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Implant placement in bone formed beyond the skeletal envelope by means of guided tissue regeneration: an experimental study in the rat

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Abstract

Objectives: The aim of the present study was to evaluate whether the placement of implants in bone formed by means of guided tissue regeneration (GTR) beyond the skeletal envelope may influence bone volume and/or structure.

Material and Methods: Rigid, hemispherical, Teflon capsules were placed with their open part facing the lateral surface of the ramus in both sides of the mandible in 18 rats. After 1 year, the capsules were removed by a re-entry operation, and a custom-made titanium implant was placed in the augmented ramus in only one side of the jaw. Six animals were sacrificed shortly after implant surgery, another six after 3 months, and the last six after 6 months. Histological specimens of the augmented sites including the implants were prepared, and the volumes of (1) the newly formed bone (mineralized bone and marrow) (2) the soft connective tissue, and (3) the implant, in the space originally created by the capsule were estimated by a point-counting technique. Additionally the height of the augmented bone was measured.

Results: One year after capsule placement, the major portion of the space originally created by the capsules was filled with newly formed bone. In the test specimens, implant placement seemed to result in a denser arrangement of the augmented bone, but this event did not influence its long-term stability. Although some resorption occurred after 3 and 6 months, the vast portion of the generated bone remained stable over time in both tests and controls, and there were no differences between tests and controls at any observation periods.

Conclusion: It is concluded that large amounts of bone can be formed beyond the skeletal envelope by means of GTR, and that this bone remains stable on a long-term basis both with and without the placement of titanium implants.

Key words: guided tissue regeneration, GTR; guided bone regeneration/augmentation; GBR/GBA; implants; long-term; osseointegration; titanium

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The ''guided tissue regeneration'' (GTR) technique involves the placement of a physical barrier (membrane) to cover a periodontal- or bone defect during surgery so that a secluded space is created and the neighbouring soft tissues are prevented from participating in the healing process. This ensures that cells with the capacity to regenerate the particular type of lost tissues can populate the defect during healing (for a review see Karring et al. 1993). The GTR principle, which was originally intended for the treatment of periodontal lesions, has also been applied successfully, often under the terms ''guided bone regeneration'' (GBR) or ''guided bone augmentation'' (GBA), for the treatment of various types of bone defect and for increasing the bone volume of atrophic alveolar ridges, thereby making the placement of dental implants feasible (for a review see Hämmerle & Karring 1998). Further-

more, experimental studies in rodents have shown that by means of GTR, bone can be formed rather predictably beyond the genetically determined ''skeletal envelope'', i.e. in a place where it has not existed before (Linde et al. 1993, Kostopoulos et al. 1994, Lundgren et al. 1995, Stavropoulos et al. 2003b). Results from a histological case-series report support the view that such ''extra-skeletal'' bone formation by means of GTR is possible also in humans (Hämmerle et al. 1996).

Several reports have demonstrated that the results obtained after the clinical application of the GTR principle for the treatment of periodontal lesions can be maintained on a long-term basis (Cortellini et al. 1999, Ehmke et al. 2003, Stavropoulos & Karring 2004). Data from experimental studies (Buser et al. 1995, Fritz et al. 2000) and the success and survival rates of implants inserted in bone regenerated by means of GTR (for reviews see Hämmerle et al. 2002, Fiorellini & Nevins 2003) suggest that the results of GBR/GBA are also preserved over extended periods of time. Similarly, studies in rodents on bone formed beyond the skeletal envelope on the lateral surface of the mandibular ramus by means of GTR have demonstrated that only a minor resorption of the new bone occurs shortly after barrier removal, while the major portion is stable on a long term basis (Lioubavina et al. 1999, Stavropoulos et al. 2004). There is only limited information, however, on what influence the placement of dental implants may have on this situation (Rasmusson et al. 1997).

The aim of the present study was to evaluate whether the placement of implants in bone formed by means of GTR beyond the skeletal envelope may influence bone volume and/or structure.

Materials and Methods

Surgical procedures

A total of 18, 3-month-old, albino rats of the Wistar strain were used in the study. The animals were anaesthetized by a subcutaneous injection of 0.6 ml of Immobilon™ (Pherrovet, Malmö, Sweden). Horizontal incisions were made along the inferior border of the mandible, and by elevating muscle-periosteal flaps, the lateral aspect of the mandibular ramus was exposed. Four holes, 0.5 mm in diameter, placed as corners in a square (with sides of approximately 6 mm, and one side being parallel to the base of the ramus), were made through the ramus with a small round bur. A custom-made rigid, hemispherical Teflon capsule, with an internal diameter of 6 mm and a 1 mm peripheral collar, was placed with its open part facing the lateral surface of the mandibular ramus (Fig. 1a), in both sides of the jaw. The capsules were fixed on the ramus by means of interrupted 4.0 silk sutures (Ethicon, 2000, Norderstedt, Germany) through the four holes in the bone (Fig. 1b and c). The soft tissues were re-positioned over the capsule and sutured with mattress 4.0 Vicryl sutures (Ethicon, 2000). The anaesthesia was terminated by a subcutaneous injection of 0.6 ml of the antagonist Revivon™ (Pherrovet). During the experiment, the animals were fed ad libitum with standard laboratory food pellets.

