Murine calvarial bone but not DBBM shows signs of resorption under inflammatory conditions

Ulrike Kuchler (1), Gabriel Mulinari dos Santos (2, 3, 4), Patrick Heimel (3, 6), Alexandra Stähli (4, 5), Franz Josef Strauss (4, 7), Stefan Tangl (3, 6), Reinhard Gruber (4, 5, 6)

1. Department of Oral Surgery, School of Dentistry, Medical University of Vienna, Austria
2. Department of Oral Surgery and Integrated Clinic, Universidade Estadual Paulista "Júlio de Mesquita Filho", Araçatuba Dental School, Araçatuba, Brazil
3. Core Facility Hard Tissue and Biomaterial Research, Karl Donath Laboratory, School of Dentistry, Medical University of Vienna
4. Department of Oral Biology, School of Dentistry, Medical University of Vienna, Austria
5. Department of Periodontology, School of Dental Medicine, University of Bern, Switzerland
6. Austrian Cluster for Tissue Regeneration, Austria
7. Department of Conservative Dentistry, School of Dentistry, University of Chile, Santiago, Chile

Objective

Deproteinized bovine bone matrix (DBBM) is considered a slow resorbing bone substitute that is visible after more than a decade in biopsies of augmented sites. Our group discovered in a minipig augmentation model that when the occlusive protection of the augmented site is displaced, severe signs of resorption of DBBM are initiated (Busenlechner et al. 2012). Since then, the findings were not reproduced, likely because bone augmentation usually works predictably in vivo and clinicians accept that a certain percentage of bone resorption occurs.

Nevertheless, our observations have led to speculations on the impact of the microenvironment on the resorption of DBBM and possibly other biomaterials. There is an unproven theory that DBBM placed in a biomechanical unstable ectopic soft tissue is subjected to resorption. However, once DBBM is entombed in an orthotopic bone environment, no clear signs of osteoclastic resorption are visible. To gain a better understanding of the underlying cellular and molecular mechanisms, we performed a rat calvaria osteolysis model.

Material and Methods

Calvarial osteolysis model was performed as follows. Periosteum was elevated off the external cortex of the calvarium and three treatments modalities were randomly applied: i) DBBM alone, ii) together with polyethylene particles (1%; Ceridust VP 3620; Clariant, Gersthofen, Germany) was implanted into the skin pouches. Alternatively, in the third group after the DBBM augmentation iii), inflammation was induced by local injection of LPS from E. coli serotype O55: B5 (25 mg/kg). Mice were euthanized on day fourteen and each calvarium was subjected to histological (Figure 1) and µCT analysis (Figure 2).

Results

Injection of LPS caused an expected almost 5-fold increase of the median void volume with 23.56% ($p<0.001$). Ceridust also increased the median void volume around 2-fold over controls to 13.19% ($p=0.062$). In support of these observations, there was a decrease of cortical BV/TV from 81.14% in the untreated group to 73.32% ($p=0.006$) in the LPS group and 77.32% ($p=0.062$) in the Ceridust group, respectively (Figure 3). Taken together, LPS and Ceridust caused severe remodeling of the frontal calvaria bone indicated by a substantial increase of pores and seams and a decrease of the BV/TV.

Micro CT analysis showed a DBBM volume of 14.07% in the untreated group. No changes in DBBM volume were observed with LPS and Ceridust with 11.02% and 11.16%, respectively ($p=0.057$). The DBBM void volume/TV in the augmented site was 46.35% in the untreated group. Injection of LPS and the use of Ceridust caused no significant changes of DBBM volume/TV with 50.99% and 51.93%, respectively ($p=0.092$; Figure 4). The site distribution of the DBBM particles was not affected by the inflammatory conditions (Figure 5)

Conclusion

The results presented herein demonstrate that LPS and Ceridust cause an intense remodeling activity in the mouse calvaria bone. The size distribution and the overall volume of DBBM particles was, however, unaffected by LPS and Ceridust in the observation period. We therefore conclude that the inflammatory response to LPS and Ceridust that causes severe remodeling of the calvaria bone did not result in catabolic changes of DBBM particles. This observation is relevant considering that is provides insight into the behavior of DBBM under inflammatory conditions. This inflammatory scenario cannot be ruled out in a clinical setting, for example when socket preservation in a patient with periodontitis is performed.

References

Busenlechner D, Tangl S, Arnhart C, Redl H, Schuh C, Watzek G, Gruber R. 2012. Resorption of deproteinized bovine bone mineral in a porcine calvaria augmentation model. Clin Oral Implants Res. 23(1):95-99.

