Evaluation of a Collagen-Glycosaminoglycan Dermal Substitute in the Dog Palate


ABSTRACT

Tissue shortage complicates surgery of cleft lip and palate. The healing of defects on the palate impairs growth of the dentoalveolar complex because of scar tissue formation. Implantation of a matrix into the wound might overcome this adverse effect.

Integra with and without a silicone top layer was implanted into standardized full-thickness wounds (Ø 6 mm) in the palatal mucoperiosteum in beagle dogs. In some wounds, the silicone layer was removed after 14 days. Control wounds did not have an implant. At 2 and 4 weeks post-surgery, the wounds were assessed for epithelialization, inflammation (hematoxylin and eosin, leucocyte protein L1), number of myofibroblasts (alpha smooth muscle actin), and general histological characteristics.

Wounds filled with Integra without the silicone layer showed fewer myofibroblasts and inflammatory cells than the sham wounds. Collagen fibers were more randomly orientated in these wounds than in the sham group. Wound closure was found to be retarded, and many inflammatory cells were present when Integra with silicone was implanted. The silicone layer was lost within 4 weeks in these wounds.

We conclude that, in the moist oral environment, the silicone of Integra is not required. Re-epithelialization and tissue integration proceed more favorably without it. Further research in the dentoalveolar development with Integra will be conducted in a simulated cleft palate repair in the dog model.

INTRODUCTION

LIKE SURGICAL RECONSTRUCTIONS OF THE SKIN, a shortage of tissue often complicates reconstructions of the oral mucosa. During closure of a palatal cleft using the Von Langenbeck method, for example, there is a lack of mucosal tissue, which leaves areas of denuded bone on the palate. Generally, no autologous grafts are used to cover these wounds. Instead, they are left to heal by secondary intention, which means that proliferation and migration of cells, as well as wound contraction, occurs. This results in extensive scar tissue, which is firmly anchored to the palatal bone.1 Intraoral wound contraction and scar tissue formation during the growth of the maxillofacial complex, as is the case in cleft palate repair, counteracts normal development of the face because of long-term impairment of skeletal growth and the development of dentition.2–4

Studies have been performed to prevent the attachment of scar tissue to the palatal bone after palatal surgery by modifying the surgical techniques. Surgical techniques that reduce the denudation of the palatal bone show some beneficial effects,5,6 but the use of biodegradable poly-(L-lactic) acid membranes or tissue expansion did not show any significant beneficial long-term effect in animal models.7,8 Another generation of biodegradable membranes composed of collagen and glycosaminoglycans has been described for the reconstruction of large-surface-area burns and for neck contraction therapy with promising results.9,10 The implantation...
of such a collagen–glycosaminoglycan matrix in burn wounds yields a functionally and aesthetically highly acceptable scar. A few animal studies indicate that these materials may also be suitable for intraoral surgery.

Burke et al. first described a collagen–glycosaminoglycan matrix for dermal substitution in 1981. They developed an acellular, biodegradable collagen–glycosaminoglycan matrix of which the porosity, the glycosaminoglycan content, and the cross-link density were all within the normal physiological range. With slight modifications, this material is commercially available (Integra, Integra Life Sciences Corporation, Plainsboro, NJ). It provides a matrix for cellular invasion and undergoes remodeling while newly synthesized extracellular matrix replaces it. This process may require 1 to 2 y to complete. The matrix has a mean pore size of 70 to 200 μm and a glycosaminoglycan content of 8%. It is used to cover large burn wounds. To prevent fluid loss, Integra is provided with a top layer of silicone, which is usually removed 7 to 14 days later in a second operation after the formation of a neodermis, as described in the manufacturer’s instructions, and replaced by a thin epidermal autograft. It has been shown that the newly formed dermis in these grafted areas was indistinguishable from the unwounded tissue, and there were no clinical signs of hypertrophic scar tissue or keloids. Therefore, it was concluded that Integra substantially minimizes long-term postoperative contractures.

