Osseointegration of subperiosteal implants using bovine bone substitute and various membranes


An earlier study revealed incomplete osseointegration of individually made titanium subperiosteal implants covered by ePTFE membranes and fixated to the rabbit tibial bone surface. In addition, the newly-formed bone was dominated by large marrow spaces. In this subsequent study, subperiosteal implants were also fixated on the bone surface of both tibia of 9 Copenhagen White rabbits. Bio-Oss® particles were packed densely covering the entire implant surface. One of 3 different membranes covered the implant and the particles. The membranes used were the degradable Polylactin 910® mesh, a degradable bilayer collagen membrane and the non-degradable ePTFE membrane. Undecalcified sections were prepared for histologic evaluation after a 12 weeks' observation period. All 18 subperiosteal implants were completely osseointegrated. In addition, the marrow spaces were reduced compared to our previous study. The Bio-Oss® particles proved to be biocompatible and osteoconductive. The ePTFE membranes revealed neither signs of collapse nor adjacent infiltration of inflammatory cells. The Polylactin 910 mesh and the bilayer collagen membranes collapsed slightly. There were signs of resorption of the surface of the newly-formed bone under the degradable membranes. The cause of resorption can not be documented.

The prognosis of implant installation in atrophic jaws is generally very good (Albrektsson et al. 1986). However, a certain amount and quality of bone is necessary. Autogenous bone grafts are widely used for augmentation of extremely atrophic jaws, before or simultaneous with implant placement. However, preliminary experimental studies have indicated that the earlier abandoned subperiosteal implants could be of interest (Schmid et al. 1991) in the treatment of extremely atrophic jaws (Hjort-Hansen et al. 1995; Schmid et al. 1991). Subperiosteal implants covered by ePTFE membranes (Gore-Tex®, W.L. Gore and Associates, Flagstaff, AZ, USA) were used in these studies to prevent undesired cells from invading the tissues surrounding the implant. Complete osseo-integration of the subperiosteal implants was noted in the study by Schmid and co-workers (1991), whereas the implants were only partly covered by newly-formed bone in our study (Hjort-Hansen et al. 1995). In addition, large marrow spaces in the space between the implant and the membrane were noted.

Bone substitutes have been used to improve repair of bone defects (Klinge et al. 1992; Thaller et al. 1993) and in various augmentation procedures (Block & Kent 1985; Berglundh & Lindhe 1997; Frame 1987; Gotfredsen et al. 1991; Moy et al. 1993; Wetzel et al. 1995; Zitzman et al. 1997). Several experimental and clinical studies have documented the highly biocompatible and osteoconductive characteristics of Bio-Oss® par...
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particles (Geistlich Pharma, Wolhusen, Switzerland) (Klinge et al. 1992; Storgaard-Jensen et al. 1996; Thaller et al. 1993). Consequently, concomitant use of subperiosteal implants and Bio-Oss® covered by a membrane may establish the optimal conditions for osseointegration of subperiosteal implants.

The purpose of the present experiment was therefore to study whether complete osseointegration of individually made titanium subperiosteal implants could be obtained concomitant with prevention of large marrow spaces formation in the newly-formed bone by using a bovine bone substitute and various membrane types.

**Material and methods**

A license to perform the study was obtained from the Danish National Experimental Animal Inspectorate.

**Experimental animals**

Nine adult female Copenhagen White rabbits (*Oryctolatus Cuniculus*) with closed epiphyseal plates were used. The animals were kept in single cages and fed a dried standard rabbit diet (Slangerup Foderstofforretning, Slangerup, Denmark) and water ad libitum.

**Anesthesia and drug administration**

The animals received streptomycin-benzylpenicillin (Streptocillin Vet®, 200,000 i.u./ml, Novo Industries, Denmark, 0.25 ml/kg, i.m.) just before the surgical procedure. General anesthesia was induced by fluanison (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium, 0.5 ml/kg, i.m.) and diazepam (Stesolid®, Dumex, Copenhagen, Denmark, 0.4 ml/kg, i.m.). The site of surgery was infiltrated preoperatively with 1 ml lidocaine adrenaline (Xylocain® adrenaline, 20 mg/ml + 12.5 µg/ml, Astra, Södertälje, Sweden). Postoperatively, streptomycin-benzylpenicillin (Streptocillin Vet®, 200,000 i.u./ml, Novo Industries, Denmark, 0.125 ml/kg, i.m.) were given for the subsequent 3 days.

