Bone regeneration following socket preservation using different bone substitute materials. A pilot study in dogs.

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Purpose: The aim of the present study was to histologically evaluate hard tissue healing following application of bone substitutes into fresh extraction sockets in dogs.
Methods: The following groups were tested: (i) Ceramisys granulated HA nanopaste [GNP] (ReproBone HA/TCP granulates 20% combined within ReproBone Nano HA bone paste 80%, Ceramisys Ltd, Sheffield, UK) (ii) self-hardening bone cementum [OC] (Bone Cement, AAP biomaterials, Dieburg, Germany), (iii) $CaSO_4$-Pellets [POS] (PerOssal, botiss dental GmbH, Berlin, Germany). The materials were randomly inserted in the extraction sockets immediately after tooth extraction. Untreated extraction sites served as control (C). After eight weeks, the animals were sacrified and dissected blocks were prepared for histomorphometrical analysis (n=4).
Results: During the entire study period, healing was uneventful for all animals. Histological analysis revealed a high variability of material resorption and osteoconductive properties of the applied materials. Similar to POS, the nanopaste part of GNP was completely resorbed within eight weeks, whereas the granular part of GNP and OC could still be identified within the extraction sockets. In contrast to the GNP granules being osseoconductively integrated into the newly formed bone, major parts of the OC remnants displayed an fibrous encapsulation and less hard tissue regeneration.
Conclusions: Within the limits of the present study it was concluded that POS and GNP supported, whereas OC inhibited hard tissue healing following tooth extraction. GNP may be generally considered as suitable material for use in bone tissue repair and augmentation.

Key words - animal study, hydroxyapatite, wound healing, extraction, implant therapy, socket preservation, nanopaste

INTRODUCTION

Sufficient bone dimensions are necessary for oral rehabilitation with dental implants. A compensation of the physiological atrophy occuring during the dynamic bone regeneration of the extraction socket simplifies implant insertion and improves predictability without the need of additional augmentative therapy. This may entail an advantage concerning therapy duration but also in regard to aesthetics and stress for the patient.
The physiological processes of wound healing after tooth extraction may be described in 5 steps:

1. formation and maturation of a blood coagulum (day 1)
2. organization of the coagulum by capillaries (4-5 days)
3. formation of a temporary matrix (14-16 days)
4. osteoblastic reorganization of the temporary matrix and complete epithelial closure of the alveolus (until 6 weeks)
5. bone modeling/ remodeling (5-10 weeks)[1, 2]

The morphological changes of the alveolar process in apical-coronal as well as buccal-lingual direction after extraction were determined in many pre-clinical and clinical studies.[3-5] Concerning dimensional changes of the bone after dental extraction a loss of vertical as well as horizontal bone volume could be observed. Especially for the maxilla resorptional changes of the buccal bone wall of the alveolus have been described.[3, 6] Hereby, it comes to an inevitable oral shift of the alveolar process.[6] The dimension of atrophy seems to be particularly distinct during the first 3 months after dental extraction.[5] It was shown that the regular resorption of the bone matrix depends on the localisation of the extracted tooth[5], on defect size and composition of the surrounding bone.[7, 8] As negative parameters for wound healing severe periodontitis of the extracted tooth, uncareful extraction methods but also wound infections have been reported.[9, 10] Also, high age initially had an inhibitory effect on the development of the provisional bone matrix. However, this influence was not evident after a healing period of 40 days.[11]

Calcium phosphate ceramics are widely established as the alloplastic biomaterial of choice for bone repair and reconstruction. With over 30 years of clinical use, they have developed an excellent reputation for good biocompatibility and efficacy. Injectable resorbable paste and putty materials have attracted significant interest in recent years as they offer increased applicability and ease of handling. Particularly nanometer sized products may potentially possess advantageous properties not seen in standard materials.
It seems obvious that the insertion of a bone substitute material into the fresh extraction socket may have a positive effect on wound healing and avoid the atrophy of the alveolar bone. It has to be mentioned that any applied

material will alter the normal sequence of healing. Depending on material properties and the size and shape of the inserted particles, resorption, osseous integration/organization and soft tissue encapsulation can be observed. It was considered that the process of biodegradation is directly influenced by the type of material crystallisation.[13] Hereby, size of biphasic calcium phosphate particles affected the cellular degradation activity but not the bone filling ability.[14] In a study comparing hydroxyapatite in solid and particulate form, both materials could not completely prevent bone resorption after tooth extraction. Nevertheless, particles were better physically maintaining ridge height and width than the solid root forms.[12]

Preliminary experimental studies have shown that nanosized ceramics may represent a promising class of bone graft substitutes due to their improved osseointegrative properties.[15,16] Whilst other small hydroxyapatite (HA-) particles are very slowly resorbed by osteoclast-like-giant cells[17-19], nanosized soluble ceramics are considered to be directly incorporated by resorptive cells. However, the application of nanosized HA paste alone in extraction socket was followed by unpredictable material resorption and disturbances of blood vessel ingrowth[20]. A combination of the nanopaste with granular materials might maintain the desired space for bone regeneration. A ready-to-use paste in a syringe, containing a combination of both granular and paste, may support osseous formation in the extraction socket even better than nanosized HA paste alone.

