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Evaluation of a cell-permeable barrier for guided tissue regeneration combined with demineralized bone matrix

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Abstract: Aim: To evaluate whether bone formation by guided tissue regeneration (GTR) and demineralized bone matrix (DBM) can be enhanced by the use of a cell-permeable Teflon barrier allowing the penetration of undifferentiated mesenchymal cells from the surrounding soft tissues.
Material and methods: DBM was produced from the long bones of rats, and its bone-inductive properties were tested in three rats prior to the study by intramuscular implantation. Thirty, 4-month-old, male albino rats of the Wistar strain were used. Following surgical exposure of the mandibular ramus, a cell-permeable Teflon capsule, loosely packed with DBM, was placed with its opening facing the lateral surface of the ramus (test side). At the contralateral side, serving as control, a non-perforated (cell-occlusive) Teflon capsule, loosely packed with the same amount of DBM, was placed. 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.
Results: Computer-assisted planimetric measurements on the histological sections disclosed similar amounts of newly formed bone in both test and control capsules. After 4 months, the new bone in the control capsules occupied 45.0% of the cross-sectional area of the capsule, while it was 50.5% in the test capsules. This difference was not statistically significant ($P < 0.05$).
Conclusion: Similar amounts of bone formed in cell-permeable and cell-occlusive capsules grafted with DBM, suggesting that invasion of undifferentiated mesenchymal cells from the surrounding soft tissues into the barrier-protected area is unnecessary for bone formation with GTR.

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The biological concept of guided tissue regeneration (GTR) is based on the observation that a desired wound healing result can be achieved by creating a secluded space that will be populated by the type of cell with the capacity to regenerate the particular type of tissue that has become lost (Karring et al. 1993). With respect to the regeneration of osseous defects by GTR, the treatment involves the placement of a cell-occlusive barrier over a bone defect in such a way that the proliferation/collapse of the surrounding soft tissues into the barrier-protected area is prevented, thereby allowing bone-forming cells from the existent bone edges to invade the space and produce bone (Lang et al. 1997). GTR has been used successfully in humans for the treatment of periodontal defects (Karring et al. 1997; Karring & Cortellini 1999) and alveolar

bone defects, both with and without dental implants (Lang et al. 1997; Hämmerle & Karring 1998).

GTR combined with the implantation of demineralized bone matrix (DBM) was used successfully for the regeneration of bone or peri-implant defects in humans (Nevins & Mellonig 1992; Simion et al. 1994, 1996; Brugnami et al. 1996), and of calvarial (Kleinschmidt et al. 1993; Mardas et al. 2002), long-bone (Nielsen et al. 1992), and peri-implant bone defects in experimental animals (Stentz et al. 1997). However, other investigators failed to demonstrate any added effect of DBM implantation on the amount of bone generated in peri-implant defects in dogs (Becker et al. 1992, 1995; Hürzeler et al. 1995; Caplanis et al. 1997) and in extraction sites in humans (Dies et al. 1996).

DBM allografts are supposed to enhance bone formation by osteoinduction (Urist et al. 1967). According to this principle, undifferentiated mesenchymal cells, under the stimulation of specific growth-regulatory factors such as bone morphogenetic proteins (BMPs) contained in the DBM, migrate by chemotaxis, increase their number by mitosis, and differentiate, first into chondrocytes producing cartilage and later into osteoblasts producing bone (Reddi et al. 1987).

In a recent study in rats using a capsule model, which is capable of discriminating the efficacy of bone-promoting materials, DBM failed to enhance bone formation as an adjunct to GTR, despite the fact that the bone-inductive capacity of the material was verified by heterotopic implantation (Mardas et al. 2003). Non-permeable capsules filled with DBM were placed on the mandibular ramus on one side of the animals, while empty control capsules were placed on the other side. The amounts of bone in test and control capsules were similar, and it was suggested that the use of an occlusive GTR device might have prevented the migration of inducible undifferentiated mesenchymal cells (Reddi et al. 1987) from the surrounding soft tissues into the barrier-protected area, and thereby reduced a possible bone-inducing effect of the DBM material. Therefore, the aim of the present study was to evaluate whether bone formation by GTR and DBM can be enhanced by the use of a cell-permeable Teflon barrier.

