

4-1999

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Repository Citation

Toung, James S.; Ogle, Roy C.; Morgan, Raymond F.; and Lindsey, William H., "Insulinlike Growth Factor 1- and 2-Augmented Collagen Gel Repair of Facial Osseous Defects" (1999). *Medical Diagnostics & Translational Sciences Faculty Publications*. 2. https://digitalcommons.odu.edu/medicaldiagnostics_fac_pubs/2

Original Publication Citation

Toung, J.S., Ogle, R.C., Morgan, R.F., & Lindsey, W.H. (1999). Insulinlike growth factor 1- and 2-augmented collagen gel repair of facial osseous defects. *Archives of Otolaryngology--Head & Neck Surgery*, 125(4), 451-455. doi: 10.1001/archotol.125.4.451

Insulinlike Growth Factor 1- and 2-Augmented Collagen Gel Repair of Facial Osseous Defects

James S. Toung, BA; Roy C. Ogle, PhD; Raymond F. Morgan, DDS, MD; William H. Lindsey, MD

Background: Defects of the facial bone structure are common problems for the facial plastic surgeon. Native type 1 collagen gels (T1CGs) have been shown to mediate repair of facial critical-size defects in rat models.

Objective: To evaluate the efficacy of T1CG augmented with insulinlike growth factor (IGF) 1, IGF-2, and a combination of IGF-1 and IGF-2 on the repair of facial critical-size defects in a rodent model.

Methods: Twenty-four retired male breeder Sprague-Dawley rats were divided into 4 groups of 6 animals. Facial critical-size defects were created by removing the nasal bones with a bone-cutting drill. Defects were treated with 300 μ g of type 1 collagen gel (T1CG), T1CG augmented with 3 μ g of IGF-1, T1CG augmented with 3 μ g of IGF-2, or T1CG augmented with a combination of 3 μ g of IGF-1 and 3 μ g of IGF-2. After 30 days the animals were examined at necropsy with precise planimetry, histological analysis of new bone growth, and radiodensi-

tometric analysis of bone thickness.

Results: Radiodensitometric measurements showed that IGF-2 augmentation resulted in greatest osseous healing, with measurements being statistically significant over those of all other groups ($P \leq .03$). Combination IGF-1 and IGF-2 had osseous healing that was intermediate between IGF-1 augmentation and IGF-2 augmentation alone, with measurements being statistically significant over those of unaugmented gels ($P < .001$) and IGF-1 augmentation ($P \leq .03$). Augmentation with IGF-1 resulted in healing that was significant over that of unaugmented gels ($P \leq .04$).

Conclusion: Collagen gels augmented with IGF significantly enhance the osteoconductive repair of nasal critical-size defects in a rodent model, with IGF-2 showing highest efficacy.

Arch Otolaryngol Head Neck Surg. 1999;125:451-455

OSSEOUS DEFECTS of the nasal and other facial bones can result from many processes, including congenital malformations, trauma, neoplasms, and infections. Current techniques for the repair of such defects have major limitations or risks. Autogenous bone grafts using the iliac crest or calvaria as a donor site are widely used in reconstructive surgery; however, the amount of graft material that can be obtained is limited and such techniques have risks of donor site morbidity.¹ Cadaveric or allogenic bone is also available for grafts, but there may be the potential for transferring viral pathogens.² Demineralized bone does promote bone repair but has unpredictable resorption rates, which may be influenced by local vasculature, inflammation, and poor HLA match between host and donor.³ Alloplastic materials such as Silastic and silicone can be displaced and can foster infections.

Critical-size defects (CSDs) are osseous defects that show less than 10% healing in 6 months or within the life of the animal.⁴ With a CSD, bone regeneration would only take place in the presence of materials that are osteogenic. A CSD therefore provides a simple model for evaluating the osteogenic capabilities of materials and methods used in bone repair, without the intervening variables found in models that prevent osseous union by inhibiting the healing process. The rat nasal defect fulfills criteria to be used as a CSD.⁵ Type 1 collagen gels (T1CGs) have previously been shown to mediate the total surface repair of osseous nasal CSDs in Sprague-Dawley rats.⁶

Insulinlike growth factors (IGFs) have been shown to promote proliferation and protein synthesis in osteoblasts and are believed to be the route by which growth hormone mediates the growth of bone. Thaller et al⁷ demonstrated that supplying IGF-1 systemically through an

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MATERIALS AND METHODS

Twenty-four 6-month-old, retired male breeder Sprague-Dawley rats (450-500 g) were randomly assigned to 4 groups of 6 rats to receive collagen (group 1), collagen plus IGF-1 (group 2), collagen plus IGF-2 (group 3), and collagen plus IGF-1 plus IGF-2 (group 4).

