Published in final edited form as:

Otolaryngol Head Neck Surg. 2016 October ; 155(4): 612–623. doi:10.1177/0194599816645772.

MERS versus Standard Surgical Approaches for Porcine Vocal Fold Scarring with Adipose Stem Cell Constructs

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Abstract

Objective.—Cells, scaffold, and surgical approaches are important for regeneration of the lamina propria of the scarred vocal fold (VF). Microendoscopy of Reinke’s space (MERS) is a surgical approach used to access the lamina propria. The present study evaluated MERS in the treatment of VF scarring as compared with standardized approaches for the treatment of VF scarring with adipose stem cell constructs.

Study Design.—Animal study.

Setting.—Academic center.

Subjects and Methods.—VF injury was performed bilaterally to induce scarring in 20 pigs. Eight weeks after injury, pigs were classified into no treatment, minithyrotomy, VF injection, VF incision/dissection, and MERS. All groups (except control) were implanted with adipose stem cell and hyaluronan. Four weeks after treatment, histology for collagen, hyaluronan, and fibronectin; mRNA expression for α -smooth muscle actin, tumor growth factor β 1, collagen 1 α 1, collagen

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This article was presented at the 2015 AAO-HNSF Annual Meeting & OTO EXPO; September 27–30, 2015; Dallas, Texas.

Competing interests: Henry Hoffman, Cook Medical—research consultant, purchased rights on patent procured through University of Iowa Research Foundation.

3 α 1, matrix metalloproteinase 2, basic fibroblast growth factor, and hepatocyte growth factor; and tissue rheology were evaluated.

Results.—Differences were measured among surgical approaches for protein levels of collagen, hyaluronan, and fibronectin ($P = .0133$, $P < .0001$, and $P = .0025$, respectively). Fibroblast growth factor, collagen 1 α 1, and matrix metalloproteinase 2 transcript levels were different among treatment groups ($P = .003$, $P = .0086$, and $P = .014$, respectively), while no differences were measured for α -smooth muscle actin, tumor growth factor β 1, hepatocyte growth factor, and collagen 3 α 1. Rheologically, significant differences were not measured between groups.

Conclusion.—MERS is a promising surgical approach for the treatment of VF scarring, optimizing the placement of implanted biomaterials.

Keywords

vocal fold; scar; Reinke's space; adipose tissue–derived stromal cells; hydrogel

Vocal fold (VF) scarring is the fibrous change of the layered structure of VF resulting from physical trauma, chronic vocal misuse, or a complication of surgical removal of lesions.¹ It causes impairment of the VF mucosal wave and hoarseness. Histologic characterization of VF scarring in animal models has reported increased and disorganized collagen, increased fibronectin (FN), and decreased elastin with concomitant changes in tissue viscoelasticity.^{2,3} Current surgical approaches and pharmaceutical interventions are often unsatisfactory in treating VF scar, resulting in inconsistent outcomes.^{4,5}

Regenerative therapies have been studied for use in the superficial lamina propria (LP) as a strategy for treating VF scar.⁶ In traditional tissue engineering paradigms, cells and scaffolds assist with initial construct development and maintain function after implantation.⁶ Studies exploiting injections of fibroblasts or bone marrow stem cells seeded in hyaluronic acid (HA)–based gels into scarred VF in various animal models reported elevated collagen or procollagen, elevated FN, and conflicting improvements in viscoelasticity.^{7,8} An in vitro investigation demonstrated that adipose tissue–derived stromal cells (ASCs) have several advantages for treatment of VF scarring. When fibroblasts from scarred VF were cocultured with ASCs, collagen production, cell proliferation, and α -smooth muscle actin (α -SMA) expression were significantly decreased, whereas HA production increased.⁹ Coculture increased hepatocyte growth factor (HGF) secretion from ASCs, indicating that implanted ASCs potentially ameliorate VF scarring by acting as long-term intrinsic sources of HGF. HA is a high molecular weight glycosaminoglycan that is present in the extracellular matrix of tissue, including VF LP.¹⁰ The viscoelastic properties of HA are closest to native VF as compared with other injectable materials that have been evaluated, thus offering excellent biocompatibility.¹¹ HA hydrogels in combination with ASCs are a promising construct for the treatment of scarred VF.¹²

To date, optimal delivery approaches of biomaterials and/or cells to the LP have not been established. To access the scarred LP, several surgical approaches have been studied, including VF injection (INJ), VF incision and dissection (VI), minithyrotomy (MT), and, most recently, microendoscopy of Reinke's space (MERS).^{7,8,13} Unlike previous surgical

techniques, MERS utilizes a microendoscope to access the LP through an anterior external approach at the inferior border of the thyroid cartilage. This approach provides imaging of the interior space of the VF with a device offering a working channel for instrumentation to clear fibrotic tissue from the muscle and from an area for precise injection of biomaterial constructs, without disrupting the epithelial cover of the VF.^{13,14}