The aim of this study was to assess bone healing following application of Ceramisys granulated HA nanopaste (ReproBone HA/TCP granulates 20% combined within ReproBone Nano HA bone paste 80%) in comparison with two commercial control materials using an extraction socket model. The acceptance criteria for the study was that the Ceramisys granulated nanopaste shows substantially similar or superior performance to the commercial control materials and that the Ceramisys nanopaste shows good evidence of biocompatibility with no evidence of incompatibility.

Material and Methods

All experiments were performed in the animal research institute of the University of Duesseldorf, Germany. Four 18-months old beagle dogs were used in the study. The researchers are well established in testing of biomaterials using this specific animal model. All animals exhibited a fully erupted, healthy, permanent dentition. After tooth extraction, the dogs were fed twice a day with soft-food diet and water.

Study design

Study design was orientated on a study protocol published by Block and Kent in the year of 1986.[12] In four adult dogs, the second molar teeth of the lower jaws were extracted carefully and the extraction sockets were either filled with

(i) Ceramisys granulated HA nanopaste [GNP] (ReproBone HA/TCP granulates 20% combined within ReproBone Nano HA bone paste 80%)
(ii) self-hardening bone cementum [OC] (OsteoCem Inject, AAP biomaterials, Dieburg, Germany)
(iii) pre-formed socket preservation HA-$CaSO_4$-Pellets [POS] (PerOssal, botiss dental GmbH, Berlin, Germany)

The fourth extraction site remained untreated and served as control (C). Healing period was set at eight weeks.

Anaesthesia and animal care

After intramuscular sedation with 0.17 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit, Duesseldorf, Germany), anaesthesia was started using 21.5 mg/kg thiopental-sodium (Trapanal 2.5%, Altana GmbH, Konstanz, Germany). For surgical procedures inhalation anesthesia was performed by use of oxygen, nitrous oxide and isofluorane. To maintain hydration, animals received a constant rate infusion of lactated Ringer's solution while being anaesthetized. Intraoperative analgesia was done by intravenous injection of 0.4 mg/kg piritramid (Dipidolor®, Janssen-Cilag GmbH, Neuss, Germany) and 4.5 mg/kg carprofene (Rimadyl®, Pfitzer Pharma GmbH, Karlsruhe, Germany). For postoperative treatment, piritramid and carprofene were applied subcutaneously for three days in the same dose as described before. Additionally, prophylactic administration of clindamycine (11.0 mg/kg body weight, Clerobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 3 days.

Surgical procedure

The second molar teeth (M2) of the lower jaws were carefully removed after vertical tooth separation. Three single root extraction sockets were filled with the test materials selected at random, whereas the control sites remained unfilled. Wounds were closed after vertical flap elevation using resorbable mattress sutures (Resorba, Nurnberg, Germany). After a healing period of eight weeks, the animals were sacrified (overdose of sodium pentobarbital, 200 mg/kg i.v.) after intravenous injection of 50 000 i.E. Heparine. After preparation and catherization of the carotide arteries, a decapitation was performed and the head of the animal was intra-arterially fixed with 1000 ml of 4% buffered formaline solution. Following this primary fixation procedure, the jaws were dissected in order to obtain the blocks containing the experimental specimens or corresponding control sites. The specimens were fixed in 4% neutral buffered formalin solution for 7 days. Then the samples were

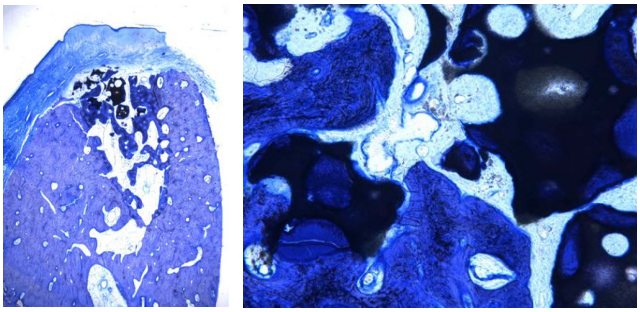

Fig. 1a, 1b: Representative histology of extraction socket eight weeks following socket preservation with GNP (a: 12,5x/b: 100x)

