Comparative Study of Guided Bone Regeneration Using Absorbable and Permanent Barrier Membranes: A Histologic Report

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In an experimental study using the Göttingen minipig, Gore-Tex, Gore Resolut, and Vicryl barrier membranes were tested for their efficacy in guided bone regeneration. The results were compared to those of autologous periosteum. The strongest reossification was seen in the bone defects covered with Gore-Tex; however, time-dependent disintegration phenomena, which had already been observed in preliminary examinations, were clearly established. After investigations of cell cultures to which human macrophages were added, the latter findings can be interpreted as the result of a physicochemical process, since a direct attack by the macrophages was not seen in vitro. Of the membranes used, the absorbable ones tended to collapse, depending on the size of the defect that was used, and they did not enhance reossification as much as did the permanent membranes. The periosteum-covered defects showed a satisfactory degree of regeneration, and no differences were observed between freely transplanted and pedunculated periosteum.
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Key words: absorbable/nonabsorbable barrier membranes, disintegration phenomena, Gore-Tex membranes, guided bone regeneration

The principle of guided bone regeneration has been used widely since the end of the last decade, particularly in periodontics and implant dentistry.[1–9] After preliminary experiments with semipermeable millipore filters,[10] the clinical application of expanded polytetrafluoroethylene (e-PTFE) was established.[11–13]

Some investigators have attempted to avoid the necessity of a reentry operation to remove this permanent membrane system by using absorbable membrane systems such as lactid or glycolid polymers.[14–17] However, to the authors' knowledge, a direct comparison of the usefulness of absorbable and nonabsorbable membranes in guided bone regeneration has not been completely presented in the literature. In this animal test using the Göttingen miniature pig under standardized conditions, the efficacy of Gore-Tex (e-PTFE), Gore Resolut, and Vicryl membranes and of autologous periosteum in guided bone regeneration was examined.

In a previous study under the described experimental conditions, reossification of artificial desmal bone defects was not satisfactory after 8 weeks using an absorbable membrane (Ethisorb). The strongest reossification took place with e-PTFE membranes, which also proved to be permeable to cells. However, time-dependent disintegration phenomena of this permanent membrane system, which is believed to behave inertly, were examined.[18] Periosteum-covered defects also displayed a satisfactory degree of regeneration; no differences between freely transplanted and pedunculated periosteum appeared after 8 weeks.

Identical standardized conditions were used to compare these properties of Vicryl and Gore Resolut membranes directly with those of Gore-Tex membranes. However, a 4-month observation period was

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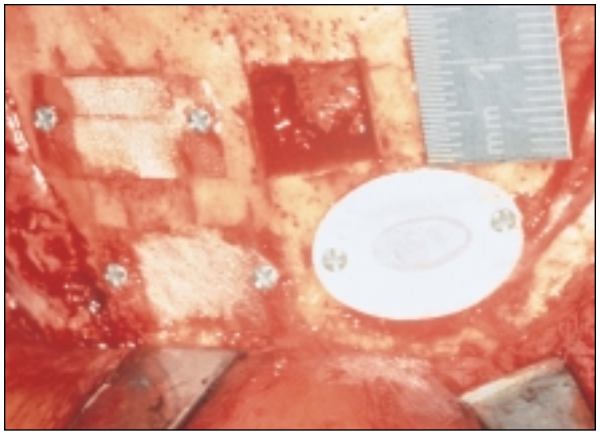


Fig 1a Size and shape of the desmoid bone defects in the os frontale. The defects were covered with various absorbable and permanent membranes, and with autogenous periosteum. One defect was left uncovered to serve as a control. Microscrews were placed into the rim of the defect centers to secure the membranes and for radiologic marking of the center of the defect.