The aim of the current study is to compare outcomes of 4 commonly used surgical approaches (INJ, VI, MT, MERS) with an ACS-hydrogel construct in a scarred porcine VF model. While we recognize that differences exist between human and porcine models, there has been extensive research indicating their similarities in wound healing¹⁵ and scarring.¹⁶ A recent study also supports similarities in the dissection plane of the pig and human LP,¹⁷ and porcine LP has human-like distributions of elastin, collagen, and HA,^{18,19} critical for tissue biomechanical properties.²⁰

Materials and Methods

Study Design

All aspects of this study were in accordance with the Institutional Animal Care and Use Committee (University of Wisconsin Madison).²¹ Twenty-four female Landrace pigs (9 weeks old) were included in this investigation, based on an estimate of sample size determined from tissue viscoelasticity^{7,8} and calculated per a 2-tailed *t* test at a significance level of 0.05. To achieve at least 80% power, 4 pigs per group were required. To induce VF scarring, bilateral VF injuries were performed in 20 pigs. VF injuries were performed via direct suspension laryngoscopy with a 30° telescope; the larynx was imaged while the animal was in a supine position. Four-millimeter cup biopsy forceps were used to create a single injury bilaterally in the middle membranous portion of the VF. Four pigs did not undergo injury and were designated as normal control (NC). Eight weeks after injury, 20 injured pigs were classified into 5 groups: positive control (PC; untreated animals), INJ, VI, MT, and MERS. Allogenic porcine ACSs (pASCs) combined with HA-hydrogel scaffold (approximately 2.0×10^5 pASCs with 0.2 mL of hydrogel) were infused in the subepithelial area of each scarred VF. Four weeks after treatment, all pigs were euthanized.

Mesenchymal Stromal Cell Isolation

Subcutaneous abdominal fat was collected from Landrace pigs and fully characterized as described previously.²² pASCs were cultured with *α*-MEM (Lonza, Walkersville, Maryland) supplemented with 5% fetal bovine serum, 0.1% nonessential amino acids, and 0.1% L-glutamine (Sigma-Aldrich, St Louis, Missouri). Cells were subcultured until passage 2 and cryopreserved in culture media supplemented with 15% fetal bovine serum and 10% dimethyl sulfoxide.

Hystem-C

A chemically modified hyaluronan-gelatin hydrogel (Hystem-C) was previously customized for use in the VF LP.^{7,8} The hydrogel consists of 8.2% polyethylene glycol diacrylate, 1.4% crosslinked thiol-modified HA, and 1% thiol-modified gelatin.

In Vivo Transplantation

Immediately before use, pASCs at passages 3 to 4 were harvested with trypsin-EDTA (Life Technologies, New York, New York) and filtered with a 40- μ m strainer (BD Falcon, Billerica, Massachusetts). After centrifuge at 1100 revolutions per minute for 6 minutes and aspiration of supernatant, glycosyl supplemented with Gelin-S (1% thiol-modified gelatin) was mixed with the pellet of pASCs, and 8.2% polyethylene glycol diacrylate was added to a final concentration of 1×10^6 cells/mL. Constructs were incubated in a 1-mL syringe for 5 minutes prior to use to allow partial gelation.

Therapeutic Procedures

Anesthetic induction was completed with Telazol (tiletamine/zolazepam, 2–7 mg/kg, intramuscular), xylazine (1–2.2 mg/kg, intramuscular), and atropine (0.05 mg/kg, intramuscular). Animals were intubated and placed on isoflurane.

INJ was performed under direct suspension laryngoscopy; pASCs-hydrogel was injected into the submucosal area just lateral to the VF scar, with a laryngeal injector and a 24-gauge needle (Medtronic Xomed Surgical Products Inc, Jacksonville, Florida).

VI was performed under direct suspension laryngoscopy; an anterior-posterior incision into the upper surface of VF was performed with sharp and sickle knives (MicroFrance; Metronics, Grand Rapids, Michigan). Dissection was performed on the scarred LP to create a subepithelial pocket, and pASCs-hydrogel was injected into the pocket. All wounds were covered with fibrin sealant (Tisseel; Baxter, Deerfield, Illinois).

MT was performed as described previously.²³ Once a pocket in the scarred LP was created, it was filled with the pASCs-hydrogel through the holes of the MT through a 1-mL syringe with 24-gauge needle. The hole in the cartilage was plugged with fat tissue and the wound closed with absorbable sutures.

MERS was performed per previous studies.^{13,14} Microdissection of the intra-VF was performed via forceps placed through the working channel of the sialendoscope, providing direct visualization of the fibrotic LP. Following microdissection within the LP, pASCs-hydrogel was injected into the newly developed pocket through the open channel on the sialendoscope, which was connected with a 1-mL syringe. The cartilage defect was closed by suturing the surrounding soft tissue, and the wound was closed with absorbable sutures.

