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A histological evaluation for guided bone regeneration induced by a collagenous membrane

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Abstract

This study was designed to evaluate the histological changes during ossification and cellular events including osteogenic differentiation responding to collagenous bioresorbable membranes utilized for GBR. Standardized artificial bony defects were prepared at rat maxillae, and covered with a collagenous bioresorbable membrane. These animals were sacrificed at 1, 2, 3 and 4 weeks after the GBR-operation. The paraffin sections were subject to tartrate resistant acid phosphatase (TRAP) enzyme histochemistry and immunohistochemistry for alkaline phosphatase (ALP), osteopontin (OP) and osteocalcin (OC). In the first week of the experimental group, woven bone with ALP-positive osteoblasts occupied the lower half of the cavity. The collagenous membrane included numerous ALP-negative cells and OP-immunoreactive extracellular matrices. At 2 weeks, the ALP-, OP- and OC-immunoreactivity came to be recognizable in the region of collagenous membrane. Since ALP-negative soft tissue separated the collagenous membrane and the new bone originating from the cavity bottom, the collagenous membrane appeared to induce osteogenesis in situ. At 3 weeks, numerous collagen fibers of the membrane were embedded in the adjacent bone matrix. At 4 weeks, the membrane-associated and the cavity-derived bones had completely integrated, showing the same height of the periosteal ridge as the surrounding alveolar bones. The collagen fibers of a GBR-membrane appear to participate in osteogenic differentiation.
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1. Introduction

Guided tissue regeneration (GTR) introduced by Nyman et al. [1] and Gottlow et al. [2] is a technique that uses a barrier membrane, which can allow the repopulation of periodontal ligament-derived cells onto the dental root surface. Dahlin et al. [3] originally applied this technique to bone regeneration in a bone loss area to establish the concept of "osteopromotion". Based on Dahlin's technique, Buser et al. [4] proposed the term of "guided bone regeneration (GBR)", which aims to promote bone augmentation by a barrier membrane. This technique has been applied in clinical dentistry to various cases including dental implant therapy with an insufficient volume of bone in recipient site. The most popular membrane currently utilized for GBR is an expanded polytetrafluoroethylene (e-PTFE) membrane, which is a biologically inert material and can

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be safely applied clinically. Many investigators have demonstrated successful bone regeneration by using this material alone [5,6] or in combination with other filling materials—bone grafts [7,8]. Despite the many advantages of this membrane, however, it must be removed by a secondary operation that is required to repair dehiscence because of its natural unresorbability.

Recently, bioresorbable membranes have been highlighted because of their permitting a single-stage procedure, and have come to be widely used in conjunction with GTR and GBR [9–11]. The major benefit of bioresorbable membranes is the absence of the necessity of a second surgery to remove the membrane. Bioresorbable collagenous membranes are currently available for dental clinics [10,12–14], and have been employed for various areas of basic research including its application for GTR and GBR [15,16]. Many researchers have paid attention to the final volume of newly formed bone achieved by GBR with a bioresorbable membrane, especially in comparison with GBR using an e-PTFE membrane [9,13–15]. Despite the plethora of studies on bioresorbable membranes, little has been reported about histological changes of new bone formation and the cellular events of bone cells when these membranes were applied for GBR.

There are two types of bioresorbable membranes: synthetic polymers and natural biomaterials [17]. A collagenous membrane is a natural biomaterial of a bioresorbable membrane. Bio-Gide® is composed of porcine type I and type III collagen fibers without any organic components and/or chemicals, and has a bilayer structure composed of a "compact" and "porous" layer. The compact layer of the membrane possesses a smooth and condensed surface to protect against connective tissue infiltration, while the porous layer permits cellular invasion. When used for GBR, the porous and compact layers may enable osteogenic cell migration and prohibit connective tissue intrusion, respectively. In host animals, mesenchymal cells can differentiate into osteogenic cells under preferential circumstances [18]. Collagen fibers are the most abundant components in a bone matrix [19], and may act as a reservoir of many local factors and in the cell–matrix attachment of osteogenic cells. Despite the absence of bone-specific proteins, collagen fibers of GBR-membranes may serve as a physical scaffold for osteogenic cells in bone defects as well as function as a barrier against an infiltration of surrounding connective tissues.

The purpose of the present study was therefore to investigate chronological changes in new bone formation in bone defects when applied with a collagenous membrane of GBR, and to verify cellular events in situ responding to the collagenous membrane of the GBR from a histological point of view.

2. Materials and methods

All animal experiments in this study were conducted under the Niigata University Guidelines for Animal Experimentation.

