TISSUE ENGINEERING: Part A
Volume 00, Number 00, 2010

DOI: 10.1089/ten.tea.2008.0571

Original Article

Development and Preclinical Evaluation of Acellular Collagen Scaffolding and Autologous Artificial Connective Tissue in the Regeneration of Oral Mucosa Wounds

Lady Espinosa, B.Sc.,[1,2] Alejandro Sosnik, Ph.D.,[3] and Marta R. Fontanilla, Ph.D.[1]

This work assessed wound healing response in rabbit oral lesions grafted with autologous artificial connective tissue or acellular collagen scaffolds. Autologous artificial oral connective tissue (AACT) was produced using rabbit fibroblasts and collagen I scaffolds. Before implantation, AACT grafts were assayed to demonstrate the presence of fibroblasts and extracellular matrix components, as well as the expression of characteristic genes and secretion of chemokines, cytokines, and growth factors. AACT grafts were tested in the rabbits from which the fibroblasts were obtained, whereas acellular collagen type I scaffolds (CS) were evaluated in a separate group of rabbits. In both cases, contralateral wounds closed by secondary intention were used as controls. In a separate experiment, AACT-grafted wounds were directly compared with contralateral CS-grafted wounds in the same animals. Wound contraction and histological parameters were examined to evaluate closure differences between the treatments in the three animal experiments performed. Contraction of wounds grafted with AACT and CS was significantly lower than in their controls ($p < 0.05$). Additionally, AACT significantly lowered wound contraction when compared with CS ($p < 0.05$). Intriguingly, it was observed that AACT-grafted wounds initially displayed a significantly higher ($p < 0.05$)—albeit transient—inflammatory response than seen in CS-grafted wounds and secondary healed wounds. This suggests that an early inflammatory component may contribute to tissue regeneration. Altogether, the results suggest that AACT- and CS-grafted wounds favor regeneration of oral mucosa.

Introduction

Both oral and dermal wound healing processes go through hemostasis, inflammation, proliferation, and remodeling of the extracellular matrix.[1] From a clinical perspective, oral mucosa wound healing is faster and scarring is less pronounced. In terms of the cellular response, lower levels of macrophage, neutrophil, and T-cell infiltration are apparent in oral mucosa healing. Also, a more limited production of inflammatory cytokines (e.g., IL-6) is observed, indicating that constrained inflammation is a key difference between both phenomena.[1] A common approach to treat discontinuities in the oral mucosa is the use of autografts and pedicle flaps.[2] Tissue grafting has been used for reconstructive surgery, vestibuloplasty, preprosthetic surgery, and periodontal procedures.[3–5] Large oral mucosa defects can stem from congenital (e.g., cleft palate) or pathological conditions (e.g., malignant tumor resection). In this context, the shortage of donor areas and the generation of wounds that heal by secondary intention result in tissue contracture, cosmetic deformity, functional compromise, and reduction of donor tissue for further interventions.[6–8] These drawbacks motivated the search for tissue engineering strategies to treat large mucosa defects using autologous engineered tissue. In developing countries, there is a critical need for the development of in-site methodologies for the generation of autologous engineered mucosa to provide patients with the most appropriate treatment at a reasonable cost, regardless their socioeconomic status.

Our research group is presently focused on the development of a cost-effective and highly accessible tissue substitute that would improve the regeneration process of oral mucosa defects through the modulation of contraction, inflammatory response, cell migration, and proliferation. In this framework, a three-dimensional *in vitro*-produced tissue based on the combination of collagen type I scaffolds and second/third passage autologous fibroblasts was developed.[9–11] To gain further insight into the regeneration process, in this study, oral

[1]Pharmacy Department and [2]Graduate Program in Biotechnology, Faculty of Sciences, National University of Colombia, Bogotá D.C., Colombia.
[3]Department of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry and National Science Research Council (CONICET), University of Buenos Aires, Buenos Aires, Argentina.

connective autologous tissue-engineered implants autologous artificial oral connective tissue (AACT) was produced using rabbit fibroblasts and characterized *in vitro*. In addition, the healing process of oral mucosa wounds covered with AACT, acellular collagen type I scaffolds (CS), or left to heal by secondary intention was determined. The results suggest that AACT maintains the ability to secrete a variety of proangiogenic, tissue-remodeling, and immune-modulating effectors and promoted tissue regeneration and wound healing to a greater extent than CS. However, CS enhanced wound healing when compared with secondary intention. Our observations support the use and development of AACT for the therapy of oral mucosa wounds.

Materials and Methods

Ethical considerations

Animal procedures were carried out following the guidelines of the Colombian Ministry of Health. All surgical procedures were performed in aseptic conditions and under anesthesia (ketamine, 35 mg/kg; xylazine, 5 mg/kg; local lidocaine, 20 mg/mL; and epinephrine, 0.02 mg/mL).

Manufacture of CS

Collagen I was isolated from tendons taken from rat tails supplied by the School of Veterinary Medicine Central Animal Facility (National University of Colombia). Rat tails were washed and disinfected (2% sodium hypochlorite), and tendons were removed and cut into small pieces. They were suspended in 0.5 M acetic acid (4°C, 24 h), the suspension was centrifuged, and the supernatant was neutralized (1 M NaOH). The precipitated collagen was dissolved in 0.1 M acetic acid to a final concentration of 2 mg/mL, stirred and poured in tissue culture dishes (60 mm×15 mm), frozen at −20°C, and freeze-dried to yield collagen scaffolds (4.5 cm diameter). The collagen scaffolds were rehydrated and then crosslinked with glutaraldehyde (0.02%, 4°C, and 24 h). They were subsequently washed with water until no crosslinker residues were detected (2,4-dinitrophenylhydrazine test), freeze-dried again, sterilized with ethylene oxide, and stored at least 48 h prior to their evaluation.

Cell isolation and culture

Partial-thickness specimens were harvested from the upper incisive vestibules of male New Zealand rabbits. Each sample was used to produce the fibroblast-loaded scaffold for the animal that was the source of the cells. The interdental papilla and the gingival margin were preserved intact. Six-week-old animals (2–3 kg) were kept under conditions approved by the Ethics Committee (Faculty of Sciences, National University of Colombia). The samples were washed with phosphate-buffered saline, 200 IU/mL penicillin, 200 μg/mL streptomycin, and 0.5 μg/mL amphotericin. To promote separation of epithelial and connective tissues, cleaned tissues were incubated overnight with 0.4% dispase (from *Bacillus polymyxa*, 1.17 units/mg; Gibco/Invitrogen, Carlsbad, CA), at 4°C. Isolated connective tissue was cut into small pieces (2 mm×2 mm×1 mm) and used as explants for establishing the fibroblast cultures in 25 cm^2 flasks. Explants were covered with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acids, 1% sodium pyruvate, 1% vitamins, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 100 μg/mL amphotericin. Cultures were incubated (37°C, 5% CO_2) and the culture medium was replaced twice a week.

The fibroblast phenotype of cultured cells was determined by assessing vimentin expression using a monoclonal mouse antivimentin antibody (clone Vim 3B4, final concentration 0.25 μg/mL; Dako, Glostrup, Denmark). A biotinylated anti-mouse immunoglobulin in phosphate-buffered saline containing stabilizing protein and 0.015 mol/L sodium azide, and streptavidin conjugated to horseradish peroxidase containing stabilizing protein and antimicrobial agents were used as staining reagents.

Preparation and characterization of AACT

Collagen I scaffolds (35 mm diameter) were placed, one per well, in six-well plates, preincubated with FBS overnight, and seeded with second/third passage fibroblasts (3×10^5 cells/mL Dulbecco's modified Eagle's medium). Cell-seeded scaffolds were placed on a shaking plate (300 rpm) and incubated at 37°C (5% CO_2, 3 h). Plates were left steady for 1 week under the described conditions. Acellular collagen scaffolds were produced following the same procedure, except for the seeding of the cells.

To analyze the AACT histological appearance after 1 week *in vitro*, cultured scaffolds were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin–eosin and alcian blue. Using trizol, RNA was extracted from pieces taken from each individual AACT immediately before grafting. This was done to qualitatively assess the expression of elastin, collagen type I and III, metalloproteinase-1, and metalloproteinase-2 genes at 7 days. The integrity of the RNAs was verified using gel electrophoresis. Oligonucleotide pairs were designed and synthesized by Invitrogen (Table 1). A one-step SuperScript III Platinum RT-PCR kit was used for evaluating the expression of studied genes at 7 days, including β-actin as a control of the reaction.

Secretion of characteristic chemokines (MCP-3, MCP-4, UPAR, PECAM-1), cytokines (IL-1A, IL-1B,IL-2, IL-4, IL-6, IL-8, IFN-γ, TNF-α), growth factors and their receptors (FGF-2, VEGF, VEGF-D, VEGF-R2, VEGF-R3, PDGF-BB), and extracellular matrix metalloproteinase inhibitors (TIMP-2) was assessed in medium from AACT cultures at 0 h, 4 h, and 7 days postseeding using a protein array system (RayBio Human Angiogenesis Antibody Array, C Series 1000; RayBiotech, Norcross, GA), following the manufacturer's instructions. Culture medium deprived of FBS was used in these experiments. To assure that the secreted compounds were exclusively produced by AACT fibroblasts, culture medium and medium from CS incubated for 7 days were used as blank and control, respectively.

