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Methyl cellulose gel obstructed bone formation by GBR: an experimental study in rats

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Abstract

Aim: To evaluate whether bone formation under Teflon capsules may be enhanced by concomitant implantation of recombinant human platelet-derived growth factor-BB/insulin-like growth factor-I (rhPDGF-BB/IGF-I) incorporated into a methyl cellulose gel.

Materials and Methods: Fifty-five male 6-month-old albino rats of the Wistar strain were used in the study. The lateral aspect of the mandibular ramus was exposed on both sides of the jaw. In 70 sites, the periosteum was removed from the ramus, leaving the bone denuded, while in 35 sites, it was preserved. On 10 non-periosteal (P−) sites and five periosteal (P+) sites, an empty rigid teflon capsule ($d = 7$ mm), serving as control, was placed on the ramus. In the 40 test animals, the capsule placed on the one side of the jaw was filled at random with one of three different concentrations (1200, 600, 150 μg/ml) of rhPDGF-BB/IGF-I gel. The capsules placed on the contralateral side of the jaw contained a placebo methyl cellulose gel. Each growth factor group, defined according to the gel concentration, and the placebo group contained 10 capsules placed on the P− side and five capsules placed on the P+ side. Two months after surgery, all animals were sacrificed.

Results: Histologic analysis revealed that in the non-filled control capsules, the amount of new bone including the bone marrow was 29.9% and 39.7% of the capsule area on the P− and P+ sides, respectively. In the test capsules with the growth factor gel and placed on the P− sides, the amounts of new bone ranged from 5.6% to 6.3%, which were similar ($p > 0.05$) to that formed in the capsules filled with the methyl cellulose gel (5.5%). New bone formation was larger in the capsules on the P+ sides than in those on the P− sides but was similar in the capsules with different growth factor concentrations (range 17.9–19.6%) and in those with placebo gel (21.0%). In all groups, the carrier gel was poorly absorbed and occupied most of the capsules.

Conclusion: Local application of a methyl cellulose gel obstructed bone formation under Teflon capsules placed adjacent to uninjured cortical bone in the mandibular ramus of rats. These data suggest that another material should be utilized to deliver growth factors under Teflon membranes for guided bone regeneration.

Key words: bone formation; GTR; guided tissue regeneration; insulin-like growth factor; platelet-derived growth factor; rats.

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Guided tissue regeneration (GTR) involves the placement of a physical barrier (membrane) to provide space for invasion of cells with the capacity to regenerate the particular type of tissue that has been lost. At the same time, other types of cells are prevented from entering the wound during healing after surgery. This treatment principle was originally developed for the regeneration of periodontal tooth support lost because of periodontitis (Karring et al. 1993, 1997). Subsequently, the same principle, sometimes under the term of guided bone regeneration (GBR) or guided bone augmentation (GBA), was applied successfully in both experimental animals and humans for the treatment of different types of bone defect (Dahlin et al. 1988, 1990, 1995, Jovanovic et al. 1992, Kostopoulos & Karring 1994, Hämmerle et al. 1995, Matzen et al. 1996) and for augmentation of atrophic alveolar ridges (Buser et al. 1990, 1991, Cortellini et al. 1993, Lang et al. 1994b). In addition, it was documented in both experimental animals and humans that

new bone could be produced beyond the skeletal envelope by GTR (Linde et al. 1993, Kostopoulos et al. 1994, Lundgren et al. 1995, Hämmerle et al. 1996, Lioubavina et al. 1999).

In vivo and in vitro experiments have demonstrated that the application of growth-regulatory factors, such as platelet-derived growth factor (PDGF) may stimulate proliferation and chemotaxis of pre-osteogenic cells (Lynch et al. 1987, Ross et al. 1990). In vitro, PDFG or insulin-like growth factor-I (IGF-I) enhanced the proliferation of osteoprogenitor cells and the synthesis of bone collagen (Canalis 1981, 1985, 1989, Pfeilshifter et al. 1990, Zhang et al. 1991). In addition, a synergistic effect of a combination of PDGF-BB and IGF-I on the enhancement of collagen production and wound healing has been reported (Lynch et al. 1987). Studies have shown that local application of recombinant human PDGF-BB (rhPDGF-BB) alone or in combination with rhIGF-I in a gel can enhance bone regeneration in periodontal (Lynch et al. 1989, Rutherford et al. 1992 Howell et al. 1997), and peri-implant (Lynch et al. 1991a) defects in dogs, monkeys and humans. These studies were conducted without the use of GTR membranes.