All underwent an operative procedure to produce a CSD as described previously.⁵ Animals were anesthetized with pentobarbital sodium, 17 mg/kg, and 10 mg of ketamine hydrochloride. The hair over the nose and frontal skull was shaved and treated with a depilatory agent. The head was placed in a cephalostat and the skin was incised from the nasal tip to the supraorbital glabella. The periosteum was incised and separated from the underlying bone for lateral retraction. With the use of a bone-cutting burr with a handheld drill, the nasal bones were removed and a rectangular defect measuring 5 × 15 mm was created. The floor of this defect was the deep side of the superior nasal mucosal membranes. A diamond burr was used to shape the defect to the precise measurements. The nasal cavity was not violated. Copious saline irrigation was used to remove bone dust and debris. After the defect was created and cleaned of debris, a collagen gel (with or without IGF augmentation) was placed into the defect. The periosteum was closed with a 6-0 nylon suture. The skin was closed with 4-0 polypropylene. After recovery from anesthesia, the animals were returned to the vivarium for routine housing.

Thirty days after surgery, the rats were killed by intracardiac lidocaine injection. The entire nasal dorsum of each rat was removed by using a cutting burr and was cleaned. The defects then underwent radiography (Faxitron

Series model 43085N; Hewlett-Packard, Palo Alto, Calif) with an exposure time of 30 seconds at 30 kV (X-OMAT-AR film; Eastman Kodak Co, Rochester, NY). The developed x-ray films were then scanned into a computer by means of a densitometer (Molecular Dynamics Personal Densitometer; Molecular Dynamics, Sunnyvale, Calif), and the radiodensity of the defects was quantified with ImageQuant NT version 4.3 software (Molecular Dynamics). The defects were then treated with decalcifying solution (J. T. Baxter Inc, Deerfield, Ill) and embedded into paraffin for sectioning and hematoxylin-eosin staining to histologically evaluate new bone growth.

Rat tail collagen was prepared as previously described.^{6,10} Rat tail collagen (2.8 mg/mL) in ice-cold 0.5-mol/L acetic acid was dialyzed against unbuffered Dulbecco modified Eagle medium and sterilized by addition of 0.1% chloroform during dialysis. To form gels, type 1 collagen (2.8 mg/mL) in 0.5-mol/L acetic acid was mixed with 5× concentrated Dulbecco modified Eagle medium in purified water and buffered to a final pH of approximately 7.5, as indicated by phenol red, with the use of sodium bicarbonate; the volume was adjusted to a final concentration of 1 mg/mL with deionized water before incubation. Type 1 collagen gels were cast from 300- μ L volumes of cold 1 mg/mL into 15 × 5-mm rectangular forms. Gels containing human recombinant IGF-1 and/or IGF-2 were created by adding 3 μ g of IGF-1 and/or IGF-2 (Upstate Biotechnology Inc, Lake Placid, NY) in 0.5-mol/L acetic acid before the sodium bicarbonate was added. The doses of IGFs were based on hypothetical local distribution of IGFs, assuming equal distribution through a 500-g rat, using doses equivalent to those showing skeletal effects when delivered by osmotic infusion pumps in studies by Thaller et al.⁷

osmotic infusion pump accelerated the repair of calvarial CSDs in rats.⁷ It was also previously shown that 3-dimensional T1CGs augmented with IGF-1 result in significantly increased healing over that of unaugmented collagen gels ($P < .04$).⁸ Fewer studies have been conducted on the effects of IGF-2; however, in vitro studies on bone cell cultures seem to indicate that IGF-2 and IGF-1 have the same efficacy in stimulating protein synthesis but that higher levels of IGF-2 are necessary to achieve the same effects, indicating that IGF-2 has less potency.⁹ This study used a nasal CSD to determine the effect of 3-dimensional T1CGs augmented with IGF-2 and combination IGF-1 and IGF-2 on the repair of endochondral bone defects.

