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Investigation of Surgical Adhesives for Vocal Fold Wound Closure

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Objectives: Surgical adhesives are increasingly used for vocal fold microsurgery to assist wound closure and reduce the risks of scar formation. Currently used vocal fold adhesives such as fibrin glue, however, have thus far not been found to promote wound closure or reduce scarring. The objectives of this study were to investigate the mechanical strength and the cytotoxicity of three commercially available adhesives (Glubran 2, GEM, Viareggio, Italy; BioGlue, CryoLife, Kennesaw, GA; and Tisseel, Baxter Healthcare, Deerfield, IL) for vocal fold wound closure.

Methods: Shear and tension tests were performed on 150 porcine larynges. The cytotoxicity of the adhesives to immortalized human vocal fold fibroblasts was investigated using neutral red uptake assays.

Results: The average shear adhesive strength for Tisseel, BioGlue, and Glubran 2 was 13.86 $\pm$ 5.03 kilopascal (kPa), 40.92 $\pm$ 17.94 kPa, and 68.79 $\pm$ 13.29 kPa, respectively. The tensile adhesive strength for Tisseel, BioGlue, and Glubran 2 was 10.70 $\pm$ 6.42 kPa, 34.27 $\pm$ 12.59 kPa, and 46.67 $\pm$ 12.13 kPa, respectively. The vocal fold cell viabilities in extracts of Tisseel, BioGlue, and Glubran 2 were 99.27%, 43.05%, and 1.79%, respectively.

Conclusion: There was a clear tradeoff between adhesive strength and toxicity. The maximum failure strength in shear or tension of the three surgical adhesives ranked from strongest to the weakest was: 1) Glubran 2, 2) BioGlue, and 3) Tisseel. Tisseel was found to be the least toxic of the three adhesives, whereas Glubran 2 was the most toxic.

Key Words: Surgical adhesives, vocal folds.

Level of Evidence: NA

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INTRODUCTION

Background

Vocal fold wound closure following phonosurgery may necessitate sutures or surgical adhesives in order to minimize the risks of re-injury or scar formation. Wound closure techniques can directly affect the wound-healing process. The use of sutures has been reported to cause scar formation.[1] Surgical adhesives provide a viable alternative to sutures for wound closure. They have been increasingly used in clinical practice due to their ease of application, reduced inflammation and infection rates, excellent cosmetic outcome, and improved prevention of fluid leakage through the wound.[2] Despite the increasing popularity of surgical adhesives in cardiovascular and general surgery, their application in phonosurgery is significantly less common.

Surgical adhesives have been mainly used in phonosurgery for vocal fold microflap closure.[3–5] To date, only fibrin tissue adhesives (Tisseel, Baxter Healthcare, Deerfield, IL) have reportedly been used for vocal fold tissue. Although fibrin glue has been used since the 1990s, its efficacy for wound closure and wound healing has only recently been investigated.

Portes et al.[6] investigated the influence of fibrin glue for vocal fold wound healing using porcine models in 2012. They conducted an animal study on pigs (n = 6) to study the in vivo wound healing of vocal fold microflaps closed with fibrin glue versus those allowed to heal via secondary intention. Histological studies revealed that there was a greater collagen concentration in the vocal fold tissue of microflaps closed with fibrin glue (27.8% collagen) than those left to heal via secondary intention (20.4% collagen). Consequently, they reported that fibrin glues may increase the risk of fibrinogenesis.[6]

Maunsell et al.[7] compared vocal fold microflap healing for groups treated with fibrin glue, sutures, and no treatment in a 90-day rabbit study in 2013. For each animal, microflap incisions were made bilaterally. One vocal fold was not treated and served as control. The contralateral vocal fold was treated with either fibrin glue (n = 19 rabbits) or 8-0 Vicryl suture (n = 18 rabbits). The results showed that the suture treatment group had the highest collagen concentration, and the fibrin

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treatment group had the highest number of inflammatory cells. The study concluded that neither method was effective in either reducing scar formation or promoting wound healing relative to the no-treatment group.[7]

Myer et al.[8] measured the tensile strength of fibrin glue for vocal fold microflap wound closure in 2015 in comparison with that of sutures and no treatment. The animal models included six excised bovine vocal folds and five porcine vocal folds. A universal testing system was used to perform tensile tests. The results showed that sutures had the strongest tensile strength, whereas the fibrin glue and no-closure groups did not differ significantly in tensile strength. Hence, Myer et al. concluded that fibrin glue provided little mechanical support for vocal fold wound closure.[8]

