

THE OILY CALCIUM HYDROXYDE SUSPENSIONS IN BONE REGENERATION

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REZUMAT

S-a demonstrat recent că suspensia uleioasă de hidroxid de calciu (Osteoinductal®, Osteoinductal GmbH, München, Germania) ameliorează regenerarea osoasă, atât în defectele închise, cât și în defectele periodontale intraosoase. Se pare că acțiunea sa se bazează pe proprietățile osteoformatoare, bacteriostatice, antiinflamatoare și analgetice, imediate și de durată, la intrarea în contact cu suprafața osoasă expusă. Efectul osteoformator al suspensiei a fost confirmat prin experimente pe culturi de celule, prin studii pe animale, precum și prin studii clinice pe subiecți umani. Studii clinice controlate au demonstrat accelerarea vindecării osoase și reducerea efectelor adverse post-chirurgicale după chistectomii, apicoectomii, extracții de molari impactați, în intervențiile de conservare ale creștelor alveolare, dovedind efecte benefice și în tratamentul alveolitei. Cercetări suplimentare sunt necesare în vederea elucidării mecanismelor de acțiune ale produsului, a stabilirii celei mai bune tehnici chirurgicale și a determinării posibilelor combinații cu diferite materiale pentru grefare.

ABSTRACT

An oily Calcium hydroxide suspension (OCHS – Osteoinductal®, Osteoinductal GmbH, München, Germany) has been lately documented to enhance the bone regeneration both in closed defects, as in intrabony periodontal defects. Its action seems to rely on immediate and longterm osteoformative, bacteriostatic, antiinflammatory and analgetic properties, when coming into contact with exposed bone surface. The osteoformative effect of the OCHS was confirmed in cell culture experiments, in animal studies and in clinical trials on humans. Controlled clinical studies have demonstrated accelerated bone healing and reduced postsurgical side-effects after cystectomies, apicoectomies, removals of impacted third molars, in alveolar ridge preservation procedures and beneficial effects for the treatment of the dry alveolitis. Further basic and clinical research is needed in order to elucidate the ways of action of the product, the most appropriate surgical technique and the effectiveness of its possible combinations with various grafting materials.

INTRODUCTION

Over the last decades, numerous attempts of grafting bone defects have been made, using materials of various origins. Failures such as recurrent dehiscences, infections or low-quality ossifications were often reported in relation with these attempts.¹ As the already known and successfully used biologic bone substitutes still raise problems of compatibility/availability/cost effectiveness, synthetic materials with osteopromotive capacities increasingly become of clinical interest, being therefore more and more investigated and demanded. Recently, a new treatment option has been developed, based on oily Calcium hydroxyde suspension (OCHS), that, in contact with the exposed bone surface has an immediate and long-term osteoformative, bacteriostatic, antiinflammatory, and subsequently analgetic action.

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Basic biochemical quantitative researches on human bone tissue cell cultures have been carried out in order to establish the stimulative action of the OCHSs. The researches, performed by Roecher et al. at the Biochemical Institute of the Witten-Herdecke University¹⁻⁵, were based on the clinical experience with an analogous material used for a long time in endodontics (Gangraena-Merz®). It was shown that a particular oily Calcium hydroxyde formulation (Osteoinductal®, Osteoinductal GmbH, Muenchen, Germany) had an increased influence over 15 basic biochemical parameters involved in the bone regeneration. On human bone cell cultures, Osteoinductal® had a stimulative effect on the metabolism, on the growth and differentiation of the osteoblasts.^{1,2,4}

OILY CALCIUM HYDROXYDE SUSPENSIONS VS. AQUEOUS SOLUTIONS OF CALCIUM HYDROXYDE

The oily formulation, available under the name Osteoinductal® (Osteoinductal GmbH, Muenchen, Germany), contains the following natural components: Calcium hydroxide (CH), liquid and solid

carbohydrate chains, and fatty acids (myristoleic, oleic, palmitoleic, gadoleic, margaric, pentadecanic, myristic, linolenic, stearic, palmitic, arachidic, lauric and linolic) esterified with glycerol. The oily parts consist of a natural product of porcine origin - *oleum pedum*, which was demonstrated to have a low cytotoxic effect on human fibroblasts (Klaiber et al. 1981),⁶ and *vaselinum album*.

