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Evaluation of a novel nanocrystalline hydroxyapatite paste and a solid hydroxyapatite ceramic for the treatment of critical size bone defects (CSD) in rabbits

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Abstract The purpose of our study was to test the effectiveness of Ostim nanocrystalline hydroxyapatite paste and Cerabone ceramic by treating a critical size bone defect (CSD) on the right foreleg of a white New Zealand rabbit. Evaluation was carried out by comparing four groups each with a different CSD filling: an only OSTIM bone filling, an only Cerabone filling, an OSTIM–Cerabone combination, and a control group with no filling of the CSD. The results of this study display a rapid and uniform bone ingrowth following the CSD filling with Ostim. The histological and histomorphometrical data have shown similarly excellent results for both the Ostim and Cerabone–Ostim groups. The control group faired poorly in comparison, as three cases of non-union were observed and none of the defects were totally refilled with fresh bone within 60 days. The successful bone healing with osseous consolidation verifies the importance of the nanocrystalline hydroxyapatite in the treatment of metaphyseal osseous volume defects in the metaphyseal spongiosa.

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Introduction

The autogenous bone graft has established itself as the ideal material for the integration into metaphyseal bone defects and is seen today as the 'golden standard' in the field of osteoreconstructive surgery. The lack of the body's own spongiosa resources, especially common in older patients, and the morbidity associated with the removal of spongiosa from the iliac crest are, however, still two major drawbacks of the autogenous bone graft [1–6]. Many research groups are, therefore, currently researching to develop adequate bone substitutes, which fulfil the biological and physical requisites needed for a successful defect filling.

So far calcium phosphate-based bone substitutes of synthetic and biological origin have been particularly useful alternatives to autogenous bone grafts in traumatic surgery and orthopaedics [7–12]. Hydroxyapatite (HA) ceramics have also established themselves as useful bone substitutes since their introduction in the 70s. In the last 10 years many newly developed resorbable HA-materials have also shown a great deal of potential as bone substitutes [9, 11–24].

OSTIM is currently the only licensed innovative nanocrystalline HA-compound in Germany which can be used as a bone substitute in humans. There have, however, so far

only been a limited number of clinical and experimental studies conducted to test the feasibility of this compound [25–31].

The purpose of our study was to test the effectiveness of the Ostim nanocrystalline hydroxyapatite paste and Cerabone ceramic by treating a critical size bone defect (CSD) on the right foreleg of a white New Zealand rabbit. Evaluation was carried out by comparing four groups each with a different CSD filling: an only OSTIM bone filling, an only Cerabone filling, a OSTIM–Cerabone combination, and a control group with no filling of the CSD [32].

Materials and methods

Material properties of the hydroxyapatite compounds used

OSTIM® is a newly developed fast resorbable pure hydroxyapatite in nanoparticle form made by Osartis/Obernburg in Germany (Fig. 1, Table 1).

CERABONE® ist eine solid hydroxyapatite ceramic made by Osartis/Obernburg—Germany (Fig. 2, Table 2).

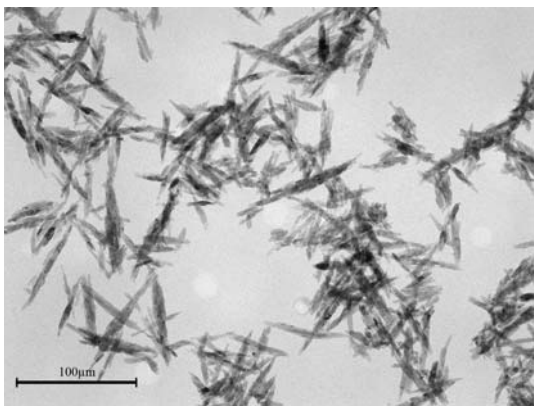

Fig. 1 OSTIM®—Transmission electron microscopy image of hydroxyapatite crystal agglomerates

Table 1 OSTIM—Material properties

OSTIM®
Empirical formula $[Ca_{10}(PO_4)_6(OH)_2]$
Single-phase hydroxyapatite
Synthetically produced
100% inorganic
100% absorbable
Calcium/Phosphate ratio = 1.67
Surface area/mass = 106 m^2g^{-1}