González-Herranz et al.[9] conducted a clinical study of the efficacy of fibrin glue for microflap closure in 2017. They performed a retrospective study on 32 patients who underwent phonosurgery using a microflap technique. One group of patients (n = 22) received fibrin glue for microflap closure, and another group (n = 10) received no treatment. Preoperative and postoperative (6 months postoperation) assessments of the Voice Handicap Index 10 (VHI) were completed, along with a videostroboscopic exam. The stroboscopic exams revealed that patients treated with fibrin glue had significantly increased mucosal wave amplitude in comparison to patients with no treatment. The patients' self-reported VHI surveys, however, showed no significant difference between the two treatment groups. The authors concluded that microflap closure via secondary intension provided better postoperative voice outcome.[9] Other studies have reported that the performance of fibrin glue for vocal fold wound closure is unsatisfactory.[6–8]

Recent developments in biomaterials and biotechnology have yielded a number of alternatives to fibrin glue. This motivated the current research work to identify, characterize, and test other surgical adhesives for vocal fold wound closure. An adhesive's performance is influenced by wound size and the amount of mechanical stress present in the tissue. Ideal vocal fold adhesives must provide adequate wound closure strength to withstand vocal folds' vibratory motion, as well as low cytotoxicity to avoid inflammation. During phonation, the vocal fold tissues experience tensile stress from muscle contraction and a combination of shear and normal stress associated with the mucosal wave.[10,11] The mucosal wave is a superposition of shear and compressional surface waves on two vocal fold surfaces.[12,13] Hence, it is clinically important to evaluate an adhesives' strength in shear and tension-loading situations. Moreover, in vitro cytotoxicity tests were performed in the present study to evaluate the vocal fold tissue's possible immune response to the adhesives.

Objectives

The objective of this study was to investigate the adhesive strength and the cytotoxicity of three commercially available surgical adhesives for vocal fold wound closure.

MATERIALS AND METHODS

Surgical Adhesives

The commercially available surgical adhesives were selected because they are representative of three different classes of adhesives and were readily available in Canada: Tisseel (Baxter Healthcare), BioGlue (CryoLife, Kennesaw, GA), and Glubran 2 (GEM, Viareggio, Italy).

Mechanical Characterizations: Lap Shear Test and Tension Test

During phonation, the vocal fold's vibratory motion exerts forces from different directions on the microflap wound, including tension and shear loading. Shear and tension tests were performed on 150 pairs of porcine vocal folds to quantify adhesive strength following standardized procedures (American Society for Testing and Materials [ASTM] F2255-05 & ASTM F2258-05).[14,15] Minor changes were made to adapt the standard protocol for the relatively small vocal fold tissue samples, as described below. The distribution of the test samples is shown in Table I.

Tissue Sample Preparation. Fresh porcine larynges were obtained from a local slaughterhouse immediately postmortem. The samples were snap frozen and stored in a deep freezer at −80°C. On the day of the experiment, each thawed larynx was sectioned into two hemilarynges via a midsagittal cut through the cricoid cartilage. The lamina propria and epithelial layer of the true vocal fold were carefully peeled off from the vocalis muscle. Figure 1 shows details of the dissection procedures.

Aluminum supporting blocks with dimensions of 8 × 4 × 50 mm and 8 × 10 × 50 mm were manufactured for lap shear and tension tests, respectively. The block surfaces were coated with a thin masking tape to improve adhesion between tissue and block surface. The dissected vocal folds were mounted onto the blocks using superglue (ASI M60, Adhesive Systems, Inc., Frankfort, IL), a medical grade superglue that does not penetrate through tissue. The epithelium side of the fold was in contact with the block, as illustrated in Figure 1E. For tension tests, thin nylon threads were added to secure the edges of the tissue samples onto the blocks.

TABLE I.
Distributions of the Number of Vocal Fold Tissue Pairs Tested for Lap Shear Tests and Tension Tests.

Test Type	Surgical Adhesive	Curing Time	Vocal Fold Tissue Pairs Per Group	Vocal Fold Tissue Pairs Tested for Each Adhesive	Total
Lap shear test	Glubran 2	5 minutes	15	30	75
		60 minutes	15		
	BioGlue	5 minutes	15	30	
		60 minutes	15		
	Tisseel	5 minutes	0	15	
		60 minutes	15		
Tension test	Glubran 2	5 minutes	15	30	75
		60 minutes	15		
	BioGlue	5 minutes	15	30	
		60 minutes	15		
	Tisseel	5 minutes	0	15	
		60 minutes	15		

BioGlue (CryoLife, Kennesaw, GA).
Glubran 2 (GEM, Viareggio, Italy).
Tisseel (Baxter Healthcare, Deerfield, IL).