RESULTS

At necropsy, samples from all groups grossly appeared to have total surface healing, with the samples from the augmented groups appearing grossly thicker than samples of the unaugmented group. Bone densities and thicknesses of the excised nasal dorsums were compared by means of radiodensitometric values as explained previously.⁸ Briefly, the samples were x-rayed and the developed films scanned into a computer. ImageQuant converts the radiopacity from the scanned x-ray image into arbitrary volume units, with greater radiopacity corre-

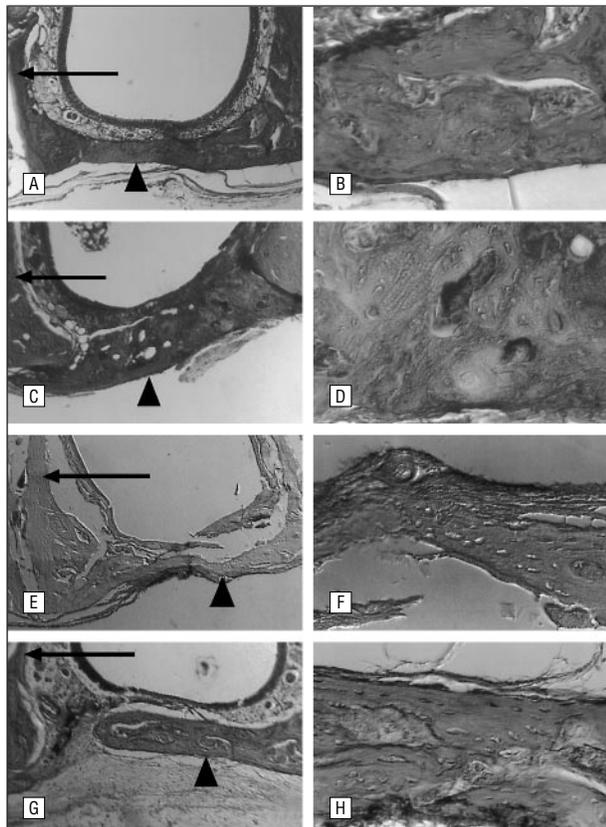
lating with smaller volume units. Because these units of volume negatively correlate with radiopacity, we have designated these volume units as radiodensitometric values; therefore, smaller radiodensitometric values are obtained with greater bone healing.⁸ Radiodensitometric values obtained from ImageQuant analysis of the x-ray films are presented in the **Table**. The mean (\pm SEM) for the collagen-only group was 5593.5 \pm 61.9; the mean for the IGF-1 augmented group was 5175.0 \pm 104.9. The mean for the IGF-2-augmented group was 4451.3 \pm 87.3. The mean for the combination IGF-1 and IGF-2 group was 4826.2 \pm 77.7. For comparison, a defect from a rat that was killed immediately after surgery yielded a value of 6128. A nasal bone harvested from a rat with no surgical defect yielded a value of 2880. Statistically significant differences were shown between paired groups by the Student *t* test.

Radiodensitometric measurements showed that the IGF-2 augmentation resulted in greatest osseous healing, with measurements being statistically significant over that of unaugmented gels ($P < .001$), IGF-1 augmentation ($P < .001$), and combination IGF-1 and IGF-2 augmentation ($P \leq .003$). Combination IGF-1 and IGF-2 had osseous healing that was intermediate between IGF-1 augmentation and IGF-2 augmentation alone, with measurements being statistically significant over those of unaugmented gels ($P < .001$) and IGF-1 augmentation

Radiodensitometric Values of X-rayed Nasal Defects*

No Defect	No Healing	Collagen	Collagen + IGF-1	Collagen + IGF-2	Collagen + IGF-1 and -2
2880	6128	5423	5287	4392	4678
		5528	5339	4198	4662
		5730	5363	4766	4896
		5741	4695	4275	4643
		5711	5071	4425	5079
		5428	5295	4652	4999
		5593.5 ± 61.9†	5175.0 ± 104.9†	4451.3 ± 87.3†	4826.2 ± 77.7†

*IGF indicates insulinlike growth factor.
†Mean ± SEM.



Defect treated with collagen augmented with combination insulinlike growth factor (IGF) 1 and IGF-2 at ×10 (A) and ×40 (B) magnification; collagen augmented with IGF-2 at ×10 (C) and ×40 (D) magnification; collagen only at ×10 (E) and ×40 (F) magnification; and collagen augmented with IGF-1 at ×10 (G) and ×40 (H) magnification (hematoxylin-eosin). Arrows represent the nasal septum; arrowheads, new bone growth.

