A Histomorphometric Evaluation of Factors Influencing the Healing of Bony Defects Surrounding Implants

J. A. Pretorius[1]/B. Melsen[2]/J. C. Nel[1]/P. J. Germishuys[1]

Purpose: *The authors' aim was to perform a histomorphometric study of the healing of bone defects created adjacent to titanium and hydroxyapatite (HA) -coated implants and covered with either a resorbable or a nonresorbable membrane in combination with different filler materials and to evaluate to what degree coating, membrane, and/or filler influenced the healing of the defects.* ***Materials and Methods:*** *Posterior teeth were extracted from the mandibles of 10 baboons, and 12 implants were placed in each animal in the edentulous areas. The implants were either titanium or HA-coated, the membranes were either Vicryl, Gore-Tex, or Resolut, and the filler was either demineralized freeze-dried bone (DFDB), autogenous bone, or Biocoral. The implants were observed for either 3, 6, 9, 12, or 18 months. The volume of newly generated tissue and the relative contribution of bone, marrow, and filler were evaluated, as was relative extension of resorption, formation, and quiescent surface.* ***Results:*** *The results indicated that autogenous bone is still the gold standard, but both the DFDB and Biocoral compared favorably to it. Both filler materials were being gradually replaced by bone; this process was not yet finished at 18 months postsurgery.* ***Discussion:*** *Since even the sterilization of DFDB cannot exclude the possibility of a disease transmission, it is important to find an appropriate substitute. Both filler and membranes contributed to the re-establishment of the original volume; better results were achieved with the Vicryl and Gore-Tex membranes than with the Resolut. Biocoral can be considered an effective material.* ***Conclusion:*** *A bony defect is not necessarily a contraindication for the placement of an implant.* (More than 50 references.) INT J ORAL MAXILLOFAC IMPLANTS 2005;20:387–398

Key words: *bone augmentation, bone regenerative membranes, bony defects, dental implants, implant coatings*

Since endosseous implants are used in the reconstruction of degenerating dentition, the clinician frequently encounters situations in which implants must be placed in areas where the alveolar process is reduced in height and width. However, sufficient bone volume is a prerequisite for esthetics and functional long-term implant success and stability.[1] The lack of horizontal and vertical bone volume has been considered an important factor in relation to potential implant failure.[2] Several techniques have been used to induce vertical and horizontal bone augmentation of bone around implants.[3–13]

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Two different principles have been applied for generating the desired new bone formation: guided tissue regeneration (GTR) using membranes and the transplant of osseoinductive or -conductive materials. These principles have been used separately or in combination. Although fresh autogenous grafts are considered the "gold standard," a search for other more readily available graft materials is ongoing.[14–16]

The advantages and disadvantages of using nonresorbable or resorbable membranes for GTR have been studied repeatedly, with varying results.[16–19] Since a principle of GTR is to maintain space for migration of the cell population, collapse of the membrane is a recognized reason for failure. This can be avoided by titanium reinforcement or by supporting the membranes with filler materials. Graft materials used to keep the barriers from collapsing against the implants have been studied.[9,15] Implants can help support the membrane as, for example, in the case of immediate implant placement in extraction sockets with a bony defect around the implant. Cortellini and Tonetti[16] compared the results of

different membranes in the treatment of such cases and found positive results with both resorbable and nonresorbable membranes.

The mechanical ability of a membrane to resist collapse is crucial; however, stiffness renders clinical management difficult. The unavoidable second-stage surgery for removal of the nonresorbable membrane can be another drawback.[16] On the other hand, softer resorbable membranes may need support from filler material,[17–21] and the physico-chemical process involved in the degradation of a membrane may affect the environment for bone regeneration.[20] Becker and associates[22] found greater bone formation around implants with the use nonresorbable of expanded polytetrafluoroethylene (e-PTFE) membranes than in controls without membranes. Simion and colleagues[19] reported less bone regeneration with resorbable than with nonresorbable membranes; but when combined with autogenous bone chips, resorbable polylactic acid/polyglycolic acid (PLA/PGA) membranes were as effective as e-PTFE membranes.

The questions regarding which graft or filler materials can be used to aid bone augmentation have not been fully elucidated. The bone inductive potential of demineralized freeze-dried bone allograft (DFDBA) has been demonstrated,[22] but no adjunctive effect of DFDBA as a filler for use with GBR was found by Caplanis and coworkers.[23–26] In a survey article, Sanz and Giovannoli[27] drew attention to the inconsistency of the different results and pointed out that more clinical research is needed.

In addition to coverage and the filler materials, implant surface has been reported to play a crucial role in the healing of a defect around an implant. HA-coated implants have shown better results than noncoated implants, particularly with regard to bone-to-implant contact (BIC), shear strength, and fixation.[28–31]

The authors' aim was, therefore, to perform a histomorphometric study of the healing of a bone defect created adjacent to titanium and hydroxyapatite (HA) -coated implants covered with nonresorbable and resorbable membranes in combination with different filler materials and to address the following questions:

- Does an HA coating influence the healing of a defect adjacent to the implant?
- To what degree is healing of a bone defect adjacent to an implant dependent on the utilization of GBR?
- Does the use of a nonresorbable membrane or a resorbable membrane make a difference?
- Do different filler materials enhance bone regenera tion and augmentation differently?

This protocol was approved by the Ethical Committee of the H. A. Grove Animal Research Center of the University of Pretoria, South Africa.

