

Influence of hydrogel mechanical properties and mesh size on vocal fold fibroblast extracellular matrix production and phenotype

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Abstract

Current clinical management of vocal fold (VF) scarring produces inconsistent and often suboptimal results. Researchers are investigating a number of alternative treatments for VF lamina propria (LP) scarring, including designer implant materials for functional LP regeneration. In the present study, we investigate the effects of the initial scaffold elastic modulus and mesh size on encapsulated VF fibroblast (VFF) extracellular matrix (ECM) production toward rational scaffold design. Poly(ethylene glycol) diacrylate (PEGDA) hydrogels were selected for this study since their material properties, including mechanical properties, mesh size, degradation rate and bioactivity, can be tightly controlled and systematically modified. Porcine VFF were encapsulated in four PEGDA hydrogels with degradation half lives of ~25 days, but with initial elastic compressive moduli and mesh sizes ranging from ~30 to 100 kPa and from ~9 to 27 nm, respectively. After 30 days of static culture, VFF ECM production and phenotype in each formulation was assessed biochemically and histologically. Sulfated glycosaminoglycan synthesis increased in similar degree with both increasing initial modulus and decreasing initial mesh size. In contrast, elastin production decreased with increasing initial modulus but increased with decreasing initial mesh size. Both collagen deposition and the induction of a myofibroblastic phenotype depended strongly on initial mesh size but appeared largely unaffected by variations in initial modulus. The present results indicate that scaffold mesh size warrants further investigation as a critical regulator of VFF ECM synthesis. Furthermore, this study validates a systematic and controlled approach for analyzing VFF response to scaffold properties, which should aid in rational scaffold selection/design.

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1. Introduction

Although no firm statistics exist, voice disorders, including scarring of the vocal fold (VF) lamina propria (LP), are estimated to affect over 3% of the population to various degrees [1]. The VFs are paired, multi-layered structures (Fig. 1), each consisting of underlying muscle, followed by the LP and overlying epithelium [2]. When the VFs are brought together by the intrinsic laryngeal muscles, they can be set into vibratory motion by airflow

from the lungs. Ordered oscillation yields efficient cycle-to-cycle closure of the VFs and high-quality voice [3]. The human LP is generally subdivided into superficial (SLP), intermediate (ILP) and deep (DLP) layers (Fig. 1) [4,5]. At normal pitch and loudness, the SLP is believed to “slide” over the ILP, undergoing the high frequency and strain excursions required for cyclic VF closure [4,5]. When the pliability and physical volume of the VF SLP are reduced by scarring, voice changes ranging from hoarseness to complete voice loss result, depending on the severity of scar [3,6–8].

VF scar has proven difficult to treat with current surgical techniques and standard augmentation substances (e.g.

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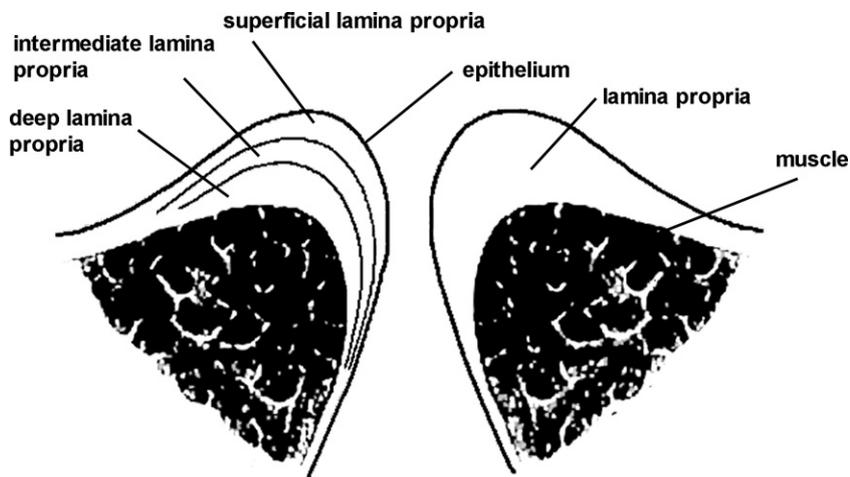


