Optimization of a Biomolecule-Based Therapy to Induce Motor Endplate Formation for Treatment of Vocal Fold Paralysis

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Background and Hypothesis:
Vocal fold paralysis (VFP), often caused by recurrent laryngeal nerve (RLN) damage from surgery, scarring, or malignancy, can impair speech, swallowing, and breathing. Current treatments—surgery, injections, and voice therapy—offer symptom relief but do not restore function. Previous work in our lab showed that muscle progenitor cells (MPCs) can be induced into motor endplate-expressing cells (MEEs) using a biomolecule cocktail of agrin, neuregulin (NRG1), and acetylcholine (ACh). The formation of motor endplates triggers the release of neurotrophic and angiogenic factors like endothelial nitric oxide synthase (eNOS). Injecting MEEs into porcine larynges improved neuromuscular junction (NMJ) function, and direct injection of the biomolecule cocktail showed similar effects in mice. However, the optimal concentrations for therapeutic effects remain unknown. We hypothesized that specific combinations or doses of these biomolecules would significantly increase motor endplate formation as demonstrated by upregulated nicotinic cholinergic receptor subunit alpha 1 (CHRNA1) RNA and protein expression, with resultant upregulation of eNOS RNA.

Methods:
Using two human skeletal muscle cell lines, we tested 51 biomolecule treatment variations. CHRNA1 and eNOS levels were quantified using Dot blots and qPCR.

Results:
Three biomolecule combinations notably increased expression of CHRNA1 and eNOS, suggesting enhanced potential for NMJ restoration and muscle regeneration.

Conclusion and Impact:
Select biomolecule cocktails significantly upregulate key markers for muscle repair and angiogenesis. These findings support their potential as therapeutic agents for VFP and related neuromuscular disorders.