After 1 year, the experimental sites were re-entered (Fig. 2a) and the capsules were removed, revealing a dome-shaped bone-like tissue formed on the lateral surface of the ramus in both sides of the mandible in all animals (Fig. 2b and c). In one side of the mandible, chosen at random, a custom-made screw-shaped titanium implant (2 mm Ø × 5 mm long) with a sandblasted and acid-etched surface (Osseotite®, *3i* Implant Innovations, Palm beach, FL, USA) was inserted at the centre of the dome (test side) (Fig. 3a) until the head of the implant came in contact with the coronal part of the augmented tissue (Fig. 3b and c). In the contralateral

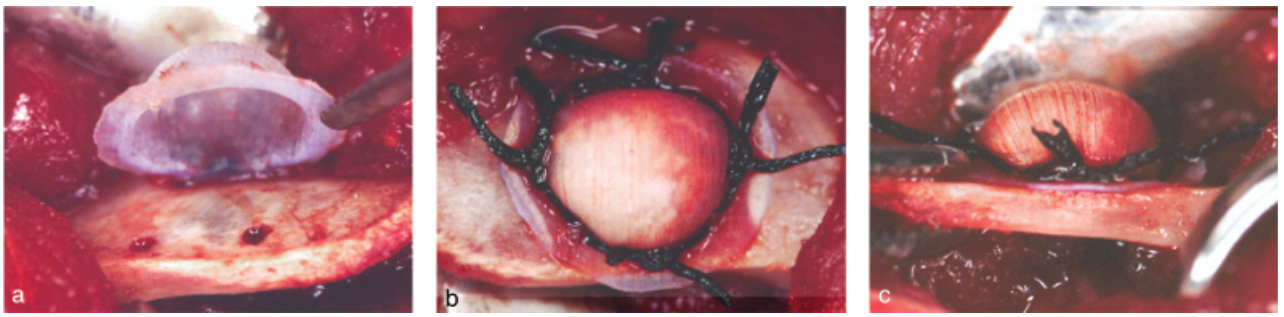

Fig. 1. Empty Teflon capsules (a) are placed on the lateral surface of the mandibular ramus and fixed by means of silk sutures (b), so that a tight adaptation on the bone surface is ensured (c).

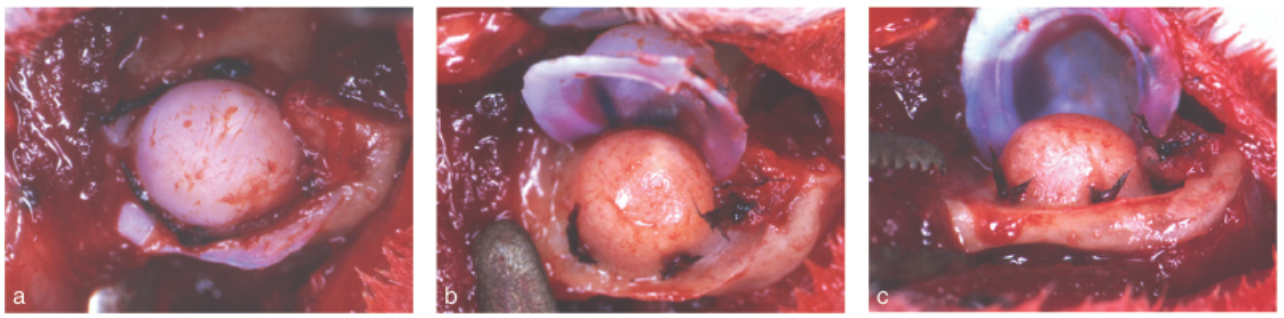

Fig. 2. After 1 year in place (a), the capsule is removed. Note that a dome-shaped bone-like tissue has formed under the capsules (b, c).

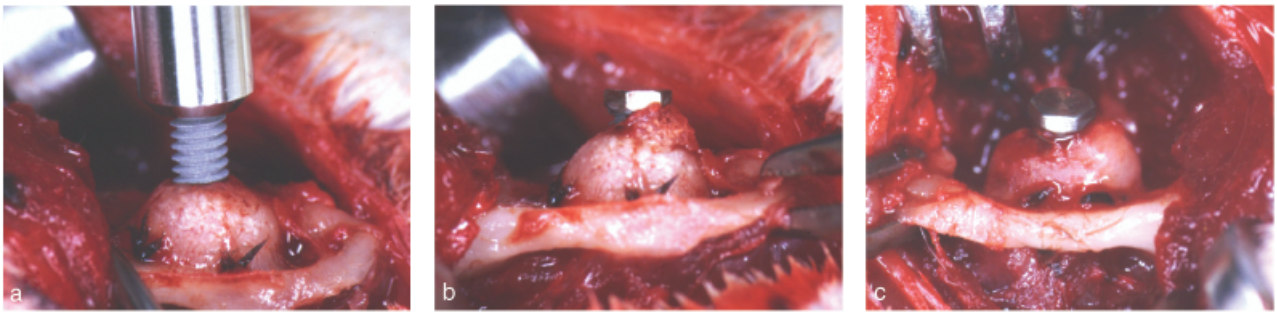

Fig. 3. A screw-shaped titanium implant was inserted at the centre of the dome (test side) (a) until the head of the implant came in contact with the coronal part of augmented tissue. Occasionally, because of an eccentric insertion of the implants, only part of the implant head was in contact with the augmented tissue (b) or, in few occasions, a minor part of the augmented tissue broke off (c).

