Osseous alterations at the interface of hydrogel expanders and underlying bone

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SUMMARY. Introduction: In plastic and reconstructive surgery, self-activating hydrogel expanders are used to augment soft-tissue space. The purpose of this study was to investigate the morphological response of underlying bone to the constant pressure exerted by a hydrogel expander. Methods: Eighteen Lewis rats were randomly divided into three groups. In group 1, a hydrogel expander was placed subperiosteally directly onto the calvaria of the rats. In group 2, the expander and the underlying bone were separated by a polydioxanone (PDS) foil. Group 3 animals served as controls. Before and 14 days after the insertion of the expanders, micro-computed tomography (CT) images were obtained and fused. We analysed hydroxyapatite density beneath and at the periphery of the expander and performed a histomorphometric bone analysis. Results: Whereas there were no significant differences (p < 0.05) (groups 1 and 2) in bone density at the periphery of the expanders between the study groups, a significant decrease in hydroxyapatite density beneath and at the periphery of the expander and performed a histomorphometric bone analysis. Results: Whereas there were no significant differences (p < 0.05) (groups 1 and 2) in bone density at the periphery of the expanders between the study groups, a significant decrease in hydroxyapatite density beneath the expanders was observed in those animals in which the devices were placed directly onto the calvaria (group 1). Whereas bone thickness was unaffected at the periphery of the expanders in all groups, it was significantly decreased beneath the expanders in all implanted animals. A morphological examination revealed resorption lacunae with a diameter of 218.4 ± 56 µm in those rats in which the expanders had been placed directly onto the calvaria. Conclusion: This study shows the direct influence of hydrogel expanders on underlying bone. Whereas bone resorption and connective tissue formation also occur underneath hydrogel expanders, these effects can be avoided if the expander and the underlying bone are separated by PDS foil. The key to success is to ensure the appropriate placement of expanders and thus to avoid bone resorption. © 2009 European Association for Cranio-Maxillofacial Surgery

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INTRODUCTION

Expanders are an established method to generate soft tissue during plastic and reconstructive surgery. The tissue is exposed to persistent tensile stress during the expansion process, which will result in tissue gain after an initial soft-tissue elongation (Antonyshyn et al., 1988; Bennett and Hirt, 1993). In most cases, the underlying bone serves as a counter-bearing for the expansile stress applied.

In order to achieve an optimum soft-tissue increase, subperiosteal implantation of the expander is usually preferred over extraperiosteal implantation (Rucker et al., 2005). However, the advantage for soft-tissue generation goes hand in hand with considerable limitation of the nutritional supplies to the bone, which depends significantly on the periosteum (Chanavaz, 1995). The new hydrogel expanders are self-expanding, thus decreasing the need for traditional external filling. Moreover, an almost constant tensile stress is exerted (Wiese, 1993). The hydrogel expansion is based on the absorption of interstitial liquid and is determined by the composition of the expanders material (Wiese et al., 2001).

Although hydrogel expanders are already in clinical use (Berge et al., 2001; Ronert et al., 2004), the reactive changes in the underlying bone have hardly ever been analysed. While changes in bone can be represented using various methods, radiological and histological methods have proved to be especially useful in animal experiments. Hydroxyapatite is the main crystalline component of bone and accordingly, conclusions as to reactive changes in bone density can be drawn from radiological investigations (Buirago-Tellez et al., 1997). Image fusion of the preoperative and postoperative pictures permits a spatial allocation to the individual bone to be examined by way of a direct comparison (Grosu et al., 2003). As described by Luebbers et al., image fusion using anatomical landmarks that correspond with specific regions of the untreated skull is a useful method for accurately investigating morphological changes (Luebbers et al., 2008).

Starting from a certain threshold value, especially the influence of persistent pressure results in bone resorption due to reduced perfusion (Saito et al., 1998). This effect has already been described in the relevant literature based...
on animal experiments using balloon expanders (Schmelzeisen et al., 1999). The following study is designed to show whether using hydrogel expanders in animal experiments will result in changes in the underlying bone and what consequences fluid absorption have on it.

Q4 MATERIAL AND METHODS

Hydrogel expander

The expander (Cupola 0.7 ml, Osmed Co., Ilmenau, Germany) was a hydrogel expander with a silicone shell (Fig. 1). The physiologically compatible hydrogel consisted of a copolymer based on methyl methacrylate and N-vinyl pyrrolidone. The vinyl shell was perforated at two points by 0.5 mm diameter holes to slow expansion. The expander’s initial volume was 0.113 cm$^3$. In physiological saline solution, it was possible to expand the device to six times its initial volume (according to Osmed Co.).

Food and care

The experiments were performed on Lewis rats (male, 300 g, $n = 18$). The animals had free access to food (Altromin, no.1324, Germany) and water. They were kept in single cages under constant physiological conditions. The three groups were designated as follows: group 1 ($n = 6$) was used as a control group. In group 2 ($n = 6$), the hydrogel expander was applied directly to the bone, while in group 3 ($n = 6$), it was separated from the bone by a polydioxanone (PDS) foil (0.25 mm, Johnson & Johnson Int., Ethicon GmbH & Co KG, D-Norderstedt).