Gross Assessment of pASCs-Hydrogel

Twelve weeks after VF injury, larynges were harvested. The position and amount of pASCs-hydrogel construct that remained in the VF were noted. Location was noted as glottis or subglottal space. Amount of remaining construct was noted as >50% retained, <50% retained, or none.

Real-time Polymerase Chain Reaction

The posterior half of the left VF was submerged in RNAlater (Qiagen, Valencia, California) and incubated at 4°C. Tissues were disrupted with a homogenizer. Total RNA was

subsequently extracted with the RNeasy Mini Kit (Qiagen) and concentration measured by spectrophotometric analysis via a NanoDrop1000 (Thermo Scientific, Fremont, California). Complementary DNA was synthesized from 1.48 µg of total RNA by reverse transcription (Invitrogen Superscript II; Invitrogen, Carlsbad, California). Quantitative polymerase chain reaction was carried out with the Light Cycler 1.5 System (Roche Diagnostics Corp, Indianapolis, Indiana) to quantify mRNA expression of α -SMA, tumor growth factor β 1 (TGF β 1), collagen type 1 α 1 (COL1), collagen type 3 α 1 (COL3), matrix metalloproteinase 2 (MMP2), basic fibroblast growth factor (bFGF), and HGF. Housekeeping gene hypoxanthine phosphoribosyltransferase 1 was quantified for each complementary DNA sample. Primers used are described in Table 1. Quantitative polymerase chain reaction reactions were performed as previously described.²⁴ Each individual sample was tested in triplicate.

Histopathology and Immunohistochemistry

Left VFs were removed from harvested larynges. The left VF was divided at midline; the anterior half was embedded in paraffin; and 5-µm coronal sections were cut. Routine processing was performed, and slides were stained with hematoxylin and eosin to assess morphology. Trichrome was used to determine collagen content. Alcian blue was used to stain HA by incubating serial slides with and without bovine testicular hyaluronidase (Sigma-Aldrich).

Immunohistochemistry was performed to detect FN. Sections were incubated with proteinase K antigen retrieval for 5 minutes (1:9; EMD Millipore, Billerica, Massachusetts), 10% bovine serum albumin in phosphate-buffered saline for 1 hour at room temperature for blocking, polyclonal antibody (1:250; Thermo Scientific) at 37°C for 1 hour, anti-rabbit IgG secondary with peroxidase reagent (Vector Laboratories, Burlingame, California) for 30 minutes, and then 3,3'-diaminobenzidine horseradish peroxidase substrate for 2 minutes and counterstained with hematoxylin.

Slides were analyzed by microscopy (Eclipse E600; Nikon, Melville, New York) equipped with a digital camera (DP-71; Olympus, Center Valley, Pennsylvania). MetaMorph software (Molecular Devices Corporation, Sunnyvale, California) was used to measure collagen, HA, and FN. Thresholds of HA and FN were established in the *Set color threshold*. The same threshold was carried out consistently across all samples. Percentage of positively stained pixels per total pixels in the regions of interest was determined for each sample.

Rheometric Evaluation

Right VF mucosa was carefully dissected from underlying vocal muscle, immediately flash frozen, and stored at -80°C. Sections were thawed and hydrated in a 1% phosphate-buffered saline solution at room temperature. Rheologic measurements were carried out through a controlled stress rheometer (Bohlin Gemini-150; Malvern Instruments, Malvern, UK) by employing a parallel plate with a stationary lower plate and rotating upper plate (8-mm diameter). Wet-dry sand-paper (220 grit; Norton Abrasives, Worcester, Massachusetts) was affixed to plates. Temperature during testing was maintained at 37°C $\pm$ 0.5°C. Gap distances between plates was determined according to the normal force exerted on the tissue (0.1–0.6

mm). Shear stress, shear strain, and strain rate associated with the oscillatory shear deformation were computed from prescribed torque and measured angular velocity, and viscoelastic data were obtained from these functions.

Statistical Analysis

One-way analysis of variance and Tukey's multiple comparison tests were used to identify differences among treatment groups for histology, immunohistochemistry, and transcript measurement. $P < .05$ was considered statistically significant for 1-way analysis of variance. Tukey's multiple comparison test represented the mean difference between 2 groups and the 95% confidence interval (95% CI) of the difference. If the 95% CI excluded zero, the difference between the means was considered statistically significant ($P < .05$). Rheometric data were analyzed through a repeated measures analysis of covariance, assuming a first-order autoregressive error structure within a subject. Independent variables were log frequency and treatment group. $P < .05$ was considered statistically significant.

Results

All pigs survived the surgical procedures without complications and survived until euthanized.

Gross Assessment

Table 2 reviews the gross appearance of the pASCs-hydrogel constructs in all specimens. For INJ, 6 of 8 VFs had migration of the pASCs-hydrogel construct into the subglottal space; constructs were >50% retained in all samples. For MT, the construct was present in the glottis of all VF, with >50% retention in 4 of 8 VFs. For VI, no construct was found in 5 of the VF; <50% of the construct was found in the remaining 3 VFs (2 glottis, 1 subglottal space). For MERS, <50% of the construct was retained and found in the glottis of all samples.

