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Histological evaluation of a biodegradable polyactive®/ hydroxyapatite membrane

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Guided tissue regeneration (GTR) is a technique which is used for the treatment of bone defects associated with periodontal disease or enossal dental implants. In most experimental studies on GTR, non-degradable membranes are used. A drawback inherent to such devices is that at the end or in the course of the wound healing they have to be removed. Therefore, the aim of the present study was to investigate a new biodegradable membrane material for use in GTR, which also has excellent mechanical properties and is biocompatible. The material is a composite consisting of poly(ethyleneglycol terephthalate) and poly(butylene terephthalate) segmented copolymer (PEG/PBT), which for the experiments was used in pure form and also mixed with hydroxyapatite (HA) grains. Subcutaneous and subgingival implantation studies in goats were performed to determine the biocompatibility and biodegradability characteristics of several of these materials. Differences between materials were introduced in the production process, PEG/PBT ratio, material thickness and presence of HA. Implantation periods were 3, 6 and 12 wk. The histological results indicated that all investigated materials were biocompatible with the surrounding tissue. Degradation of the membranes was attended by a mild cellular reaction. The degradation process was mainly influenced by the PEG/PBT ratio. A higher PBT content resulted in a decreased degradation.

Keywords: Guided tissue regeneration, biocompatibility, biodegradation, membrane, periodontology

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Bone defects associated with periodontal disease or enossal dental implants may be regarded as risk sites for periodontal destruction. Although bone defects can be reduced by means of subgingival curettage and periodontal surgery, both methods may present an inherent disadvantage of tissue loss as well as the formation of a long epithelial attachment. Recently, physical barriers in the form of a membrane have been placed between the mucogingival flap and the bone and tooth/implant surfaces during surgery. The membrane acts as a barrier. It deflects the gingival tissue away from the root surface and creates a protected space over the defect that allows the remaining periodontal ligament fibroblasts to selectively repopulate the root surface. These fibroblasts are thought to account for the formation of a new fibrous attachment which prevents the epithelial migration. This method is called guided tissue regeneration (GTR)[1].

In addition to periodontal defects, this GTR principle has also been used in a series of experimental studies for the treatment of implant/bone defects and alveolar ridge augmentation[1–6]. The concept of GTR in these applications is to exclude the rapidly repairing tissues (epithelium and gingival connective tissue) and to allow the migration of regenerative bone cells from the surrounding alveolar bone into the defect.

Currently, non-resorbable materials are used for the fabrication of the membranes. Examples are: Millipore® filter and expanded poly(tetrafluoroethylene) (ePTFE, Gore-Tex®)[7–10]. However, a disadvantage of non-resorbable membranes is that they require a second surgical session for the removal of the membrane after the initial healing has taken place. In addition to time and cost, this second operation can again cause damage to gingival tissues. Therefore, the use of biodegradable membranes has been advocated more recently, and has been investigated in experimental animal studies[11–15]. The materials tested up to now are: polylactic acid (PLA), polyurethane, polyglycolic acid and Type 1 collagen. Although these materials showed some promising results, it has to be concluded that the ideal resorbable membrane still needs to be developed. The challenge is that a resorbable membrane must meet very specific human factor and biological requirements. For example, it should be firm to the extent of not

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restricting the space for bone or periodontal ligament formation by gingival pressure, but it should also have a certain flexibility to allow ease of handling. In addition, the wound healing must not be impeded by excessive inflammatory reactions associated with the decomposition and resorption of the membrane itself.

Recently, a very promising material has been developed which has excellent mechanical properties and is biocompatible and biodegradable. In addition, it should be mentioned that the degradation properties can be varied according to the anticipated needs. The material is a composite, consisting of Polyactive® (generic name: poly(ethyleneglycol terephthalate) poly(butylene terephthalate) segmented copolymer, PEG/PBT)[16–18] of which some compositions were mixed with 30 wt% hydroxyapatite (HA) grains. A suggested property[19] of this calcium phosphate ceramic is that it stimulates new bone formation in large defects.

Within the framework of the present study the biocompatibility and biodegradability characteristics of these newly developed Polyactive membranes were investigated in two experimental animal studies.

MATERIALS AND METHODS

Membranes

Both unfilled Polyactive and Polyactive/hydroxyapatite (PAHA) membranes were manufactured by HC Implants b.v. (Leiden, The Netherlands) using two different methods:

1. Membranes which were produced by a thin film solvent casting procedure. During the evaporation process the HA grains are subsided to one side. However, the surface of the HA grains is still covered with Polyactive.
2. Membranes which were produced by thin film solvent casting, but after evaporation were heat treated by pressing between two plates. This procedure resulted in a one-sided exposure of the HA grains.

In addition to the manufacturing, further differences between membranes were introduced in PEG/PBT ratios, material thickness and presence of HA. In total, 16 different membranes were synthesized (*Table 1*).

Before implantation, the membranes were cleaned in pure ethanol, dried and sterilized by γ-irradiation (2.5 Mrad). Subsequently, randomly chosen samples of all materials were inspected by scanning electron microscopy.

Animals and surgical procedure

In the first study, eight samples of each membrane were inserted subcutaneously into the dorsum of eight healthy adult female goats for either 3, 6 or 12 wk. The choice of these implantation periods was based on the final application of the membranes for GTR. A total of 384 implants were placed, 48 in every goat. The implants measured 1 cm × 2 cm.

Surgery was performed under general anaesthesia, induced by intravenous pentobarbital (25 mg kg^{-1} and atropine (0.5 mg per animal). After oro-tracheal intubation, anaesthesia was maintained by ethrane (2–3%) through a constant volume ventilator.

Table 1 Review of all implant parameters

PEG/PBT ratio	Hydroxy-apatite presence	Production process	Material thickness (μm)	Abbreviation
80/20	Present	Solvent-cast	200	80/200S+
80/20	Present	Solvent-cast	350	80/350S+
80/20	Present	Heat-treated	200	80/200H+
80/20	Present	Heat-treated	350	80/350H+
80/20	Absent	Solvent-cast	200	80/200S
80/20	Absent	Solvent-cast	350	80/350S
80/20	Absent	Heat-treated	200	80/200H
80/20	Absent	Heat-treated	350	80/350H
60/40	Present	Solvent-cast	200	60/200S+
60/40	Present	Solvent-cast	350	60/350S+
60/40	Present	Heat-treated	200	80/200H+
60/40	Present	Heat-treated	350	80/350H+
60/40	Absent	Solvent-cast	200	80/200S
60/40	Absent	Solvent-cast	350	80/350S
60/40	Absent	Heat-treated	200	80/200H
60/40	Absent	Heat-treated	350	80/350H

For the insertion of the experimental membranes, the back of the goats was shaved, washed and disinfected with iodine. Implantation of the 3, 6 and 12 wk implants was performed in three separate surgical sessions. In the first session, at both sides of the spinal column eight longitudinal incisions of about 3 cm were made through the full thickness of the skin. Subsequently, lateral to the incisions subcutaneous pockets were created by blunt dissection with scissors. One implant was inserted in each pocket. Finally, the wounds were carefully closed. In the second and third session, after 3 and 9 wk, the implants for the 6 and 3 wk study were placed at a row above or below the 12 wk implants (*Figure 1*). To assure a complete and reliable randomization and to exclude the influence of implantation site, the various implants were allocated according to a split plot design[20]. Balancing was done by using the method of latin squares.

In the second study four PAHA membranes were selected. The chosen membranes consisted of 80/20 or 60/40 PAHA with a thickness of 200 μm (80/200 H+, 80/200S+, 60/200H+ and 60/200S+). The materials were applied in the upper jaw of goats around the first premolar and the edentulous area mesial to the first premolar. For this study four goats were used. After anaesthesia, for the insertion of the membrane a longitudinal incision was made on the palatal side of the alveolar ridge mesial to the first premolar. In addition, an incision was made in the gingival sulcus of the first premolar. Subsequently, a mucoperiosteal flap was raised. After placement of the membrane on the exposed alveolar bone, the wound was carefully closed. Each animal received two different materials, one in each jaw side. Eight weeks after surgery, the animals were killed. During this session, the gingival tissues were inspected for clinical signs of inflammatory reaction and perforations of the membrane. To reduce the perioperative infection risk, prophylactic antibiotic Albipen® was administered to all goats for 3 d starting 1 h postoperatively.

