Elastin Mimetic Hybrid Polymers for Vocal Fold Tissue Engineering

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Statement of Purpose: The human vocal folds are consisted of a pliable vibratory layer of connective tissue (lamina propria, LP) that controls the production of sound in humans and can sustain up to 30% strain at high frequencies of 100 to 1000 Hz.[1] Mechanical stresses, deleterious environment factors and pathological conditions can disrupt the natural pliability of the vocal folds, resulting in a wide spectrum of voice disorders. Tissue engineering methods hold promise for the restoration of functional vocal folds, but the unique viscoelastic properties of vocal folds pose significant challenges in designing synthetic scaffolds. Traditional synthetic scaffolds employed for vocal fold tissue engineering usually exhibit inferior mechanical properties for these applications, tending to fail catastrophically and demonstrating insufficient ability to recover from mechanical failure.

Elastin is one of the major fibrous components of the LP ECM, proving tissue with high elasticity and mechanical robustness.[1] The useful mechanical properties of elastin arise largely from highly flexible hydrophobic segments, which have many transient structures that easily change conformation when stretched; as well as from alanine- and lysine-rich α-helical segments that form covalent cross-links between adjacent molecules to support the elastic network.[2] Our aim is to develop a novel means to regenerate functional vocal fold tissue by creating an elastomeric matrix that not only emulate the native elastin but also exhibit improved stability and flexibility.

To this end, we have synthesized elastin mimetic hybrid polymers (EMHP) with an alternating multiblock architecture composed of alanine-rich, lysine-containing peptide and synthetic polymer that is flexible and biocompatible.[3] Cell adhesive EMHP was synthesized using RGD-containing peptide as the building blocks. The crosslinked EMHPs (xEMHP) exhibit mechanical properties similar to that of a commercial polyurethane elastomer. Porcine vocal fold fibroblasts (PVFFs) cultured on RGD-containing xEMHP were able to attached to the substrate and proliferate normally.

Methods: The synthesis of azide-functionalized PEG (N_3-PEG-N_3) and alkyne-terminated peptide with the amino acid sequence $X(AKAAAKA)_2X$ (X = Fmoc-L-propargylglycine) can be found in our previous publication.[3] RGD-containing peptides with the sequence $X(AKAAAKA)_2XGRGDG$ were synthesized in the same manner. Multiblock hybrid copolymers were constructed by condensation polymerization of N_3-PEG-N_3 and alkyne-functionalized RGD-containing peptide using Cu(I)-catalyzed alyne-azide cycloaddition (CuAAC). The resulting hybrid copolymers were crosslinked by bis(sulfosuccidimidyl)suberate (BS3) at room temperature. Compression and tensile tests were conducted with an RSA III instrument (TA Instruments). PVFFs were seeded on the cross-linked materials for 3 days. Cell viability and proliferation were monitored by live/dead assay and Cell Titer Blue assay, respectively. Cell morphology was evaluated by actin staining.

Results/Discussion: Multiblock EMHPs were synthesized by a condensation polymerization method involving CuAAC between the azide end groups of PEG and the alkyne end groups of peptides containing an $X(AKAAAKA)_2XGRGDG$ repeats, as shown in Figure 1. ^1H NMR analysis of the resulting RGD-containing EMHPs showed the presence of both PEG and peptide domains. FT-IR analysis indicated the disappearance of the azide peak at 2100 cm^{-1} and the appearance of Amide I and Amide II bands in EMHP. The molecular weight increase was shown by SEC and SDS-PAGE. It was found that the presence of the RGD sequences in the peptide domains increased the molecular weight of the EMHPs that were formed compared to EMHPs synthesized using peptides without RGD. The swelling ratio of the RGD-containing EMHPs was also up to two times higher than that of EMHPs without RGD sequences. The water-swollen xEMHPs exhibit compressive modulus similar to that for TecoflexTM SG80A).

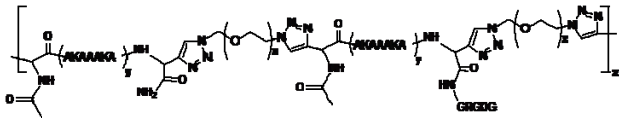

Figure 1. Synthesis of RGD-containing elastin mimetic PEG-peptide multiblock copolymers.

Cells were seeded on cross-linked EMHP hydrogels with or without RGD, and the cell viability, attachment, and proliferation were closely monitored. Our preliminary data show that PVFFs were able to attach and proliferate on the crosslinked, RGD-containing hybrid copolymers. The mechanical properties of the crosslinked hydrogels are comparable to a commercially available polyurethane elastomer (TecoflexTM SG80A).

Conclusions: Cell-adhesive, EMHPs were synthesized using RGD-containing peptides and oligomeric PEG by condensation polymerization using CuCAAC. Covalent crosslinking of the resulting polymers afforded an elastomeric material that facilitates PVFF attachment and proliferation. The tunability in mechanical and biological properties is a useful feature of EMHPs that makes them attractive candidates for use as tissue scaffolds for vocal fold tissue engineering.

References:

(1).Gray, S. et al. *Ann. Otol., Rhinol., Laryngol.* **1995**, *104*, 13. (2). Bellingham, C. M. et al. *Curr. Opin. Solid State Mat. Sci.* **2004**, *8*, 135. (3) Grieshaber, S. E. et al. *Macromolecules* **2009**, *42*, 2532.