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Denaturation of demineralized bone matrix significantly reduces bone formation by guided tissue regeneration

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Key words: bone formation, demineralized bone matrix, guided tissue regeneration, osteoinduction, rats

Abstract: Aim: To examine in a discriminating capsule model whether denaturation of demineralized bone matrix (DBM) by heating may influence bone formation.
Materials and methods: DBM was produced from the long bones of rats. Half the portion of DBM was denatured by heating in distilled water for 20 min at temperatures between 70°C and 90°C. Prior to the study, the destruction of the osteoinductive properties of the DBM was confirmed in three rats following intramuscular implantation. Thirty, 4-month-old, male albino rats of the Wistar strain were used in the study. Following surgical exposure of the mandibular ramus, a hemispherical Teflon capsule (internal diameter = 5.0 mm) was placed, with its open part facing the lateral aspect of the ramus. On one side (test side), the capsule was loosely packed with denatured DBM, while on the contralateral side, serving as control, the capsule was loosely packed with the same amount of non-denatured DBM. After healing periods of 30, 60, and 120 days, groups of 10 animals were killed and 40–70 μm thick undecalcified sections of the capsules were produced. Three sections from each specimen, representing the mid-portion of the capsule, were subjected to histological analysis and computer-assisted planimetric measurements.
Results: Increasing amounts of newly formed bone were observed in both test and control capsules during the experimental period. At 4 months, the new bone formed in the control capsules occupied 46.7% of the cross-sectional area of the capsules, while it was only 19.1% in the test capsules ($P < 0.05$).
Conclusion: Denaturation of DBM by heating significantly reduces bone formation by guided tissue regeneration.

Demineralized bone matrix (DBM) has been used in humans to induce bone formation in periodontal (Bowers et al. 1989a; Bowers et al. 1989b), peri-implant (Simion et al. 1994), craniofacial (Glowacki et al. 1981), and jawbone (Kaban et al. 1982) osseous defects, and in extraction sockets (Brugnami et al. 1996). The osteoinductive properties of DBM (Urist et al. 1967) are attributed to low molecular weight proteins that are contained in small quantities in the matrix (Mizutani & Urist 1982; Urist et al. 1982). It is presumed that these proteins, which are primarily bone morphogenetic proteins (BMPs), are capable of inducing the transformation of undifferentiated mesenchymal cells into bone-forming cells (Reddi & Cunningham 1991).

Encouraging data with respect to the bone-inducing property of DBM were mainly provided in experimental studies in rodents (Urist 1965; Urist et al. 1967; Urist & Strates 1970). Subsequently, successful results were reported following the treatment of calvarial (Kleinschmidt et al.

1993; Mardas et al. 2002a) and long-bone defects (Nielsen 1992) with DBM combined with guided tissue regeneration (GTR) in rats and rabbits. The results in higher ranking species such as dogs, monkeys, and humans, on the other hand, are controversial (Bowen et al. 1989; Bowers et al. 1989a; Bowers et al. 1989b; Oreamuno et al. 1990; Becker et al. 1992, 1995b; Meadows et al. 1993; Pinholt et al. 1994; Simion et al. 1994; Hürzeler et al. 1995; Caplanis et al. 1997). As an explanation of these discrepancies, it was suggested that an increased metabolic rate or a higher reactivity to osteoinductive stimuli possibly enhances the osteogenic potential of DBM in rodents as compared with that in higher animals (Sato & Urist 1985; Pinholt et al. 1990). However, a recent study in rats (Mardas et al. 2003), using a capsule model capable of discriminating the osteopromotive effect of grafting materials, also failed to demonstrate increased bone formation with DBM when compared with inherent bone formation in the same animal, despite the fact that the bone-inducing capacity of the material was documented by implantation in heterotopic sites. As another explanation of the varying results following DBM grafting, it has been suggested that commercially available DBM allografts may have limited or no osteoinductive capacity because of the denaturation of the BMPs during the processing of the material (Becker et al. 1995a; Schwartz et al. 1996). Therefore, the aim of the present study was to examine, in a discriminating capsule model, whether denaturation of DBM by heating may influence bone formation.

Materials and methods

Production of Teflon capsules

Standardized, rigid, hemispherical Teflon capsules with an internal diameter of 5.0 mm (height 2.5 mm) were produced (W.L. Gore & Associates, Flagstaff, AZ, USA). The capsules had a 1.0 mm wide collar at their periphery for stabilization of the capsule on the bone surface.