Fig. 1. Schematic of a coronal section through the human vocal folds.

collagen and fat) [3,6,9]. As such, researchers are actively exploring alternative treatment routes, including the development of designer implants for functional LP regeneration [10–15]. Although it is understood that scaffold properties, such as bioactivity [16], mesh size [17], mechanical properties [18–20] and degradation rate [21] critically impact cell behavior, it has proven difficult to directly attribute alterations in VFF fibroblast (VFF) response to specific changes in scaffold parameters. This situation has hampered rational selection/design of implant materials for LP regeneration. The aim of the present study is to validate an approach for the systematic and quantitative assessment of the influence of scaffold properties on VFF behavior. Specifically, the current work focuses on the impact of initial scaffold mesh size and mechanical properties on VFF extracellular (ECM) production and phenotype. Ultimately, these data will be applied toward the rational design of LP regeneration matrices.

Central to these studies is the selected scaffold material, poly(ethylene glycol) diacrylate (PEGDA). PEGDA hydrogels have several properties which make them appropriate for systematic exploration of cell response to specific alterations in scaffold material properties. Pure PEGDA hydrogels function as biological “blank slates”, meaning that they do not significantly adsorb bioactive plasma proteins. Thus, these hydrogels do not promote cell interaction without the specific conjugation of biochemical stimuli to the scaffold [22]. This is significant since most synthetic and natural scaffolds adsorb a range of bioactive proteins from serum (in the *in vitro* setting) or from plasma (in the *in vivo* setting). These adsorbed proteins are often major determinants of cell behavior, in addition to any bioactive moiety deliberately conjugated or adsorbed to the scaffold [23,24]. In contrast, the biological “blank slate” nature of PEGDA hydrogels permits the controlled and defined investigation of bioactivity on cell behavior. In the present study, we explored the effects of initial PEGDA scaffold modulus and mesh size in the presence of constant initial levels of cell adhe-

sion peptide, RGDS, thus removing initial scaffold bioactivity as a design variable [25].

An additional benefit of PEGDA hydrogels is the ability to tune their initial mesh size and mechanical properties over a broad range simply by varying the molecular weight (mol. wt.) and/or concentration of PEGDA [26]. Moreover, the degradation rate of PEG-based hydrogels can be systematically tailored. Pure PEGDA hydrogels degrade by hydrolytic cleavage of the ester bonds between the aliphatic PEG polymer backbone and the crosslinking units [27,28]. Variations in this degradation rate can be achieved by conjugating α -hydroxy acids or enzymatically cleavable peptides to the PEG macromer backbone [27,29]. Thus, PEG-based hydrogels have the property that their bioactivity, mesh size, modulus and degradation rate can each be systematically tuned [17,21,26,30], a critical property for the proposed studies.

In the present work, porcine VFF were encapsulated in four PEGDA hydrogel formulations with initial elastic compressive moduli ranging from ~ 30 to 100 kPa and with initial mesh sizes ranging from ~ 9 –27 nm. To simplify the investigation of the dependence of VFF ECM synthesis and phenotype on scaffold properties, PEGDA hydrogel formulations with similar degradation rates were selected [28]. After 30 days of static culture, VFF collagen, elastin and sulfated glycosaminoglycan (sGAG) production as well as VFF phenotype in each hydrogel formulation were analyzed using biochemical and histological techniques.

2. Material and methods

2.1. Polymer synthesis

PEGDA was prepared as previously described [31] by combining 0.1 mmol ml⁻¹ dry PEG (8 or 10 kDa, Fluka), 0.4 mmol ml⁻¹ acryloyl chloride and 0.2 mmol ml⁻¹ triethylamine in anhydrous dichloromethane (DCM) and stirring under argon overnight. The resulting solution was washed with 2 M K₂CO₃ and separated into aqueous

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