2.1. Animals and experimental procedures

Twenty 4-week-old male Wistar rats were used in this experimental study. Under anesthesia by an intraperitoneal injection of 8% chloral hydrate (400 mg/100 g body weight), the first and second molars were extracted from the left-hand and right-hand sides of the maxillae according to Ohnishi et al. [20]. Briefly, at 4 weeks after the tooth extraction, palatal and buccal full-thickness flaps were elevated in the extracted regions. A standardized artificial bony defect (width: 1.0 mm, depth: 1.0 mm, mesiodistal length: 2.5 mm) was formed by drilling with a low-speed dental handpiece at 500 rpm equipped with a round bur (ϕ: 1.0 mm). A profuse irrigation with physiological saline was maintained throughout the drilling. Following a brief washing in physiological saline, the defect on the experimental side was covered with a bioresorbable membrane (Bio-Gide®, Geistlich AG, Wolhusen, Switzerland) composed of porcine type I and type III collagen fibers. Bio-Gide® has a bilayer structure of "compact" and "porous" layers. Each membrane was trimmed off to be rectangular (4×5 mm) enough to cover the bony defects. The compact membrane layer has a smooth and condensed surface to prohibit an undesirable connective tissue infiltration, while the porous layer permits cellular migration. Thus, the porous surface of the membrane was placed facing the bone cavity. The membrane was stably positioned by periosteal flaps which were sutured with silk threads. As a control group, the contra-lateral site received the same experimental procedure without any covering with Bio-Gide®. After these procedures, the animals were housed with free access to water and provided a powder diet. No antibiotic was given to the operated rats. At 1 week after the operation, all sutures were removed.

2.2. Histochemical procedure

The operated rats were sacrificed at 1, 2, 3 and 4 weeks after the GBR operation ($n = 5$ each). Under deep anesthesia as described above, they were fixed with a transcardiac perfusion with a fixative of 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.4). Following fixation, the maxillae were removed en bloc, immersed in the same fixative for an additional 16 h, and decalcified with 10% EDTA-2Na solution for 4 weeks at 4 °C. After demineralization, the specimens were dehydrated through an ascending ethanol series prior to embedment in paraffin.

Paraffin sections of a 5 μm thickness were used for immunocytochemistry with antisera against tissue non-specific alkaline phosphatase (ALP) [21], osteopontin (IBL Co., Japan) (OP), osteocalcin (Biomedical Technologies. Inc., Stoughton, MA) (OC), and ED1 that can recognize rat monocyte/macrophage [22], as well as for tartrate resistant acid phosphatase (TRAP) enzyme histochemistry. The immunoreactions were visualized with diaminobenzidine development. For TRAP detection, the sections were rinsed with PBS and processed for the azo-dye method according to Burstone [23]. The sections were faintly counter stained with methyl green.

3. Results

3.1. General condition and socket healing after tooth extraction

The operated animals showed no remarkable changes in condition including feeding habits. At 4 weeks after the extraction, the tooth sockets were completely filled with newly formed bone, which was impossible to distinguish from the peripheral alveolar bone (data not shown). No infiltration of inflammatory cells was found in the operated area including the oral epithelium, connective tissue, extraction sockets, and peripheral alveolar bone. The alveolar ridge including the previous tooth socket had features of compact bone.

3.2. Control group

At 1 week after the preparation of an artificial bone cavity, the lower half of the cavity was filled with newly formed bone, which was easily distinguishable from the surrounding maxillary bone (Fig. 1A). Oral epithelium and underlying connective tissue completely covered the bony defects without an infiltration of inflammatory cells. This newly formed bone manifested a correspondence to woven bone, as evidenced by the arrangement of many plump osteocytes and thin bony spicules in a random direction. A considerable number of ALP-immunopositive cuboidal osteoblasts appeared on the periosteal surface of the newly formed bone without any accompanying TRAP-reactive osteoclasts (Fig. 1B). In contrast, a few TRAP-reactive osteoclasts were recognizable in the inner region of the newly formed bone (Fig. 1C).