Preparation of DBM

DBM was produced according to the method described by Glowacki & Mulliken (1985) as follows. Long bones were harvested from 3-month-old, male albino rats of the Wistar strain. The bones were cleaned for adherent soft tissues and washed in cold deionized water. Extraction of the cleaned bone tissue was carried out with frequent changes of absolute ethanol for at least 1 h. The bone was dehydrated by anhydrous ethyl ether in a fume hood for 1 h and stored at room temperature. The dehydrated material was frozen and pulverized in a liquid hydrogen impacting mill. The pulverized bone particles were demineralized with 0.5 M HCl for 3 h at room temperature. The acid and the free minerals were washed away with deionized water, and centrifugation was used to remove the residual water from the particles. The demineralized bone particles were extracted with changes in absolute ethanol for 1 h, and subsequently extracted in a fume hood with changes of anhydrous ethyl ether for 1 h. The material was left in the hood overnight in order to let the rest of the ethyl ether evaporate. Half of the portion of the produced DBM was denatured by heating in distilled water for 20 min at temperatures between 70°C and 90°C.

Testing of the bone-inductive properties of DBM

The bone-inductive properties of both normal and denatured DBM were tested by implantation of 0.025 g of each type of DBM particles into pockets produced in the thoracic muscles of three, 3-month-old albino rats of the Wistar strain. Histological sections produced 3 weeks following implantation were used to examine whether bone had formed on the DBM particles.

Surgical procedures

Thirty, 4-month-old, male albino rats of the Wistar strain were used in this part of the study. The animals were anesthetized with a subcutaneous injection of Immobilon™ (Pherrovet, Malmö, Sweden). A skin incision was performed at the inferior border of the mandible. The underlying tissues were prepared in layers and the masseter muscle was exposed. A deep incision was then made through the muscle down to the underlying inferior border of the mandible, and a full-thickness muscle periosteal flap was elevated. Following complete exposure of the lateral and medial surface of the mandibular ramus, a capsule loosely packed with a standardized preweighed amount (0.025 g) of denatured DBM particles was placed with its open part facing the lateral aspect of the ramus on one side (test side) of the jaw, chosen at random. At the contralateral side of the jaw, serving as control, a capsule loosely packed with the same amount (0.025 g) of normal DBM particles was placed. The capsules were fixed with 4-0 silk sutures passing through the collar of the capsule and through holes made in the ramus. Care was taken to adapt the capsule to the lateral bone surface of the ramus as closely as possible. The wound was closed by suturing the muscle and subcutaneous tissues with 5-0 resorbable sutures (Resolut™, W.L. Gore & Associates), while the skin incision was closed with 4-0 silk sutures. At the end of the surgical procedure, the anesthesia was terminated with a subcutaneous injection of Revivon™ (Pherrovet).

Histology

After healing periods of 30, 60, and 120 days, groups of 10 animals were killed. The jaws were removed and fixed in 5% neutral buffered formalin. The specimens were dehydrated in alcohol and embedded in Technovit 7200 VLC (Kulzer, Bereich Technic, Wehrheim/Ts, Germany). Undecalcified sections, 40–70 μm thick, were obtained through the capsules perpendicular to the lateral surface of the mandible by means of the Exakt™ (Exakt-Apparatebau, Norderstedt, Germany) cutting–grinding technique. Half the number of sections (every second) were stained with a tetrachrome stain consisting of Sudan black, toluidine blue, basic fuchsin, and light green. The remaining sections were stained with toluidine blue and basic fuchsin. The sections were subjected to histological analysis and planimetric measurements.

Planimetric measurements

The cross-sectional areas of (1) the space created by the capsule, (2) the newly formed bone (i.e. bone trabeculae including marrow spaces), (3) the DBM particles, (4) the loose connective tissue, as well as the height of the capsule and that of the newly formed bone, were measured on three sections of each specimen, 0.5 mm apart and representing the midportion of the capsule. For this purpose, the Image I/Metacolor™ digital image analysis system (Universal Imaging Corporation, West

Chester, PA, USA) was used, which was connected to a transmission light microscope. The mean of the measurements was calculated and expressed as a percentage of the mean cross-sectional area or of the mean central height of the space created by the capsules. The results were subjected to statistical analysis with the Wilcoxon test for paired observations. The probability level of $P<0.05$ was considered as the level of statistical significance.

Reproducibility of the measurements

The above-mentioned parameters were recorded twice on 40 randomly selected control and test sections within a 2-week interval. The differences between the two recordings were tested for normality with the Kolmogorov–Smirnoff goodness-of-fit test, and they were statistically analysed with the Student's *t*-test for paired observations. No statistically significant differences were found between the two recordings of any of the measured parameters ($P>0.05$).