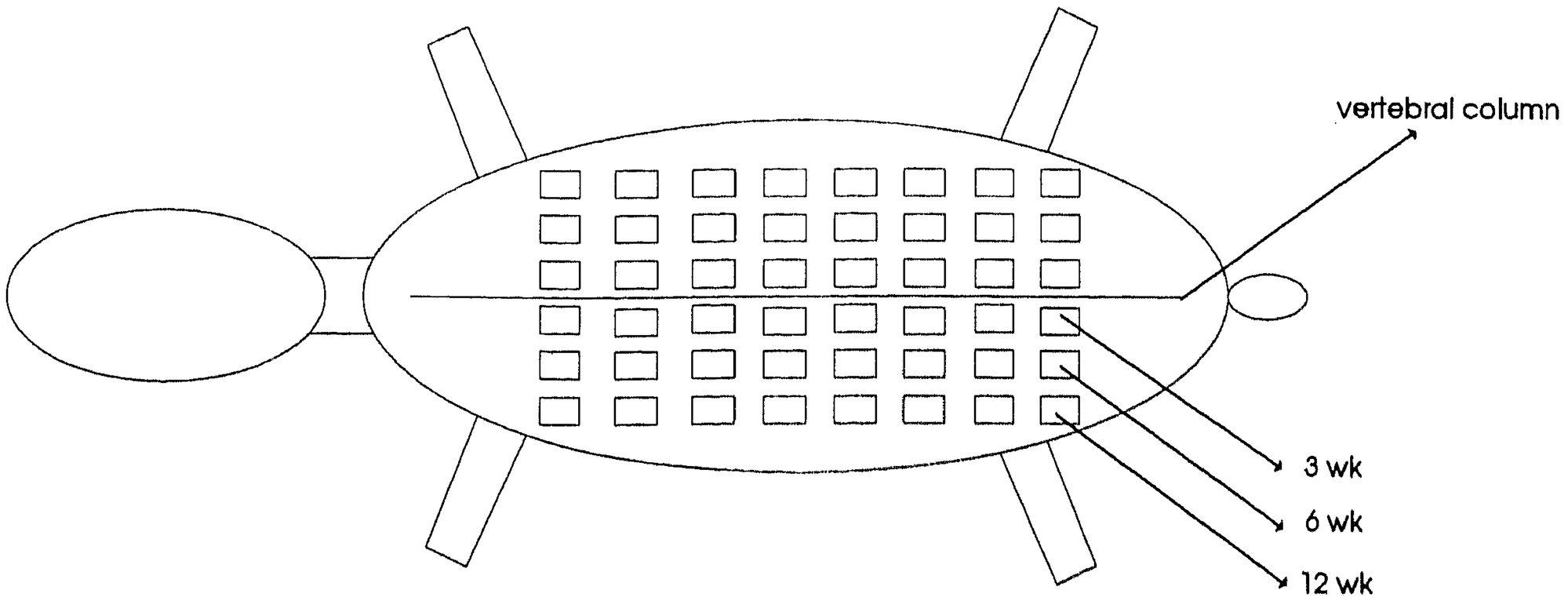


Figure 1 Schematic drawing of the subdermal implant positions.

Histological evaluation techniques

At the end of the implantation periods the goats were killed by an overdose of Nembutal®. Then, the implanted membranes, including all surrounding tissues, were removed, fixed in 10% buffered formalin and embedded in methylmethacrylate. After polymerization, thin serial sections were cut using a Jung-K microtome. The sections were stained with basic fuchsin and methylene blue and investigated by light microscopy. The light microscopical sections of the subcutaneous implants were assessed both histologically and histomorphometrically for:

1. The type of cellular response to the investigated materials.
2. The degree of degradation of the investigated materials.

Histological evaluation was performed on an ordinal level using a histomorphometrical grading scale as developed by Jansen *et al.*[21] (*Table 2*). In summary, the evaluation of the capsule was semiquantitative and semiqualitative, while the evaluation of the interface was only semiqualitative. The semiquantitative capsule classification consisted of a capsule thickness measurement on the basis of the observed number of fibroblasts. The semiqualitative rating of the capsule and interface consisted of numerically rating the tissue morphology (fibrous tissue, fat tissue, maturity) and cellularity (presence of fibroblasts, macrophages, giant cells and other inflammatory cells). Quantification of the degradation rate was based on numerical evaluation of the number of fragments.

The histological sections of the subgingival implants were only evaluated by thorough description of the observed tissue reaction and membrane degradation behaviour.

RESULTS

Membrane morphology

Figures 2 and *3* are scanning electron micrographs, which show the characteristic morphological aspects of the various membrane materials. All PAHA membranes produced by thin film casting and additional heat treatment demonstrate a one-sided distribution with exposure of the HA grains. This was in contrast to the membranes without heat treatment, where the HA grains are still covered with Polyactive. The Polyactive membranes without HA revealed a smooth surface appearance.

Histology and histomorphometry

During the post-implantation period, in both studies, all animals showed an unevoked healing without any

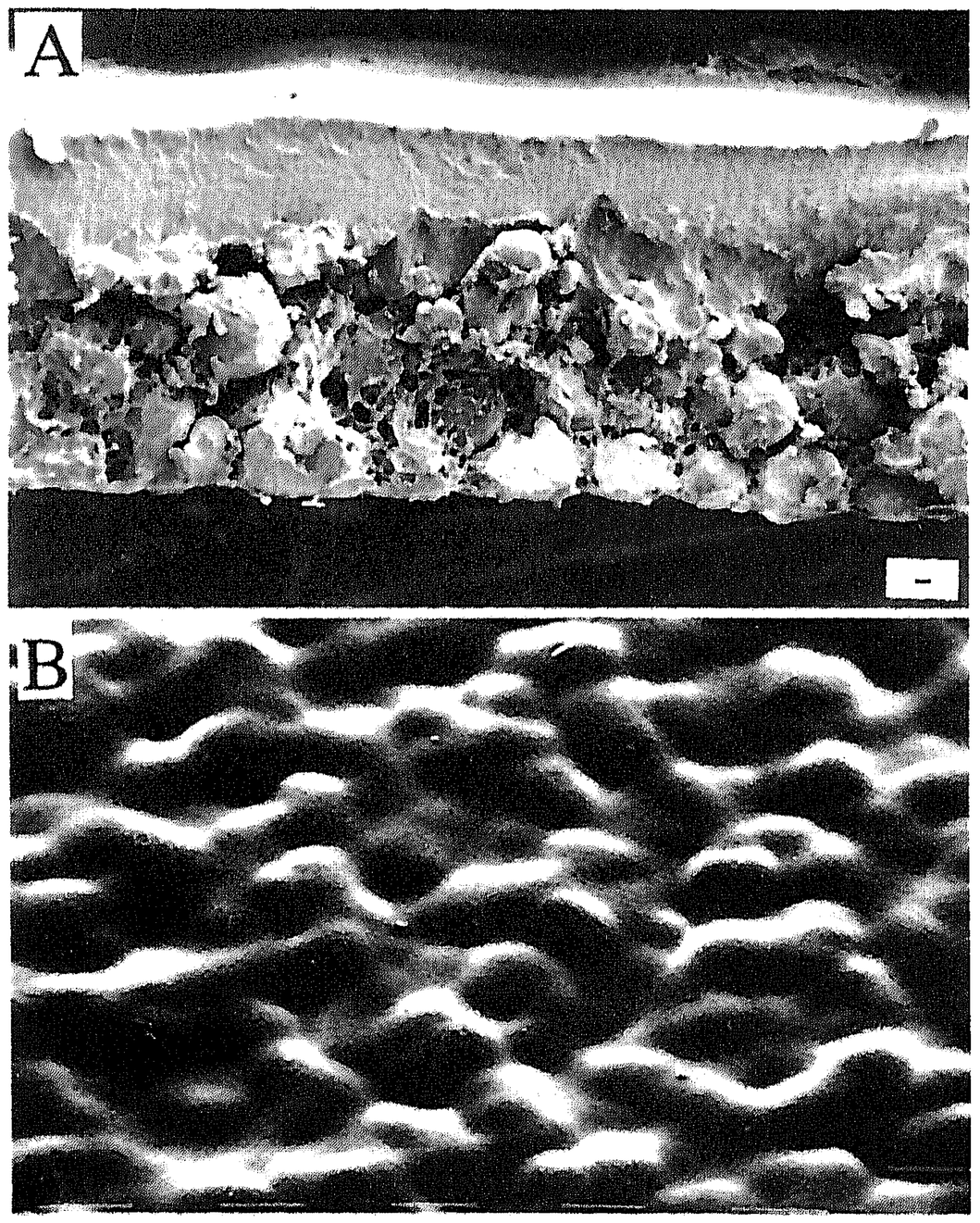


Figure 2 Scanning electron micrographs of Polyactive®/hydroxyapatite membrane produced by thin film casting: **A**, cross-section showing the one-sided distribution of the HA grains, original magnification ×160, bar = 10 μm; **B**, the surface of the HA side, original magnification ×80, bar = 100 μm.