Comparison of AACT- or CS-grafted oral mucosa wounds with contralateral wounds left to heal by secondary intention

Our experimental model only permits evaluation of two contralateral wounds in one rabbit mouth. Therefore, therapeutic efficacy of AACT versus control and CS versus control was evaluated in independent experiments utilizing three different batches of 14 rabbits each, which were assessed at 2, 4, and 8 weeks for histomorphometric analyses. Clinical

Table 1. Oligonucleotides Used for Reverse Transcription–Polymerase Chain Reaction

Col 1A1	Forward	CTG ACT GGA AGA GCG GAG AGT
	Reverse	ACG CTG TTC TTG CAG TGG TAG
Col3A1	Forward	TAC ACA GTT CTG GAG GAT GG
	Reverse	ATT TAG TTG GTC ACT TGT ACT GGT
Elastin	Forward	GGC TTT TGC TGG AAT CCC A
	Reverse	ATG CCT CCA ATT CCA GGA A
MMP-1	Forward	CTG AAA CCC TGA AGA TGA TGA AG
	Reverse	CAA AGG GAG AAT TGT CAC GAT GA
MMP-2	Forward	AGA TCT TCT TCT TCA AGG ACC GGT T
	Reverse	GGC TGG TCA GTG GCT TGG GGT A

follow-ups of cicatrisation were carried out at 0, 4, 7, and 11 days.

The animals were anesthetized and two contralateral split-thickness upper wounds ($\sim 1.5\,cm \times 0.8\,cm$) were made on both vestibular sides of the mouth in the alveolar mucosa from the mucogingival line toward the apical zone of the incisors and premolars. Randomly, one of the generated wounds was covered with one of the two types of substitutes (AACT or CS, seven animals for each kind of graft) and the contralateral wound was left to heal by secondary intention as a control. Vicryl® 5-0 (Ethicon GmbH, Norderstedt, Germany) horizontal and vertical suspensory sutures, one in each corner of the implant, were used to stabilize it. The medium of artificial connective tissue cultures was replaced by serum-free medium 24 h before grafting. After the surgical procedure, animals were treated with a single dose of oxytetracycline (11 mg/kg, intramuscular [i.m.]) and meloxicam (1.5 mg/kg, i.m.). Antiseptic clorhexidine solution was sprayed daily to prevent infections. Except for fibroblast seeding, both scaffold substitutes were subjected to identical treatments during manufacturing and during the preparation for grafting. Digital images of grafted and control areas were taken at 0 (procedure day), 4, 7, and 11 days post-surgery, using a metallic ruler for reference. Images were analyzed using ScionImage (version beta 4.0.3.2; Scion Corporation, Frederick, MD) to determine wound area contraction. The percentage of wound contraction (W) was calculated using the following equation: $W = (A - B/A) \times 100$, where B is the wound area at 4, 7, and 11 days and A is the wound area at 0 days.

To perform the histomorphometric analysis, the animals were sacrificed at 2, 4, and 8 weeks postsurgery with sodium pentobarbital (390 mg/mL, i.v., 1 mL/kg body weight) and sodium diphenylhydantoin (50 mg/mL). Samples of central and peripheral parts of the wounds including a margin of 3 mm of healthy surrounding mucosa were harvested. Biopsies were fixed with 10% formaldehyde, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin–eosin. The analyses were carried out by two pathologists who were not informed about the origin of the tissue. Stained cuts were examined under a light microscope outfitted with a digital camera (DSF 1-U2; Nikon, Tokyo, Japan). Epithelium and connective tissues were evaluated qualitatively and quantitatively. Quantitative analysis was used to assess epithelium thicknesses, blood vessels, and inflammatory infiltration of healing wounds. Digital images of each histological cut were analyzed using the program NIS ELEMENTS AR2.30 Build 309 (Nikon). The epithelium thicknesses were measured in the grafted and control wounded areas, as well as in the healthy surrounding mucosa. Ten epithelium thickness measurements were carried out randomly in each wound cut image (magnification: 100×), and mean values of grafted wounds were compared with the mean values of the controls. A comparison of grafted and control areas with healthy surrounding mucosa was also performed. Three consecutive regions of the grafted wounded area were examined for counting blood vessels (magnification: 400×) and mean values were compared with the control mean values. Inflammation was graded at 1000× magnification, counting inflammatory cells/field in the wounded area and healthy surrounding mucosa; five fields were counted and the cell number mean value was used for the analysis. Normal tissue was considered to have less than 10 inflammatory cells/field, mild inflamed tissue to have 10–30 cells/field, moderately inflamed tissue to have 30–50 inflammatory cells/field, and severe inflamed tissue to have more than 50 cells/field.[12] For the calculation of the parameters described above, all the 84 wounds monitored (2 per animal) in this study were evaluated.

Comparison of AACT- with CS-grafted oral mucosa wounds

A further comparison of AACT- and CS-grafted wound closure in the same animal was undertaken to test whether grafting wounds with AATC leads to a more adequate closure than that of CS-grafted oral mucosa wounds. Assays were performed on nine animals following the protocols previously described. Clinical follow-ups of cicatrisation at 0, 4, 7, and 11 days and histomorphometric analyses at 2 weeks were performed as described when AACT or CS were grafted and compared with their controls.

Statistical analysis

The statistical significance of differences in contraction, number of newly formed blood vessels, epithelium thickness, and inflammation was determined by a Wilcoxon signed rank test for paired samples using Statgraphics (version 5.1) and [R] software. Wilcoxon signed rank test for paired samples is recommended when normality and homogeneity of variances cannot be established, as in biological data. In all cases, a p-value of <0.05 was considered to be statistically significant.

Results

AACT evaluation in vitro

As the essential part of this study was the development of fibroblast-containing collagen I scaffolds (AACT), the

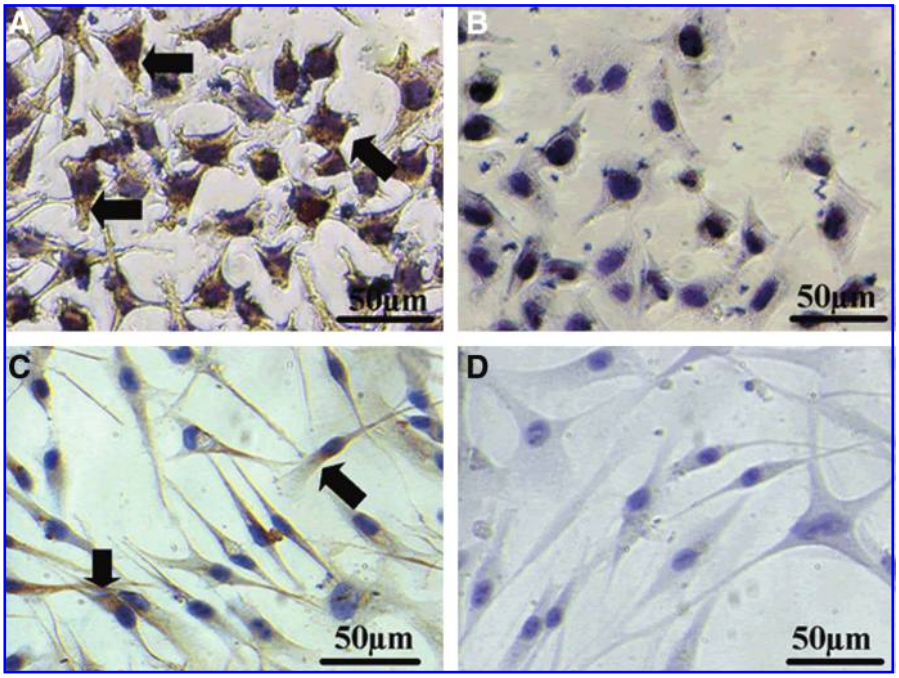

FIG. 1. Characterization of isolated oral cells. The phenotype of cells isolated from rabbit oral mucosa was determined by evaluating cell morphology and assessing vimentin expression by immunohistochemistry. Cultured NCTC 929 fibroblasts incubated with anti-vimentin antibody (**A**). Cultured NCTC 929 fibroblasts without anti-vimentin antibody (**B**). Cultured oral fibroblasts incubated with anti-vimentin antibody (**C**). Cultured oral fibroblasts without anti-vimentin antibody (**D**). Scale bar = 50 μm. Arrows in both panels (**A, C**) point to immune-stained cells. Color images available online at www.liebertonline.com/ten.

nature of the cells used for scaffold seeding was assessed. Immunostaining of second-passage oral autologous fibroblasts and a fibroblast cell line used as control showed the characteristic fibroblast morphology and the expression of the structural protein vimentin (Fig. 1). Figure 2A and B shows histological cuts of AACT grafts stained with hematoxylin–eosin and alcian blue at the time of implantation, revealing the presence of fibroblasts and extracellular matrix components (e.g., glycosaminoglycans). In both micrographs, cell migration into the porous structure of the scaffold and