side, there were no implants placed (control side). Then, the soft tissues were re-positioned and sutured over the augmented tissues. Prior to the re-entry operations, six animals were randomly allocated for sacrifice shortly after capsule removal and implant placement (baseline), or after 3 or 6 months.

Histomorphometry

After sacrifice of the animals, the tissue formed under the capsules including the surrounding tissues were dissected free and fixed in 10% neutral-buffered formalin, dehydrated in a series of ascending concentrations of alcohol, and embedded in glycolmethacrylate (Technovit 7200 VLC, Kulzer GmbH, Bereich Technic, Wehrheim/Ts, Germany). Undecalcified sections of approximately 200 μm thickness were obtained almost perpendicular to the lateral surface of the ramus, which had firstly been randomly rotated. In the specimens containing the implants, care was taken that the sectioning plane was parallel to the long axis of the screw. The sections were then reduced to a thickness of 20–30 μm by means of the Exact™ (Exact-Apparatebau, D-200, Norderstedt, Germany) sawing–grinding technique (Donath & Breuner 1982). Finally, every second section was stained with a modified Goldners' trichrome stain and the rest of the sections with van Giesons' picro-fuchsin stain.

From each specimen, the most central section was sampled for analysis. By means of a computer-assisted stereological toolbox (CAST, Visiopharm, Hørsholm, Denmark) connected to a BH-50 Olympus light microscope (Olympus Denmark AS, Ballerup, Denmark) via a video camera and a frame grabber, a grid of test points and cycloids was superimposed on the section image. Then, the margins of the augmented bone tissue on the ramus were identified and the space originally created by the capsule was delineated (traced and/or reproduced) on the computer screen by means of a mouse. Data were generated by counting separately test points ''hitting'' (i.e. superimposed on) newly formed bone (bone trabeculae or marrow spaces), implant, and loose connective tissue inside the traced capsule area (Gundersen & Jensen 1987). The number of points ''hitting'' inside the capsule space was also recorded. The data for each specimen were expressed as percentage of the space originally created by the capsule for each of the above-mentioned parameters. In order to evaluate possible differences between groups and/or observation periods regarding the traced/reproduced capsules, the number of points ''hitting'' inside the capsule space of each specimen was multiplied by the corresponding ''area per point'' (Gundersen & Jensen 1987). In order to evaluate osseointegration in the test specimens, the number of intersections of the cycloids with the implant when the implant was in direct contact with mineralized bone were measured and expressed as percentage of the total number of cycloid–implant intersections. Additionally, the height of the newly formed bone (corresponding to the width of the ramus) was measured linearly at the midpoint of the base of the traced capsule area in the control specimens and to the highest point adjacent to the implant (from the left or the right) in the test specimens. The same researcher (A. S.) made all measurements.

Statistical analysis

The differences between tests and controls at the various observation times were analysed with Wilcoxon signed-rank test for paired observations, while the differences between the various time points for tests and controls were tested with Mann–Whitney's test for non-paired observations.

The SPSS 10.0 (SPSS Inc., Chicago, IL, USA) statistical program was used for all the analyses, and the level of significance was set to $p < 0.05$.

Results

Healing following the surgical procedures was uneventful in all the animals.

Clinical findings

At re-entry for removal of the capsules after 1 year it was observed that a dome-shaped bone-like tissue, which filled the space provided by the capsules almost entirely, had formed on the lateral surface of the mandibular ramus (Fig. 2b and c) in all the specimens, except for two controls where approximately 1/2 of the coronal part of the dome was lacking. These two specimens (one belonging to the baseline group and one to the 6-month group) were excluded from the calculations.

