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Regeneration of the tympanic membrane using fibroblast growth factor-2

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Review Article

Dr Z-C Lou takes responsibility for the integrity of the content of the paper

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Abstract

Objective. A systematic review was conducted to investigate the effectiveness of fibroblast growth factor-2 on the regeneration of tympanic membrane perforation.

Methods. The PubMed database was searched for relevant studies. Experimental studies, human randomised controlled trials, prospective single-arm studies and retrospective studies reporting acute and chronic tympanic membrane perforations in relation to two healing outcomes (success rate and closure time), were selected.

Results. All 11 clinical studies investigating the effect of fibroblast growth factor-2 on traumatic tympanic membrane perforations in humans reported a success rate of 89.3–100 per cent, with a closure time of around 2 weeks. Three studies of fibroblast growth factor-2 combined with Gelfoam showed that the success rate of chronic tympanic membrane perforation was 83–98.1 per cent in the fibroblast growth factor-2 group, but 10 per cent in the gelatine sponge groups.

Conclusion. Fibroblast growth factor-2 with or without biological material patching promotes regeneration in cases of acute and chronic tympanic membrane perforation, and is safe and efficient. However, the best dosage, application time and administration pathway of fibroblast growth factor-2 are still to be elucidated.

Introduction

Tympanic membrane perforation is a common entity in otology clinics. Most traumatic tympanic membrane perforations heal spontaneously in a few weeks. However, if the lesion does persist, surgical intervention is needed. Surgical techniques are invasive, require hospitalisation, can lead to complications and are associated with higher medical costs.

In recent years, exogenous growth factors (e.g. epidermal growth factor and fibroblast growth factor-2) have been adopted to repair tympanic membrane perforations. Among them, fibroblast growth factor-2 is commonly used to repair the eardrum. Fibroblast growth factor-2 was the first cellular growth factor to be evaluated for wound repair and regeneration.[1,2] Although not initially present at the wound site, the mitogenic and angiogenic properties of this cytokine[3] have suggested that it could have properties associated with the augmentation of granulation tissue formation, and the promotion of organ and connective tissue repair. Although the concept of growth factors or cytokines as mediators of this process has been espoused for many years, only in the last decade has there been sufficient biochemical characterisation of these activities to permit their application, as recombinant gene products, to the process of wound repair.

In this mini-review, we focus on the role of fibroblast growth factor-2 in the repair of tympanic membrane perforations (Figure 1). In addition, some questions concerning fibroblast growth factor-2 are discussed.

Materials and methods

A comprehensive search of the literature was conducted using the PubMed (US National Library of Medicine) database. The key words used were: 'tympanic membrane perforation', 'fibroblast growth factor-2' and 'basic fibroblast growth factor'. Only English-language literature was included, but there was no restriction on the study design or date of publication. The final search was conducted on 30 January 2017. The inclusion criteria were: (1) English language; (2) inclusion of an abstract; (3) inclusion of a healing outcome (closure rate and/or closure time); (4) description of complications; and (5) acute or chronic tympanic membrane perforation. The exclusion criteria were: (1) performance of a histological or morphological study only; and (2) a review or commentary.

The titles and abstracts were screened independently by two researchers to identify potentially relevant articles, and the full-text articles were then obtained. The bibliography of each article was also searched for further potentially relevant studies. All articles that met the inclusion criteria were reviewed for data extraction and quality assessment.

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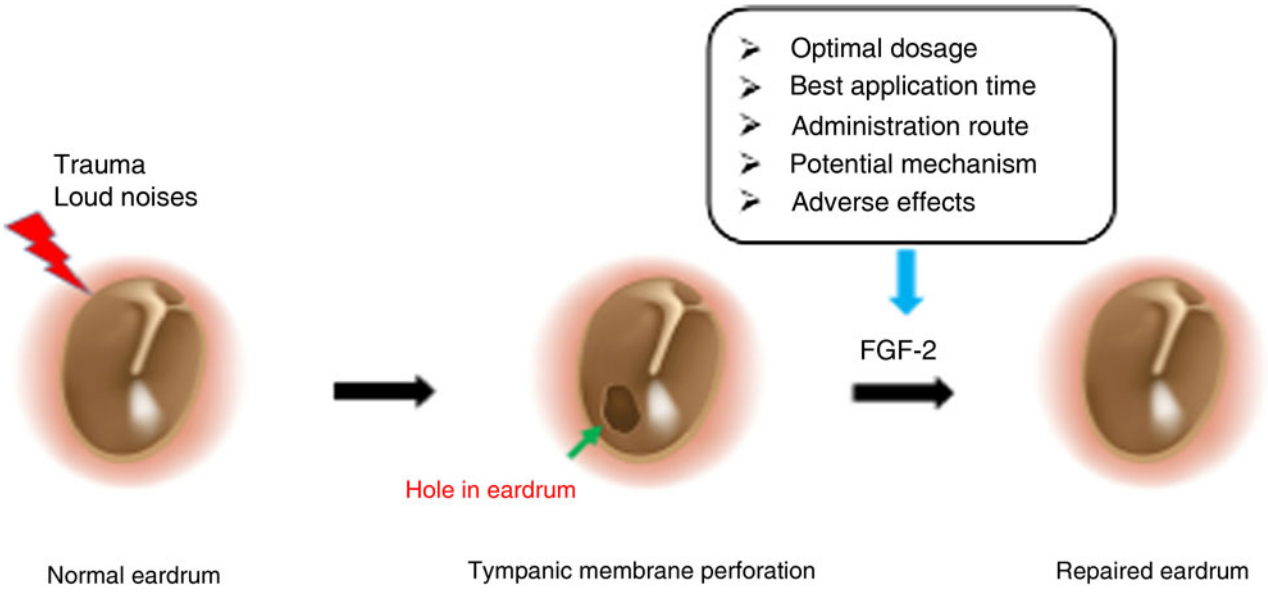