Material and methods

Construction of Teflon capsules

Standardized, rigid, hemispherical Teflon capsules with an internal diameter of 5.0 mm (height 2.5 mm) were produced. The capsules had a 1.0 mm wide collar at their periphery for stabilization of the capsule on the bone surface. In half the number of capsules (test capsules), nine through and through perforations were made by means of a needle with a diameter of 0.3 mm.

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 then dehydrated by anhydrous ethylether 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. Subsequently, the demineralized bone particles were extracted with changes in absolute ethanol for 1 h, and with changes of anhydrous ethylether for another 1 h in a fume hood. The material was left in the hood overnight in order to let the rest of the ethylether evaporate.

Testing of the bone-inductive properties of DBM

The bone-inductive properties of DBM were tested by implantation of 0.025 g of DBM particles into pouches 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 along 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 perforated, cell-permeable capsule (test capsule), loosely packed with a standardized preweighed amount (0.025 g) of DBM particles, was placed with its open part facing the lateral aspect of the mandibular ramus at one side of the jaw, chosen at random. At the contralateral side of the jaw, serving as control, an occlusive capsule loosely packed with the same amount (0.025 g) of 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 as close as possible to the lateral aspect of the ramus. The surgical wound was closed by suturing the muscle and subcutaneous tissues with 5-0 resorbable sutures (Resolut™, W.L. Gore & Associates, Flagstaff, AZ, USA), 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, Malmö, Sweden).

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, and 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 from 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, connected to a transmission light microscope. The mean of the above-mentioned 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 for any of the measured parameters ($P>0.05$).

Results

Testing of the bone-inductive properties of DBM

At 3 weeks following implantation of 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.

Thirty-day specimens

In both the control (Fig. 1) and test (Fig. 2) specimens, new bone had formed on the surface of the mandibular ramus, and on DBM particles near the surface. Newly formed bone was also observed on DBM particles away from the surface, but DBM particles embedded in loose connective tissue occupied the major portion of the capsules. In some test specimens, connective tissue seemed to have invaded the capsule from surrounding tissues through the perforations made in the capsule. This was observed mainly in the upper part of the capsules (Fig. 2). In both control and test capsules, a narrow acellular space appearing empty was located at the periphery of the dome-shaped tissue formed in the capsule.

The newly formed bone amounted to 2.9% (range 0.6–7.5%) of the cross-sectional area of the capsules in the control sides and 2.8% (range 1.2–7.1%) in the test sides (Table 1).

The height of the newly formed bone was 0.1 mm in both test and control capsules (Table 2). No statistically significant differences were observed between test and control capsules ($P>0.05$).

Sixty-day specimens

In the control capsules, immature trabecular bone had formed in continuity with the surface of the mandibular ramus and directly on the DBM particles near the surface (Fig. 3). Newly formed bone was also observed on DBM particles away from the surface. Similarly, in the test (perforated) capsules bone formation had

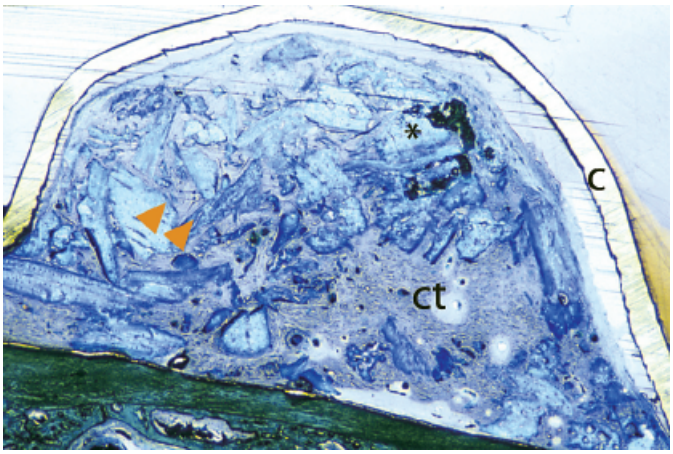

Fig. 1. Photomicrograph of a control specimen at day 30. The major part of the capsule (c) is filled with DBM particles (▶) embedded in loose connective tissue (ct). New bone has formed on DBM particles (*) away from the lateral surface of the mandibular ramus (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