Other investigators have reported a periodontal regenerative therapy termed P-GTR (PDGF-BB-modulated GTR) to be effective in promoting periodontal regeneration in horizontal Class III furcation defects in canines (Cho et al. 1995, Park et al. 1995). The authors of these studies concluded that PDGF-BB delivered in solution directly onto conditioned tooth root surfaces yielded significantly better results than GTR alone when treating periodontal furcation defects.

Bone formation by GBR often requires that a membrane is maintained for a long period of time with an increasing risk of complications such as membrane exposure and infection (Simion et al. 1994). Therefore, it is reasonable to presume that enhancement of bone formation by a growth factor may improve the predictability of the GBR technique. In fact, in a study in dogs, Becker et al. (1992) showed that local application of PDGF-BB/IGF-I in a gel in GBR-treated peri-implant defects resulted in a higher percentage of newly formed bone and bone-to-implant contact than GBR alone.

The purpose of the present study was to investigate in a discriminating Teflon capsule model whether bone formation by GBR may be enhanced by concomitant implantation of PDGF-BB/IGF-I in a methyl cellulose gel.

Material and Methods

Growth factors

A combination of purified recombinant human PDGF-BB and IGF-I (rhPDGF-BB/IGF-I; Institute of Molecular Biology, Boston, MA, USA) in a 1:1 weight ratio and in three different concentrations (1200, 600 and 150 µg/ml) contained in a methyl cellulose gel (Methocel A4 M, Dow Chemical Co., Midland, MI, USA) was used. The methyl cellulose gel alone served as a control. The gels containing the different concentrations of growth factors were coded, and the information about the coding was not available until the results had been analysed.

Surgical procedure

Fifty-five male 6-month-old albino rats of the Wistar strain were used in the study. During the surgical procedures, the animals were anaesthetized with a subcutaneous injection of Immobilon® (Pherrovet, Malmö, Sweden). In 10 rats, serving as controls, percutaneous incisions were made along the inferior border of the mandible in one side. The underlying tissues were prepared in layers until the masseter muscle became