**Surgical procedure**

The hind legs of the rabbits were initially shaved and washed with 0.1% aqueous chlorhexidine digluconate. The medial part of both tibia were exposed via a skin incision and careful subperiosteal dissection. To facilitate later precise implant localization, a sign was made in the bone surface by a burr. An impression of the bone surface was made with Impregum® (ESPE, Seefeld, Germany). The periosteum and muscular layers were repositioned and sutured by non-degradable sutures (Mira-lene®, Braun Melsungen, Germany). The skin was sutured by degradable sutures (Vicryl®, Ethicon, Norderstedt, Germany). The impressions were used to make individual commercially pure titanium frameworks within 48 h. The thickness of the framework was 0.9 mm. Two central posts with a height of 2 mm were placed to secure space between the membrane and implant and to imitate abutments. A hole was made in both ends for fixation screws. After casting, the framework was acid treated, washed, and sterilized.

Two days after the first operation, the animals were re-anesthetized. Both tibia were exposed through the original incision and the titanium implant was placed unambiguously according to the previously made localization sign with complete congruence with the bone surface. One implant was placed on each tibia. Perforations in the underlying cortex were made with a 1-mm burr according to the fenestrations of the implant (Fig. 1). The space between the posts was packed with a bovine bone substitute soaked in blood (Bio-Oss®, spongiosa, 1–2 mm, Geistlich Pharma, Wolhusen, Switzerland) (Fig. 2). The implant and Bio-Oss® particles were covered at random by an expanded polytetrafluoroethylene (ePTFE) augmentation membrane (Gore-Tex®, Gore, Flagstaff, AZ, USA), a degradable Polyglactin 910 Mesh (Vicryl®, Ethicon, Norderstedt, Germany) (PG), or a degradable bilayer collagen membrane (Biogide®, Geistlich, Wolhusen, Switzerland) (BC). There were 6 observations for each membrane, 18 in total. The size of the membrane was adjusted to cover the implant extending 5 mm beyond the implant edges. The implant and membrane were fixed with two 5-mm screws (W. Lorens Micro System, Martin Medizin-Technik, Tuttingen, Ger-

![Fig. 1. An individually made commercially pure titanium subperiosteal implant placed on the bone surface. The cortical bone is perforated through the fenestrations of the implant.](image-url)
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Fig. 2. Implant covered by Bio-Oss® particles soaked in blood. This implant is due to be covered by an ePTFE membrane.

Fig. 3. Osseointegration of a subperiosteal titanium implant covered by Bio-Oss® particles (BO) and an ePTFE membrane (EM). Original magnification ×23.

Fig. 4. Bio-Oss® particles surrounded by woven bone. Area A shows woven bone, area B shows parallel fibered bone. Cutting cones (C) are noted. Original magnification ×58.

Furthermore, the majority of Bio-Oss® particles were also integrated in woven bone (Fig. 4). The remaining particles were integrated in marrow spaces and covered by osteoid and osteoblast seams. The surface of the woven bone surrounding implants and particles were covered by osteoid and irregular osteoblast seams. The woven bone was in some areas replaced by parallel fibered bone without a clear pattern. However, resorption of the woven bone could be noted on one side and apposition of bone on the other side in other areas. At the level of the posts, marrow spaces were noted. The hematopoietically active marrow consisted of a large number of blood-forming cells and some fat cells. Numerous vessels were seen in the newly-formed bone.

Some of the surgically created perforations of the cortical bone were occupied by Bio-Oss® particles, hematopoietic tissue in contact with the marrow of the tibia, or minor amounts of woven bone (Fig. 5).

Histology

Expanded polytetrafluoroethylene membrane covered implants. All subperiosteal implants were completely osseointegrated in woven bone (Fig. 3). The periosteum, muscular layer, and skin were repositioned and sutured as previously described. A similar procedure was performed at the contralateral tibia.

No control group, covering an implant with a membrane but no Bio Oss® particles, was included due to the results obtained in our earlier study (Hjorting-Hansen et al. 1995). The result of the former study was incomplete osseointegration of subperiosteal implants covered by ePTFE membranes and no integration of implants not covered by a ePTFE membrane.

Tissue processing

The animals were sacrificed after 12 weeks with an overdose of pentobarbital (Mebumal®, University Hospital (Rigshospitalet), Denmark). Both Tibia were resected 5 mm mesially and distally from the ends of the implant and fixed in 4% neutral buffered formaldehyde for 4 weeks after the bone marrow was exposed by removing the lateral cortical bone and dividing the implant into 2 parts facilitating fixation, dehydration, and embedding in methacrylate. At least 3 to 4 ground sections with a thickness of 25–30 μm were prepared from each implant parallel with the long axis of the tibia. All sections were stained by basic Fuchsin and light green (Gotfredsen et al. 1989).

Results

The postoperative period of the 9 animals was uneventful.

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Fig. 5. A former surgically created perforation (arrows) with a mixture of woven bone and hematopoietic tissue. The perforation provided communication between the marrow of the tibia and the experimental area. Original magnification \( \times 23 \).

