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Review

Tissue engineering-based therapeutic strategies for vocal fold repair and regeneration

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ABSTRACT

Vocal folds are soft laryngeal connective tissues with distinct layered structures and complex multi-component matrix compositions that endow phonatory and respiratory functions. This delicate tissue is easily damaged by various environmental factors and pathological conditions, altering vocal biomechanics and causing debilitating vocal disorders that detrimentally affect the daily lives of suffering individuals. Modern techniques and advanced knowledge of regenerative medicine have led to a deeper understanding of the microstructure, microphysiology, and micropathophysiology of vocal fold tissues. State-of-the-art materials ranging from extracecullar-matrix (ECM)-derived biomaterials to synthetic polymer scaffolds have been proposed for the prevention and treatment of voice disorders including vocal fold scarring and fibrosis. This review intends to provide a thorough overview of current achievements in the field of vocal fold tissue engineering, including the fabrication of injectable biomaterials to mimic *in vitro* cell microenvironments, novel designs of bioreactors that capture *in vivo* tissue biomechanics, and establishment of various animal models to characterize the *in vivo* biocompatibility of these materials. The combination of polymeric scaffolds, cell transplantation, biomechanical stimulation, and delivery of antifibrotic growth factors will lead to successful restoration of functional vocal folds and improved vocal recovery in animal models, facilitating the application of these materials and related methodologies in clinical practice.

1. Introduction

The human vocal folds (VF) consist of a pliable vibratory layer of connective tissue called the lamina propria (LP) that controls the production of sound, can sustain up to 30% strain at frequencies of 100–1000 Hz (Hz), and can reversibly recover after transient stretch to high strain [1–4]. Mechanical stresses, deleterious environmental factors and pathological conditions can disrupt the natural pliability of the vocal folds, resulting in a wide spectrum of voice disorders [5–7]. Voice disorders are the most common communication disorder across the lifespan and dysphonic suffering in individuals causes significant negative influences on their social interactions and daily personal lives [6]. While surgical techniques and behavioral treatments are currently employed to treat voice disorders, surgical interventions can cause scarring and yield inconsistent results [5,8]. Thus, tissue regeneration methods combining the use of bioactive factors, injectable scaffolds, and stem cell therapy have remained of significant research interest in the development of methods to treat vocal fold scarring and vocal fold replacement [9,10], although challenges in integrated treatment of the mechanical and biological properties of materials has limited the success of these approaches. In this article, we review recent advances and progress in vocal fold repair and regeneration

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from a tissue engineering-based therapeutic perspective, focusing on (1) biomaterials intended to mimic the mechanical properties of native VF tissues; (2) bioreactor designs that capture dynamic vocal biomechanical properties, and (3) both *in vitro* and *in vivo* applications of these biomaterials, informed by results from bioreactor studies, in vocal fold tissue engineering.

2. Vocal fold

2.1. Vocal fold scar and societal significance

Numerous and common stimuli can lead to vocal fold dysfunction and damage, including voice overuse, chemical exposure (e.g., smoke inhalation), gastroesophageal reflux, allergies, intubation, traumas, radiation and inflammation [7,11,12]. Benign vocal fold lesions such as nodules and polyps that can result from these stimuli are usually limited to the SLP, and although temporary relief can be achieved by rest or voice therapy, surgical intervention may be required, which can lead to scar formation, reduced flexibility of the LP, and impaired voice production [6,12,13].

Voice disorders are the most common communication disorder across the lifespan and dysphonic suffering in individuals causes significant negative impact on their social interactions and daily lives. There is a paucity of epidemiological data in the literature that is specific to vocal fold scarring and its impact on vocal use. It is, however, well documented that up to 9% of the general population has some type of voice abnormality and that 29% of the general population will develop a voice disorder during their life [8,12,13]. Approximately 10% population of the United States work force involves heavy voice use for their occupation; reaching roughly 28 million people suffering daily voice discomfort causing them to miss work, apply for disability insurance or change occupations [8,12–14]. When costs related to lost work days and treatment expenses, excluding other monetary costs related to pharmaceutical treatment, workman's compensation and change/loss of job are considered, societal expenditures of voice problems in teachers alone has been estimated to be of the order of $2.5 billion annually in the United States [12,15,16]. Optimal treatment for voice disorders has not yet been realized, however, and new approaches warrant consideration for regeneration of functional vocal fold tissue.