Histopathology and Immunohistochemistry

Tissue levels of collagen, HA, and FN were measured to determine morphology of the VF LP. NC had normal alignment of collagen throughout the LP, while PC had hypertrophic collagen bundles (Figure 1). Significant differences were measured among surgical approaches for protein levels of collagen, HA, and FN ($P = .0133$, $P < .0001$, and $P = .0025$, respectively; Figure 2). Both PC and VI had significantly higher collagen levels relative to NC (95% CIs: -39.85 to -2.497 for NC vs PC, -41.05 to 23.702 for NC vs VI; Table 3). For HA (Figures 1 and 2), NC had significantly higher levels than all treatment groups (95% CIs: $44,441$ – $154,148$ for NC vs PC, $44,924$ – $154,631$ for NC vs VI, $44,305$ – $154,012$ for NC vs INJ, $32,015$ – $141,722$ for NC vs MT, $16,031$ – $125,738$ for NS vs MERS). Figure 1D–1Y demonstrates FN staining. NC showed faint brown staining, whereas scarred VFs (PC, VI, INJ, MT, and MERS) showed definitive staining. VI showed significantly higher FN levels than NC, MT, and MERS (95% CIs: $-314,851$ to $-57,317$, $56,558$ to $314,093$, $13,574$ to $271,109$, respectively).

Real-time Polymerase Chain Reaction

In wound repair, α -SMA and TGF β 1 were not only markers of fibrosis but also contributors.²⁵ MMP2, bFGF, COL1, and COL3 play a crucial role in extracellular matrix remodeling.²⁶ bFGF, COL1, and MMP2 gene expression levels were significantly different among treatment groups ($P = .003$, $P = .0086$, and $P = .014$, respectively). bFGF mRNA was significantly greater for MERS relative to all other groups (95% CIs: -181.7 to -23.79 for NC vs MERS, -182.9 to -24.94 for PC vs MERS, -182.0 to -24.02 for VI vs MERS, -200.8 to -7.292 for INJ vs MERS, and -182.9 to -24.90 for MT vs MERS; Table 4, Figure 3). COL1 mRNA was significantly greater for MERS as compared with PC, VI, and MT (95% CIs: -711.9 to -74.45 for PC vs MERS, -684.4 to -47.02 for VI vs MERS, -704.7 to -67.34 for MT vs MERS). MMP2 mRNA was significantly greater for MERS as compared with PC, VI, and MT (95% CIs: -284.3 to -1.307 for PC vs MERS, -284.0 to -1.050 for VI vs MERS, -293.2 to -10.23 for MT vs MERS). No significant differences were measured between groups for α -SMA, TGF β 1, HGF, and COL3.

Rheometric Evaluation

Measurement of tissue viscoelasticity is a measure of tissue function. Figure 4 demonstrates elastic (G') and viscous modulus (G'') of the tissue samples as a function of oscillation frequency, on a log-log scale. For G' , all samples increased monotonically with frequency. Overall for G' , MT-treated VFs were the stiffest, followed by INJ, PC, VI, MERS, and NC, with NC having the highest elasticity. There was considerable variability within groups, but no statistical difference was measured ($P = .4772$). Viscosity decreased in order from MT to INJ, PC, VI, NC, and MERS. For G'' , there was considerable variability noted within groups, with no significant differences measured among groups ($P = .0936$).

Discussion

Regeneration of the VF LP requires a biomimetic approach in combination with a surgical technique that allows placement of biomaterials without instigating additional injury. INJ, VFI, VI, and MT are current standard surgical approaches for the placement of materials into the VF LP. Recently, MERS was introduced as a new surgical technique to provide direct real-time visualization of the LP and an ability to manipulate surgical instruments parallel to the VF edge while maintaining an intact epithelium. The current study was designed to compare MERS with those approaches presently in clinical practice.

To date, INJ is the most commonly performed technique in animal studies for the treatment of VF scarring.^{7,8} INJ is simple and easy to perform, yet it is difficult for even a well-experienced surgeon to know precisely where the needle tip is located and specifically where injected material will infuse. Another limitation of INJ is the high incidence for malpositioning of the biomaterial.^{27,28} In this investigation, migration of injected material into the subglottal space was noted in 75% of the animals. VI has been used in only 1 animal study,²⁹ in which the authors made an incision on the upper surface of scarred canine VF, dissected LP, implanted cells/scaffold, and covered the incised VF with Tisseel, reporting good results. However, in the present study, dissection of the LP was difficult due to fibrosis, and the incised VF mucosa was perforated because of tethering of the scarred mucosa.