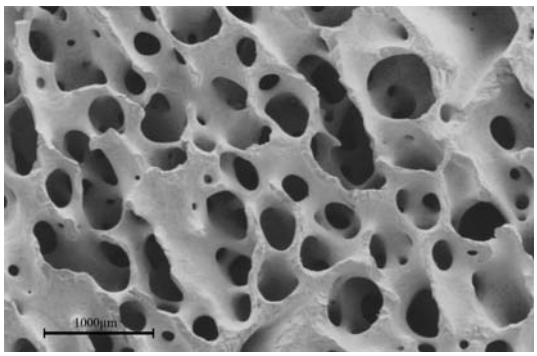

Fig. 2 Cerabone®—Honeycomb-structure

Table 2 Cerabone—Material properties

CERABONE®
Empirical formula $[Ca_{10}(PO_4)_6(OH)_2]$
Pentacalcium-hydroxide-[tris]-phosphate
Bovine origin
pH: 8.2
CaO content: 0.005 (w/w)%
Calcium/Phosphate ratio: 1.67
Average pore diameter: 800 µm

Experimental protocol

Permission was granted from the ethics committee in Karlsruhe to conduct all parts of our animal experiments we applied for (AZ 35-9185.81/54/01, Regierungspräsidium Karlsruhe). All operative procedures on the rabbits were performed under general anaesthesia, which was initiated with Atropin (0.1 mg kg^{-1}s.c.), Rompun (4 mg kg^{-1} s.c.) and Ketanest (60 mg kg^{-1} i.m.) and was further maintained with an isoflurane–nitrous oxide–oxygen-mixture. The forelegs were shaved and sterilised with Octenisept. An anterolateral incision of approx. 3 cm was made over the ulna of the right foreleg between the flexion and extension musculature. Following exposure of the bone a cut of 7 mm length and 5 mm depth was made using an oscillating bone saw. The bone marrow was then removed with a sharp spoon leaving a CSD surrounded by a thin layer of cortical bone (Fig. 3).

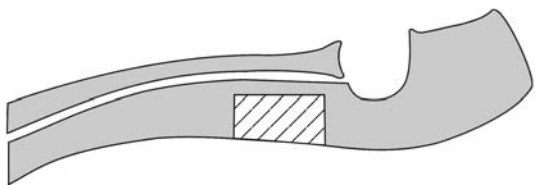

Fig. 3 CSD cross section of the foreleg

Thirty-two animals were divided into four groups of eight. In the first group the CSD was left without a filling and served as the control group. In the second group the CSD was filled with the non-resorbable hydroxyapatite ceramic Cerabone®. The third group received the nanocrystalline paste Ostim as a filling. Finally a combination filling of a Cerabone core, measuring $5 \times 4 \times 4$ mm, surrounded by the Ostim paste was used for the CSD in the fourth group (Fig. 4a–b).

Following the CSD filling, a three-hole titanium miniplate was fixed onto the bone with two bicortical screws of 1.5 mm diameter and 6 mm length to ensure the stability of the defect (Fig. 4b) (Synthes/Oberndorf-Switzerland). The multilayer closure of the wound was done with fast resorbable 3.0-BIOSYN® sutures (Brau-Dexon GmbH/Tuttlingen). A bandage was then placed onto the leg and free movement of the animal was allowed immediately after the operation.

Conventional radiographs were conducted postoperatively and after sacrificing the animals. Two sagittal slices were also obtained after sacrificing the animals from the treated CSD and processed for histology with the Donath method [33]. The slices were embedded in PMMA and ground down to a thickness of 10 μm and stained with toluidine blue for microscopical evaluation. A histomorphometric assessment was then conducted using the ZEISS KS 300 system (Carl Zeiss Jena GmbH/Jena) [34, 35]. The statistical analyses were conducted using the software package SPSS®. Quantitative results of the histomorphometry are presented as box and whisker plots.

