When implants are considered for patients with deficient alveolar bone, reconstruction of the alveolar ridge prior to or simultaneously with implant placement is mandatory to achieve complete osseointegration of the implants, a prerequisite for long-term success. For the repair of osseous defects, fresh autogenous bone has been widely used in combination with implants and still constitutes the first choice. Since considerable morbidity may be associated with graft procurement procedures, alternative means of replacing lost bone aimed mostly at the enhancement of local bone regeneration to obviate the need for autogenous bone transfer have been considered. The most recent concepts in this regard are the use of membranes for guided bone regeneration, or the application of an increasing number of growth factors both in combination with and without membranes to induce bone formation in defect areas.

Recent experimental results indicate that the use of barrier membranes can direct bone fill not only to contour defects of the alveolar ridge, but also to grow beyond the level of the surrounding bone, thus forming excess bone to a considerable extent. An earlier pilot study in a small sample of laboratory animals showed that excess bone formed within 5 months under a degradable polylactic membrane in a block-shaped scaffold of pyrolyzed bovine bone on the lateral aspect of the mandible. This bone could be harvested and transferred for onlay grafting to the mandible. The successful outcome of this pilot study has encouraged a larger series investigation to

**Mandibular Onlay Grafting Using Prefabricated Bone Grafts with Primary Implant Placement: An Experimental Study in Minipigs**

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The aim of this experimental study was to evaluate the use of prefabricated autogenous bone grafts as onlay grafts to the mandible. Excess bone of 10 × 12 × 40 mm was produced inside blocks of pyrolyzed bovine bone under a polylactic membrane coverage on the outside of the mandible in 15 adult Göttingen minipigs. After 5 months, this bone was harvested and transferred to the premolar region of the mandibular body in 10 animals. Onlay grafts of mandibular bone were used as controls for the transplanted prefabricated grafts. All grafts were fixed by primary placement of one titanium implant each. Five animals served as ungrafted controls. Evaluation was performed after 3 months and 5 months, respectively. Two animals were lost to evaluation, and one scaffold became infected. Eleven of the remaining 12 scaffolds showed sufficient bone ingrowth for grafting. Three months after transplantation, bone volume of the prefabricated grafts was almost completely preserved, with only minimal resorption in the superficial pores of the scaffolds, while the control grafts exhibited partial resorption. The titanium implants, which had been placed at the time of onlay grafting, exhibited direct bone-implant contact. Five months after grafting, all titanium implants showed complete osseointegration, with direct bone-implant contact. The grafted bone exhibited a significant increase in bone density by appositional bone formation. The control grafts were nearly completely resorbed at that time.

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Key words: guided bone regeneration, onlay grafts, prefabricated bone grafts, titanium implants
further elucidate the potential of these prefabricated grafts, which are cultivated in vivo on the facial skeleton under membrane coverage for subsequent reconstruction of osseous defects.

The present experimental study uses prefabricated bone grafts, which were cultivated on the mandible by guided bone regeneration, for onlay grafting with primary implant placement. The aim of this investigation was to analyze revascularization of these grafts and to determine structural changes and volume maintenance at different post-transplantation intervals. Finally, osseointegration of titanium implants was assessed.