As an alternative to the second-stage skin grafting, Integra can be seeded with autologous keratinocytes before application. However, from this study it cannot be concluded whether the epithelium is derived from these seeded keratinocytes or from keratinocyte ingrowth from the wound edges. An earlier in vitro study conducted by our group in which we showed that the seeding of keratinocytes on top of Integra did not result in the formation of a stratified epithelium in vitro suggests the latter. If Integra is to be used in the oral environment, it is possible that the application can be simpler than in the case of dermal wounds. First, the oral cavity is a moist environment, and the need to prevent fluid loss from the wound bed therefore is far less. This means that the silicone sheet might not be required. Second, intraoral wound healing generally proceeds faster than the healing of skin wounds, and the formation of a continuous epithelium can probably occur by the ingrowth of keratinocytes from the wound edges. Therefore, we hypothesize that the outer layer of silicone on top of Integra might not be required for intraoral applications such as cleft palate repair. The aim of this study was to investigate the intraoral tissue response to Integra with and without the silicone top layer.

**MATERIAL AND METHODS**

Four beagle dogs (aged 2–3 years) were used in this study. The animals were kept under normal laboratory conditions and were fed standard dog chow and water ad libitum. The Board for Animal Experiments of the Radboud University Nijmegen Medical Centre, The Netherlands approved the experiment.

**Surgical procedure**

Before surgery, the animals were premedicated with 0.5 mL Thalamonal (0.05 mg fentanyl + 2.5 mg/mL droperidol; Janssen Pharmaceutica, Beerse, Belgium) and 0.5 mL atropine intramuscular (0.5 mg/mL atropine sulphate). Subsequently, they were anesthetized with an intravenous injection of 30 mg/kg of 60 mg/mL sodium pentobarbital (Apharmo, Arnhem, The Netherlands). After intubation, anesthesia was maintained with 15 mg/m enfuran (Abbott, Amstelveen, The Netherlands). The palatal mucosa was cleaned with povidone iodine (Dagro-Pharma, Diemen, The Netherlands). In addition, approximately 2 mL of 0.4 mg/mL lidocaine-hydrochloride + 0.0125 mg/mL adrenaline (Astra Chemicals, Rijswijk, The Netherlands) was injected into the palatal mucosa to avoid excessive bleeding during the procedure.

In each dog, 4 standardized full-thickness wounds were created in the medial region of the palate using a 6-mm biopsy punch (Stiefel Laboratorium, Offenbach am Main, Germany). The circular soft tissue defects were made distally to the canines. The defects were assigned to one of the following treatments according to a Latin square scheme. In each dog, one wound was left open and served as a sham wound (C). A second wound was filled with Integra with the top layer of silicone (S+), a third wound was filled with Integra without the layer of silicone (S–), and the last wound again was filled with Integra with the outer layer of silicone, but the top layer was gently removed with a forceps after 14 days (S14). Every matrix was carefully fixed in place using 4 stitches (4-0 Vicryl, Ethicon; Johnson & Johnson Company, Amersfoort, The Netherlands). After surgery, the animals were medicated with 1.5 mL of ampicillin anhydride 150 mg/mL (Mycofarm, de Bilt, The Netherlands). All animals received a normal diet after surgery. All experimental conditions were evaluated clinically and histologically at 2 and 4 weeks post-surgery.

**Histology**

For histological evaluation, 2 animals were killed at 2 weeks, and the other 2 were killed at 4 weeks post-surgery. At the time of death, the animals were anesthetized using 30 mg/kg of 60 mg/mL sodium pentobarbital. After some minutes, a lethal dose of sodium pentobarbital was injected intravenously. A small mucosal biopsy (Ø 4 mm) was taken from the margin of each wound. These biopsies, containing tissue from the experimental wound and some normal palatal mucosa, were fixed for 4 h in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) at room temperature and embedded in paraffin. These samples were used for immunohistochemical staining.
INTEGRA IN THE DOG PALATE

Subsequently, the maxillae were dissected and immersed in 4% paraformaldehyde in 0.1 M phosphate buffer at room temperature. After fixation, they were sawn into 4 smaller blocks, each containing one biopsy wound. The tissue blocks were decalcified in 20% formic acid and 5% sodium citrate. Decalcification was checked radiographically. Then the blocks were dehydrated through a graded series of ethanol and embedded in paraffin. Serial paraffin sections 7 μm thick were made through the entire wound. Each tenth section was mounted onto a Superfrost (Menzel-Gläser, Braunschweig, Germany) slide and stained with hematoxylin and eosin (H&E) for general tissue survey. One of the authors (RO) blindly evaluated the wound area of each specimen for the degree of inflammation and epithelial regeneration (rete ridges and the number of epithelial cell layers), using an ordinal scale from 0 to 4, as shown in Table 1. Twelve consecutive sections from the center of each wound were scored. The mean score and standard error of these sections were used for evaluation of the total wound area.

