Injectable Biomaterials Restore Vocal Fold Biomechanics and Muscle Volume after Laryngeal Nerve Injury

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Statement of Purpose: Vocal Fold augmentation (VFA) using injectable biomaterials is the clinical technique of choice to address glottal insufficiency. Injectable biomaterials have been developed for VFA to provide temporary (Eg. biodegradable polymers such as Carboxymethylcellulose (CMC)) or long term action (Eg. calcium hydroxyapatite (CaHa) and autologous fat) [1, 2]. While VFA can improve voice quality after nerve crush injuries; muscle volume restoration and mechanical properties of vocal fold after the augmentation are not fully characterized. In this study, CaHa (long term action) and CMC (short term action) were injected for VFA in swine models after nerve crush injury to test whether they reverse atrophy after recurrent laryngeal nerve (RLN) injury, maintain VF stiffness and restore native VF biomechanics.
Methods: Swine models with vocal fold atrophy were developed by performing a nerve excision of 2cm of the left RLN near the cricothyroid joint. Animals were divided into no augmentation (control), CMC, or CaHa augmentation groups at 14 days, then observed at 28, 56, or 84 days (n=3 per group/time/treatment). Biomechanical measurements (normal force, structural stiffness, and displacement at 1.96mN) were calculated using automated microindentation mapping with a grid overlay. Fixed specimen histological slides were matched to measurement locations. Histological evaluation was conducted using Hematoxylin and Eosin staining. Immunohistochemistry analysis was performed by staining with FluoroMyelin™ Red and DAPI to visualize the thyroarytenoid (TA) muscle, and its cross sectional area was quantified. Statistical analysis was conducted using two-way ANOVA (treatment and side) with Tukey's post hoc test. Figure 1 shows RLN model development and augmentation through local injection of biomaterials into the target muscle.

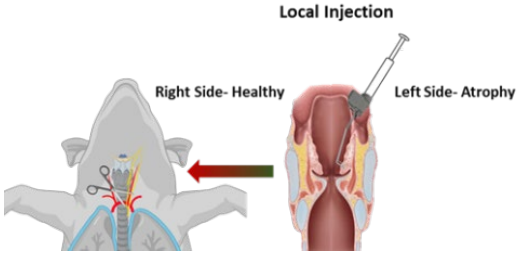

Figure 1. Schematic pictures of nerve injury and augmentation using injectable biomaterials.

Results: Across the VF mid-section, structural stiffness (mean, SEM) on the right (53.2mN/mm, 5.04) was greater than the left (25.5mN/mm, 5.04) after 28 days (p=0.003). After 28 days, increased left VF stiffness was seen with augmentation with CMC (64.1mN/mm, 9.9, p=0.049) and CaHa (66.7mN/mm, 8.1, p=0.020) compared to no intervention (Figure 2). Stiffness after CMC or CaHa augmentation was similar to uninjured VFs (p>0.84). Left TA muscle area decreased by 57.3% at 28 days and 45.5% at 84 days and did not vary between study groups (Figure 3 and 4).

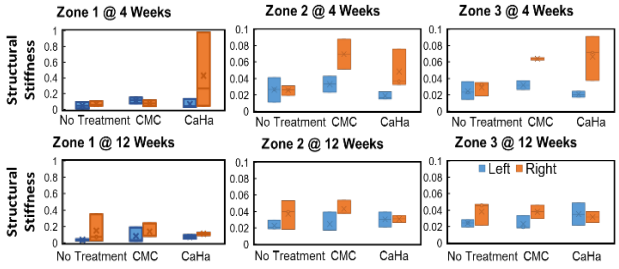

Figure 2. Local Structural Stiffness: Local mechanics changes with treatment after 28 and 84 days (zones 1-3 lie posterior-anterior along VF).

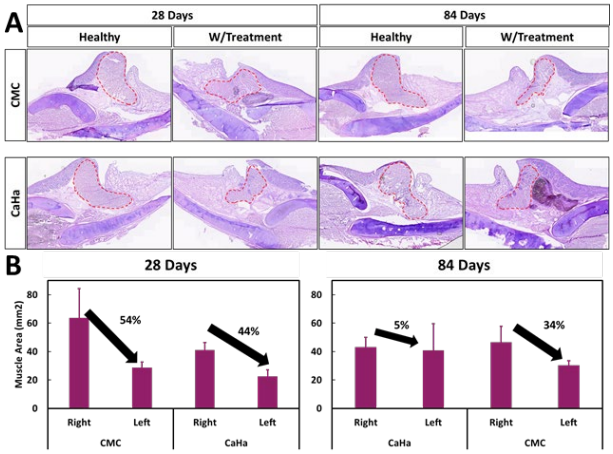

Figure 3. Histological Evaluation: VF Cross Sections H&E stained. (B) TA muscle area quantification of all groups after 28 and 84 days.

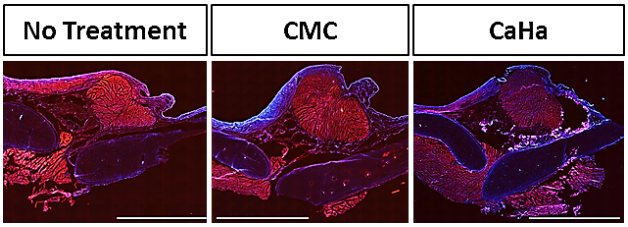

Figure 4. IHC Evaluation of tested groups after 84 days. Red and blue represent Myelin and DAPI, respectively.

Conclusions: VF biomechanical properties after CMC or CaHa augmentation were comparable to native VFs in this RLN injury model after 12 weeks. Improved restoration of muscle area was seen with CaHa. These data suggest VF augmentation restores native VF biomechanics and both CMC and CaHa result in similar outcomes.

Acknowledgements: This effort was supported in part by the US Air Force 59th Clinical Research Division and the Jacobson Endowment for Innovation & Entrepreneurship.

References: [1] Cohen, J. T., et al., " J Laryngol Otol J Laryngol Otol. 134.3 (2020): 263-269. [2] Lee, Mi-Sun, et al. Surg Radiol Anat 31.9 (2009): 649-655.