Some implants were unintentionally not placed exactly in the centre of the dome-shaped augmented tissue and/or exactly perpendicularly to the mandibular ramus. The result was that only part of the implant head was in contact with the augmented tissue (Fig. 3b) and/or, in a few occasions, that a minor part of the augmented tissue broke off (Fig. 3c). Therefore, in the calculations regarding the height of the newly formed bone in the test specimens, only the measurement with the highest value was used for each implant, assuming that this was the side where the implant head was

Table 1. Mean values in % (CV) of the space originally created by the capsule for bone (bone trabeculae, bone marrow, and total amount of bone), connective tissue, and implant, and mean bone height in mm (CV) and mean bone-to-implant (BIC) contact in % (CV)

	N	Bone								Connective tissue		Implant			
		trabeculae (%)	*p*	marrow (%)	*p*	total (%)	*p*	height (mm)	*p*	fill (%)	*p*	fill (%)	*p*	BIC (%)	*p*
Baseline															
Control	5	51.9 (0.24)		38.9 (0.41)		90.8 (0.07)		2.56 (0.10)		9.2 (0.57)					
Test	6	31.5 (0.09)		15.7 (0.28)		47.2 (0.12)		2.36 (0.11)		12.9 (0.43)		39.9 (0.10)		22.9 (0.34)	
3 months*															
Control	6	50.1 (0.18)	0.71	26.08 (0.30)	0.14	76.21 (0.15)	0.03	2.07 (0.10)	0.01	24.6 (0.47)	0.02				
Test	6	31.6 (0.22)	0.75	10.9 (0.55)	0.15	42.5 (0.23)	0.34	1.84 (0.11)	0.01	18.4 (0.17)	0.08	39.1 (0.22)	0.63	83.4 (0.07)	<0.01
6 months†															
Control	5	44.9 (0.05)	0.35	26.9 (0.32)	0.17	71.8 (0.11)	0.01	1.96 (0.17)	0.05	28.2 (0.26)	0.01				
Test	6	32.9 (0.16)	0.75	8.4 (0.52)	0.04	41.3 (0.17)	0.05	1.80 (0.22)	0.02	22.1 (0.15)	0.01	36.6 (0.13)	0.20	88.3 (0.07)	<0.01

*p-values from 3 months *versus* baseline.
†p-values from 6 months *versus* baseline, analyzed by Mann–Whitney's test.
CV, coefficient of variation.

originally (i.e. at the time of insertion) in contact with the augmented tissue.

Histological findings

Histometric analysis confirmed that there were no statistically significant differences between the different observation times or the different groups regarding the traced/reproduced capsule area (data not shown).

Control specimens

At capsule removal (i.e. 1 year after the capsule was placed) abundant amounts of new bone (91%) were observed in the control specimens (Table 1 and Fig. 4). The new bone had formed in continuity with the host bone, extending on average 2.6 mm (Table 1 and Fig. 5) from the lateral surface of the ramus, and had a dome-shaped configuration, which apparently conformed the internal surface of the hemispherical capsules (Figs 6a and 8, upper row). At all three observation periods, the newly formed bone consisted of lamellar mature bone with a trabecular appearance and marrow spaces composed largely of adipose cells, but in the 3- and 6-month specimens (Fig. 6b and c, respectively) the dome-shaped bone tissue appeared denser (especially in the periphery of the dome) and somewhat flattened (Fig. 8, middle row and lower row, respectively) when compared with baseline. The amounts (relative volumes) of mineralized bone and bone marrow did not differ between the three observation periods, but the total amount (relative volume) of new bone (i.e. mineralized bone+bone marrow) at 3 and 6 months was statistically significant less than at baseline ($p = 0.03$ and 0.01, respectively) (Table 1). The ratio of bone marrow to bone trabeculae at baseline, 3, and 6 months was 83.3% (CV: 0.56), 54% (CV: 0.37), and 58.1% (CV: 0.33), respectively. There were no statistically significant differences between baseline and 3- ($P = 0.4$), or 6-month ($p = 0.4$) specimens regarding the ratio of bone marrow to bone trabeculae. Additionally, after 3 and 6 months some reduction had occurred in the height of the new bone (2.1 and 2.0 mm, respectively) as compared with baseline (Table 1 and Fig. 5). There were no significant differences between the 3- and 6-month control specimens regarding any of the evaluated parameters ($p > 0.05$). In all three-observation periods, areas undergoing re-modelling could be identified.

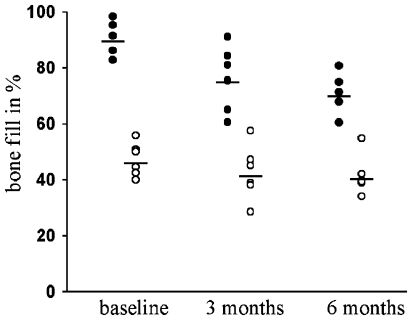

Fig. 4. Bone fill as a percentage of the space originally created by the capsule for tests (white dots) and controls (black dots), at baseline (capsule removal) and after 3 and 6 months. Horizontal lines indicate mean values.

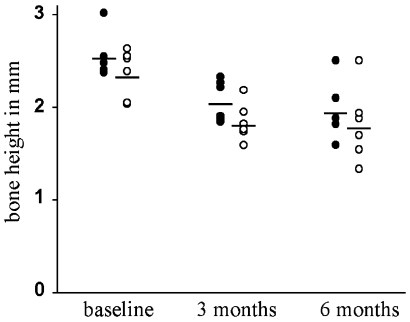

Fig. 5. Bone height in mm for tests (white dots) and controls (black dots) at baseline (capsule removal) and after 3 and 6 months. Horizontal lines indicate mean values.