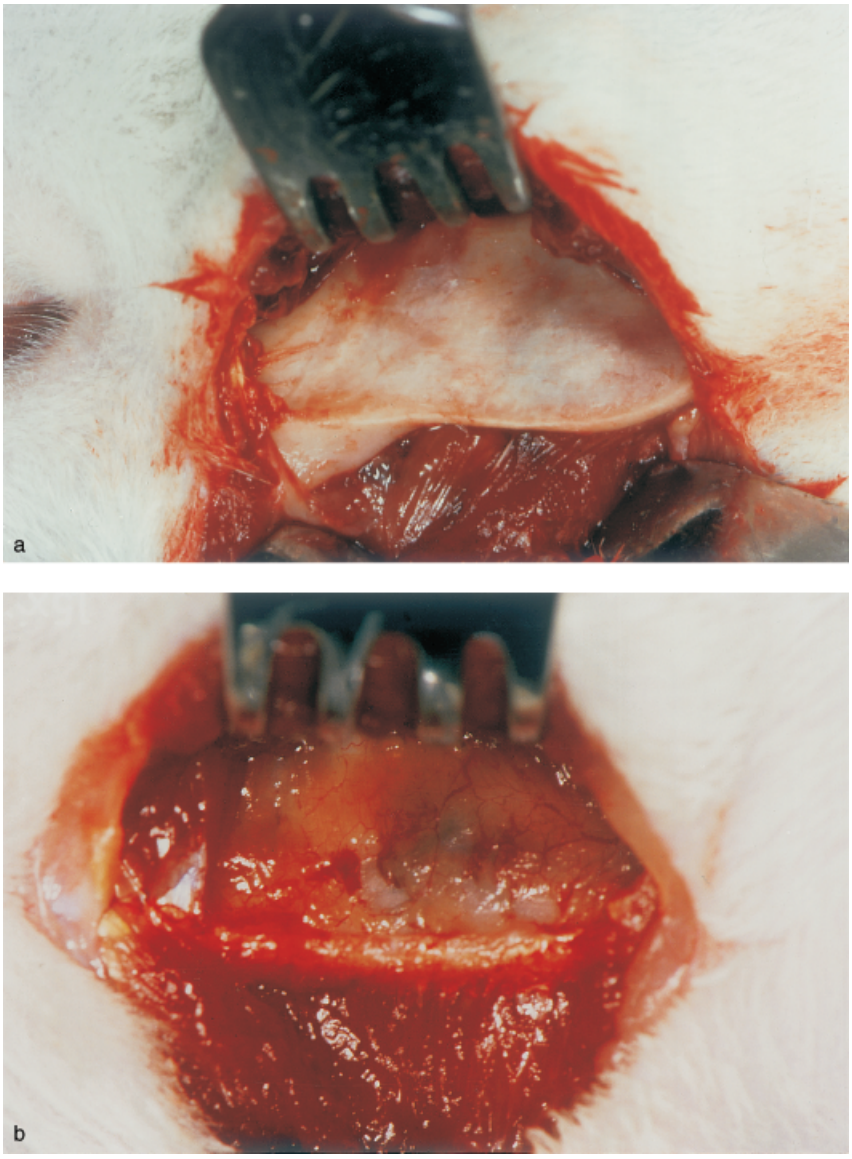

Fig. 1. (a) Denuded bone at the lateral aspect of the mandibular ramus. (b) Lateral aspect of the ramus with preserved periosteum.

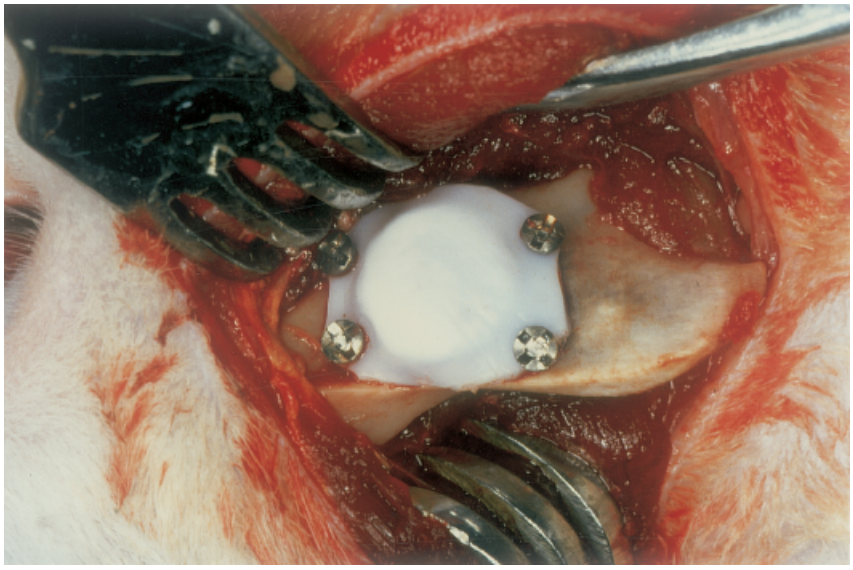

Fig. 2. Teflon capsule fixed to the ramus with 4 mini-screws.

exposed. Subsequently, a deep incision was made through the muscle down to the underlying inferior border of the mandible, and a full-thickness muscle-periosteal flap was elevated, leaving the bone denuded (non-periosteal sides) (Fig. 1a). In five of these rats, a similar surgical procedure was also performed on the other side of the jaw, but on this side the periosteum was preserved on the mandibular ramus following elevation of the masseter muscle (periosteal sides) (Fig. 1b). In both sides of these five animals, and in the one side of the remaining five control animals, an empty rigid non-porous dome-shaped Teflon capsule with a diameter of 7 mm was placed on the denuded or periosteum-covered mandibular ramus, and fixed with four titanium miniscrews (Leibinger GmbH, Freiburg, Germany) (Fig. 2).