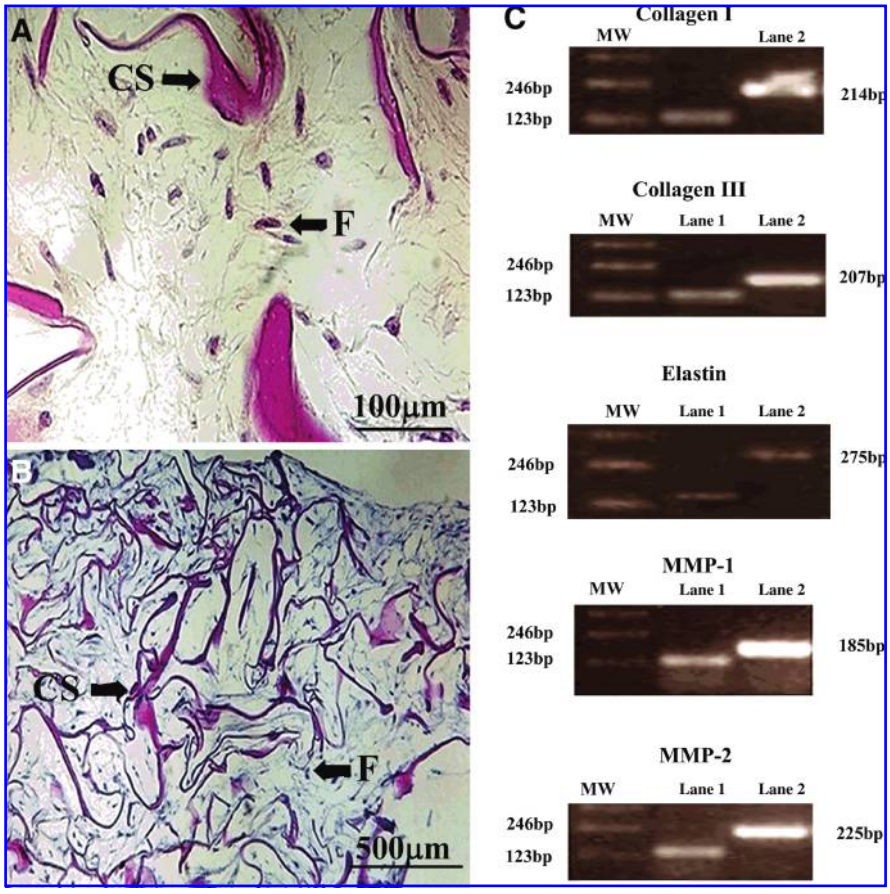

FIG. 2. Autologous artificial connective tissue (AACT) evaluation. AACT section stained with H&E (**A**); AACT section stained with alcian blue for glycosaminoglycan detection (**B**). (**C**) AACT reverse transcription (RT)-PCR extracellular matrix products made from total RNA extracted at 7 days: molecular weight marker (MW), β-actin (Lane 1), AACT RNA at 7 days (Lane 2). AACT, autologous artificial oral connective tissue; CS, collagen type I scaffolds; F, fibroblasts; H&E, hematoxylin and eosin. Scale bar = 100 μm (**A**), 500 μm (**B**). Color images available online at www.liebertonline.com/ten.

Table 2. AACT Secreted Proangiogenic, Tissue-Remodeling, and Immune Modulating Effectors

	AACT arbitrary units/μg of protein	
	4 h	*7 days*
Growth factors and receptors		
FGF-2	0.0	22.7
PDGF-BB	15.0	19.9
VEGF	0.0	550.5
VEGF-D	50.8	37.9
VEGF-R2	56.9	66.6
VEGF-R3	0.0	44.0
Tissue inhibitor matrix metalloproteinase TIMP-2	15.0	513.1
Cytokines		
IL-1α	0.0	43.6
IL-1β	0.0	23.9
IL-2	0.0	33.0
IL-4	73.1	61.0
IL-6	0.0	0.0
IL-8	0.0	0.0
IL-10	0.0	0.0
IFN-γ	33.0	0.0
TNF-α	82.3	0.0
Chemokines		
MCP-3	0.0	37.6
MCP-4	124.1	52.3
PECAM-1	0.0	16.9
UPAR	64.3	43.9

AACT, autologous artificial oral connective tissue.

adhesion to the collagen-based substrate were apparent. Expression of matrix synthesis and matrix remodeling genes was qualitatively assessed *in vitro* by reverse transcription (RT)-PCR. Figure 2C shows representative RT-PCR products of total RNA extracted from an AACT cultured for 1 week. As observed, fibroblasts expressed RNAs encoding for elastin, collagen I and III, metalloproteinase-1, and metalloproteinase-2, indicating the maintenance of the expected phenotype and suggesting fibroblast remodeling of the collagen scaffold. Data from detection of growth factors, cytokines, chemokines and some of their receptors, and metalloproteinase inhibitors present in medium from cultured AACT at 4 h and 7 days are shown in Table 2. Secretion of FGF-2, VEGF, VEGF-R3, TIMP-2, IL-1α, IL-1β, IL-2, MCP-3, and PECAM-1 increased at 7 days, whereas secretion of IFN-γ, TNF-α, and MCP-4 were reduced. Secretion of PDGF-BB, VEGF-R2, IL-4, and UPAR were unaltered. These compounds were not detected in culture medium and medium from CS incubated for 7 days used as blank and control, respectively. It is noteworthy that albeit TNF-α expression was initially detected (4 days), its secretion decreased to undetectable levels at 7 days. Additionally, IL-6 and IL-8 did not accumulate in the supernatants of AACT cultures at any studied time. Taken together, these results suggest that at the moment of implantation (7 days), fibroblasts in AACT systems secrete proangiogenic, tissue-remodeling, and immune-modulating effectors, while the production of TNF-α and IFN-γ is decreased.

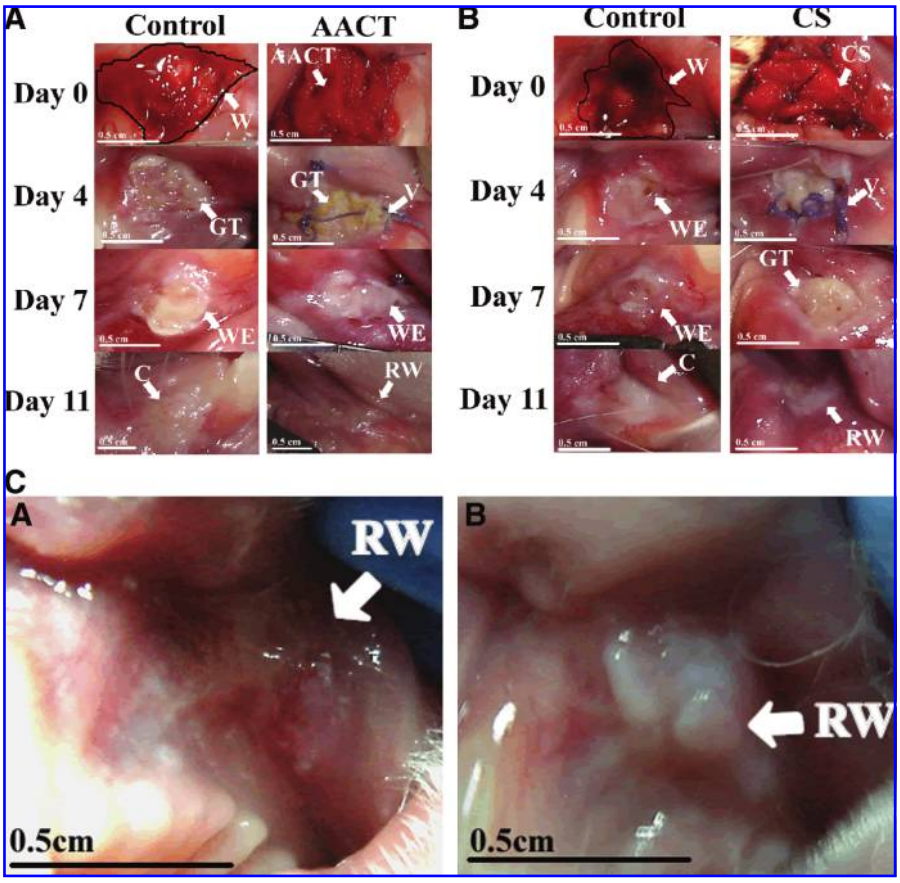

FIG. 3. (**A**) Cicatrisation follow-ups of AACT-grafted and control wounds. Comparison of AACT-grafted and control wounds at 0, 4, 7, and 11 days posttreatment. Arrows in both panels point to the following: wound (W), AACT-grafted, Vicryl stitches (V), wound contracture (C), granulation tissue (GT), wound edges (WE), repaired wound (RW). Scale bar = 0.5 cm. (**B**) Cicatrisation follow-ups of CS-grafted and control wounds. Comparison of CS-grafted and control wounds at 0, 4, 7, and 11 days posttreatment. Arrows in both panels point to the following: wound (W), grafted acellular-CS, Vicryl stitches (V), wound contracture (C), GT, WE, and RW. Scale bar = 0.5 cm. (**C**) Cicatrisation follow-ups of AACT-grafted and CS-grafted wounds. Comparison of AACT-grafted (A) and CS-grafted (B) wounds at day 11 is presented. Arrows in both panels point to RW. Scale bar = 0.5 cm. Color images available online at www.liebertonline.com/ten.