Immunohistochemistry

Paraffin sections of the 4-mm mucosal biopsies were collected on Superfrost Plus slides (Menzel-Gläser), deparaffinized, and rehydrated again. Before staining, the slides were treated with 0.1% trypsin 250 (DIFCO Laboratories, Detroit, MI) for 10 min and rinsed with PBS. Thereafter, the slides were treated with 3% hydrogen peroxide in PBS for 30 min to block endogenous peroxidase and rinsed in PBS.

α–Smooth muscle actin was used to detect myofibroblasts. The sections were pre-incubated with 5% bovine serum albumin (BSA, Sigma Chemical Co., St. Louis, MO) in PBS buffer (5% PBSA). After pre-treatment, the sections were incubated with a mouse alpha-smooth muscle actin antibody (1:1600) (Sigma Chemical Co.) overnight at 4°C. After washing with PBS, the sections were incubated with a biotinylated donkey anti-mouse antibody (1:500) (Jackson Labs, West Grove, PA) for 60 min at room temperature. After washing with PBS, the sections were treated with ABC-peroxidase (Vector Lab, Burlingame, CA). Peroxidase activity was visualized using a standard DAB technique (Sigma). The sections were counterstained with Delafield’s hematoxylin. For negative controls, the primary antibodies were omitted. All negative controls were blank.

Leucocyte protein L1, to detect inflammatory cells, was detected using a mouse anti-L1 antibody (1:800) (Abcam Ltd, Cambridge, UK). In dogs, it is known that cells express leucocyte protein L1 at an early stage of monocyte and macrophage differentiation.23 This protein is also present in the epithelium. The staining method was the same as described for α–smooth muscle actin. The secondary antibody was a biotinylated donkey anti-mouse antibody (1:500) (Jackson Labs).

Semi-quantitative analysis of the distribution of positive leucocyte protein L1 cells was done at a magnification of 200 times. The scoring system used for the presence of protein L1 was on a scale from 0 (none) to 4 (abundant). A random area was chosen for each of the sections α stained with α-smooth muscle actin, and stained cells within each area were counted. Representative sections were photographed using a Leitz DMRD Microscope (Leica, Wetzlar, Germany).

Statistics analysis

Differences in the means of degree of inflammation (H&E), epithelial regeneration, leucocyte protein L1, and the number of myofibroblasts between the groups were tested. To investigate the tissue response to Integra with and without the silicone top layer, the groups were pooled, because no differences existed between the wounds with a silicone top layer (S+ and S14) and those without silicone (C and S–) siliconeS14. Differences in the number of myofibroblasts, inflammation, epithelial regeneration, and leucocyte protein L1 between siliconeS–wounds with a silicone top layer (S+ and S14) and those without (C and S–) were tested using a Mann-Whitney rank sum test, because all the data were normally distributed. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Clinical observations

Wound closure was completed uneventfully after 2 weeks in the sham wounds (C) and the wounds filled with Integra without the silicone (S–). The wounds with Integra plus the silicone top layer (S+ & S14), however, showed slower healing and had not completely re-epithelialized after 2 weeks. Parts of the wound surface in the latter two groups appeared inflamed, and a fibrin clot was still evident. The top silicone layer was still visible through the coagulum in all of the S+ and S14 wounds. Parts of the silicone layer protruded from the tissue, indicating possible ongoing sequestration. The silicone top layer was removed intentionally in the S14 wounds. After four weeks, all experimental wounds including

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<th>Table 1. Histometric Scoring System Used to Evaluate Degree of Inflammation and Epithelial Regeneration</th>
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the wounds with the silicone top layer (S+) were closed. None of the wounds without the silicone layer (S– & C) showed any signs of inflammation. However, all other experimental wounds (S+ & S14) still showed some mild inflammation; parts of the wound surface still appeared inflamed. Although the silicone layer in this group was not removed intentionally, it fell away spontaneously between 2 and 4 weeks post-surgery.