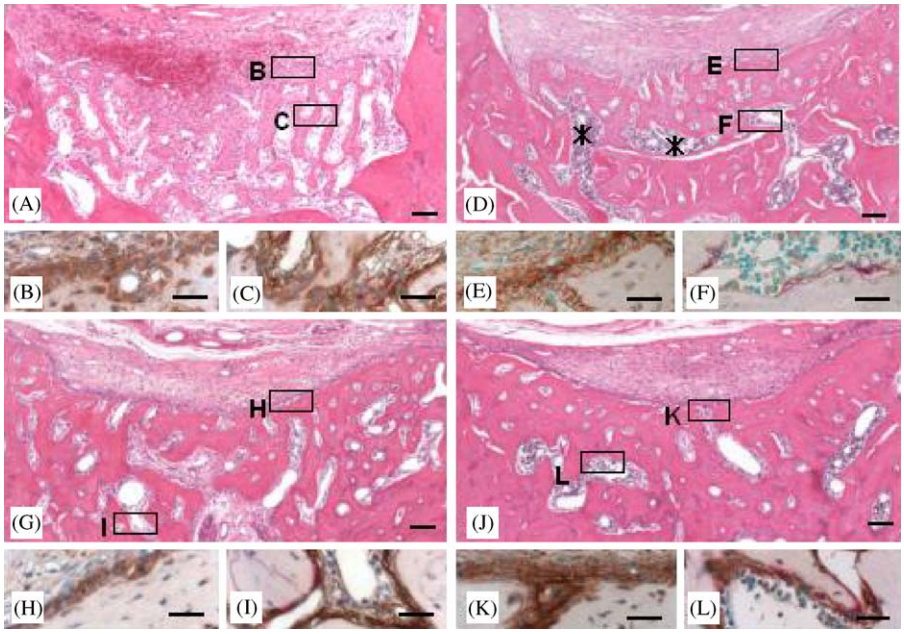

Fig. 1. Control group at postoperative 1 week (A–C), 2 weeks (D–F), 3 weeks (G–I) and 4 weeks (J–L). Stained with hematoxylin and eosin (A, D, G, J) and double staining with ALP and TRAP activities (B, C, E, F, H, I, K, L). (A) The lower half of the cavity shows woven bone. (B) A thick osteoblastic layer with an intense ALP-positivity (brown color) covers the periosteal surface of the new bone. (C) A TRAP-reactive osteoclast (red color) exists among numerous osteoblasts located on the thin bony spicules. (D) The periosteal ridge reaches two-thirds of the height of the peripheral bone, appearing as concaved periosteal surface. Asterisks indicate bone marrow in the intertrabecular region. (E) Numerous intense ALP-positive osteoblasts exist on the periosteal surface without TRAP-reactive osteoclasts. (F) In the inner portion, some TRAP-reactive osteoclasts (red color) are located on the trabecular surface. (G) The newly formed bone possesses thick trabeculae, but does not extend to the same height of the periosteal ridge of peripheral alveolar bones. (H) The periosteal surface underlies ALP-positive osteoblasts without TRAP-reactive osteoclasts. (I) In contrast to the ridge region, there are optimal amounts of ALP-positive osteoblasts and TRAP-reactive osteoclasts on the trabeculae of the inner portion. (J) Trabeculae of the newly formed bone become thicker compared with that at 3 weeks. (K) Numerous ALP-positive osteoblasts cover the periosteal surface of the new bone. (L) In the inner region, several TRAP-reactive osteoclasts are recognizable on the trabeculae. Original magnification: ×95 (A, D, G, J), ×500 (B, C, E, F, H, I, K, L), bars = 100 μm (A, D, G, J), 20 μm (B, C, E, F, H, I, K, L).

At 2 weeks after the cavity preparation, the newly formed bone showed thicker trabeculae, though revealing woven bone (Fig. 1D). The periosteal ridge developed to the point of filling up to two-thirds the height of the previous bone, appearing as a concaved configuration. The inner region of the newly formed bone had bone marrow, and many ALP-positive osetoblasts and several TRAP-reactive osteoclasts localized on the trabecular surfaces (Fig. 1F). In contrast to the inner portion, the ridge region of newly formed bone comprised intense ALP-positive osetoblasts (Fig. 1E).

At 3 and 4 weeks, the newly formed bone displayed thicker trabeculae, but the periosteal surface of new bone remained concave (Figs. 1G and J). Osteoblasts still existed predominantly on the ridge region (Figs. 1H and K). In contrast, the inner portion of the newly formed bone underlay many ALP-positive osteoblasts along with some TRAP-reactive osteoclasts (Figs. 1I and L).

3.3. Experimental group

At 1 week after the cavity preparation and the subsequent placement of a resorbable membrane, the membrane consisting of porous and compact collagenous layers covered the bone defect (Fig. 2A). New bone extended from the bottom region of the cavity as was seen in the control group. Identical to the control group, the newly formed bone showed a profile of woven bone; numerous osteocytes embedded in thin bony spicules and many ALP-immunopositive osteoblasts (Fig. 2B). Although abundant cells invaded the porous layer of the membrane, attenuate cell popularity was found in the compact layer facing the oral epithelium. Despite the absence of any remarkable ALP-immunoreactivity (Fig. 2C), the surfaces of exogenous collagens in the porous layer of the membrane showed OP-immunoreactivity (Fig. 2D). Broad immunoreaction for OP was also found in both newly formed and pre-existing bones (Fig. 2E). No inflammatory cell was noticeable, and consistently, a few ED1-immunopositive monocyte/macrophages were dispersed in the perimeter of the resorbable membrane (data not shown).