Table 2 Grading scale for soft-tissue implants

Reaction zone	Response	Score
capsule quantitatively	thickness rating:	
	1–4 fibroblasts	4
	5–9 fibroblasts	3
	10–30 fibroblasts	2
	>30 fibroblasts	1
	Not applicable	0
capsule qualitatively	capsule tissue is fibrous, mature, not dense, resembling connective or fat tissue in the non-injured regions	4
	capsule tissue is fibrous but immature, showing fibroblasts and little collagen	3
	capsule tissue is granulous and dense, containing both fibroblasts and many inflammatory cells	2
	capsule consists of masses of inflammatory cells with little or no signs of connective tissue organization	1
	cannot be evaluated because of infection or other factors not necessarily related to the material	0
interface qualitatively	fibroblasts contact the implant surface without the presence of macrophages or foreign body giant cells	4
	scattered foci of macrophages and foreign body cells are present	3
	one layer of macrophages and foreign body cells is present	2
	multiple layers of macrophages and foreign body cells are present	1
	cannot be evaluated because of infection or other factors not necessarily related to the material	0
degradation rate	degradation into more than 20 fragments	4
	degradation into 11–20 fragments	3
	degradation into 2–10 fragments	2
	no degradation	1

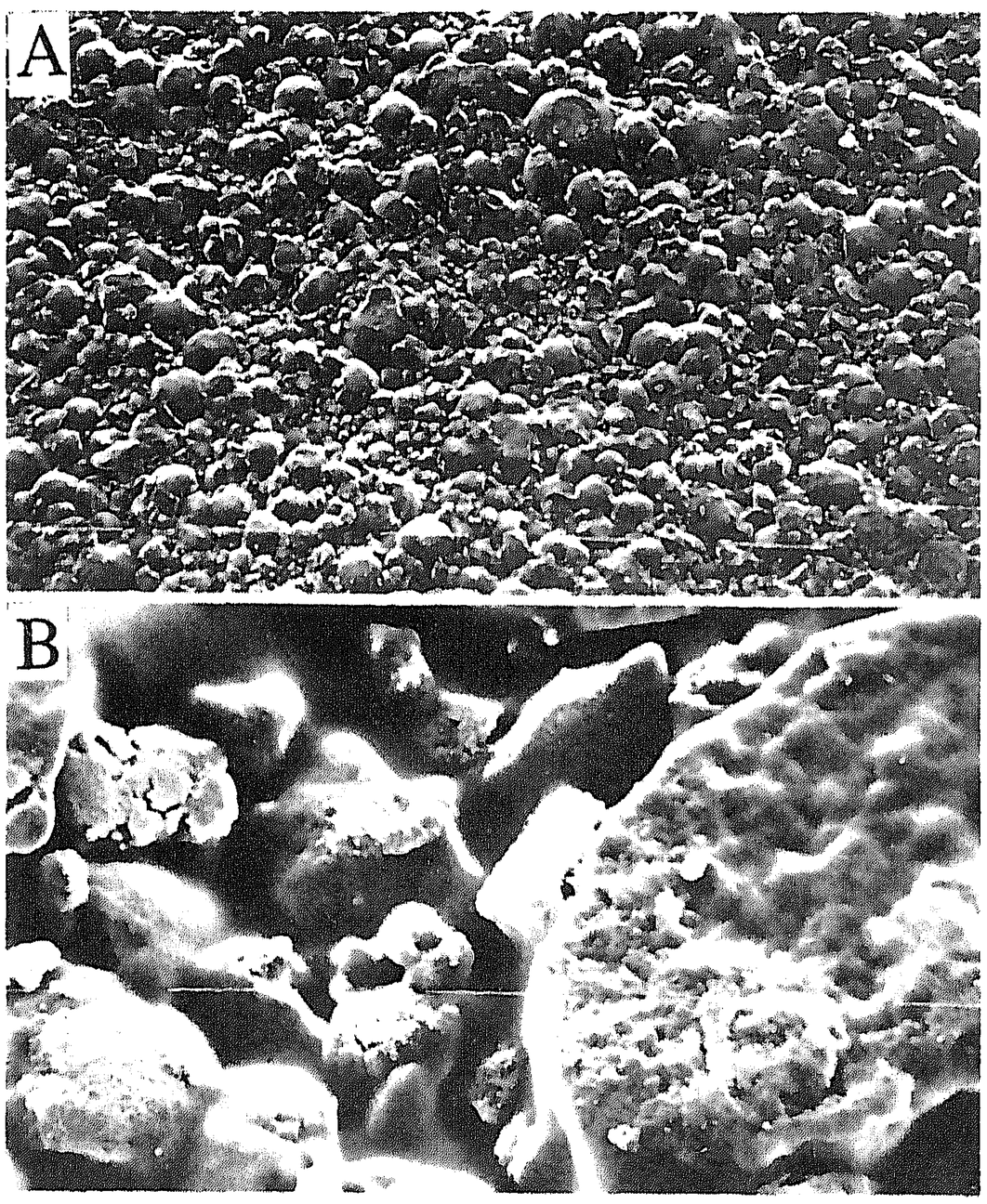


Figure 3 Scanning electron micrographs showing the surface of the HA side of Polyactive®/hydroxyapatite produced by thin film casting and additional heat treatment. Compared with *Figure 2A, B* the surface is rougher; the HA grains are exposed by the heat treatment: **A**, original magnification ×80, bar = 100 μm; **B**, original magnification ×1250, bar = 10 μm.

Figure 4 Light micrographs of solvent-cast 60/40 Polyactive® membrane, thickness 350 μm, at 6 wk. The membrane shows no sign of degradation and is surrounded by a fibrous capsule approximately 20 cell layers thick. In some areas the capsule made direct contact with the membrane surface **A**, while in other areas, **B**, only a single layer of phagocytic cells was present at the membrane/capsule interface: **A**, original magnification ×50, bar = 50 μm; **B**, original magnification ×50, bar = 50 μm.

disturbance of the wound healing process. The clinical examination at the end of the experimental periods demonstrated that the membranes did not cause perforation or inflammation of the covering skin/gingiva tissue.

Subcutaneous tissue response

Examination of the histological sections revealed a characteristic and fairly uniform tissue response for all tested materials. At all implantation periods, the membranes were surrounded by a fibrous capsule approximately 10–30 cells thick (*Figure 4*). The capsule contained fibrocytes and was usually free from an inflammatory reaction. The connective tissue maturity of the capsule around the 60/40 membranes appeared to be higher than that seen around the 80/20 membranes.

Further, it was observed that the fibrous capsule surrounding the 80/20 membranes was separated from the membrane surface by a single or multiple layer of mono- and multinucleated phagocytic cells (*Figure 5*). Usually, more foreign body giant cells were found at the HA side of the 80/20 PAHA membranes compared with the bulk Polyactive side (*Figures 6* and *7*).

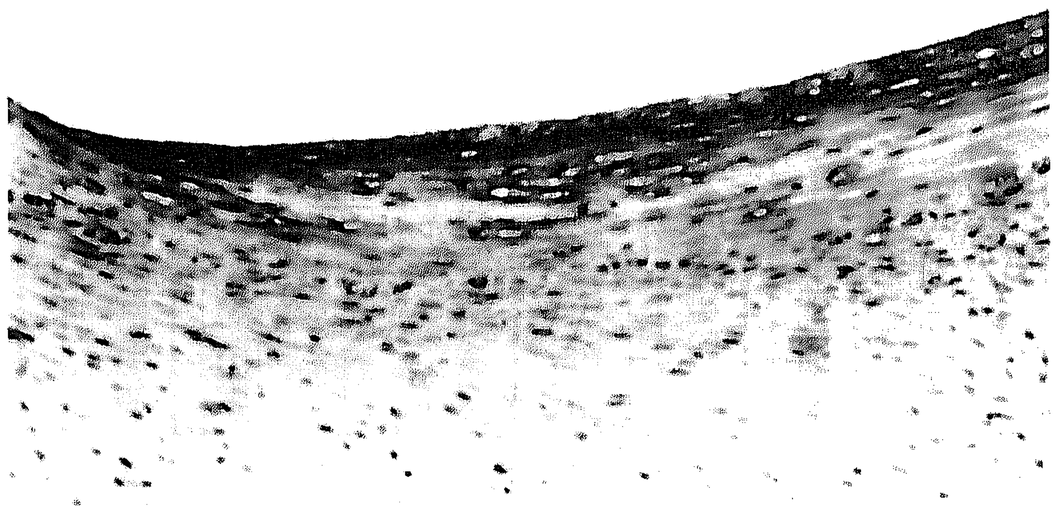


Figure 5 Light micrograph of solvent-cast 80/20 Polyactive® membrane, thickness 200 μm. The membrane is fragmented. The fibrous capsule is separated from the membrane fragments by a single layer of phagocytic cells. Original magnification ×50, bar = 50 μm.