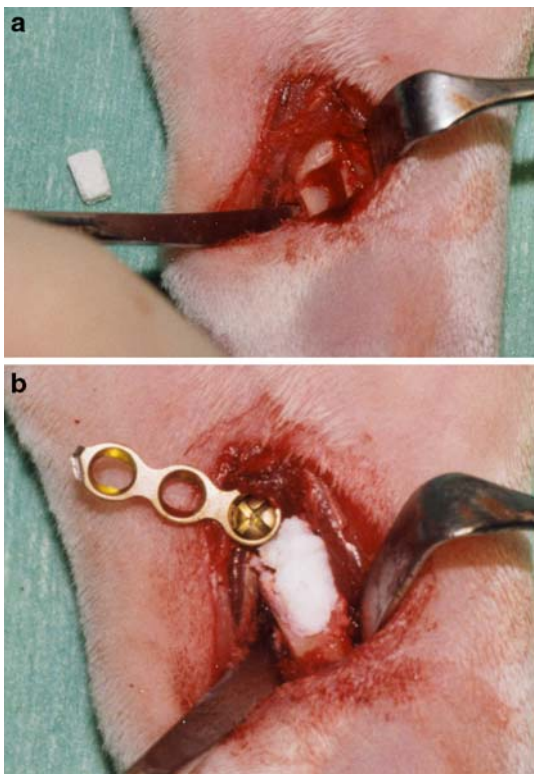

Fig. 4 (**a**) CSD—intraoperative situs without a filling. (**b**) CSD—intraoperative situs with the Ostim–Cerabone filling, The bony defect has been filled with the solid Cerabone ceramic core surrounded by the Ostim paste. The CSD is then stabilised with the three hole mini plate

Results

Subjective observations

The bone filling together with the plate osteosynthesis could be carried out on all animals as planned. The clinical progression of the rabbits was free of complications. Only in two cases was delayed wound healing observed due to bandage friction.

Qualitative radiological analysis

Sixty days following the operation three cases of non-union were observed in the control group. Four of the eight animals presented a subtotal bone ingrowth into the CSD. The Cerabone group presented good integration of the filling with the surrounding bone. The radiographs of all cases in the Ostim group displayed uniform bone growth into the CSD as well as good integration. The Ostim–Cerabone combination group showed similar good integration as with the other fillings (Fig. 5a–d).

Qualitative histologic evaluations

Non-union was observed in three of the rabbits in the control group.

The good bony integration of the whole Cerabone group could also be confirmed histologically. The honeycomb structure of the ceramic was filled to various extents with newly formed bone and bone marrow without any signs of fibrosis.

In the Ostim group the residual particles were covered by newly formed bone, which filled the whole defect in all cases. Well structured cortical and trabecular bone tissue was seen among the implanted material. Residual particles of the HA-paste were detected in the newly formed bone marrow as fragmented round structures of different size. These were seen 60 days after surgery. Osteoblasts were mostly functionally active cells with osteoid production. They were regularly distributed on the surface of the bone trabecle. No inflammatory reactions, osteofibrosis or osteolysis were observed (Fig. 6a–b).

The Ostim–Cerabone combination group presented good integration with the surrounding bone with a pronounced presence of bone growth into the ceramic. Within some