2.2. Vocal fold anatomy and composition

The larynx comprises a number of types of tissue including, but not limited to, cartilage, muscles, epithelium, nerve and stroma and is positioned above the trachea and esophagus. The vocal folds are positioned within the larynx and their primary role is to protect the airway, whereas their secondary role is to produce voice. Arytenoid cartilages adduct and abduct to bring the vocal folds together and apart; the vocal folds are abducted (open) during breathing and adducted (closed) during phonation [11]. The tissues vibrate in a wave-like motion during sound production [1,17–20], and one of the keys to this mechanical versatility lies in their unique structure and multi-layered composition.

Each vocal fold consists of a stratified squamous epithelium, matrix-rich lamina propria, and thyroarytenoid muscle (Fig. 1) [1,10]. The epithelium is composed of stratified squamous cells and has a primary role of protecting the deeper layers of tissue, and as a result this layer undergoes repeated turnover [21]. The lamina propria (LP) is a collagen and elastin-rich, pliable vibratory layer of connective tissue positioned between epithelium and muscle tissue [22], sub-categorized into three distinct layers: superficial lamina propria (SLP), intermediate lamina propria (ILP), and deep lamina propria (DLP). The outermost layer of the LP, the SLP, sustains the majority of stress during vocal fold vibration [2]. However, these three layers are not distinctly separated to the naked eye in human and animals. The SLP is believed to move freely over the layers beneath it. Deeper layers become increasingly dense with collagen and elastin with the ILP and DLP containing the highest concentration of elastin and collagen [23,24]. The thyroarytenoid muscle is positioned next to the DLP. Vocal fold scar disrupts the mucosal wave, or the movement of the LP, due to increased stiffness and viscosity and can also result in incomplete vocal fold closure [5]. The combined effects of increased stiffness and viscosity cause impaired vibration resulting in voice changes including hoarseness and fatigue [25,26].

2.3. Phonation physiology and mechanical properties of vocal folds

Vocal folds can be described as two biomechanical layers: the body (thyroarytenoid muscle and DLP) and the cover (epithelium and SLP) [26,28,29], with a main difference between the cover and body layers arising from differences in their oscillation properties. The cover is characterized by its pliability and propagation of oscillatory waves in response to the contraction of the stiff body layer [30,31]. The physiology of phonation that arises from the motion of the vocal folds was first explained by the myoelastic theory of voice production, a theory first developed by Johannes Muller and later modified by Van de Berg [26]. This theory describes the build-up of subglottic air pressure created by the lungs to drive the vocal folds apart. The minimum amount of lung pressure to initiate vocal fold abduction is known as the phonation threshold pressure (PTP) [32,33]. In simple terms, the Bernoulli effect causes a drop in pressure in the glottis which, in combination with their elastic properties, serves to drive the vocal folds back together [26,34]. More recently, Titze expanded this theory, demonstrating that the Bernoulli effect is only a minor contributor to the initiation of vocal fold oscillation. Rather, flow induced oscillation allows for sustained oscillation secondary to a continual transfer of energy from the glottal airstream to the tissue, overcoming frictional energy losses in the vocal folds [33]. Mass and stiffness combined with the geometric properties of the vocal folds control self-sustained oscillation. Changes in air pressure across the glottis with convergent and divergent configurations results in asymmetry of air pressures necessary for sustained oscillation. Further comprehensive reviews are recommended for interested readers [17,33,35].

3. Current clinical approaches and evaluation of biomaterials for vocal treatment

Several approaches exist for the treatment of voice disorders including voice therapy, surgery, or some combination of the two. The clinical approach targets for treatment depends on factors such as the pathology's severity and impact as well as the patient's individual needs and motivation for improved voice.

3.1. Voice therapy and surgical approaches

Behavioral voice therapy may be offered to balance the respiratory, phonatory, and resonance subsystems, optimize vocal quality, and reduce vocal effort/fatigue. Providing education about vocal hygiene can also assist the patient in management of secondary contributing factors (reflux, hydration, excessive voice use, etc) [6]. Currently, there is no research validating a specific method of voice therapy for treatment of vocal fold scarring. Behavioral voice therapy may be implemented as a preventative measure to limit further damage to the scarred tissue and voice quality. The patient may develop compensatory hyperfunctional behaviors (e.g.