Figure 6 Solvent-cast Polyactive®/hydroxyapatite 80/20 membrane, thickness 350 μm, at 12 wk. The membrane is fragmented. Phagocytic cells are present at the HA side of the fragments. Original magnification ×50, bar = 50 μm.

Finally, the histological evaluation showed that at the interface of the majority of 60/40 membranes, only scattered foci of monolayers of giant cells or macrophages were present (*Figure 8*). Interestingly, there was no difference in occurrence of foreign body

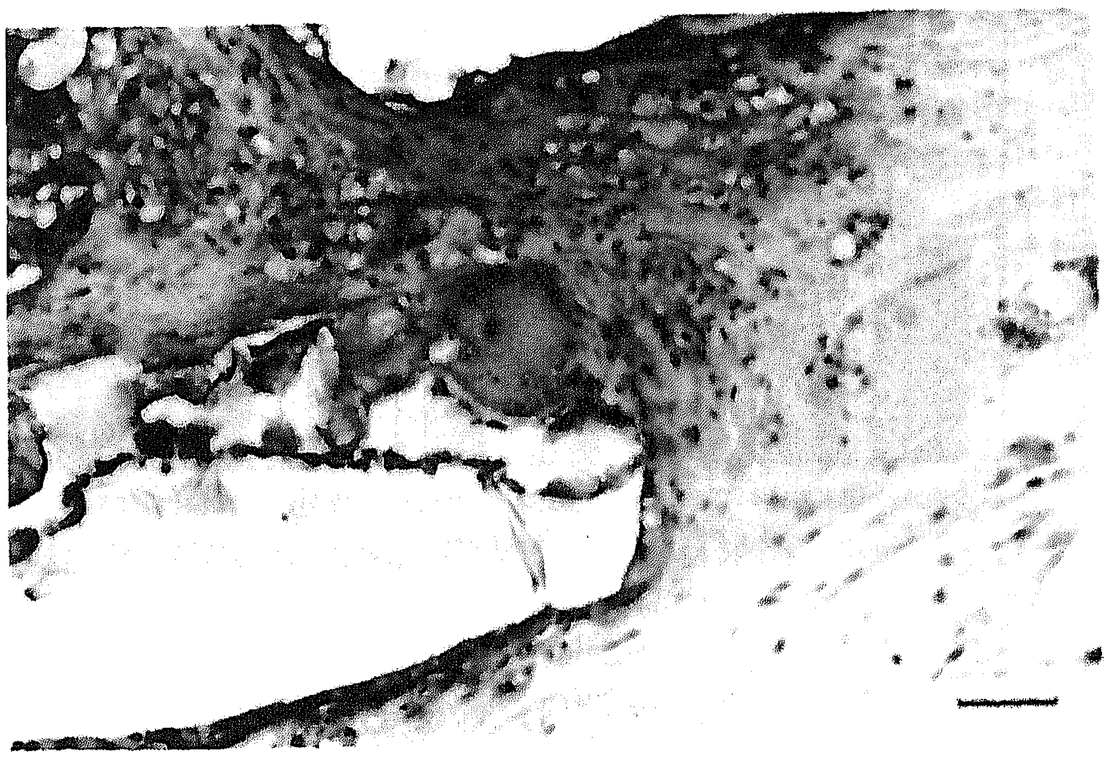


Figure 7 Heat-treated Polyactive®/hydroxyapatite membrane, thickness 200 μm, at 12 wk. The membrane is fragmented. Foreign body giant cells were observed at the HA/capsule interface. Original magnification ×50, bar = 50 μm.

