Acta Odontologica Scandinavica, 2013; 71: 1501–1507

informa
healthcare

ORIGINAL ARTICLE

Quantitative evaluation of myofibroblast apoptosis during wound healing in rat palate after post-operative administration of basic fibroblast growth factor (bFGF)

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Abstract

Objective. Excessive wound contraction apparently inhibits maxillary growth; thus, myofibroblast apoptosis needs to be accelerated in mucoperiosteal denudation after palatoplasty. The aim of this study was to evaluate myofibroblast apoptosis during wound healing in mucoperiosteal denudation of rat palates immediately after post-operative administration of basic fibroblast growth factor (bFGF). ***Materials and methods.*** A total of 100 male Wistar rats aged 20 days were divided into control, scar, sham and bFGF groups ($n = 25$ each). In the scar, sham and bFGF groups, mucoperiosteum was removed from the palate and fibrin glue was applied to the exposed bone surface immediately after surgery. In the bFGF group, 10 μL of 2 μg/μL bFGF solution was injected into the operated area beneath the fibrin glue. At 2, 5, 7, 14 and 28 days post-operatively, myofibroblast apoptosis during the wound healing process was investigated by double immunofluorescence staining. The apoptotic area of myofibroblasts was measured using image software. ***Results.*** In the bFGF group, at 2 days, apoptosis of myofibroblasts in the lamina propria and submucosa was marked, as compared with the other three groups and apoptosis of myofibroblasts was scarcely seen at 5 days. At 5 and 7 days, the apoptotic area of myofibroblasts in the bFGF group was statistically significantly smaller when compared to the scar and sham groups. ***Conclusion.*** The results confirmed that bFGF injection immediately after surgery accelerated apoptosis of myofibroblasts in mucoperiosteal denudation of rats. This may reduce maxillary growth retardation due to excessive wound contraction.

Key Words: *basic fibroblast growth factor, cleft palate, wound healing, myofibroblast, apoptosis*

Introduction

The pushback method including mucoperiosteal denudation [1,2], a method for palatoplasty in cleft palate patients, is advantageous for improvement of swallowing and speech functions. However, both wound contraction and scar tissue formation in the remaining palatal wounds induce maxillary growth retardation and Class III malocclusion [3–13]. Numerous cleft lip and palate patients show under-development of the maxilla in not only the sagittal but also vertical and transverse dimensions following surgical repair involving mucoperiosteal denudation [14,15].

With regard to pharmacological control of wound healing using animal models, it has been reported that interferon-gamma (IFN-gamma) is able to reduce the number of myofibroblasts, which are considered to be responsible for scar tissue contraction [16]. Funato et al. [17,18] suggested that transforming growth factor-beta 1 (TGF-β1) induces the formation of myofibroblasts in the early stages of palatal wound healing, while basic fibroblast growth factor (bFGF) may induce apoptosis of myofibroblasts after re-epithelialization of the wound. The application of bFGF as solution [19] and as collagen scaffolds [20] in animal mucosal injury has also been examined.

We previously reported that bFGF administration onto the rat palate at 1 week after removing mucoperiosteum recovered bone growth retardation [21,22]. In our previous histological and immunohistochemical

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(Received 15 June 2012; revised 14 December 2013; accepted 30 January 2013)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2013.773370

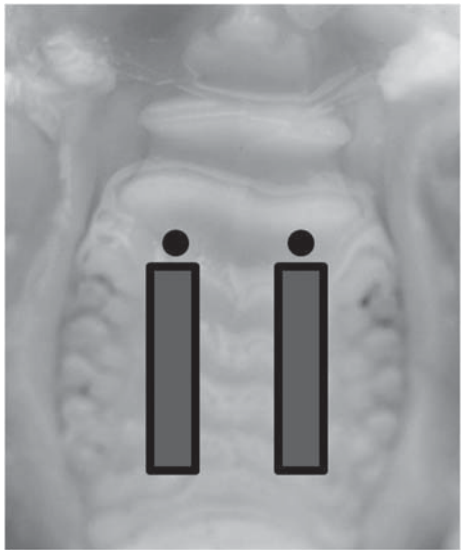

Figure 1. Rat palate. Rectangle indicates surgically treated areas in the scar, sham and bFGF groups. Dots indicate syringe insertion site for bFGF/distilled water injection.

studies, bFGF injection into the rat palate at 1 week after surgery reduced the number of thick bundles of collagen fibers [23], which was attributed to recovery of the vascular number and size [21,24] and recovery of endothelial cell number [24]. The findings suggest that bFGF is a potential modulator that reduces maxillary growth retardation due to post-operative scar formation in cleft palate patients.