Materials and Methods

Surgical Procedures. The experiments were carried out on 15 adult female Göttingen minipigs, (average weight 37.5 kg) under general intravenous anesthesia (Methomidate and Fentanyl, Janssen, Neuss, Germany) and endotracheal ventilation (5 L nitrous oxide, 2 L oxygen). After undergoing exposure of the lateral aspect of the mandible through an extraoral submandibular approach, each of the animals received subperiosteal implantation of three blocks of pyrolyzed bovine bone, each 10 × 12 × 12 mm (Endobon, M erck, D armstadt, Germany), in the area of the mandibular angle (Figs 1a and 1b). This material is fabricated from cancellous bovine bone from which all organic components are removed by heat treatment (1100 to 1300°C), resulting in a pure hydroxyapatite scaffold with interconnecting porosity of 30 to 80% and pore diameters between 100 and 1500 µm. The total implanted scaffold volume approximated 4.5 cm³ (Fig 1c). Before placement of the blocks, the surface of the cortical bone was punctured with a small bur. The blocks were then covered by a polylactic membrane (70/30 L/DL, inherent viscosity 3.3 g/dL, thickness 500 µm). Prior to implantation, the stiff membrane was contoured by a custom-made device over a heated metal phantom whose dimensions corresponded to the volume of the blocks (Figs 1a and 1b). Fixation of the membrane coverage and the blocks was then accomplished by two titanium miniplates each (Synthes, Waldenburg, Switzerland), followed by wound closure in three layers with resorbable sutures (E thicon, N orderstedt, Germany).

The 15 animals were divided into three groups of five animals each according to the following classifications:

Group 1: Implantation of scaffolds for cultivation of prefabricated bone grafts for 5 months. Evaluation after harvesting without subsequent transfer.

The International Journal of Oral & Maxillofacial Implants
Group 2: Implantation of scaffolds for cultivation of prefabricated bone grafts for 5 months. Harvesting and transfer as onlay grafts to the mandible. Evaluation 3 months after onlay grafting.

Group 3: Implantation of scaffolds for cultivation of prefabricated bone grafts for 5 months. Harvesting and transfer as onlay grafts to the mandible. Evaluation 5 months after onlay grafting.

In groups 2 and 3, harvesting of the grafts was performed following exposure of the implanted scaffolds through a submandibular approach after 5 months of cultivation. The scaffold volume was then detached from the underlying mandibular bone by means of fine drills and chisels and separated into two halves. One of these halves was used for bridging of mandibular defects on the opposite side of the mandible. The results of this part of the study may be found elsewhere.23 The second half was transferred to the premolar area of the mandibular body for onlay grafting (Fig 2). To allow for histologic evaluation of bone formation at the time of grafting, a 1-mm slice was obtained from each graft before transplantation. Fixation of the prefabricated grafts was accomplished by simultaneous placement of a screw-type titanium implant (standard 15-mm length, Nobel Biocare, Gothenborg, Sweden), which was placed through the graft into the underlying mandibular bone according to routine procedures.24

Control onlay grafts of approximately 10 × 12 × 20 mm were obtained from the opposite side of the mandible, where segmental resections had been carried out during creation of a mandibular defect. These grafts were also fixed to the mandibular bone as onlay grafts by means of titanium implants (Nobel Biocare). Wound closure was again performed in three layers using resorbable sutures.

After completion of surgery, intravital polychrome fluorescence labeling was undertaken in groups 2 and 3 to monitor revascularization and remodeling of the prefabricated and control grafts. Four fluorochrome stains were administered subcutaneously in a series of 5 days each (tetracycline, 12 mg/kg body weight; calcein blue, 30 mg/kg; alizarin complexon, 30 mg/kg; and calcein green, 20 mg/kg; all stains manufactured by Fluka Chemie, Neu-Ulm, Germany). The animals in group 2 received labeling during postoperative weeks 2, 4, 8, and 10, while the animals in group 3 were labeled after postoperative weeks 4, 8, 13, and 17.

**Histologic Evaluation.** At the end of the scheduled interval, all grafts and implants were obtained, together with the surrounding bone, immediately fixated in buffered formalin (4%), and embedded into methylmethacrylate resin. Undecalcified serial sections of approximately 70-µm thickness were then made perpendicular to the long axis of the grafts using a saw with a diamond-edged blade (Leika, Hamburg, Germany). Sections 1, 4, 7, and so forth, were submitted to surface staining with alizarin/methylene blue and Giemsa solution; sections 2, 5, 8, and so forth, were left unstained for fluorescence microscopy; sections 3, 6, 9, and so forth, were used for contact microradiography (Tungsten anode, 15 kV, 65 mA, 20-minute exposure, Faxitron, Rhode & Schwartz, Cologne, Germany) on glass plates (emulsion grain 1 µm) (Eastman Kodak, Rochester, NY). The microradiograms were then submitted to automated computerized morphometry (IBAS, Contron, Erching, Germany) of bone volume density and structural parameters (thickness of bone trabeculae, bone-covered scaffold surface) through a video camera (DXC-M2; Sony, Cologne, Germany) by means of microdensitometry, the principle of which has been previously described.25 Quantitative results were tested for statistical difference between the groups by a Mann-Whitney U test at a significance level of .05.