Although Tisseel was applied to cover the incision, pASCs-hydrogel could not be found in 62.5% of the samples. For VI, collagen was significantly higher when compared with the other groups, and FN levels were significantly higher when compared with NC, MT, and MERS. Given these results, we cannot recommend VI of biomaterials as an approach for the treatment of VF scarring.

In the MT and MERS groups, pASCs-hydrogel could be found in all VFs. Both approaches provide a pocket in the LP where the pASCs-hydrogel could support matrix regrowth. Of note, for MT and MERS, <50% of the construct was frequently retained, in comparison with that injected. It is possible that leakage occurred from the pocket of the LP at the entrance site at thyroid cartilage. The position of cartilage hole is different for each procedure. For MT, the center of the hole was 3 to 4 mm lateral to midline and 3 to 4 mm superior to the inferior edge of the thyroid cartilage. This position made it for an easier closure after injection of the pASCs-hydrogel. For MERS, drilling holes at the inferior border of thyroid cartilage connecting the cricothyroid membrane made closing the defect more difficult and time-consuming. Modifying the entrance location and overinjecting to account for leakage are possible solutions to consider. Retention of material placed by MERS is anticipated to improve by limiting disruption of the anterior opening into the LP by placing the sialendoscope at the junction of the cricothyroid membrane and anterior-inferior thyroid cartilage without removal of cartilage. Advances in fine-needle fiberoptics to enhance positioning of instrumentation through transillumination are anticipated to refine this approach.³⁰

With MERS, a pocket can be made for biomaterials similar to MT and VI. Proper placement maximizes the potential for regeneration of scarred LP. MERS also provides direct visualization of the intra-VF, optimizing dissection within the scarred LP without perforation of the mucosa. MERS showed significantly higher bFGF gene expression and the highest expression of HGF mRNA. Previous studies demonstrated that bFGF and HGF stimulate HA production and promote organization of collagen bundles.^{9,31,32} Our findings showed significantly higher expression of COL1 mRNA without measurable differences in collagen production at the protein level for MERS. Results suggest that cells in the VF are actively promoting synthesis and degradation of matrix. Our findings are similar to previous studies with the use of a synthetic HA-hydrogel. Thibeault et al⁷ reported increases in procollagen and collagen genes 4 months after autologous fibroblasts-HA hydrogel scaffolds were injected into a scarred rabbit VF. Johnson et al⁸ also reported increases in procollagen 1 month after injection of bone marrow stem cells-HA hydrogel scaffolds immediately after VF injury. These results are suggestive of a remodeling phase, where the synthesis of the extracellular matrix and regulation of growth factors are imperative to regrowth of the tissue. HA is a major component of the VF extracellular matrix.³³ Therefore, HA scaffolds could act as an extracellular matrix itself—beyond just supporting survival and growth of implanted cells—if properly instilled into the scarred LP. As mentioned above, in MERS, the pASCs-hydrogel was located in the glottis in all harvested VFs, whereby we could expect MERS to amplify the synergistic effect of instilled pASCs-HA hydrogel by letting them localize in the proper position.

In summary, this study applied pASCs-HA hydrogel for the treatment of VF scarring in a porcine model, comparing MERS with several standardized surgical approaches. While the INJ, MT, and MERS groups demonstrated similar histologic results for collagen, HA, and FN, the MERS-treated group had high expression of bFGF and HGF. Future investigations are warranted for long-term outcomes with the inclusion of vibratory mechanical consequences prior to initiation of human trials.

Acknowledgments

Funding source: National Institutes of Health, National Institute on Deafness and Other Communication Disorders (R01 DC04336, R01 DC013508).

Sponsorships: None.

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Figure 1.

Histology and immunohistochemistry: trichrome (A, E, I, M, Q, V; magnification 403; 2-mm scale bar), alcian blue (B, F, J, N, R, W); alcian blue with hyaluronidase digestion (C, G, K, O, S, X; magnification 1003; 1-mm scale bar), and fibronectin (D, H, L, P, T, Y; magnification 203; 200- μ m scale bar). INJ, vocal fold injection; MERS, microendsocopy of Reinke's space; MT, minithyrotomy; NC, normal control; PC, positive control; VI, vocal fold incision and dissection.

Figure 2.

Quantitative measurement of collagen, hyaluronic acid (HA), and fibronectin (FN) was significantly different among groups: PC and VI had significantly higher collagen than that of NC; NC had significantly higher HA than other treatments; VI had significantly higher FN than that of NC, MT, and MERS. * $P < .05$. INJ, vocal fold injection; MERS, microendoscopy of Reinke's space; MT, minithyrotomy; NC, normal control; PC, positive control; VI, vocal fold incision and dissection.

Figure 3.

Gene expression for injured vocal folds following treatment with porcine adipose tissue–derived stromal cells–hydrogel constructs. bFGF, COL1, and MMP2 showed significant differences among treatment groups, while no differences were measured for α-SMA, TGFβ1, HGF, and COL3 transcript levels. * $P < .05$. COL3, collagen 3α1; HGF, hepatocyte growth factor; INJ, vocal fold injection; MERS, microendoscopy of Reinke's space; MT, minithyrotomy; NC, normal control; PC, positive control; α-SMA, α-smooth muscle actin; TGFβ1, tumor growth factor β1; VI, vocal fold incision and dissection.