Wound healing is generally classified into three consecutive, partly overlapping phases: inflammation, proliferation and tissue remodeling [25]. In the later phases of inflammation, myofibroblasts appear in the wound and start to promote wound contraction [26,27]. After wound contraction, myofibroblasts disappear due to apoptosis. The timing is consistent with the tissue remodeling phase [25]. Excess wound contraction appears to inhibit maxillary growth; thus, the period of myofibroblast apoptosis should be accelerated in mucoperiosteal denudation after palatoplasty.

The timing of bFGF injection in our previous study (1 week after removing mucoperiosteum on rat palate) appears to correspond to the proliferation phase. However, bFGF administration immediately after surgery is more effective for evaluating the effects against wound healing due to inhibition of excess myofibroblast proliferation and subsequent wound contraction. It is impossible to directly inject bFGF immediately after surgery in the rat palate because bFGF solution leaks out to the oral cavity. However, fibrin glue can be applied to the exposed bone surface of both animal models and patients immediately after surgery, and it is then possible to inject bFGF underneath the fibrin glue just after removing mucoperiosteum from the rat palate.

The aim of present study was to evaluate myofibroblast apoptosis during wound healing in mucoperiosteal denudation of rat palates immediately after post-operative administration of bFGF.

Materials and methods

Animals

A total of 100 male Wistar rats aged 20 days were used in this study. The mean weight was 50 g. All animals were kept under normal laboratory conditions and provided with *ad libitum* access to standard rat chow and water. Animals were divided into four groups: control; scar; sham; and bFGF (n = 25 each). No significant differences in weight were seen among the four groups throughout the experimental period. The experiment was approved by the Board for Animal Experiments at Fukuoka Dental College.

Surgical procedure of scar formation on palate and bFGF injection

For scar formation on the palate, in the scar, sham and bFGF groups, mucoperiosteum was removed from the palate as described by Kim et al. [28] (Figure 1). Rectangular strips of the bilateral one-third of palatal mucoperiosteum were excised under general anesthesia induced by intraperitoneal injection of 7 mg/kg of sodium pentobarbital (Somnopentyl; Kyouritsu Seiyaku, Tokyo, Japan). The greater palatine neurovascular bundles were not avulsed during surgery. The exposed bone surface was wiped with a cotton pellet for complete removal of the periosteum. In the scar group, sham group and bFGF group, fibrin glue was applied to the exposed bone surface immediately after surgery. Control animals did not undergo any surgical procedures.

Recombinant bFGF was provided by Kaken Pharmaceutical (Tokyo, Japan). In the bFGF group, 10 μL of bFGF solution (20 μg bFGF/10 μL distilled water) was injected into each side of the treated area using a syringe (Hamilton, Reno, NV) beneath the fibrin glue. The sham group received an injection of 10 μL of distilled water. In the present study, the dose of bFGF was determined based on the results of previous studies [21–24].

At 2, 5, 7, 14 and 28 days post-operatively, animals were carefully anesthetized by intraperitoneal injection of 7 mg/kg sodium pentobarbital (Somnopentyl; Kyouritsu Seiyaku, Tokyo, Japan) after performing inhalation anesthesia with ether. A polyethylene catheter tube was introduced into the aorta for perfusion.

Histological preparation

After perfusion, the maxilla was removed, immersed in 10% formalin, decalcified in 10% EDTA, dehydrated in a graded series of ethanol and embedded in paraffin. Serial frontal sections of 7 μm were taken

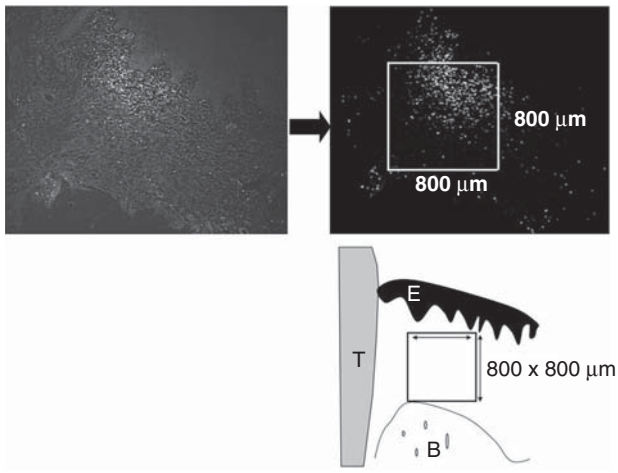

Figure 2. Figure and scheme of measurement of alpha-SMA-positive cell apoptotic area by image processing software. Square indicates measurement area. T, tooth; B, palatal bone; E, epithelium.

around the disto-lingual root of the 1st molar for hematoxylin and eosin (HE) staining and double immunofluorescence staining for apoptosis in myofibroblasts.