In the 45 test rats, the mandibular ramus was exposed as described above for the control animals. In 30 of these rats, the periosteum was removed from the ramus on both sides of the jaw, while in the remaining 15, the periosteum was preserved on both sides. Capsules, similar to those placed in the control animals, were filled with the placebo methyl cellulose gel, and fixed on the ramus in one side of the jaw, chosen at random. The capsules placed on the ramus in the other side were filled at random with one of the three different concentrations of PDGF-BB/IGF-1 gel (1 ml per capsule). Each growth factor test group, defined according to the growth factor concentration, comprised 10 test capsules placed on a denuded ramus (non-periosteal site) and five capsules placed on a periosteal covered ramus (periosteal sites). At the end of the surgical procedure, the wound was sutured with 5–0 Vicryl® (Ethicon, Norderstedt, Germany) sutures, and the anaesthesia was terminated by injection of Revivon® (Pherrovet). No penetrations in the bone surface were performed in any of the animals.

After 2 months, all animals were sacrificed, and the mandibles were dissected free. The specimens containing the capsules and the surrounding tissues were fixed in 10% neutral-buffered formaline, dehydrated in alcohol and subsequently embedded in methylmethacrylate. Undecalcified sections with a thickness of 60–80 µm were cut through the capsule perpendicular to the mandibular ramus. Four sections from each site, representing the mid-portion of the capsule, were stained with Toluidine Blue and subjected to histological analysis.

Histological measurements

In each coded histological section, the area created by the capsule, and the area of newly formed bone including the bone marrow were measured in mm^2, using a Leitz DM-RBE® microscope (Leica, Wetzlar, Germany) equipped with an image system (Quantiment 500-MC®, Leica). The amount of the new bone was expressed as the percentage of the capsule area. The mean value of the newly formed bone was calculated from four sections taken from each site that was used as the statistical unit. The Wilcoxon ranking test for unpaired data and the Kruscal–Wallis test were used to determine the differences between the various treatment groups. The α error was set at 0.05.

Results

Out of the 105 operated sites, 21 became infected within the first month. Among the infected sites, two had received the gel with a growth factor concentration of 1200 µg/ml, six with a concentration of 600 µg/ml and three with a concentration of 150 µg/ml. Eight sites had received the methyl cellulose gel without any growth factors, and two sites were the empty control capsules. All the infected sites were excluded from the evaluation, thereby leaving 84 sites for histological analysis.

Non-periosteal sites

In the control capsules placed empty on the denuded bone, new bone was seen in continuity with the mandibular ramus and comprised lamellar and woven bone (Fig. 3). Osteoid seams were frequently observed adjacent to large marrow spaces filled with fat cells. A layer of connective tissue containing osteoblastic and fibroblastic cells was always covering the newly formed bone. The rest of the capsule area appeared empty.

The amount of new bone including the bone marrow occupied 29.9% (mean) of the capsule area, which was significantly ($p < 0.05$) more than that seen in the test capsules containing either the growth factor gels or the methyl cellulose gel alone. The amounts of new mineralized and non-mineralized bone in the growth factor-treated sites were similar (range 5.6–6.3%) and did not differ from those of the gel-alone sites (5.5%) (Table 1).

In all gel capsules placed on the denuded bone, the methyl cellulose gel was poorly absorbed and occupied most of the capsule area. Sparse amounts of new woven bone were seen in some specimens, usually near the edges of the capsule. In four specimens, new bone had formed as a thin layer on top of the gel (Fig. 4). The top layer of the newly formed bone was always covered with a connective tissue containing osteoblastic cells and a few small islands of woven bone. In three test capsules, which were almost completely filled with the remaining gel, no signs of new bone formation were found.