General histology

Unwounded tissue. The superficial layer of the dog's palatal mucoperiosteum consists of a parakeratinized stratified epithelium with many ridges protruding into the underlying connective tissue (Fig. 1D). This connective tissue layer, immediately below the epithelium, consists of a 3-dimensional network of thick collagen fibers. Throughout the mucoperiosteum, large blood vessels and sinuses are present in the deeper layers of the connective tissue. Between the sinuses and close to the bone, the major palatine arteries and branches of the palatine nerves are organized into neurovascular bundles located mainly at the lateral aspect of the bone. The palatal bone is of the lamellar type.

Two weeks post-surgery. The sham wounds (C) were covered with up to 10 epithelial cell layers, and some ridges had started to form (Fig. 1A). The wound was completely filled with granulation tissue rich in fibroblasts, granulocytes, some blood vessels, and thin collagen fibers. The collagen fibers had a mainly transverse orientation. Superficial osteoclastic bone resorption was found in all samples (not shown).

The epithelium covering the wounds filled with Integra without silicone (S–) was also continuous (Fig. 1B). The thickness of the epithelium and the ridge formation was similar to that of the control wounds. All the matrices were easily recognized in the wounds because of their characteristic network structure. The granulation tissue underneath the epithelium was still hyperemic, and some inflammatory cells were present. Thin collagen fibers were aligned in transverse direction and seemed to enclose the matrices (Fig. 1B).

The inflammatory response (minimal to mild) outside the implant (S–) was comparable to the sham wounds (Fig. 1A).

The wounds filled with Integra with silicone (S+ and S14) were not yet closed, but the epithelium had already migrated from both wound edges underneath the matrix (Fig. 1C). The silicone layer was still present in both groups at this time. Underneath the newly forming epithelium, as well as in the wounds, hyperemic granulation tissue with many fibroblasts and granulocytes surrounded the Integra, and moderate bone resorption was found. The bone was of the lamellar type. The inflammatory response was mild to moderate, with many neutrophil granulocytes.

The inflammatory response in all the groups (C, S–, and S+) seemed to be the same at this point in time. No significant differences were found between these groups for the inflammatory response at 2 weeks post-surgery. After pooling silicone layer presence (S+ and S14) and absence

![FIG. 1.](http://www.liebertpub.com/ten)

Hematoxylin and eosin staining of the experimental wounds at 2 and 4 weeks post-surgery. (A) Sham wound at 2 weeks post-surgery. (B) Integra without a top layer of silicone (S–) at 2 weeks post-surgery. (C) Integra with a top layer of silicone (S+) at 2 weeks post-surgery. (D) Normal palatal mucosa of a beagle dog. (E) Sham wound at 4 weeks post-surgery. (F) S–at 4 weeks post-surgery. (G) S+ at 4 weeks post-surgery. (H) Integra with the top layer gently removed with a forceps after 14 days (S14) at 4 weeks post-surgery. m, Integra matrix; e, epithelium; s, submucosa; p, periosteum; b, bone; T, wound margin (the wound is to the right of the arrow. The original magnification 50×; the bar represents 150 μm. Color images available online at www.liebertpub.com/ten.)
(C and S–) of these top layer groups S–, no significant differences were observed for the inflammatory response or the epithelial regeneration (not shown).

Four weeks post-surgery. The quantitative data are summarized in Figure 2 for the inflammatory response and in Figure 3 for the epithelial regeneration of the wound tissue. The epithelium had become thicker in the sham wounds (C), although it was still thinner than the normal oral epithelium (Fig. 3). A thick, cornified layer had formed, and ridges were protruding into the underlying connective tissue (Fig. 1E). The inflammatory reaction had almost ceased, and only a few inflammatory cells were found (Fig. 2). Density of fibroblasts was also less than at 2 weeks post-surgery. The connective tissue showed thinner collagen fibers than the tissue outside the wound area, and the collagen fibers were clearly aligned. Bone apposition was found at some sites where the biopsy punch had probably damaged the bone.

The epithelium covering the wounds filled with Integra without silicone (S–) showed many ridges penetrating into the connective tissue (Fig. 1F). The thickness of the epithelial layer was more or less comparable with the epithelial layer of the sham wounds (C) at the same stage (Fig. 3). The orientation of the collagenous bundles in the superficial layer of the dermis was more random, with fewer transverse thick bundles. In the deeper layers of the wound, however, they were more oriented in a more transverse direction with more dense collagenous bundles. The matrices were still easily recognized within the wounds, and they contained many fibroblasts. The inflammatory reaction was comparable in the control wounds (Fig. 2).