After 2 weeks, the newly formed bone had developed further than that seen at 1 week, but could not reach the same height of the peripheral alveolar ridge (Fig. 3A). Abundant ALP-positive cells were present in the porous

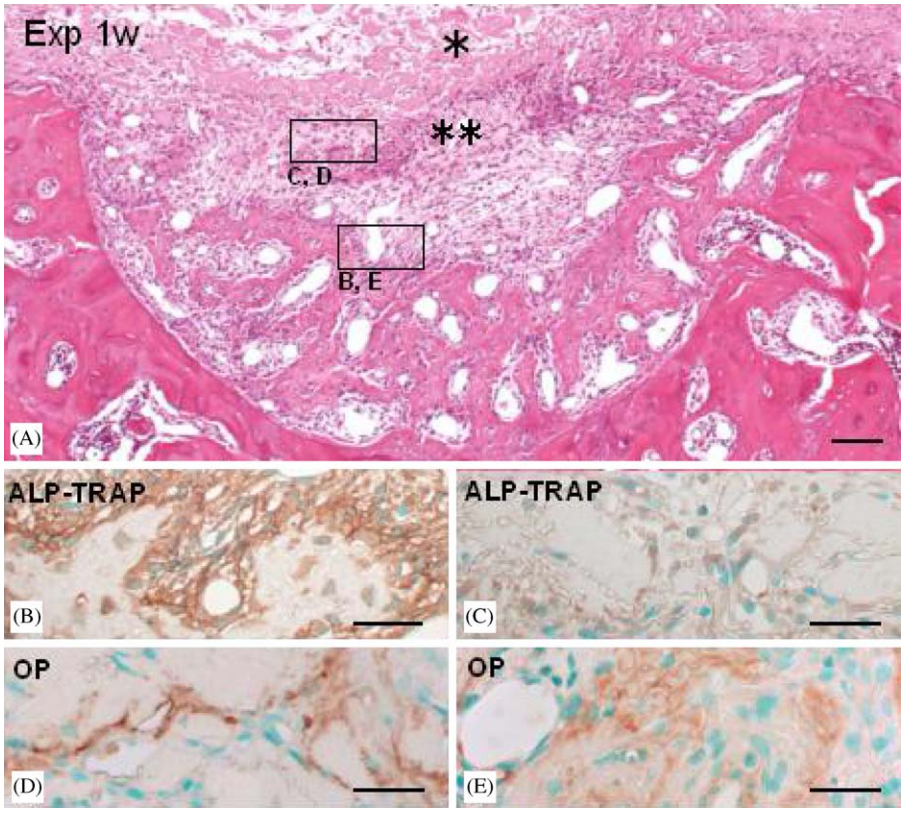

Fig. 2. Experimental group at postoperative 1 week. Stained with hematoxylin and eosin (A), double staining with ALP and TRAP activities (B, C) and osteopontin (OP)-immunoreaction (D, E). (A) The membrane over the cavity is composed of porous (double asterisks) and compact (asterisk) collagenous layers. (B) A double staining with ALP and TRAP of the boxed area (B) in Fig. 2A. Numerous ALP-positive osteoblasts exist on the thin spicules of bone at the inner portion of the new bone. (C) In contrast, abundant cells are found in the porous layer of the membrane, but have no ALP-reactivity. (D) Despite the absence of ALP (see Fig. 2C), OP is detectable on the surfaces of exogenous collagens in the porous layer (double asterisks) of the membrane. Note the absence of immunoreactivity of OP in the stout exogenous collagen. (E) OP-immunoreaction is localized widely in the matrix of the new bone. Original magnification: $\times 95$ (A), $\times 500$ (B–E), bars = 100 μm (A), 20 μm (B–E).

