Development of A Tissue Engineered Vocal Fold Cover Replacement from Rabbit Adipose Derived Stem Cells

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Abstract

Tissue engineering of a vocal fold replacement is a promising potential treatment for severe vocal fold scarring. Development and testing of such a tissue construct is an ongoing project. We have previously demonstrated that human adipose derived stem cells (ASC) can produce a bilayered construct with suitable properties for implantation and phonation. This phase of the project developed a similar construct for pre-clinical animal studies, using rabbit cells.

Rabbit adipose derived stem cells were isolated, cultured and embedded in fibrin gels under air-liquid interface conditions with epidermal growth factor. After culture periods of one to four weeks, constructs were harvested, sectioned and examined.

Results: Rabbit cells attached and survived within rabbit fibrin gels which were capable of handling and suturing. rASC and human cryoprecipitate were not capable of handling. Osteogenic and adipogenic differentiation was induced in cultured ASC, confirming their multipotency. Differentiation to these unwanted lineages was not seen in vocal fold tissue constructs.

Conclusions: Rabbit ASC are suitable for use in a tissue-engineered vocal fold replacement. This model will be used in future implantation trials in rabbits.

Introduction

The specialized extracellular matrix of the vocal fold lamina propria and its attached epithelium are, thus far, irreplaceable after severe scarring. Tissue engineering of the vibratory vocal fold has been proposed as a treatment for severe vocal fold scarring. This laboratory previously developed a human ASC-populated three-dimensional tissue-engineered construct intended for vocal fold cover layer replacement. Here, we attempt to take the next step toward human application by creating an animal model.

We set out to develop a tissue-engineered construct suitable for implantation in rabbit vocal folds. Rabbits are a favorable animal model for study of the larynx due to their histologic and size similarities to human larynxes, easy animal husbandry, ability to phonate in an excised setting and inherently silent behavior.

Rabbit ASC have been shown to differentiate into an epithelial phenotype when exposed to an air liquid interface and multiple growth factors. Here, we report the production of a tissue-engineered mucosal replacement from rabbit ASC that will allow for preclinical construct implantation studies. Key differences in the in vitro development between human and rabbit cells are highlighted.

Methods and Materials

rASC collection: rASC were isolated from inguinal fat pads, treated with collagenase and centrifuged. The stromovascular fraction was collected and cultured.

rASC Differentiation: osteogenic differentiation was induced with 1.25 dihydroxy vitamin D3, ascorbate 3 phosphate and B-glycerophosphate. Adipogenic differentiation was induced by dexamethasone, insulin and indomethacin.

Fibrin-ASC constructs: human cryoprecipitate and rabbit fibrinogen were used as fibrin sources and rASC were seeded onto both in a trans well 3D air liquid interface with and without EGF (Fig 2 & 3).

Results

rASC differentiate into mesenchymal phenotypes.

Light microscopy demonstrated that cultures induced to differentiate into adipogenic (left panel) and osteogenic (center panel) types showed morphological changes after 2 weeks in culture (control media only, right).

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Discussion

The construct developed here is suitable for implantation as a vocal fold mucosa graft in rabbits. In vivo microenvironment may direct differentiation of the superficial layer of cells to a more mature epithelial phenotype; this will be tested further in implant studies. ASC or bone marrow derived stem cell injections into animal vocal folds in vivo decrease scarring and dense collagen deposition; improve viscoelasticity and mobility; and increase elastin production, vocal fold smoothness and collagen organization. Furthermore, adding a microrized acellular dermal matrix or hydrogel scaffold to the injected decreased scarring.

The wound healing properties of ASC may be adequate to prevent scar formation after implantation, while the fibron scaffold may further support ASC survival and differentiation. These aspects will be tested in a rabbit implantation trial.

Conclusions

Rabbit adipose-derived stem cells were used to create a three-dimensional tissue-engineered construct suitable for vocal fold cover replacement. The three dimensional construct was bilayered and resembled the vocal fold lamina propria and mucosa in microstructure although not in cell phenotype. The construct was able to withstand handling and suturing similar to a native rabbit vocal fold cover layer. This construct will be used for future in vivo implantation studies in rabbits.

References