Results

Testing of the bone-inductive properties of DBM

At 3 weeks following implantation of the normal DBM into the thoracic muscles, newly formed immature bone was observed in contact with the DBM particles in all specimens. The induced bone was characterized by thin trabeculae and large marrow spaces filled with cells and vascular elements. At 3 weeks following intramuscular implantation of the denatured DBM particles, no new bone formation was seen in any of the specimens. The denatured DBM particles were encapsulated in connective and muscle tissue, and presented signs of resorption.

Thirty-day specimens

One animal was excluded from the statistical analysis because the capsule was displaced at the test side.

In the control specimens, woven bone had formed in continuity with the original lateral surface of the mandibular ramus and on DBM particles near the surface. Bone formation was also observed on DBM particles placed away from the surface. The remaining capsule area was occupied by DBM particles embedded in loose connective tissue. An acellular space appearing empty was present at the periphery of the dome-shaped tissue in the capsule (Fig. 1).

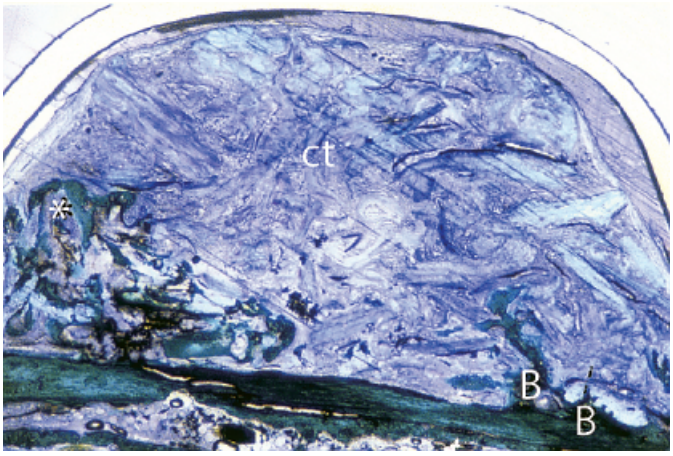

Fig. 1. Photomicrograph of a control specimen at day 30. Newly formed bone (B) is seen adjacent to the original lateral surface of the mandibular ramus as well as on DBM particles (*) away from the surface. Some DBM particles are embedded in loose connective tissue (ct) (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

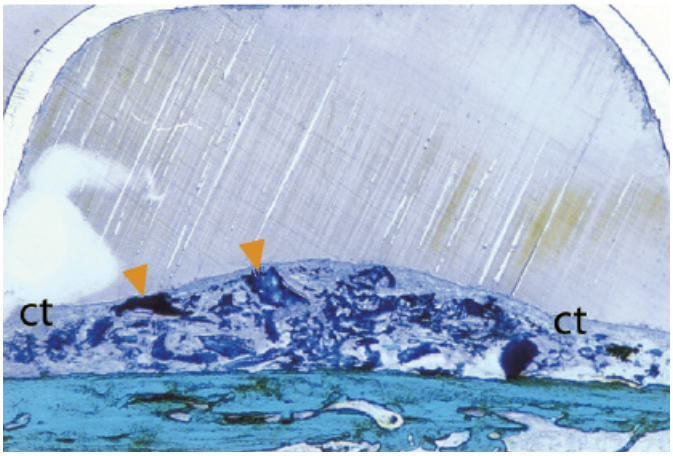

Fig. 2. Photomicrograph of a test specimen at day 30. No new bone formation is observed. A small number of denaturated DBM particles (►), embedded in loose connective (ct), are present adjacent to the surface of the mandibular ramus. The remaining space underneath the capsule is acellular and appears empty (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

Limited amounts of newly formed bone were found in the test capsules. Bone formation had occurred in continuity with the surface of the mandibular ramus, but never in contact with the denatured DBM particles away from the surface. The surface of the particles appeared rough due to the presence of resorption lacunae with multinucleated cells. The remaining space of the test capsules was acellular and appeared empty (Fig. 2).

The cross-sectional area of the denatured DBM particles (17.9%) in the test capsules was significantly smaller ($P<0.05$) than that of the normal DBM particles (62.9%) in the control capsules (Table 1). The newly formed bone amounted to 6.9% (range 2.6–12.2%) of the cross-sectional area of the capsules in the control sides and 4.1% (range 1.2–7.8%) in the test sides. This difference between control and test specimens was statistically significant ($P<0.05$) (Table 1). The height of the newly formed bone was 0.1 mm in both the test and control sides (Table 2).