MATERIALS AND METHODS

Ten adult baboons (*Papio ursinus*) were utilized in this investigation. They were housed in the research center. The animals were fasted the night before the experimental procedures. They were immobilized with 100 mg ketamine (Centaur Labs Animal Health/Bayer, Isando, South Africa) at a dose of 10 mg/kg. A blowpipe and Telinject darts (Telinject, Jukskei Park, Johannesburg, South Africa) were used for the immobilization. After the immobilized animal was weighed, a cuffed endotracheal tube and a urethral catheter were placed. Ringer's lactate was given at a rate of 150 mL/h. During the surgery the animals were kept under anesthesia using a mixture of halothane and oxygen.

All the posterior mandibular teeth were removed under general anesthesia in an operation theater. The bony margins were slightly trimmed, and the wound areas were sutured with resorbable sutures to control bleeding and enhance healing. No unnecessary bone was removed. Periapical radiographs were obtained before and after tooth extraction. Immediately after the operation, the animals were given a single injection of 20 mg/kg cefazolin (Eli Lilly, Indianapolis, IN) and 0.02 mL/kg buprenorphine (Shering-Plough, Kenilworth, NJ). Further treatment consisted of 0.5 mL of flunixin meglumine (Schering-Plough) given once daily for 3 days and 10 mg/kg cephalexin (Aspen Pharmaceutical, Gallo Manor, Johannesburg, South Africa) twice daily for 5 days.

Recovery and healing were monitored for 3 months. The animals were kept on a well-balanced diet prescribed by a dietician and a veterinary surgeon. Diagnostic casts of the edentulous mandible were obtained after tooth extraction and before implant placement. The spacing, depth, and size of the bony defects were planned on the casts prior to implant placement. Each experimental animal received 12 custom-made threaded implants (Nobel Biocare, Yorba Linda, CA), 6 on each side in the edentulous posterior area of the mandible. One side of the mouth was randomly selected for the placement of uncoated titanium implants; HA-coated implants were placed on the other side (Fig 1a). Prior to placement of the implant, a bony defect relative to the implant position was created with a vulcanite bur. This resulted in the exposure of 5 threads on the buccal aspects of the implants. The lingual aspect of each implant was engaged in intact mandibular bone.

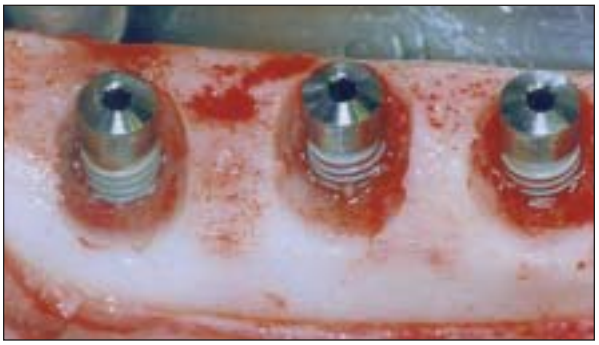

Fig 1a Implants placed. Defects have not yet been created.

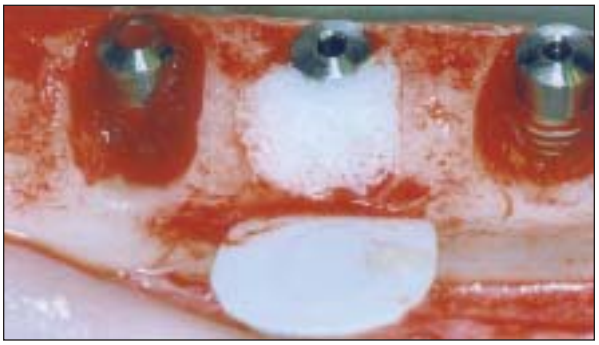

Fig 1b Defects created after placement of implants. About 5 threads were exposed.

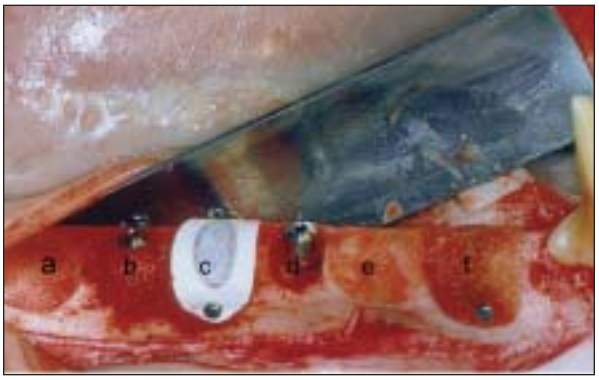

Fig 1c The treatment options: *(a)* Vicryl membrane and Biocoral, *(b)* Biocoral with no membrane, *(c)* Gore-Tex membrane and Biocoral, *(d)* control, *(e)* Vicryl and autogenous bone, and *(f)* R4 membrane and DFDB.

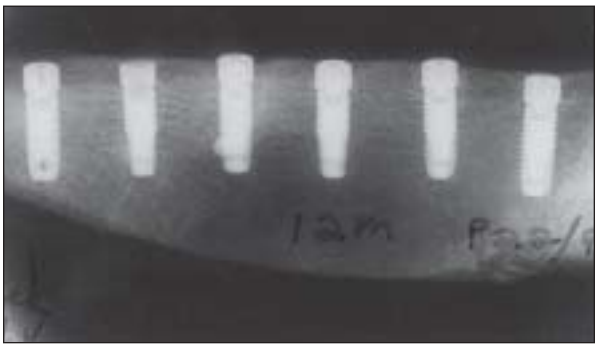

Fig 1d Intraoral radiograph following implant placement.

In each animal, the following graft materials and membranes were used. Site numbers were assigned randomly and changed from animal to animal (Figs 1b to 1d). Each site was subjected to the same treatment procedure.