Double immunofluorescence staining for apoptosis in myofibroblasts

For double immunofluorescence staining, TUNEL staining was performed according to the manufacturer's protocol and a commercial kit (*In situ* Cell Death Detection Kit, Fluorescein; Roche, Mannheim, Germany). After TUNEL staining, sections were incubated overnight at 4°C with mouse anti-human alpha-smooth muscle actin (alpha-SMA) monoclonal antibody (DAKO, Tokyo, Japan) at a dilution of 1:100. Sections were then incubated with a Texas Red-conjugated goat anti-mouse immunoglobulin antibody (IgG) (Molecular Probes Inc., Eugene, OR) for 40 min. Finally, sections were mounted with mounting media and observed by fluorescence microscopy (BZ-8100; KEYENCE, Tokyo, Japan).

In order to confirm the presence of myofibroblasts in scar tissue, laser-scanning confocal microscopy was performed on anti-alpha-SMA-stained sections of rat palate that exhibited scar tissue at 5 days after surgery. Formalin-fixed paraffin-embedded sections were immersed in xylene and ethanol and were washed in 10% PBS. Sections were exposed to mouse anti-human alpha-SMA monoclonal antibody (DAKO, Tokyo, Japan) and Texas Red-conjugated goat anti-mouse IgG (1 μg/ml) (Molecular Probes Inc., Eugene, OR) after blocking with 1% goat serum in PBS. Nuclei were counterstained with DAPI (Sigma-Aldrich, Tokyo, Japan), mounted in 50% polyvinylpyrrolidone solution and examined by laser-scanning confocal microscopy (LSM710, Carl Zeiss, Jena, Germany) with a PlanApochromatic 60× oil immersion objective lens (numerical aperture ×1.3).

Quantification of apoptosis in myofibroblasts

The apoptotic area of myofibroblasts was measured with NIH ImageJ (available as freeware from http://rsbweb.nih.gov/ij/). Apoptotic cells are stained green on TUNEL staining, while cells expressing alpha-SMA are stained red. Apoptotic myofibroblasts are positive for both TUNEL and alpha-SMA expression and are stained yellow. To quantify the apoptotic area of myofibroblasts, images were loaded into ImageJ. The type was set to Black & White. The image threshold was then set from 72 to 254, referring to the yellow stained area on the double immunofluorescence staining image, which only shows the apoptotic area of myofibroblasts. The number of thresholded pixels was measured in an 800 × 800-μm area above the crest of palatal bone (Figure 2).

In each animal, the apoptotic area of myofibroblasts was calculated as a mean value from two sections with an interval of five serial sections. At each stage from the 2nd to 28th post-operative day in the four groups, the mean value and standard deviation of the apoptosis area of myofibroblast was determined from five individuals.

Data from the four groups at every time point were subjected to one-way analysis of variance with Scheffé multiple comparison post-hoc test. All statistical analyses were performed using StatView (Hulinks Inc., Tokyo, Japan).

Results

HE staining

In the control group, the palates were covered with stratified squamous epithelium, which was slightly parakeratotic with numerous rete pegs. Beneath the epithelium, there was a network of coarse collagen fibers, including numerous blood vessels in the lamina propria and submucosa. There were no apparent changes throughout the experimental period.

In the scar and sham group, inflammatory cell infiltration was observed in the submucosa at 2 days. It became more obvious at 7 days. At 14 days, collagen fibers showed a somewhat irregular arrangement. At 28 days, collagen fibers at the submucosa were increased in number and were arranged in thicker bundles than in the control group.

In the bFGF group, inflammatory cell infiltration was observed in the submucosa at 2 days. The image in the bFGF group was similar to those in the scar and the sham groups. Inflammatory cell infiltration was more widespread at 7 days and reduced at 14 days. At 14 and 28 days, collagen fibers showed a somewhat irregular arrangement, but the fibrous tissue was less dense and the collagen bundles were thinner than in the scar and sham groups. The image was more similar to the control group than the scar or sham groups (Figure 3).