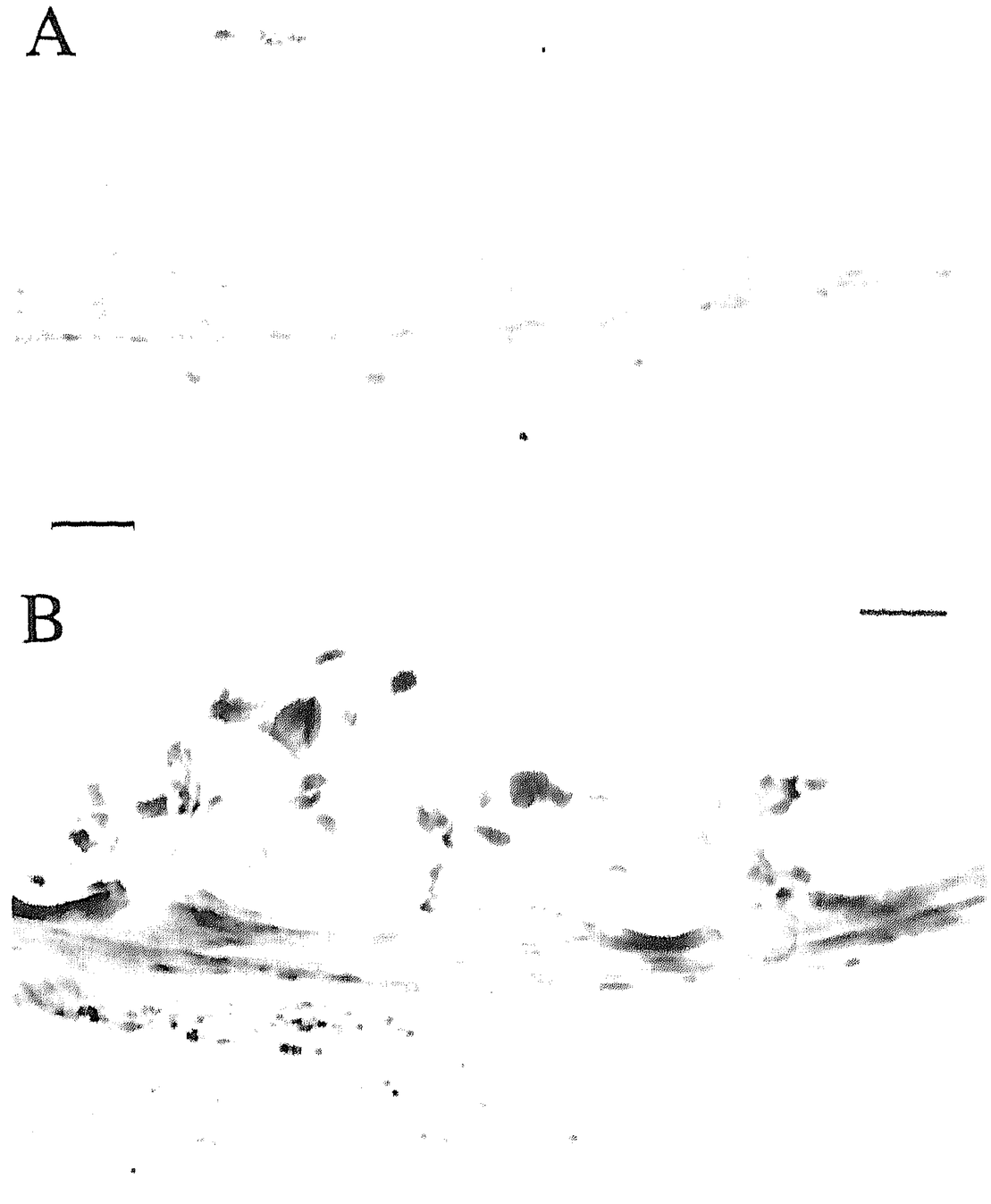
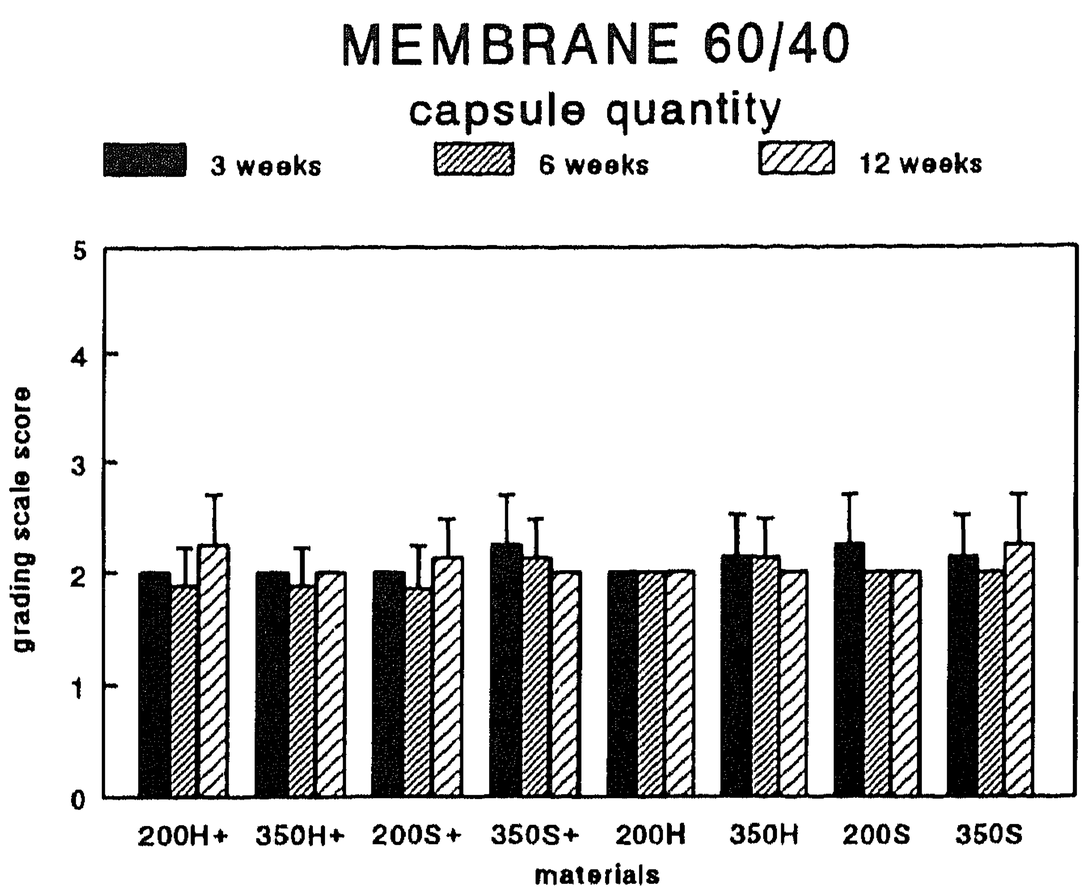
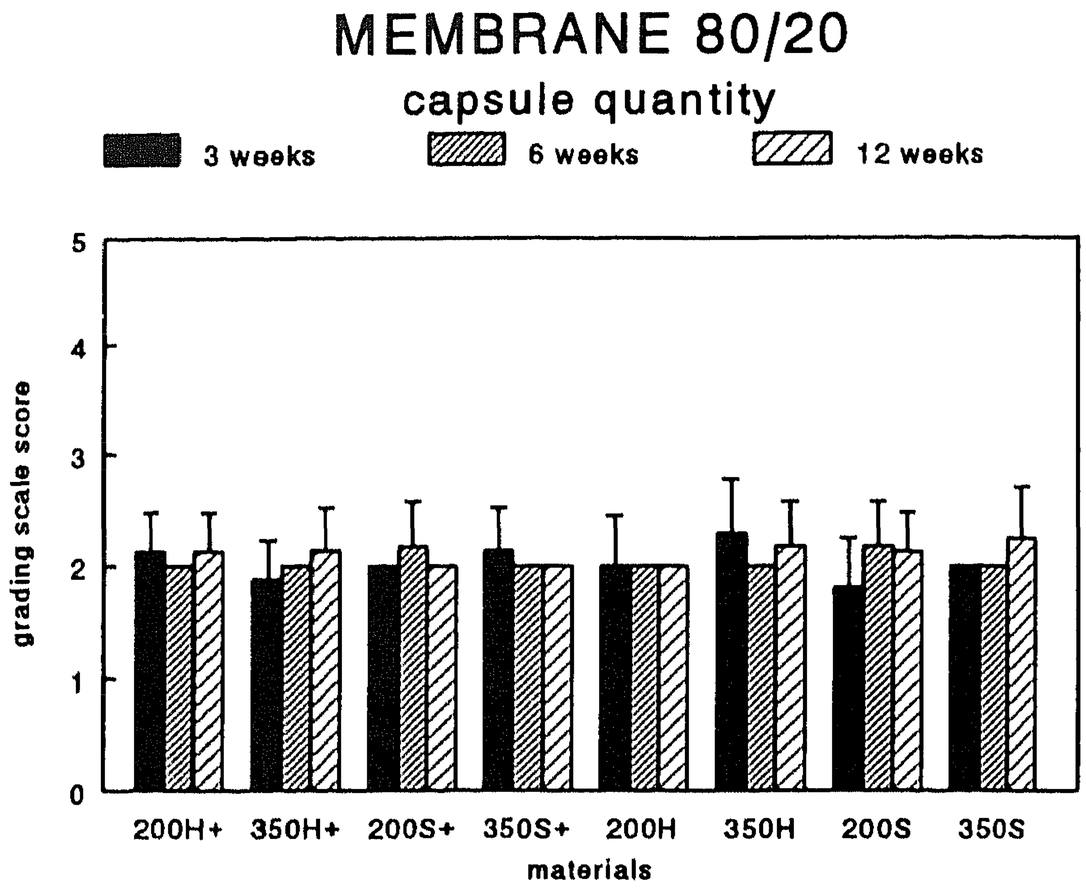
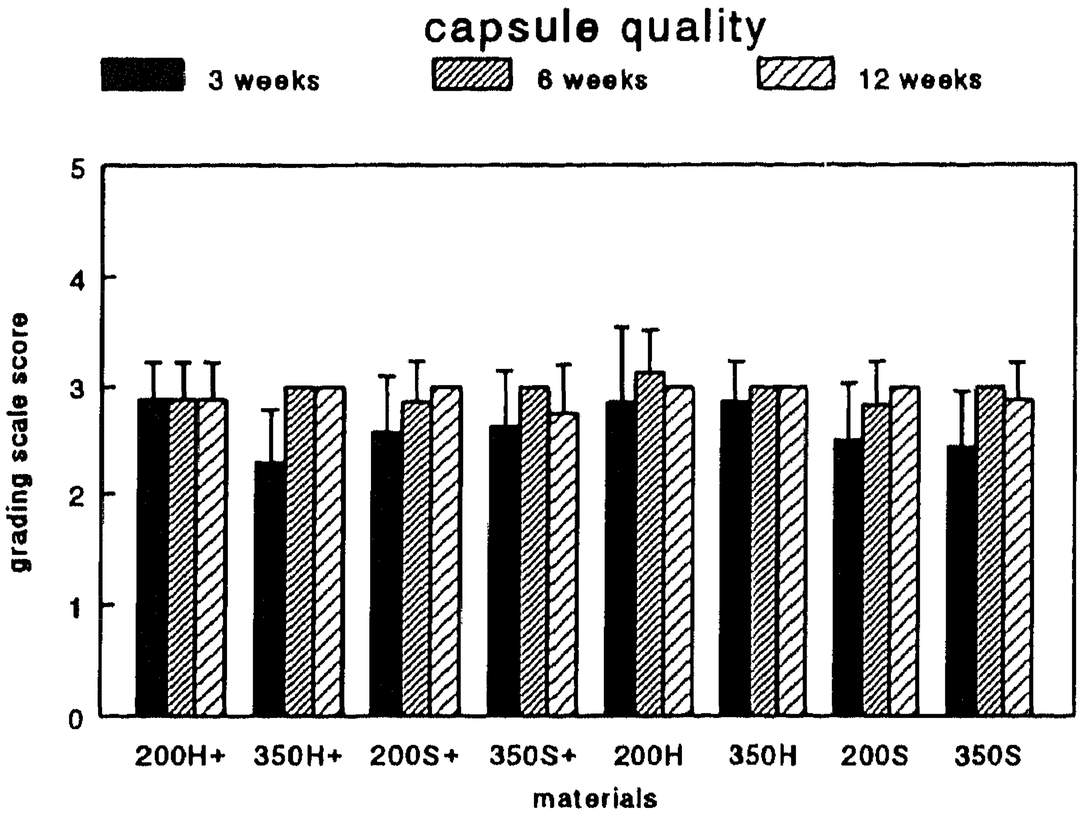
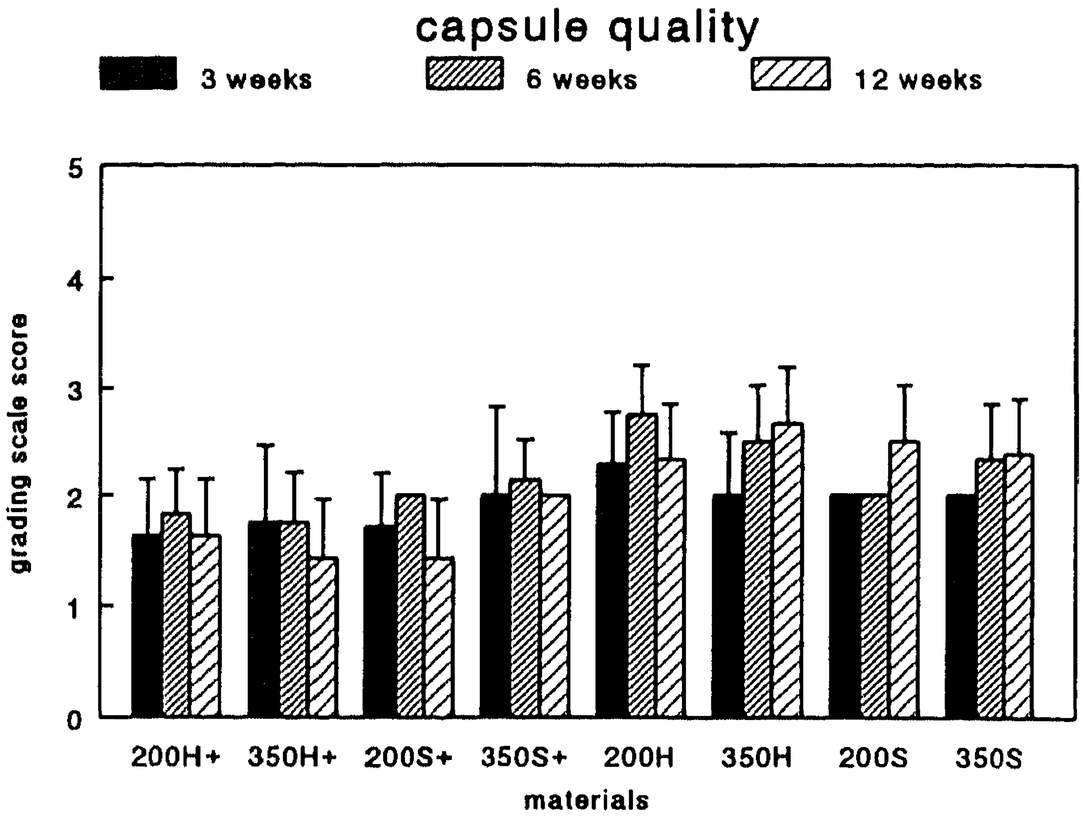