Grafts follow-up

Figure 3A shows the follow-up of the cicatrisation process in wounds grafted with AACT versus those healed by secondary intention (controls). After AACT grafting, no dehiscence between the implant and the resection borders was found. Moreover, AACT hemostatic activity and ability to tolerate suturing were also visible. At 4 days, AACT was partially degraded and replaced by granulation tissue, while stitches remained in place. Control wound appeared whiter and thicker than the surrounding healthy mucosa and borders were higher than those seen in the grafted region. By 7 days, AACT was replaced and a white region circumscribed by the margins of the wound was seen in the grafted area. The control wounds appeared whiter and thicker than the grafted region and showed higher wound margins. On 11th day, the grafted wounds appeared to be epithelialized and they presented similar red coloration and volume as the surrounding healthy mucosa, suggesting neovascularization. No wound contractures or edges were apparent in the grafted wounds. In addition, healed tissue was soft and resilient under palpation. In contrast, in the control, wound contracture was clearly apparent.

Similar hemostatic function, lack of dehiscence, and suture tolerance were found during the follow-up of CS-grafted wounds (Fig. 3B). Clinically, evidence of contracture was observed in both the CS-grafted and the control wound at 11 days. However, contracture appeared to be less pronounced in the grafted area than in the control. A head-to-head comparison of AACT- and CS-grafted wounds revealed that the former (Fig. 3C; panel A) were epithelialized, presented similar red coloration and volume as the surrounding healthy mucosa, and no wound contractures or edges were visible, whereas in the latter (Fig. 3C; panel B), scarring and contracture were evident.

Evaluating wound contraction

Figure 4A and B reveals that the progressive wound contraction at 4, 7, and 11 days gradually reduced the wound area. In the AACT- and CS-grafted wounds, contraction was significantly lower than in defects closed by secondary intention ($p < 0.05$). Figure 4C shows that when AACT and CS were compared, a statistically significant increase in contraction percentages was seen in CS-treated areas versus AACT grafts ($p < 0.05$).

Histological analysis of healing wounds

Figure 5 shows representative images stained with hematoxylin–eosin of the biopsies taken from wounds grafted with AACT and controls at 2, 4, and 8 weeks after surgery. At 2 weeks, all wounds grafted with AACT (panel A) showed pseudoepitheliomatous hyperplasia, high cellularity, and graft remains. Additionally, inflammatory infiltrates and the presence of granulation tissue having numerous blood vessels indicated an acute—albeit moderate—inflammatory response according to the inflammation grading scale used in this study. In contrast, in the control wound (Panel B) the acute inflammatory reaction was mild, connective tissue was profuse, and coagulum remains were observable as well as parakeratosis and hyperkeratosis. At 4 weeks, AACT-grafted wounds con-

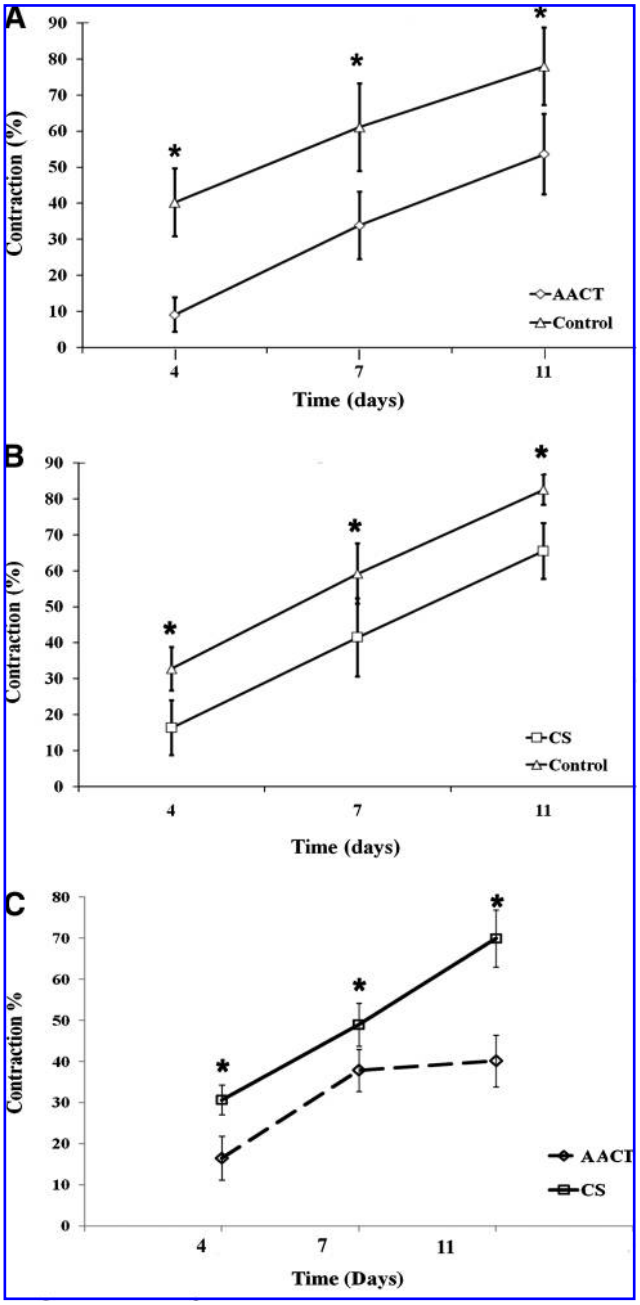

FIG. 4. Contraction percentages at 0, 4, 7, and 11 days posttreatment. Contraction of oral mucosa wounds grafted with AACT versus control, oral mucosa wounds grafted with CS versus control, and oral mucosa wounds grafted with AACT versus CS was determined in three different experiments as described in Materials and Methods section. (**A**) Contraction percentages of AACT-grafted wounds and their controls ($p = 0.001$). Contraction percentages of CS-grafted and control wounds ($p = 0.002$) are presented in (**B**). Contraction percentages of AACT and CS-grafted regions ($p = 0.009$) are shown in (**C**). Wound contraction percentage mean values ± standard error of both treatments are shown. *Significant difference in each single time for the pooled data was established using a Wilcoxon signed rank test.

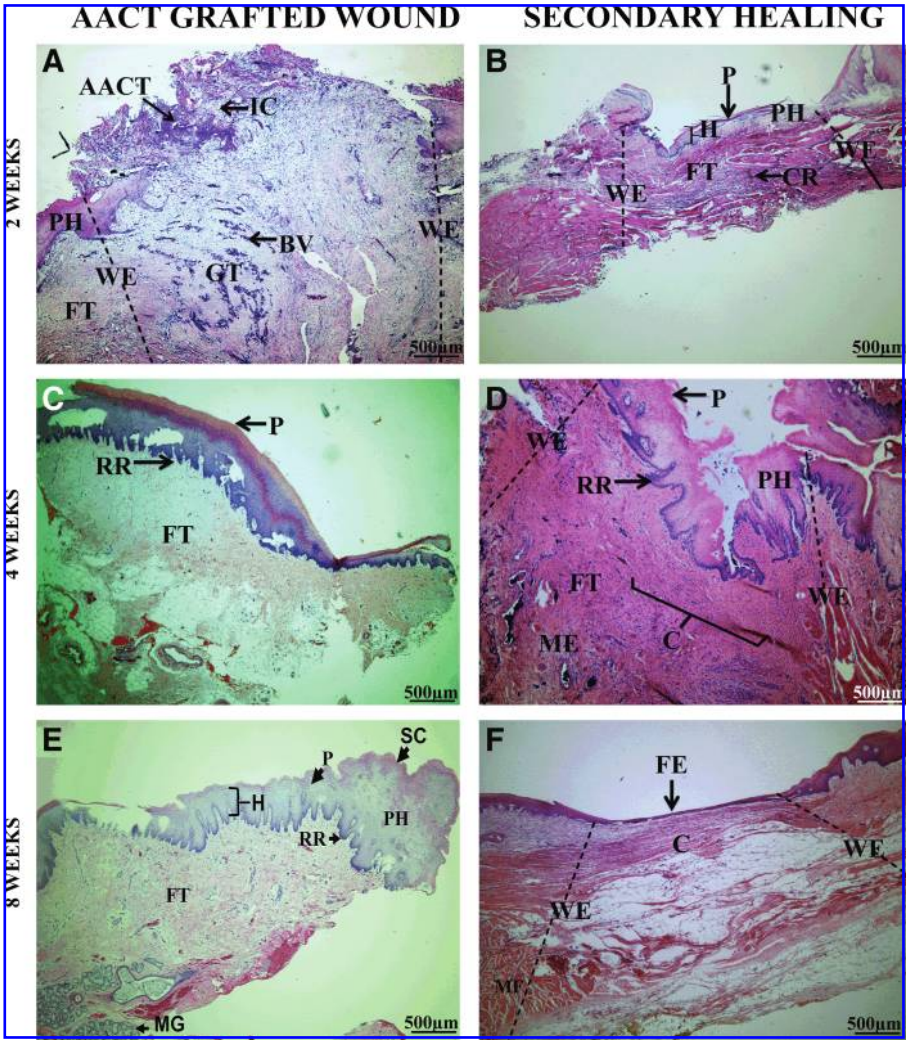

FIG. 5. Comparison of AACT-grafted and control wound tissue samples stained with H&E. Grafted and control wounds at 2 weeks (**A** and **B**, respectively), and grafted and control wounds at 4 weeks (**C** and **D**, respectively) and at 8 weeks (**E** and **F**, respectively). Dotted lines mark the wound edges. BV, blood vessels; C, contraction; CR, coagulum remains; FE, flattened epithelium; FT, fibroconnective tissue; GT, granulation tissue; H, hyperkeratosis; IC, inflammatory cells; MF, muscular tissue; MG, mucous glands; P, parakeratosis; PH, pseudoepitheliomatous hyperplasia; RR, rete ridges; SC, stratum corneum; WE, wound edges. Scale bar = 500 µm. Color images available online at www.liebertonline.com/ten.