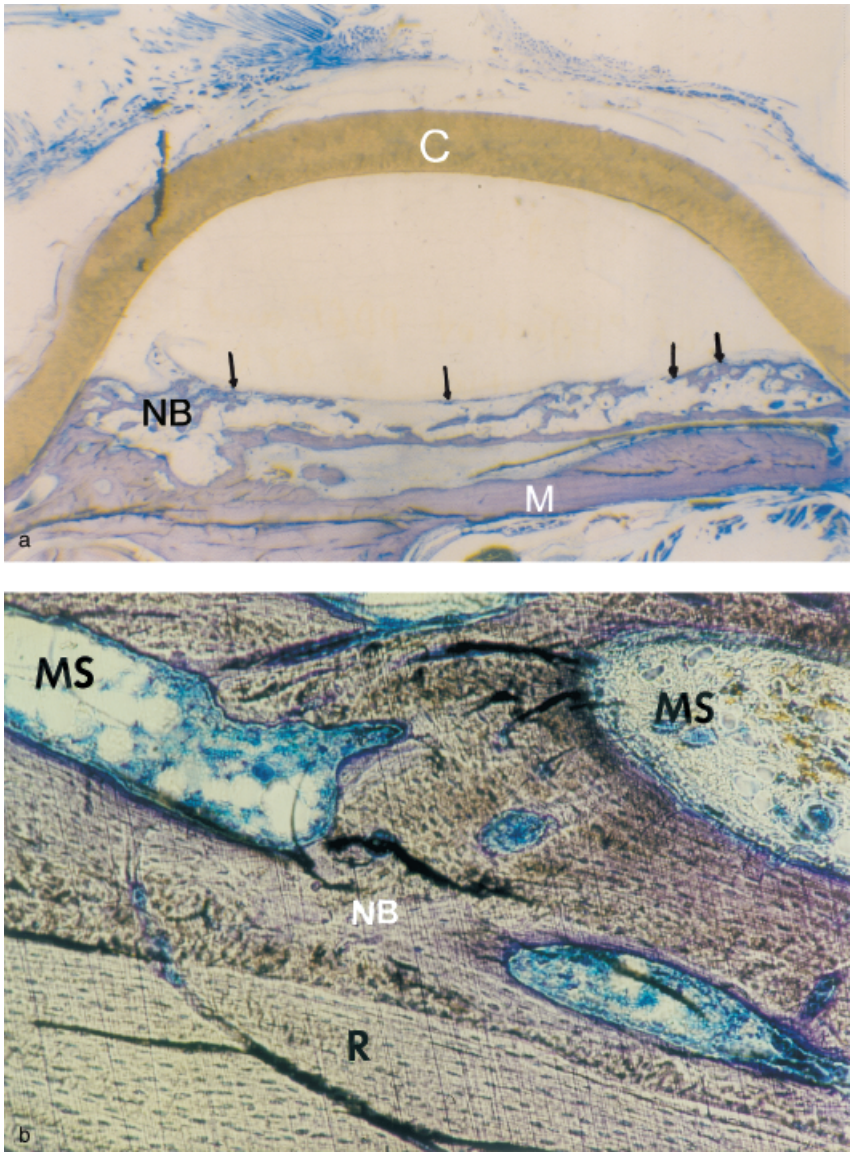

Fig. 3. Microphotograph of a control capsule (C) placed on denuded bone (a). New bone (NB) is seen adjacent to the mandibular (M) ramus. The top of the newly formed bone is covered with a thin layer of connective tissue (arrows) (Toluidine Blue, magnification × 18). High magnification (b) of the capsule seen in (a) shows new bone (NB) in continuity with the mandibular ramus (R). The marrow spaces (MS) are filled with fat and osteoblastic cells (magnification × 100).

Table 1. Percentage of newly formed bone in non-periosteal sites

Treatment	Sites *N*	Mean	Median	Lower quartile	Upper quartile	*p*-value T/C
CGF – 1200 μg/ml	8	6.3	5.7	3.3	9.7	0.0004
CGF – 600 μg/ml	6	6.1	5.7	3.2	9.6	0.0011
CGF – 150 μg/ml	8	5.6	4.2	1.0	8.6	0.0004
methyl cellulose gel	24	5.5	4.5	3.1	6.7	0.0000
Control	10	29.9	31.1	24.3	36.9	

CGF, concentration of growth factor in methyl cellulose gel; T/C, *p*-values between the corresponding test group and the control; *p*-values between the test groups and those and the placebo were always >0.5.

Periosteal sites

In the control periosteal sites (Fig. 5), the amount of newly formed bone including the bone marrow occupied 39.7% of the capsule area (Table 2), which was greater than that observed in the control non-periosteal sites (29.9%). The histologic appearance of this new bone was similar to that formed in the control capsules placed on the denuded bone.