Fig. 1. Diagram of traumatic tympanic membrane perforation repaired with fibroblast growth factor-2 (FGF-2).

Data extracted from each article included: patient demographics, study design, type of surgical intervention performed, length of follow up and measured outcomes.

Results

A total of 42 articles were obtained from the initial literature search. After reviewing their citations and abstracts, 10 studies did not meet the inclusion criteria. Thirty-two studies were deemed to be potentially relevant and the full texts of these articles were reviewed. After further review of the remaining articles ($n = 32$), three were found to be reviews and were therefore removed.

A total of 29 studies were available for the final review and analysis; of these, 10 were experimental studies (Table I),[4–13] 11 were human traumatic tympanic membrane perforation studies (Table II)[14–24] and 8 were human chronic tympanic membrane perforation studies (Table III).[25–32] Of the 10 experimental studies, 7 examined the effect of fibroblast growth factor-2 on acute tympanic membrane perforations[4–10] and 3 focused on the effect of fibroblast growth factor-2 on chronic tympanic membrane perforations.[11–13] Of the 11 studies on traumatic tympanic membrane perforation in humans, 3 were randomised controlled trials (RCTs),[14,19,23] 4 were prospective controlled studies,[15,16,18,22] 1 was a single-arm and exploratory clinical trial,[20] 2 were prospective, quasi-randomised, controlled clinical studies,[17,24] and 1 was a retrospective cohort study.[21] Of the eight studies on chronic tympanic membrane perforation in humans, one was an RCT,[25] four were prospective single-arm studies,[26–29] one was a prospective controlled study[30] and two were retrospective studies.[31,32]

Of the 10 experimental studies, 5 showed that subjects in the fibroblast growth factor-2 group with or without a patch (Gelfoam or gelatine hydrogel) had a significantly shortened closure time and improved closure rate for acute tympanic membrane perforations compared with the control group (saline, phosphate-buffered saline, or glycerol with or without a patch).[4–8] Chauvin *et al.* showed that fibroblast growth factor-2 reduced the closure rate of acute tympanic membrane perforations compared with 1 per cent hyaluronic acid and epidermal growth factor, but improved the closure rate compared with subjects who spontaneously healed.[8] However, collagen membrane integrated with collagen-binding basic fibroblast growth factor promoted the healing rate at an early stage (within 7 days) and reduced the healing time in acute tympanic membrane perforations.[10] In addition, two studies on chronic tympanic membrane perforations showed that fibroblast growth factor-2 with or without Gelfoam facilitated the closure rate of chronic tympanic membrane perforations compared with a saline solution.[11,12] However, one comparative study of chronic tympanic membrane perforations found that the closure rate in the fibroblast growth factor-2 group was lower than that of the heparin-binding epidermal growth factor-like growth factor group, but was similar to the polymer-only group.[13]

All 11 clinical studies investigating the effect of fibroblast growth factor-2 on traumatic tympanic membrane perforations in humans were reported by Lou and colleagues (Table II), between 2012 and 2016, at the same medical institution.[14–24] The authors found that the topical application of fibroblast growth factor-2 with or without Gelfoam significantly shortened the closure time and improved the closure rate of large traumatic tympanic membrane perforations in humans compared with the control group, regardless of the cause of trauma (penetrating perforations or blast injury) and the duration of trauma (acute or subacute). The closure rate of large perforations ranged from 89.3 to 100 per cent, and the closure time was around two weeks in most large perforation cases.[14–23] However, surprisingly, they also found, in a prospective, quasi-randomised, controlled clinical study, that the closure rate and closure time in the fibroblast growth factor-2 group were similar to those of the ofloxacin eardrops treatment group.[24]

