Is Osteoinductal® an osteostimulative bone replacement material?

A summary of relevant scientific findings –
Part 1: Biochemical, animal-experimental, and clinical studies

G.-H. Dietz jr., L. Lazzerini, M. Brunelli, S.-I. Stratul, Munich, Germany

With the aim of providing scientific evidence for the osteoinductive and osteostimulative effect of Osteoinductal®, biochemical, animal-experimental, clinical, and human histological studies of the bone replacement material were carried out. The results show that Osteoinductal® strongly stimulates bone metabolism and thereby leads to accelerated bone regeneration. The question of why it demonstrates a similar effect to bone morphogenic proteins (BMP) while itself being protein-free, is still in need of detailed explanation.

With the aim of providing scientific evidence for the osteoinductive and osteostimulative effect of Osteoinductal®, quantitative biochemical tests were conducted on human bone tissue cultures under the influence of oleaginous calcium hydroxide suspensions. On account of its years-long particularly good clinical experience with an analog preparation (“Gangraena-Merz”) in endodonty, studies were carried out at the Biochemical Institute at the University of Witten/Herdecke.

One special oleaginous calcium hydroxide preparation in particular (trade name: Osteoinductal®) demonstrated a heightened influence of the 15 most important biochemical parameters of bone regeneration. It could be shown using live human bone tissue cultures that Osteoinductal® strongly stimulates the metabolism of human spongiosa and, beyond this, greatly increases osteoblast differentiation and osteoblast growth in the cell culture. There followed many studies by 6 universities in 4 countries.

Although they were carried out entirely independently of one another, there was an astonishing level of agreement in their findings, namely that Osteoinductal® strongly induces and stimulates bone regeneration.

Aim of this article
The aim of this article is to provide a summary of relevant studies and case reports on the theme of Osteoinductal®. On account of the reducibility of their results, only studies are mentioned that strictly followed the manufacturer’s guidelines on use. This article aims to show that according to the scientific findings Osteoinductal® should be designated as an osteoinductive and osteostimulative bone replacement material.

Composition of the oleaginous calcium hydroxide suspension (Osteoinductal®)
The main components of Osteoinductal® are calcium hydroxide and oleum pedum of porcine origin.

Mode of action of the suspension
The preparation is applied intraoperationally as a white cream to freshened, healthy bone surfaces that are not isolated by coagula. Here a depot effect is realized, since the calcium hydroxide can be released only at the phase boundary (oil – tissue fluid) only to the extent to which the oleaginous phase is resorbed by the body. The simple but very important difference between the well-known aqueous calcium hydroxide suspensions and those that are oil-based is that the latter lead to a gradual, long-lasting alkalinization of the milieu, which after hours in contact...
with the bone reaches a pH of 10.5. This change of the milieu leads to a strong stimulation of the metabolism of osteoblasts and fibroblasts and in this way inhibits the metabolism of osteoclasts and bacteria. Aqueous calcium hydroxide suspensions, in contrast, abruptly bring about a pH value of 12-13, thereby killing all bacteria, but also all the somatic cells with which they come in immediate contact.

**Indications for the suspension**

On account of its osteogenetic effect on traumatically and surgically exposed bone surfaces and its antibiotic and inflammation inhibiting effects, Osteoinductal is indicated in the following cases: It has a use after every traumatic or surgical exposure of bones or bone surfaces, particularly however during periodontal curettage or flap operations or augmentations. Use of the suspension is also indicated after implantations and extractions, as well as after dental surgical interventions. According to the manufacturer, damage the size of a cherry caused by a cystectomy can also be treated therapeutically with Osteoinductal®.

**Biochemical Studies**

Biochemical research into clinical questions is very rare in dentistry despite the fact that it delivers extremely significant, quantifiable results. It is conducted with human, live tissue in vivo and under practically physiological conditions. This is how Röcher [33] investigated the influence of oleaginous calcium hydroxide suspensions on bone metabolism at the bone research center of the University of Witten/Herdecke under the direction of the late Professor Peter Bartholmes and with the aid of a 5-year-long series of extensive experiments. With the use of typical bone metabolism markers, the effect of this preparation on bone tissue was studied. In the foreground of this work stood the observation of bone generation, which was quantified via measurement of the collagen neo-synthesis activity. The bone tissue used came from the femoral heads of 31 male and female patients between 13 and 82 years old, with a cluster of 40- to 60-year-olds, explanted in the context of an implantation. In order to study the bone metabolism in conditions as close as possible to in vivo conditions, a tissue culture system was established that allowed the maintenance of human bone material in culture for up to a week and the subjection of that material to biochemical tests. The vitality of the particular bone tissue could be checked in advance with quantitative measurements of oxygen consumption so that invariably healthy tissue could be studied. The established bone tissue culture can, on account of its closeness to natural physiological conditions, be viewed as an in-vivo system.

The bone metabolism markers typical of bone growth are stimulated by the oleaginous calcium hydroxide suspension, whereas bone growth is impaired by proteolytic enzymes (such as collagenase).