CH is a product of lime slaking from quick lime. The slurry of CH takes up carbon dioxide from the air and hardens with the formation of Calcium carbonate and water. CH is not soluble in organic acids, has a marginal solubility in water and an improved solubility in glycerine or syrup. The pH value of the saturated aqueous solution is 12.4 - so strong alkalic - and, as a result of this strong alkalization, it has a bactericidal action.

There is a long history of the use of CH in odontology, starting with the Hermann's formula (1920). The properties of the CH in relation to the hard tissue formation were summarized in a study by Kohal et al. (1996):⁷ several experimental studies demonstrated the antimicrobial and anti-inflammatory effect of CH and the possibility of inducing hard tissue repair; CH can reduce the bone resorption by neutralizing the acidic metabolic products of macrophages and osteoclasts, by reducing the metabolic exchanges of the resorptive cells, by activation of the osteoblastic alkaline phosphatase and by increasing the metabolic activity of the osteoformative cells (Tronstad 1988, 1991; Staehle & Pioch 1989; Sundqvist 1993; Stock & Nehammer 1994);⁸⁻¹² CH is a nonspecific tissue irritant without antigen properties (Stuart et al. 1979);¹³ it has a certain antibacterial effect and can induce hard tissue formation (Cvek et al. 1976; Bystroem et al. 1985);^{14,15} when applied on the amputated dental pulp or into the root canal close to the apex, CH resulted in a destruction of the vital tissue, with formation of a necrotic layer and with subsequent formation of a hard tissue barrier below the exposure site (SCHROEDER 1985; HOLLAND et al. 1977; TEPEL et al. 1994);¹⁶⁻¹⁸ CH has a beneficial effect on the periapical lesions (Dietz 1978; 1980; 1981, 1986; 1985; 1987);¹⁹⁻²³ (Darwisch et al., 1994)²⁴. Dietz's formulation from the early 80's was the first oily CH paste in form of a temporary root canal medication (Gangraena - Merz®). Observations made on the behavior of large apical lesions overfilled with Gangraena-Merz® led to the assumption that an OCHS could promote and enhance bone regeneration.²²⁻²⁵ The beneficial effect of Gangraena-Merz® in endodontic applications could be also noted in relation to large combined (endodontic-periodontal) lesions (Sigusch 2000).²⁶

When aqueous solutions of CH contacts living tissues, the initial high pH value at the interface of the blood-supplied tissue results in proteolysis of all present proteins, bacteria, non-vegetative forms included, for example on an exposed dental pulp. The calcite „m” membrane which is created (Knappwost A, Wuestfeld D, 1979)²⁷ however, prevents caustic burns from having a deep impact, and is rather responsible for the creation of a favourable gradient in living tissue from pH 8.8 to pH 7.3 at a distance of a few millimeters. The pH gradient drives hydroxyl ions into the inflammation environment by diffusion. They tend to neutralize the hydrogen ions that are responsible for pain by direct action on nervous terminations. The result is a significant pain relief and suppression of inflammation.¹

While in case of aqueous solution the deep impact of caustic burns in living tissues is prevented by the hastened and imperfect formation of calcite (CaCO₃) “membranes”, in case of the OCHS this protective function is assumed by the stable oily component, because oil does not combine with water. In the oily suspension, proteolysis is prevented by the oily protective layer in contact with the blood-supplied tissue i.e., the bone. A stable, long lasting pH gradient which does not irritate the tissue is thus created. In aqueous CH solutions, this pH gradient is only created after the caustic phase. This caustic phase happens in seconds and looks rather like a pH shock, a real caustic burn, caused by the rapid increase of the pH from 7.5 to 12-13 in the tissue area where it was applied. The alkalization action of the OCHS, in contrast, is slow and gradual. After three hours, it increases to pH 8-9 and finally reaches a plateau, which is maintained for days, until the suspension is completely resorbed. Thus, at the treated site, a gradient is created, which can range between 7 and 11, varying according to the intensity of contact with the bone tissue and with the presence of various amounts of tissular fluid that contains tissue buffers, which can influence the pH value.¹ The bactericidal and alkalinizant properties of the OCHS have been challenged by STAEHLE & PIOCH (1989), however based only on observations made on an *in vitro* model.¹⁰