Patching with biological materials (gelatine sponge and fibrin glue, an atelocollagen/silicone bilayer membrane patch) was applied in eight clinical studies investigating the effect of fibroblast growth factor-2 on chronic tympanic membrane perforation in humans.[25–32] However, no studies reported the effect of applying fibroblast growth factor-2 alone. Three studies examining fibroblast growth factor-2 combined with Gelfoam showed that the success rate of treating chronic tympanic membrane perforations was 83–98.1 per

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TABLE I. SUMMARY OF FIBROBLAST GROWTH FACTOR-2 EFFECTS ON EXPERIMENTAL TYMPANIC MEMBRANE PERFORATION

Study (year)	Study subject	Treatment strategy	Healing outcome
Mondain *et al.*[4] (1991)	Sprague Dawley rats	3 doses of bFGF (2000 ng, 400 ng & 200 ng) were applied; control ear received saline alone	At higher dose of 2000 ng of FGF-2, all animals obtained complete healing in average of 3.16 days. At 400 ng of FGF-2, mean closure time was 6.1 days, while it was 8.86 days in placebo-treated ears. At lower dose of 200 ng, mean closure time was 6.33 days in FGF-2 group & 6.44 days in placebo ears. Myringitis was observed with 2000 ng & 200 ng of FGF-2, while no myringitis was present with 400 ng of FGF-2
Fina *et al.*[5] (1991)	Guinea pigs	1 µg FGF-2 group & PBS-only group	Closure rate was 10% in PBS ears & over 55% in FGF-2 group at 3 days for 1 mm TMPs; no PBS-treated ears showed closure & repair at 5 days, while 87.5% closed with FGF-2 treatment for 2 mm TMPs
Fina *et al.*[6] (1993)	Guinea pigs	FGF-2 or placebo was applied either directly or to Gelfoam pledget	In group 1, closure rate was 60% in FGF-2 group & 30% in placebo group for 1 mm TMPs, & closure rate was 100% in FGF-2 group & 33% in placebo group for 2 mm TMPs. In group 2, all 2 mm flapped TMPs achieved complete healing in FGF-2 or placebo groups
Vrabec *et al.*[7] (1994)	Rats	TMPs were treated using FGF-2; control ears received glycerol	Mean closure time was 9.74 ± 2.31 days in FGF-2 treated ears, & 13.74 ± 4.93 days in control ears
Chauvin *et al.*[8] (1999)	Guinea pigs	1 mg of 1% hyaluronic acid, 0.4 µg FGF-2 or 1.0 µg EGF; control ears received 0.1 ml Vasocidin	Complete closure was obtained in: 100% of ears treated with hyaluronic acid & EGF, 85.7% of FGF-2 ears at day 21, & 63.6% of controls at day 32
Hakuba *et al.*[9] (2014)	Guinea pigs	bFGF-gelatine hydrogel group, saline-gelatine hydrogel group & control group	Closure rates in bFGF hydrogel, saline-hydrogel & control groups were 100%, 62.5% & 0%, respectively, after 30 days
Zhang *et al.*[10] (2017)	Sprague Dawley rats	Collagen membrane integrated with collagen-binding bFGF	Collagen-binding bFGF-integrated collagen membrane promoted healing rate at an early stage (<7 days) & reduced TMP healing time
Kato & Jackler[11] (1996)	Chinchillas with chronic TMP	FGF-2 + Gelfoam group, & Gelfoam & buffer solution only group	Complete closure of TMP was observed in 81% of FGF-2-treated ears by 4 weeks, but in only 41% of controls by 6.5 weeks
Ozkaptan *et al.*[12] (1997)	Guinea pigs with chronic TMP	400 ng FGF-2 group & saline only solution group	13 of 15 perforations treated with FGF-2 had closed at 20 days
Santa Maria *et al.*[13] (2015)	Mice with chronic TMP	HB-EGF (5 µg/ml) group, FGF-2 (100 µg/ml) group, EGF (250 µg/ml) group & control (polymer-only) group	Healing rates at 4 weeks were: HB-EGF, 83.3% (15 out of 18); FGF-2, 31.6% (6 out of 19); EGF, 15.8% (3 out of 19); & controls, 27.8% (5 out of 18)

bFGF = basic fibroblast growth factor; FGF-2 = fibroblast growth factor-2; PBS = phosphate-buffered saline; TMP = tympanic membrane perforation; HB-EGF = heparin-binding epidermal growth factor-like growth factor; EGF = epidermal growth factor