Sixty-day specimens

In the control capsules, immature trabecular bone had formed on the surface of the mandibular ramus and directly on the DBM particles near the surface. Newly formed

Table 1. **Newly formed bone (BONE), DBM particles (DBM), and connective tissue (CT) expressed as a percentage (mean ± SD) of the cross-sectional area (mm^2) of the capsules (CAP) in test (t) and control (c) specimens at various observation times**

Time (*N*)	30 days (9)	60 days (10)	120 days (10)
BONE c	6.9 ± 2.7*	24.8 ± 8.1**	46.7 ± 11.0**
BONE t	4.1 ± 2.1*	5.9 ± 4.5**	19.1 ± 14.3**
CT c	20.6 ± 4.2*	19.8 ± 9.3	11.2 ± 5.0
CT t	14.5 ± 7.3*	11.5 ± 8.7	20.8 ± 11.5
DBM c	62.9 ± 3.9**	44.7 ± 7.2**	35.0 ± 8.8**
DBM t	17.9 ± 4.6**	6.3 ± 2.6**	2.7 ± 3.0**
CAP c	10.3 ± 1.5	10.2 ± 0.9	10.4 ± 0.8
CAP t	9.9 ± 1.5	10.2 ± 1.0	9.9 ± 1.3

*Statistically significant difference ($P<0.05$).
**Statistically significant difference ($P<0.01$).

Table 2. **Height (mm) of the cross-sectional area (mean ± SD) of the capsules (CH) and of the newly formed bone (BH) in test (t) and control (c) specimens at various observation times**

Time (*N*)	30 days (9)	60 days (10)	120 days (10)
CH c	2.6 ± 0.3	2.7 ± 0.2	2.7 ± 0.2
CH t	2.5 ± 0.3	2.7 ± 0.3	2.6 ± 0.4
BH c	0.1 ± 0.01	0.8 ± 0.4*	1.6 ± 0.5*
BH t	0.1 ± 0.01	0.2 ± 0.1*	0.7 ± 0.5*

*Statistically significant difference ($P<0.01$).

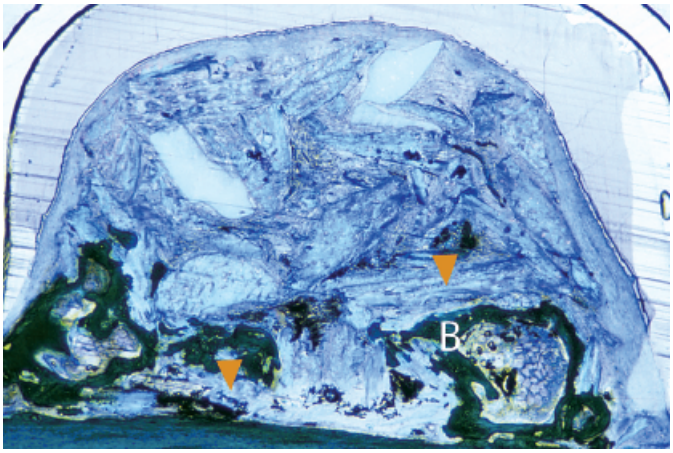

Fig. 3. Photomicrograph of a control specimen at day 60. New immature trabecular bone (B) is present on the surface of the mandibular ramus and on the DBM particles (▶) near the surface (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

bone was also observed on DBM particles away from the surface. The rest of the DBM particles were embedded in loose connective tissue (Fig. 3).

In the test capsules, limited amounts of new bone were observed on the surface of the mandibular ramus (Fig. 4). A small number of denatured DBM particles were present in the capsules. Their outline was rough due to the presence of resorption lacunae with multinucleated cells. The denatured DBM particles were embedded in loose connective tissue containing inflammatory cells. The remaining space under both test and control capsules was acellular and appeared empty.

The denatured DBM particles amounted to 6.3% of the cross-sectional area of the test capsules, while the normal DBM particles amounted to 44.7% of the cross-sectional area of the control capsules (Table 1). This difference was statistically significant ($P<0.01$). The newly formed bone occupied 24.8% (range 10.0–39.3%) of the cross-sectional area of the capsules in the control group, while it comprised only 5.9% (range 0.0–15.3%) in the test capsules (Table 1).

The mean height of the newly formed bone was 0.8 mm (range 0.3–1.6 mm) in the control specimens vs. 0.2 mm (range 0.0–0.4 mm) in the test specimens (Table 2). This difference between test and control capsules was statistically significant ($P<0.01$).

One hundred and twenty-day specimens

Significant amounts of newly formed bone were observed in the control capsules. This newly formed bone appeared dense and consisted of thick trabeculae and small marrow spaces. Only a few DBM particles could be distinguished inside the new bone. Some particles embedded in a layer of loose connective tissue were observed on top of the newly formed bone. A narrow acellular space was observed at the periphery of the dome-shaped tissue in the capsules (Fig. 5).