The wounds filled with Integra with silicone (S+) showed a continuous epithelium of approximately 8 cell layers. The thin epithelium showed some small ridges protruding into the underlying connective tissue at this stage (Fig. 1G). Although the top layer was not removed in this group, it was not present any more, but the Integra was always easily recognized because of its typical structure. The granulation tissue underneath the epithelium was still hyperemic, and many inflammatory cells were present. The inflammatory response (mild to moderate) outside the implant was comparable to that in the sham wounds after 2 weeks (Fig. 2).

The wounds filled with Integra with the top layer removed after 14 days (S14), were closed with a continuous epithelium consisting of a few cell layers (Fig. 1H and Fig. 3). Underneath the epithelium, hyperemic granulation tissue with leukocytes and macrophages were found within and surrounding the matrix. All the matrices were easily recognized in all of the wounds. The inflammatory response was mild to moderate, with many neutrophil granulocytes still present (Fig. 2).

The inflammatory response in the groups with the silicone layer present (S+ and S14) was significantly greater than in the groups without a silicone layer (C and S–) (Fig. 2). Furthermore, after pooling of the data, epithelial regeneration was significantly greater in the group without the silicone layer at 4 weeks post-surgery (Fig. 3).

Immunohistochemistry

α-Smooth muscle actin. The quantitative data on the number of myofibroblast in the wound tissue are summarized in Figure 4.

α–smooth muscle actin staining was used to detect myofibroblasts. There were evident differences between normal, unwounded, and wounded mucosa. In normal palatal mucosa, α–smooth muscle actin was exclusively found in blood vessel walls (Fig 5A, D outside the wound area, indicated by

**FIG. 2.** Inflammatory response at 4 weeks. Each bar represents one individual wound. The values are means ± standard errors of 12 consecutive sections from the center of each wound. *Significant difference for the pooled data (p < 0.05) using the Mann-Whitney rank sum test.

**FIG. 3.** Epithelial regeneration at 4 weeks. Each bar represents one individual wound. The values are means ± standard errors of 12 consecutive sections from the center of each wound. *Significant difference for the pooled data (p < 0.05) using the Mann-Whitney rank sum test.
an arrow head). In contrast, myofibroblasts were found in considerable numbers in the granulation tissue in all wounds at 2 weeks post-surgery. In general, more myofibroblasts were present in the deeper layers of the submucosa than just below the epithelium. The sham wounds (C) (Fig. 5A) showed myofibroblasts with a mainly parallel orientation, in contrast to the other wounds (S– and S+) (Fig. 5B, C). The wound area of the latter wounds (S– and S+) contained more randomly orientated myofibroblasts. In some samples, the myofibroblasts showed a focal clustering.

At 4 weeks post-surgery, the number of myofibroblasts had decreased in the sham wounds (C) (Fig. 5D) and in the wounds filled with Integra without silicone (S–) (Fig. 5E). The staining for α-smooth muscle actin in these wounds was less dense, with a tendency to a more random orientation of these cells than in the sham wounds (C).

The wounds filled with Integra (S+) (Fig. 5F) and those in which the top layer was removed (S14) (Fig. 5G) showed the same characteristics. Intense staining for myofibroblasts was found in these wounds. The density of myofibroblasts was higher than in the other wounds (S– and C) (Fig. 4), and the number of myofibroblasts was comparable with that of the 2-week post-surgery groups. After pooling, the number of myofibroblasts in the groups with the silicone layer present (S+ and S14) was significantly higher than in the groups without the silicone (C and S–) (Fig. 4).

Leucocyte protein L-1. The quantitative data are summarized in Figure 6 for the leucocyte protein L1 reaction of the wound tissue.

The L-1 staining was used to detect neutrophils and macrophages and is also known to stain mucosal epithelium. In normal palatal mucosa, leucocyte protein L1 localizes to the granular layers of the epithelium, but it is not found in the basal or cornified cell layers.

At 2 weeks post-surgery, L-1 positive cells were found in large numbers in the sham wounds (C) (Fig. 7A) and wounds filled with Integra without silicone (S–) (Fig. 7B). The wounds filled with Integra with silicone (Fig. 7C) showed numerous leucocyte protein–

FIG. 4. Number of myofibroblasts at 4 weeks. Each bar represents one individual wound. The values are means ± standard errors of 12 consecutive sections from the center of each wound. *Significant difference for the pooled data (p < 0.05) using the Mann-Whitney Rank sum test.