Mechanical Test Protocol. The vocal folds from the same larynx were tested in pairs. A volume of 30 microliter of surgical adhesive was uniformly applied onto the lamina propria side of one vocal fold tissue. Then, the second tissue substrate was placed over the first one to bond together. Figure 2 illustrates the differences in sample preparation and test procedures between shear and tension tests. The samples were stored in the environmental chamber for curing for either 5 minutes or 60 minutes at 37°C. The Tisseel samples were only tested at the 60-minute curing time because preliminary tests showed that the adhesive bond created by Tisseel was too weak for testing after 5 minutes of curing.

The dimensions of each sample and the contact area were measured using a high-precision caliper (Absolute Super Caliper Series 500, Mitutoyo, Aurora, IL) prior to the test. After the desired curing time, the glued sample was clamped to a universal traction test machine (ADMET expert 5000, ADMET Inc., Norwood, MA) and pulled at a rate of 5 mm/minute until failure. The applied load, displacement, and test time were recorded for further analysis.

Data Analysis. The surgical adhesive's adhesive strength was calculated by dividing the maximum failure force and the contact area. The total energy required to fracture the glued joint was calculated by integrating the area under the stress versus strain curve. Data analysis was performed using MatLab 2017b (MathWorks, Natick, MA) and SAS 9.4 software (SAS Institute Inc., Cary, NC).

Biocompatibility Test: Neutral Red Uptake Assay

A neutral red uptake assay was used to measure the toxicity of the surgical adhesives to immortalized human vocal fold fibroblasts. This assay allows the detection of live cells via monitoring the cell's neutral red dye uptake. Damaged or dead cells have a lower ability to uptake neutral red dye than live healthy cells.

Extract Preparation. Surgical adhesives are irregularly shaped solids. Their extracts were prepared with a concentration of 200 mg/mL following the standard protocols in International Organization for Standardization (ISO) 10993-12[16] in 12-well cell culture-treated polystyrene microplates. A volume of 200 mg of surgical adhesive was dropped in the center of the well, and 1 mL of Dulbecco's Modified Eagle's medium (DMEM) (D5546, Sigma-Aldrich, St. Louis, MO) was added to completely cover the adhesive. The samples were incubated at 37°C for 24 hours. Table II