In the test capsules, immature trabecular bone was found in continuity with the lateral surface of the mandibular ramus. The new bone consisted of thin trabeculae and large marrow spaces with fat cells. The newly formed bone was covered with a layer of loose connective tissue, while the remaining space underneath the capsule was acellular and appeared empty. denatured DBM particles were observed only occasionally (Fig. 6).

In the control specimens, the newly formed bone averaged 46.7% (range 27.1–65.3%) of the cross-sectional area of the capsule space, while in the test specimens, it was 19.1% (range 5.3–39.3%) (Table 1).

The height of the newly formed bone averaged 1.6 mm (range 0.8–2.2 mm) in the control capsules and 0.7 mm (range 0.1–1.5 mm) in the test capsules (Table 2). These differences between the control and test specimens were statistically significant ($P<0.01$).

Discussion

The present study demonstrated that denaturation of the DBM particles by heating significantly reduced bone formation. This finding corroborates the results of a recent study by Wang & Glimcher (1999), who found that denatured DBM failed to induce bone or cartilage in calvarial defects in rats.

The reason for the reduced amounts of bone produced in the capsules with dena-

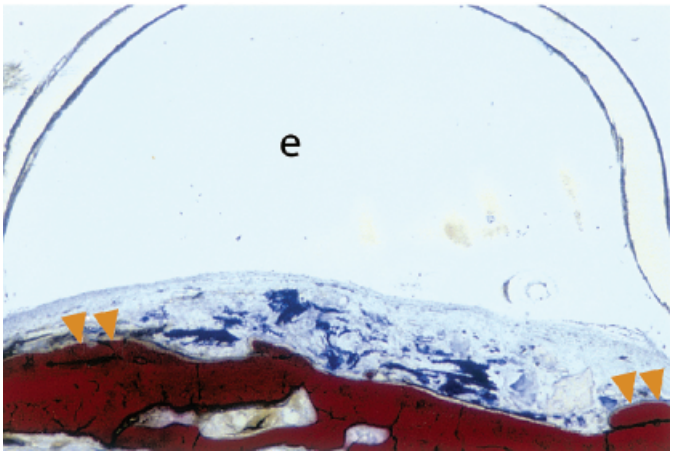

Fig. 4. Photomicrograph of a test specimen at day 60. Limited amounts of newly formed bone are seen on the surface of the mandibular ramus. A small number of denaturated DBM particles in loose connective tissue can be identified. The remaining space under the capsule is acellular (e) and appears empty (toluidine blue, basic fuchsin; magnification: × 10).

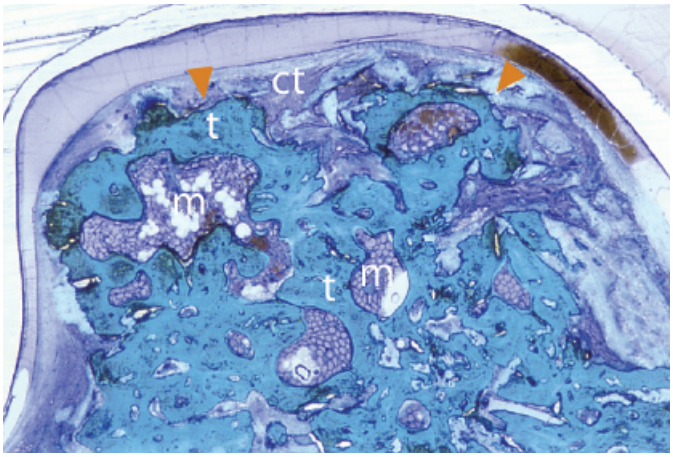

Fig. 5. Photomicrograph of a control specimen at day 120. The capsule is almost filled with new dense bone with thick trabeculae (t) and small marrow spaces (m) with fat cells. DBM particles (►) are seen on top of the new bone embedded in loose connective tissue (ct) (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