tinued to show pseudoepitheliomatous hyperplasia and lax fibroconnective tissue. The grafted area showed parakeratosis, elongated rete ridges, collagen fibers randomly oriented, and no contraction (panel C). In contrast, in control healed tissue at 4 weeks, the collagen fibers were oriented parallel to the wound surface and contraction was noticeable. Acanthosis, muscular entrapment, dense fibroconnective tissue, and low cellularity were also observed in control wounds (panel D). At 8 weeks, the grafted area was covered by stratified epithelium exhibiting a stratum corneum layer and showing parakeratosis, pseudoepitheliomatous hyperplasia, and elongated rete ridges. Mucous glands, lax fibroconnective tissue, collagen fibers randomly oriented, and no contraction were apparent (panel E). In contrast, in 8-week-old control wounds, muscular tissue was sparse and it appeared entrapped within fibroconnective tissue. The epithelium covering the control wound was devoid of rete ridges, flattened and thinner than the normal surrounding epithelia. Lastly, thick fibers of hyalinized collagen parallel-oriented to the wound surface were found in control wounds (panel F). A higher magnification of this figure is presented in Supplemental Fig. S1; supplemental figure S1 available online at www.liebertonline.com/ten.

Figure 6 shows representative histological samples stained with hematoxylin–eosin from the areas grafted with CS and controls at 2, 4, and 8 weeks after surgery. At 2 weeks, CS-grafted wounds showed elongated rete ridges, parakeratosis and hyperkeratosis, mild inflammation, granulation tissue, lax fibroconnective tissue, collagen fibers randomly oriented, and no contraction (panel A). In wounds healed by secondary intention the epithelium was devoid of rete ridges and exhibited parakeratosis and hyperkeratosis. Also, contraction was evident, the granulation tissue was abundant, coagulum remains were still observed, and the inflammation was graded as none (panel B). In general, CS-grafted wounds (panels C and E) displayed similar histological patterns to those found in AACT-grafted wounds at 4 and 8 weeks (panels D and F). A higher magnification of this figure is presented in Supplemental Fig. S2; supplemental Fig. S2 available online at www.liebertonline.com/ten.

Figure 7 presents the histological comparison of contralateral healed wounds grafted with AACT (panel A) and CS (panel B) at 2 weeks. Both grafted areas exhibited stratified epithelium and parakeratosis; however, rete ridge elongation was more pronounced in AACT grafts. Lax fibroconnective tissue and granulation tissue were observed in both graft types; however, scaffold remains were only present in the CS-grafted wounds. Curiously, acute inflammation appeared to be higher in AACT grafts than in CS-grafted regions.

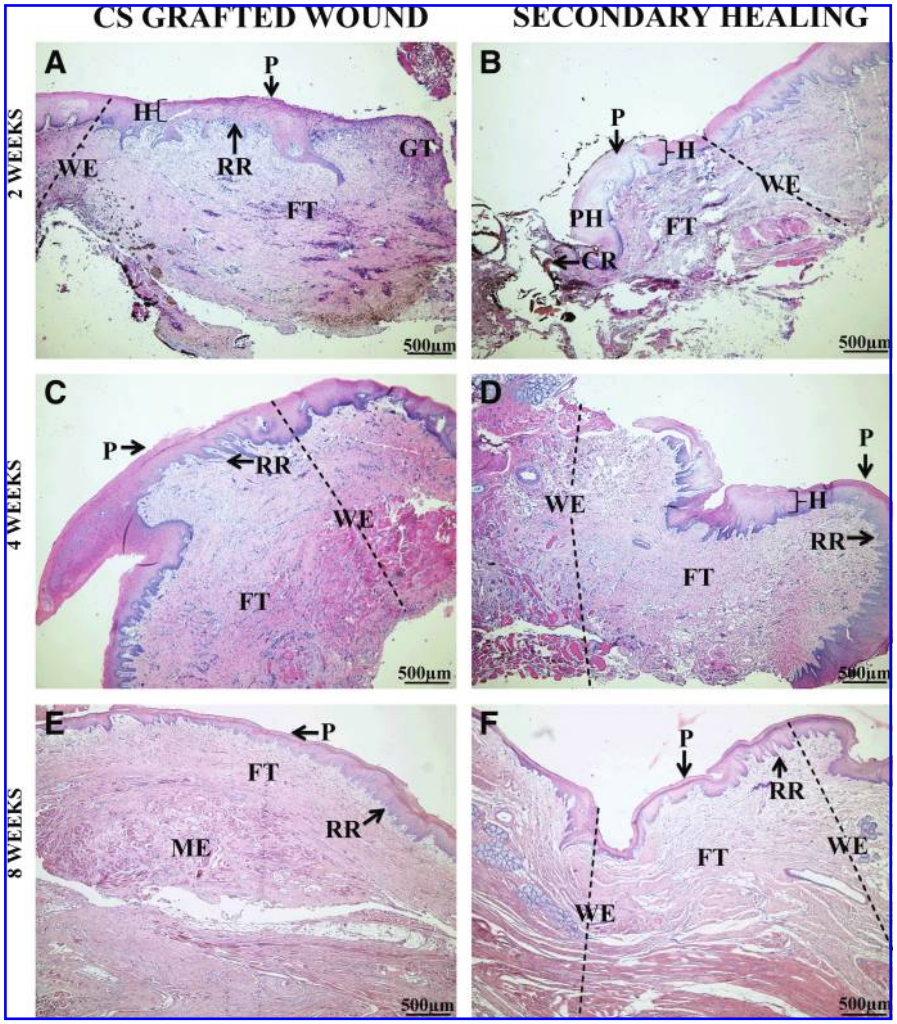

FIG. 6. Comparison of CS-grafted and control wound tissue samples stained with H&E. Grafted and control wounds at 2 weeks (**A** and **B**, respectively), 4 weeks (**C** and **D**, respectively), and 8 weeks (**E** and **F**, respectively). Dotted lines mark the wound edges. BV, blood vessels; C, contraction; CR, coagulum remains; FT, fibroconnective tissue; GT, granulation tissue; H, hyperkeratosis; IC, inflammatory cells; ME, muscular entrapment; MT, muscular tissue; P, parakeratosis; PH, pseudoepitheliomatous hyperplasia; RR, rete ridges; WE, wound edges. Scale bar = 500 µm. Color images available online at www.liebertonline.com/ten.

Evaluating epithelium thickness, inflammation, and newly formed blood vessels

Figure 8 shows the average thickness of the epithelium covering the wounds at 2, 4, and 8 weeks of AACT-treated (panel A), CS-treated (panel B), and their respective control areas. Statistical analysis demonstrated that the thickness of epithelial tissue was significantly different in wounds treated with AACT versus control wounds at 4 weeks ($p = 0.0227$). In contrast, epithelium thickness was not significantly different in wounds treated with CS versus control wounds ($p = 1000$). Comparison of AACT- and CS-grafted wounds at 2 weeks showed that AACT-treated wounds had thicker epithelium than CS-grafted wounds ($p = 0.003$; data not shown).