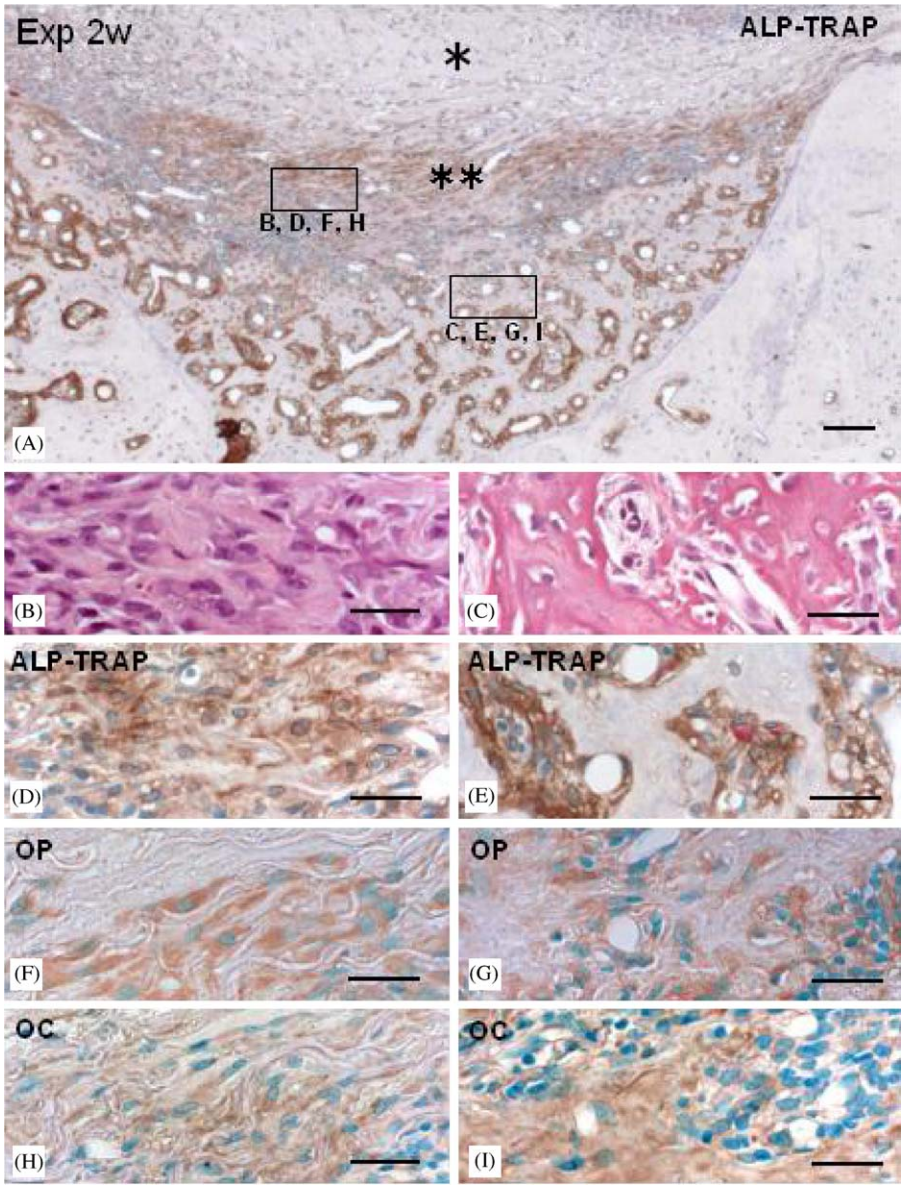

Fig. 3. Experimental group at postoperative 2 weeks. Double staining with ALP and TRAP activities (A, D, E), stained with hematoxylin and eosin (B, C), immunohistochemistry for osteopontin (OP) (F, G) and osteocalcin (OC) (H, I). (A) The newly formed bone still shows thin trabeculae. (B) Abundant cells infiltrate in the porous layer. Note stout collagen fibers stained faintly with eosin. (C) Newly formed bone extends from the cavity bottom showing irregularly shaped trabeculae which include many osteocytes. (D) The porous layer of the membrane contains abundant ALP-positive cells, but no TRAP-reactive osteoclast. (E) In the inner region of the newly formed bone, numerous ALP-positive osteoblasts arrange on the trabeculae, accompanied by some TRAP-reactive osteoclasts. (F) OP-immunoreactivity is discernable in cells and surrounding extracellular matrices. (G) In contrast, the newly formed bone displays a broad distribution of OP-immunoreaction. (H) The porous layer represents OC-immunopositivity, especially in the extracellar matrices among collagen fibers. (I) In contrast to the porous layer, the new bone matrix is uniformly positive for OC. Original magnification: $\times 95$ (A), $\times 500$ (B–I), bars = 100 μm (A), 20 μm (B–I) asterisk: compact layer of collagenous membrane, double asterisks: porous layer of collagenous membrane.

layer of the membrane (Figs. 3B and D). The newly formed bone still displayed woven bone (Fig. 3C), and localized numerous ALP-positive osteoblasts and several TRAP-reactive osteoclasts in the inner portion (Fig. 3E). Consistent with the high cellularity of ALP-positive cells, both OP- and OC-immunopositivity were recognizable in the porous layer of the collagenous membrane; e.g., when observed at a high resolution, the majority of infiltrated cells and extracellular matrices in this layer exhibited OP- and OC-immunoreactivity (Figs. 3F and H). Interestingly, neither remarkable ALP-, OP- nor OC-reactivity was detectable in the region interconnecting the exogenous collagenous membrane and the newly formed bone derived from the cavity, which demonstrated the membrane-associated ossification independent of the newly formed bone

coming up from the cavity bottom. A few of the ED1-positive cells were sparsely found in the region of the resorbable membrane (data not shown).