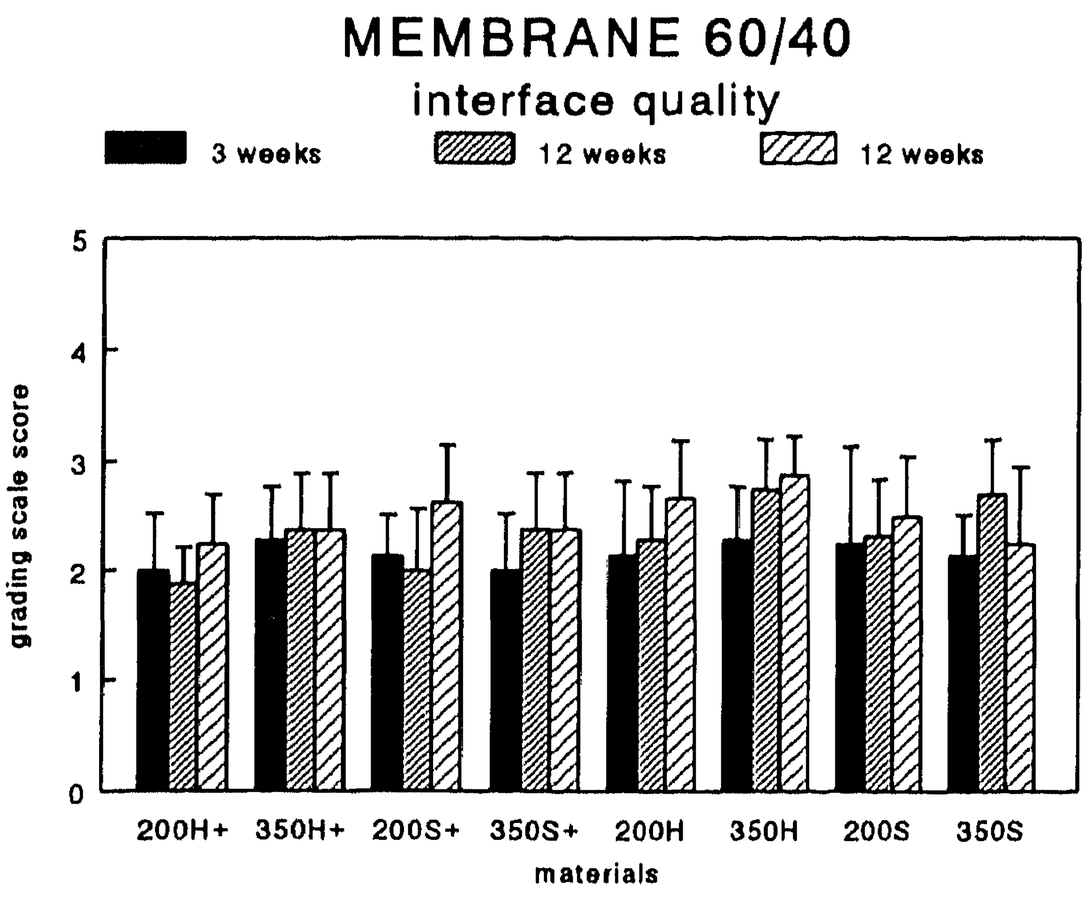
Figure 8 Light micrograph of heat-treated 60/40 Polyactive®/hydroxyapatite membrane, thickness 350 μm, at 12 wk. There is minimal occurrence of foreign body reaction in the interface at the HA, **B**, as well as the bulk Polyactive® side, **A**, of the membrane: **A**, original magnification ×50, bar = 50 μm; **B**, original magnification ×50, bar = 50 μm.