In the capsules containing the gel with growth factors the amounts of new bone including the bone marrow ranged from 17.9% to 19.6%, and were similar to that of the sites receiving the gel without factors (20.5%) (Fig. 6). All gel-filled capsules presented less bone formation than the control capsules that were placed empty. However, it was obvious that all periosteal sites always presented more new bone formation than the corresponding non-periosteal sites (Tables 1 and 2).

The periosteal-treated sites contained thicker mineralized new bone and better-organized marrow spaces than the non-periosteal sites with a similar growth factor concentration. The new bone was always in continuity with the mandibular ramus and was covered by a loose connective tissue. This soft tissue contained fibroblastic and osteoblastic cells and several islands of new woven bone. The amount of the various cells was always larger in the periosteal than in the non-periosteal sites, which contained larger areas of non-absorbed gel inside the capsule.

Discussion

The present study demonstrated that methyl cellulose gel inhibits bone formation under a Teflon capsule when used for GBR adjacent to the uninjured ramus of rats. This inhibitory effect appeared to block any stimulatory effect of local application of PDGF-BB/IGF-I on bone formation by GBR. After 2 months, similar and only small amounts of new bone were found in all the capsules containing methyl cellulose gels regardless of the presence of growth factors. Substantial amounts of non-absorbed gel were consistently present inside the capsules. In contrast, the control capsules, placed empty on the mandibular ramus, presented considerably larger amounts of newly formed bone. This finding is in agreement with those reported previously in experimental studies using the capsule model (Kostopoulos et al. 1994, Lioubavina et al. 1999, Stavropoulos et al. 2001).

The findings of the present study are in contrast with other reports, where PDGF-BB/IGF-I in methyl cellulose gel used without the membrane techni-

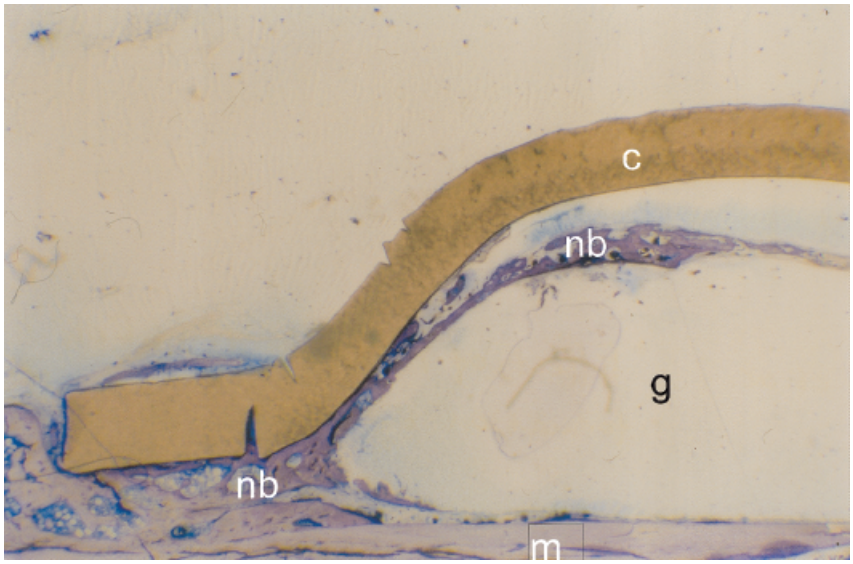

Fig. 4. Microphotograph of a capsule placed on denuded mandible (m) and containing the growth factor gel (concentration: 1200 μg/ml). A limited amount of new bone (nb) is seen close to the edge of the capsule (c) but also on top of the non-absorbed gel (g), which occupies most of the capsule area (Toluidine Blue, magnification × 18).