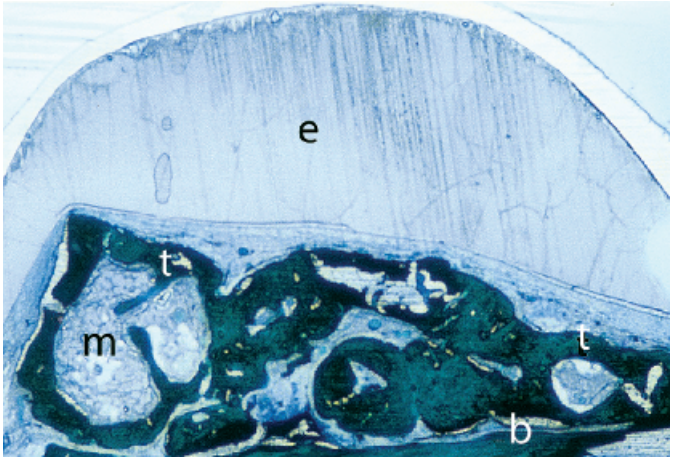

Fig. 6. Photomicrograph of a test specimen at day 120. Immature trabecular bone is seen on the surface of the mandibular ramus (b). The new bone consists of thin trabeculae (t) and large marrow spaces (m) with fat cells. The remaining space under the capsule is acellular (e) and appears empty. No denaturated DBM particles can be observed in the capsule (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

tured DBM as compared with those with non-denatured DBM may be the loss of osteoinductive properties. The fact that the denatured DBM, in contrast to the normal DBM, failed to induce new bone formation after intramuscular implantation supports this view. This is also in agreement with the findings of Urist et al. (1967), who reported that temperatures approaching 100°C completely destroy the bone induction properties of DBM, and with the observation of Urist & Iwata (1973) that wet rat bone exposed to air lost its BMP activity after heating at temperatures between 70°C and 100°C for 1 h. On the other hand, it is also likely that the denaturation by heating may have induced structural changes of DBM, which may have reduced the osteoconductive capacity of the material. In fact, it was reported by Urist et al. (1967) that heating at temperatures above 60°C results in shrinkage of the matrix collagen. This view is supported by the fact that bone formation never occurred directly on the denatured DBM particles. In addition, the number of denatured DBM particles was reduced considerably after 30 days and had almost disappeared after 120 days following their implantation (Table 1). The DBM particles may have been resorbed solely by multinucleated cells, but it is also possible that they became dissolved due to increased solubility of the collagen matrix caused by the heating.

Controlled clinical trials have provided convincing evidence, both clinically and histologically, that periodontal defect sites treated with freeze-dried demineralized bone matrix (DFDBA) present significantly more bone fill and attachment gain than non-grafted sites (Pearson et al. 1981; Mellonig 1984; Bowers et al. 1989a; Bowers et al. 1989b; Meadows et al. 1993). Other studies, comparing the effect of grafting of DFDBA in intraosseous periodontal defects with that of grafting of non-demineralized bone matrix (FDBA) or porous hydroxyapatite (PHA), which do not possess any osteoinductive properties (Urist & Strates 1970), showed similar clinical improvements with the various materials (Bowen et al. 1989; Rummelhart et al. 1989). In fact, in a similar clinical trial, Oreamuno et al. (1990) found that PHA produced even more gain in the clinical attachment level and defect fill than grafting with DFDBA. Similarly, the grafting of

DBM did not present any effect on bone formation when used for alveolar ridge augmentation in dogs and goats (Pinholt et al. 1992, 1994) or when placed in extraction sockets in humans (Becker et al. 1994).

DBM was used in combination with GTR in the treatment of peri-implant bone defects to support a membrane so that it does not collapse into the defect. At the same time, the graft was supposed to promote bone formation by osteoinduction (Mellonig & Nevins 1995). Successful results were reported following this combined treatment of bone- or peri-implant defects in dogs (Stentz et al. 1997) and humans (Nevins & Mellonig 1992; Simion et al. 1994, 1996), but other investigators failed to demonstrate any added effect of DBM grafting on bone formation in peri-implant defects in dogs (Becker et al. 1992, 1995b; Hürzeler et al. 1995; Caplanis et al. 1997) and in extraction sites in humans (Dies et al. 1996).

Based on the findings of the present study and those in the study by Wang & Glimcher (1999), it is reasonable to presume that alterations of the osteoconductive or osteogenic potential of DBM during the manufacturing process, e.g. sterilization, may explain the inconsistent results obtained in the studies mentioned above (Becker et al. 1992, 1995b; Hürzeler et al. 1995; Dies et al. 1996; Caplanis et al. 1997). This suggestion is also supported by the studies of Becker et al. (1995a) and Schwartz et al. (1996), who reported that commercially available DBM allografts may have limited or no osteoinductive capacity because of the denaturation of the BMPs during the normal processing of the material. Heating (Urist et al. 1967; Urist & Iwata 1973), irradiation for sterilization (Urist et al. 1967; Munting et al. 1988), pulverization (Syftestad & Urist 1979), sonification (Bang & Johannessen 1972), and chemical agents for sterilization (Urist et al. 1967; Munting et al. 1988) are some factors involved in DBM processing that are capable of causing denaturation of the matrix BMPs, resulting in the reduction or loss of bone-inductive properties.

