Introduction

Large bony defects show often a delayed healing and have an increasing risk of infection. Several materials are used for coverage of large defects. These materials must be biocompatible, easy to use, and must have an appropriate stability to present a mechanical hindrance. Aim of this study was to test two new developed biodegradable membranes for defect coverage in a sheep model. Round cranial defects (1.5 mm diameter) were performed in mature sheep under protection of the dura. Six 1.5 cm full-thickness critical size defects were performed in the parietal and occipital skull of each animal. The holes were drilled into the cranium using a cutting Burr under protection of the dura. The defects were randomly dedicated to the six defect treatments. The membranes were fixed with three resorbable polylactide pins (Synthes, USA).

Material and Methods:

Mature female merino mix sheep were used in this study (n=16). Six 1.5 cm full-thickness critical size defects were performed in the parietal and occipital skull of each animal. The holes were drilled into the cranium using a cutting Burr under protection of the dura. The defects were randomly dedicated to the six defect treatments. The membranes were fixed with three resorbable polylactide pins (Synthes, USA).

Empty defects (E) Spongiosa filled defects (Sp)
D1: without coverage D4: without coverage
D2: 70/30 PL/DLLA D5: 70/30 PL/DLLA
D3: PLLA D6: PLLA

The animals were sacrificed after 12 or 24 weeks.

n=8 per group and time point

Radiology: After sacrifice quantitative coronal CT-scans (0.625 mm slice thickness) of the calvaria were performed and Bone Mineral Density (BMD) was measured.

Histochemistry: After CT-scan the tissue was fixed in 10% normal buffered formaldehyde and embedded undecalcified in methylmethacrylate (Technovit 9100). The anterior, central and posterior part of each defect was cut in 5 µm sections. For histological investigation following stains were performed: Safranin-O/von Kossa, or Light green/Safranin-O. The tissue was stained for TRAP to detect osteoclasts.

Statistic: The data are expressed as mean +/- standard deviation. For multiple group comparison the ANOVA-test was used and corrected by Bonferroni (SPSS 10.0).

Results

No macroscopical reactions due to the implants were seen in any animal. Residues of the membranes were preparable in all membrane covered defects at both time points.

Radiology

Comparing the unfilled and the spongiosa filled defects, as expected, a significant higher BMD was measured in the spongiosa groups. No differences in the bony filling were detectable when the three unfilled defect types (D1, D3, D5) or the three spongiosa filled defect types (D2, D4, D6) were compared to each. An increase in the BMP from week 12 to week 24 was seen in the unfilled defects independently from the coverage.

Histology

The anterior, the central and the posterior part of the defects were analyzed. In accordance to the radiological findings, more bony tissue was seen in the spongiosa filled defects (Fig. 1).

Discussion

The necessity of a second operation for removal of metallic implants leads to an increasing research on biodegradable materials for surgical use. Most of the experimental and clinical results reveal a good biocompatibility of the materials and a satisfactory clinical outcome. However, due to reports of severe foreign body reactions resulting in the worst case in osteolysis, biocompatibility tests are necessary before clinical application of newly developed polymer materials. This study investigated the biocompatibility of two biodegradable membranes with different characteristics. The amorphous poly(D,L-lactide) membrane degrades faster than the semi-crystalline 70/30 poly(L/D,L-lactide) membrane. Both membranes served as a mechanical hindrance to prevent the prolapse of soft tissue into the defect and also the migration of undesired cells. The presented data of the in vivo biocompatibility test revealed no differences in the amount and distribution of osteoclasts at the two investigated time points and between the investigated groups. There was a foreign body reaction detectable around the two membrane types, however, no effect on the tissue regeneration between the investigated groups was detectable which was related to the membranes. As expected, a significantly higher amount of bone was detectable in the groups with spongiosa filled defects. In the membrane covered defects the spongiosa showed a progressed remodeling to the native bony structure of the cranium. The groups without spongiosa revealed partly new bone formation, without complete bridging in any group or at any time point. Comparing the 12 and 24 weeks groups, an increased bone formation was detectable at the later time point.

In conclusion, the results of the present in vivo study reveal a good biocompatibility and prevention of soft tissue prolapse of the two used membranes without differences between the membranes. No osteopromoting effect was detectable due to the membranes, but an enhanced remodeling of the spongiosa into native bony structures under the membranes.

Fig. 1 Percentage of bony filling of the defect (histomorphometrical analysis of the central section).