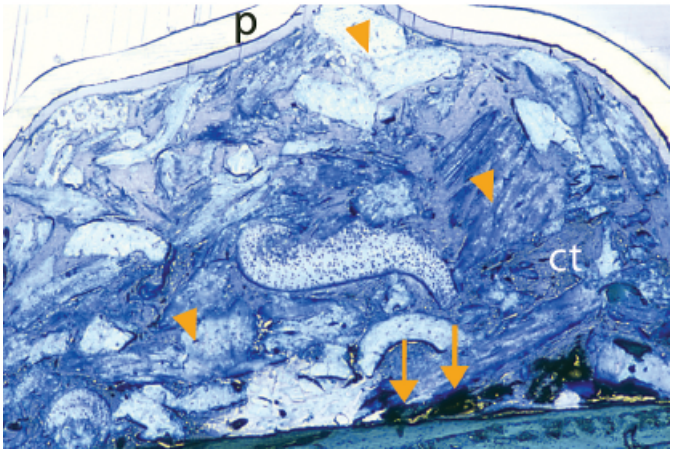

Fig. 2. Photomicrograph of a test specimen at day 30. New bone (→) has formed on the surface of the mandibular ramus. Connective tissue seems to have proliferated through the perforations (p) in the capsule. DBM particles (▶) can be seen embedded in loose connective tissue (ct) (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

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 (10)	60 days (9)	120 days (10)
BONE c	2.9 ± 2.1	17.2 ± 9.6	45.0 ± 9.1
BONE t	2.8 ± 1.7	18.7 ± 6.8	50.5 ± 13.0
CT c	27.9 ± 5.2	26.8 ± 6.0	18.6 ± 3.4
CT t	30.8 ± 6.6	28.8 ± 6.6	17.5 ± 5.4
DBM c	59.4 ± 6.6	50.5 ± 2.9	32.5 ± 10.5
DBM t	58.9 ± 6.3	46.8 ± 4.6	27.0 ± 1.4
CAP c	10.3 ± 0.4	10.0 ± 0.9	10.4 ± 1.1
CAP t	10.2 ± 0.8	10.3 ± 1.4	10.1 ± 1.2

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 (10)	60 days (9)	120 days (10)
CH c	2.6 ± 0.1	2.5 ± 0.2	2.6 ± 0.2
CH t	2.6 ± 0.3	2.6 ± 0.2	2.5 ± 0.3
BH c	0.1 ± 0.1	0.5 ± 0.3	1.6 ± 0.5
BH t	0.1 ± 0.01	0.4 ± 0.4	1.6 ± 0.5

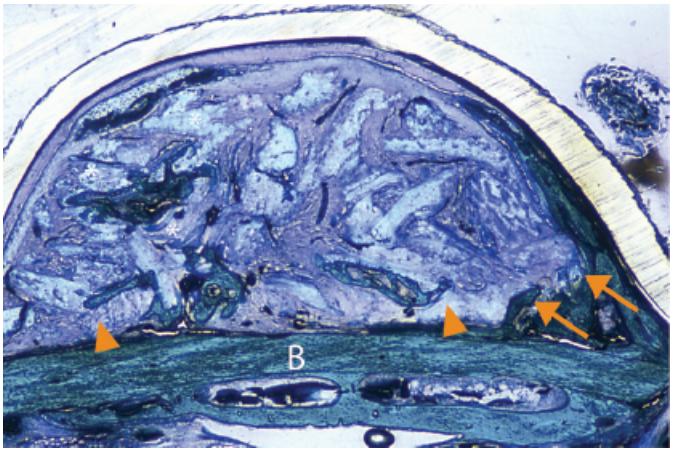

Fig. 3. Photomicrograph of a control specimen at day 60. Newly formed bone (→) is present on the surface of the mandibular ramus adjacent to the capsule wall, and on DBM particles (►) near the surface (B), and away from the surface (*) (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