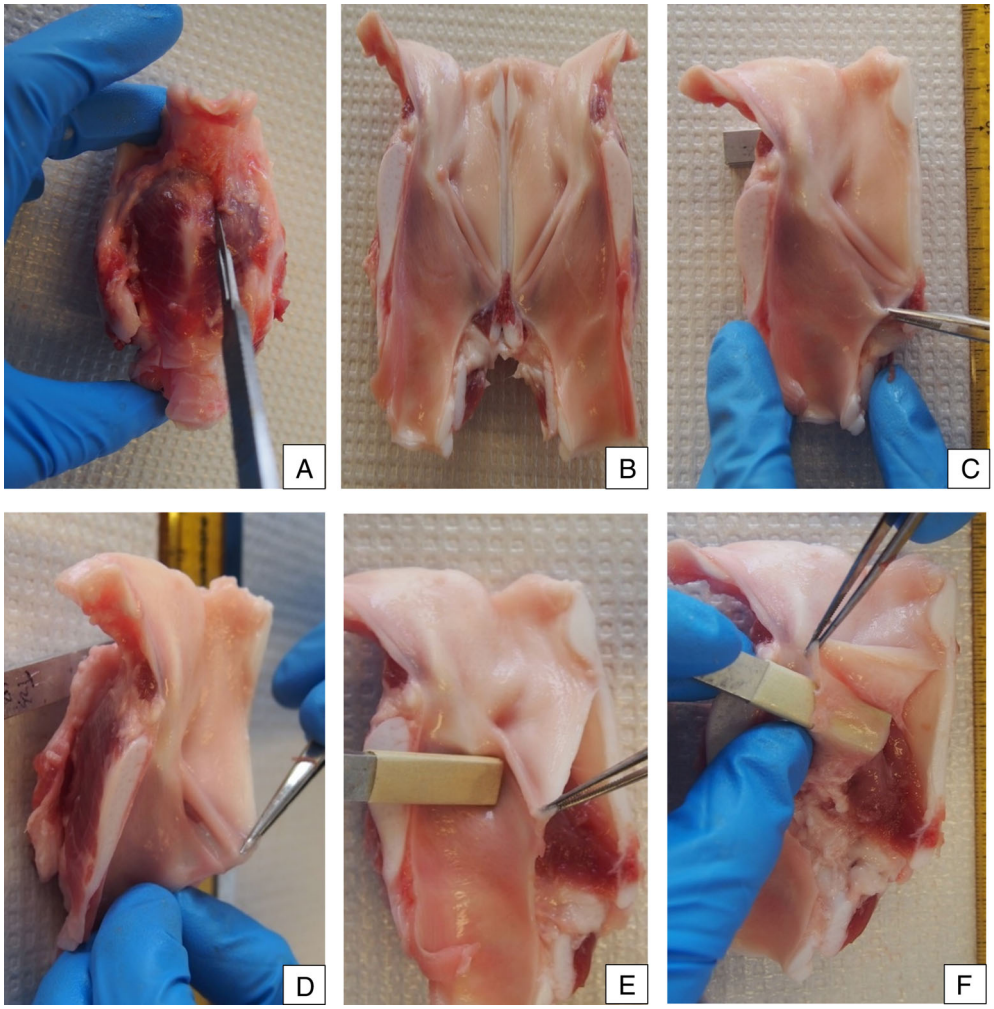

Fig. 1. Tissue dissection for lap shear tests: (A) Porcine larynx after removal of connective tissue surrounding the larynx. A midsagittal incision was made in the cricoid cartilage. (B) Porcine larynx sectioned into two hemilarynges. (C) Incision made at the cartilage to separate the vocal fold tissue-cartilage connection. (D) Lamina propria peeled off from the muscle layer. (E & F) Lamina propria glued onto the aluminum blocks. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

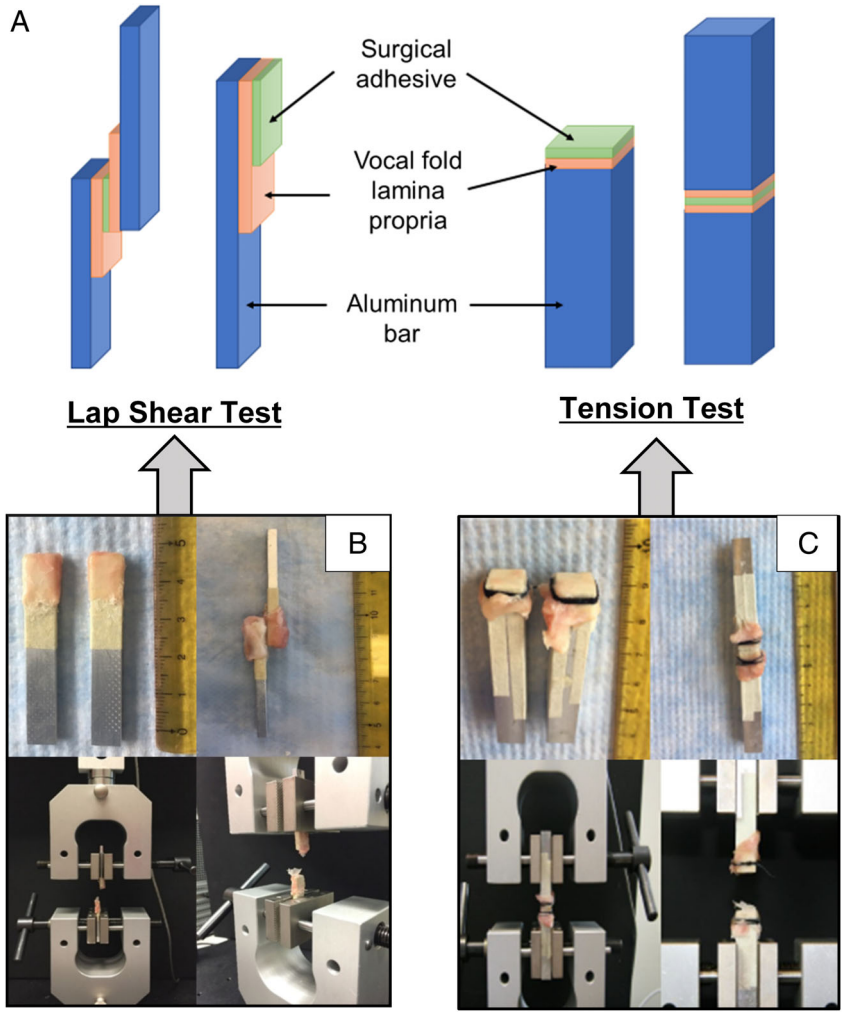

Fig. 2. Illustrations of the sample preparation procedures and the test protocols of shear and tension tests: (A) a sketch of the differences between lap shear test and tension test, (B) lap shear test sample preparation and test procedures, and (C) tension test sample preparation and test procedures. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

shows a list of the adhesive extracts and control solutions prepared for this study. The extracts were prepared on the same day that cells were seeded into 96-well microplates. After 24 hours of incubation, the extracts were collected into small vials and supplemented with 1% nonessential amino acid (M7145, Sigma-Aldrich), 1% penicillin-streptomycin solution (Wisent Inc., St-Bruno, Canada), 5% fetal bovine serum (FBS) (Wisent Inc., St-Bruno, Canada), and 1% L-glutamine (Wisent Inc., St-Bruno, Canada). The supplemented extracts were placed in the incubator until use.