Test specimens

As in the controls, the new bone in the test specimens had formed in continuity with the host bone, and consisted of lamellar mature bone with a trabecular appearance and marrow spaces with fat cells. The dome-shaped new bone in the test specimens extended on average 2.4 mm (Table 1 and Figs 4 and 5) from the lateral surface of the ramus (Fig. 7a). At baseline, mineralized bone-to-implant contact (BIC) was scarce and the major portion of the implant surface was covered by a blood coagulum and/or a loose connective tissue rich in vascular structures. In the 3- and 6-month specimens, the augmented bone appeared somewhat denser, but the amounts (relative volumes) of mineralized bone and bone marrow, and the total amount (relative volume) of new bone (i.e. mineralized bone+bone marrow) did not differ significantly from

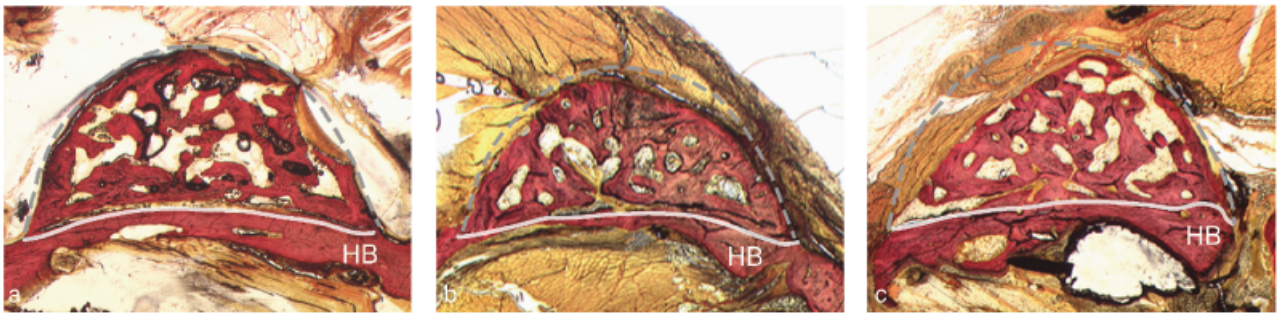

Fig. 6. Photomicrographs of tissues formed in control capsules at baseline (capsule removal) (a), and after 3 (b) and 6 months (c). New bone occupies the major portion of the space originally created by the capsule (dashed line) at all observation times, although some resorption of the augmented bone can be observed 3 and 6 months after capsule removal. The new bone has a trabecular appearance with marrow spaces with fat cells. The white line delineates the host bone surface. Van Giesons' stain.

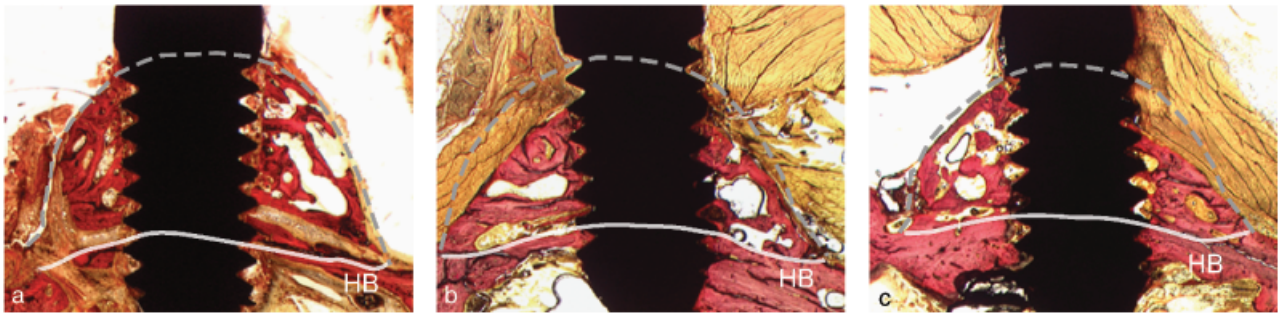

Fig. 7. Photomicrographs of test specimens at baseline (shortly after implant placement) (a), and after 3 (b) and 6 months (c). New bone occupies the major portion of the space originally created by the capsule (dashed line) at all observation times, although some resorption of the augmented bone can be observed 3 and 6 months after capsule removal. The new bone has a trabecular appearance with marrow spaces with fat cells. Osseointegration of the implants can be observed at the 3- and 6-month specimens. The white line delineates the host bone surface. Van Giesons' stain.