The extent of inflammation is described in Table 3. At 2 weeks, a significant increase in the number of inflammatory cells in wounds grafted with AACT ($p = 0.0025$) or CS ($p = 0.0025$) was observed in comparison to their controls. Additionally, the number of inflammatory cells was significantly higher ($p = 0.0010$) in AACT healed tissue than in CS

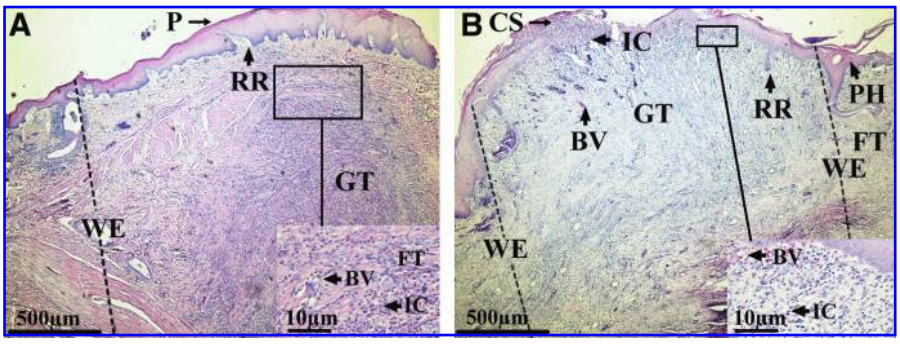

FIG. 7. Comparison of AACT and CS-grafted wound tissue samples stained with H&E. AACT-grafted and CS-grafted wounds at 2 weeks are shown in (**A**) and (**B**), respectively. Dotted lines mark the wound edges. BV, blood vessels; FT, fibroconnective tissue; GT, granulation tissue; IC, inflammatory cells; P, parakeratosis; PH, pseudoepitheliomatous hyperplasia; RR, rete ridges; WE, wound edges. Scale bar: 500 µm (**A** and **B**), 10 µm (**A** and **B**, insets). Color images available online at www.liebertonline.com/ten.

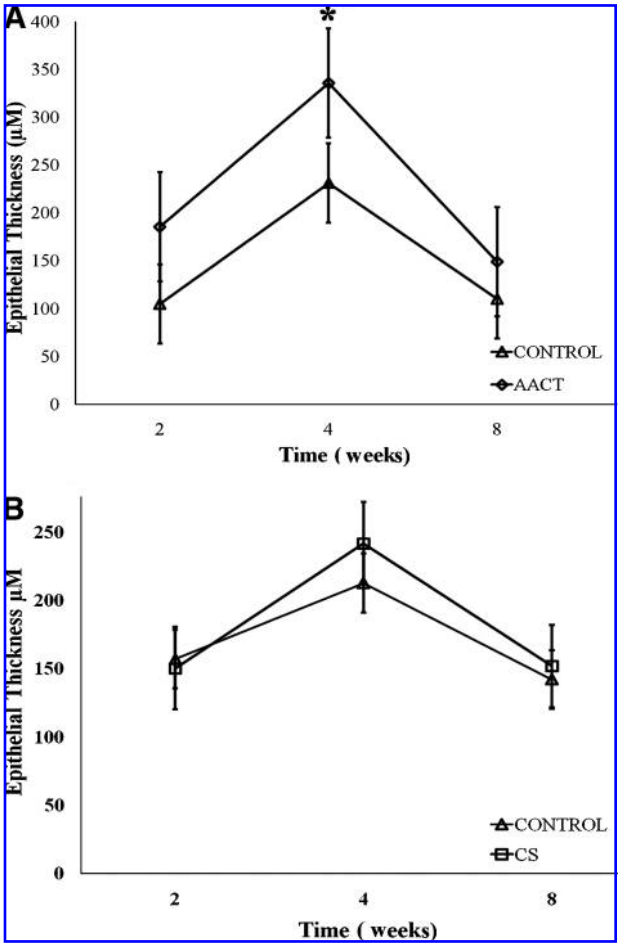

FIG. 8. Epithelial thickness of wounds grafted and their respective controls at 2, 4, and 8 weeks posttreatment. (**A**) Epithelial thicknesses of AACT-grafted wounds and their controls ($p < 0.05$). Epithelial thicknesses of CS-grafted wounds and their controls are presented in (**B**) ($p > 0.05$). *Significant difference in each single time for the pooled data ($p < 0.05$) established using Wilcoxon signed rank test.

healed tissue. Applying the grading scale stated in the table, inflammation in the AACT-grafted wounds was moderate, inflammation in the CS-grafted wounds was mild, and the controls did not display signs of inflammation. By 4 and 8 weeks, inflammation in AACT- and CS-grafted wounds had decreased and was not significantly different from control wounds. Table 4 shows the number of newly formed blood vessels in healed tissues. A significant increase in the number of vessels was observed in wounds grafted with AACT compared with their controls at 4 and 8 weeks ($p = 0.0089$ and $p = 0.0158$, respectively). Similar results were observed when CS-grafted wounds were compared with their controls at 4 weeks ($p = 0.0178$). When a comparison between AACT and CS-grafted contralateral wounds was conducted, AACT-treated regions showed a significantly higher number of blood vessels ($p = 0.0010$).

Discussion

Although oral mucosa heals sooner than skin[1,13] the treatment of large oral mucosa defects, such as cleft-lip palate and cancer-originated ones, without significant scarring and wound contraction, still constitutes a scientific and clinical challenge.[14] Currently, the autograft of split-thickness oral mucosa is the desired treatment, though autologous oral mucosa can be limited. When autologous oral mucosa for grafting is scarce (e.g., large defects), tissue-engineered products can be an alternative tissue source. In fact, epithelial sheets,[15–21] acellular dermal allografts,[22–27] oral mucosa equivalents,[4,5,28] bioabsorbable polyglactin meshes,[29,30] collagen–glycosaminoglycan membranes,[31] hyaluronic acid,[32] collagen and silicon bilayer membranes,[33] and collagen scaffolds[34–35] have been preclinically and clinically evaluated. Each of the aforementioned approaches has advantages and disadvantages, and depending on the characteristics of the oral wound, one product might be preferred over the others.[29] In one report, collagen type I gels seeded with autologous gingival fibroblasts were used to cover partial-thickness gingival wounds and found to augment attached gingiva and promote regeneration of keratinized tissue[34]; however, the low mechanical strength of this type of gel required gauze and foil to implant and secure the scaffold.

This preclinical study evaluated autologous tissue-engineered implants (AACT), based on collagen type I scaffolds and gingival fibroblasts, and acellular CS in the treatment of partial-thickness gingival open wounds. As suggested by the AACT characterization results, it was observed that oral fibroblasts cultured onto collagen type I scaffolds penetrated and adhered to collagen fibers, synthesizing an eosinophilic glycosaminoglycan-containing extracellular matrix with the potential for extracellular matrix remodeling. Additionally, antibody array data showed that at the moment of implantation the fibroblasts in AACT secrete proangiogenic growth factors, tissue-remodeling effectors, and immune modulators and decrease the production of TNF-α and IFN-γ. Interestingly, it has been reported that excessive concentrations of TNF-α result in impaired wound healing.[36] It is important to point out that the decrease in TNF-α and interferon-γ production in AACT does not imply that these two factors—or other immune-modulating cytokines—are not being expressed in the wound site. The above data suggest that at the time of implantation, AACT produces proangiogenic and immune-modulating effectors that may contribute to tissue regeneration.[37–39]

An added advantage of the evaluated products is their mechanical stability, which allows their trimming with surgical scissors and their suturing to the wound bed, thereby overcoming the need for gauze and foil. Additionally, it was observed that wound contracture was higher in secondary healed than in grafted wounds and in CS- than in AACT-treated areas. Histological analysis revealed the existence of important differences between grafted and secondary intention healed areas and suggested that rete ridge formation, neovascularization, and early inflammation were enhanced in AACT- compared with CS-grafted contralateral wounds. Moreover, although both products decrease wound contraction compared with secondary intention, AACT was significantly better at reducing wound contraction than CS. Based on our *in vitro* observations (antibody array) showing AACT secretion of reepithelialization, angiogenesis, and inflammation mediators, it is tempting to speculate that the paracrine component of this scaffold contributes to the observed therapeutic benefit. However, additional experiments are needed to evaluate this hypothesis.

Table 3. Extent of Inflammation in Wounds Grafted with AACT or CS, and Controls

	Week 2 (n = 7)		*Week 4* (n = 7)		*Week 8* (n = 7)	
Inflammation	*AACT*	*Control*	*AACT*	*Control*	*AACT*	*Control*
Non	—	7	7	7	7	7
Mild	—	—	—	—	—	—
Moderate	7	—	—	—	—	—
Severe	—	—	—	—	—	—
p-*Value*	*0.0025*		*1.000*		*0.1282*	
	Week 2 (n = 7)		*Week 4* (n = 7)		*Week 8* (n = 7)	
Inflammation	*CS*	*Control*	*CS*	*Control*	*CS*	*Control*
Non	—	7	7	7	7	7
Mild	7	—	—	—	—	—
Moderate	—	—	—	—	—	—
Severe	—	—	—	—	—	—
p-*Value*	*0.0025*		*0.3051*		*0.3173*	
	Week 2 (n = 9)					
Inflammation	*AACT*	*CS*				
Non	—	—				
Mild	—	9				
Moderate	9	—				
Severe	—	—				
p-*Value*	*0.0010*					

CS, collagen type I scaffolds.