Figure 4. Elastic modulus (G') and viscous modulus (G'') for treatment groups. MERS, microendoscopy of Reinke's space; VF, vocal fold.

Table 1.

Real-time Polymerase Chain Reaction Targets and Primer Sequences for the Porcine Genome.

Target	Leading	Lagging	Size, bp
α -SMA	5'-TGTGACAAATGGTTCTGGGCTCTGT-3'	5'-TTCGTACCCACGTAGCTGTCTTT-3'	144
TGF β 1	5'-CGATTAAAGGTGGAGAGAGGACTG-3'	5'-AATGAATGGTGGACAGACACAGG-3'	128
COL1	5'-AGAAAGAGACATCCCAACAGTCA-3'	5'-AGATCACGTATCGCACAAACA-3'	131
COL3	5'-CCTGCTGGAAGAATGGTGAC-3'	5'-ACGTTTACCGGTTTACCTT-3'	132
MMP2	5'-TTCCCGGAGATGTCGCCCCC-3'	5'-CCTGTCTGGGGAGCCCCGAA-3'	151
bFGF	5'-CTGCTCCTTGCCATTCTAC-3'	5'-AAGAGCCATCTACACACTTATG-3'	89
HGF	5'-ATGCTACTTGGTGTCATTGTTCT-3'	5'-TTGATGTAAAGAGAGTTGTGTTAATGG-3'	183
HPRT1	5'-AAGGACCCCTCGAAGTGTG-3'	5'-CACAAACATGATTCAGTCCCTG-3'	122

Abbreviation: bp, base pairs.

Table 2.

Gross Appearance of the Porcine Adipose Tissue Drive Stromal Cells–Hydrogel Construct in VF Specimens.

Group: ID	Localization of Construct		Remained Amount of Construct	
	Left	Right	Left	Right
INJ				
2332	Subglottal space	Subglottal space	>50% retained	>50% retained
2335	Glottis	Subglottal space	>50% retained	>50% retained
2339	Subglottal space	Subglottal space	>50% retained	>50% retained
2340	Subglottal space	Glottis	>50% retained	>50% retained
MT				
2342	Glottis	Glottis	<50% retained	<50% retained
2343	Glottis	Glottis	>50% retained	>50% retained
2351	Glottis	Glottis	<50% retained	<50% retained
2352	Glottis	Glottis	>50% retained	>50% retained
VI				
2353	None	None	None	None
2354	Glottis	Glottis	<50% retained	<50% retained
2355	Subglottal space	None	<50% retained	None
2356	None	None	None	None
MERS				
2359	Glottis	Glottis	<50% retained	<50% retained
2360	Glottis	Glottis	<50% retained	<50% retained
2361	Glottis	Glottis	<50% retained	<50% retained
2362	Glottis	Glottis	<50% retained	<50% retained

Abbreviations: INJ, VF injection; MERS, microendoscopy of Reinke's space; MT, minithyrotomy; VF, vocal fold; VI, VF incision and dissection.

Table 3.

Statistical Comparisons of Protein Expression.

Protein	P Value ^a	Tukey's Multiple Comparisons Test	Mean Difference	95% CI (Difference)
Collagen	.0133 ^b	NC vs PC	-21.17	-39.85 to 22.497 ^c
		NC vs VI	-22.38	-41.05 to 23.702 ^c
		NC vs INJ	-11.92	-30.59 to 6.754
		NC vs MT	-11.61	-30.28 to 7.064
		NC vs MERS	-17.91	-36.58 to 0.7664
		PC vs VI	-1.205	-19.88 to 17.47
		PC vs INJ	9.251	-9.424 to 27.92
		PC vs MT	9.561	-9.113 to 28.24
		PC vs MERS	3.263	-15.41 to 21.94
		VI vs INJ	10.46	-8.219 to 29.13
HA	<.0001 ^b	VI vs MT	10.77	-7.909 to 29.44
		VI vs MERS	4.468	-14.21 to 23.14
		INJ vs MT	0.3102	-18.36 to 18.98
		INJ vs MERS	-5.987	-24.66 to 12.69
		MT vs MERS	-6.297	-24.97 to 12.38
		NC vs PC	99.294	44.441 to 154.148 ^c
		NC vs VI	99.778	44.924 to 154.631 ^c
		NC vs INJ	99.158	44.305 to 154.012 ^c
		NC vs MT	86.868	32.015 to 141.722 ^c
		NC vs MERS	70.885	16.031 to 125.738 ^c
		PC vs VI	483.5	-54,370 to 55,337
		PC vs INJ	-135.8	-54,989 to 54,718
		PC vs MT	-12,426	-67,280 to 42,427
		PC vs MERS	-28,410	-83,263 to 26,444
		VI vs INJ	-619.3	-55,473 to 54,234
		VI vs MT	-12,910	-67,763 to 41,944

