# Vibratory Stimulation for Anti-Fibrotic Therapy

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**Statement of Purpose:** During phonation, the human vocal folds are subjected to substantial mechanical stresses [1,2]. The presence of macrophages and myofibroblasts in histological samples from healthy patients suggests that normal vocal usage results in repetitive microtrauma that can be repaired without scarring or alterations in vocal quality [1,2]. Several recent studies have shown that externally applied vibration influences fibroblast expression of selected target genes associated with matrix metabolism [3-6]. However, the broader effects of vibration on fibroblast transcriptional activity have not been investigated. The objective of this study was to use microarray analysis to test the hypothesis that exposure to vibration activates an anti-fibrotic pattern of gene expression in fibroblasts.

**Materials and Methods:** Adult normal human dermal fibroblasts (NHDF, Lonza) were seeded on porous Tecoflex sponges (25 mm long × 3 mm thick × 5 mm wide) at $1.2 \times 10^6$ cells/sample. Substrates (n=4/group) were incubated under static conditions for 4 days, then transferred to bioreactors, subjected to a one-time 20% axial strain, and exposed to vibration (100 Hz, 1 mm amplitude) cultured for 6 hrs/day for 1 to 7 days. Samples maintained under static (floating) conditions or exposed to the one-time 20% strain for initial tensioning were included as controls. After vibratory stimulation, total RNA was isolated using RNeasy Mini kit (Qiagen), and gene expression profiles were examined by cDNA microarray. Microarray results were confirmed at the mRNA level by real time RT-PCR and at the protein level by ELISA and Western blotting. As a preliminary functional model, samples were subjected to cyclic strain or cyclic strain with vibration under tension. Collagen deposition was quantified by hydroxyproline assay and substrate mechanical properties were tested using an MTS Synergie 100 (MTS Systems Corp.) after 7 days of culture. Cell density was measured by Picogreen assay.

**Results:** Microarray analysis showed that 1350 genes were differentially expressed (fold change>2, $p<0.05$) between the vibration and the one-time strain control groups at 6 hours. Among these, vibration strongly induced the expression of the fibrotic cytokines TGFβ2, CTGF, and endothelin-1 (Fig. 1A). However, vibration also significantly affected a number of genes associated with the TGFβ signaling pathway, with a net effect of antagonizing TGFβ signal transduction. SMAD3 and TGFβR3 were significantly downregulated, while the inhibitory SMAD7 was significantly upregulated in response to vibration (Fig. 1 A and B). In addition, inhibitory transcription factors, SKIL and SIK1, were significantly activated, and TGFβR1 was simultaneously reduced over 7 days under vibratory loading (Fig. 1B). In general, cyclic strain activates the TGFβ pathway and promotes increased collagen synthesis and development of increased mechanical properties. Accordingly, when NHDF-seeded substrates were exposed to cyclic strain, significant increases were observed in hydroxyproline (Fig. 1C) and elastic modulus (Fig. 1D) relative to static controls. However, exposure to cyclic strain in combination with vibration reversed these effects-hydroxyproline and mechanical properties did not significantly differ from static controls. Cell density at 7 days was not significantly different between static, cyclic strain, and cyclic strain plus vibration groups (not shown).

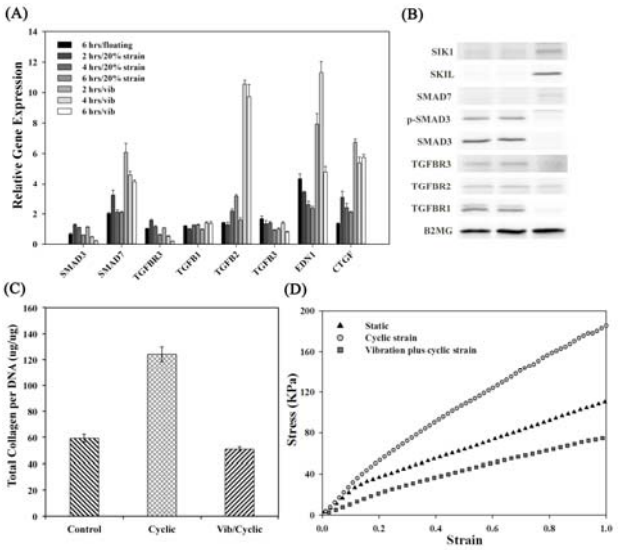


Figure 1. The effects of vibratory stimulation on gene/protein expression using real time RT-PCR (A) and Western blot analysis (B), and on collagen expression (C) and mechanical properties (D).

**Conclusions:** Exposure of fibroblasts to vibratory stimulation produced significant changes in matrix-related gene expression. Many of the new targets are consistent with the tissue physiology and anti-scarring activity of the vocal mucosa with upregulation of multiple proteases and downregulation of collagens. The strong induction of the inhibitory activities in TGFβ signaling pathway was observed, while pro-fibrotic cytokines exhibited strong sensitivity responsive to vibratory amplitude. Our future efforts will be focused on the development of novel therapies for fibrotic disease.

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**References:** 1. Catten et al. *Otolaryngol Head Neck Surg* 1998;118:663–7. 2. Gray et al. *Otolaryngol Clin North Am* 2000;33:679–98. 3. Titze et al. *J Biomech* 2004;37:1521–9. 4. Wolchok et al. *Biomaterials* 2009;30:327–35. 5. Kutty et al. *J Tissue Eng Regen Med* 2010;4:62–72. 6. Farran et al. *J Tissue Eng Regen Med* 2013;7:213–25.