- A resorbable Vicryl mesh membrane (Polyglactin 910; Johnson & Johnson/Ethicon, Somerville, NJ) and Biocoral 450 filler material (Inoteb, le Guernol, Saint Connery, France)
- No membrane and Biocoral 450 filler material
- A nonresorbable Gore-Tex membrane (Gore, Elkton, MD) and Biocoral 450 filler material
- A resorbable Vicryl mesh membrane and autogenous bone graft filler material. Autogenous bone was harvested from the drilling sites and collected from an osseous coagulum trap in the aspirating system
- A resorbable membrane made of Resolut R4 Regenerative Material (lactide and glucolide polymers) (Gore) and DFDB xenograft from human bone (S.A. Bone, Johannesburg, South Africa)
- No membrane and no filler (control)

All sites were totally covered with a full-thickness mucoperiosteal flap sutured with resorbable sutures. No second-stage surgery for uncovering was performed.

At the end of the experiment, the baboons were anesthetized and subsequently sacrificed by perfusion with buffered neutral formalin. The experimental sites were excised. Undecalcified sections of the sites were prepared and embedded in a mixture of glycol methacrylate resin and Technovit 7200 VLC (Heraeus Kulzer, Wehrheim, Germany). Five buccolingual sections parallel to the long axis of the implants were cut with a diamond band saw and polished on an exact microgrinding system to a thickness of approximately 60 µm. The sections were then stained alternatively with hematoxylin-eosin (h&e) and toluidine blue. The following parameters were subsequently evaluated using an Olympus microscope (Olympus, Tokyo, Japan) at the section representing the central area of the implant at a 100× magnification by means of SigmaScan measuring software (Systat, Richmond, CA):

Table 1 Exposure of the Implant Threads According to Membrane Type

Membrane	No. of Threads Exposed													
	0		1		3		4		6		8		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
None	32	80.0	4	10.0	1	2.5	3	7.5					40	100
Gore-Tex	11	55.0	7	35.0	2	10.0							20	100
Resolut	15	75.0	3	15.0			1	5.0			1	5.0	20	100
Vicryl	30	75.0	7	17.5					3	7.5			40	100
Total	88	73.3	21	17.5	3	2.5	4	3.3	3	2.5	1	.8	120	100

Table 2 Relative Volume of Newly Formed Tissue Expressed as Area of the Healed Zone (mm^2)

	Filler	N	Subset for alpha = .05		
			1	2	3
Student-Newman-Keuls (1,2)	No filler	40	2.8584		
	Biocoral	40		3.6888	
	DFDB	20		4.1268	
	Autogenous bone	20			5.6341

- Degree of BIC as a percentage of the implant surface
- Height of the bone healing (ie, percentage of the total coverage of the exposed threads of the implants) (Table 1)
- Volume of the healed defect as reflected in the area filling the defect, calculated in mm^2 (Table 2)

The following parameters were determined at a magnification of 160× with a Zeiss II integrating reticle (Carl Zeiss, Oberkochen, Germany): quality of the tissue in the healed defect, fractional resorption surface area, fractional formation surface area, fractional resting surface area. A total of 150 intersections were counted when evaluating surfaces and 200 intersections when evaluating areas. The quality of the tissue in the healed defect was expressed as the relative amount of marrow, bone, and filler, and was evaluated according to Gundersen and associates[32] (Table 3). The microscopic fields were chosen in equidistant steps parallel to the surface of the implants. The orientation of the reticle was changed through random rotation of the reticle between fields.

The activity of the bone in the healed defect was reflected in the

- **Fractional resorption surface area** ($S_{fract(r)}$) (μm^2): The extent of resorption lacunae as a percentage of the total trabecular bone surface within the defect. Resorption lacunae were identified as scalloped defects in the trabecular surface showing distinct erosion of the lamellar system in polarized light.
- **Fractional formation surface area** ($S_{fract(f)}$) (μm^2): The extent of osteoid-covered surface area as a percentage of the total trabecular bone within the defect.
- **Fractional resting surface area:** 100% minus the fraction recorded as resorptional or appositional (Table 4).

Statistics

In the statistical evaluation, the following *independent variables* were included:

- **Animal:** 10 animals, 5 parasagittal sections (duplications) per animal
- **Implants:** 120 implants
- **Surface preparations:** uncoated titanium and HA-coated titanium
- **Membranes:** Gore-Tex, Vicryl, Resolut (R4)
- **Filler:** Biocoral, autogenous bone, and DFDB
- **Time:** 5 observation periods

The *dependent variables* comprised the parameters obtained by the histomorphometric analysis.

The statistical analysis was carried out in five steps. The first step concerned the influence of surface preparations. In this respect, the experimental design was completely balanced. Because an analysis of variance (ANOVA) demonstrated that the only parameter influenced by the coating was the BIC, the results obtained for the 2 types of implants were