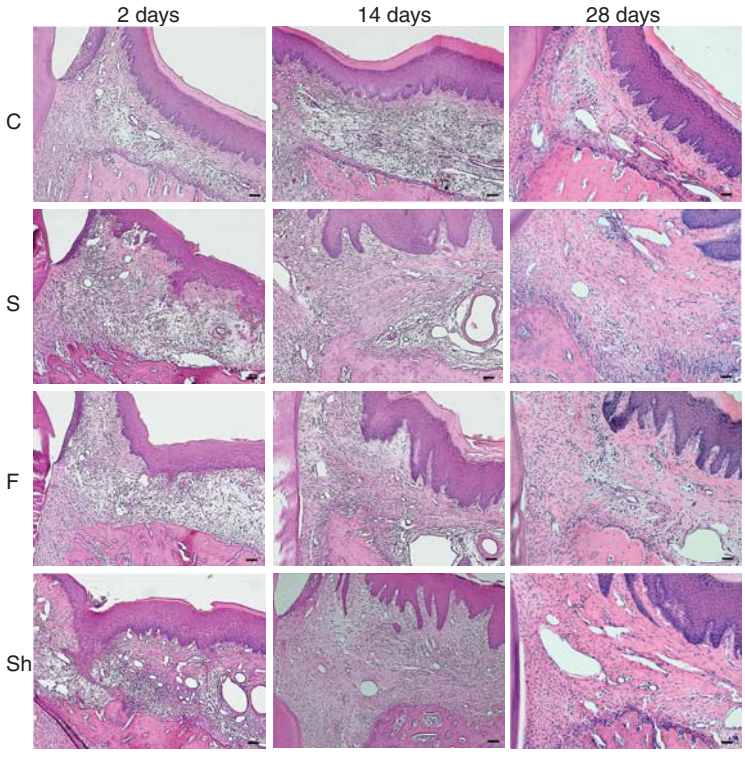

Figure 3. HE staining showing frontal section of rat palate at 2, 14 and 28 days post-operatively (C, control group; S, scar group; F, bFGF group; Sh, sham group). Scale bars represent 100 μm.

Double immunofluorescence staining

Laser-scanning confocal microscopy of rat scar tissue showed that blood vessel walls were immunostained with anti-alpha-SMA and that the scar tissue contained fibroblast-like cells expressing alpha-SMA in the cytoplasm. There were no cross-reactions of anti-alpha-SMA to the nuclei in alpha-SMA-positive fibroblast-like cells (Figure 4).

In the control group, alpha-SMA-positive cells were seen on blood vessels in the lamina propria and submucosa. Apoptosis of alpha-SMA-positive cells in the submucosa was scarcely seen. There were no apparent changes throughout the experimental period.

In the scar and sham group at 2 days, expression of alpha-SMA and apoptosis of alpha-SMA-positive cells were widespread in the lamina propria and submucosa. At 5 and 7 days, both alpha-SMA-positive cells and apoptosis of alpha-SMA-positive cells were more obvious than at 2 days. At 14 days, expression of alpha-SMA was still seen in lamina propria and submucosa, but there was little apoptosis of alpha-SMA-positive cells. At 28 days, there was no apparent expression of alpha-SMA or apoptosis of alpha-SMA-positive cells.

In the bFGF group, at 2 days, apoptosis of alpha-SMA-positive cells in the lamina propria and submucosa was marked, as compared with the other three groups. At 5 days, apoptosis of alpha-SMA-positive cells was scarcely seen, whereas expression of alpha-SMA cells was obvious in the lamina propria and submucosa. At 7 and 14 days, alpha-SMA-positive cells gradually disappeared from the lamina propria and submucosa. At 28 days, there was no apparent apoptosis of alpha-SMA-positive cells or expression of alpha-SMA (Figures 5A and B).

Quantitation of apoptosis in myofibroblast

Tables I and II show a comparison of apoptotic area (pixels) in myofibroblasts in the four groups throughout the experiment period. The apoptotic area of alpha-SMA-positive cells in the control group showed the lowest value among the four groups throughout the experimental period.

There were no significant differences between the scar and sham groups at any experimental time point. The apoptotic area of alpha-SMA positive cells in the scar and sham groups was significantly larger than in the control group at 2, 5 and 7 days. At 14 days, there were no significant differences between the scar and control groups; nevertheless, the sham group showed significantly larger values than in the control group. At 28 days, there were no significant differences between the control, scar and sham groups.