Grafting of AACT or CS appears to be better than secondary oral wound healing because of the diminishing of wound contraction. Epithelial thickness, neovascularization, histological characteristics, and the quality of healed connective tissue indicate that AACT grafting more effectively promotes the regeneration of physiologic oral mucosa. These findings are in full agreement with previous reports by Yannas on skin wound healing where grafting of appropriate extracellular matrix analog templates privileged tissue regeneration over repair.[40] The significant differences in epithelium thickness found at 2 weeks suggest that AACT forms connective tissue *in vivo* capable of supporting further epithelium regeneration. These results are in agreement with data found by others.[41,42] Because cross-talk between wound keratinocytes and fibroblasts is important during reepithelialization of an injured region,[43,44] the AACT fibroblast secretion of reepithelialization chemoattractants such as FGF-2, MCP-4, and UPAR at the time of implantation may stimulate epithelium growth and maturation.[45,46] In addition, because fibroblast migration from the wound bed during natural wound healing also leads to the synthesis and remodeling of the provisional matrix and to the formation of a disorganized collagen network,[47] it will be interesting to investigate *in vivo* the importance of these cells in AACT promoted tissue regeneration.

Inflammation is an important phase of the wound healing process,[44–46] and the present work demonstrates that at 2 weeks the wounds grafted with AACT or CS display in-

Table 4. Number of Newly Formed Blood Vessels in Healed Tissues

	Acellular collagen scaffolds[a]			*AACT*[a]		
Weeks	*Scaffold*	*Control*	p-*Value*	*Scaffold*	*Control*	p-*Value*
2	6.2 ± 2.5	4.9 ± 1.5	>0.05	5.6 ± 2.1	4.9 ± 1.6	>0.05
4	12.6 ± 3.8	7.7 ± 1.7	<0.05	10.1 ± 2.4	5.9 ± 1.5	<0.05
8	10.9 ± 4.6	9.3 ± 3.6	>0.05	9.8 ± 1.5	7.4 ± 1.9	<0.05
	Acellular collagen scaffolds versus AACT[b]					
	CS	*AACT*	p-*Value*			
2	21.8 ± 3.2	29.9 ± 3.9	<0.05			

[a] $n = 42$. Data are reported as mean ± SD.
[b] $n = 9$. Data are reported as mean ± SD.

creased numbers of inflammatory cells compared with controls. Thereafter, inflammation decreased and the differences between grafted and control areas were not significant at 4 and 8 weeks. Moreover, when AACT- and CS-grafted wounds in the same animals were compared, the number of inflammatory cells observed was also significantly higher in the AACT grafts than in the CS-treated wounds. These results support the notion that the extent of mucosal regeneration is associated with an inflammatory response early in the healing process.

Mechanistically, collagen, collagen-derived peptides from the scaffolds, and fibroblasts are modulators of inflammation during wound healing and repair. Indeed, collagen activates platelet release and aggregation. The role of collagen I scaffold grafts in improving healing processes by promoting interactions with cells from the wound bed and cell haptotaxis, cell invasion, and matrix remodeling has been reviewed elsewhere.[46,48,49] As this work demonstrated, autologous fibroblasts cultured into collagen I scaffolds synthesize and secrete growth factors and cytokines that may orchestrate the recruitment and the activation of inflammatory cells, additional fibroblasts, and epithelial and endothelial cells from the wound bed.[50–53] After migration and proliferation, these cells likely synthesize proteins involved in deposition and remodeling of the extracellular matrix contributing to the enhancement of the healing process. Thereafter, our results suggest that the number of inflammatory cells in the regenerating tissue decreases, perhaps avoiding the establishment of an undesired chronic inflammation process.[44,45,54] It is important to note that a similar acute but not sustained inflammation response in oral wounds implanted with collagen-based scaffolds in palatal mucosa has been previously reported.[31,35] Although the mechanisms orchestrating this regulated immune response remain to be elucidated, a recent report suggests that acute and transient infiltration of inflammatory cells promotes the regeneration of mucosal epithelium via IL-11 and IL-22-dependent activation of epithelial STAT3.[55] It would be of great interest to determine if a similar mechanism is operative in the observed AACT and CS regenerative effects.

In conclusion, the data presented here demonstrate that treatment of oral mucosa wounds with AACT promotes tissue regeneration and minimizes wound contraction to a greater extent than CS, although both scaffolds demonstrate far superior wound healing capacity than secondary intention and possess adequate mechanical properties for surgical manipulation. The therapeutic effects of AACT may be mediated by a paracrine component that favorably modulates the immune response, promotes the recruitment of epithelial and endothelial cells, and supports neovascularization of the mucosal tissue. Taken together our results lend support to the clinical use of AACT for the therapy of oral mucosa wounds.

Acknowledgments

This work was supported by the Colombian Science Agency "COLCIENCIAS" (grants 13081216738 and 110145921544), the National University of Colombia (DIB), and the Foundation for Promotion of Research and Technology of the Colombian Republic Bank. We are particularly grateful to Julio Cesar Rios for his contribution to the experiment shown in Fig. 1, and to former M.Sc. student Alejandro Bello and pathologist Carmen L. Roa for their contribution to the experiment shown in Fig. 2A and B. The authors thank Drs. Luis Alberto Perez, Ramon Pereira, and Pedro E. Morales for invaluable help in animal experiments and Drs. Enrico Nasi, Maria Gomez, and Ismael Samudio for critical reading of the manuscript. The authors are also grateful to Maria M. Posada, Juan F. Diaz, Edward Suesca, and Universidad El Bosque for their help in this research and to Lily Cohen for editing the manuscript.

Disclosure Statement

No competing financial interests exist.

References

1. Szpaderska, A.M., Zuckerman, J.D., and DiPietro, L.A. Differential injury responses in oral mucosa and cutaneous wounds. J Dent Res **82,** 621, 2003.
2. Yadav, P. Reconstructive surgery in oral cancers. Indian J Plast Surg **40,** 22, 2007.
3. Ophof, R., Maltha, J.C., Von den Hoff, J.W., and Kuijpers-Jagtman, A.M. Histologic evaluation of skin-derived and collagen-based substrates implanted in palatal wounds. Wound Repair Regen **12,** 528, 2004.
4. Sauerbier, S., Gutwald, R., Wiedmann-Al-Ahmad, M., Lauer, G., and Schmelzeisen, R. Clinical application of tissue-engineered transplants. Part I: mucosa. Clin Oral Implants Res **17,** 625, 2006.
5. Izumi, K., Feinberg, S.E., Iida, A., and Yoshizawa, M. Intraoral grafting of an *ex-vivo* produced oral mucosa equivalent: a preliminary report. Int J Oral Maxillofac Surg **32,** 188, 2003.
6. Rahmani, M.E., and Lades, M.A. Comparative clinical evaluation of acellular dermal matrix allograft and connective tissue graft for the treatment of gingival recession. J Contemp Dent Pract **7,** 63, 2006.
7. Butler, C.E., Navarro, F.A., Park, C.S., and Orgill, D.P. Regeneration of neomucosa using cell-seeded collagen-GAG matrices in athymic mice. Ann Plast Surg **48,** 298, 2002.
8. Fujioka, M., and Fujii, T. Maxillary growth following atelocollagen implantation on mucoperiostal denudation of the palatal process in young rabbits: implication for clinical cleft palate repair. Cleft Palate Craniofac J **34,** 297, 1997.
9. Pérez, S., Doncel, A., Roa, C.L., and Fontanilla, M.R. Estandarización de un método para la elaboración de un análogo de dermis humana. Rev Colomb Cienc Quim Farm **30,** 9, 2001.
10. Bello, S.A., and Fontanilla, M.R. Evaluation of autologous artificial connective tissue as grafting materials on rabbit oral mucosa lesions. Abstract presented at the 6th Annual Meeting of the Tissue Engineering Society International (TESI), Orlando, FL, 2003. Abstract no. P090.
11. Bello, S.A., Pereira, R., and Fontanilla, M.R. Elaboración de tejido conectivo artificial autólogo de mucosa oral y evaluación de su desempeño como cobertura biológica, en lesiones mucosas inducidas en conejos. Rev Fed Odontol Colomb **20,** 20, 2004.
12. Morton, S.R., and Dongari-Bagtzoglou, A.I. Cyclooxygenase-2 is upregulated in inflamed gingival tissues. J Peridontol **72,** 461, 2001.
13. Häkkinen, L., Uitto, V.J., and Larjava, H. Cell biology of gingival wound healing. Periodontol **24,** 127, 2000.