Protein	P Value ^a	Tukey's Multiple Comparisons Test	Mean Difference	95% CI (Difference)
FN	.0025 ^b	VI vs MERS	-28,893	-83,747 to 25,960
		INI vs MT	-12,290	-67,144 to 42,563
		INI vs MERS	-28,274	-83,127 to 26,580
		MT vs MERS	-15,984	-70,837 to 38,870
		NC vs PC	-73,989	-202,756 to 54,778
		NC vs VI	-186,084	-314,851 to 257,317 ^c
		NC vs INJ	-64,641	-193,408 to 64,127
		NC vs MT	-758.3	-129,526 to 128,009
		NC vs MERS	-43,743	-172,510 to 85,025
		PC vs VI	-112,095	-240,862 to 16,672
		PC vs INJ	9348	-119,419 to 138,116
		PC vs MT	73,231	-55,537 to 201,998
		PC vs MERS	30,247	-98,521 to 159,014
		VI vs INJ	121,443	-7324 to 250,211
		VI vs MT	185,326	56,558 to 314,093 ^c
		VI vs MERS	142,342	13,574 to 271,109 ^c
INI		INI vs MT	63,883	-64,885 to 192,650
		INI vs MERS	20,898	-107,869 to 149,666
		MT vs MERS	-42,984	-171,752 to 85,783

Abbreviations: 95% CI, 95% confidence interval; bFGF, basic fibroblast growth factor; FN, fibronectin; HA, hyaluronic acid; INJ, injection; MERS, Microendoscopy of Reinke's space; MT, minithyrotomy; NC, normal control; PC, positive control; VI, vocal fold incision and dissection.

^aOne-way analysis of variance.

^b $P < .05$.

^cThe 95% CI excludes zero in Tukey's multiple comparison test.

Table 4.

Statistical Comparisons for Gene Expression.

Gene	P Value ^a	Tukey's Multiple Comparisons Test	Mean Difference	95% CI (Difference)
bFGF	.003 ^b	NC vs PC	1.151	-77.83 to 80.13
		NC vs VI	0.2277	-78.75 to 79.21
		NC vs INJ	1.253	-95.48 to 97.98
		NC vs MT	1.108	-77.87 to 80.09
		NC vs MERS	-102.8	-181.7 to 223.79 ^c
		PC vs VI	-0.9235	-79.90 to 78.06
		PC vs INJ	0.1022	-96.63 to 96.83
		PC vs MT	-0.04310	-79.02 to 78.94
		PC vs MERS	-103.9	-182.9 to 224.94 ^c
		VI vs INJ	1.026	-95.70 to 97.76
		VI vs MT	0.8804	-78.10 to 79.86
		VI vs MERS	-103.0	-182.0 to 224.02 ^c
		INJ vs MT	-0.1453	-96.88 to 96.59
		INJ vs MERS	-104.0	-200.8 to 27.292 ^c
		MT vs MERS	-103.9	-182.9 to 224.90 ^c
COL1	.0086 ^b	NC vs PC	74.84	-243.9 to 393.5
		NC vs VI	47.40	-271.3 to 366.1
		NC vs INJ	39.97	-350.4 to 430.3
		NC vs MT	67.73	-251.0 to 386.4
		NC vs MERS	-318.3	-637.0 to 0.3832
		PC vs VI	-27.43	-346.1 to 291.3
		PC vs INJ	-34.86	-425.2 to 355.5
		PC vs MT	-7.110	-325.8 to 311.6
		PC vs MERS	-393.2	-711.9 to 274.45 ^c
		VI vs INJ	-7.430	-397.8 to 382.9
		VI vs MT	20.32	-298.4 to 339.0

Gene	P Value ^a	Tukey's Multiple Comparisons Test	Mean Difference	95% CI (Difference)
MMP2		VI vs MERS	-365.7	-684.4 to 247.02 ^c
		INJ vs MT	27.75	-362.6 to 418.1
		INJ vs MERS	-358.3	-748.6 to 32.04
		MT vs MERS	-386.0	-704.7 to 267.34 ^c
	.014 ^b	NC vs PC	93.35	-48.13 to 234.8
		NC vs VI	93.10	-48.39 to 234.6
		NC vs INJ	86.62	-86.66 to 259.9
		NC vs MT	102.3	-39.21 to 243.8
		NC vs MERS	-49.44	-190.9 to 92.05
		PC vs VI	-0.2569	-141.7 to 141.2
		PC vs INJ	-6.729	-180.0 to 166.6
		PC vs MT	8.926	-132.6 to 150.4
		PC vs MERS	-142.8	-284.3 to 21.307 ^c
		VI vs INJ	-6.472	-179.8 to 166.8
		VI vs MT	9.183	-132.3 to 150.7
		VI vs MERS	-142.5	-284.0 to 21.050 ^c
		INJ vs MT	15.65	-157.6 to 188.9
		INJ vs MERS	-136.1	-309.3 to 37.22
α-SMA		MT vs MERS	-151.7	-293.2 to 210.23 ^c
		NC vs PC	136.6	-378.0 to 651.2
		NC vs VI	80.84	-433.8 to 595.5
		NC vs INJ	207.6	-422.7 to 837.9
		NC vs MT	224.8	-289.8 to 739.4
		NC vs MERS	-66.43	-581.1 to 448.2
		PC vs VI	-55.77	-570.4 to 458.9
		PC vs INJ	70.97	-559.3 to 701.3
		PC vs MT	88.17	-426.5 to 602.8
		PC vs MERS	-203.0	-717.7 to 311.6
		VI vs INJ	126.7	-503.5 to 757.0