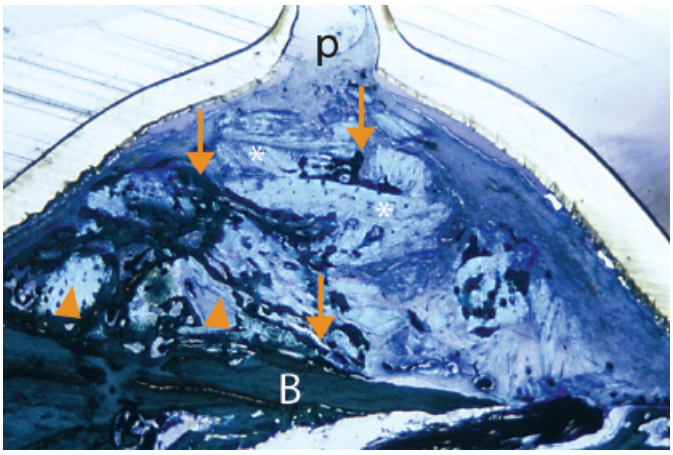

Fig. 4. Photomicrograph of a test specimen at day 60. New bone (→) has formed on the surface of the mandibular ramus (B), and on DBM particles (►) near the surface. New bone (→) is also seen around DBM particles (*) away from the surface. Connective tissue seems to have proliferated through the perforations in the capsule (p) (toluidine blue, basic fuchsin; magnification: × 10).

occurred on the ramus and directly on DBM particles near and away from the surface (Fig. 4). Connective tissue, which appeared to have proliferated through the perforations in the capsules, was limited to the upper part of the capsules. In both the test and control sides, the major portion of the capsule was occupied by DBM particles embedded in loose connective tissue.

The newly formed bone occupied 17.2% (range 4.9–35.3%) of the cross-sectional area of the capsules in the control specimens and 18.7% (range 8.0–28.3%) in the test capsules (Table 1).

The mean height of the newly formed bone was 0.5 mm (range 0.1–1.0 mm) in the control specimens, and 0.4 mm (range 0.1–1.1 mm) in the test specimens (Table 2). These differences between the test and control specimens were not statistically significant ($P > 0.05$).

One hundred and twenty-day specimens

Significant amounts of newly formed bone with similar histologic features were observed in both control and test capsules. This newly formed bone consisted of thick trabeculae and small marrow spaces, and was in continuity with the original surface of the mandibular ramus (Figs 5 and 6). A few DBM particles embedded in newly formed bone could be identified. Some DBM particles were present on top of the newly formed bone, embedded in a layer of loose connective tissue (Figs 5 and 6).

In the control specimens, the newly formed bone occupied 45.0% (range 33.1–67.5%) of the cross-sectional area of the capsules, while it was 50.5% (range 35.2–74.2%) in the cell-permeable test capsules (Table 1).

The height of the newly formed bone was 1.6 mm (range 0.8–2.6 mm) in the control specimens and 1.6 mm (range 0.8–2.2 mm) in the test specimens (Table 2). No statistically significant differences were found between the test and control capsules ($P > 0.05$).

Discussion

The present study demonstrated that similar amounts of bone formed in cell-permeable and cell-occlusive capsules grafted with DBM. The rationale of the present study was that the perforations in the

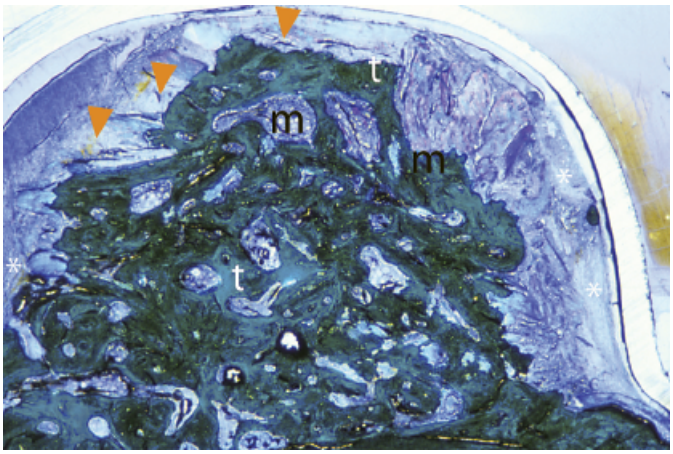

Fig. 5. Photomicrograph of a control specimen at day 120. Considerable amounts of newly formed bone can be observed in the capsule. The new bone consists of thick trabeculae (t) and small marrow spaces (m) with fat cells. DBM particles (▶) embedded in loose connective tissue (*) are present on top of the new bone (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