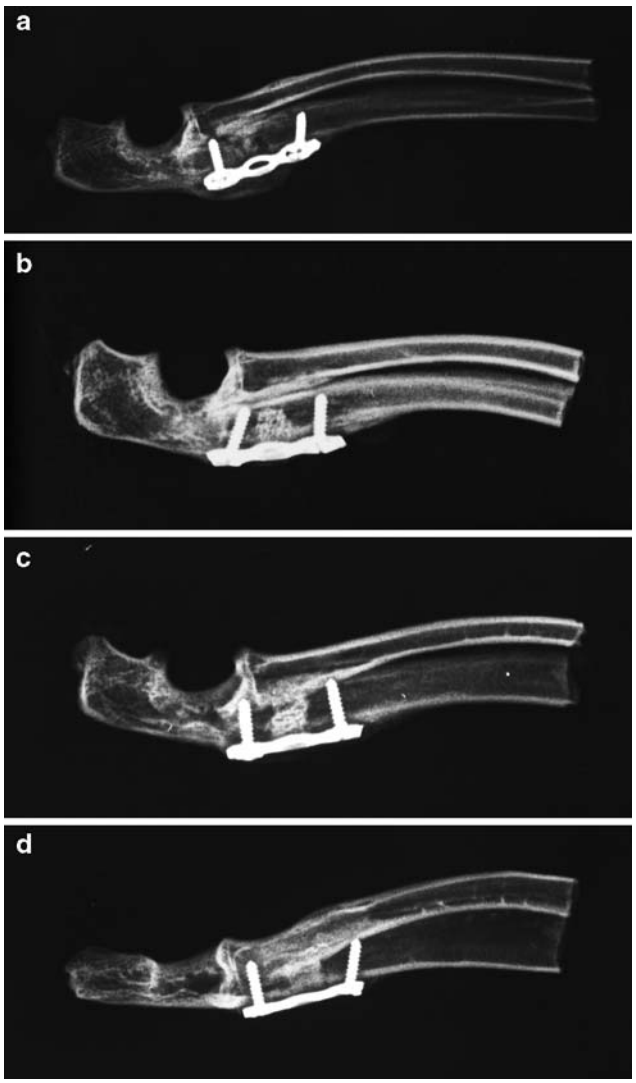

Fig. 5 (**a**) Control group. Subtotal bone ingrowth into the CSD and reactionary callus growth around the plate. (**b**) Cerabone group. A good integration of the solid ceramic can be observed. (**c**) Ostim–Cerabone group. An even better integration of the combination implant can be seen compared to the Cerabone implant alone. (**d**) OSTIM group, Uniform bone ingrowth can be seen

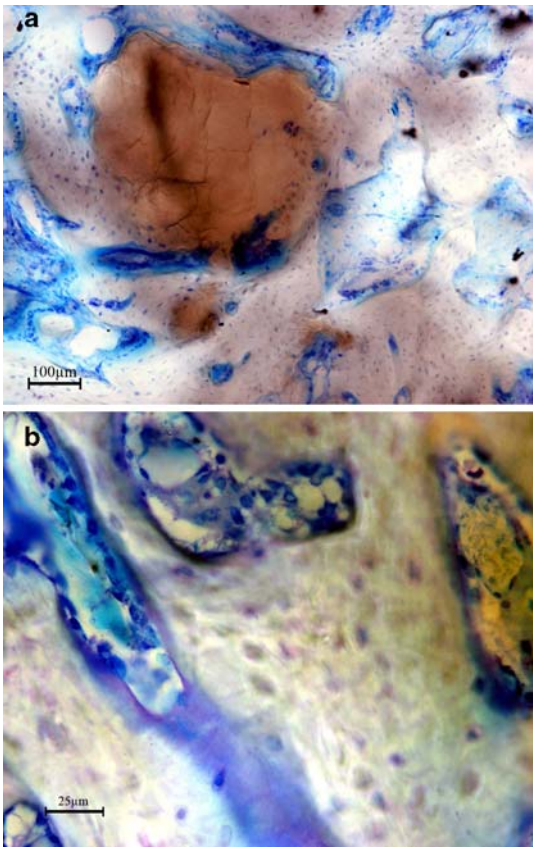

Fig. 6 CSD filling with OSTIM. (**a**) Staining with toluidin blue. Rest particles of the HA-nanocrystalline paste are seen as round structures of different size in the bone marrow and within the bone trabecular tissue. (**b**) Sixty days after implantation blood vessels are visible which are surrounded by circumferential lamellae of bone and closely resemble evolving haversian systems. Ostim fragments can also be seen

medullary space remaining particles of amorphic HA-paste was seen. There were no signs of inflammation or fibrosis (Fig. 7a–b).

Histomorphometry

The *bone coverage* over the implant area was calculated as the percentage of surface occupied by bone ingrowth. The best result was attained in the Ostim group with a median of 41% although the Cerabone–Ostim group presented a similar result of 36%. The Cerabone group also faired well with 26% and was still much better than the control group with only 16% bone coverage (Fig. 8).

The *bone ingrowth* was calculated as the percentage of the ceramic pore space filled with new bone. The CSDs of the Cerabone® group were replaced with a median of 55 of newly formed bone. The Cerabone–Ostim group was even better with 75 ($p < 0.05$) (Fig. 9).

Discussion

Wide experience has been gained with the use of Endobone in both clinical and experimental fields [14, 16, 22, 36–38]. Such experience has yet to be gained with the newly developed HA-ceramic Cerabone. According to the manufacturer, Cerabone is very similar to Endobone as it is of bovine origin and also has a very similar porous structure with a mean pore diameter of 800 µm and a range of 100–1500 µm. The main difference lies within the reduced calcium oxide content and the improved structural stability of approx. 70% (Coripharm, Dieburg/Germany). The ability of a HA-ceramic to integrate within the vital bone