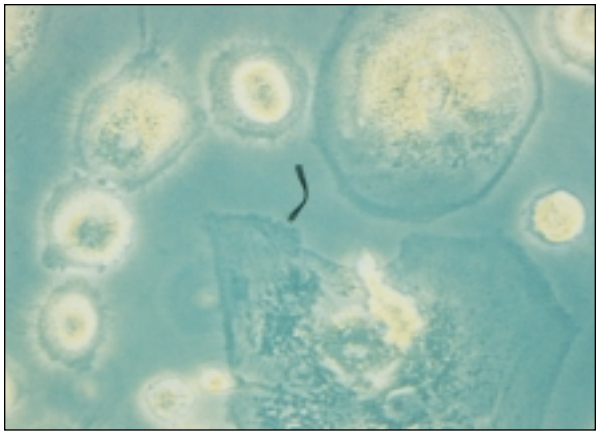


Fig 1b Macrophage control for demonstration of vital macrophages after 2 months (videoprint from the cell culture).

added. Furthermore, the dissolving process of the e-PTFE membranes, which had become apparent in the previous examinations, was more carefully examined by means of subjecting the membranes to human macrophages in tissue culture.

Materials and Methods

Animal Test Examination. Eight adult female Göttingen miniature pigs were chosen as test animals. For the bone regeneration study, examination was carried out in the area of the os frontale, with artificial desmoid defects matching the size of the critical size defects called for by Schmitz and Hollinger.[19] After bicortical access to the os frontale was achieved under general anesthesia, the periosteum was resected, and $1 \times 1 \times 0.5$ cm defects were created in each animal. The defects were covered with Vicryl (Ethicon, Norderstedt, Germany), Gore Resolut, and Gore-Tex (W. L. Gore, Flagstaff, AZ) membranes, and with autogenous periosteum. One defect was not covered and served as a control (Fig 1a). Microscrews were placed into the rim of the defects to secure the membranes and to serve as a radiologic marker of the center of the defect. Since, in the previous study, a considerable degree of degradation of the absorbable membrane systems became visible after only 2 weeks,[18] this period was the minimum observation period (two minipigs). An 8-week (three minipigs) and a 4-month study (three minipigs) also were conducted. The antibiotic streptomycin (10 mg/kg) was given for the first 3 postoperative days.

Within the intervals of observation, a polychromous sequence marking was carried out using tetracycline, calcein green, xylenol orange, and alizarin-complexon in the concentrations reported by Rahn.[20] Finally, intra-arterial angiography was performed with a suspension of Berlin blue and barium sulfate. For fixation of the specimens, 2.5% glutaraldehyde was used. Nondecalcified thin-sliced slides of bone were examined by means of light and fluorescence microscopy.[21] Furthermore, disintegration phenomena of the e-PTFE membranes were studied by means of scanning electron microscopy. The processes affecting the degradable membranes were quantified polarization-optically. The percentage of reossification of the center of the specimen was evaluated planimetrically by point counting. The results are given as the mean ± standard deviation. Because of the small number of animals tested in this study, a statistical analysis was not performed.

Studies on Cell Cultures. Human monocytes from the Buffy coat were purified over a lymphoprepgradient (Nycomed, Oslo, Norway; Immuno, Heidelberg, Germany). Adherence occurred on hydrophilic and hydrophobic petri plates (Heraeus, Hanau, Germany), after which the cells were put into RPMI 1640 cell culture medium (Biochrom, Berlin, Germany) and 15% human serum, and the Gore-Tex specimens were added. The medium was renewed every week. After 4 weeks, new monocytes were added, and the study was completed after 2 months. Figure 1b shows a macrophage control. In all, 12 Gore-Tex pads were studied in vitro. The specimens

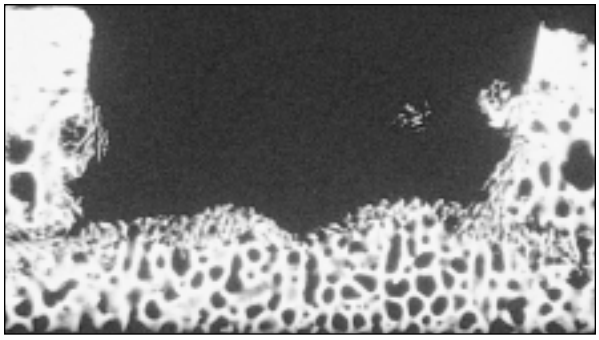


Fig 2 Microradiograph showing only sparse bone regeneration basally and on the margins of the defect after 2 weeks, independent of the material used (contact microradiograph, nondecalcified, 150-µm thick, 20 minutes, 2.5 mA, 10 kV, magnified × 2.5).

