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Review

Cell therapy and scarred vocal folds

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ABSTRACT

Vocal fold microstructure is complex and can be affected by laryngeal microsurgery, inducing scarring that prevents mechanical uncoupling of epithelium and muscle, leading to vibration disorder and disabling dysphonia. Treatment options presently are few, and often without efficacy for vibration, having only an impact on volume to reduce glottal closure defect. The present review of the literature had two aims: (i) to report the current state of the literature on cell therapy in vocal fold scarring; and (ii) to analyze the therapeutic interest of the adipose-derived stromal vascular fraction in the existing therapeutic armamentarium. A PubMed® search conducted in September 2016 retrieved English or French-language original articles on the use of stem cells to treat vocal fold scarring. Twenty-seven articles published between 2003 and 2016 met the study selection criteria. Mesenchymal stem cells were most widely used, mainly derived from bone marrow or adipose tissue. Four studies were performed in vitro on fibroblasts, and 18 in vivo on animals. End-points comprised: (i) scar analysis (macro- and micro-scopic morphology, viscoelastic properties, extracellular matrix, fibroblasts); and (ii) assessment of stem cell survival and differentiation. The studies testified to the benefit of mesenchymal stem cells, and especially those of adipose derivation. The stromal vascular fraction exhibits properties that might improve results by facilitating production logistics.

1. Introduction

Vocal fold microstructure [1] is complex, particularly due to its foliated organization. According to Hirano's body-cover theory [2], the vocal folds comprise a superior layer ("cover") composed of epithelium, basal membrane and the superior part of the lamina propria, and an inferior layer ("body") composed of the deep lamina propria and thyroarytenoid muscle, the two being separated by the intermediate layer of the lamina propria. This specific architecture allows the two functional units to vibrate independently, and is found in the mid-part of the vocal folds; the anterior and posterior regions, which are the site of maculae flavae, show a different architecture which acts as a buffer. The proportions and organization of the extracellular matrix components largely determine the mechanical properties of the vocal folds. The superficial layer of the lamina propria is mainly composed of amorphous material poor in collagen fibers and elastin; the intermediate layer contains more elastic fibers, and the deep layer more collagen fibers.

Following laryngeal microsurgery, vocal fold scarring is sometimes observed, due to partial disappearance of the lamina propria, with the superficial and/or intermediate layer replaced by fibrous tissue, preventing mechanical uncoupling of the epithelium and muscle and thereby inducing vibration disorder [1]. Scar tissue may also be found without iatrogenic etiology: congenital or, more often, acquired, via a mechanism similar to that of cutaneous vergetures or as a result of trauma or chronic inflammatory phenomena.

Pathological scarring comprises 3 phases [3]: inflammation, proliferation and remodeling. The first phase implicates inflammation factors such as interleukin 1β or TNFα, synthesized 4 to 8 hours after injury. There is then increased expression of hyaluronan synthase 1 and 2, procollagen I and III and cyclo-oxygenase 2, followed by massive recruitment of cells, mainly with fibroblastic characteristics, derived from the macula flava or remote tissue such as bone marrow. Density peaks at day 5-7, but may fail to fully restore the vocal fold [1]. The fibroblasts then differentiate into myofibroblasts;

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the collagen and elastin bundles become disorganized, losing parallelism; the density of elastin, hyaluronic acid, fibromodulin and decorin diminishes; fine type III collagen is replaced by type I; and fibronectin density increases [1]. This is often accompanied by loss of volume and glottal defect.

The vocal impact is disabling, especially for professional communicators, while current treatment possibilities are limited as the great complexity of vocal fold microstructure hinders the development of effective therapy. Resection of fibrous tissue often has poor results due to potential re-adhesion. Injecting hyaluronic acid has a positive short-term effect on viscoelasticity, but with limited tissue duration [4,5]. Autologous graft, essentially of fat and fascia, has excellent biocompatibility: fat is easy to harvest and is well tolerated, but fatty tissue injected using Coleman's technique is liable to resorb. These techniques do not restore the suppleness of the vibrating part of the fold and serve only to augment volume to counter glottal closure defect.

The sliding plane could be restored by cell therapy techniques, injecting stem cells into the vocal folds. A search of PubMed Central® in September 2016 retrieved articles in English or in French concerning the laryngeal impact of stem cells, with 27 articles published between 2003 and 2016. The search-terms were "fibrosis", "stem cells", "adipose-derived stem cells", "mesenchymal stem cells", "scarred vocal folds", "vocal cords", "cicatrix", and "tissue engineering". Articles focusing on acellular proregenerative substances were excluded, as were those dealing with laryngeal cell therapy for conditions other than vocal fold scarring. Only original articles reporting an experimental protocol, and not literature reviews, were analyzed.

The aim of the present review was two-fold:

- to describe the progress of cell therapy in vocal fold scarring;
- to assess the therapeutic interest of the adipose-derived stromal vascular fraction compared to other cell therapy substances.