At 3 weeks after the operation, the periosteal ridge of the newly formed bone reached almost the same height of the peripheral alveolar bone, though

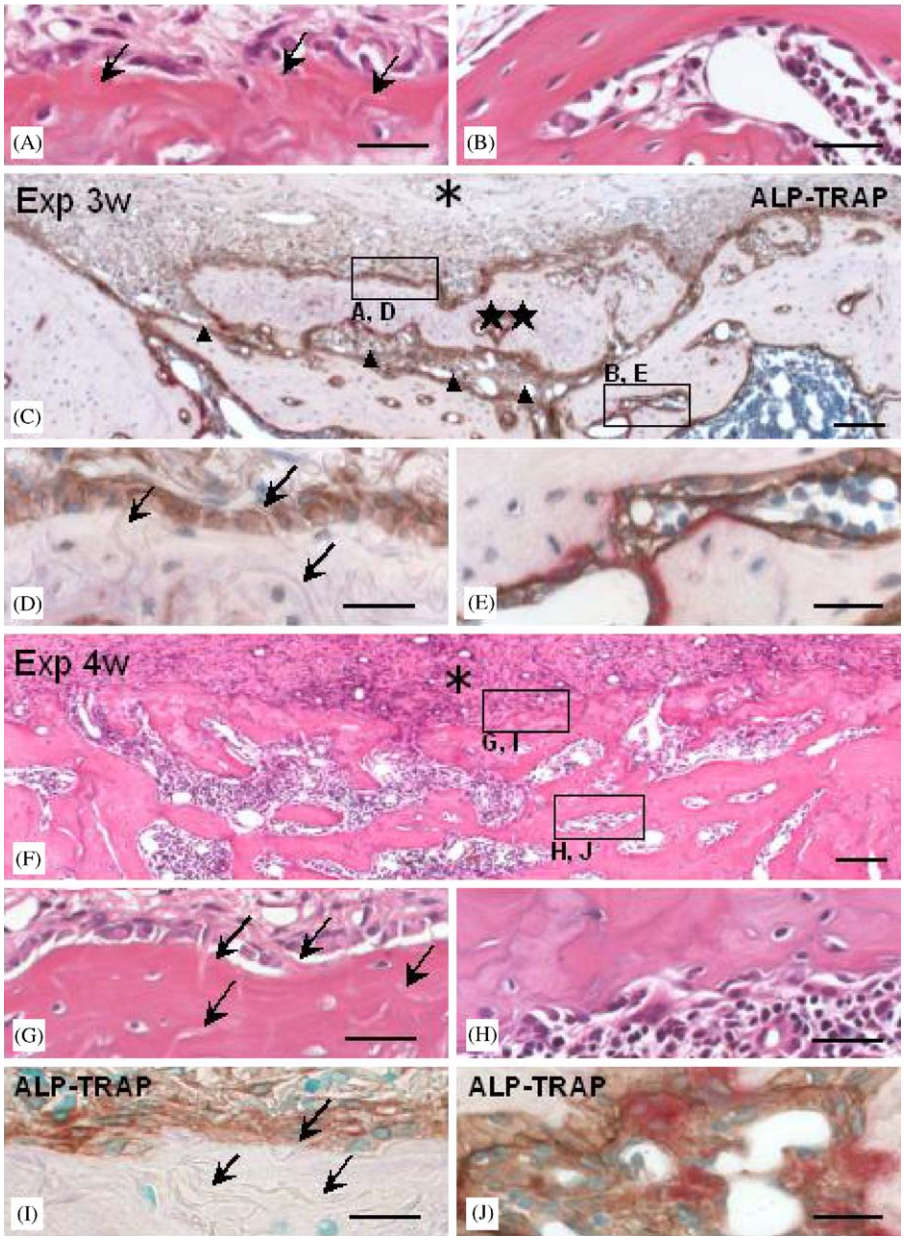

Fig. 4. Experimental group at postoperative 3 weeks (A–E) and 4 weeks (F–J). Stained with hematoxylin and eosin (A, B, F–H) and double histochemistry for ALP and TRAP activities (C–E, I, J). (A) At the ridge region adjacent to the membrane, numerous osteoblasts gather on the periosteal surface of the new bone. Many stout translucent collagen fibers (arrows) are incorporated into the bone matrix. (B) In contrast, in the inner portion of the new bone, trabeculae underlie an optimal number of osteoblasts and face the bone marrow. (C) ALP-positive osteoblasts are arranged on the surface of the newly formed bone, but TRAP-reactive osteoclasts are scattered. The newly formed bone adjacent to the membrane (double stars) is separated from the cavity-derived bone by the presence of the thin soft tissue (arrowheads). (D) In the ridge region of the newly formed bone, a predominant localization of osteoblasts, compared with TRAP-reactive osteoclasts, is recognizable. Stout collagen fibers of the membrane (arrows) penetrate into osteoblastic layers and are incorporated into the bone matrix adjacent to the membrane. (E) In contrast, both ALP-immunopositive osteoblasts and TRAP-reactive osteoclasts are localized on the surfaces of the cavity-derived new bone. Note the lack of exogenous collagen fibers in the new bone here. (F) The new bone entirely fills the previous cavity, and represents the configuration matching the surrounding alveolar bone. (G) There are many osteoblasts on the alveolar ridge, which includes the membrane-derived collagen fibers (arrows). (H) Both osteoblasts and osteoclasts cover the trabecular surfaces at the inner portion of the new bone. (I) The membrane-derived collagen fibers (arrows) are inserted into the bone matrix which underlies many osteoblasts. (J) There are many osteoclasts on the trabeculae along with an optimal number of ALP-positive osteoblasts. Asterisk: collagenous membrane. Original magnification: $\times 95$ (C, F), $\times 500$ (A, B, D, E, G–J), bars = 100 μm (C, F), 20 μm (A, B, D, E, G–J).