($P \leq .03$). Augmentation with IGF-1 resulted in healing that was significant over that of unaugmented gels ($P \leq .04$).

Histological examination of all groups showed complete surface coverage with a thin layer of immature bone. Visual comparison of bone thicknesses seen on histological examination (**Figure**) appeared to correlate with the radiodensitometric analysis (IGF-2 > combination IGF-1 and IGF-2 > IGF-1 > collagen only). No evidence of inflammatory reactions was apparent histologically.

COMMENT

Augmentation of T1CG with IGF resulted in osseous healing that was significant over that of unaugmented collagen gels. Augmentation with IGF-2 resulted in greatest osseous healing, with measurements being statistically significant over those of unaugmented gels ($P < .001$), IGF-1 augmentation ($P < .001$), and combination IGF-1 and IGF-2 augmentation ($P \leq .003$). Combination IGF-1 and IGF-2 had osseous healing that was intermediate between IGF-1 augmentation and IGF-2 augmentation alone, with measurements being statistically significant over those of unaugmented gels ($P < .001$) and IGF-1 augmentation ($P \leq .03$). Augmentation with IGF-1 resulted in healing that was significant over that of unaugmented gels ($P \leq .04$).

The technique of radiodensitometric analysis was used in this and 1 other previous study for estimating bone regeneration.⁸ Other techniques were considered. Nuclear scanning for bone density is used for estimating large defect densities but is impractical for analyzing these small defect densities. Computed tomographic scans were used in our original study on type 1 collagen-mediated repair of nasal defects but were not used in this study because of limitations with defects less than 3 mm in depth.⁶ The use of ultrasound in estimating bone density has been used by other investigators but is unavailable at our institution. In any study, analyzing bone density and regeneration is a potential weakness. We believe that this roentgenographic technique provides a good way to compare bone densities, particularly when used to compare radiodensities between flat bones such as the nasalis, which is more suitable for this technique than more complex-shaped bones. This method of analysis does not give a direct value of bone density, but the planar geometry of the excised dorsum does allow the 2-dimensional roentgenographic projections to be used for the comparison of healing between groups.

It has previously been shown that untreated nasal CSDs had less than 7% surface area healing during a 6-week time span, while repair with the use of unaugmented type 1 collagen resulted in 100% surface area healing.⁶ Type 1 collagen is a major component of the extracellular matrix of bone. By laying down the 3-dimensional T1CG into the defect, the major component of the extracellular matrix is provided. A possible mechanism of increased healing, therefore, could be that the gel

provides a scaffold for osteoblast migration.⁶ The addition of IGF-1 and IGF-2 confers additional osteoinductive properties and promotes healing of bone that is significantly greater than that of collagen gels alone. The use of IGF-1- and IGF-2-augmented T1CG offers advantages over currently used techniques in nasal reconstruction. Because the type 1 collagen used in the gels is part of the extracellular matrix of bone, it is readily incorporated into the healing defect and does not have the potential for extrusion at a later time. Many of the current techniques used in facial reconstruction possess notable risks of infection. This study and previous studies using T1CGs showed no signs of infection or inflammation.^{6,10}

The increased healing with locally delivered IGF-1 and IGF-2 augmentation was not unexpected. Systemically released IGF-1 is believed to mediate the effects of growth hormone by stimulating synthesis of glycogen in the liver and the synthesis of collagen in bones.¹¹ During the development of children, growth hormone is believed to play a key role in the growth of long bone; the effects of growth hormone on bone growth are believed to be mediated by IGF-1 production.¹² Rats with calvarial CSDs that were administered IGF-1 with 14-day osmotic infusion pumps showed nearly complete healing after 6 weeks, while control rats showed nearly no healing after 8 weeks.⁷ Both IGF-1 and IGF-2 have been shown to stimulate cell proliferation and collagen synthesis by osteoblasts as well as the synthesis of DNA, collagen, and noncollagen proteins in cultured rat calvaria.¹¹ In addition, IGF-1 and IGF-2 have been shown to decrease collagenase transcripts by more than 80% and thus decrease collagen breakdown.¹³ While IGF-1 and IGF-2 are circulating hormones and can act systemically, local production of IGF-1 and IGF-2 by skeletal tissue is also an important source of these factors and may act as a paracrine or autocrine regulator of bone formation. In fact, it has been postulated that systemic circulating IGF-1 mediates some of its actions at the growth plates by stimulating the local production of IGF-1.¹⁴