Gene	P Value ^a	Tukey's Multiple Comparisons Test	Mean Difference	95% CI (Difference)
TGFβ1	.0648	VI vs MT	143.9	-370.7 to 658.6
		VI vs MERS	-147.3	-661.9 to 367.4
		INJ vs MT	17.21	-613.1 to 647.5
		INJ vs MERS	-274.0	-904.3 to 356.3
		MT vs MERS	-291.2	-805.8 to 223.4
		NC vs PC	7.051	-0.7406 to 14.84
		NC vs VI	4.019	-3.772 to 11.81
		NC vs INJ	5.298	-4.244 to 14.84
		NC vs MT	6.694	-1.097 to 14.48
		NC vs MERS	1.783	-6.008 to 9.575
		PC vs VI	-3.031	-10.82 to 4.760
		PC vs INJ	-1.753	-11.29 to 7.790
		PC vs MT	-0.3568	-8.148 to 7.434
		PC vs MERS	-5.267	-13.06 to 2.524
		VI vs INJ	1.278	-8.264 to 10.82
		VI vs MT	2.674	-5.117 to 10.47
		VI vs MERS	-2.236	-10.03 to 5.555
		INJ vs MT	1.396	-8.146 to 10.94
		INJ vs MERS	-3.515	-13.06 to 6.028
HGF	.0725	MT vs MERS	-4.910	-12.70 to 2.881
		NC vs PC	6.321	-116.1 to 128.7
		NC vs VI	-40.64	-163.0 to 81.76
		NC vs INJ	-30.60	-180.5 to 119.3
		NC vs MT	-16.02	-138.4 to 106.4
		NC vs MERS	-111.2	-233.6 to 11.21
		PC vs VI	-46.96	-169.4 to 75.44
		PC vs INJ	-36.92	-186.8 to 113.0
		PC vs MT	-22.34	-144.7 to 100.1
		PC vs MERS	-117.5	-239.9 to 4.884
		VI vs INJ	10.04	-139.9 to 160.0
		VI vs MT	24.62	-97.78 to 147.0

Gene	P Value ^a	Tukey's Multiple Comparisons Test	Mean Difference	95% CI (Difference)
COL3	.2625	VI vs MERS	-70.56	-193.0 to 51.85
		INJ vs MT	14.59	-135.3 to 164.5
		INJ vs MERS	-80.60	-230.5 to 69.32
		MT vs MERS	-95.18	-217.6 to 27.22
		NC vs PC	101.5	-88.09 to 291.2
		NC vs VI	11.61	-178.0 to 201.2
		NC vs INJ	59.63	-172.6 to 291.9
		NC vs MT	103.7	-85.92 to 293.3
		NC vs MERS	115.8	-73.87 to 305.4
		PC vs VI	-89.92	-279.5 to 99.70
		PC vs INJ	-41.90	-274.1 to 190.3
		PC vs MT	2.168	-187.5 to 191.8
		PC vs MERS	14.23	-175.4 to 203.8
		VI vs INJ	48.02	-184.2 to 280.3
		VI vs MT	92.09	-97.53 to 281.7
		VI vs MERS	104.1	-85.47 to 293.8
INJ		INJ vs MT	44.07	-188.2 to 276.3
		INJ vs MERS	56.12	-176.1 to 288.4
MT		MT vs MERS	12.06	-177.6 to 201.7

Abbreviations: 95% CI, 95% confidence interval; bFGF, basic fibroblast growth factor; COL1, collagen 1 α 1; COL3, collagen 3 α 1; HGF, hepatocyte growth factor; INJ, injection; MERS, microendoscopy of Reinke's space; MMP2, matrix metalloproteinase 2; MT, minithyrotomy; NC, normal control; PC, positive control; α -SMA, α -smooth muscle actin; TGF β 1, tumor growth factor β 1; VI, vocal fold incision and dissection.

^aOne-way analysis of variance.

^b $P < .05$.

^cThe 95% CI excludes zero in Tukey's multiple comparison test.