In four animals, degradation of the polylactic membranes was investigated on specimens obtained at the time of harvesting 5 months after implantation. This was done using gel permeation chromatography. The specimens from the polylactic membrane were
dissolved in 5 mg/mL chloroform solution (Fluka Chemie) and driven through gel piles, SDV 300 mm, 5 µm, 1000 Å/10^3 Å/10^6 Å (Polymer Standard Service, Mainz, Germany) by a pump (Spectra-Physics P2000, Thermo Separation Products GmbH, Darmstadt, Germany) in quantities of 100 µL. Eluates were analyzed by means of a detector (Shodex RI-71, Thermo Separation Products), and the molecular weight of the polylactic remnants was assessed against polystyrol standards (Polymer Standard Service) as a mean value of four individual measurements. Results were compared to the original molecular weight by means of analysis of variance (ANOVA).

Results

Two animals died from pneumonia after implantation of the blocks. In the remaining animals, healing was uneventful, with the exception of one animal from group 1, in which infection of the implanted material occurred 13 weeks after surgery. At the time of harvesting of the grafts, the polylactic coverage was still present. Milky discoloration of the membrane indicated some incorporation of water. However, the material itself did not show further macroscopic signs of resorption, and appeared to be as mechanically stable as it was prior to implantation.

During harvesting of the prefabricated grafts in groups 2 and 3, in most cases the mandibular canal was opened, indicating that the material had sunk 1 to 2 mm into the underlying bone. The grafts exhibited variable density and hardness, owing to variable bone density inside the scaffolds. However, bone formation had bridged the gaps between the individual blocks and had formed a stable bar of approximately 10 × 12 × 40 mm. With the exception of one graft, which showed insufficient stability for onlay grafting, all other grafts could be transferred to the mandibular body as onlay grafts and secured by titanium implants.

Clinical and Radiographic Findings. All blocks from group 1, which were not transplanted, were firmly fixed to the underlying bone. Those blocks that had become infected also appeared to be stable, but macroscopically no bone was found in the superficial pores of the scaffold. The onlay grafts from groups 2 and 3 were all stable on clinical examination at the time of retrieval. The control onlay grafts from the mandible had lost considerable volume compared to the prefabricated grafts. Plain radiographs showed that the gaps between the individual scaffold blocks had almost completely disappeared. No pathologic features, such as osteolysis around titanium implants or sequestration of prefabricated grafts, were found both 3 and 5 months after transplantation. The mandibular bone onlay grafts were hardly recognizable after 3 months and were virtually invisible after 5 months. The location of these grafts could be identified only by the titanium implants.

Histologic and Microradiographic Findings. Group 1. The infected blocks showed no bone ingrowth into the pores of the scaffold and were isolated from the underlying bone by fibrous tissue. In the remaining blocks, bone ingrowth had invaded the entire height of the blocks, thoroughly penetrating the entire scaffold. The surface of the scaffold facing the polylactic membrane container was covered with a small seam of fibrous connective tissue. Bone tissue underneath had a young and immature appearance, and a highly trabecular structure (Fig 3), and it was partially lined by osteoblasts and osteoclasts in some locations. Intertrabecular soft tissue was highly cellular and richly vascularized. In areas of minor bone ingrowth, degradation of the scaffold was observed, with phagocytized particles inside multinuclear giant cells and deposition of these degradation products in the adjacent soft tissues.