The finding in the control capsules that approximately 50–60% of the capsule volume became filled with bone after 4 months is in agreement with the results of a previous study using the same capsule model (Mardas et al. 2003). In this study, non-denatured DBM failed to enhance bone formation as compared with that observed in empty control capsules, despite the fact that the osteoinductive properties of the material were documented by implantation at heterotopic sites. It is unlikely that the lack of effect of DBM in this study can be due to a poor potential for bone regeneration since rodents are considered to possess a higher capacity for regeneration than higher ranking animals (Sato & Urist 1985; Pinholt et al. 1990). In fact, it has been demonstrated in several studies (Kostopoulos et al. 1994, 2001; Kostopoulos & Karring 1995; Lioubavina et al. 1999; Stavropoulos et al. 2001) that empty capsules as the ones used in the present study consistently become filled with bone within 4–6 months after placement on the mandibular ramus of rats. This capsule model, showing continuous bone formation within a defined space during this time period, possesses superior discrimination to appraise the bone-promoting potentials of various bone grafts or bone graft substitutes when a capsule with the test material is placed on one side and an empty capsule on the other side of the same animal. It should be noticed in this context that except for DBM (Mardas et al. 2003), testing of bone graft substitutes in this capsule model has consistently resulted in less bone formation in grafted capsules as compared with empty capsules (Kostopoulos et al. 2001; Stavropoulos et al. 2001, 2003), indicating that the mere filling of a secluded space adjacent to bone with a biomaterial does not necessarily enhance, but may rather inhibit, bone formation (Kostopoulos et al. 2001). The results of the present study show that denaturation of DBM by heating obstructs bone formation by GTR.

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Résumé

Le but de cette étude a été d'analyser dans un modèle de capsule discriminatoire une éventuelle influence de la dénaturation par le réchauffement de la matrice osseuse déminéralisée (DBM) sur la formation osseuse. DBM a été produite à partir d'os longs de rats. La moitié de la production de DBM a été dénaturée par la chaleur dans de l'eau distillée pendant 20 min à des températures entre 70 et 90°C. Avant l'étude, la destruction des propriétés ostéoconductives de DBM avait été confirmée chez trois rats suite à des implantations intramusculaires. Trente rats albinos mâles de quatre mois de la souche Wistar ont été utilisés pour cette étude. A la suite de l'exposition chirurgicale de la branche montante, une capsule en téflon hémisphérique (diamètre interne de 5.0 mm) a été placée avec sa partie ouverte contre la partie latérale de la branche montante. D'un côté (côté test) la capsule a été remplie sans tassement de DBM dénaturée tandis que dans le côté contralatéral qui servait de contrôle la capsule a été remplie sans tassement avec la même quantité de DMB non-dénaturée. Après des périodes de guérison de 30, 60 et 120 jours des groupes de dix animaux ont été tués et des coupes non-décalcifiées de 40 à 70 µm d'épaisseur ont été effectuées. Trois sections de chaque spécimen représentant la portion moyenne de la capsule ont été soumises à l'analyse histologique et aux mesures planimétriques par ordinateur. Des quantités croissantes de nouvel os ont été observées tant dans les capsules tests que contrôles. Après quatre mois, l'os néoformé dans les capsules contrôles occupaient 47% des zones examinées seulement 19% dans les capsules tests ($P<0.05$). La dénaturation de DBM par la chaleur réduit de manière significative la formation osseuse par régénération tissulaire guidée.

Zusammenfassung

Eine Denaturierung der demineralisierten Knochenmatrix (DBM) verringert bei der GTR die Knochenneubildung signifikant

Ziel: Die Studie hatte zum Ziel, im Tiermodell zu untersuchen, ob die Hitzedenaturierung von DBM die Knochenneubildung beeinflussen kann.