OSTEOINDUCTAL® - ITS PROPERTIES AND INDICATIONS IN THE BONE SURGERY

The material has a white creamy appearance, does not harden and does not change consistency. Due to its excellent histo-compatibility and its bio-inert behaviour, it is slowly resorbed without causing side effects, and is an excellent support for the active

substance, the Calcium hydroxyde. Moreover, it is very stable up to high temperatures (188°C) and, subsequently, it can be sterilized very well. In gamma radiations it remains stable up to 37 kGy. It can be stored indefinitely at 5°C. No other organic oil has with comparable stability.¹ Osteoinductal® was developed for application in bone surgery. The material is designed for application on fresh vital bone surfaces, without interposition of a coagulum. At local level, the material develops a deposit action, as only the CH at the interface between the liquid/oily phase is released, while the oily components are slowly resorbed by the macrophages. In contrast to the aqueous CH solution, the oily suspension produces a long-term constant, mild alkalization of the environment, with a slow local gradual increase of the pH up to 9–10.5. This pH plateau seems to stimulate the local metabolism of the osteoblasts and fibroblasts, while the osteoclastic and bacterial activity seem to be inhibited. In contrast, aqueous solutions of CH produce rapid pH increases to 12–13, resulting both in instantaneous bactericidal effect and cellular death at the contact area.¹ According to the producer and based on its osteostimulative effect on surgically exposed bone surfaces, as on its antibacterial and antiinflammatory properties, the OCHS Osteoinductal® is indicated in any situation that requires an enhanced bone healing: for socket preservation, for the treatment and prevention of the dry alveolitis, after apical surgeries, after removal of third molars and cystic formations that result in closed bone defects, in the implant surgery. There are also indications for the periodontal surgery, and a couple of isolated clinical case presentations, displaying a certain amount of radiographic bone fill in deep intrabony defects could be found in the literature.¹ However, the bone regeneration induced by Osteoinductal® in periodontal defects has not been documented in controlled clinical studies until recently.^{28,29}

A technology or a material must fulfill the following criteria, in order to be classified as “regeneration-promoting” in periodontology:³⁰

1. In vitro studies, which confirm the effect mechanism.

2. Controlled histological animal studies, which exhibit demonstrate a new formation of root cementum, periodontal ligament and alveolar bone.

3. Human biopsies, which prove a new formation of root cementum, periodontal ligament and alveolar bone on a plaque- infected root surface.

4. Controlled clinical studies, which prove a gain of clinical attachment and radiological new bone formation.

THE OCHS IN CELL CULTURE EXPERIMENTS

The influence of various OCHSs on the bone metabolism has been studied on living tissues in a row of experiments carried out by Roecher et al. between 1980–1985 at the University Witten-Herdecke^{1–5}, Germany.^{1–5} The studies focused on the impact of the product on the bone metabolic markers. In a first experiment, the bone metabolism was evaluated by assessing the type-I collagen production in an in vitro system specially designed to reproduce as much as possible the original tissular conditions.^{1,2,5} In another cell cultures experiment,^{1,3,4} the bone tissue isolate was obtained from hip joint replacement surgeries.^{1,3,4} Thirty-one healthy patients aged 13–82 underwent hip joint surgery. The bone tissue cultures were tested for vitality by assessing the oxygen consumption, so that only perfectly healthy tissue samples entered the experiment. The samples were kept in vitro for one week. In the test group, it was found that, under the influence of the OCHSs, the typical metabolic markers for the bone reconstruction were stimulated, while the bone resorption was inhibited by inhibition of the proteolytic enzymes like the collagenases. It could be also noted that the OCHSs strongly influence the differentiation of the primary osteoblasts. (Table 1)

Table 1. A summary of the influence of the oily Calcium Hydroxyde suspension Osteoinductal® on the most important bone markers (after Dietz et al., 1998)¹