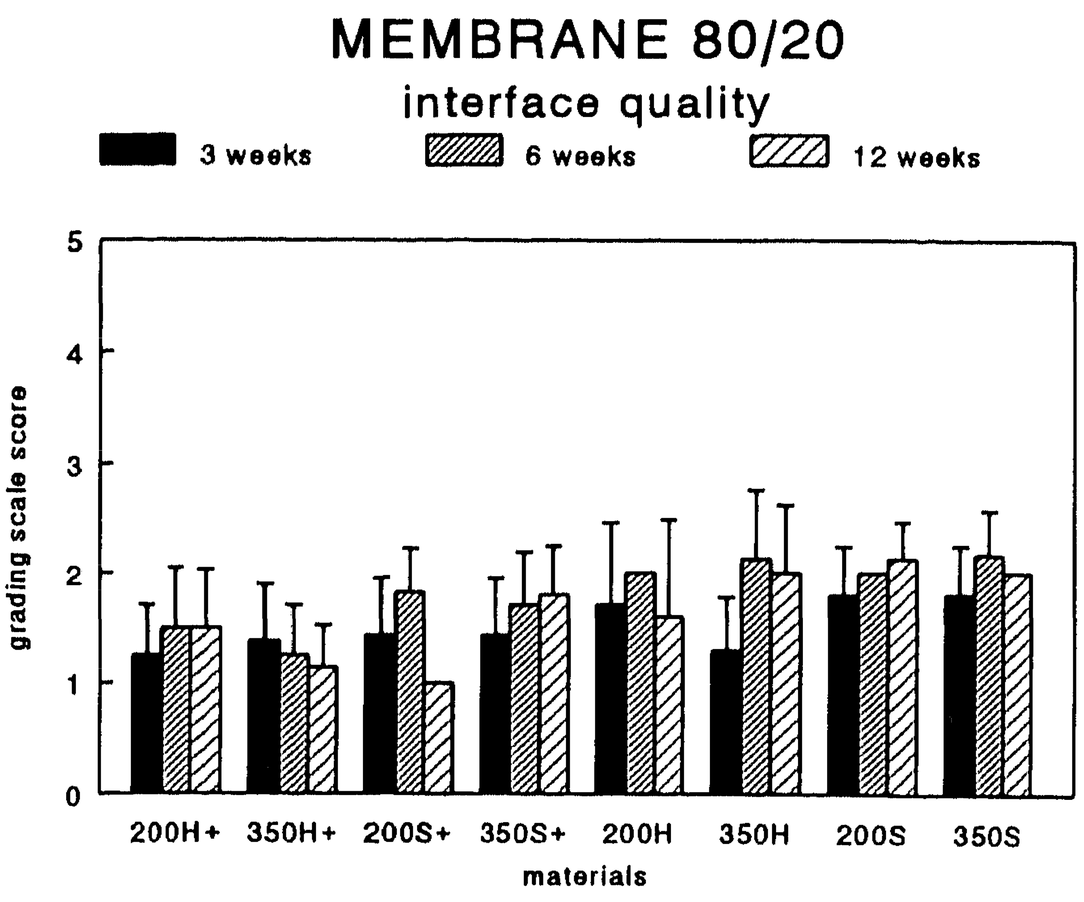


Figure 9 Comparative rating of capsule quantity, capsule quality and interface quality of the various membranes and implantation periods. 200 resp. 350 = thickness in μm; S = solvent-cast; H = heat-treated; + = Polyactive^{R}/hydroxyapatite membrane.

reaction between the HA and bulk Polyactive side. Further, around many membranes there were areas where the fibrous capsule made apparent direct contact with these membrane surfaces without separating the reactive cell layer in the interface (*Figure 4*).

Figure 9 shows graphically the 3, 6 and 12 wk data of the histomorphometrical evaluation. Statistical testing of the data, using a one-way analysis of variance (ANOVA) and a multiple comparison procedure (Newman–Keuls), revealed that no significant difference existed in capsule quantity between the various membranes and implantation periods. Considering the capsule quality, statistical testing demonstrated only a significant difference ($P < 0.001$) between 60/40 and 80/20 200 μm heat-treated (200H+), 350 μm heat-treated (350H+) and 200 μm solvent-cast (200S+) PAHA membranes for all implantation periods. A similar observation was made for the interface quality, statistical testing showed only a significant difference ($P < 0.001$) between 60/40 and 80/20 200 μm heat-treated (200H+), 350 μm heat-treated (350H+) and 200 μm solvent-cast (200S+) PAHA membranes for all implantation periods.

Concerning the membrane degradation behaviour, 3 wk postoperatively, fragmentation of all 80/20 membranes was visible (*Figures 5*, *6* and *7*), irrespective of the material thickness (220–350 μm) and production process (heat-treated vs solvent-cast). Fragmentation was attended by an increase in the number of macrophages, foreign body giant cells and blood vessels. With time, the 80/20 membranes exhibited progressive fragmentation, with a slightly higher fragmentation rate for the PAHA composite membranes. Most of the 60/40 membranes showed no or minimal signs of fragmentation (2–6 fragments), even after 12 wk of implantation.

The data regarding the quantitative evaluation of the degradation behaviour are given in *Figure 10*. Statistical testing, using ANOVA and the Newman–Keuls multiple comparison procedure, revealed that all 80/20 membranes had significantly higher degradation rates than 60/40 membranes ($P < 0.001$). The fragmentation of the 80/20 materials started within 3 wk postoperatively. The rate of degradation of the 60/40 membranes was much slower. Compared with the degradation process of the 80/20 membranes had significantly higher degradation rates than 60/40 membranes ($P < 0.001$). The fragmentation of the 80/20 materials, after 12 wk implantation the degradation of some of the 60/40 membranes was still not started.

To examine the possible existence of a relationship between interface quality and degradation behaviour a simple linear regression test was applied, using the data as given in *Figures 9* and *10*. The statistical significance of the relationship between the two variables was determined by performing a *t*-test. The analysis revealed that a strong negative correlation existed. The computed correlation coefficient (r) for interface quality and degradation behaviour was -0.8 ($P < 0.01$).

Subgingival tissue response

The clinical examination at the end of the experimental period demonstrated that only one implant was exposed through the soft tissue at the distal end of the alveolar incision. In none of the animals was an adverse gingiva reaction observed.

Histological analysis of the PAHA membranes confirmed practically completely the preclinical findings and the histological results of the subdermal-placed membranes. After 8 wk implantation, all PAHA membranes that were used appeared to be fragmented (*Figure 11*). The fragments were surrounded by a medium thin fibrous tissue capsule, which was separated from the membrane surface by a single and/or multiple layer of macrophages and foreign body cells. Occasionally, it was even impossible to find the membranes in the sections again. In addition, in the 60/40 heat-treated specimen it was observed that the degradation was attended by an accumulation of the fragments.

DISCUSSION AND CONCLUSIONS

In the last decade, the technique of GTR has been developed for the regeneration of periodontal tissues and bone around natural teeth and dental implants[1–6]. In a series of animal and human experiments the efficacy of various surgical techniques and membrane materials has been tested. On the basis of these studies, currently, ePTFE is the material mostly used for GTR procedures. However, the application of ePTFE has several disadvantages of which the most important are its clinical manageability and non-degradable nature[22]. For these reasons, present research is focusing on the evaluation of new biodegradable and functionally satisfactory membrane materials. The aim of our study was to evaluate the subdermal and subgingival tissue and clinical behaviour of a newly developed composite membrane material made of synthetic polymer and HA grains.

The experiments confirmed the results of other investigators[16–18] regarding the biocompatibility of the Polyactive polymer used in the composite. The observed tissue reaction around all 60/40 materials has all the characteristics of the classical fibrous encapsulation of non-toxic implants[23,24]. Especially around the 80/20 polymers containing HA grains, a greater degree of cellular reaction was observed due to the faster degradation and more adverse release of degradation products and HA grains. This increase in cellular response was statistically confirmed by the linear regression test showing the existence of a relation between interface quality and degradation behaviour. Despite this increased reaction, it should be emphasized that, characteristic of hydrolysable polyesters, the cellular response was still remarkably mild[25].

It should also be noted that the increased degradation rate of 80/20 membranes is in agreement with earlier descriptions of the degradative mechanisms of this kind of copolymer. The main factor inducing the degradation of PEG/PBT copolymer is considered to be hydrolysis of the ester bonds[18]. The degradation rate will be affected by crystallinity, molecular weight, additives and fabrication conditions. In the case of PEG/PBT copolymers, the ester linkage present in the water-soluble PEG segment is most susceptible to

hydrolysis[26]. Therefore, a higher content of crystalline hydrophobic PBT will result in decreased degradation[27]. The thickness used, addition of HA grains and fabrication technique appeared to be of minor influence on the degradation of the PEG/PBT membranes used.

An important factor for the GTR technique is a sufficient length of healing period. At present, dependent on the application, minimum healing periods between 6 wk and 6 months are recommended[1]. Considering *Figure 10*, it can be concluded that the degradation of 80/20 membranes is too fast. Apparently, the biodegradable characteristics of 60/40 membranes are more suited for GTR. In addition, it should be emphasized that it is possible to optimize the degradation rate for each specific application by appropriate adjustment of the proportions of PEG and PBT.