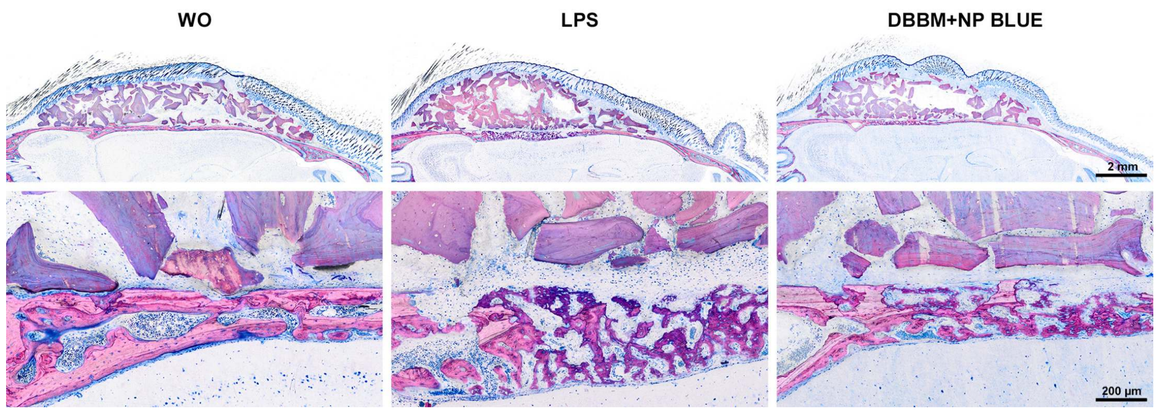

Figure 1: Periosteum was elevated and the calvaria bone was augmented with DBBM. Inflammation was induced by polyethylene particles (Ceridust) or by local injection of LPS from E. coli serotype O55: B5. Mice were euthanized on day fourteen. Note the severe remodeling of the frontal calvaria bone in the LPS and Ceridust groups.

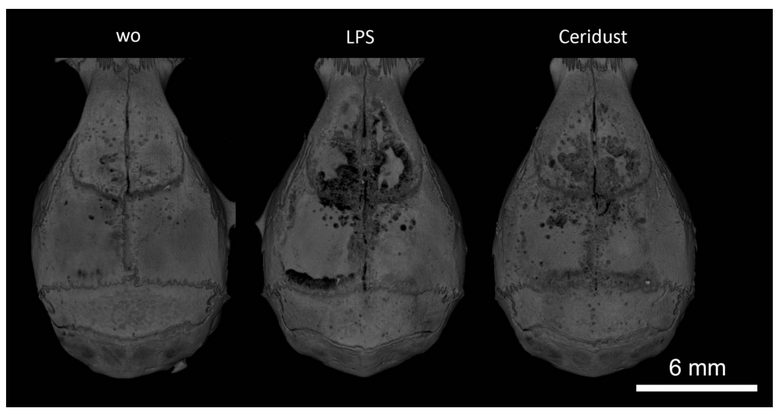

Figure 2: MicroCT analysis of the calvaria bone subjectd toby LPS from E. coli serotype O55: B5. and polyethylene particles (Ceridust). Strong resorption signs are visible in the LPS and the Ceridust group.

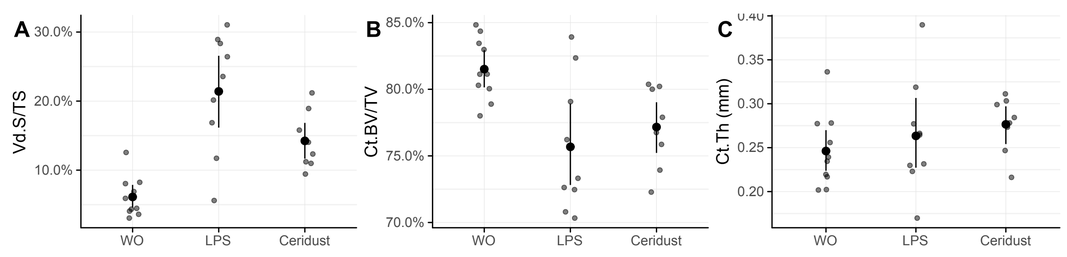

Figure 3: Relative void surface of the calvaria bone increased by LPS and Ceridust (A). Relative cortical bone volume decreased (B), while the absolute cortical thickness was not changed by LPS or Ceridust.

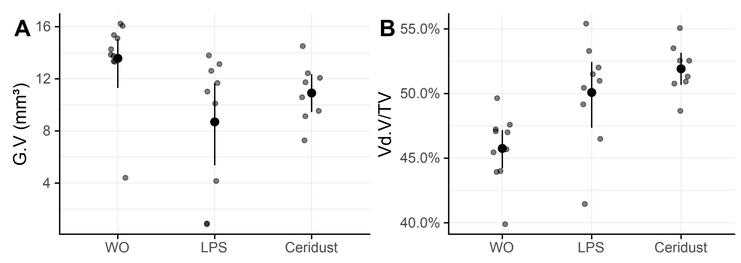

Figure 4: The changes of decreasing the absolute volume of DBBM was not significant (A), and also void volume of DBBM did not reach the level of significance.

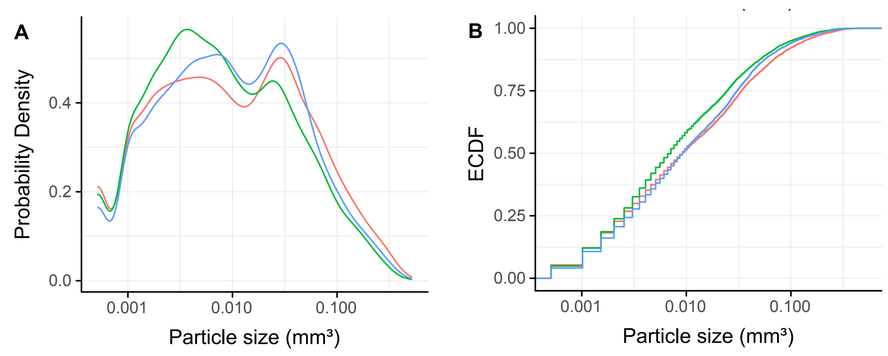

Figure 5: LPS and Ceridust did not significantly affect the volume distribution of DBBM particles (red WO, green LPS, blue Ceridust)

Contact information

Reinhard Gruber, Department of Oral Surgery, School of Dentistry, Medical University of Vienna, Austria; reinhard.gruber@meduniwien.ac.at