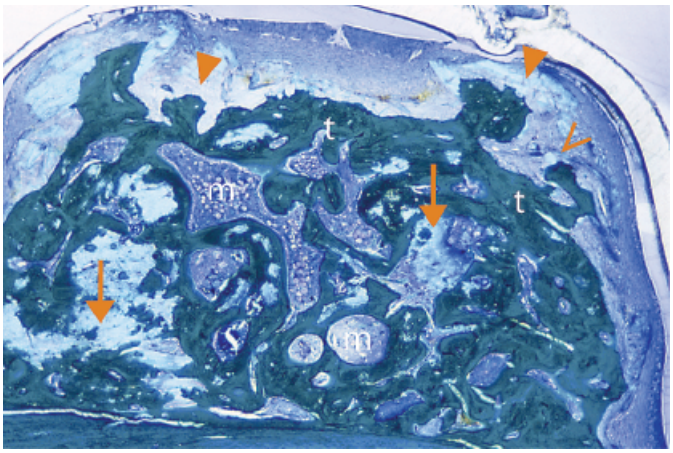

Fig. 6. Photomicrograph of a test specimen at day 120. New dense trabecular bone occupies the major portion of the capsule. The new bone presents thick trabeculae (t) and marrow spaces (m) with fat cells. Some DBM particles (▶) embedded in loose connective tissue or in contact (▶) with the new bone can be seen. DBM particles (→) incorporated in bone can also be seen (Sudan black, toluidine blue, basic fuchsin, and light green; magnification: × 10).

capsule wall would allow undifferentiated mesenchymal cells residing in the surrounding soft tissues to migrate into the space created by the capsule and be induced to differentiate into osteoblasts by DBM, thereby enhancing osteogenesis.

Undifferentiated mesenchymal cells are present in the periosteum, endosteum and marrow spaces (Maniatopoulos et al. 1988; Owen & Friedenstein 1988; Nakahara et al. 1990; Haynesworth et al. 1992), in subcutaneous connective tissue or muscles in the proximity of bone surfaces (Friedenstein 1973), and among endothelial (Trueta 1963; Collin-Osdoby 1994), capillary and perivascular cells (*pericytes*) of newly formed blood vessels (Brighton et al. 1992). In the occlusive control capsules, assuming that they were adequately sealed, only cells deriving from marrow spaces, endosteum, endothelium, and perivascular tissues of the mandibular bone could participate in the bone induction process. It was assumed that the perforations of a diameter of 0.3 mm in the test capsules would also allow undifferentiated mesenchymal cells from subcutaneous connective tissue and muscles to migrate into the barrier-protected area. However, the present study does not provide evidence that this actually occurred, although the histological examination suggested that surrounding soft tissues proliferated through the perforations. In fact, it has never been examined as to which maximal pore size will prevent cells from invading a barrier-protected area. Therefore, it cannot be excluded that similar amounts of bone were formed in the cell-permeable and cell-occlusive capsules due to a lack of migration of undifferentiated mesenchymal cells from the surrounding soft tissues. Another reason for the similar amounts of bone found in the perforated and cell-occlusive capsules could be that there are a limited number of mesenchymal cells in the surrounding soft tissues. This possibility is supported by the findings of Caplan (1987) that during mammalian evolution, a reduced number of pluripotential cells is maintained and that their number at the same time becomes reduced with increasing age. The failure to enhance bone formation in the perforated test capsules as compared with the cell-occlusive control capsules cannot be attributed to a lack of osteoinductive capacity of the DBM particles since this was confirmed by bone formation following extraskeletal implantation. The considerable amounts of bone formed in both cell-occlusive and cell-permeable capsules, on the other hand, indicate that the highly vascularized granulation tissue that invades the capsules from the mandibular bone constitutes a sufficient source of bone-forming cells. This view is supported by the results of Schmid et al. (1997) showing the importance of angiogenesis in neogenetic bone formation under occlusive dome-shaped capsules in experiments where no osteoinductive materials were used. Thus, whether angiogenesis or cells from sources other than bone in fact possess the potential to induce osteogenesis were not answered by the present investigation, but the results suggest that they are unnecessary for bone formation by GTR.