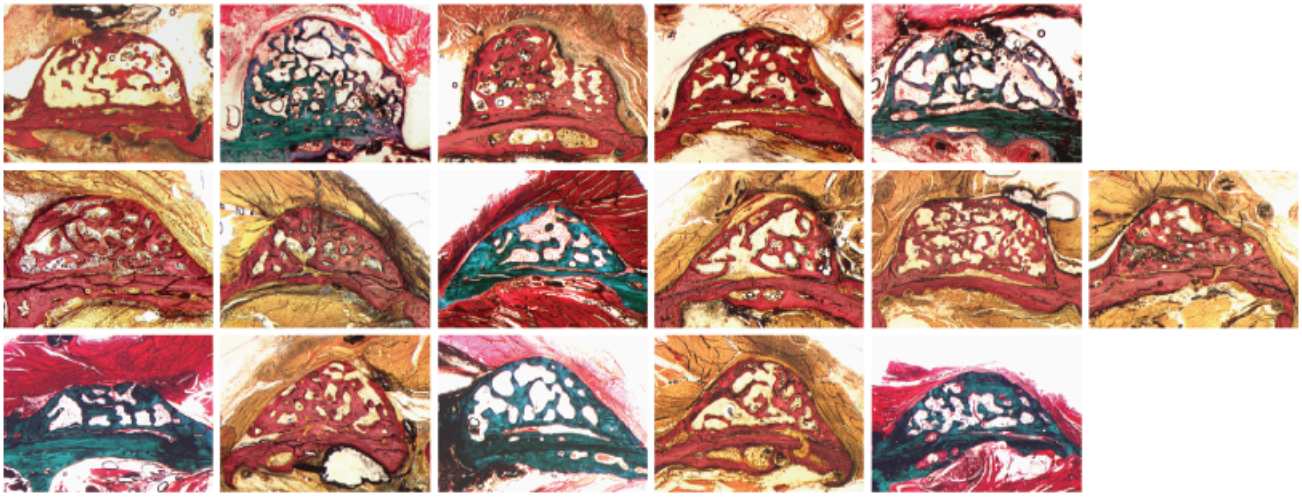

Fig. 8. Photomicrographs of all the evaluated control specimens at baseline (upper row), 3 (middle row), and 6 months (lower row).

those observed at baseline. However, after 3 and 6 months some reduction had occurred in the height of the new bone (1.8 mm) as compared with baseline ($p = 0.01$ and 0.02, respectively) (Table 1 and Fig. 5), and the dome-shaped bone tissue appeared somewhat flattened (Figs 7b and c, and 9). In the 3- and 6-month specimens, variable amounts of mineralized BIC (i.e. osseointegration) were observed. The ratio of bone trabeculae to bone marrow at baseline, 3, and 6 months was 50% (CV: 0.26), 35.4% (CV: 0.56), and 25.9% (CV: 0.52), respectively. The difference between baseline and the 6-month group regarding the ratio of bone trabeculae to bone marrow was statistically significant ($p = 0.03$). There were no significant differences between the 3- and 6-month test specimens

Fig. 9. Photomicrographs of all the evaluated test specimens at baseline (upper row), 3 (middle row), and 6 months (lower row).

regarding any of other evaluated parameters ($p > 0.05$). At all three-observation periods, areas undergoing remodelling were observed.

There were no statistically significant differences ($p > 0.05$) in the height of the newly formed bone and in the amount of connective tissue between control and test specimens, at any observation period.

Discussion

The present experimental study evaluated the effect of placement of screw-shaped titanium implants in bone formed beyond the skeletal envelope by means of GTR. Obviously, a direct comparison between control and test specimens regarding the amount of the generated bone would not be appropriate, as the implant occupied a large portion of the capsule space in the test specimens. Instead, the amount of connective tissue fill was used as a surrogate variable when evaluating changes in bone fill in the present study. The finding that, at all observation times, the amount of connective tissue inside the space originally created by the capsules was similar in control and test specimens, along with the fact that the height of the generated bone was practically the same in both groups at the three time points, indicates that implant placement did not influence significantly the amount of bone that was present after capsule removal. An earlier study has suggested that a solid surface (e.g. an implant) may have a stabilizing effect on bone augmented beyond the skeletal envelope (Rasmusson et al. 1997), i.e. implant placement results in a better preservation of the augmented bone volume. In this experimental study, the authors placed implants obliquely (leaving four to five implant threads exposed) in the tibia of rabbits and provided them with a titanium space-keeper covered with a Teflon membrane, so that bone formation was allowed to occur beyond the original skeletal envelope. After 8 weeks of healing a re-entry procedure for removal of the space-keeper and the membrane was performed, and groups of animals were sacrificed 8 and 16 weeks after the re-entry operation. By means of computerized coordinate measurements on plaster models, evaluating changes in the height of the augmented tissues, the authors observed larger amounts of bone height reduction beside (2 and 3 mm laterally to) the implant than directly above the implant body. However, the above-mentioned study suffers from the lack of a control group, i.e. an augmented group without an implant was not included in the study.