the week-matched control group could not attain the same height. Although the entire bony cavity seemingly occupied the newly formed bone, the newly formed bone associated with the membrane was separated from the cavity-derived bone by virtue of the presence of the thin soft tissue. Noticeably, the collagen fibers of the membrane, which were stout compared with authentic rat collagen fibers, penetrated the thick osteoblastic layers and were incorporated into the bone matrix adjacent to the membrane (Fig. 4A). No exogenous collagen fibers were embedded in the newly formed bone that derived from the cavity bottom (Fig. 4B). Thus, the membrane-derived collagen fibers came to be a component of the new bone matrices adjacent to the membrane. A large number of ALP-positive osteoblasts assembled on the periosteal surface of the membrane-associated newly formed bone, implying active bone formation (Figs. 4C and D). In contrast, both ALP-immunopositive osteoblasts and TRAP-reactive osteoclasts occurred on the surfaces of the cavity-derived new bone (Figs. 4C and E). Similar to the situation at 2 weeks, a few ED1 reactive macrophages were discernible (data not shown).

After 4 weeks, the newly formed bone with thick trabeculae filled the previous cavity, and recovered the configuration that matched the surrounding alveolar bone (Figs. 4F and H). Consistent with the findings at 3 weeks, the bone matrix at the ridge region included the membrane-derived collagen fibers, and underlay numerous osteoblasts (Fig. 4G). Unlike the observations at 3 weeks, however, it was difficult to distinguish the membrane-derived new bone from that generated in the cavity, due to its complete integration. In contrast to the predominant osteoblastic population on the ridge region (Fig. 4I), the thick trabeculae of the inner region had both many ALP-positive osteoblasts and TRAP-reactive osteoclasts (Fig. 4J). The collagenous membrane remained above the ridge region, and only a few ED1-immunopositive cells were discernible in the region of the resorbable membrane (data not shown).

4. Discussion

To date, clinical [6,7,9,12–14] and basic [8,15,16] investigations have paid attention to the final volume of bone augmentation achieved by GBR. In this research, we have attempted to investigate the histological changes of newly formed bone induced by GBR with a resorbable collagenous membrane, and verified whether collagen fibers of the resorbable membrane could affect its biological function in osteogenic cells. As expected, our observations revealed the following: first, the collagenous membrane permitted the alveolar ridge of the newly formed bone to reach the same height as the pre-existing bone; second, the ALP-positive cells and OC- and OP-immunopositive bone matrices appeared early at the second week in the porous layer of the membrane, indicating the induction of osteoblastic differentiation; third, the membrane-derived collagen fibers were incorporated into the matrix of the new bone neighboring the membrane; and fourth, the membrane-associated bone integrated with bone extended from the cavity. To our knowledge, this is the first report confirming that a collagenous membrane per se participates in osteogenesis, and provides exquisite ridge augmentation. This has engendered considerable interest in developing bone augmentation.

In the experimental group, the periosteal ridge of the newly formed bone recovered to the same height of the pre-existing bone at 3 weeks, while the control group could not attain that level even after 4 weeks. The compact layer of the Bio-Gide® must have prevented the invasion of undesirable connective tissue, and therefore, may preserve enough space for osteogenic cells to generate bone in the cavity. However, this was expected to happen, as well when an e-PTFE membrane would be applied. The significance here lies in the fact of the osteogenesis in close association with the collagenous membrane. As shown in Fig. 3, intense ALP-reactive cells and OP/OC-immunopositive matrices in the porous layer of the Bio-Gide® indicate the induction of osteoblastic differentiation at early 2 weeks. This is a striking finding on the properties of collagenous membranes, and may account for a well-augmented ridge with the use of this membrane in our study. As shown in Figs. 2C and D, however, the presence of numerous ALP-negative cells and the OP-immunoreactivity in the porous layer at 1 week leads to the postulation that this membrane serves as a physical scaffold for migrating cells and traps bone matrix proteins, rather than serving for osteoinduction at this stage. The new bone thereafter originated from the porous layer of the membrane independent of bone from the cavity. Hence, the membrane-associated bone appears to participate in committing in situ previously migrated cells to an osteogenic lineage. It seems, therefore, unlikely that ALP-positive osteoblasts emanated up to the porous layer of the membrane from the new bone in the cavity.

