh BMP-2 itself. Nevertheless the use of rh BMP-2 on collagen scaffolds should be limited to experimental studies. The combination of mineralized collagen scaffold with the cyclic RGD-peptide, which usage is confined to experimental studies so far, stimulated the spondylodesis as well in this animal model. This occurred without any side effects, like foreign body reactions, within the observation period. The combination of cyclic RGD-peptide with an osteoconductive matrix showed very good results for spondylodesis and was superior to the autologous transplant. This showed impressively, that cRGD is able to accelerate osseointegration and bone modeling, without side effects within a 12-weeks observation period. This peptide should be examined in long term studies to exclude any side effects.