Fig 3a In double polarization, a partial collapse of the Vicryl membrane into the defect occurred after 2 weeks (nondecalcified thin section, 20 µm, Giemsa, × 10).

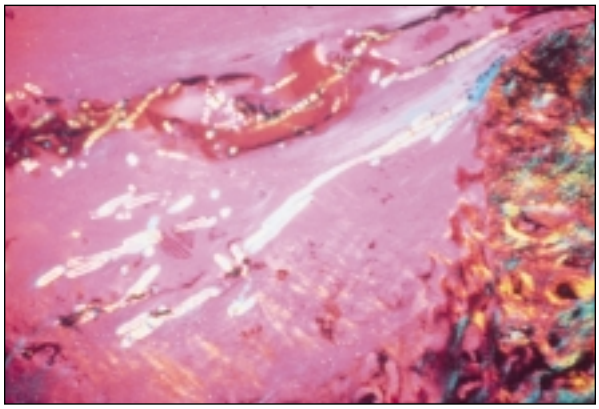


Fig 3b Partial collapse and initial degradation of the Gore Resolut membrane after 2 weeks (nondecalcified thin section, 20 µm, Giemsa, double polarization, × 10).

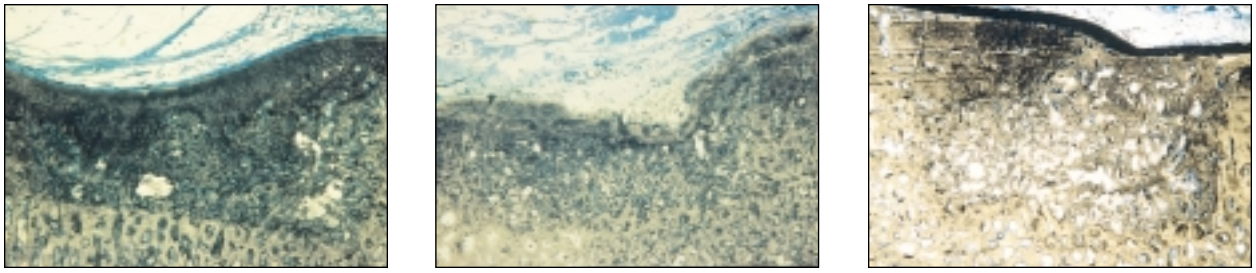
were then subjected to a series of solutions containing alcohol of increasing concentrations, dried by the critical point drying method with liquid carbon dioxide, and coated with gold palladium. The specimens were studied by scanning electron microscopy (Zeiss DSM 960, Bena, Germany).

Results

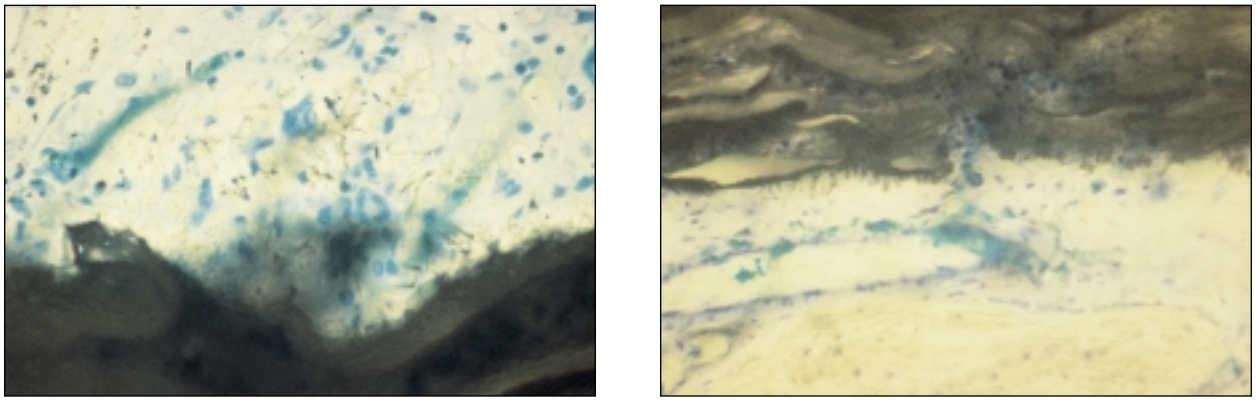
Within the observation interval of *2 weeks*, initially a blood clot formed, and vessels from the widened bone marrow space sprouted into it. Microradiographically, as expected, only sparse bone regeneration occurred basally and on the sides, and there was no dependence on the material used (Fig 2).