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At 2 weeks post-surgery, L-1 positive cells were found in large numbers in the sham wounds (C) (Fig. 7A) and wounds filled with Integra without silicone (S–) (Fig. 7B). The L-1–positive cells were equally distributed throughout the entire wound area. The wounds filled with Integra with silicone (Fig. 7C) showed numerous leucocyte protein–

FIG. 5. Immunohistochemical staining of α-smooth muscle actin containing cells in 2- and 4-week-old wounds. (A) Sham wound at 2 weeks post-surgery. (B) Integra without a top layer of silicone (S–) at 2 weeks post-surgery. (C) Integra with a top layer of silicone (S+) at 2 weeks post-surgery. (D) Sham wound at 4 weeks post-surgery. (E) S– at 4 weeks post-surgery. (F) S+ at 4 weeks post-surgery. (G) Integra with the top layer gently removed with a forceps after 14 days (S14) at 4 weeks post-surgery. The specific protein of α-smooth muscle actin is stained brown and indicated by an arrowhead (V). m, Integra matrix; 1, wound margin; the wound is right to this arrow. The original magnification 50×; the bar represents 150 μm. Color images available online at www.liebertpub.com/ten.
The leucocyte protein L1 response to all the groups (C, S–, and S+) seemed to be the same at this point in time. Moreover, after pooling silicone layer presence (S+ and S14) and absence of these top layer groups (C and S–), no significant differences were observed for this reaction at 2 weeks post-surgery (not shown).

At 4 weeks post-surgery, only few L1-positive cells remained in the sham wounds (Fig. 7D) and the wounds without silicone (S–) (Fig. 7E). The positive cells were found throughout the whole healing wound, and their distribution was comparable with that of the unwounded tissue (Fig. 7D, outside the wound area). At this point in time, the number of positive cells in the wounds filled with Integra with silicone (S+) seemed to be higher (Fig. 7F), although there was no significant difference from the groups without this top layer (C and S–). However, the wounds with Integra where this top layer was removed after 14 days (S14) were filled with numerous L1-positive cells (Fig. 7G). The staining was not equally distributed but was more pronounced close to the epithelium. After pooling, the response in the groups with the silicone layer present (S+ and S14) was significantly higher than in the groups without silicone (C and S–) (Fig. 6). The general histology for the inflammatory response between these groups also confirmed this (Fig. 2).

**DISCUSSION**

The overall aim of our research is to develop a mucosal substitute for implantation on the palate after cleft palate surgery. The implantation of a suitable graft material into an

**FIG. 6.** The leucocyte protein L1 staining at 4 weeks. Each bar represents one individual wound. The values are means ± standard errors of 12 consecutive sections from the center of each wound. *Significant difference for the pooled data (p < 0.05) using the Mann-Whitney rank sum test.

**FIG. 7.** Immunohistochemical staining of leucocyte protein L1 containing cells in 2- and 4-week-old wounds. (A) Sham wound at 2 weeks post-surgery. (B) Integra without a top layer of silicone (S–) at 2 weeks post-surgery. (C) Integra with a top layer of silicone (S+) at 2 weeks post-surgery. (D) Sham wound at 4 weeks post-surgery. (E) (S–) at 4 weeks post-surgery. (F) S+ at 4 weeks post-surgery. (G) Integra with the top layer gently removed with a forceps after 14 days (S14) at 4 weeks post-surgery. The specific protein is stained brown. m, Integra matrix; w, wound margin; the wound is to the right of the arrow. In normal, unwounded palatal mucosa, leucocyte protein L1 is localized to the spinous and subcorneal layers of the stratum spinosum. The original magnification 50x; the bar represents 150μm. Color images available online at www.liebertpub.com/ten.
open wound might reduce wound contraction and scarring. The aim of this specific study was to investigate the in vivo response to Integra with and without the silicone top layer after implantation on the palate of beagle dogs. We hypothesize that this top layer may not be required for intraoral applications such as cleft palate repair because of the moist environment.