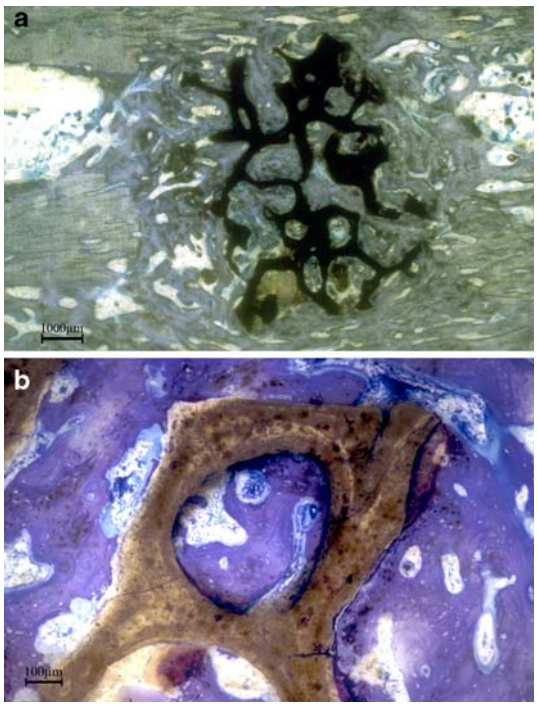

Fig. 7 CSD-filling with Ostim–Cerabone combination. (**a**) The good integrated HA-ceramic and Ostim fragments are visible macroscopically. (**b**) Staining with toluidin blue. Well structured trabecular bone with numerous osteoblasts on the trabecular and ceramic surface

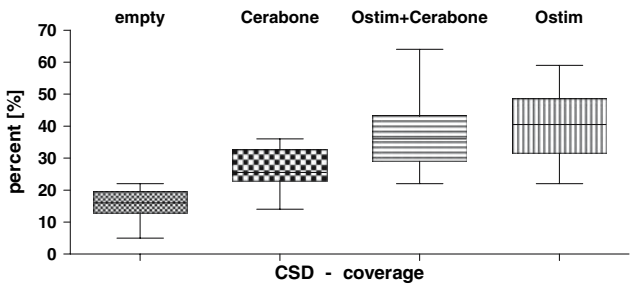

Fig. 8 Percentage of bone replacement in the CSD

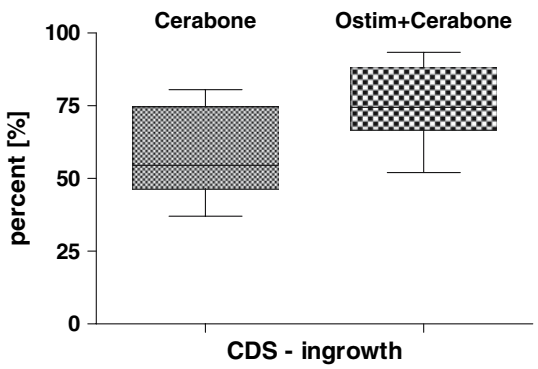

Fig. 9 Percentage of bone filling into the honeycomb structure of the HA-ceramic

depends both on its osteoconduction properties and geometrical porous structure [38–40]. Osteoconduction needs to be rapid and the pore diameter more than 100 µm to allow for adequate osteo- and angiogenesis. Eight weeks postoperatively the results gained from our Cerabone group were similarly favourable to those of Hing, who described full integration three months after implantaion of the ceramic Endobone [38]. Holmes and Renooij on the other hand used HA-ceramics with a maximum pore diameter of 150 µm and described poor vascularisation into the ceramic as well as little bone growth [41, 42].

We observed even further bone growth into the CSD with the fully resorbable nanocrystalline paste OSTIM. This is a hydroxyapatite compound with a Calcium/Phospate ratio of 1.675 making it equivalent to that of the pure hydroxyapatite present in human bone. The specific surface area of the bone substitute is 106 m^2g^{-1}. According to the hypotheses put forward by Constanz and Knaack such HA-compounds accelerate the bony ingrowth into the CSD as they closely mimic the required resorptive and osseointegrative properties of poor crystalline apatitic structure of natural bone [10, 43, 44]. This in turn allows for rapid osteogenesis and angiogenesis to take place, both critical steps for successful bone defect augmentation [11, 13]. The first experimental results of Grigoryan in 2000 confirmed the rapid bone ingrowth at a low complication rate following the treatment of jaw defects with Ostim in dogs [26]. Comprehensive clinical experience of using the hydrated HA-paste as a void filling only exists in the field of maxillofacial surgery. Various stomatology publications describe an accelerated fracture healing and bone density increase at a high degree of tolerance [11, 25–27, 30, 31, 45]. In 1996 Zuev treated 395 patients with jaw defects and peridontal abscesses. The complication rate of the 200 patients in the Ostim group was at 1.5% compared to 3.6% in the group with 195 patients treated with allografts [31]. Bezrukow achieved excellent results in 1998 after treating 49 patients with Ostim following cystectomy of benign cyst-tumours of the jaw. The defects in all 49 patients were replaced with fresh bone three months following the defect filling [25]. Ostim in combination with angularly stable osteosynthesis has also been successfully used in the field of traumatology. Particularly good results were achieved in a prospective study with 21 patients for the treatment of type C2 and C3 radial fractures [46].