In the bFGF group, the apoptotic area of alpha-SMA-positive cells was significantly larger than in the control or sham groups at 2 days. At 5 and 7 days, the bFGF group showed a significantly smaller value than the scar and sham groups, while there were no significant differences between the bFGF and control groups. At 14 and 28 days, there were no significant

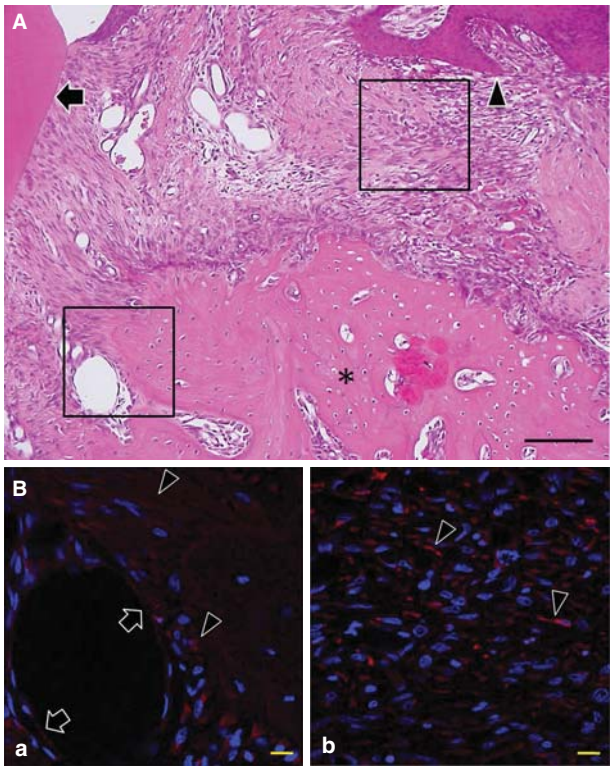

Figure 4. Immunohistochemical analysis for expression of alpha-SMA in rat palate. (A) HE-stained section of rat palate at 5 days post-operatively. Scar connective tissue has formed under the palatal mucous epithelia. Arrow, left M1; asterisk, palatal bone; arrowhead, epithelial peg. Scale bar represents 100 μm. (B) Anti-alpha-SMA-immunostained section adjacent to the HE section. (a) Laser-scanning confocal microscopy for the area near palatal bone highlighted by a box in (A). Reaction products with anti-alpha-SMA (visualized in red) are present on endothelial cells of blood vessel wall (arrows) and in the cytoplasm of fibroblast-like cells of scar connective tissue (arrowheads). (b) Laser-scanning confocal microscopy for the area under palatal mucous epithelia highlighted by a box in (A). Strong linear reaction products with anti-alpha-SMA (visualized in red) are found around the nuclei in the cytoplasm of fibroblast-like cells of scar connective tissue (arrowheads). There are no cross-reactions to nuclei. Scale bars represent 10 μm.

differences between bFGF and the other three groups, except between the bFGF and sham groups at 14 days.

Discussion

Myofibroblasts play an important role in the wound-healing process, promoting wound closure and matrix deposition. Myofibroblasts appear to be involved in wound contraction and reductions in myofibroblasts decrease wound contraction [16]. The number of myofibroblasts is drastically decreased following the completion of re-epithelialization [29]. In the scar and sham groups, alpha-SMA-positive cells appeared at 2 days. At 5 and 7 days, expression-positive areas were more obvious and remained observable at 14 days. Cornelissen et al. [16] reported that, during wound healing, myofibroblasts appeared in the control group from 5–22 days after surgery, with peak numbers being observed at 8 days after surgery. The time frame in the scar and sham groups thus appears to be consistent with the results of Cornelissen et al. [16].

Tobita and Inokuchi [30] reported that bFGF-positive macrophages are present at 14 days post-operatively, when the number of myofibroblasts increases. Funato et al. [17,18] reported that bFGF is a potential stimulator of apoptosis in myofibroblasts after re-epithelialization in the palatal wound healing process.

Our previous histological and immunohistochemical findings have shown that bFGF administration on rat palate 1 week after removing mucoperiosteum reduces the number of thick bundles of collagen fibers [23] and this was attributed to the recovery of vascular number and size [21,22,24] and endothelial cell number [24].

Wound healing can be divided into three phases; inflammation, proliferation and tissue remodeling [25]. Wound contraction by myofibroblasts begins in the late phase of inflammation. Therefore, using fibrin glue to enable bFGF administration immediately after surgery is more effective for evaluating its effects on myofibroblasts, in contrast to previous studies, in which the timing of administration was 1 week after removing the mucoperiosteum [21–24].

In the present study, we evaluated myofibroblast apoptosis during wound healing in the rat palate immediately after post-operative administration of bFGF, although the method of bFGF administration was already determined based on previous studies [21–24].

The expression of alpha-SMA was confirmed both in blood vessel walls and in the cytoplasm of fibroblast-like cells in the scar connective tissue by laser-scanning confocal microscopy, and thus alpha-SMA-positive fibroblast-like cells appearing to be myofibroblasts were present in scar tissue.