Cell Seeding. Immortalized human vocal fold fibroblast cells[17] were incubated in DMEM (D5546, Sigma-Aldrich) at 37°C, 95% relative humidity, and 95% CO_2 atmosphere until confluent. Supplements added into the cell culture media were the same as those in the surgical adhesive extract, with 10% FBS added. A volume of 200 μL of cell culture solution was seeded into a flat bottom 96-well microplate at 1×10^4 cells/well. The plate was incubated for 24 hours.

Cell Treatment. After 24 hours, the media in the microplates were discarded, and 200 μL treatment solutions listed in Table II were added to the cells. The cells were then incubated for another

TABLE II.
Treatment Solutions Used for Neutral Red Uptake Assay.

Solution Name	Function	Preparation
Fresh DMEM	Growth control	Freshly prepared DMEM, not incubated
DMEM extracted in polystyrene well	Negative control	DMEM extracted in polystyrene well for 24 hours
Phenol (0.64% v/v)	Positive control	
Industrial cyanoacrylate adhesive extract, ASI M60	Reference material: industry adhesive	
Glubran 2 extract	Adhesive extract	
BioGlue extract	Adhesive extract	
Tisseel extract	Adhesive extract	

BioGlue (CryoLife, Kennesaw, GA).
Glubran 2 (GEM, Viareggio, Italy).
Tisseel (Baxter Healthcare, Deerfield, IL).
DMEM = Dulbecco's Modified Eagle's medium; v/v = volume/volume.

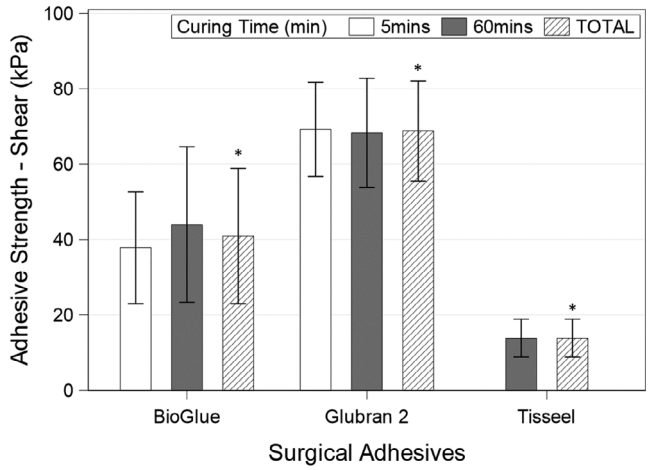

Fig. 3. The average adhesive strength of BioGlue (CryoLife, Kennesaw, GA) (N for 5-minute group = 15, N for 60-minute group = 15, N for total group = 30); Glubran 2 (GEM, Viareggio, Italy) (N for 5-minute group = 15, N for 60-minute group = 15, N for total group = 30); and Tisseel (Baxter HealthCare, Deerfield, IL) (N for 5-minute group = not available, N for 60-minute group = 15, N for total group = 15) under shear loading. The total group for each adhesive includes both 5-minute curing time and 60-minute curing time samples. Error bars represent the standard deviation of the mean. *Represents groups with statistically different mechanical properties ($P < 0.0001$).

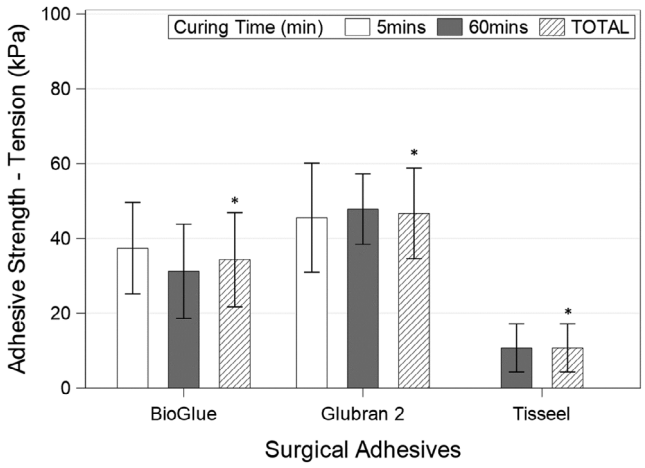

Fig. 4. The average adhesive strength of BioGlue (CryoLife, Kennesaw, GA) (N for 5-minute group = 15, N for 60-minute group = 15, N for total group = 30); Glubran 2 (GEM, Viareggio, Italy) (N for 5-minute group = 15, N for 60-minute group = 15, N for total group = 30); and Tisseel (Baxter Healthcare, Deerfield, IL) (N for 5-minute group = not available, N for 60-minute group = 15, N for total group = 15) under tension loading. The total group for each adhesive includes both 5-minute curing time and 60-minute curing time samples. Error bars represent the standard deviation of the mean. *Represents groups with statistically different mechanical properties ($P \leq 0.0002$).