TABLE II. SUMMARY OF FIBROBLAST GROWTH FACTOR-2 EFFECTS ON HUMAN TRAUMATIC TYMPANIC MEMBRANE PERFORATION

Study (year)	Study object	Study design	Treatment strategy	Healing outcome
Lou[14] (2012)	94 large TMPs	Prospective, randomised, controlled trial	FGF-2, FGF-2 via Gelfoam & conservative treatment	Closure rates in FGF-2, FGF + Gelfoam, & observation groups were 100%, 97% & 55%, respectively
Lou *et al.*[15] (2012)	147 TMPs	Prospective clinical study	Gelfoam + FGF-2	Closure rates were 98.6%, 97.6%, 96.3% & 100%, respectively, at following times: <3 days, 4–7 days, 8–14 days & 2–4 weeks after injury
Zhang & Lou[16] (2012)	104 patients with small penetrating perforations	Prospective non-blinded controlled study	Spontaneous healing ($n = 51$) & FGF-2 treatment ($n = 53$)	Closure rates at 3 months in spontaneous healing & FGF-2 treatment groups were 77% & 100%. Closure time was 43.1 ± 2.5 days for control patients & 12.6 ± 1.2 days for FGF-2 patients
Lou & Wang[17] (2013)	58 large TMPs with inverted edges	Prospective, sequential allocation, three-armed, controlled clinical study	No intervention ($n = 18$), edge approximation alone ($n = 20$) & FGF-2 ($n = 20$)	Closure rate was 100% in FGF-2, 60% in edge approximation & 56% in no intervention groups ($p < 0.05$). Average closure time was 12.4 ± 3.6 days in FGF-2, 46.3 ± 8.7 days in edge approximation & 48.2 ± 5.3 days in no intervention control group
Lou *et al.*[18] (2014)	126 TMPs	Prospective clinical study	Higher & lower doses of FGF-2	Closure rate was 92% in low dosage & 100% in high dosage for large perforations; closure rate was 100% in low dosage & 93% in high dosage for medium perforations
Lou & Wang[19] (2015)	93 TMPs	Prospective randomised clinical study	Observation & FGF-2 treated groups	bFGF-treated group exhibited significantly higher total closure rate (97.8 *vs* 82.5%) & shorter closure time (12.5 ± 3.4 *vs* 34.0 ± 5.9 days; $p < 0.05$) compared with spontaneous healing group
Lou *et al.*[20] (2015)	17 total or near-total TMPs	Single-arm & exploratory clinical trial	FGF-2 treated	Complete TMP closure was achieved in 16 out of 17 patients, with mean closure time of 28.4 ± 10.9 days
Lou *et al.*[21] (2015)	99 TMPs	Retrospective cohort study	FGF-2	Total closure rate was 92.9% (92 out of 99) at 6 months; mean closure time was 10.59 ± 6.81 days
Lou *et al.*[22] (2016)	29 subacute TMPs	Prospective clinical study	Observation group & FGF-2 group	Closure rate was 91.7% in FGF-2 group, with closure time of 18.1 ± 11.4 days, & 52.9% in spontaneous healing group
Zhengcai-Lou *et al.*[23] (2016)	86 large TMPs	A randomised, prospective clinical study	EGF, FGF-2 group & observation group	Closure rates in EGF, bFGF & observation groups were 86.2%, 89.3% & 72.4%, respectively. Closure times in EGF, bFGF & observation groups were 12.5 ± 7.1 days, 13.7 ± 7.6 days & 28.1 ± 12.2 days, respectively
Lou *et al.*[24] (2016)	185 TMPs	Prospective, quasi-randomised, controlled clinical study	FGF-2, 0.3% ofloxacin eardrops & Gelfoam patching	Closure rates in observation, bFGF, Gelfoam & ofloxacin groups were 82.2%, 93.2%, 85.7% & 92.3%, respectively. Mean closure times were 25.6 ± 13.32, 12.3 ± 8.15, 14.3 ± 5.44 & 13.97 ± 8.82 days for observation, bFGF, Gelfoam patch & ofloxacin groups, respectively

TMP = tympanic membrane perforation; FGF-2 = fibroblast growth factor-2; bFGF = basic fibroblast growth factor; EGF = epidermal growth factor

TABLE III. SUMMARY OF FIBROBLAST GROWTH FACTOR-2 EFFECTS ON TYMPANIC MEMBRANE PERFORATION WITH CHRONIC SUPPURATIVE OTITIS MEDIA