Parameter	Osteoinductal®	Control
Bacteria	major reduction	no influence
Osteoblastic differentiation	major increase	reduction
Osteoblastic growth	major increase	no influence
Osteoblastic DNA synthesis	reduction	no influence
Osteoblastic collagen synthesis	extreme increase	no influence
Tissular collagen synthesis	extreme increase	no influence
Osteoblastic LDH activity	major increase	no influence
Tissular LDH activity	extreme increase	no influence
Osteoblastic PGE2 activity	increase	increase
Tissular PGE2 synthesis	increase	no influence
Tissular ALP activity	major increase	no influence
Tissular AP activity	no influence	no influence
Tissular osteocalcine synthesis	major increase	no influence
Tissular collagenase activity	reduction	major increase
Respiratory activity	no influence	no influence

ANIMAL EXPERIMENTS

A preliminary study on animal model was conducted by Merten and Dietz jr. (1999).^{31,32} Critical size defects of cca. 4ml (the size of the defect that would not benefit of complete spontaneously healing under normal circumstances) were created in the diaphysis of adult minipigs (an experimental breed created at the University of Goettingen, Germany) by removing first a rectangular cortical plate. In the experiment, the interior of the diaphysis were excavated and replaced/filled with Osteoinductal®. The cortical covers were re--placed and the wound were sutured. The operated limb was immediately functionally loaded under normal conditions. Subjects showed no sign of pain. No inflammation or infection of the soft tissues was present. At three weeks after from the surgery fluorescent markers were injected. Animals were sacrificed at four weeks after the surgery. Diaphysis were isolated and the samples were histologically processed. Non-decalcified sections were obtained. The sections underwent macroscopical, light microscopical, microradiographical and fluorometric analysis. Light microscopy examination of the samples revealed an almost complete reossification of the bone cavity. The new bone reached from the vital margins of the defect concentric towards the center. A layer of active osteoblasts covered the new trabeculae, while in the center of the defect a small unconsumed amount of the OCHS could be observed. Macroscopic and microradiographical analysis showed an early bone regeneration in the excavated diaphysial spaces, that were subtotally filled with new trabeculae relatively poorly organized, but with clear orientation towards the center of the defect (centripetal orientation). Morphometrical analysis of the samples revealed circumferencial bone gains of 4-5 mm at 4 four weeks under the influence of the OCHS Osteoinductal®. The fluorescent microscopy analysis of the samples revealed, according to the authors, exuberant woven bone early formation, starting from the margins of the experimental diaphysial cavity. The bone regeneration observed seemed to reproduce an angiogenetic pattern. However, the so-called polytopic (multilocular) new bone formation, characteristic for osteoinductive materials, could not be observed in this experiment.

Ito, Shibukawa, Kawai, Amano, Aand Yamada (2001) have investigated the effect of the OCHS on the post-extractonal alveolar bone formation.^{33,34} In this study, 21 Wistar rats were randomly distributed in two experimental groups. In both groups, lateral teeth

of the animals were extracted. The control group underwent no further treatment, while in the test group Osteoinductal® was applied in the fresh alveolae after the extraction. Animals were sacrificed at 4 weeks and at 8 weeks, and jaw specimens from the operated sites were removed. After embedding in paraffin, tissues were sliced at 5 ì thickness and stained with HE. In each specimen, the proportions of bone dimensions were calculated using a microscope system (MS-803, Moritex, Tokyo, Japan). At 4 four weeks, in the control group the new bone formation was discrete, and a pit formed by the invasion into the tooth lacunae of the gingival connective tissue was clearly present. In the test group, considerable new bone was formed. The test group had significantly more bone formation at 4 four weeks. However, at eight weeks, the difference between the control and the test group was slightminor. The results of the study suggested that Osteoinductal® may speed up postoperative bone induction.

The effect of the OCHS on the wound healing and osseointegration of dental implants has been also investigated in a pilot study in Beagle dogs (KOHAL Kohal et al., 1997).⁷ In this study, implant osteotomies were performed on both sides of the mandible of 8 eight beagle dogs in which premolars were previously extracted. Osteoinductal® was applied into the osteotomies on one side before placement of the implants, whereas the other side did not received Osteoinductal®. The dogs were sacrificed at 1, 2 and 4 weeks and 3 months after implant placement, and the specimens were evaluated histologically and histomorphometrically. The study indicated that the use of the Calcium Hydroxide suspension Osteoinductal® has a detrimental effect on wound healing and osseointegration of dental implants.