The results of the present study are in accordance with findings in other experimental studies, where placement of titanium screw-shaped implants, inserted in bone regenerated within the genetically determined skeletal envelope by means of GTR had no effect on the regenerated bone (Buser et al. 1995, Fritz et al. 2000). For instance, Fritz et al. (2000) extracted the mandibular molars of monkeys and after a period of healing, titanium screw-shaped implants were placed. After osseointegration was accomplished, the implants were furnished with prosthetic restorations and loaded for a period of 12 months. Then, block biopsies including the implants were harvested, leaving a large rectangular defect. After an additional 6-month period, the chronic alveolar ridge defects were covered with titanium reinforced Teflon membranes, and when bone regeneration had occurred, titanium implants were inserted once again. Following osseointegration, these implants were also furnished with prosthetic restorations and loaded for a period of 12 months before sampling. Comparison between the two regimens failed to reveal any significant differences regarding the clinical, radiographical and histomorphometric characteristics of the bone/implant relationships. For both the implants placed in pristine and those placed in regenerated bone, a similar minimal amount of bone loss took place (0.3 *versus* 0.5 mm, respectively) and a similar amount of osseointegration (59% *versus* 69%, respectively) was observed after 12 months of loading. Similarly, Buser et al. (1995) observed by using a canine model that bone regenerated by means of GTR responded to implant placement like pristine bone, and that this bone was capable of sustaining functional load. Thus, based on these results and those of the present study, as well as on the results from clinical studies (for reviews see Hämmerle et al. 2002, Fiorellini & Nevins 2003), showing similar success and survival rates for implants placed in sites augmented by means of or in association with GTR and implants placed without the need for GTR procedures, it is reasonable to suggest that regenerated/generated bone responds to implant placement in the same (physiologic)

manner as pristine bone, irrespective of its ''topographical'' location, i.e. within or beyond the genetically determined skeletal envelope.

On the other hand, the ratio of bone marrow to bone trabeculae at 6 months was significantly less than that in baseline (25.9% *versus* 50%, respectively) in the test specimens, while there were no statistically significant differences between the three time points regarding bone marrow/bone trabeculae ratio in the controls, suggesting that implant placement resulted in a denser arrangement of the augmented bone. A similar observation can be made at the histological level in the above-mentioned study of Buser et al. (1995). However, this event does not seem to influence the long-term stability of the augmented tissue.

The results of the present experiment, confirm that large amounts of bone (three to five times the width of the ramus in the present study) can form predictably in rats beyond the genetically determined skeletal envelope by means of GTR, and that most of this ''extraskeletal'' bone remains stable on a long-term basis. These findings are in accordance with the results of previous experiments (Lioubavina et al. 1999, Stavropoulos et al. 2004), although a larger reduction of the bone volume was found in the present experiment 3 and 6 months after capsule removal (16% and 22%, respectively). Lioubavina et al. (1999) observed that bone tuberosities formed on the lateral aspect of the rat mandible 6 months after the placement of originally empty Teflon capsules, were stable at least 1 year after membrane removal. In this study, only a small (4–8%), although statistically significant, reduction in the amount of the newly formed bone was observed at 3 months after capsule removal, while no further resorption occurred during the remaining observation period. Similar results were presented by Stavropoulos et al. (2004), with the same experimental model as in the present study, where only a minor insignificant reduction/resorption of the augmented bone tissue was observed 6 months after capsule removal.

The accuracy and the reproducibility of bone volume estimations by the method of analysis used in the present experiment was documented previously in studies using capsules of similar size (Stavropoulos et al. 2001, 2003a). The capsules, however, in those studies were not removed prior to the preparation of the specimens while in the present experiment there were no capsules present in the histological sections (the capsules were removed at baseline). Although the margins of the augmented osseous tissue on the ramus were easily recognizable at all observation periods and the position of the capsule readily identifiable at baseline (just after capsule removal), the original outline of the capsules in the 3- and 6-month groups had in several occasions to be reproduced (by means of a mouse on the images of the sections viewed on a monitor). As all data regarding the amount of the various tissues formed under each Teflon capsule were generated in relation to – and expressed as a percentage of – the capsule space (i.e. as ratios), it was mandatory to evaluate whether the traced/reproduced capsule space was similar in both groups and at all observation periods, in order to avoid falling into ''the reference trap'' (Braendgaard & Gundersen 1986). The term ''reference trap'' is coined for cases where wrong conclusions have been drawn from densities alone. The fact that there were no significant differences regarding the traced/reproduced capsule space neither between the various observation periods in both tests and controls nor between test and control specimens at the various time points, excludes the possibility that we have been caught in such a ''reference trap''.

In conclusion, the findings in the present study suggest that large amounts of bone can be formed beyond the skeletal envelope by means of GTR, and that this bone remains stable on a long-term basis both with and without the placement of titanium implants. This observation is important, for instance, for the treatment of patients with congenital bone deficiencies where the ''genetically'' determined skeletal envelope is insufficient for the placement of implants. These results, however, need to be validated in larger animal models and under more clinically relevant conditions (e.g. in load-bearing parts of the jaws and/or involving loading of the implants).

Acknowledgements

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References

Braendgaard, H. & Gundersen, H. J. (1986) The impact of recent stereological advances on quantitative studies of the nervous system. *Journal of Neuroscience Methods* **18**, 39–78.