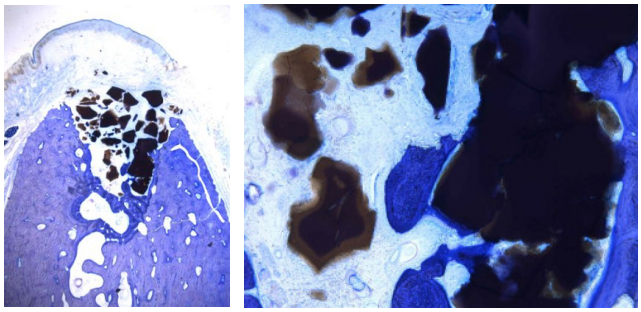

Fig. 2a, 2b: Representative histology of extraction socket eight weeks following socket preservation with OC (a: 12,5x/b: 100x)

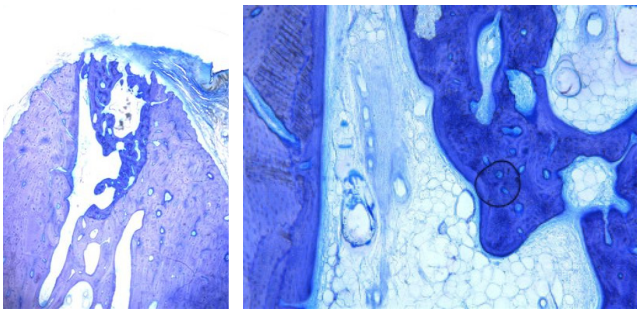

Fig.3a, 3b: Representative histology of extraction socket eight weeks following socket preservation with POS (a: 12,5x/b: 100x)

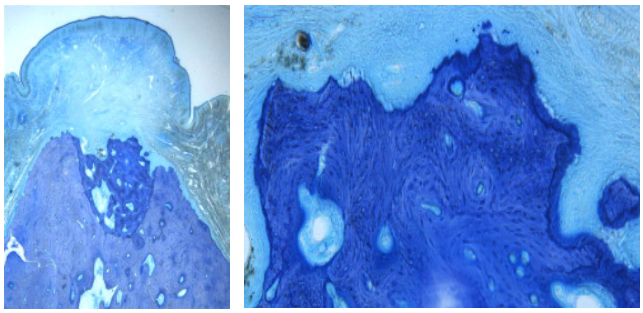

Fig. 4a, 4b: Representative histology of control sites (a: 12,5x/b: 100x)

dehydrated using ascending grades of alcohol, and infiltrated and embedded in special glycol methacrylate resin (Technovit 7200VLC, Heraeus Kulzer, Wehrheim, Germany) for nondecalcified sectioning. Each specimen was cut in bucco-oral direction and along the axis of the extraction socket using a diamond band saw (Exakt, Apparatebau, Norderstedt, Germany), resulting in three sections of approximately 200 µm thickness.[21] Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer, Wehrheim, Germany) to opaque plastic and ground to a final thickness of 40 µm. All sections were stained with toluidine blue after superficial etching and decalcification with 10 % hydrogene peroxide and 4% formic acid.

Histological analysis

Microscopic observations were performed by an experienced investigator masked to the specific experimental conditions. For documentation, images were obtained using a light microscope (BX50, Olympus, Hamburg, Germany), associated with a camera (SIS Color View3, Soft imaging System, Muenster, Germany).

RESULTS

All extraction sockets healed uneventfully. No infections or wound dehiscences were observed during the entire study period.
Eight weeks after treatment extraction sites of the GNP-filled alveolar sockets (Fig. 1) histologically showed a sufficiently regenerated woven bone with smaller parts of the amorphous, osseoconductive integrated bone substitute material (Fig. 1a). The newly formed bone could clearly be distinguished based on the stronger staining compared to its environment, displaying smaller areas of cortical and cancellous remodeling. No inflammatory reaction could be detected. The healing of the over-lying soft tissue appeared finished and closed with normal epithelium. The material was obviously biocompatible with good evidence of osteoconductive activity to regenerate the bone (Fig. 1b).
The extraction sockets of OC (Fig. 2) were dominated by large solid bone cement particles of different dimensions. The alveolus was mainly not osseous organized (Fig. 2a). Only the particles directly exposed to the former alveolar walls showed a partly osseous integration. Within the other regions, bone cement remnants showed encapsulation with parallel connective tissue fibres (Fig. 2b). Due to the superposition of the granulas compared to the alveolar walls, alveolar crest resorption occurred mainly on the buccal aspect. The mucosa was slightly thickened, but without significant signs of inflammation.
The absorption of POS (Fig. 3) was the most advanced. There were no longer any noticeable remnants of the substitute after 8 weeks (Fig. 3a). A fat-cell-rich bone marrow and a high number of blood vessels were evident within the socket sites (Fig. 3b). In the crestal part a more dense bone layer, covering the defect area had been built. This new bone crest - as to be expected after 8 weeks – seemed to be still under remodeling and dominated by woven bone.
In all cases, control specimens (Fig. 4) displayed osseous organization of the extraction sites. Sockets were filled with woven bone showing first signs of remodellation