Group 2. The specimens, which had been obtained from the grafts at the time of transplantation, exhibited bone formation with ingrowth into the superficial pore layers in most of the sections. The bone was partially immature and highly cellular but also exhibited areas of compactness. Bone formation appeared to have commenced mainly on the surface of the scaffolds, but also exhibited patterns of primary perivascular osteogenesis and secondary deposition of bone onto the pore surfaces in some areas (Fig 4). Three months after transplantation, bone volume was almost completely preserved, with only minimal resorption of bone in the superficial pores of the scaffolds (Fig 5a).
Marrow spaces inside the transplanted bone did not appear to be as highly cellular as at the time of harvesting. In cases of incomplete bone fill of the scaffolds before grafting, fibrous soft tissue filled the superficial pore layers, and no substantial increase in the amount of bone was observed. The titanium implants, which had been placed at the time of onlay grafting, exhibited direct bone-implant contact in those sections of the scaffolds that had been filled with bone tissue before grafting (Fig 5b). Two of the three implants from this group were completely osseointegrated, while one exhibited bone anchorage of only two thirds of the implant height. The control grafts from the mandible likewise exhibited good volume preservation and rounding of the contours after 3 months. Only the edges of the graft facing the lower mandibular border appeared to be subject to rapid resorption. The cortical structure of the grafted mandibular bone had remained unchanged, and the titanium implants were completely osseointegrated (Fig 5c).

Group 3. The prefabricated grafts from this group exhibited similar features both before and 5 months after transplantation when compared to group 2. One of the five scaffolds had exhibited incomplete bone ingrowth prior to transplantation; however, all five titanium implants showed complete osseointegration, including direct bone-implant contact (Fig 6a), and the volume of the grafted bone inside the scaffolds was preserved after 5 months. The only appreciable difference in comparison to group 2 was an increase in bone density by appositional bone formation and a decrease in cell content and volume of marrow spaces in between (Fig 6b). In contrast, the control onlay grafts showed considerable resorption when compared to the 3-month period, with the titanium implants protruding far beyond the considerably reduced graft height (Fig 6c).
Fluorescence Microscopy. Group 2. The 2-week label (tetracycline) was present only in the basal portions of the graft and in the superficial layers facing the overlying soft tissue (Fig 7a), while the subsequent labeling after 4, 8, and 10 weeks exhibited fluorescence over the entire graft. These labels were arranged in a concentric manner, indicating remodeling and continuing bone apposition 4 weeks after transplantation (Fig 7b). Bone formation next to the titanium implants took place in a similar manner. In the basal portions, tetracycline was present immediately adjacent to the implant surface, while in the central and superficial parts of the grafts, the 10-week label (calcein green) predominated at the implant surface.
Group 3. As in group 2, the 4-week label (tetracycline) was present over the entire graft, with concentric deposition and partial replacement by subsequently administered labels. Bone formation and remodeling at the implant surface reflected the sequence of labels inside the graft: tetracycline was present all along the implant surface, but was intermittently replaced by subsequent fluorochrome labels (Fig 7c). The presence of the 17-week label over the entire graft indicated active remodeling and bone formation until the end of the 5-month observation period.

Quantitative Results. The average amount of bone formation inside the scaffolds under the polyactic membranes that were evaluated without transplantation was 47.6% of the implanted volume, which corresponds to a 64.6% fill of the pore volume. The average thickness of the newly formed bone was 102.7 µm, covering 87.5% of the scaffold surface (Fig 8).

Average volume density of bone tissue inside the prefabricated grafts 3 months after transplantation had increased to 55.7%. However, compared to group 1, this difference was not significant (P = .2752). The increased bone volume apparently resulted from a slightly increased thickness of bone apposition. Mean trabecular bone thickness in group 2 had increased to 128.2 µm; however, this difference likewise was not significant when compared to group 1 (P = .2850). The percentage of scaffold surface covered by bone tissue had increased to an average of 93.7%.