Buser, D., Ruskin, J., Higginbottom, F., Hardwick, R., Dahlin, C. & Schenk, R. K. (1995) Osseointegration of titanium implants in bone regenerated in membrane-protected defects: a histologic study in the canine mandible. *International Journal of Oral Maxillofacial Implants* **10**, 666–681.

Cortellini, P., Stalpers, G., Pini, P. G. & Tonetti, M. S. (1999) Long-term clinical outcomes of abutments treated with guided tissue regeneration. *Journal of Prosthetic Dentistry* **81**, 305–311.

Donath, K. & Breuner, G. (1982) A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage–Schliff (sawing and grinding) technique. *Journal of Oral Pathology* **11**, 318–326.

Ehmke, B., Rudiger, S., Hommens, A., Karch, H. & Flemmig, T. (2003) Guided tissue regeneration using a polylactic acid barrier. *Journal of Clinical Periodontology* **30**, 368–374.

Fiorellini, J. P. & Nevins, M. L. (2003) Localized ridge augmentation/preservation. A systematic review. *Annals of Periodontology* **8**, 321–327.

Fritz, M. E., Jeffcoat, M. K., Reddy, M., Koth, D., Braswell, L. D., Malmquist, J. & Lemons, J. (2000) Guided bone regeneration of large mandibular defects in a primate model. *Journal of Periodontology* **71**, 1484–1491.

Gundersen, H. J. & Jensen, E. B. (1987) The efficiency of systematic sampling in stereology and its prediction. *Journal of Microscopy* **147** (Part 3), 229–263.

Hämmerle, C. H., Jung, R. E. & Feloutzis, A. (2002) A systematic review of the survival of implants in bone sites augmented with barrier membranes (guided bone regeneration) in partially edentulous patients. *Journal of Clinical Periodontology* **29** (Suppl. 3), 226–231.

Hämmerle, C. H. & Karring, T. (1998) Guided bone regeneration at oral implant sites. *Periodontology 2000* **17**, 151–175.

Hämmerle, C. H., Schmid, J., Olah, A. J. & Lang, N. P. (1996) A novel model system for the study of experimental guided bone formation in humans. *Clinical Oral Implants Research* **7**, 38–47.

Karring, T., Nyman, S., Gottlow, J. & Laurell, L. (1993) Development of the biological concept of guided tissue regeneration–animal and human studies. *Periodontology 2000* **1**, 26–35.

Kostopoulos, L., Karring, T. & Uraguchi, R. (1994) Formation of jawbone tuberosities by guided tissue regeneration. An experimental study in the rat. *Clinical Oral Implants Research* **5**, 245–253.

Linde, A., Thoren, C., Dahlin, C. & Sandberg, E. (1993) Creation of new bone by an osteopromotive membrane technique: an experimental study in rats. *Journal of Oral Maxillofacial Surgery* **51**, 892–897.

Lioubavina, N., Kostopoulos, L., Wenzel, A. & Karring, T. (1999) Long-term stability of jaw bone tuberosities formed by ''guided tissue

regeneration''. *Clinical Oral Implants Research* **10**, 477–486.

Lundgren, D., Lundgren, A. K., Sennerby, L. & Nyman, S. (1995) Augmentation of intramembraneous bone beyond the skeletal envelope using an occlusive titanium barrier. An experimental study in the rabbit. *Clinical Oral Implants Research* **6**, 67–72.

Rasmusson, L., Sennerby, L., Lundgren, D. & Nyman, S. (1997) Morphological and dimensional changes after barrier removal in bone formed beyond the skeletal borders at titanium implants. A kinetic study in the rabbit tibia. *Clinical Oral Implants Research* **8**, 103–116.

Stavropoulos, A., Kostopoulos, L., Mardas, N., Nyengaard, J. R. & Karring, T. (2001) Deproteinized bovine bone used as an adjunct to guided bone augmentation: an experimental study in the rat. *Clinical Implant Dentistry and Related Research* **3**, 156–165.

Stavropoulos, A., Kostopoulos, L., Mardas, N., Nyengaard, J. R. & Karring, T. (2003a) Gentamicin used as an adjunct to GTR. *Journal of Clinical Periodontology* **30**, 455–462.

Stavropoulos, A., Kostopoulos, L., Nyengaard, J. R. & Karring, T. (2003b) Deproteinized bovine bone (Bio-Oss®) and bioactive glass (Biogran®) arrest bone formation when used as an adjunct to guided tissue regeneration (GTR): an experimental study in the rat. *Journal of Clinical Periodontology* **30**, 636–643.

Stavropoulos, A. & Karring, T. (2004) Long-term stability of periodontal conditions achieved following guided tissue regeneration with bioresorbable membranes. Case series results after 6 to 7 years. *Journal of Clinical Periodontology* **31**, 939–944.

Stavropoulos, A., Kostopoulos, L., Nyengaard, J. R. & Karring, T. (2004) Fate of bone formed by guided tissue regeneration with or without grafting of Bio-Oss® or Biogran®. An experimental study in the rat. *Journal of Clinical Periodontology* **31**, 30–39.

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