Study (year)	Study object	Study design	Treatment strategy	Healing outcome
Kanemaru *et al.*[25] (2011)	63 chronic TMPs	Randomised controlled trial	Gelatine sponge & fibrin glue with or without FGF-2	TMP closure was achieved in >98.1% (52 out of 53) in FGF-2 group & 10% (1 out of 10) in control group
Acharya *et al.*[26] (2015)	12 children	Prospective single-arm & exploratory clinical trial	FGF-2 + Gelfoam	Overall rate was 83% (10 out of 12) after 12 months
Omae *et al.*[27] (2017)	11 chronic TMPs	Prospective, multicentre, open-label, single-arm & exploratory clinical trial	FGF-2 + Gelfoam	TMP closure was achieved in 88.9% patients after 12 weeks
Hakuba *et al.*[28] (2003)	14 chronic TMPs	Prospective cohort study	FGF-2 combined with atelocollagen/silicone bilayer membrane patch	TMP closure was achieved in all 9 cases in bFGF group within 3.7 weeks, but in only 2 of 5 cases in control group
Hakuba *et al.*[29] (2010)	87 chronic TMPs	Single-arm & exploratory clinical trial		TMP closure was achieved in 92.0%, & hearing improved by 13.6 dB
Hakuba *et al.*[30] (2013)	116 chronic TMPs	Retrospective cohort study		TMP closure was achieved in 62% patients after 1 year, epithelial pearl formation was observed in 5% patients, with an average time of 7.3 months
Hakuba *et al.*[31] (2015)	10 children	Single-arm & exploratory clinical trial		TMP closure was achieved in 81.8% ears 1 year post-operatively
Hakuba *et al.*[32] (2015)	153 chronic TMPs	Retrospective cohort study		TMP closure was achieved in 66.0% ears after 1 year of follow up

TMP = tympanic membrane perforation; FGF-2 = fibroblast growth factor-2; bFGF = basic fibroblast growth factor

cent in the fibroblast growth factor-2 group, but 10 per cent in the gelatine sponge and fibrin glue group.[25–27] Another five studies evaluating the effect of fibroblast growth factor-2 on chronic tympanic membrane perforation in humans were reported by Hakuba and colleagues, during the period 2003–2015, at the same medical institution.[28–32] They reported a success rate of 62–100 per cent in the fibroblast growth factor-2 combined with an atelocollagen/silicone bilayer membrane patch group, compared to a success rate of only 40 per cent in the control group.

Discussion

Experimental tympanic membrane perforation

First, we consider the effect of fibroblast growth factor-2 on the regeneration of an experimental tympanic membrane perforation. Historically, the growth factor activity of fibroblast growth factors was the first to be identified.[1] Within the fibroblast growth factor family, fibroblast growth factor-2 has been the most investigated.[33,34] Fibroblast growth factor-2 is an 18 kD protein. At physiological pH and temperature, the *in vitro* half-life time of fibroblast growth factor-2 activity is approximately 12 hours. The normal function of basic fibroblast growth factor is unknown to date. When the vascular wall is damaged, fibroblast growth factor-2 can be released through several mechanisms. Fibroblast growth factor-2 is a potent stimulator of endothelial cells and neovessel formation in *in vitro* and *in vivo* models of angiogenesis.[3,33] Similarly, fibroblast growth factor-2 stimulates the proliferation of endothelial cells, fibroblasts, smooth muscle cells and chondrocytes, but also induces the production of proteases, including collagenases, *in vitro*.[34–36]

The mechanism underlying fibroblast growth factor-2 facilitation of eardrum healing is unclear. A previous study showed that fibroblast growth factor-2 is produced after tympanic membrane perforation and facilitates perforation closure through various mechanisms. The progress of tympanic membrane healing is also accelerated, but the basic healing process is unchanged after fibroblast growth factor-2 treatment.[37] Fibroblast growth factor-2 mainly activates mitosis in fibroblast and endothelial cells, induces neovascularisation and better arrangement of collagenous fibres, and facilitates the proliferation of the fibrous layer, thereby providing the scaffold of epithelial migration and preventing eardrum atrophy.[5,8,37,38] However, although the average area of blood vessels in the eardrum was greater after fibroblast growth factor-2 application, the number of vessels remained unchanged.[5,37]

Some scholars reported that the fibroblast growth factor-2 group showed a significantly improved closure rate and shorted the closure time in subjects with acute tympanic membrane perforations compared with the phosphate-buffered saline treated group or glycerol group.[4,5,7–9] In addition, fibroblast growth factor-2 treatment also promotes the healing of glucocorticoid-induced tympanic membrane perforations.[39,40] Two studies demonstrated that the topical application of fibroblast growth factor-2 facilitated the closure of chronic tympanic membrane perforations.[11,12] Kato and Jackler treated chronic tympanic membrane perforations by applying 25 µl fibroblast growth factor-2 to a Gelfoam pledget every other day for 6 days in an experimental group, and administered 25 µl phosphate-buffered saline alone to a control group.[11] The closure rate was 81 per cent in the fibroblast growth factor-2 group and 41 per cent in the control group. However, in an experimental study of chronic tympanic membrane perforations similar to human chronic tympanic membrane perforations with chronic suppurative otitis media, Santa Maria *et al.* found that the success rate in the fibroblast growth factor-2 group was only 31.6 per cent at four weeks, which was similar to that of the control group (27.8 per cent).[13]