2. Discussion

2.1. Mesenchymal stem cells: an option of choice for the treatment of vocal fold scarring

The presence of mesenchymal stem cells has been reported in many tissues in adults. These are pluripotent cells, able to generate various tissues from the same embryonic germ layer. They were first isolated in bone marrow [6], and the term "mesenchymal stem cell" (MSC) was introduced in 1990, with reference to the mesenchyme, derived from the mesoderm, one of the 3 embryonic germ layers from which bone marrow develops.

Our literature search found that the most widely used stem cells were mesenchymal, derived from two main sources: bone marrow (BM-MSC: bone marrow mesenchymal stem cells) in 13 cases, and adipose tissue (ADSC: adipose-derived stem cells) in 14 cases. Large amounts of adipose tissue can be obtained by simple liposuction, making harvesting less demanding or invasive. MSCs are about 8 times as frequent in adipose tissue as in bone marrow: 5000/mL versus 600/mL [7]. Two studies compared BM-MSCs versus ADSCs [8,9], using an embryonic stem cell line [10,11]. More originally, another study used MSCs harvested from canine epiglottis [12]: the vocal folds contain MSCs (stellate cells), especially in the maculae flavae [1], and the authors hypothesized that they are also present in the epiglottis, which presents a larger harvesting area than in the vocal folds.

Four of the 27 studies were exclusively in vitro (Table 1), focusing on MSC impact on animal or human vocal fold fibroblasts. The other 23 studies included in vivo experimentation, with stem cell injection to the vocal folds (Table 2), exclusively in small animals (mouse, rat) or large animals (rabbit, dog); no injections have yet been reported in humans.

2.1.1. In vitro experimentation

Kumai et al. [13,14] showed that scar tissue fibroblasts produce more collagen and proliferate more rapidly than normal fibroblasts. He then showed that these fibroblasts, co-cultured with ADSCs, proliferate less and express less α-smooth-muscle actin (SMA), a myofibroblast differentiation marker. They produce an extracellular matrix with less collagen and more hyaluronic acid and hepatocyte growth factor (HGF). HGF neutralization inhibits the effect of the ADSCs on collagen production, confirming the known antifibrotic properties of HGF. Its role in the larynx has been the focus of several studies [15,16]. Chen and Thibeault [17] co-cultured healthy and scarred vocal fold fibroblasts with BM-MSCs associated to hyaluronic acid hydrogel; fibroblast proliferation was inhibited, without impact on morphology or viability. Transcriptome study confirmed increased HGF and *vascular endothelial growth factor* (*VEGF*) gene expression. Unlike Kumai et al., Chen et al. found increased type I collagen production in the presence of BM-MSCs. Hiwatashi et al. [18] studied the impact of TGFβ1 expression by co-culturing normal vocal fold fibroblasts with ADSCs or BM-MSCs with or without TGFβ1. TGFβ1 is a cytokine that plays a major role in the inflammatory cascade triggered by vocal fold injury. Hiwatashi et al. found that MSCs regulated extracellular matrix composition, notably by reducing type I and III collagen levels, and inhibited TGFβ1 expression and differentiation toward myofibroblasts, by reducing α-SMA levels.

2.1.2. In vivo experimentation

Most in vivo preclinical studies (Table 2) used stem cell xenografts (11 studies) of human origin (bone marrow or embryo) or from mouse or rabbit (bone marrow). Some used tacrolimus immunosuppression to avoid rejection [10,11,19,20]. Fewer used autograft (8 studies) or conspecific stem cell allograft (4 studies). Several used bioengineered products to potentiate MSC effects or promote MSC survival: hydrogels (collagen, atelocollagen, fibrin, hyaluronic acid, associated or not [21–26]), atelocollagen sponge [9,27], synthetic extracellular matrix [28] or acellular gel derived from pig jejunum submucosa [29]. One study associated HGF to MSCs [23]. Only 1 team systematically administered antibiotics with corticosteroids by injection [30].

Overall, animal models were similar in the various studies: a scar was performed by laser, electrocautery or cold instruments in one or both folds up to the thyroarytenoid muscle. Only De Bonnecaze et al. [31] performed cervicotomy with median laryngotomy. The vocal folds were usually injected with conditioned or non-conditioned cells or control solution immediately after injury, but at a longer interval in some studies: from 4 days to 18 months in Angelou et al.'s study [30]. The latter more closely matched clinical practice in humans, where vocal fold scarring should not be treated for at least 6 months after surgery [32]. In 2 studies, injection was performed preventively, 4 days before injury [24,25]. When cells were associated to an atelocollagen sponge, injection was replaced by vocal fold incision, positioning the sponge within the subepithelial space [9,27]. The interval between injection and endoscopy and/or euthanasia, on the other hand, varied widely between 2 weeks and 12 months. Study objectives comprised:

- assessment of the effect of MSC injection on scarring by macro- and micro-scopic study of the vocal folds, and assessment of viscoelasticity, extracellular matrix and fibroblast proliferation, differentiation and survival;
- assessment of differentiation and survival of the injected cells, using an immunofluorescent marker.

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