(Fig. 4a). The bumpy crestal surface, standing for an ongoing superficial bone resorption (Fig. 4b), was covered by a thick soft tissue and a completely healed epithelium. Alveolar bone resorption was mainly localized on the buccal aspect of the alveolus.

DISCUSSION

On the basis of the data generated, it was observed that the three materials tested exhibited varying degrees of bone regeneration. Direct bone apposition was evident to the implanted materials on a different level. POS revealed complete resorption within eight weeks. For GNP the nanopaste paste was completely resorbed, whereas the granular material remained. The lowest resorption was found for OC, in line with the lowest bone regeneration.
Comparing GNP to the other groups, it was substantially similar to the commercial POS material with respect to biocompatibility, and similar to the control group and POS with respect to healing time. Extraction socket regeneration was superior to OC.
The paste element of the GNP resorbed quicker than the granules resulting in some solid HA/ß-TCP components still being identified after 8 weeks. Comparing the area of newly formed bone within eight weeks, alveolar defect healing of GNP, POS and the untreated control group was superior to OC. Hereby, the hard and soft tissues accepted the presence of osteoconductively integrated GNP with no evidence of any adverse effect. In contrast to OC, the remaining granulate residues did not interfere with the natural bone healing or re-densification, leading to a partly fibrous encapsulation.
Researching the current literature only few histological studies on nanosized HA (NHA), the main component of GNP, can be found. Huber et al.[22] evaluated the bone regeneration after insertion of NHA in cancellous bone in a human traumatological study. The human bone biopsies taken in progress of the removal of osteosynthetic plates showed good regeneration in the allocated areas with regular bone turnover. In line with our results, neither severe inflammatory reactions, nor osteofibrosis or osteonecrosis caused by the bone substitute material were observed.[22] In contrast to the present study, small parts of the bone substitute were detected as amorphous material in the adjacent tissue. Hereby, only the HA/ß-TCP particles remained stable, whereas the nanosized HA revealed complete resorption (Fig. 1). Arts et al.[15] observed non-resorbed NHA remnants in a study of acetabular bone impaction grafting. NHA served in a 10% addition to mixtures of morselised cancellous bone (MCB) and/or Tricalcium phosphate – Hydroxyapatite (TCP-HA) granules as reductor for cement penetration during acetabulum reconstructions. In the histological sections, most of the nanosized ceramic was osseous integrated with MCB or TCP-HA granules after 8 weeks. In contrast to our results, specificly stained sections revealed that some NHA remnants were actively resorbed by multinucleated giant cells and macrophages.[15] This was also observed in one of our former studies evaluating HA-nanopaste alone for socket preservation.[20]
In line with the present results, an experimental study in pigs revealed a complete resorption of the hydroxyapatite-nanopaste within 12 weeks.[23] Different to other nanosized HA-pastes, GNP consists of a fastly resorbable HA-nanopaste part and a granular biphasic calciumphosphate ceramic. The granules are important for space-maintenance and application stability, whereas the nanosized part is resorbed very early not disturbing the blood vessel ingrowth, since other studies indicated that HA pore sizes smaller than 100 µm inhibit blood vessel ingrowth and bone formation.[24, 25]

It may be assumed that the present histological findings can be transferred to other indications like lateral bone augmentation, sinus graft procedures and periimplant defect filling. Hereby, an influence of periodontal ligament cells has to be considered. Cardiaopoli et al.[26] investigated the role of the periodontal ligament for extraction socket healing. No difference was found between socket healing after periodontal ligament removal and control sites within 3 months of healing period. In respect of these findings, the role of the periodontal ligament seems to be inessential for the healing procedure of extraction socket healing, indicating a transferability of the present data to other bone regeneration procedures.

CONCLUSION

Within the limits of the present study it was concluded that POS and GNP supported bone defect regeneration following tooth extraction. The results achieved the acceptance criteria set out for the test. OC seemed to be not useful for socket preservation procedures. In respect to other indications, GNP may be considered as a suitable material also for other bone regeneration applications.

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