The results of this study also display a rapid and uniform bone ingrowth following the CSD filling with Ostim. The histological and histomorphometrical data have shown similarly excellent results for both the Ostim and Cerabone–Ostim groups. The control group faired poorly in comparison as three cases of non-union were observed and none of the defects were totally refilled with fresh bone within 60 days.

We found Ostim easy to use as even the most irregular defect zones could be effortlessly filled. For this reason we see further application possibilities for this substance in the field of traumatology, e.g. in the treatment of tibia head impression fractures, comminuted radius fractures and pronounced calcaneal impression fractures. The application of Ostim always needs to be combined with some form of stable osteosynthesis, preferably with an angularly stable plate, due to its lack of dimensional stability.

Conclusion

The successful bone healing with osseous consolidation verifies the importance of the nanocrystalline hydroxyapatite in the treatment of metaphyseal osseous volume defects in the metaphyseal spongiosa.

Reference

1. E. D. ARRINGTON, W. J. SMITH, H. G. CHAMBERS, A. L. BUCKNELL and N. A. DAVINO, *Clin. Orthop. Relat. Res.* **329** (1996) 300
2. J. A. GOULET, L. E. SENUNAS, G. L. DESILVA and M. L. GREENFIELD, *Clin. Orthop. Relat. Res.* **339** (1997) 76
3. S. W. LAURIE, L. B. KABAN, J. B. MULLIKEN and J. E. MURRAY, *Plast. Reconstr. Surg.* **73** (1984) 933
4. C. S. UBHI and D. L. MORRIS, *Injury* **16** (1984) 202
5. M. E. BLAKEMORE, *Injury* **14** (1983) 519
6. E. M. YOUNGER and M. W. CHAPMAN, *J. Orthop. Trauma* **3** (1989) 192
7. F. D. BURSTEIN, *Cleft Palate Craniofac J.* **37** (2000) 1
8. J. O. HOLLINGER, J. BREKKE, E. GRUSKIN and D. LEE, *Clin. Orthop. Relat. Res.* **324** (1996) 55
9. J. O. HOLLINGER and G. C. BATTISTONE, *Clin. Orthop. Relat. Res.* **207** (1986) 290
10. D. KNAACK, M. E. GOAD, M. AIOLOVA, C. REY, A. TOFIGHI and P. CHAKRAVARTHY, et al., *J. Biomed. Mater Res.* **43** (1998) 399
11. R. SCHNETTLER, J. P. STAHL, V. ALT, T. PAVLIDIS, E. DINGELDEIN and S. WENISCH, *Eur. J. Trauma* **30**(4) (2004) 219
12. M. SZPALSKI and R. GUNZBURG, *Orthopedics* **25** (2002) s601
13. T. J. BLOKHUIS, M. F. TERMAAT, F. C. DEN BOER, P. PATKA, F. C. BAKKER and H. J. HAARMAN, *J. Trauma* **48** (2000) 179
14. D. BRIEM, W. LINHART, W. LEHMANN, N. M. MEENEN and J. M. RUEGER, *Unfallchirurg* **105**(2) (2002) 128
15. C. CASSIDY, J. B. JUPITER, M. COHEN, M. DELLI-SANTI, C. FENNELL and C. LEINBERRY, et al., *J. Bone Joint Surg. Am.* **85** (2003) 2127
16. H. GIERSE and K. DONATH, *Arch. Orthop. Trauma Surg.* **119** (1999) 349
17. W. G. HORSTMANN, C. C. VERHEYEN and R. LEEMANS, *Injury* **34** (2003) 141