In the bFGF group, the apoptotic area of alpha-SMA-positive cells was significantly larger than that in the control or sham groups at 2 days. At 5 and 7 days, however, the bFGF group showed significantly smaller areas than the scar and sham groups, while there were no significant differences between the bFGF and control groups. Thus, apoptosis of alpha-SMA-positive cells in the bFGF group was strongest at 2 days, but was strongest at 5 days in the scar and sham groups. This suggests that bFGF injection immediately after surgery accelerates apoptosis of myofibroblasts in mucoperiosteal denudation of rat and that palatal wound contraction is reduced by promotion of apoptosis of myofibroblasts with bFGF injection immediately after surgery.

From the pathological findings at 28 days, collagen fibers in the submucosa of the scar and sham groups were elevated in number and were arranged in thicker bundles when compared with the control group. Injection of bFGF just after surgery slightly relieved the changes in collagen fibers in the scar and sham groups at 28 days. Based on this result, it is unclear whether immediate injection of bFGF just after surgery directly

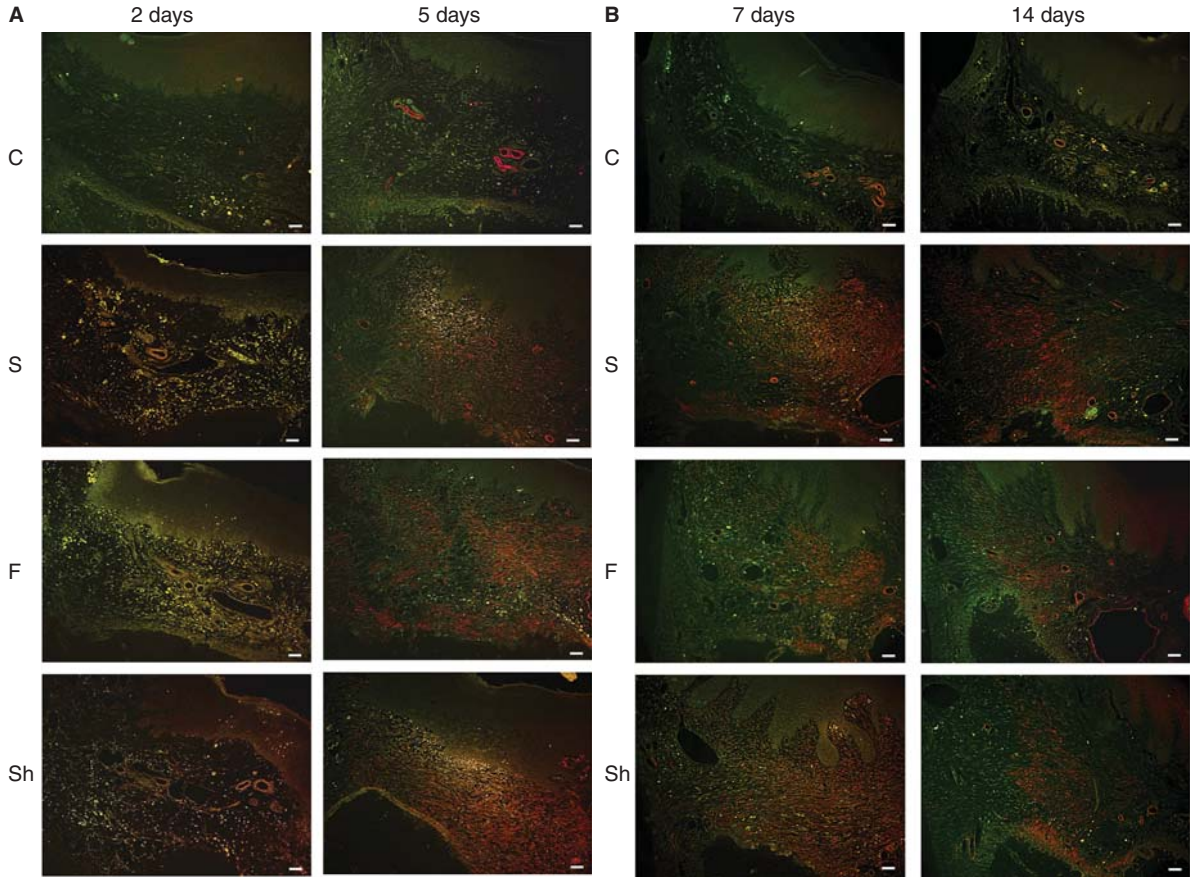

Figure 5. (A) Double immunofluorescence staining showing frontal section of rat palate at 2 and 5 days post-operatively (C, control group; S, scar group; F, bFGF group; Sh, sham group). Cells positive for both TUNEL and alpha-SMA expression were stained yellow and are considered to be apoptotic myofibroblasts. Scale bars represent 100 μm. (B) Double immunofluorescence staining showing a frontal section of the rat palate at 7 and 14 days post-operatively (C, control group; S, scar group; F, bFGF group; Sh, sham group). Cells positive for both TUNEL and alpha-SMA expression were stained yellow and are considered to be apoptotic myofibroblasts. Scale bars represent 100 μm.

recovers thick bundles of collagen fibers at the tissue remodeling phase of wound healing.