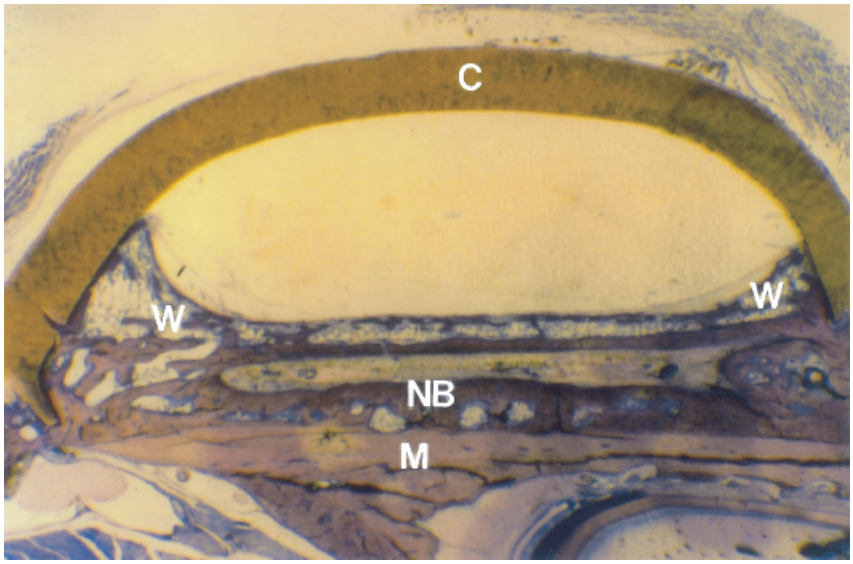

Fig. 5. Microphotograph of a control capsule placed on periosteum-covered bone. Approximately 40% of the capsule is filled with new bone (NB) in direct contact with the mandibular (M) ramus. The lower portions of the new bone contain thick trabeculae, while the upper portion comprises of woven bone (W) (Toluidine Blue, magnification × 18).

Table 2. Percentage of newly formed bone in periosteal sites

Treatment	Sites *N*	Mean	Median	Lower quartile	Upper quartile
CGF – 1200 μg/ml	5	19.6	19.8	13.2	21.9
CGF – 600 μg/ml	3	18.7	25.3	4.2	27.0
CGF – 150 μg/ml	5	17.9	17.4	16.9	18.2
methyl cellulose gel	13	21.0	20.5	19.4	22.8
Control	3	39.7	37.8	21.9	59.3

CGF, concentration of the growth factor in methyl cellulose gel.

que enhanced bone regeneration in periodontal (Lynch et al. 1989, 1991b, Giannobile et al. 1994) or peri-implant (Lynch et al. 1991a) bone defects in dogs and monkeys, and with results obtained in monkeys or humans following treatment of periodontal defects with PDGF-BB incorporated into a collagen matrix (Rutherford et al. 1992) or into a methyl cellulose gel (Howell et al. 1997). It was reported that in such non-membrane-covered defects, the carriers were disintegrated fast by cells penetrating into the wound, thus resulting in the release of 96% of growth factors within 4 days after application (Lynch et al. 1991b).

The effect of rhPDGF/IGF-I in a methyl cellulose gel combined with GBR was evaluated in buccal dehiscences on implants in a study in dogs (Becker et al. 1992). A higher percentage of new bone in contact with the implant surface was observed following the combined therapy than with GBR alone at 18 weeks. Similarly, in studies in dogs, where the GTR therapy was used in conjunction with the application of a solution of PDGF-BB onto previously acid-conditioned root surfaces, significantly more new bone formation was observed than with GTR alone (Cho et al. 1995, Park et al. 1995). Furthermore, GBR/rhPDGF-BB treatment favoured bone formation in rabbit calvarial defects to a greater extent than GBR combined with a placebo gel after 8 weeks (Vikjaer et al. 1997).

It is possible that in such surgically created bone defects, more cells are available for bone formation and absorption of the carrier gel than in the present capsule model, thereby allowing release of the growth factors and providing more space for bone apposition underneath the membrane. An explanation for the findings in the present study of more bone being found in the gel-filled capsules placed on the ramus with the remaining periosteum than in those placed on the denuded bone may also be that the number of cells available for bone formation and resorption of the methyl cellulose gel are larger in the situation with the initially preserved periosteum. The amount of various cells inside the capsules was always larger in the periosteal sites than in the non-periosteal sites, suggesting that those cells may have facilitated the absorption of the carrier gel. This is supported by the finding that smaller amounts of the remaining gel were found in the periosteal than in non-periosteal sites.