Table 3 Change in Composition of the Healed Defects in Relation to Time

	Healing time (mo)									
	3		6		9		12		18	
Membrane	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Bone (%)										
Vicryl/Biocoral	39.87*	2.09	56.51*	3.09	56.95*	2.16	71.92*	3.94	72.62*	3.69
No membrane/Biocoral	39.10*	1.55	62.77*	2.10	55.54*	4.61	63.47*	3.44	65.56*	2.56
Gore-Tex/Biocoral	39.60*	2.29	48.80**	2.41	53.88*	1.74	58.80*	3.40	69.23*	2.26
Vicryl/autogenous bone	38.04*	2.91	59.79*	4.62	54.40*	4.34	51.92**	2.67	66.63*	4.35
R4/DFDB	58.46**	2.21	49.13**	3.87	53.17*	1.53	68.33*	2.96	59.75*	5.38
Control	34.50*	4.26	59.20*	6.97	69.63*	3.83	71.42*	4.42	60.19*	5.54
Marrow (%)										
Vicryl/Biocoral	38.24*	7.11	32.52*	4.30	29.91*	2.68	21.09*	3.57	13.66*	1.78
No membrane/Biocoral	33.90**	2.11	23.54*	1.70	31.37*	4.68	20.51*	2.68	33.38**	4.35
Gore-Tex/Biocoral	28.68**	2.15	28.72*	2.45	31.77*	1.68	30.32*	2.76	39.81**	5.54
Vicryl/autogenous bone	61.96***	2.91	40.21*	4.62	45.60**	4.34	48.08**	2.67	18.31*	2.38
R4/DFDB	41.54*	2.21	50.88**	3.86	46.83**	1.53	31.67*	2.96	21.85*	2.87
Control	65.50***	4.26	40.80*	6.97	30.38*	3.83	28.58*	4.42	40.25**	5.38
Filler (%)										
Vicryl/Biocoral	21.89*	5.61	10.97*	2.41	13.14*	4.65	6.99*	0.69	13.72*	2.25
No membrane/Biocoral	27.00*	2.57	13.69*	1.29	13.10*	1.92	16.02*	1.21	12.59*	1.42
Gore-Tex/Biocoral	31.73*	2.19	22.48**	2.24	14.34*	1.33	10.88*	1.47	12.46*	0.62
Vicryl/autogenous bone	NA		NA		NA		NA		NA	
R4/DFDB	NA		NA		NA		NA		NA	
Control	NA		NA		NA		NA		NA	

The samples labeled with the same number of asterisks belonged to the same subgroup. Those with 1 asterisk differed significantly from those with 2 ($P < .05$).

Table 4 Change in the Dynamic Parameters in Relation to Time

	Healing time (mo)									
	3		6		9		12		18	
Membrane	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Resorption (%)										
Vicryl/Biocoral	21.52*	3.13	25.68*	2.24	19.38*	1.22	22.21*	2.73	22.31*	1.12
No membrane/Biocoral	22.30*	1.72	21.79*	1.19	21.50*	2.75	20.83*	1.30	18.56*	1.21
Gore-Tex/Biocoral	20.54*	1.83	23.73*	2.05	22.21*	0.77	28.60*	2.16	18.93*	0.71
Vicryl/autogenous bone	18.52**	1.50	20.73*	1.06	18.77*	1.63	14.32**	2.24	15.98**	1.21
R4/DFDB	25.08*	2.33	18.92*	1.67	19.98*	0.96	28.33*	0.85	20.88*	1.83
Control	17.12**	1.80	16.24**	1.32	19.46*	1.03	17.56**	2.74	15.72**	1.03
Resting (%)										
Vicryl/Biocoral	39.59*	2.38	33.21*	2.62	42.58*	2.31	44.06*	3.57	52.27*	3.54
No membrane/Biocoral	38.75*	3.05	38.46*	2.47	49.38*	1.13	56.27*	4.19	60.08*	2.53
Gore-Tex/Biocoral	35.97*	3.13	32.75*	2.49	36.38*	1.26	46.92*	4.75	64.26*	1.91
Vicryl/autogenous bone	35.60*	1.40	43.33*	3.19	47.50*	4.41	70.68**	3.43	56.19*	2.75
R4/DFDB	41.38*	1.51	46.99*	2.44	43.05*	3.58	42.21*	2.09	53.81*	1.79
Control	36.08*	1.63	60.97*	2.32	45.86*	1.90	60.42*	9.40	69.11**	0.97
Formation (%)										
Vicryl/Biocoral	38.89*	4.51	41.11*	2.68	38.04*	1.54	33.73*	2.18	25.42*	3.99
No membrane/Biocoral	38.96*	3.59	39.75*	1.74	29.13*	1.62	22.90*	4.25	21.35*	1.70
Gore-Tex/Biocoral	43.50*	4.35	43.52*	2.08	41.42*	1.34	24.48*	3.31	16.81*	1.97
Vicryl/autogenous bone	45.88**	2.01	35.94*	2.91	33.73*	4.40	15.00*	1.36	27.83*	2.18
R4/DFDB	33.54*	1.86	34.09*	1.97	36.97*	3.07	29.46*	2.80	25.31*	3.29
Control	46.80**	2.89	22.79**	1.37	34.68*	2.43	22.02*	6.76	15.17*	1.07

The samples labeled with the same number of asterisks belonged to the same subgroup. Those with 1 asterisk differed significantly from those with 2 ($P < .05$).

pooled in the following analysis. In the second step, the influence of the different types of filler on the volume of the newly formed tissue for each time point was evaluated by a 2-way repeated-measurements ANOVA. The differences were evaluated by multiple paired *t* tests (Table 2). In the third step, a statistical description including the means and SE of the dependent variables was obtained for the available treatment combinations, and for each time point an ANOVA was performed, testing the impact of the specific treatment. Significant differences were detected by an a-posteriori test, and the subgroups were indicated in Tables 3 and 4.

Subsequently, healing over time was analyzed by a regression analysis, and the slopes, which represented the different treatment modalities, were compared using a 2-way ANOVA evaluating the impact of healing time and site preparation (Figs 2a to 2d).

Finally, the relationship between filler and membrane was analyzed using an ANOVA. Because of the lack of balance in the design, only the relationship between Biocoral and membrane could be analyzed.

RESULTS

In regard to the influence of the implant surface, it was found that both surface types demonstrated satisfactory BIC. BIC increased significantly over time in the case of both surfaces. The BIC contact was consistently higher in the case of HA-coated implants, and at the last observation time, the BIC for the HA-coated surface was significantly than that of the titanium surface (86.6% vs 73.3%; $P < .05$).

The vertical level of healing was satisfactory in most cases. However, there were some cases in which the implant remained exposed. This was most frequently seen at sites where a Gore-Tex membrane was used (Table 1).