The experiments were performed and animals were kept in accordance with the German Animal Protection Act (section 8, subsection 1 of 25 May 1998) (Federal Law Gazette I, p. 1105), as amended by Article 2 of the Act of 12 April 2001 (Federal Law Gazette I, p. 530). The projects dated 1 Jan 2007 were authorised by the Lower Saxony Consumer Protection and Food Safety Office, Section 33/Animal Protection, Oldenburg.

Surgical procedure

The animals were prepared for expander implantation by nuchal incisions, at a safe distance from the later position of the expander. Following subcutaneous preparation, the periosteum was cut transversely directly at the caudal edge of the occipital bone. Anterior to this incision, the periosteum was dissected from the calvaria area on a surface of 7 mm diameter and was prepared subperiostially in the anterior direction. In the control group (group 1), the periosteum was then repositioned and the wound closed with fouratraumatic interrupted sutures using resorbable Ethicon Vicryl® sutures (polyglycin 910, size 4.0, Johnson & Johnson Int., St.-Stevens-Woluwe, Belgium), whereas in groups 2 and 3 the expander was subperiostially placed anterior to the coronal suture. It was to be located in the median sagittal plane, exerting no tension. While the perforation on top of the expander shell touched the periosteum, the opposite perforation in the centre of the expander base had direct contact with the calvaria in group 2, whereas in group 3 it was separated from the bone by a PDS foil (Ethicon, Johnson & Johnson Int., St.-Stevens-Woluwe, Belgium).

Then, wound closure was performed atraumatically in two layers, with four interrupted sutures per layer using resorbable Ethicon Vicryl® sutures (polyglycin 910, size 4.0, Johnson & Johnson Int., St.-Stevens-Woluwe, Belgium).

Micro-computed tomography (CT)

Before surgery, a microCT (XtremeCT, SCANCO Medical AG, Bruettisellen, Switzerland) of the rats’ skulls was performed under ketamine/Dormicum anaesthesia (Ketavet®, 100 mg/kg body weight, Parke-Davis, Germany; Rompun®, 5 mg/kg body weight, Bayer Healthcare, Germany).

Twenty-one days after the implantation, another microCT was done under anaesthesia. For evaluation purposes, the CT images of all animals were superimposed (Voxim, IVS Solutions, Chenninz, Germany) to permit the exact spatial allocation of bone areas before and after surgery and to identify the changes which had occurred beneath the expander. Furthermore, hydroxyapatite density beneath the expander and in the peripheral area of contact was determined based on the radiological images and using software computer programme (Evaluation Program, SCANCO Medical AG, Switzerland).

Histology

For histological processing, the preparations of the experimental group and the control group were fixed for 24 h in 3.5% formaldehyde (pH 7.4) and rinsed in water. Then they were decalcified in 10% EDTA (0.3 M

Fig. 1 — Vinyl-coated hydrogel expander before (A) and 21 days after in vivo placement (B) with an expansion coefficient of six.
In accordance with the distribution of Q5 Tris-m sectioned in 5 μm intervals. The sections obtained were stained based on standard protocols with haematoxylin-eosin.

Immunohistochemistry

Immunohistochemistry was used to detect osteoblasts in the samples. Paraffin sections (5 μm) were washed in phosphate buffered Salt Solution (PBS, Biochrome, Berlin, Germany). After blocking nonspecific binding with 2% normal goat serum, the slides were incubated for 1 h in a humidified chamber at room temperature with the primary antibodies rabbit anti-rat collagen I (BIOLOGO, Kronshagen, Germany) and mouse anti-rat osteocalcin (Dianova, Hamburg, Germany). Goat isotype-matched IgG serum (Dianova, Hamburg, Germany) served as isotype control, and an additional negative control without primary antibody was used. Slides were rinsed in PBS and incubated for 45 min with biotinylated goat anti-rabbit antibody (Dianova, Hamburg, Germany) or biotinylated goat anti-mouse antibody (Dianova, Hamburg, Germany), respectively. To block endogenous peroxidases, the sections were washed with PBS and treated with 0.3% H₂O₂ in 100% methanol for 30 min. Subsequently, the slides were rinsed in PBS and incubated with peroxidase conjugated streptavidin for 30 min. The sections were incubated with 3-amino-9-ethylcarbazole-substrate (AEC, Vector Laboratories, Burlingame, CA, USA) at room temperature. After 10–20 min, the colour development was stopped after microscopic examination by rinsing the slides with PBS, then the slides were mounted (Aquatex; Merck, Darmstadt, Germany).

Statistical analysis

Results are expressed as means ± Standard Error of Mean (SEM). In accordance with the distribution of data, differences between the groups were assessed using the parametric Student’s paired t test or the non-parametric Wilcoxon matched-pairs signed-rank test. Statistical differences were considered significant at $p < 0.05$.

RESULTS

During the entire observation period, no negative impact on vital parameters could be identified in any of the rats. Consequently, all 18 animals completed the study were involved in the examination.