It is not known whether during healing the periosteum became completely replaced by the newly formed bone or was pushed further away from the man-

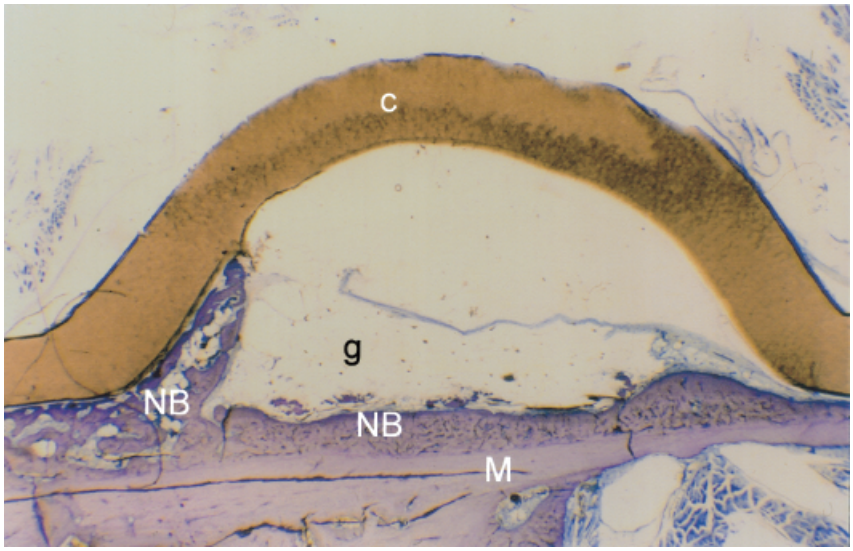

Fig. 6. Microphotograph of a capsule placed on periosteum-covered bone and containing growth factor gel (concentration: 150 μg/ml). New bone (NB) is seen in the capsule (C) adjacent to the mandibular (m) ramus and isolated islands of woven bone are seen within the gel (g) (Toluidine Blue, magnification × 19).

dibular ramus by the new bone. In all periosteal sites, the new bone was in direct continuity with the mandibular ramus and was covered with a layer of loose connective tissue containing fibroblastic and osteolastic cells. Whether this soft tissue is the periosteum or a layer of not yet mineralized newly formed bone is still unknown.

The observation of the present study that the gel-filled capsules presented less new bone formation than the empty control capsules suggests that the presence of the gel has prevented bone formation. In three tests and 1 gel specimen, new bone formation was observed on top of the non-absorbed gel, indicating that the gel was highly biocompatible but not osteoconductive. Possibly, it is this biocompatibility that may have caused a very limited inflammatory reaction towards the gel to induce its degradation. Thus, the persistence of the carrier gel inside the capsules may have obstructed bone formation by occupying the space provided by the capsules. This, in fact, is a violation of a main principle of GTR that a secluded ''empty'' space should be available for ingrowth of the desirable tissue (Karring et al. 1993). The finding that a non-absorbed carrier may obstruct new bone formation by GTR is also supported by a study in dogs, where grafting of a transforming growth factor (TGF-β-1) in a $CaCO_3$/hydroxyethyl starch carrier into GTR-treated supra-alveolar mandibular defects failed to enhance new bone formation (Wikesjö et al. 1998). Non-resorbable implant material occupied most of the area underneath the membrane, thereby reducing the space for bone regeneration. Similar observations of obstructions of regeneration by grafted implants have been reported in studies evaluating GTR with prostaglandin E1 analogue and methacrylate composite (Trombelli et al. 1999) or GTR with chitin balls (Kostopoulos et al. 2001). Thus, this finding can suggest that in GTR the use of growth factor carriers that can be readily absorbed and/or are osteoconductive is essential. This view is also supported by studies showing that the incorporation of PDGF-BB into bioresorbable membranes resulted in stimulation of bone formation in rat calvarial defects probably because the growth factors were readily released during degradation of the membrane (Chung et al. 1997, Park et al. 1998). Further research should be conducted to find suitable carriers for local application of growth factors in combination with GTR.

Within the limitations of this study, the histologic observations indicate that local application of the methyl cellulose gel prevented new bone formation by GBR and that this inhibitory effect could not be overcome by the growth factors. The initial presence of the periosteum most likely improved absorption of the carrier gel, and thereby favoured new bone formation.

Acknowledgements

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