The relative volume of newly formed tissue was significantly larger when the defect had been filled with autogenous bone than with any of the other fillers, and in the case of no filler (control), the volume was significantly smaller than when the defect was filled with Biocoral or DFDB (Table 2).

The statistical description of the relative percentages of bone, filler, and marrow appears in Table 3, and the significant differences were subsets disclosed by the a-posteriori test performed after the ANOVA performed for each time point. In relation to all the treatment groups, apart from the cases where the defect was filled with DFDB, it was found that the relative percentage of bone increased from 35% to 40% to 60% to 73% (Table 3). The DFDB was difficult to differentiate from the surrounding bone, but it seemed encapsulated and inactive over the total observation period. After 18 months, the relative percentage of bone did not differ within or between the treatments. The percentage of marrow was largest in the control defects and in those filled with autogenous bone at 3 months. With time, the relative part of the defect filled with marrow decreased and after 18 months was smallest in the defects that had been filled with Biocoral. There was a tendency toward a reduction in the relative percentage of filler over the observation period in the Gore-Tex group. The increase at 18 months was not significant. In the no-membrane group, there was a drop between 3 and 6 months, after which no significant changes were observed.

According to the ANOVA and the regression analysis used to evaluate the change of the parameters over time, the slopes deviated significantly from 0 in the cases of relative bone density and relative amount of filler. The slopes representing these parameters were, on the other hand, not influenced by the type of treatment (Table 3 and Fig 2a).

Not only the quality of the tissue filling the defect but also the ongoing remodeling of the defect is of importance. When the dynamic of the bone evaluated by the relative extension of the resorption and apposition was estimated, it was found that the extent of resorption exhibited large variation. However, there was no significant change over time, although the control and the defects with autogenous bone demonstrated less resorption than the defects filled with Biocoral and DFDB. There was no difference in regard to the membrane used. The fractional resorption surface area averaged 23% and ranged from about 16% to 28% (Table 4). The extension of apposition was largest in the control defects and in the defects filled with autogenous bone after 3 months, but this difference disappeared after a longer observation period. The extension of apposition decreased, however, evenly over time, starting at 34% to 47% and ending between 15% and 27% after 18 months. Correspondingly, the extension of the inactive surface did increase over time; this increase was unrelated to the type of treatment.

Because of the design of the study, it was only possible to evaluate the interaction in the case of Biocoral as filler. The results of the analysis did not favor any of the membranes. If the defects did not open and the Biocoral was maintained, the addition of a membrane did not change the healing pattern.

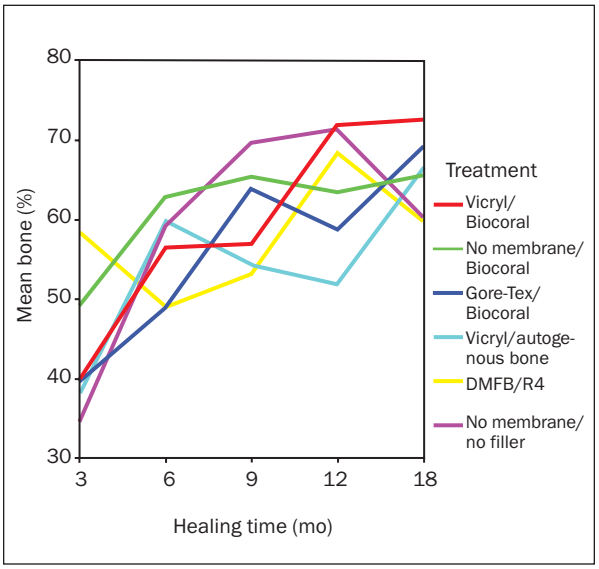

Fig 2a Bone percentage in relation to time and treatment.

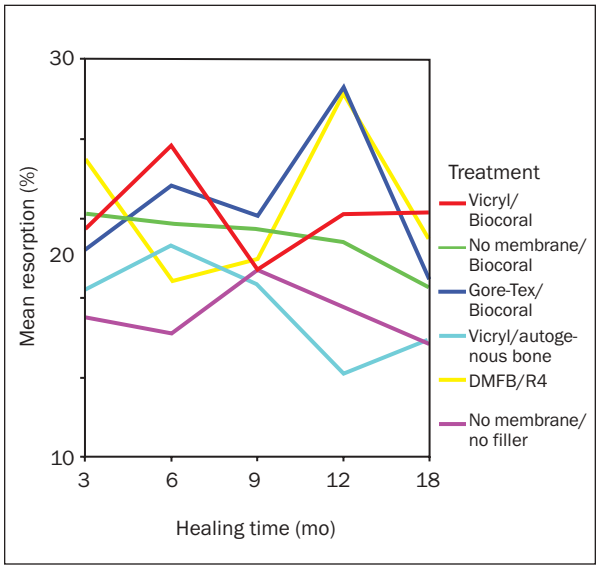

Fig 2b Relative extension of resorption surface in relation to time and treatment.

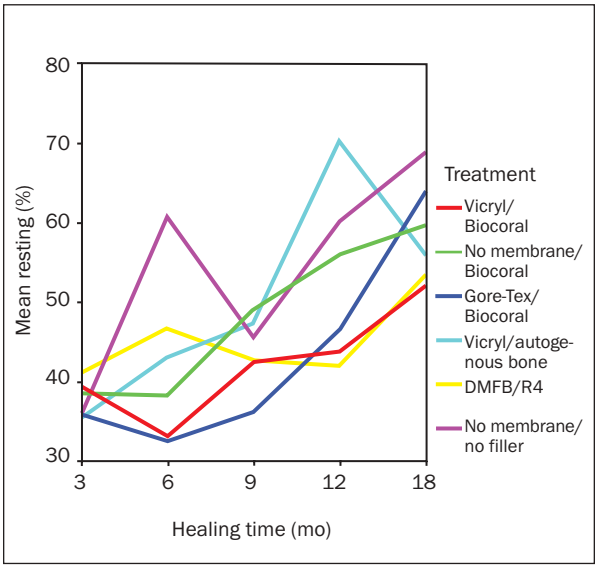

Fig 2c Relative extension of resting surface in relation to time and treatment.