14. Izumi, K., Song, J., and Feinberg, S.E. Development of a tissue-engineered human oral mucosa: from the bench to bed side. Cell Tissues Organs **176,** 134, 2004.
15. de Luca, M., Albanese, E., Megna, M., Cancedda, R., Mangiante, P.E., Cadoni, A., and Franzi, A.T. Evidence that human oral epithelium reconstituted *in vitro* and transplanted onto patients with defects in the oral mucosa retains properties of the original donor site. Transplantation **50,** 454, 1990.
16. Lauer, G. Autografting of feeder-cell free cultured gingival epithelium: method and clinical application. J Craniomaxillofac Surg **22,** 18, 1994.
17. Ueda, M. Formation of epithelial sheets by serially cultivated human mucosal cells and their applications as a graft material. Nagoya J Med Sci **58,** 13, 1995.
18. Raghoebar, G.M., Tomson, A.M., Scholma, J., Blaauw, E.H., Witjes, M.J.H., and Vissink, A. Use of cultured mucosal grafts to cover defects caused by vestibuloplasty. An *in vivo* study. J Oral Maxillofac Surg **53,** 872, 1995.
19. Tsai, C., Ueda, M., Hata, K., Horie, K., Hibino, Y., Sugimura, Y., Toriyama, K., and Torri, S. Clinical results of cultured epithelial cell grafting in the oral and maxillofacial region. J Craniomaxillofac Surg **25,** 4, 1997.
20. Lauer, G., Schimming, R., and Feinberg, S.E. Tissue-engineered mucosa graft for reconstruction of the intraoral lining after freeing of the tongue: a clinical and immunohistologic study. J Oral Maxillofac Surg **59,** 169, 2001.
21. Bodner, L., and Grossman, N. Autologous cultured mucosal graft to cover large intraoral mucosal defects: a clinical study. J Oral Maxillofac Surg **61,** 169, 2003.
22. Novaes, A.B., Jr., Grisi, D.C., Molina, G.O., Souza, S.L., Taba, M., Jr., and Grisi, M.F. Comparative 6-month clinical study of a subepithelial connective tissue graft and acellular dermal matrix graft for the treatment of gingival recession. J Periodontol **72,** 1477, 2001.
23. Aichelmann-Reidy, M.E., Yukna, R.A., Evans, G.H., Nasr, H.F., and Mayer, E.T. Clinical evaluation of acellular allograft dermis for the treatment of human gingival recession. J Periodontol **72,** 998, 2001.
24. Wei, P.-C., Laurell, L., Lingen, M.W., and Geivelis, M. Acellular dermal matrix allografts to achieve increased attached gingival. Part 2. A histological comparative study. J Periodontol **73,** 257, 2002.
25. Wagshall, E., Lewis, Z., Babich, S.B., Sinensky, M.C., and Hochberg, M. Acellular dermal matrix allograft in the treatment of mucogingival defects in children: illustrative case report. ASDC J Dent Child **69,** 39, 2002.
26. Gath, H.J., Hell, B., Zarrinbal, R., Bier, J., and Raguse, J.D. Regeneration of intraoral defects after tumor resection with a bioengineered human dermal replacement (Dermagraft). Plast Reconstr Surg **109,** 889, 2002.
27. Raguse, J.D., and Gath, H.J. A metabolically active dermal replacement (Dermagraft) for vestibuloplasty. J Oral Rehabil **32,** 337, 2005.
28. Hotta, T., Yokoo, S., Terashi, H., and Komori, T. Clinical and histopathological analysis of healing process of intraoral reconstruction with *ex vivo* produced oral mucosa equivalent. Kobe J Med Sci **53,** 14, 2007.
29. McGuire, M.K., and Nunn, M.E. Evaluation of the safety and efficacy of periodontal applications of a living tissue-engineered human fibroblasts-derived dermal substitute. I. Comparison to the gingival autograft: a randomized controlled pilot study. J Periodontol **76,** 867, 2005.
30. Wilson, T.G., Jr., McGuire, M.K., and Nunn, M.E. Evaluation of the safety and efficacy of periodontal applications of a living tissue-engineered human fibroblast- derived dermal substitute. II. Comparison to the subepithelial connective tissue graft: a randomized controlled feasibility study. J Periodontol **76,** 881, 2005.
31. Ophof, R., Maltha, J.C., Kuijpers-Jagtman, A.-M., and Von den Hoff, J.W. Evaluation of a collagen-glycosaminoglycan dermal substitute in the dog palate. Tissue Eng **13,** 2689, 2007.
32. Prato, G.P., Rotundo, R., Magnani, C., Soranzo, C., Muzzi, L., and Cairo, F. An autologous cell hyaluronic acid graft technique for gingival augmentation: a case series. J Periodontol **74,** 262, 2003.
33. Omura, S., Mizuki, N., Horimoto, S., Kawabe, R., and Fujita, K. A newly developed collagen/silicone bilayer membrane as a mucosal substitute: a preliminary report. Br J Oral Maxillofac Surg **35,** 85, 1997.
34. Mohammadi, M., Shokrgozar, M.A., and Mofid, R. Culture of human gingival fibroblasts on a biodegradable scaffold and evaluation of its effect on attached gingival: a randomized, controlled pilot study. J Periodontol **78,** 1897, 2007.
35. Jansen, R.G., Kuijpers-Jagtman, A.M., van Kuppevelt, T.H., and Von den Hoff, J.W. Collagen scaffolds implanted in the palatal mucosa. J Craniofac Surg **19,** 599, 2008.
36. Rumalla, V.K., and Borah, G.L. Cytokines, growth factors, and plastic surgery. Plast Reconstr Surg **108,** 719, 2001.
37. Werner, S., and Grose, R. Regulation of wound healing by growth factors and cytokines. Physiol Rev **83,** 835, 2003.
38. Gillitzer, R., and Goebeler, M. Chemokines in cutaneous wound healing. J Leukoc Biol **69,** 513, 2001.
39. Bartold, P.M., and Narayanan, A.S. Inflamation and wound healing. In: Strem, C., ed. Biology of the Periodontal Connective Tissue. Hanover Park, IL: Quintessence Publishing Co. Inc., 1998, pp. 27–116.
40. Yannas, I. Regeneration templates. In: Bronzino, J.D., ed. The Biomedical Engineering Handbook, second edition. Boca Raton, FL: CRC Press LLC, 2000, Chapter 113.
41. Winning, T., and Townsend, G.C. Oral mucosal embryology and histology. Clin Dermatol **18,** 499, 2000.
42. Locke, M., Hyland, P.L., Irwin, C.R., and Mackenzie, I.C. Modulation of gingival epithelial phenotypes by interactions with regionally defined populations of fibroblasts. J Periodont Res **43,** 279, 2008.
43. Clark, R.A.F. Epithelial-mesenchymal networks in wounds: a hierarchical view. J Invest Dermatol **120,** ix, 2003.
44. Eming, S.A., Krieg, T., and Davidson, J.M. Inflammation in wound repair: molecular and cellular mechanism. J Invest Dermatol **127,** 514, 2007.
45. Anderson, J.M. Inflammation, wound healing, and the foreign-body response. In: Rather, B.D., Hoffman, A.S., Schoen, F.J., and Lemons, J.E., eds. Biomaterials Science: An Introduction to Materials in Medicine. San Diego, CA: Elsevier Academic Press, 2004, pp. 296–303.
46. Clark, R.A.F. Wound repair: lessons for tissue engineering. In: Lanza, R.P., and Chick, W.L., eds. Principles of Tissue Engineering. Georgetown, TX: R.G. Landes Company and Academic Press, 1997, pp. 737–779.
47. Enoch, S., Moseley, R., Stephens, P., and Thomas, D.W. The oral mucosa: a model of wound healing with reduced scarring. Oral Surg **1,** 11, 2008.
48. Rosso, F., Marino, G., Giordano, A., Barbarisi, M., Parmeggiani, D., and Barbarisi, A. Smart materials as scaffolds for tissue engineering. J Cell Physiol **203,** 465, 2005.

49. Ruszczak, Z. Effect of collagen matrices on dermal wound healing. Adv Drug Deliv Rev **55,** 1595, 2003.
50. Wong, T., McGrath, J.A., and Navsaria, H. The role of fibroblasts in tissue engineering and regeneration. Br J Dermatol **156,** 1149, 2007.
51. Deuel, T.F., and Chang, Y. Growth factors. In: Lanza, R., Langer, R., and Vacanti, J., eds. Principles of Tissue Engineering. Burlington, MA: Elsevier Academic Press, 2007, pp. 193–201.
52. Lamme, E.N., van Leeuwen, R.T.-J., Jonker, A., van Marle, J., and Middelkoop, E. Living skin substitutes: survival and function of fibroblasts seeded in a dermal substitute in experimental wounds. J Invest Dermatol **111,** 989, 1998.
53. Lamme, E.N., van Leeuwen, R.T., Brandsma, K., van Marle, J., Middelkoop, E. Higher numbers of autologous fibroblasts in an artificial dermal substitute improve tissue regeneration and modulate scar tissue formation. J Pathol **190,** 595, 2000.
54. Fivenson, D.P., Faria, D.T., Nickoloff, B.J., Poverini, P.J., Kunkel, S., Burdick, M., and Strieter, R.M. Chemokine and inflammatory cytokine changes during chronic wound healing. Wound Repair Regen **5,** 310, 1997.
55. Eckmann, L., Nebelsiek, T., Fingerle, A.A., Dann, S.M., Mages, J., Lang, R., Robine, S., Kagnoff, M.F., Schmid, R.M., Karin, M., Arkan, M.C., and Greten, F.R. Opposing functions of IKKbeta during acute and chronic intestinal inflammation. Proc Natl Acad Sci USA **105,** 15058, 2008.

Address correspondence to:
Marta R. Fontanilla, Ph.D.
Pharmacy Department
Faculty of Sciences
National University of Colombia
Av Carrera 30 # 45-10
Bogota D.C. 00571
Colombia

E-mail: mrfontanillad@unal.edu.co

Received: October 16, 2008
Accepted: December 15, 2009
Online Publication Date: February 3, 2010