24 hours. Three to five replicates of each treatment solution were added to the cells, and the experiment was repeated twice.

Neutral Red Uptake Assay. The neutral red uptake assays and data analyses were performed following the same protocol as described in the literature.[18] The cell viability of the negative control was arbitrarily set as 100%. The percent cell viability was determined by a relative colorimetric analysis of the treatment group with respect to the negative control group. The data analyses were performed with Microsoft Excel (Microsoft Office 2016, Microsoft Corporation, Redmond, WA), MatLab 2017b (MathWorks), and SAS 9.4 (SAS Institute Inc.).

RESULTS

Adhesive Strength

Results indicated that, for the same amount of strain, Glubran 2 could withstand greater stress than BioGlue or Tisseel at both time points tested. The averages of the maximum adhesive strength at failure (henceforth designated *adhesive strength*) of the adhesives in shear and tension are shown in Figure 3 and Figure 4. Figure 5 shows the three adhesives' failure mechanisms under tension loading. Glubran 2 and Tisseel dominate in adhesive failure where the adhesive fails along the interface between the tissue and the glue. BioGlue dominates in cohesive failure where the failure plane propagates within the adhesive. The post hoc pairwise *t* tests with Bonferroni adjustment of the analysis of variance (ANOVA) test indicated that, for both shear and tension tests, the three adhesives' adhesive strength values were significantly different ($P \leq 0.0002$). Similar trends were observed for tension tests and lap shear tests. The adhesive strength of all adhesives did not vary significantly with curing time ($P > 0.05$).

Biocompatibility

The cellular response of the vocal fold cells to the surgical adhesives is shown in Figure 6. Notably, Tisseel had the greatest cell viability, whereas Glubran 2 had the lowest cell viability. The industrial adhesive and the positive control had cell viability less than 8%. The growth control was greater than 97% and was very similar to the negative control. These two observations support the validity of the neutral red uptake assay. Because the viability of both Glubran 2 and BioGlue was lower than 70%, those two adhesives were considered cytotoxic. Indeed, biocompatibility tests showed that only Tisseel is nontoxic to vocal fold cells for the standard concentrations.

DISCUSSION

Adhesive Strength

Mechanical testing results in the current study are comparable to similar results reported in the literature. Kull et al.[19] tested the shear and tensile adhesive strength of Glubran 2 and Tisseel using the same protocols as in our study, with porcine skin as the tissue substrate. They reported an average shear strength of 32.6 ± 89 kPa and 2.2 ± 1.3 kPa for Glubran 2 and Tisseel, respectively.[19] They reported an average adhesive tensile strength of 21 ± 60 kPa and 0.7 ± 0.6 kPa for Glubran 2 and Tisseel, respectively.[19] Sidle and Maas[20] investigated the shear strength of BioGlue for attachment of periosteum on bones using human cadavers. They found BioGlue's shear strength was 45.9 ± 27.4 kPa.[20] Furthermore, Mehdizadeh et al.[21] have performed lap shear tests using fibrin glue on acellular porcine small intestine submucosa. They reported the adhesive shear