THE OCHS IN HUMAN CLINICAL STUDIES

The results from animal experiments have been further corroborated by the observation from human case reports (Lazzerini, 2001).³⁵ Eight patients, each with one tooth to be extracted for various reasons and to be replaced by dental implants, entered this study. After tooth extraction, a small fragment of the alveolar bone of each patient underwent instant histological examination, so that only patients with alveolar bone evaluated as healthy entered the study. Subsequently, the alveolae were irrigated with saline solution at low temperature and filled with the OCHS. The gingiva at the opening of the alveolae was sutured and control X-rays were performed. Patients were

advised to avoid smoking. Antibiotics were administered in order to standardize the healing conditions. Sutures were removed after seven days, when a clinical evaluation of the healing process was performed. Healing occurred uneventful in all patients. There were no signs of inflammation or pain, and the gingiva seemed to heal optimally. After a time that varied from 4 to 8 weeks, the sites underwent reopening in order to receive dental implants. During the preparation of the site, samples of the bone were harvested and were histologically evaluated to determine the quality of the regeneration. Samples were decalcified in EDTA, included in paraffin and underwent H-E staining. The histological analysis revealed a minimal or absent foreign body reaction, that demonstrated the high tolerability of the OCHS. The degree of maturation of the new bone increased with the time of the harvesting. Although few osteoclasts were observed, it was supposed their presence was due partly because of the natural remodelling processes in the new bone, and partly because of the still existing foci of necrotic bone produced during the drilling for the implantation. Authors concluded that Osteoinductal® seemed to enhance the normal bone regeneration. However, a literally osteoinductive capacity of the product could not be observed within the limits of this study.

Further human studies focused on the antiinflammatory and analgetic properties of the product. In a large clinical study, a population of 320 patients, aged between 9 and 87 years, that underwent either S/RP, apical surgeries, implantations or suffered of dry alveolitis were treated with Osteoinductal® (Merten, Dietz jr., 1999).³⁶ Each patient underwent a two-week observation time, and data were collected at the beginning of the treatment, the next day, at one week and 2 weeks after the treatment. Symptoms like pains, oedema and inflammation were evaluated by the patients on a self-assessment 4-step scale. Although of little statistical value because of the large dispersion of the population and because of the subjective assessment of the intensity, the study demonstrated the efficacy of the product in alleviating symptoms like postoperative pains, oedema and inflammation, and its very good tolerability.

Postoperative events after osteotomic removal of the impacted third molar and treatment with Osteoinductal® were investigated in several controlled clinical studies (Filippi et al., 2000; Mozzatti & Ambrogio 2000, Filippi 2001).³⁷⁻³⁹ Seventy healthy patients (33 in the control group, 37 in the test group), each presenting a mandibular impacted third molar, were included in a controlled clinical study (Filippi et al., 2000).³⁷

Investigated parameters were the postoperative pain (evaluated by patient' self-assessment on a visual analogic scale), the postoperative oedema (evaluated by measuring of the median tragus-mentale-tragus distance), the maximal mouth opening (by measuring of the interincisal distance) and the consumption of the recommended analgetic medication. Evaluation of the parameters was made on a daily basis during one week in the postoperative period. Results after seven days showed more pain-free patients (25) in the test group than in the control one (13), an initially larger oedema in the test group than in the control group but with a steeper decrease after 7 days than in the control group, as well as a steeper decrease in the analgetic consumption in the test group than in the control one. No differences in the maximal mouth opening between the two groups were observed. Similar results were reported by DOBBE in 2001 in a clinical study on the influence of the OCHS on the postextractional healing after the removal of the impacted third molar.⁴⁰ In a study conducted on 10 patients aged 18-50, 12 various alveolae underwent postextractional socket grafting with Osteoinductal® (Volkanovska, Filipovski 2002).⁴¹ In the study, 98% of the cases were reported as pain-free at the end of the first week post-treatment.