In a previous study using a cell-occlusive capsule model similar to that used in the present study, the implantation of DBM resulted in bone formation similar to that in empty control capsules (Mardas et al. 2003). It is unlikely that this lack of an added effect of DBM grafting is due to a lack of undifferentiated mesenchymal cells when assuming that the perforations in

the capsules used in the present study may have allowed such cells to invade the barrier-protected area from the surrounding soft tissues. The present findings, however, support the view that DBM cannot accelerate bone formation under already favourable bonehealing conditions, such as situations where proliferation/collapse of the surrounding soft tissues into the defect is prevented (Aspenberg et al. 1988).

It was suggested that occlusive membranes may hamper the penetration of nutrients and growth-regulatory factors into the barrier-protected site and thereby inhibit bone formation (Linde et al. 1993; Zellin & Linde 1996). The finding in the present material that the use of a highly porous capsule did not enhance bone formation as compared with an occlusive capsule does not support this view. The results of the present study, on the other hand, are in accordance with those of a study of Schmid et al. (1994) showing a lack of significance of barrier porosity on the amount of bone formed in a barrier-protected site, but besides that, the results indicate that barrier porosity has no influence on the rate of bone formation either.

Resumé

Le but de cette étude a été d'évaluer si la formation osseuse par régénération tissulaire guidée (GTR) et la matrice osseuse déminéralisée (DBM) pouvait être augmentée par l'utilisation d'une barrière en téflon perméable aux cellules permettant la pénétration des cellules mésenchymateuses non-différenciées des tissus mous aux alentours. DBM a été produite à partir d'os longs de rats et ses propriétés inductives ont été testées chez trois rats avant cette étude par implantation intramusculaire. Trente rats albinos mâles de quatre mois de la souche Wistar ont été utilisés. A la suite de l'exposition chirurgicale de la branche montante, une capsule en téflon perméable aux cellules remplies de DBM non-tassée a été placée avec son ouverture vers la surface latérale de la branche montante (site test). Au niveau contralatéral qui servait de contrôle, une capsule en téflon non-perforée ne permettant donc pas le passage des cellules et remplie sans tassement de la même quantité de DBM a été placée. 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 produites. Les mesures planimétriques par ordinateur des coupes histologiques montraient des quantités semblables d'os néformé dans les capsules tests et contrôles. Après quatre mois, l'os néoformé dans les capsules contrôles occupaient 45% des coupes de la capsule et 51% au niveau des capsules tests. Cette différence n'était pas significative ($P>0.05$). Des quantités semblables d'os s'étaient formées dans les capsules perméables ou occlusives aux cellules remplies de DBM suggérant que l'invasion des cellules mésenchymateuses provenant des tissus mous avoisinants dans la zone protégée par la barrière n'est pas nécessaire pour la formation osseuse par GTR.

Zusammenfassung

Die Untersuchung einer zelldurchlässigen Membran bei der GTR in Kombination mit DBM.

Ziel: Ziel dieser Studie war es zu untersuchen, ob bei der mit demineraliserter Knochenmatrix (DBM) unterstützten GTR mit einer zelldurchlässigen Teflonmembran, die undifferenzierten Mesenchymalzellen aus den umliegenden Weichgeweben die Penetration erlaubt, die Knochenneubildung besser abläuft.

Material und Methode: Die DBM stammte von den langen Röhrenknochen der Ratten und seine osteoinduktiven Eigenschaften testete man, indem man vor der Studie 3 Ratten die DBM 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 zelldurchlässige Teflonkapsel, die locker mit DBM aufgefüllt worden war, mit der offenen Seite gegen die Aussenseite des Unterkiefers (Testseite). Die gegenüberliegende Seite (Kontrollseite) erhielt eine mit derselben Menge DBM locker aufgefüllte, aber nicht perforiert Teflonkapsel (für Zellen undurchlässig). 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.