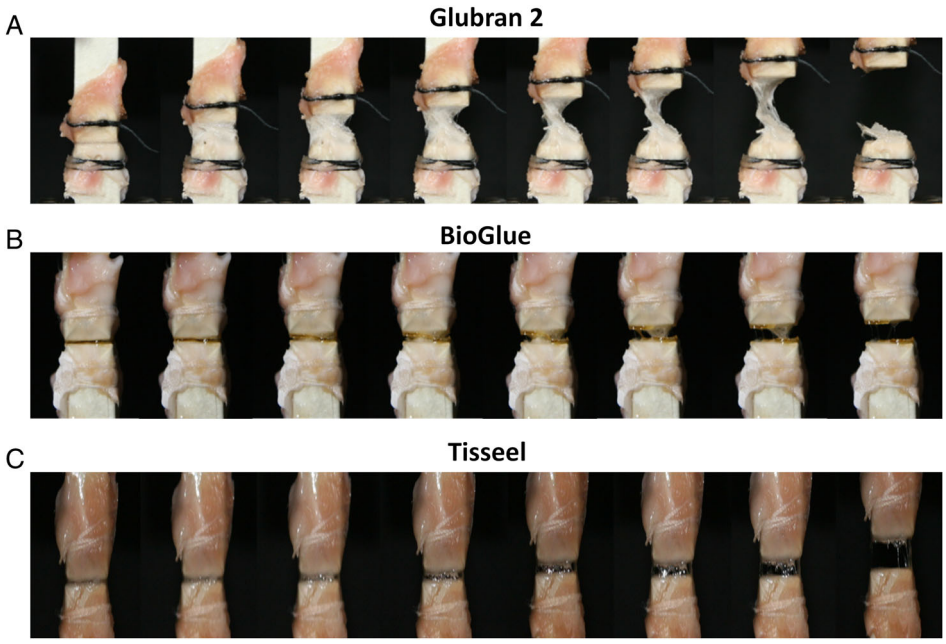

Fig. 5. Typical failure mechanisms of Glubran 2 (GEM, Viareggio, Italy), BioGlue (CryoLife, Kennesaw, GA), and Tisseel (Baxter Healthcare, Deerfield, IL) under tension loading. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

strength of fibrin glue was 15.4 ± 2.8 kPa.[21] The reported average adhesive strength in the literature for Tisseel ranges between 0.7 kPa and 15 kPa.[19,21–23]

For Glubran 2, the average shear and tensile strength in the current study is around twice the value reported by Kull et al.[19] BioGlue's shear strength was found to be very similar to that reported by Sidle and Maas.[20] For Tisseel, the average shear and tensile strength found in our study was approximately one order of magnitude greater than that from Kull et al.'s study [19] but falls within the range of values reported elsewhere.[21–23] The differences in tissue substrate are deemed to be the main factor explaining the differences.

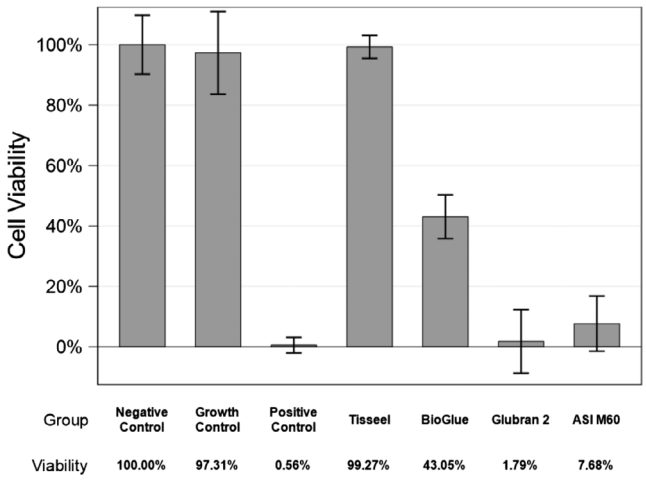

Fig. 6. Cell viability of BioGlue (CryoLife, Kennesaw, GA), Glubran 2 (GEM, Viareggio, Italy), and Tisseel (Baxter Healthcare, Deerfield, IL) for human vocal fold fibroblast cells. Error bars represent the standard deviation of the mean.

In Kull et al.'s study,[19] the standard deviations of the adhesives' average strength were approximately 60% to 270% of their mean strength values. This indicates that a large variability is not unusual in studies involving soft tissues. The wide range in the reported adhesive strength of Tisseel may be caused by differences in test tissue substrate and test protocol. Intrinsic variability in tissue and adhesive properties may also have contributed. It is possible that the adhesives are sensitive to the test environment. Changes in the external test conditions such as temperature and relative humidity may have affected the adhesives' final strength.

Influence of Curing Time

ANOVA tests yielded no significant differences between samples cured for 5 minutes and 60 minutes for BioGlue and Glubran 2. This shows that the strength of both adhesives was stable up to 1 hour after curing. For both adhesives, the manufacturers indicate that cross-linking starts within 20 to 40 seconds after application and reaches full strength within 2 minutes. Our test results confirmed that this is indeed the case. Tisseel, on the other hand, appeared to be very weak immediately after application.