18. P. KOPYLOV, K. JONSSON, K. G. THORNGREN and P. ASPENBERG, *J. Hand Surg. [Br]* **21**(6) (1996) 768
19. W. MITTELMEIER, C. BRAUN and R. GRADINGER, *Chirurg* **67** (1996) 1087
20. H. MITTELMEIER, W. MITTELMEIER and M. GLEITZ, *Orthopade* **27** (1998) 126
21. T. A. SCHILDHAUER, T. W. BAUER, C. JOSTEN and G. MUHR, *J. Orthop. Trauma* **14** (2000) 309
22. K. D. WERBER, R. B. BRAUER, W. WEISS and K. BECKER, *J. Hand Surg. [Am]* **25** (2000) 833
23. S. W. WOLFE, L. PIKE, J. F. SLADE and III L. D. KATZ, *J. Hand Surg. [Am]* **24** (1999) 816
24. R. ZIMMERMANN, M. GABL, M. LUTZ, P. ANGERMANN, M. GSCHWENTNER and S. PECHLANER, *Arch Orthop. Trauma Surg.* **123**(1) (2003) 22
25. V. M. BEZRUKOV, L. A. GRIGOR'IANTS, V. P. ZUEV, A. S. PANKRATOV, *Stomatologiia (Mosk)* **77** (1998) 31
26. A. S. GRIGOR'IAN, L. A. GRIGOR'IANTS and M. N. PODOINIKOVA, *Stomatologiia (Mosk)* **79**(4) (2000) 9
27. O. KILIAN, V. ALT, C. HEISS, T. JONULEIT, E. DINGELDEIN and I. FLESCH, et al., *Growth Factors* **23** (2005) 125
28. M. A. RAUSCHMANN, T. A. WICHELHAUS, V. STIRNAL, E. DINGELDEIN, L. ZICHNER and R. SCHNETTLER, et al., *Biomaterials* **26** (2005) 2677
29. R. SCHNETTLER, E. DINGELDEIN and G. HERR, *Orthopade* **27** (1998) 80
30. W. M. THORWARTH, K. A. SCHLEGEL, S. SROUR, S. SCHULTZE-MOSGAU and J. WILTFANG, *Implantologie* **12**(1) (2004) 21
31. V. P. ZUEV, L. A. DMITRIEVA, A. S. PANKRATOV and N. A. FILATOVA, *Stomatologiia (Mosk)* **75** (1996) 31
32. J. O. HOLLINGER and J. C. KLEINSCHMIDT, *J. Craniofac. Surg.* **1**(1) (1990) 60
33. K. DONATH and G. BREUNER, *J. Oral. Pathol.* **11** (1982) 318
34. W. A. MERZ and R. K. SCHENK, *Acta Anat (Basel)* **76** (1970) 1
35. V. SMOLEJ, WWW IndustrialHeating com, (2001)
36. M. U. HELBER and C. ULRICH, *Unfallchirurg* **103** (2000) 749
37. K. A. HING, S. M. BEST and W. BONFIELD, *J. Mater. Sci. Mater. Med.* **10**(3) (1999) 135
38. K. A. HING, S. M. BEST, K. E. TANNER, W. BONFIELD and P. A. REVELL, *J. Mater. Sci. Mater. Med.* **8** (1997) 731
39. T. ALBREKTSSON and C. JOHANSSON, *Eur. Spine. J.* **10**(suppl 2) (2001) S96
40. M. JARCHO, *Clin. Orthop. Relat. Res.* **157** (1981) 259
41. R. HOLMES, V. MOONEY, R. BUCHOLZ and A. TENCER, *Clin. Orthop. Relat. Res.* **188** (1984) 252
42. W. RENOOIJ, H. A. HOOGENDOORN, W. J. VISSER, R. H. LENTFERINK, M. G. SCHMITZ and I. H. VAN, et al., *Clin. Orthop. Relat. Res.* **197** (1985) 272
43. B. R. CONSTANTZ, I. C. ISON, M. T. FULMER, R. D. POSER, S. T. SMITH and M. VANWAGONER, et al., *Science* **267** (1995) 1796
44. A. S. POSNER, *Clin. Orthop. Relat. Res.* **200** (1985) 87
45. P. KOPYLOV, K. ADALBERTH, K. JONSSON and P. ASPENBERG, *J. Hand Surg. [Br]* **27** (2002) 538
46. F. X. HUBER, J. HILLMEIER, L. HERZOG, N. MCATHUR, H. J. KOCK and P. J. MEEDER, *J. Hand Surg. [Br]* **31**(3) (2006) 298