It is of paramount importance to determine how the membrane-derived collagen fibers could enable cell migration and osteogenic differentiation. Generally, collagen fibers can serve as scaffolds for the migration of various cell types. In addition, collagen can bind other matrix proteins including fibronectin and laminin by means of a collagen-binding domain in these molecules [24,25]. For instance, if fibronectin and OP, which have RGD sequences [26], would be bound to the membrane-derived collagen fibers, many cell types including osteogenic cells could adhere to the collagen fibers by mediating the RGD sequences of fibronectin/OP. Thus, collagen plays a pivotal role in cell–matrix and matrix–matrix interactions on cell migration.

However, it is still unclear how collagen fibers of the Bio-Gide® membrane promote osteogenic differentiation. As a conjecture, the membrane-derived collagen fibers may trap some osteoinductive factors such as bone morphogenetic proteins, transforming growth factors, insulin-like growth factors, and fibroblast growth factors that could be easily released from the bone matrix when the artificial bone cavity was prepared. Since the connective tissue facing the compact layer of the membrane did not show any osteogenesis, some osteoinductive factors might have accumulated onto collagen fibers of the porous layer that faces the cavity. Even if collagen itself has no ability to bind these osteoinductive factors, it can bind several extracellular matrices that have a high affinity for these factors [27]. If so, it is possible that several osteoinductive factors would be trapped by collagen fibers of the membrane, and make previously migrated cells differentiate into an osteoblastic lineage. In addition, type I collagen may directly play an important role in the osteoblastic differentiation [28,29]. Type I collagen can affect cell differentiation through an internal DGEA motif of its α_1 chain to cell surface $\alpha_2\beta_1$ integrin [28,30]. Since osteogenic cells express $\alpha_2\beta_1$ integrin as well, type I collagen could easily influence their differentiation. In fact, Xiao et al. reported that signaling through $\alpha_2\beta_1$ integrin enhanced the transcriptional activity of OC gene [31]. Thus, this collagenous membrane appears to possess osteoconductivity.

In the experimental groups at 3 and 4 weeks, collagen fibers of the porous layer were embedded in the bone matrix as shown in Figs. 4A, D, G and I. Osteoblasts might have recognized the membrane-derived collagen fibers as authentic collagens of host animals, and so deposit bone matrices on them. When using an e-PTFE instead of a collagenous membrane, however, PTFE components were never incorporated in bone matrices [20]. A synthetic polymer such as e-PTFE is inert, but carries the drawbacks of a second operation to remove this membrane. The newly formed bone, thereby, would more or less suffer damage with a potential risk of bone resorption of the alveolar ridge. In contrast, the collagenous bioresorbable membrane of Bio-Gide® obviates the necessity for a second operation. The collagen fibers embedded in the bone matrix found in our study would be gradually metabolized by bone remodeling in the future. In addition, the degradation of collagen fibers that were not incorporated into the matrix has been predicted to begin after 4 months postoperation in case of human clinics, a sufficient length of time for bone regeneration. The degraded collagens would finally turn into oligopeptides. Thus, the collagenous membrane is a natural biomaterial that could be partially incorporated into the bone matrix. At this point, Bio-Gide® could be a better alternative than a synthetic polymer.

Bone remodeling is crucial to maintain the long-lasting potential of once-established bone augmentation. In this study, TRAP-reactive osteoclasts were localized on the trabeculae facing bone marrow, whereas the newly formed bone adjacent to the collagenous membrane solely localized active osteoblasts without TRAP-positive osteoclasts. This implies that bone modeling takes place predominantly in the ridge region, at least, until 4 weeks after surgery, whereas the inner portion of the cavity-derived bone is subject to bone remodeling by osteoclasts and osteoblasts. To maintain well-developed ridge augmentation, preferential circumstances including well-furnished nutrients through blood vessels and neither an epithelial intrusion nor unforeseen immune response appear to be prerequisites even after the installment of dental implants. A slight inflammation could be a candidate for a stimulant of osteoclastic resorption since osteoclasts can respond to inflammatory factors such as interleukins and tumor necrosis factors [32]. However, in this study, we failed to find inflammatory cells such as ED1 immunopositive macrophages in the vicinity of the collagenous membrane, which successfully implies minimum inflammation and no interference with bone regeneration.

5. Conclusions

The present study focused on the histological changes and cellular events in osteogenesis induced by GBR with a collagenous membrane, i.e., Bio-Gide®. Bio-Gide® appears to have osteoconductivity, resulting in a well-augmented alveolar ridge, which is suitable for the subsequent installment of dental implants. This material represents an excellent choice for GBR by promoting osteoblastic activity.

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