The degrees to which IGF-1 and IGF-2 stimulate cell proliferation and collagen synthesis and suppress collagenase production *in vitro* are roughly equivalent, hence their *in vitro* efficacies appear to be comparable. Yet IGF-1 appears to be more potent than IGF-2, because it mediates these effects at lower molar concentrations. There are 2 known types of IGF receptors, type 1 and type 2, and both IGF-1 and IGF-2 interact with both types of receptors. *In vitro* evidence suggests that the stimulatory effects of both IGF-1 and IGF-2 on isolated bone and bone cell cultures are mediated by the type 1 receptors. Thus, it has been proposed that the lower biological potency displayed by IGF-2 in isolated bone and bone cell cultures results from the preference of the type 1 IGF receptor for binding IGF-1.¹⁴

In the rodent nasal CSD model, however, IGF-2-augmented collagen gels resulted in bone healing that was significantly greater than that of IGF-1-augmented collagen gels. This suggests that, *in vivo*, IGF-2 may have greater efficacy than IGF-1 in mediating the repair of osseous nasal defects in rats. Several possibilities may explain this surprising result. First, the effects of IGFs are strongly influenced by the balance of IGFs, IGF receptors, and IGF-

binding proteins.¹⁵ The *in vitro* and *in vivo* environments may cause cells to differ in the levels of expression of these proteins. Second, studies have shown that bones and bone cells taken from different parts of an organism have large differences in the local expression of IGFs, IGF-binding proteins, fibroblast growth factor, and alkaline phosphatase.^{16,17} No study to date has compared cells from the nasalis bone with those of other sites. Third, IGF-1 and IGF-2 messenger RNA is normally preferentially expressed at different times during fracture repair and growth. For example, endothelial and mesenchymal cells at the granulation tissue stage express a predominance of IGF-2 messenger RNA. At the stage of bone and cartilage formation, osteoblasts and nonhypertrophic chondrocytes express messenger RNA for both IGF-1 and IGF-2. Osteoclasts are positive for IGF-2 messenger RNA at the stage of bone remodeling. In all stages, there is a predominance of IGF-2 in the human bone matrix.¹⁸

Most surprising of all was that augmentation of the gels with a combination of both IGF-1 and IGF-2 resulted in healing that was intermediate between that of IGF-1 augmentation and IGF-2 augmentation alone. There was twice as much total IGF present in these gels (6 μ g total). The observed healing suggests that some inhibition of IGF-2 by IGF-1 might be explained if both factors act through the same receptors, as the 2 factors would compete for the same receptor binding sites. *In vitro* evidence does suggest that both IGFs interact at the same receptors but with different affinities. Another possible explanation is a biphasic response. With this type of response, increasing concentrations of the growth factor may increase growth at low concentrations, but, with greater concentrations, decreased healing may result. This type of dose-response activity is seen with transforming growth factor- β .¹⁹

An advantage of using IGF-augmented collagen gels over systemic delivery of IGFs is that the growth factors are directed to the site of the defect. This provides a more controlled dosage to the site, reduces the amount of IGFs needed, and reduces the effects of high doses of systemic IGFs. Unlike the cranial vault, the nasalis bone forms by endochondral ossification rather than intramembranous ossification. This is the first study, to our knowledge, to look at the effects of a known concentration of IGF-2 delivered locally to a CSD in a site formed by endochondral ossification. The techniques to evaluate IGF-2 in higher animals exist, and the materials are currently available for such studies. The use of IGF-2 is approved in humans, and techniques for obtaining human collagen from amnions have been developed.²⁰

CONCLUSIONS

Rat nasal defects treated with IGF-2-augmented T1CGs showed healing that was significantly greater than that of collagen only, IGF-1 augmentation, and combination IGF-1 and IGF-2 augmentation. Rat nasal defects treated with combination IGF-1- and IGF-2-augmented T1CGs showed healing that was intermediate between that of IGF-1 augmentation alone and IGF-2 augmentation alone, being significant over that of IGF-1 augmentation alone and collagen-only treatment. Rat nasal defects treated with IGF-

I-augmented collagen gels showed healing that was significant over that in rats treated with nonaugmented collagen gels. The use of IGF-augmented collagen gels in the repair of nasal defects merits further investigation.

Accepted for publication August 26, 1998.

Presented at the Seventh International Symposium on Facial Plastic Surgery, Orlando, Fla, June 18, 1998.

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