Five months after transplantation, the bone volume density had further increased to a mean value of 56.6%, which was significantly different compared to the nongrafted scaffolds of group 1 (P = .0495); it was not significant when compared to values 3 months after grafting. The same held true for mean trabecular thickness, which showed a significantly higher mean value of 156.2 µm after 5 months when compared to the nongrafted scaffolds (P = .0253), while the bone-covered portion of the scaffold surface was slightly smaller but without statistical significance (89.9%; P = .04561).

Molecular Weight of Polylactic Membrane. The molecular weight of the polylactic membranes had decreased from an initial value of 468.5 kd at the time of implantation to values between 48.7 and 67.9 kd. This decrease was highly significant (P = .0000).

Discussion

The present study has shown that the formation of excess bone beyond the level of the underlying host bone can be accomplished experimentally by providing a membrane-protected space that can be filled by proliferating bone tissue. Although two animals were lost to evaluation and one scaffold became infected, only 1 of the remaining 12 scaffolds available for transplantation showed insufficient bone ingrowth for grafting. Thus, the experimental model evaluated may be considered successful with regard to cultivation, harvesting, and transplantation of prefabricated bone grafts. The results confirm those of a previous pilot study in terms of the complications and pitfalls of this model. However, in contrast to that study, the polylactic membranes of the present study appeared to be nearly undegraded. This inconsistency may be explained by a variation in membrane thickness: the membranes used in the present study had a thickness of 0.5 mm, while those used in the pilot study had a thickness of only 0.2 mm. The significant decrease in molecular weight of the membrane material at the time of harvesting, however, indicates that degradation had already commenced, although no change in
volume or shape had occurred until that time. This reconfirms that polymers of poly-α-hydroxy acids degrade in a biphasic manner, since loss of molecular weight occurs long before mass loss and volume degradation are appreciable.26

The lack of bone deposition on the pore surfaces in areas of degradation of the scaffolds may be the result of local adverse reactions to possible contaminants on the material surface that prevented deposition of bone tissue, or it may represent a secondary phenomenon resulting from simultaneous resorption of bone tissue already laid down on the scaffold surface. The size of the phagocytized scaffold fragments corresponded well to the size of the hydroxyapatite crystals on the surface of the blocks,27 and paralleled the findings of de Brujn et al.,28 who also described superficial degradation of hydroxyapatite implants. The resorption of the cultivated bone, which was observed at the time of harvesting, may be associated with the commencing degradation of the polylactic membrane on the outside of the block, or it may be related to insufficient stability of the titanium plates, causing continuous irritation of tissue underneath the polylactic membrane.21

The question may arise whether this complex prefabrication procedure is necessary for the augmentation of alveolar ridges. Previous studies of porous alloplastic material in conjunction with endosseous implants had shown that empty porous blocks of hydroxyapatite could not be safely stabilized by means of endosseous implants.29,30 Many block fractures follow by dislocation of porous material, without ingrowth of bone tissue or substantial bone-implant contact, were encountered. Bone ingrowth did occur in some of the blocks in that study, but mainly because the material was contained in a defect of the alveolar ridge. However, the current trial used a pure onlay model, which requires reliable fixation of the entire block. This became possible by means of thorough penetration of bone tissue into the scaffolds prior to their use as onlay grafts.

The pre-existing bone tissue inside the scaffolds at the time of onlay grafting also appeared to be the major source for osseointegration of the titanium implants, since no bone-implant contact was seen in sections of the scaffolds with incomplete bone ingrowth at the time of transplantation. The structure of the bone immediately adjacent to the implant surface did not differ from the bone structure of the remaining graft. This result contrasts with previous histologic findings of the implants and bone grafts, which have shown a lamella of dense bone immediately adjacent to the implants, while the original loose cancellous structure of the remaining graft was preserved.30 The differences in the present study, however, may be explained by the relatively high volume density of bone tissue of the prefabricated grafts at the time of transplantation, which underwent further appositional bone formation and densification.