Furthermore, it could be demonstrated that calcium hydroxide preparations influence the differentiation of osteoblasts, something that not only points to a stimulation of bone regeneration but that also to the orthotopic osteoinduction of bone tissue.

The influences on the most important bone metabolism markers were summarized in a table (Table 1).

**Animal experiments**

**Tibia experiment with Göttinger miniature pigs**

Merten and Dietz jr. [26] conducted a preliminary test on adult Göttinger miniature pigs. The marrow cavity of the
animal’s tibia was cleared and filled with OSTEINDUCTAL®. A critical amount of diaphysal tibia damage of was caused, rendering it impossible for complete, spontaneous bone regeneration. The complete excavation of the marrow content in order to fill the bones with Osteoinductal® followed. Finally the cortical cap was repositioned and the wound closed. After 4 weeks the animal was killed, and after anchorage of the preparation, undecalcified thin section was created and analyzed. Signs of inflammation, connected with a swelling of the soft parts, did not at any point appear.

Weight was put on the extremity spontaneously and without pain. After application of the preparations, a centripetally oriented regeneration of the bone was detectable early, with the still relatively disorganized newly-emerging delicate bone trabecula filling out the excavated bone marrow cavity. Morphometrically, according to the radiological findings, the osteostimulative effect of the calcium hydroxide suspension led to a reossification distance amounting to 4-5 mm after 4 weeks. In total, the quick bone reparation of the clinically significant tibia damage under influence of the osteostimulative effect of the introduced bone replacement material was surprising (Image 1).

**Bone regeneration after extractions in rats**

On the occasion of an IADR (International Association for Dental Research) in San Diego, USA, ITO et al. [21, 22] (Periodontology Department, Tokyo Dental College) presented a poster on the theme of “The effect of a calcium hydroxide paste on bone formation.” The aim of their study was to study the extent to which Osteoinductal® influences alveolar bone formation after a tooth extraction. For this purpose 21 Wistar rats had their back, bottom, left molar extracted. Subsequently a drill was used to standardize the shape of alveolae, during which the septum was removed. In the test group, the alveolae were then filled with Osteoinductal®. The control group remained unchanged. The animals were killed after 4 or 8 weeks. The region to be investigated was placed in paraffin, sliced into 5µm-thick sections and then stained with hematoxylin and eosin. Then the relative size of the bone dimensions of each animal was measured. In the control group 4 weeks after extraction a groove of sparse newly-formed bone filled with gingival connective tissue was observed. In the test group, to which Osteoinductal® had been applied, considerably more new bone formation was observed. The difference between test group and control group was significant (p < 0.05).

After 8 weeks the difference between test and control group was weak. The authors conclude from the results that the acid metabolism hinders osteoclasts and that osteoblast metabolism was stimulated by Osteoinductal®. The authors come to the final conclusion that Osteoinductal®
probably accelerates the induction of postoperative bone formation.

**Clinical investigations**

On the occasion of the annual meeting of the Neue Arbeitsgruppe Parodontologie, e. V. (NagP e. V.) at the University of Gießen, Germany, Stratul [38] presented a poster with the title “Treatment of vertical bone damage with Alpha-TCP and an oleaginous calcium hydroxide suspension.” In the context of this study, 14 patients (9 men and 5 women; all non-smokers) with deep vertical damage were investigated in respect of the changes in the following parameters before and 6 months after treatment: pocket depth, attachment level and gingival recession. One month before the therapy all patients underwent an initial therapy, in which the plaque index (Silness-Löe) was lowered to < 1. All measurements were carried out at 6 defined locations per tooth with the Hu-Friedy PCP 12 periodontal testing probe. The mean value and standard deviation of all parameters was determined in order subsequently to compare the differences in the parameters at the different points in time by means of a Student’s t-test. To treat the vertical defect an intrasulcular incision was performed and a mucous membrane lobe prepared without relief cut. After removal of the granulation tissue the affected teeth were curetted thoroughly with hand instruments and ultrasound. Subsequently the damage was completely filled with a 1:1 mixture of Biobase® alpha Pore and Osteoinductal. The patients were treated postoperationally for a week with 3 times a day with 500 mg of amoxicyclin as well as twice-daily irrigations with 0.2% chlorhexidin. For two months, at two-weekly intervals, the patients had the developed plaque carefully removed. The healing phase ran consistently without pathological findings and without inflammations. After 6 months X-ray control showed that in all cases there had been conspicuous filling of the damage. The clinical measurements taken after 6 months showed a reduction in pocket depth from 7.93 mm + 1.44 mm to 3.7 mm + 1.69 mm and a change of the clinical attachment level from 8.07 mm + 1.44 mm to 4.21 mm + 1.81 mm. The gingival recessions grew from 0.14 mm + 0.53 mm to 1.29 mm + 1.38 mm. The change in pocket depth and of the clinical attachment level amounted to 3.79 mm (+ 1.89 mm). The results of this case report show that
that a 1:1 mixture of Biobase® Alpha Pore and Osteoinductal® can lead to a clinically and statistically significant decrease in pocket depth and clinical attachment level in the case of vertical bone damage. According to Stratul, the absence of any allergic or inflammatory reactions indicates that the mixture of both materials is well tolerated.