A widely accepted method for the primary closure of palatal clefts is Von Langenbeck palatoplasty. In this technique, bilateral palatal relaxation incisions are made in the mucoperiosteum adjacent to the posterior teeth. The mucoperiosteum is then elevated, mobilized, and moved to the midline region of the palate to close the cleft. This procedure results in two areas of denuded bone in the lateral region of the palate. Wijdeveld et al. created a midpalatal soft tissue cleft in dogs that was subsequently closed using the Von Langenbeck technique. Their results showed similar effects on growth as in the clinical situation. In the present study, standardized full-thickness wounds were created in the median region of the palate with a 6-mm biopsy punch. The diameter of these wounds was similar to the largest width of the wounds in the Von Langenbeck technique. Therefore, we can use the present results as a basis for a larger study to evaluate the wound healing process in growing beagle dogs after palatal repair with and without implantation of Integra.

The overall take rate of Integra in this study was 100%. This take rate exceeds that reported in burn patients in a large clinical series. However, healthy animals do not show the systemic illness and localized infection associated with severe burn injuries, which might contribute to the inconsistency of take rates in clinical practice. Grant et al. reported a take rate of approximately 96% in a histological study of skin wounds in pigs. Although their animal model and the implantation site are different from ours, their findings on the take rate are similar.

In the present study, wound closure was found to be retarded in all wounds filled with Integra with the silicone top layer. The migration of the epithelium and the connective tissue cells in these wounds (S+ and S14) is more difficult than in the wounds without a silicone top layer (C and S−). The present study has further shown that all matrices were integrated into the tissue. However, the silicone top layer had disappeared spontaneously between 2 and 4 weeks post-surgery from all wounds filled with Integra with the silicone top layer. This indicates that epithelial migration had occurred between the matrix and the silicone top layer. O’Donoghue et al. also found retarded wound healing after implantation of a silicone-coated membrane in skin wounds. Furthermore, the wounds filled with Integra with the silicone top layer showed a significantly greater inflammatory response than the other wounds. Delayed wound healing and a high inflammatory response was also found after subcutaneously implanted silicone. In general, we can conclude that wounds filled with Integra with a silicone layer showed a significant delay in wound closure and a significant greater and longer inflammatory response. The hydrophobic properties of the silicone and therefore the low adhesion of cells might underlie these effects.

The scar tissue was clearly distinguishable from the normal mucoperiosteum. A matrix with parallel-orientated collagen fibers and many myofibroblasts replaced the loosely woven matrix of the unwounded submucosa. Myofibroblasts are involved in wound contraction. These contractile fibroblasts are found in many types of contracting wounds. After contraction, the myofibroblasts disappear, probably through apoptosis, and wound contraction ceases. Therefore, reducing the number of myofibroblasts during palatal wound healing might decrease wound contraction. In our study, the density of myofibroblasts in all wounds was higher at the level of the mucosa than in the periosteum. One might thus expect more contraction in the superficial layer of the mucoperiosteum. Indeed, this was shown in beagle dogs. In this study, collagen fiber orientation was determined using conventional light microscopy. In this general observation, we did not quantitatively assess the morphology and orientation of the scar tissue. Several studies have determined the orientation of scar tissue using light microscopy in combination with Fourier analysis. The sham wounds in this study showed parallel collagen fibers and considerable numbers of myofibroblasts at 2 weeks post-surgery, which is in agreement with others. The collagen fibers in a healing wound tend to run in the direction of the contraction forces. Squier and Kremenak have shown that myofibroblasts are present in healing palatal wounds of the beagle dog from 7 days post-surgery and are most numerous between 10 and 15 days. The wounds filled with Integra without the top layer showed fewer myofibroblasts than the sham wounds, and the collagen fibers were not aligned in a transverse direction, as in the sham group. This indicates that wounds filled with Integra without the silicone top layer undergo less contraction than a sham wound.

From the results of this study, we can conclude that implantation of Integra without the silicone top layer yields a wound with fewer parallel collagen fibers and fewer myofibroblasts. In addition, wound closure was faster without the silicone top layer. Apparently, in the moist oral environment, the silicone layer is not required to prevent fluid loss. To evaluate the effect of Integra on maxillary growth, a larger study of the implantation of Integra without the silicone top layer after palatal repair in beagle dogs is required.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge gifts of Integra Artificial Skin from Integra Life Sciences Corporation (Plainsboro, NJ) that were made without obligation to facilitate these studies. They also thank R.E.M. van Rheden and M.P.A.C. Helmich for their excellent technical assistance. This work was supported by a grant from the American
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Association of Orthodontists Foundation and the European Orthodontic Society. There were no conflicts of interest.

REFERENCES


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