Using a polarization-optical technique, the mechanical instability of the two degradable membranes became apparent, in that they partially collapsed into the defects (Figs 3a and 3b). The Vicryl membranes consist of stranded fibrils, and Gore Resolut is made predominantly of amorphous material that had already begun to soak and disintegrate after only 2 weeks.

Within the observation interval of *8 weeks*, the bone defect covered with Gore-Tex was almost completely regenerated (91 ± 3% reossification of the defect), while the periosteum-covered defects showed a satisfactory degree of regeneration, and there appeared to be no differences between freely transplanted and pedunculated periosteum (63 ± 5% reossification of the defect). Reossification of only 40 ± 5% had been seen after 8 weeks using both of the degradable membranes. The membranes were not histologically detectable after 4 months. The former defect area was reossified to about 60% with considerable loss of height; the functional restructuring processes were considered to take place after 4 months (Figs 4a and 4b). The nonfortified e-PTFE membranes fulfilled their function as barriers over a period of 4 months, since the defects almost completely reossified. The competitive cells of the covering connective tissue were excluded from defect regeneration (Fig 4c). On the upper and lower surfaces of this material, however, the previously observed disintegration processes (Figs 5a and 5b),



Figs 4a to 4c Reossification of the desmal bone defects 4 months after they were covered with a Vicryl membrane *(left)* and with Gore Resolut *(center)* displaying marked loss of height. In contrast to these results, coverage with e-PTFE *(right)* resulted in nearly total reossification (nondecalcified thin slice, 20 µm, Giemsa, × 2.0).



Figs 5a and 5b Marked signs of degradation of the e-PTFE membrane after 4 months, visible on the upper surface of the membrane (*left,* × 100) with disintegration of filaments, as well as on the downward surface of the membrane *(right)*; the latter changes are slightly less pronounced because of a foreign body reaction (nondecalcified thin section, 20 µm, Giemsa, × 40).

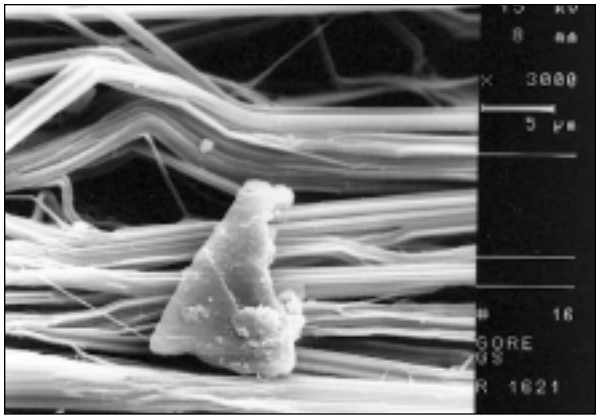
with disintegration of filaments and foreign body reaction, became apparent. This was more pronounced on the upper surface of the membrane, which was subjected to a definite mechanical stress in the animal study. These changes in the e-PTFE membranes ultimately occurred in all of the animals over time.