Osteoclasts or resorption processes could not be identified on the surface of Bio-Oss\textsuperscript{\textregistered} particles. Furthermore, no inflammatory cells were identified adjacent to the particles. However, cutting cones could be noted at the surfaces and in the center of the particles. These cones were filled with woven bone or osteoid and osteoblasts (Fig. 4).

The ePTFE membranes were in situ in all the specimens. There were no signs of collapse of the membranes. In addition, all membranes were covered by a thin layer of connective tissue on both surfaces without infiltration of inflammatory cells. Actually bone formation was noted in 2 of the 6 specimens on the outer surface of the membrane.

**Polyglactin 910 and collagen membrane covered implants.** The histologic characteristics of implants covered by PG or BC membranes were rather similar. Consequently, they will be described simultaneously.

All implants were osseointegrated in woven bone (Fig. 6a). However, the implant posts were not fully integrated in bone. The upper part of the posts was namely integrated in fibrous connective tissue. Osteoid and osteoblast seams could be noted on the surface of the woven bone. However, no osteoid and osteoblasts were identified on the surface of the newly-formed bone situated just under the former membranes. The surface was crenated, having a striking appearance of Howship's lacunae. (Fig. 6b). A few osteoclasts were identified. However, there were no signs of remodeling. At post level marrow spaces could be noted. The surgically created perforations were filled with Bio-Oss\textsuperscript{\textregistered} particles, hematopoietic tissue and woven bone. Numerous vessels were seen in areas with new bone formation as well as in the fibrous connective tissue. The Bio-Oss\textsuperscript{\textregistered} particles with close contact to the implant were surrounded by woven bone. Cutting cones with woven bone could be noted. Particles situated at post level were integrated in fibrous connective tissue and surrounded by only a small edge of osteoid. No resorption of the particles could be seen. In addition, inflammatory cells or remnants of the 2 membranes could not be demonstrated. However, a space in the overlying connective tissue indicated the space where the former membranes had been situated (Fig. 6a). There were signs of minor collapse of both membrane types. The overlying tissue seems to be able to displace the membranes down in the area between the 2 posts. There were signs of rupture of 1 of the collagen membranes.

**Discussion**

The purpose of the preliminary experiment was to study whether complete osseointegration of an individually made commercially pure titanium subperiosteal implant could be obtained concomitant with no large marrow spaces by using Bio-Oss\textsuperscript{\textregistered}.
and various membrane types. An earlier study showed incomplete osseointegration of subperiosteal implants covered by ePTFE membranes and fibrous integration of subperiosteal implants without ePTFE membranes (Hjørting-Hansen et al. 1995). The bone substitute in this study was used to occupy the space between the implant and the membrane, to support the overlying membrane, and to act as an osteoconductive material. The histological evaluation documented that all subperiosteal implants were completely osseointegrated irrespective of the used membrane type. Three stages of bone maturation were noted, namely woven bone, woven bone replaced by parallel fibered bone in some areas, and finally in other areas bone resorption of bone on one surface and bone apposition on the other surface. The occurrence of woven bone with osteoid and osteoclasts, also noted around the Bio-Oss® particles indicated that further osteogenesis could be expected if the observation period was extended.

The osteoconductive properties of Bio-Oss® have been documented in earlier studies also (Berglundh & Lindhe 1997; Klinge et al. 1992; Thaller et al. 1993; Storgaard-Jensen et al. 1996). Considerable amounts of new bone were formed in direct contact with the Bio-Oss® particles. On the surface of the particles signs of cutting cones could be noted. Although no osteoclasts were identified, as in previous studies (Klinge et al. 1992; Storgaard-Jensen et al. 1996), evidence of earlier resorption was present due to new bone formation in the central part of some of the particles and in lacunae on the surface of the particles. Consequently, it seems that the particles were participating in the general remodeling processes. Although this study is not an unbiased stereologic study, but a descriptive histologic study, the large marrow spaces as noted in an earlier study (Hjørting-Hansen et al. 1995) were considerably diminished irrespective of the covering membrane type. The combined use of Bio-Oss® covered by a membrane seems to secure more bone formation (Hürzeler et al. 1998; Hämmerle et al. 1997) compared to the use of only a membrane, as previously documented (Hjørting-Hansen et al. 1995). This is also in accordance with experiences in vertical augmentations in humans, reported by Simion and co-workers (1994, 1998). By addition of particles of autogenous bone or demineralized freeze-dried allograft, these authors succeeded in an increase of vertical augmentation of the edentulous alveolar process by nearly 100% when the above mentioned chips were used as a filler along with the use of membranes (Simion et al. 1994; Simion et al. 1998).