Human tympanic membrane perforation

Second, we consider the effect of fibroblast growth factor-2 on the regeneration of tympanic membrane perforation in humans. Fibroblast growth factor-2 has been used widely to

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repair large traumatic and subacute tympanic membrane perforations in humans, in studies conducted by Lou *et al.*[14–24] They reported a closure rate of 89.3–100 per cent in the fibroblast growth factor-2 treated group, compared to closure rates of 60 per cent in the edge approximation group and 55–72.4 per cent in the spontaneous healing group, in prospective clinical studies.[14–19,21,23,24] Topical application of fibroblast growth factor-2 also improved the closure rate of blast-induced total or near-total tympanic membrane perforations and subacute traumatic tympanic membrane perforations.[20,22]

A higher success rate has also been obtained in chronic tympanic membrane perforations in humans after fibroblast growth factor-2 administration. The efficacy of fibroblast growth factor-2 in the repair of chronic tympanic membrane perforations was reported by Hakuba *et al.*[28–32] They reported a regeneration success rate of 62–100 per cent in patients treated using fibroblast growth factor-2 combined with an atelocollagen/silicone bilayer membrane patch, whereas the success rate was only 40 per cent (two out of five) in the saline control group.[28] Kanemaru *et al.*, in a randomised controlled trial (RCT), reported a higher success rate of 98.1 per cent (52 out of 53) in the gelatine sponge and fibrin glue with fibroblast growth factor-2 group, but the success rate was only 10 per cent (1 out of 10) in the gelatine sponge and fibrin glue only group.[25] Other prospective single-arm and exploratory clinical trials recently reported success rates of 83 per cent and 88.9 per cent in the regeneration of chronic tympanic membrane perforations in humans using gelatine sponge and fibrin glue with fibroblast growth factor-2.[26,27]

Confounding factors were obvious in these clinical studies on chronic tympanic membrane perforation. In addition, some studies reported higher success rates for regeneration in cases of chronic tympanic membrane perforation using Gelfoam or an atelocollagen/silicone bilayer membrane patch alone.[41,42] Thus, the specific effects of fibroblast growth factor-2 on chronic tympanic membrane perforations have not previously been well-demonstrated. The application of fibroblast growth factor-2 only to treat clinical chronic tympanic membrane perforations has not been performed, to date.

Fibroblast growth factor-2 administration and release

Normally, growth factors enhance wound healing. However, one important consideration is that many growth factors require prolonged exposure to wound cells to stimulate a response.[43,44] The middle ear is connected to the Eustachian tube; eardrops or gel can partly flow into the nasopharynx, which could rapidly clear fibroblast growth factor-2. Several studies have suggested that fibroblast growth factor-2 should be administered once daily until the perforation is completely closed. However, daily topical application would increase the inconvenience to patients, who might even discontinue the treatment, particularly those with chronic tympanic membrane perforation in need of a long healing time.

Studies have suggested that local sustained release from a suitable matrix is the most effective method of fibroblast growth factor-2 administration *in vivo*.[43,45] In several clinical studies of chronic tympanic membrane perforations, biological materials combined with fibroblast growth factor-2 were found to be effective for sustained-release and bioactive maintenance of fibroblast growth factor-2.[25–32,46] The vehicles not only provide scaffolds for epithelial migration, but also store and offer sustained release of fibroblast growth factor-2. However, clinical studies on traumatic tympanic membrane perforations treated with fibroblast growth factor-2 found that the healing outcome was not different between fibroblast growth factor-2 with or without a vehicle.[14,16,17,19–21] Thus, an RCT investigating single and cyclic application of fibroblast growth factor-2 with or without biomaterial is needed in the future to determine the effectiveness of fibroblast growth factor-2 alone on chronic perforations.

Application time, dosage and duration

The best dosage and application time for fibroblast growth factor-2 for the regeneration of tympanic membrane perforations are unclear. The application dosage and time given in some previous studies are shown in Table IV.[4–9,11–32] The dosage in these studies was not uniform. Briefly, although fibroblast growth factor-2 promoted regeneration in cases of traumatic and chronic tympanic membrane perforation, it remains unclear whether a single or repeated dosage is best.

Lou *et al.* found that the closure rate and average closure time were not significantly different between high- and low-dosage fibroblast growth factor-2 treatments.[18,21] A high dosage of fibroblast growth factor-2 induced infection of the middle ear and discomfort, thereby prolonging the closure time. The authors suggested that the best dosage condition was the maintenance of a moist perforation edge. Kälicke *et al.* also found that the local application of basic fibroblast growth factor increases the risk of local infection after trauma.[47] A dose-dependent, sometimes highly significant increase in the infection rate occurs after soft tissue trauma.