Material und Methode: Die DBM stammte von den langen Röhrenknochen von Ratten. Die Hälfte des DBM hatte man durch Erhitzung in destilliertem Wasser während 20 Minuten bei Temperaturen von 70–90°C denaturiert. Vorgängig zu dieser Studie prüfte man, ob die osteokonduktiven Eigenschaften des DBM auch wirklich verloren gehen, indem man das Produkt bei 3 Ratten intramuskulär implantierte. Für die Studie verwendete man 30 vier Monate alte männliche Albinoratten (aus dem Wistarstamm). Nach der chirurgischen Freilegung des Unterkieferastes fixierte man eine halbkugelige Teflonkapsel (innerer Durchmesser = 5.0 mm) mit der offenen Seite gegen die Aussenseite des Unterkiefers. Auf der einen Seite (Testseite), wurde die Kapsel locker mit denaturierter DBM aufgefüllt, währenddem die gegenüberliegende Seite (Kontrollseite) mit derselben Menge locker eingebrachtem, aber nicht denaturierter DBM aufgefüllt wurde. Nach einer Heilphase von 30, 60 und 120 Tagen opferte man jeweils eine Gruppe von 10 Tieren und stellte 40–70 µm dicke nichtentkalkte Grundschnitte der Kapsel her. Histologisch und mit komputerunterstützten planimetrischen Messungen untersuchte man drei Schnittpräparate aus dem Mittelbereich jeder Biopsie.

Resultate: Während der Testphase nahm sowohl in der Testgruppe, wie auch in der Kontrollgruppe die Menge des neu gebildeten Knochens zu. Nach vier Monaten fand man in der Teflonkugel der Kontrollseite 46.7% neu gebildeten Knochen, währenddem die Kapsel der gegenüberliegenden Seite zu 19.1% aufgefüllt war ($P<0.05$).
Zusammenfassung: Die Hitzedenaturierung der DBM verringert die Knochenneubildung bei der GTR signifikant.

Resumen

Intención: Examinar en un modelo de cápsula discriminatoria si la desnaturalización de DBM por calor influiría la formación de hueso
Material y métodos: Se produjo DBM a partir de huesos largos de ratas. La mitad de la porción de DBM se desnaturalizó por calor en agua destilada durante 20 min. A temperaturas de 70 a 90°C. Antes del estudio se confirmó la destrucción de las propiedades osteoconductivas del DBM en 3 ratas tras la implantación intramuscular. Se utilizaron en este estudio 30 ratas albinas de 4 meses de edad, machos, de la cepa Wistar. Tras la exposición quirúrgica de la rama mandibular, se colocó una cápsula hemisférica de teflón (diámetro interno = 5.0 mm) con su apertura frente al aspecto lateral de la rama. En un lado (lado de prueba), la cápsula se rellenó sin forzar con DBM desnaturalizado, mientras que en lado contralateral, la cápsula se rellenó sin forzar con la misma cantidad de DBM no desnaturalizado. Tras un periodo de cicatrización de 30, 60 y 120 días, se sacrificaron grupos de 10 animales y se realizaron secciones descalcificadas de 40–70 µm de grosor de las cápsulas. Tres secciones de cada espécimen, representando la sección media de la cápsula, fueron sometidas a análisis histológicos y a mediciones planimétricas asistidas por ordenador.
Resultados: Se observaron cantidades crecientes de hueso neoformado tanto en las cápsulas de prueba como de control durante el periodo experimental. A los 4 meses, el hueso neoformado en las cápsulas de control ocupó un 46.7% del área le la sección de las cápsulas mientras que en las cápsulas de prueba fue de solo el 19.1% ($P<0.05$)
Conclusión: La desnaturalización del DBM por calor reduce significativamente la formación de hueso por GTR.

要旨
目的：脱灰骨マトリクス（ＤＢＭ）の熱変性が骨形成に影響を及ぼすかどうかを識別カプセル・モデルにおいて検討すること
材料と方法：ＤＢＭをラットの長骨から作成した。ＤＢＭの半量を温度７０－９０℃の蒸留水中で２０分間熱して変性させた。研究前に、ＤＢＭの骨誘導性特性が破壊されたことを、３匹のラットに筋内注入して確認した。３０匹の４ヶ月令、雄アルビノ、Wisterラットを同研究に用いた。下顎枝を外科的に露出させ、半球形のテフロン・カプセル（内径＝５．０ｍｍ）を、その開いた部分が下顎枝の外側面を向くように埋入した。片側で（試験部位）、カプセルには変性したDBMをゆるく充填し、反対側では変性していないDBMをゆるく充填した。３０、６０、１２０日の治癒期間後、各々１０匹ずつの動物を屠殺して、４０－７０µm厚のカプセルの非脱灰切片を作成した。各標本からカプセルの中央に相当する３つの切片を得て、組織学的分析とコンピュータによる面積測定を行った。
結果：試験カプセルでも対照カプセルでも、新生骨の量の増加が実験期間中に観察された。４ヶ月後に対照カプセル中で形成された新生骨は、カプセル断面積の４６．７％を占めたが、試験カプセルではわずか１９．１％であった（ｐ＜０．０５）。
結論：DBMの熱変性は、GTRによる骨形成を有意に減少させる。

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