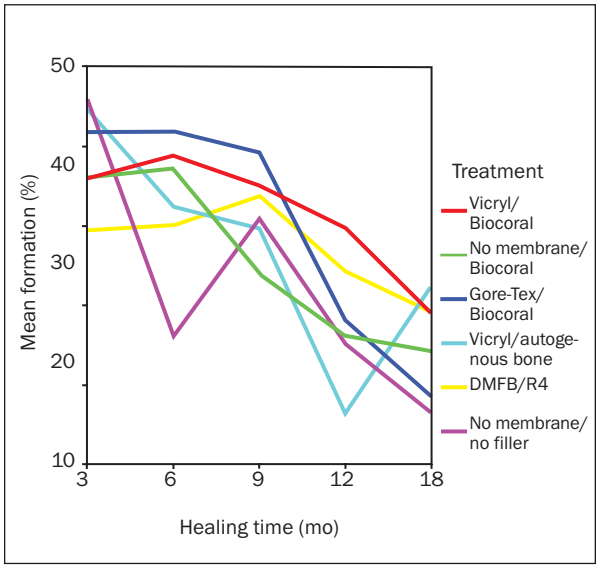

Fig 2d Relative extension of formation surface in relation to time and treatment.

DISCUSSION

The healing of 120 bony defects into which implants had been placed in the mandible of baboons was studied for between 3 and 18 months. The experimental animal model allowed for the placement of 2 × 6 implants in identical environments in the lateral alveolar process of the mandible. Thus the influence of different factors such as filler material and membranes could be compared. Another advantage of using nonhuman primates is the similarity in bone reaction to that of the human.[33–35]

Whereas the surgical procedures could be controlled and performed in an identical manner, the healing of the membrane-covered defects was complicated by exposure of the membrane and subsequent inflammation. Three different fillers and membranes were applied. However, the design of the study only allowed for analysis between Biocoral and the various membranes.

The finding that a hydroxyapatite coating significantly enhanced the approximation of bone to the implant corroborated the findings of Soballe and

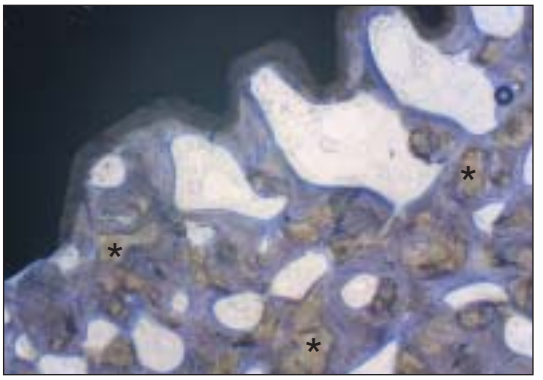

Fig 3a Micrograph 3 months following surgery of a defect filled with Biocoral adjacent to an HA-coated implant (toluidine blue; original magnification ×40). * indicates Biocoral.

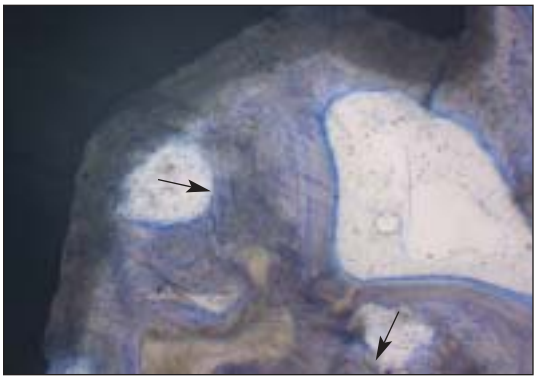

Fig 3b Image from fig 3a at a higher magnification. Arrows indicate where resorption occurred (toluidine blue; magnification ×200).

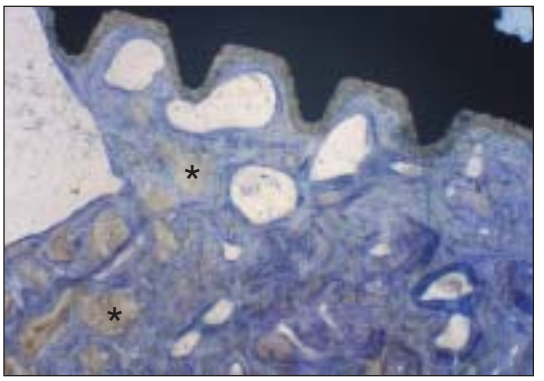

Fig 3c Micrograph 18 months following surgery of a defect filled with Biocoral adjacent to an HA-coated implant. Note the reduction in the amount of filler (toluidine blue; original magnification ×40). * indicates Biocoral.

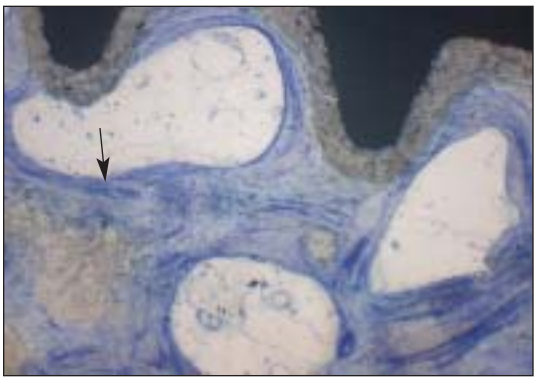

Fig 3d Image from Fig 3c at a higher magnification. Arrow indicates where resorption occurred (toluidine blue; original magnification ×100).

coworker[36,37] and Overgaard and colleagues,[38] who described bone formation even across an initial gap surrounding the implant. There seems to be general agreement on these findings.[39–41] On the other hand, the surface coating had no impact on any of the other variables, and the results obtained with the 2 types of implants were therefore pooled in subsequent analysis.