Thus, the best dosage and duration of application needs to be studied further, as fibroblast growth factor-2 solution rapidly flows into the Eustachian tube when applied as ear drops.

The best application time for fibroblast growth factor-2 has yet to be completely elucidated, particularly for acute tympanic membrane perforations. Vrabec *et al.* reported that the timing of application may influence the repair process, with a beneficial effect of fibroblast growth factor-2 when applied after the inflammatory stage of wound healing has passed.[7] Ishibashi *et al.* suggested that fibroblast growth factor-2 may mediate the connective tissue reaction in the middle layer in the proliferation stage, and indicated that the best commencement time of application for traumatic tympanic membrane perforations should be during the proliferation stage of the healing process.[48]

In clinical studies, Lou *et al.* reported that the best time to apply it was 3 days after perforation.[19,21] Fibroblast growth factor-2 had a normal biological effect only when the epithelium of the perforation edge showed signs of proliferation. The acute inflammatory response of the perforation edge occurred for 1–2 days after the perforation, and proliferation and migration of the epithelium in the germinal centre began 3–4 days after perforation, at which point the eardrum healing process entered the proliferation stage. During the proliferation stage, inflammatory cells are reduced, and fibroblasts are activated and gradually increase.[38,49]

Exogenous application of fibroblast growth factor-2 can have the best biological effect. Nevertheless, it is difficult to differentiate among the different stages of the healing process of tympanic membrane perforations in humans.

Adverse effects on regeneration

The safety of fibroblast growth factor-2 regarding the tympanic membrane has been widely investigated. Otorrhoea is a

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TABLE IV. SUMMARY OF EFFECTS FOR FIBROBLAST GROWTH FACTOR-2 DOSAGE AND APPLICATION TIME ON TYMPANIC MEMBRANE PERFORATION

Study (year)	Study subject	FGF-2 dosage or concentration	1st application time of FGF-2	Dosage frequency	Healing outcome
Mondain *et al.*[4] (1991)	Sprague Dawley rats	2000 ng, 400 ng & 200 ng	Day 0	Daily, until day of healing	Average closure times were 3.16 days, 6.1 days & 6.33 days, respectively
Fina *et al.*[5] (1991)	Guinea pigs	1 μg	Day 0	Daily	Closure rate was >55% for 1 mm TMPs at 3 days, & 87.5% for 2 mm TMPs at 5 days
Fina *et al.*[6] (1993)	Guinea pigs	1 μg	Day 0	Daily	In group 1, closure rate was 60% at 5 days for 1 mm TMP, & 100% at 14 days for 2 mm TMP in FGF-2 group
Vrabec *et al.*[7] (1994)	Rats	5 μg	Day 2	Day 5, day 7	Closure time was 9.74 ± 2.31 days in FGF-2 ears & 13.74 ± 4.93 days in control ears
Chauvin *et al.*[8] (1999)	>90% large perforation in guinea pigs	0.4 μg	Day 0	Once a week	Complete closure was obtained in 85.7% FGF-2 group by day 21
Hakuba *et al.*[9] (2014)	Guinea pig	FGF-2 concentration was unclear	Day 0	Single dosage	Closure rate in bFGF hydrogel group was 100% at post-operative day 30
Kato & Jackler[11] (1996)	Chinchillas with chronic TMP	15 μg	16 weeks after myringotomy	Every other day for 6 days; total of 3 doses to each TM	Complete closure of TMP was observed in 81% of FGF-2-treated ears at 4 weeks
Ozkaptan *et al.*[12] (1997)	Guinea pigs chronic TMP	400 ng	Day 3	Days 5 & 7	13 of 15 perforations had closed at 20 days in FGF-2 group
Santa Maria *et al.*[13] (2015)	Mice with chronic TMP	100 μg/ml	After creation of chronic perforations	Single dosage	Healing rate was 31.6 (6 out of 19) in FGF-2 group
Lou *et al.*[14–17,19–24] (2012–2016)	Human traumatic TMP	0.2–0.25 ml (21 000 IU/5 ml) of FGF-2	1st visit	Daily until day of healing	Closure rate was 89.3–100% at 6 months
Lou *et al.*[18] (2014)	Human traumatic TMP	0.1–0.15 ml of FGF-2 & 0.25–0.3 ml of FGF-2	1st visit	Daily until day of healing	Closure rate was 92% in low dosage & 100% in high dosage groups at 6 months
Kanemaru *et al.*[25] (2011)	Human chronic TMP	Gelatine sponge immersed in 5–30 μg of FGF-2 100 μg/ml	1st procedure	Treatment was repeated up to 4 times for 3 weeks after 1st treatment	Complete closure rate was 98.1% (52 out of 53)
Acharya *et al.*[26] (2015)	Paediatric patients with chronic TMP	21 000 IU/5 ml of FGF-2 solution	1st procedure	Single treatment	Successful closure was achieved in 7 of 12 children (58%) on 1st attempt
Omae *et al.*[27] (2017)	Human chronic TMP	Gelatine sponge immersed in 5–30 μg of FGF-2 100 μg/ml	1st procedure	Single treatment	Closure rate was achieved in 88.9% at 12 weeks
Hakuba *et al.*[28–32] (2003–2015)	Human chronic TMP	FGF-2 combined with atelocollagen/ silicone bilayer membrane; FGF-2 concentration was unclear	1st procedure	Single treatment	Success rate was 62–100% at 6 months