Resultate: Die komputerunterstützten planimetrischen Vermessungen der histologischen Schnitte zeigten ähnlichen Mengen neu gebildeten Knochens in den Teflonkapseln, sowohl auf der Test- wie auch auf der Kontrollseite. Nach vier Monaten füllte der neue Knochen beim vermessenen Querschnitt der Kontrollkapseln 45.0% aus, währenddem es bei den Testkapseln 50.5% waren. Dieser Unterschied war nicht statistisch signifikant ($P<0.05$).

Zusammenfassung: Sowohl in den zelldurchlässigen, wie auch den zellundurchlässigen Teflonkapseln, die vorgängig mit DBM aufgefüllt worden waren, werden ähnliche Mengen Knochen neu gebildet. Dies lässt vermuten, dass das Einwandern von undifferenzierten Mesenchymalzellen aus den benachbarten Bindegeweben in die membrangeschützte Region hinein für die Knochenneubildung gemäss den biologischen Prinzipien der GTR nicht nötig ist.

Resumen

Intención: Evaluar si la formación ósea por (GTR) y una matriz de hueso desmineralizado (DBM) pueden ser realzados por el uso de una barrera de teflón permeable a las células permitiendo la penetración de células mesenquimales indiferenciadas de los tejidos blandos circundantes.

Material y métodos: El DBM se produjo de los huesos largos de ratas y se probaron sus capacidades inductoras de hueso en tres ratas antes del estudio por implantación intramuscular. Se utilizaron 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 de teflón permeable a las células rellenada sin forzar de DBM con su abertura frente a la superficie lateral de la rama (lado de prueba). En el lado contralateral, sirviendo de control, se colocó una cápsula de teflón no perforada (oclusiva a las células), rellenada sin forzar con la misma cantidad de DBM. Tras unos periodos 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.

Resultados: Las mediciones planimétricas asistidas por ordenador de las secciones histológicas mostraron cantidades similares de hueso neoformado tanto en las cápsulas de prueba como en las de control. Tras 4 meses, el hueso neoformado en la cápsulas de control ocupó el 45.0% del área de la sección de la cápsula mientras que fue del 50.5% en las cápsulas de prueba. Esta diferencia no fue estadísticamente significativa ($P<0.05$).

Conclusión: Se formaron cantidades similares de hueso en cápsulas permeables u oclusales a las células injertadas con BDM, sugiriendo que la invasión de células mesenquimales indiferenciadas de los tejidos blandos circundantes dentro del área protegida por la barrera es innecesaria para la formación ósea con GTR.

要旨：
目的：GTR と脱灰骨マトリクス（DBM）による骨形成が、周囲の軟組織由来未分化間葉細胞の侵入を許す細胞透過性テフロン・バリヤーの使用によって促進されるかどうかを評価すること。
材料と方法：ＤＢＭをラットの長骨から作成し、本実験前にその骨誘導特性を３匹のラットに筋注して調べた。４ヶ月令、雄アルビノ・Wisterラット３０匹を同研究に用いた。下顎枝を外科的に露出させた後、DBM をゆるく充填した細胞透過性のテフロン・カプセルを、その開いた部分が下顎枝の外側面を向くようにして埋入した(試験部位)。反対側の対照部位では、同量の DBM をゆるく充填した非穿孔性（細胞閉塞性）テフロン・カプセルを埋入した。３０、６０、１２０日の治癒期間後、各々１０匹ずつの動物を屠殺して、カプセルの４０－７０μｍ厚の非脱灰切片を作成した。
結果：組織切片上のコンピュータによる面積測定によって、試験カプセルと対照カプセルの両方において類似した量の新生骨ができていることが示された。４ヶ月後に対照カプセル中の新生骨は、カプセル断面積の４５．０％を占めていたが、試験カプセルでは５０．５％であった。この差は統計的有意差ではなかった（ｐ＜０．０５）。
結論：DBM を詰めた細胞透過性と細胞閉塞性のカプセルの移植によって類似した量の新生骨が形成されたことは、周囲の軟組織由来の未分化間葉細胞がバリヤーで保護された部位に侵入することは GTR による骨形成には不必要であることを示唆している。

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