The bone volume generated in the defect was enhanced by both filler and membrane. The lowest volume was found where neither a membrane nor filler was used, and the highest where the defect had been filled with autogenous bone and covered with a membrane that did not become exposed (Table 2).

The initial healing of bony defects surrounding implants has been studied extensively.[42–45] The present study demonstrated that a steady state of turnover had not yet been reached 18 months postplacement. The regression analysis applied to the analysis of the impact on time in the different types of treatment indicated that the presence of membranes did not influence the continuous remodeling, whereas the presence of the filler, which was still under replacement, enhanced the activity level of the bone (Figs 2b and 2d). The ongoing remodeling adjacent to the implants in the absence of filler and membrane has previously been described by Steflick and associates[46,47] and Chen and coworkers.[48,49] The latter authors suggested that the phenomena reflected a mode of adaptation to the difference in stiffness of the implants and the surrounding tissues. Even left unloaded, as in this study, the strain generated during normal function differs for the implant and the surrounding bone, and it can be expected that the remodeling is ongoing, rendering bone that is better adapted to stress absorption. The present study also provided evidence for the ongoing repair of a defect at a distance from the implant.

Use of Biocoral as a filler has previously been described by Hippolyte and associates[50] and by Soost.[51] The results of the present study did indicate that the majority of the Biocoral had been replaced by bone after 6 months of observation (Fig 3). A 5-year follow-up of 16 patients in whom Biocoral had been used as a bone replacement corroborated its beneficial effect,[52] which was also described in a sur-

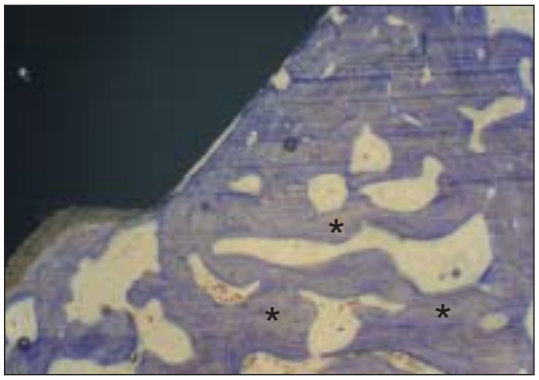

Fig 4a Micrograph 3 months following surgery of a defect filled with DFDB adjacent to an HA-coated implant (toluidine blue; original magnification ×40). * indicates the filler.

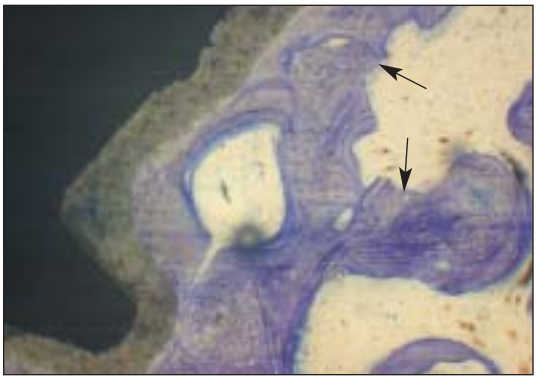

Fig 4b Image from Fig 4a shown at a higher magnification to demonstrate the resorption *(arrows)* (toluidine blue; original magnification ×200).

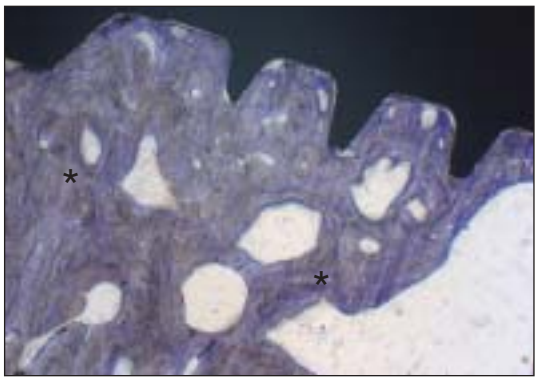

Fig 4c Micrograph 18 months following surgery of a defect filled with DFDB adjacent to an uncoated titanium implant (toluidine blue; original magnification ×40). * indicates the filler.

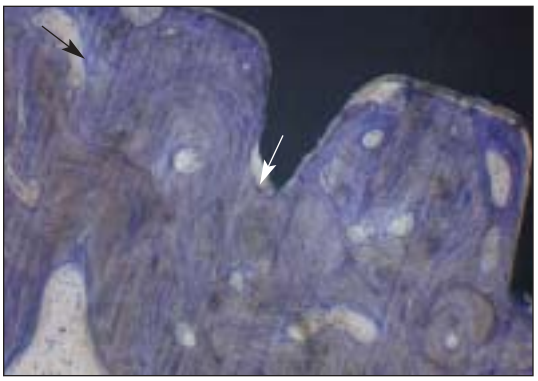

Fig 4d Image from Fig 4c shown at a higher magnification. Resorption indicated by arrows (toluidine blue; original magnification ×100).

vey by Shors.[53] In the present study, the results achieved with autogenous bone did not differ from those achieved using Biocoral (Figs 3 to 5).