FGF-2 = fibroblast growth factor-2; TMP = tympanic membrane perforation; bFGF = basic fibroblast growth factor; TM = tympanic membrane

common complication that may result from excessive application of a single dosage. However, interestingly, otorrhoea only prolonged the closure time and did not affect the closure rate.[14,21,22] Previous studies have shown that fibroblast growth factor-2 may overcome the inhibition of wound contraction associated with bacterial infection.[50,51]

Chauvin *et al.* reported hypertrophy of the external auditory canal after treatment with hyaluronic acid and fibroblast growth factor-2, but not with epidermal growth factor.[8] The mild complication may have been due to injury of the external auditory canal during the process of model work.

Mondain *et al.* reported myringitis; however, other studies have not reported similar findings.[4] Lou *et al.* found myringitis in the fibroblast growth factor-2 plus Gelfoam group, but not in the fibroblast growth factor-2 only group.[14] We speculate that the incidence of myringitis is not characteristic of fibroblast growth factor-2 treatment and is not associated with the dosage of fibroblast growth factor-2.

It is important to know if fibroblast growth factor-2 is associated with ototoxicity of the middle and inner ear. Most studies have concluded that fibroblast growth factor-2 is not likely to be a candidate causative agent of ototoxicity.[4–32] The topical application of fibroblast growth factor-2 preparation did not cause a significant reduction in the endocochlear direct current potential, and did not lead to damage of the stapes or signs of perilymph leakage when applied to the middle ear.[52] In contrast, several interesting reports have described the protective effect of fibroblast growth factor-2 on sensory hair cells, rather than ototoxicity.[53–55]

In addition, it is not completely understood whether cholesteatoma of the middle ear can develop after fibroblast growth factor-2 treatment. A few scholars have speculated that fibroblast growth factor-2 could induce the proliferation of squamous epithelial cells and ingrowth into the mucous layer, leading to the development of middle-ear cholesteatoma. However, most have suggested that fibroblast growth factor-2 strongly stimulates the regeneration of the intermediate layer composed of fibrous tissues produced by fibroblasts, thus limiting the potential for cholesteatoma formation.[56] Lou *et al.* did not find cholesteatoma of the middle ear in a series of clinical studies.[14–24]

Some scholars have reported that large doses of fibroblast growth factor-2 in cases of acute and chronic tympanic membrane perforation may result in re-perforation of the eardrum. The long-term application of a large dose of fibroblast growth factor-2 can inhibit collagen synthesis and facilitate the catabolism of collagen.[57] The inhibitory effects of fibroblast growth factor-2 on collagen deposition imply that there would be little advantage to the regeneration of tympanic membrane perforations and that it might even be detrimental, resulting in re-perforation and atrophy of the eardrum. The long-term application of other growth factors may also result in eardrum re-perforation.

Prospects and outlook for clinical application

Fibroblast growth factor-2 has been used widely to treat cases of acute and chronic tympanic membrane perforation. Traumatic tympanic membrane perforations tend to spontaneously heal and some agent solutions of non-growth factors also facilitate eardrum healing;[24] the regenerative effects of fibroblast growth factor-2 on traumatic tympanic membrane perforations may be minimal.

The important value of fibroblast growth factor-2 may be the regeneration in cases of chronic tympanic membrane perforation; however, the success rate of a single application of fibroblast growth factor-2 on chronic tympanic membrane perforations was very low. In addition, topical application of biomaterial alone could achieve a higher success rate.[41,42] Thus, the evidence for fibroblast growth factor-2 repairing chronic tympanic membrane perforations is limited.

Although the results of some previous studies have suggested that fibroblast growth factor-2 does have a positive effect on the tympanic membrane, these studies did not address the most important clinical problems: the minimal efficacious dose of fibroblast growth factor-2 for treating traumatic tympanic membrane perforations, the time of application, the total duration of application, and whether or not topical application alone without excision of the perforation edge can repair chronic tympanic membrane perforation. Randomised controlled trials of fibroblast growth factor-2 with and without biomaterial are scarce and are thus warranted.

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