The distribution of sequential fluorochrome labels showed that vascularization of the onlay grafts was complete no sooner than 4 weeks after grafting, and that revascularization started from both the underlying bone and the overlying soft tissues. The bone inside the onlay graft apparently underwent continuing remodeling and bone formation through the end of the observation period. The position of labels relative to the implant surface suggested that direct implant-bone contact with newly formed bone had been established after approximately 10 weeks but was constantly remodeled and replaced, as revealed by the fact that labels from later intervals were also found next to the implant surface.

In this way, revascularization and remodeling of prefabricated bone grafts did not differ from conventional bone grafts with regard to the time required for complete integration of the graft.31 Thus, it was unexpected that the control grafts from the mandible showed such a high degree of resorption in contrast to the prefabricated grafts. Several factors affect the maintenance of graft volume after onlay grafting, such as embryonic origin, graft structure, and mode of fixation, all of which are expected to have a direct or an indirect effect on the degree and velocity of graft revascularization.32–35 The relationship between vascularization and volume preservation appears to be somewhat inconsistent, since the results of Albrektsson and Lindner36 revealed that revascularization is also a precondition for osteoclastic resorption. However, the two types of grafts in the present study exhibited a high degree of identity with regard to the currently discussed factors: both contained mandibular, ie, membranous bone, both were used as onlay grafts to the mandible, and both were fixed to the underlying bone with titanium implants by means of an identical procedure. Besides the fact that the two grafts were placed in slightly different positions, the only difference was that the prefabricated grafts contained the scaffold or pyrolyzed bovine bone. Since it is unlikely that the scaffold had a protective effect against resorption, the reasons for this difference in volume maintenance are at present unclear.

The potential danger of bone resorption, however, highlights a problem with the use of nonresorbable alloplastic scaffolds for cultivation of prefabricated bone grafts. Since bone resorption is also likely to occur to a certain degree in prefabricated grafts, the nonresorbable porous scaffold will be left behind with empty pores, which are most likely located on the outer surface of the graft. With only thin mucosal
coverage over these empty scaffolds, the probability for mucosal dehiscence and subsequent infection may be increased, raising the question of whether alloplastic materials should be used for these purposes.

With regard to the desired bone volume, the grafted bone is obtained by regenerating bone tissue from the underlying mandible, and there is clinical evidence that the maximum obtainable increase in bone height through bone regeneration using barrier membranes without additional measures is limited to approximately 4 mm (Simion M, personal communication, 1995). This limit may be explained by the fact that the pluripotent osteoprogenitor cells that invade the empty space under the membrane and fill it with bone tissue must become attached to the surface of a matrix analogous to the marrow space with a pore size close to that of natural bone before they may proliferate and reach the final step of differentiation to bone-producing osteoblasts. In this way, the use of a scaffold seems to be necessary to enable regenerating bone tissue to occupy larger volumes, thus allowing for the cultivation of larger prefabricated bone grafts. In this way, the development of resorbable calcium phosphate materials with an interconnecting porous structure should improve the potential of this experimental model.

Conclusion

This study has shown that prefabricated bone grafts can be cultivated in vivo in a porous scaffold and transplanted as onlay grafts to the mandible. Osteointegration of simultaneously placed titanium implants was accomplished 3 months and 5 months after grafting, while the grafted bone underwent further appositional bone formation and an increase in density. To avoid problems with nonresorbable scaffolds during remodeling and resorption of the grafted bone, the use of resorbable scaffold materials of appropriate macrostructure is advocated.

Acknowledgments

The authors wish to express their gratitude to Prof L. Claes and Dr A. Ignatzius from the Department of Surgical Research and Biomechanics of the University of Ulm, Germany, for performing the determination of the molecular weight of the polyactic membrane specimens. This study was supported by the Deutsche Forschungsgemeinschaft (DFG) (Schl 368/1-1).

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