The results indicated that the slowest and smallest increase in density was seen where the filler was DFDB. The healing found in relation to DFDB was disappointing; however, it corroborated previous studies.[24,54] Both the carrier and the preparation have been demonstrated to have a significant impact on the reaction to the insertion of DFDB.[55] In addition, the environment into which the substance was placed may not be comparable with the model normally used for the study of the effect of DFDB. When Tal and coworkers[55] described the tissue reaction to DFDB, they also found that the allograft became encapsulated. This does not interfere with the positive clinical finding reported.[56,57]

The influence of the membrane on the healing process was not significant; however, poorer results were seen with the Resolut R4 membrane than with the Gore-Tex, the Vicryl, or even the control. The remodeling process leading to the healing was influenced by the filler. The present studies thus corroborated a previous study that found that the inflammatory reaction related to the dissolution of the Vicryl membrane was only of minor importance.[58] This again confirms that the dissolution process has little impact on the vital healing process taking place within the defect.

The interaction of the membrane and the filler could only be analyzed in cases where Biocoral was used; the ANOVA revealed that the membrane did not significantly change the impact of Biocoral as a filler. This fact may be ascribed the quality of the baboon periosteum; baboon periosteum, being very thick and strong, may have served to replace the effect of the covering membrane.

The study showed that some healing of a defect into which an implant is placed would take place even in the absence of filler and membrane. The healing of a defect adjacent to an implant does improve by any approach aiming at maintaining the space, whether a stiff membrane or a filler is used. Based on these findings, a bony defect cannot be considered an obstacle for the planning of a reconstruction involving implants.

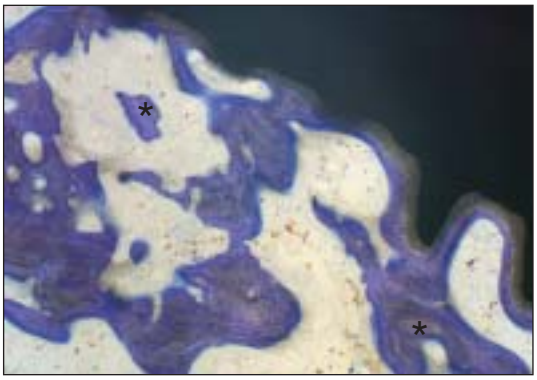

Fig 5a Micrograph 3 months following surgery of a defect adjacent to an HA-coated implant filled with autogenous bone *(*)*. The bone is already completely resorbed (toluidine blue; original magnification ×40).

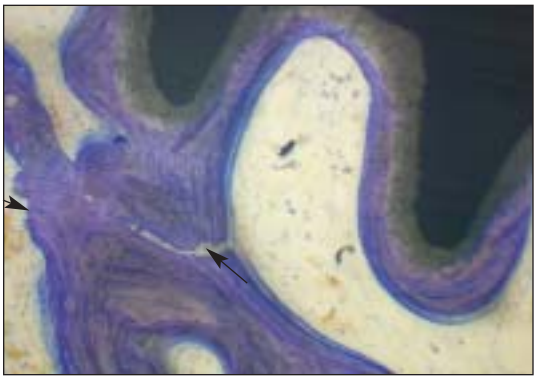

Fig 5b Image from Fig 5a shown at a higher magnification. Resorption indicated by arrow (toluidine blue; original magnification ×100).

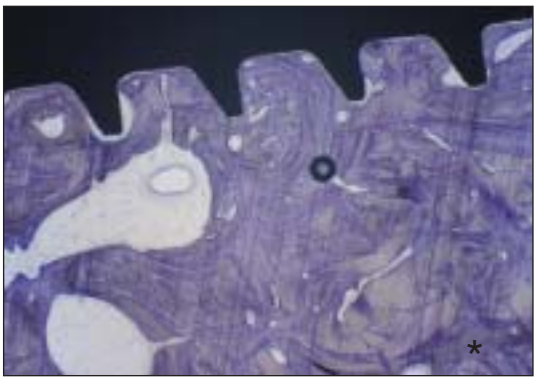

Fig 5c Micrograph 18 months following surgery of a defect adjacent to an uncoated titanium implant filled with autogenous bone. The bone turnover was low and almost no signs of the filler are present (toluidine blue; original magnification ×40).

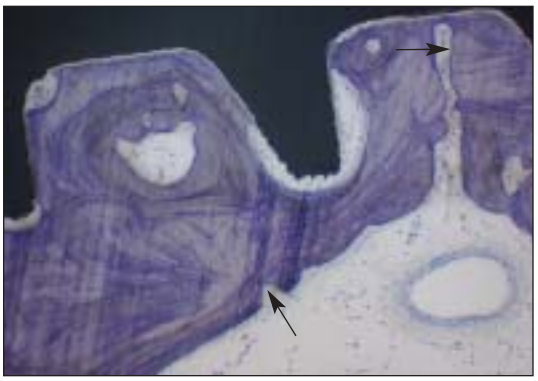

Fig 5d Image from Fig 5c shown at a higher magnification. The bone is characterized by a very low turnover *(arrow)* (toluidine blue; original magnification ×100).

CONCLUSION

The study was performed with the purpose of comparing the influence of the membrane and the filler on the healing of a bony defect surrounding a dental implant. The results supported autogenous bone as the gold standard, but both DFDB and Biocoral compared favorably. Since even the sterilization of DFDB cannot exclude the possibility of disease transmission, Biocoral may be considered a recommendable material. Both filler materials were resorbable and thus were gradually replaced by bone, a process that was still ongoing after 18 months. The filler as well as the membranes contributed to the re-establishment of the original volume. Better results were achieved with the Vicryl and Gore-Tex membranes than with the Resolut (R4). Based on these results, it is suggested that a bony defect is not a contraindication for the placement of an implant.

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