Ninan et al.[24] have studied the adhesive strength of Tisseel and cyanoacrylate adhesives for curing time between 3 hours and 48 hours on porcine skin. They reported that Tisseel's maximum strength increased more than 100-fold from 0.01 MPa to 1.29 MPa when the curing time increased from 3 hours to 48 hours. For the 12-hour to 48-hour curing period, Tisseel's maximum strength increased approximately 6-fold, whereas the cyanoacrylate's adhesive strength increased by a factor of 1.12. This trend shows that the adhesive strength of

Tisseel is curing time-dependent within the first 48 hours of application, whereas cyanoacrylate-based adhesives' strength remains relatively stable after application. Although Ninan et al.'s study results cannot be directly compared to the current study due to differences in testing methods and test tissue, the trends reported in Ninan's study are probably relevant for the current study. This suggests that a longer curing time would have been required for Tisseel. But curing times longer than 1 hour are not desirable; in fact, they are not practical for surgical wound closure. Thus, changes in adhesive strength over longer time periods were not investigated in our study. Because the human body's wound-healing mechanisms occur over days, if not months, information regarding the adhesive's strength over longer time periods is valuable. However, it was deemed irrelevant for the foreseen clinical application.

Failure mechanisms

Digital photographs were taken to identify the failure mechanisms. For Glubran 2, the adhesive mostly failed along the interface between the tissue and the glue. Once the Glubran 2 is polymerized, it forms a stiff, opaque layer. Thus internal elastic forces dominated over the bonding forces on the tissue surface. As a result, the crack propagates along the tissue-adhesive interface until failure. Failed samples typically had glue on only one side.

BioGlue and Tisseel mostly exhibited cohesive failure, with the crack within the adhesive itself. It appeared that the internal strength within BioGlue was lower than the bonding strength at the interface between glue and tissue. Failed samples thus mostly had glue on both sides. Tisseel samples failed for smaller displacement values than Glubran 2.

Cytotoxicity

The cytotoxicity profiles of the three adhesives are in line with the trends in the literature, considering the variations in adhesive concentrations used. Montanaro et al.[18] performed the neutral red uptake assay on Glubran 2 for L929 cells (ATCC, NCTC clone CCL1). The cell viability was 7.4% $\pm$ 1.1%,[18] which is comparable to our study results. Glubran 2 is a cyanoacrylate-based adhesive. Adhesives in this class are known for their high cytotoxicity.[2,25] BioGlue is an albumin protein-based adhesive. Its crosslinker, glutaraldehyde, is a tissue fixative and has also been reported as a toxic substance.[26] Fürst and Banerjee[27] reported that the glutaraldehyde release concentration for BioGlue was between 100 to 200 g/mL, which led to cell viability of less than 10% on human embryo fibroblasts (MRC5).[27] Their in vitro animal study showed severe inflammation on lung and liver tissue.[27] In contrast, other studies have reported a favorable outcome after BioGlue applications for thoracic aortic repair and the sealing of bronchial anastomosis.[28–30] Hence, it may be concluded that BioGlue's biocompatibility is tissue-specific, and our viability results shows it may not be vocal fold tissue-friendly. In contrast, Tisseel, which is a natural protein-based material that is easily metabolized, is thus associated with high cell viability.[25] Chen et al.[31] also confirmed Tisseel's good biocompatibility to corneal tissues. In summary, Tisseel is the most vocal fold-friendly tissue adhesive among the three adhesives tested. Tisseel has value as a biological dressing. Its adhesive strength may be sufficient to hold the vocal fold epithelium within 1 hour after application for regular breathing, but perhaps not for situations such as loud phonation or coughing, for which mechanical stresses within the vocal folds are significant.

Limitations

The small size of the vocal folds has introduced variations in the test results. A smaller contact area makes the measured adhesive strength more sensitive to small change in sample shape and thickness. Variations in collagen content, surface smoothness, moisture content, and size were ignored, although they may have played a role.

For cytotoxicity tests, there is no common conventional value in the literature for the amounts of adhesives to use for cytotoxicity tests. Examples of the reported adhesives' dosages used for extraction or direct contact test are 5 mL/10^5 cells,[32] 1 drop (exact volume undisclosed),[31] 10 μL/mL,[33] 6 cm^2/mL,[18] and 22 mg/mL.[34] Our study used extraction amount of 200 mg/mL, as suggested by ISO 10093-12 for irregular-shaped solids. Because the adhesive's in vitro cytotoxicity is correlated with its extraction dosage, this makes it difficult to compare reported values in the literature.

The standardized ASTM and ISO protocols may not be perfectly representative of the adhesives' in vivo performances. Future excised larynx and animal studies are needed to further evaluate the adhesives' performance in physiological conditions.

CONCLUSION

The cytotoxicity and strength of three adhesives were evaluated for vocal fold closure. Glubran 2 had the greatest mechanical strength. Tisseel had the best biocompatibility. A negative correlation was found between adhesive biocompatibility and strength. The current study's results and methods will serve as benchmarks for synthesizing future customized vocal fold tissue adhesives that are currently under development.

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