Bone density and bone thickness

The pre- and postoperative microCT data sets were merged for each animal. The density measurements of the hydroxyapatite in the area of constant bone compression were performed centrally beneath the expander and peripherally in the peripheral area of the expander contact surface (Fig. 2).

In the peripherally area no significant changes in hydroxyapatite density was identified in all groups. Bone density in the peripheral area increased slightly when the expander was located directly on the bone, while bone density decreased slightly where the bone was separated by the PDS foil.

In the central area of the expander, group 2 displayed a significant decrease in hydroxyapatite density, while the difference was not significant in group 3. The decrease in bone thickness in the central area, however, was significant in both groups (Fig. 3).

Histology

To evaluate bone changes, a qualitative comparison of the bone architecture and the osteoid formation beneath the expander with the control group was performed. In the control group, there was no histological evidence of osteoid tissue. In contrast, osteoid tissue was found in both experimental groups, both beneath the expander and peripherally, which, however, made up less than 1% of the bone in the affected area.

The control group and group 3 displayed a parallel orientation of the bone lamellae and collagen fibrils in the peripheral and central areas. In contrast, the orientation of collagen fibrils beneath the expander in group 2 was randomly structured with lacunae in the central area beneath the expander (Fig. 4). The decalcified and degraded...
Fig. 3 — Calvarial thickness 21 days after the placement of a hydrogel expander. Whereas bone thickness at the periphery of the expander (black bars) did not change after hydrogel expander placement, it decreased significantly underneath the expander (white bars), irrespective of whether or not the expander was separated from the bone using PDS foil. Unoperated calvaria served as controls. Mean ± SEM; *p < 0.05 vs controls.

fibrils were rather irregularly arranged in the resorption lacunae. These lacunae had a diameter of 218.4 ± 56 μm and connective tissue formation was seen in some parts of the bone.

DISCUSSION

The hydrogel expander presented here causes significant effects on the underlying bone. The central pressure area beneath the expander shows distinct bone resorption and connective tissue formation, phenomena that do not occur if a PDS foil separates the expander from the bone.

Depending on the expander used and the filling intervals using conventional methods, the expansion rate and the resulting pressure differ considerably (Colonna et al., 1996). In the relevant literature, there is disagreement about the optimum expansion rate (Hoffmann, 2005). In the present study, the expansion rate is determined by the expander and, therefore, comparable in the individual groups. Accordingly, it can be assumed that bone pressure is also identical and that direct comparisons can be made. A distribution of pressure caused by the PDS foil can hardly be expected in vivo, since the mechanical stability in vivo is very low (Kontio et al., 2005; Lauer et al., 2006).

In the case of persistent bone compression caused by expanders, distinct bone resorption occurs as early as 6 days. In histological terms, an osteoclastic resorption layer can be detected (Sato et al., 1998), which increases in thickness at higher pressures (Tominaga et al., 1993). Our examination revealed little osteoid tissue, which suggests low constant bone pressure.

Moreover, in the case of subperiosteal placement the microcirculation of the bone in the affected area is severely impaired (Schaser et al., 2003; Rucker et al., 2005). The results are bone resorption and restructuring processes (Hemmer et al., 1987). However, these have so far only been described in connection with traditional expanders and with no associated decrease in hydroxyapatite density (Moelleken et al., 1990; Schmelzeisen et al., 1999). In contrast, our own analyses revealed that hydroxyapatite density decreases significantly if the expander is applied directly to the bone. Any influence of the PDS foil on the bone is not to be expected in the analyses (Baumann et al., 2002; Pihlajamaki et al., 2006). Since this effect rarely occurs when there is no fluid intake by the expander and the PDS foil is applied to the bone, it can be concluded that the various restructuring processes with otherwise identical test structures are due exclusively to the local fluid depletion caused by the expander.

Gakunga et al. (2000) describe the defective bone development resulting from changes in hydrostatic pressure and the development of lacunar structures in the bone. This leads to an increased number of osteocytes under the PDS foil and when the expander is applied directly,
to connective tissue formation in the bone. This resorption process corresponds with the lower hydroxyapatite density beneath the expander (Fanghanel et al., 1988). Studies conducted by Wiese et al. have shown that inflammatory reactions in tissue to the expander are not to be expected. Our own analyses back up this statement, since there was neither clinical nor histological evidence of inflammatory reactions.

In the peripheral area, an appositional growth of bone thickness beyond the initial level was identified when the expander is applied directly. However, there is no significant correlating increase in hydroxyapatite density. Consequently, it can be assumed that the mineralisation in studies conducted by Kessler et al., who observed bone apposition following periosteal elevation of the bone in this area has not been completed yet. This overcompensation process is comparable (Kessler et al., 2007).

CONCLUSION

The hydrogel expander presented here causes significant effects on the underlying bone. The crucial point is to position the expander in such a way as to ensure that there can be no fluid resorption from the bone. This way, changes including bone resorption and connective tissue formation can be avoided.

CONFLICT OF INTEREST

There are no commercial interest and relationship of each author in connection with the submitted manuscript.

References


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