Other human studies tried to evaluate the effect of the OCHS in closed bone defects as cysts of endodontic origin. In a controlled clinical study, 34 cysts of endodontic origin (21 maxillary, 13 mandibular) were included (Stratul, Onisei 2001).⁴² Ten cysts were grafted with Osteoinductal®, 8 with the 1:1 combination of OCHS Osteoinductal® + α -TCP (BioBase® α -pore, Biovision GmbH., Ilmenau, Germany), 8 with the 1:1 combination of OCHS + Calcium sulphate (Plaster of Paris, Poneti srl, Bucharest, Romania). The control group included 8 cysts, treated only by curettage and flap closure. Results of grafting were evaluated clinically and radiologically at 3 and 6 months. Clinical evaluation included the evaluation of the following parameters: the postsurgical swelling, the postsurgical pains, the wound dehiscence during first two weeks, the complete closure of the wound at 3 months, the presence/absence of the scar at 6 months, the presence/absence of active sinus tracts. Radiological evaluation included the presence/absence of mineralized structure (trabecular bone) at 6 months, the status of the radioopaque grafting material (presence/absence of graft particles at 6 months). Briefly, the study reported postsurgical swelling in 10% of the cases in the Osteoinductal® group (90% in the control group), marked postsurgical pain in 10% of the Osteoinductal® group (38% in the control group),

no wound dehiscence in the Osteoinductal® group (38% in the control group), complete wound closure of the wound in all cases treated with Osteoinductal® (70% in the control group), presence of the scar at 6 months in 20% of the cases in the Osteoinductal® group (70% in the control group), no sinus tract present in the Osteoinductal® group (25% in the control group). Evaluation of the radiological appearance at 6 months after the surgery revealed trabecular bone images in 90% of the cases treated with Osteoinductal® (compared to 38% in the Osteoinductal® + α -TCP group, 85% in the Osteoinductal® + Calcium sulphate group, and only 25% in the control group). Bone graft particles were present on the radiographs at 6 months in 62% of the cases treated with the combination Osteoinductal® + α -TCP. The results of the radiological evaluation consisted with the results of a controlled clinical study on the effect of the OCHS in the reconstruction of postsurgical defects of the jaw bones, conducted on 69 patients (Arifi et al., 2002).⁴³

A more accurate evaluation of the quality of the radiological defect fill was made in a controlled clinical study of bone densitometry (Stratul et al., 2003).⁴⁴ In the study, 16 periapical lesions with diameters varying between 7-20 mm were randomly treated either with apicoectomy, curettage and Osteoinductal® (treatment group), or with apicoectomy and curettage alone (control group). Standardized radiographs were taken before and two months after the surgery. The Osteoinductal® group displayed an excellent clinical healing. Evaluation was made on the radiographs at two months by using EvalDens - a computer-assisted densitometric method, based on the comparison of the gray-scale shades of standard areas of sound bone with periapical areas of regenerated bone, on each same radiograph. Relative density between individual standard areas of sound bone and the periapical areas of surgically treated teeth varied between 50% and 108% within the Osteoinductal® group, with a mean value of $79 \pm 19.7\%$, and between 64% and 115% with a mean value of $89 \pm 18.7\%$ within the control group. The control group displayed higher relative densities than the group treated with Osteoinductal®. Examination of the X-rays reveals a visible defect fill in all treated cases at two months. The results of the study showed that the OCHS did not improve the relative bone density of surgically treated periapical lesions at two months, but the difference between the groups was not statistically significant. Results also indicate the limits of the densitometric analysis in groups with large dispersion of the values, and, subsequently, a strong dependence of the analysis on the initial size of the defect and on the number of cases.

A few isolated case reports about the utilisation of the OCHS in implantology have been also published (Dietz 1998, Apollaro & Toic 2000).^{1,28} There are no extensive clinical studies to evaluate the effect of the OCHS on the osseointegration of dental implants.

So far, there are few clinical studies in the literature to evaluate the effect of the oily Calcium Hydroxide suspension Osteoinductal®, alone or in various combinations with other regenerative materials, in the treatment of periodontal intrabony defects (Stratul 2004, Stratul & Sculean 2003, Dietz et al. 2003).^{28,29,46}

CONCLUSIONS

The existing evidence confirm that the oily Calcium Hydroxide suspension Osteoinductal® seems to enhance the bone healing in closed defects such as periapical lesions, cystic defects and defects post-removal of impacted molars. Its utilization significantly reduces the symptoms associated with the bone surgery. Further controlled clinical studies are needed in order to elucidate the potential of OCHS in periodontal regeneration, and to evaluate its combination with other regenerative materials in the treatment of periodontal intrabony defects.

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