The results suggest that bFGF injection in the early inflammation phase of palatal wound healing inhibits wound contraction in the later phases of inflammation. This may be attributable to the promotion of apoptosis of myofibroblasts. If bFGF is immediately injected into the palatal wounds of cleft palate patients at palatoplasty, maxillary growth retardation might be prevented due to inhibition of excessive wound contraction. Our previous findings [14–17] also suggest that bFGF injection in the proliferation phase of palatal wound healing inhibits thick collagen accumulation. This may be attributable to recovery of the vascular number and size and endothelial cell number. If bFGF is also injected into the palatal wound in cleft palate patients at 1 week after palatoplasty, maxillary growth retardation in patients with cleft palate might also be prevented due to inhibition of thick collagen accumulation and scar formation.

In conclusion, it has been shown that bFGF injection immediately after surgery accelerates apoptosis of myofibroblasts in mucoperiosteal denudation in rats. This may reduce maxillary growth retardation due to excessive wound contraction. In order to apply these results to a clinical trial, the optimal concentration and the number and timing of bFGF injections for palatal wound healing should be examined. Biochemical

Table I. Mean and standard deviation (SD) for apoptotic area (pixels) in myofibroblasts in the four groups.

	2 days		5 days		7 days		14 days		28 days	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	2 932	1626	1 939	1341	4 377	1762	2579	1900	1712	1349
Scar	13 759	4765	18 820	7303	16 773	2569	4774	2095	2149	856
bFGF	22 434	8006	7 730	1330	7 226	2461	3257	2836	1887	827
Sham	10 224	4164	20 536	5621	20 439	3860	7973	1563	3079	668

Table II. *P*-values for Scheffé multiple comparison post-hoc testing among the four groups.

	p-value				
	2 days	5 days	7 days	14 days	28 days
C vs S	0.035*	0.000*	0.000*	0.477	0.913
C vs F	0.000*	0.320	0.471	0.968	0.993
C vs Sh	0.215	0.000*	0.000*	0.010*	0.208
S vs F	0.111	0.016*	0.000*	0.744	0.979
S vs Sh	0.761	0.952	0.263	0.179	0.521
F vs Sh	0.016*	0.005*	0.000*	0.026*	0.313

C, Control; S, Scar; F, bFGF; Sh, Sham.
*$p < 0.05$.

findings of the palatal wound tissue recovered after bFGF injection should also be examined in the future.