Further, the degradation characteristics of the 60/40 membranes used in this study confirm the findings in previous studies[28,29]. For example, Radder[29], using follow-up periods of 1, 4, 12, 26 and 52 wk, observed

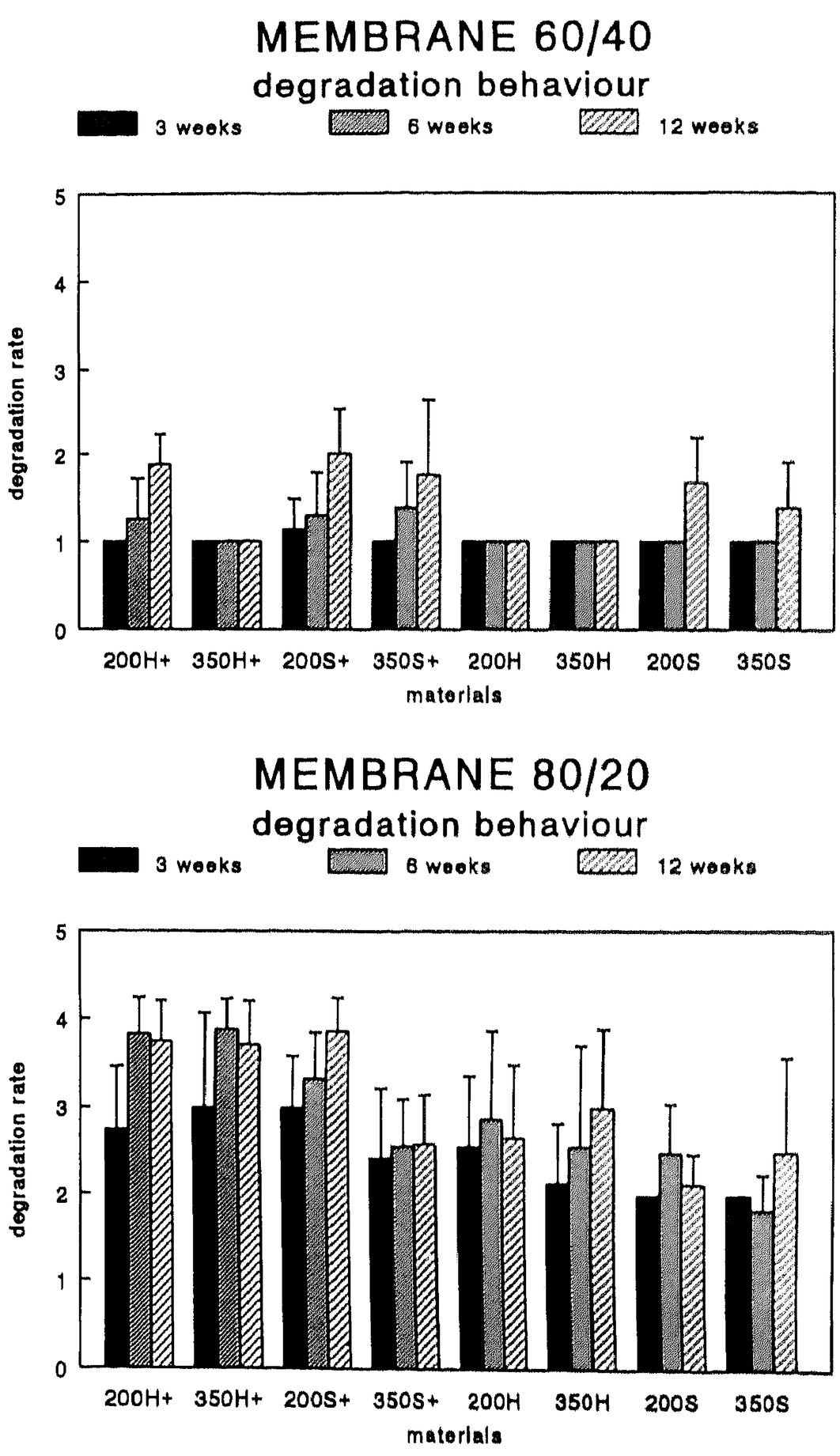


Figure 10 Comparative rating of the degradation behaviour of the membranes for different implantation periods. 200 resp. 350 = thickness in μm; S = solvent-cast; H = heat-treated; + = Polyactive[®]/hydroxyapatite membrane.

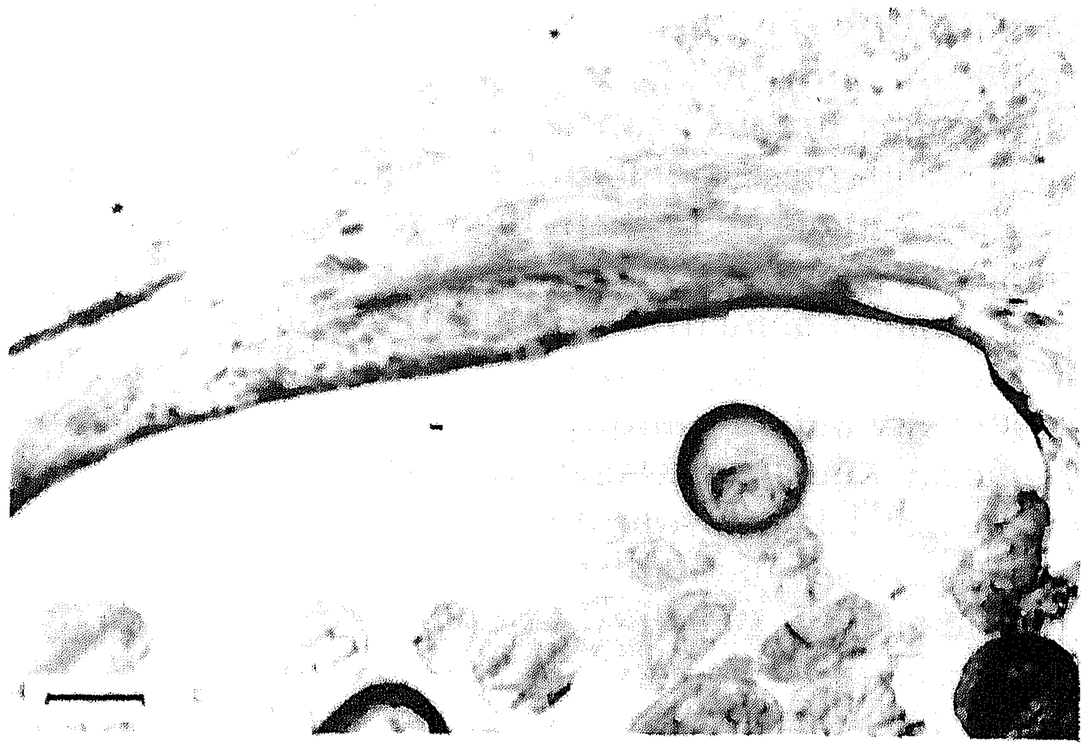


Figure 11 Light micrograph of a subgingival solvent-cast 60/40 Polyactive[®]/hydroxyapatite membrane, thickness 200 μm, at 9 wk. The membrane was fragmented. The fragments were surrounded by a fibrous tissue capsule, which was separated from the membrane surface by a layer of inflammatory cells. Original magnification ×50, bar = 50 μm.

that the degradation of subcutaneously placed 60/40 implants starts from 12 wk. Despite these observations, it is also of interest that Beumer *et al.*[30], using 40/60 bilayered Polyactive membranes with a dense top layer and a porous lower layer, observed fragmentation 4 wk after implantation. However, the thickness of the dense top layer was far less than the thickness of the membranes used in the present study. This can explain the increased degradation rate of these bilayered membranes.

Finally, subgingival placement of the PEG/PBT membranes demonstrated their clinical manageability. Except in one animal, where a modified surgical approach was used, complete soft tissue coverage of the membranes was maintained during the implantation periods. These findings are in contrast with the results of an earlier study[15] in which polylactic acid and polyurethane membranes were used for the treatment of surgically created periodontal defects. In this study exposure of the membranes was noted in almost all animals. This phenomenon was attributed to the swelling behaviour of the polyurethane membranes and to epidermal downgrowth resulting in exofoliation instead of resorption of the membranes. Therefore, as compared to this study, it seems reasonable to assume that the observations of the present study form a reliable basis for further research into the clinical applicability of PEG/PBT membranes in GTR procedures.

In summary, the results of this study have demonstrated that all tested polymeric materials showed biocompatible behaviour without extensive foreign body reaction. Histomorphometrical evaluation demonstrated that the tissue response for the tested membranes varied between composition, but not between membrane thickness, implantation time, and production process. We also observed that the membranes did not cause perforation or inflammation of the covering skin/gingival tissue. Further, from our investigations, it appeared that 3 wk postoperatively, degradation of all membranes with 80/20 PEG/PBT

ratios was visible. This period may be too short for application in GTR. Finally, we noted that some of the 60/40 materials did not degrade until 12 wk after implantation, which is in line with GTR.

On the basis of the results of the pilot study, it can be concluded that PAHA membranes appear to be of great promise for application in GTR. However, to assess the full potential of these materials for clinical application in human patients, more experimental animal studies and clinical studies have to be performed.

ACKNOWLEDGEMENT

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