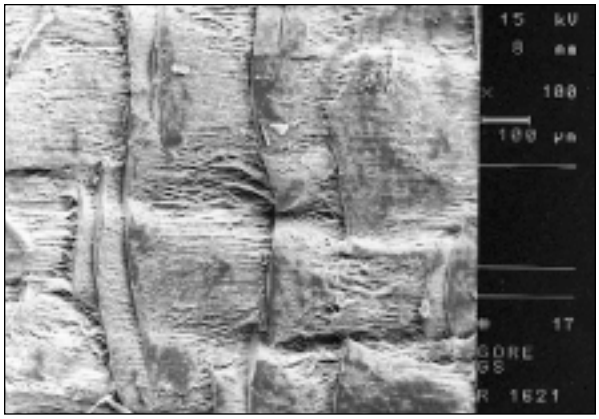
In the in vitro study, the material was taken from the macrophage culture after 2 months and examined by scanning electron microscopy. The material (12 Gore-Tex pads in total) had *not* been actively attacked by the macrophages in vitro, which makes a physicochemical process the more plausible explanation for the observed disintegration of the filaments in vivo (Figs 6a and 6b).

Discussion

After the establishment of permanent membranes for guided bone regeneration, absorbable membranes were introduced toward the end of the 1980s.[14–17] Absorbable membranes consist primarily of polyglycolid (PGA) and polylactid (PLA). After hydrolysis into lactic acid and glycolic acid, these materials are further broken down into carbon dioxide and water in the citrate cycle, commonly accompanied by a mild degree of inflammatory reaction. The absorbable membrane material tested in this study is different in structure and composition. The lactid polymers and the addition of polydioxanone, as in Ethisorb,[22] lead to a delayed degradation. The glycolid polymers provide improved mechanical features.

The essential fact in this context is that both PGA and PLA are principally hydrophobic substances, although, of the two, PGA is a little less hydrophobic, which makes hydrolysis somewhat easier. The absorption kinetics of these tested materials lead to a premature fragmentation of the membranes; thus, the physical integrity cannot be maintained over a sufficiently long time span. The premature fragmentation makes additional fixation with auxiliary devices seem unfeasible, although this may be reasonable to compensate for the mechanical weaknesses of the material. It should be noted that the defects used in





Figs 6a and 6b Substantially unaltered e-PTFE membrane surface after 2 months of exposure to the macrophage culture (*left,* × 100) without marked signs of a cellular attack *(right)* (SEM, × 3000).

this study were relatively large. Further means of supporting the membranes, especially the application of space fillers (supportive function, osteoconductive guide rail, stabilization of the blood clot, and so forth), in membrane-protected bone defects would seem reasonable in this context.[23]

The most complete bone regeneration was found under the e-PTFE membranes. However, the applied e-PTFE membranes displayed signs of disintegration over time. An immediate attack of the macrophages did not take place in vitro; thus a physicochemically mediated resolution of filaments seems more likely to be responsible. These signs of dissolution were chiefly detected on the upper surface of the membrane, which was subjected to greater mechanical strain. Comparable histologic findings have been reported after the application of e-PTFE tubes in the reconstruction of the lacrimal duct,[24] as well as in surgery where e-PTFE has been used to replace ligaments.[25] In this context, Teflon, which has been described as biocompatible, has led to synovitis via abraded particles. These inflammatory tissue reactions are compatible with the findings of Emery and Rostrup.[26]

The displayed signs of disintegration, followed by inflammatory tissue reaction found after reconstruction of lacrimal ducts and after the replacement of ligaments, were also more pronounced with progressing time. Therefore, removal of the material is recommended when used for guided bone regeneration, eg, after 8 weeks. The periosteum-covered defects showed a satisfactory degree of regeneration, and no differences were noted between freely transplanted and pedunculated periosteum.

Conclusion

In an animal test study, the efficacy of Vicryl, Gore-Tex, and Gore Resolut membranes and periosteum was compared in view of their application in guided bone regeneration. Bone defects in the os frontale (nonoral) of Göttingen miniature pigs were each covered with one of these membranes. Specimens for histologic examination were taken at 2, 4, and 8 weeks, and at 4 months. Among those tested, the degradable membranes revealed weaknesses in that they collapsed into the defect prematurely, obstructing the process of regeneration; all of this is the result of premature degradation and a lack of stiffness.

The e-PTFE membranes displayed superior results in terms of bone regeneration, and a certain degree of disintegration of filaments was observed as well.

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