Acknowledgment

This work was supported by a (MEXT) KAKENHI Grant-in-Aid for Scientific Research (B) 20390526.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Dorrance GM. Lengthening the soft palate in cleft palate operations. Ann Surg 1925;82:208–11.
[2] Wardill WEM. The technique of operation for cleft palate. Br J Surg 1937;25:117–30.
[3] Graber TM. A cepharometric analysis of the developmental pattern and facial morphology in cleft palate. Angle Orthod 1949;19:91–100.
[4] Ross RB. The clinical implications of facial growth in cleft lip and palate. Cleft Palate J 1970;7:37–47.
[5] Dahl E. Transverse maxillary growth in combined cleft lip and palate: a longitudinal roentgencephalometric study by the implant method. Cleft Palate J 1979;16:34–41.
[6] Barro WB, Latham RA. Palatal periosteal response to surgical trauma. Plast Reconstr Surg 1981;67:6–16.
[7] Ross RB. Treatment variables affecting facial growth in complete unilateral cleft lip and palate. Cleft Palate J 1987;24:5–77.
[8] Mars M, Houston WJ. A preliminary study of facial growth and morphology in unoperated male unilateral cleft lip and palate subjects over 13 years of age. Cleft Palate Craniofac J 1990;27:7–10.
[9] Wijdeveld MG, Maltha JC, Grupping EM, De Jonge J, Kuijpers-Jagtman AM. A histological study of tissue response to simulated cleft palate surgery at different ages in beagle dogs. Arch Oral Biol 1991;36:837–43.
[10] Noverraz AE, Kuijpers-Jagtman AM, Mars M, van't Hof MA. Timing of hard palate closure and dental arch relationships in unilateral cleft lip and palate patients: a mixed-longitudinal study. Cleft Palate Craniofac J 1993;30:391–6.
[11] Ishikawa H, Nakamura S, Misaki K, Kudoh M, Fukuda H, Yoshida S. Scar tissue distribution on palates and its relation to maxillary dental arch form. Cleft Palate Craniofac J 1998; 35:313–19.
[12] Ishikawa H, Kitazawa S, Iwasaki H, Nakamura S. Effects of maxillary protraction combined with chin-cap therapy in unilateral cleft lip and palate patients. Cleft Palate Craniofac J 2000;37:92–7.
[13] Berkowitz S. Cleft lip and palate - diagnosis and management. 2nd ed. Berlin: Springer-Verlag; 2006. p 285–99; 395–404.
[14] Tindlund RS, Rygh P, Boe OE. Intercanine widening and sagittal effect of maxillary transverse expansion in patients with cleft lip and palate during the deciduous and mixed dentitions. Cleft Palate Craniofac J 1993;30:195–207.
[15] Kajii TS, Alam MK, Mikoya T, Oyama A, Koshikawa-Matsuno M, Sugawara-Kato Y, et al. Congenital and post-natal factors inducing malocclusions in Japanese unilateral cleft lip and palate patients - determination using logistic regression analysis. Cleft Palate Craniofac J 2013;50; in press.
[16] Cornelissen AM, Maltha JC, Von den Hoff JW, Kuijpers-Jagtman AM. Local injection of IFN-gamma reduces the number of myofibroblasts and the collagen content in palatal wounds. J Dent Res 2000;79:1782–8.
[17] Funato N, Moriyama K, Shimokawa H, Kuroda T. Basic fibroblast growth factor induces apoptosis in myofibroblastic cells isolated from rat palatal mucosa. Biochem Biophys Res Commun 1997;240:21–6.
[18] Funato N, Moriyama K, Baba Y, Kuroda T. Evidence for apoptosis induction in myofibroblasts during palatal muco-periosteal repair. J Dent Res 1999;78:1511–17.
[19] Oda Y, Kagami H, Ueda M. Accelerating effects of basic fibroblast growth factor on wound healing of rat palatal mucosa. J Oral Maxillofac Surg 2004;62:73–80.
[20] Jansen RG, van Kuppevelt TH, Daamen WF, Kuijpers-Jagtman AM, Von den Hoff JW. FGF-2-loaded collagen scaffolds attract cells and blood vessels in rat oral mucosa. J Oral Pathol Med 2009;38:630–8.
[21] Kawanabe H, Ishikawa H, Okamura K, Taniguchi K. Basic fibroblast growth factor increases the number and size of vascular pores on bone surfaces during palatal wound healing. J Hard Tissue Biology 2004;13:73–80.
[22] Kawanabe H, Ishikawa H, Kinoshita S, Taniguchi K. Short communication: effects of basic fibroblast growth factor administration on wound healing process of rat palates with scar tissue formation. Dent Jpn (Tokyo) 2005;41:75–7.
[23] Choi W, Kawanabe H, Sawa Y, Taniguchi K, Ishikawa H. Effects of bFGF on suppression of collagen type I accumulation and scar tissue formation during wound healing after mucoperiosteal denudation of rat palate. Acta Odontol Scand 2008;66:31–7.
[24] Hata Y, Kawanabe H, Hisanaga Y, Taniguchi K, Ishikawa H. Effects of basic fibroblast growth factor administration on vascular changes in wound healing of rat palates. Cleft Palate Craniofac J 2008;45:63–72.
[25] Clark RA. Wound repair–overview and general considerations. In Clark RA, editor. The molecular and cellular biology of wound repair. New York: Plenum Press; 1996. p 3–50.
[26] Gabbiani G, Ryan GB, Majne G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia 1971;27:549–50.
[27] Clark RA. Cutaneous tissue repair: basic biologic considerations. I. J Am Acad Dermatol 1985;13:701–25.
[28] Kim T, Ishikawa H, Chu S, Handa A, Iida J, Yoshida S. Constriction of the maxillary dental arch by mucoperiosteal denudation of the palate. Cleft Palate Craniofac J 2002;39: 425–31.
[29] Moulin V, Auger FA, Garrel D, Germain L. Role of wound healing myofibroblasts on re-epithelialization of human skin. Burns 2000;26:3–12.
[30] Tobita T, Inokuchi T. Immunohistochemical study on wound healing of a muscle flap transferred into the oral cavity of rats. Oral Med Pathol 2001;6:29–36.