The study was performed by using rabbit tibia and not resorbed atrophic jaw. Therefore, bone formation would be due to neogenesis rather than regeneration. Lifting and repositioning of the periost will induce a subperiosteal bone reaction in young individuals. This does not occur in adults (Melcher 1971; Melcher & Accursi 1971). Linde and co-workers (1993) placed ePTFE domes on the surface of 3 rat calvaria. The periost was excised to reduce the tension of the overlying soft tissue. No new bone formation could be noted. In contrast, if a ePTFE dome was covered by periost, new bone formation occurred (Linde et al. 1993). This indicated that the effect of the perios- teum is necessary for bone formation. However, studies using different kinds of titanium devices have indicated, that there is no need for a perios- teum to induce bone formation (Lundgren et al. 1995; Schmid et al. 1994). In the present study perforations of the cortical bone were made to enable osteoprogenitor cells from the tibia marrow to emerge into the experimental area. This procedure is in accordance with some studies (Alberius et al. 1996; Buser et al. 1990; Hämmerle et al. 1997; Lind et al. 1993; Schmid et al. 1991; Schmid et al. 1994, Schmid et al. 1997a; Schmid et al. 1997b), while other studies reported bone formation beyond the skeletal envelope without cortical bone perforation (Kostopoulos & Karring 1994a; Kostopoulos & Karring 1994b; Lundgren et al. 1995). The surgically created perforations were filled with woven bone and hematopoietic tissue in contact with the marrow of the tibia indicating that osteoprogenitor cells had possibilities to invade the supraimplant area. No quantitative studies have to our knowledge compared the amount of newly-formed bone with or without perforating the bone surface.

The stability of the wound area and/or the stabli- lity of the membrane is a crucial point in bone formation too. Linde and co-workers concluded that the stiffer the dome material, the more the new bone formation (Linde et al. 1993). This is in accordance with titanium device studies (Lundgren et al. 1995; Schmid et al. 1994) and studies where the membrane is stabilized by a bone substitute (Hürzeler et al. 1998; Hämmerle et al. 1997; Schmid et al. 1997a; Schmid et al. 1997b).

The ePTFE membranes showed no signs of collapse and adjacent inflammatory reactions. Consequently, the material again proved to be highly biocompatible (Becmeur et al. 1990; Dahlin et al. 1988; Dahlin et al. 1990; Schenk et al. 1994). Newly-formed bone was noted occasionally on the outer surface of the membrane. The 2 degradable membranes both showed signs of minor collapse. The collapse did not impair bone formation due to the supporting Bio-Oss® particles. There were
signs of resorption of the newly-formed bone which had been in former contact with both the degradable membranes. An earlier study showed no such signs (Aaboe et al. 1998). However, the observation period was only 8 weeks in that study. The extended observation period could therefore explain the bone resorption due to a more advanced stage of degradation. Hürzeler and co-workers (1998) treated exposed implant threads in monkey degradation. However, it is not possible to document-like cells. Another reason for the resorption products creates an environment which attracts osteoclasts. An earlier study showed that transplanted autogenous bone resorb to a certain degree (Baker et al. 1979; Burchardt 1987; Swart & Allard 1985). However, the resorption of normal bone will be reduced if implants are inserted and loaded (von Wowern et al. 1990). This could be the same for bone formed around a loadable osseointegrated subperiosteal implant.

Conclusion
It should be emphasized that the present study is a preliminary, descriptive, histologic study. No statistical analysis has been made. However, the combined use of a bone substitute together with different membranes ensured complete osseointegration of the subperiosteal implants. In addition, the marrow spaces seem diminished. Further studies should focus on loading of the osseointegrated subperiosteal implants.

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Résumé

Zusammenfassung

Resumen
Un estudio anterior reveló una incompleta osteointegración de implantes de titanio subperiósticos hechos individualmente cubiertos por ePTFE y fijados a la superficie del hueso tibial del conejo. Además, el hueso neof ormado estaba dominado por grandes espacios de médula. En el siguiente estudio también se
fijaron implantes superperiósticos en la superficie ósea de ambas tibias de 9 conejos blancos de Copenhague. Se compactaron particulas de Bio-Oss® cubriendo toda la superficie implantaria. Una de tres diferentes membranas cubrió el implante y las particulares. Las membranas usadas fueron una malla de Polylactin 910® degradable, una membrana de colágeno degradable de doble capa y una membrana no degradable de ePTFE. Se prepararon secciones no descalcificadas para evaluaciones histológicas tras un período de observación de 12 semanas. Todos los 18 implantes superperiósticos estaban completamente osteointegrados, además, los espacios medulares se redujeron comparados con el estudio anterior. Las particulas de Bio-Oss demostraron ser biocompatibles y osteocondutivas. Las membranas de ePTFE no revelaron signos de colapso ni de infiltración adyacente de células inflamatorias. La malla de Polylactin 910 y la membrana de colágeno de doble capa se colapsaron ligeramente. Hubo signos de reabsorción no documentada de la reabsorción de la superficie del hueso neoformado bajo las membranas degradables. La causa de la reabsorción no se puede documentar.

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