A 3-D Organotypic Human Cochlear Culture for growth factor and drug testing.

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ABSTRACT

The irreversible loss of sensory hair cells in the cochlea is a significant cause of hearing impairment. Utilizing animal models, several studies have attempted to regenerate hair cells through gene therapy or stem cell regeneration and transplantation techniques. Although hair cells from the human cochlea and vestibular structures have been isolated in vitro, no studies to date report their tissue culture, which is the focus of this report.

Six brain dead donors were consented to donate cochlear tissue through the Center for Donation and Transplant at Albany Medical Center. A radical mastoidectomy was performed and the cochlea was blue lined prior to being recovered en bloc. The tissue samples were placed in sterile saline on ice for transport to the Neural Stem Cell Institute (NSCI) labs for culture and immunocytochemistry. Tissue was plated either as dissociated cells or as organotypic explants. The dissociated cells grew into fibroblast-like monolayers, indicating that mesenchymal rather than neural cells had been cultured. In contrast, organotypic explanted cultures grown for 14-21 days maintained a 3D resemblance of the inner cochlear structure, with evident cell layers. Alpha-tubulin immunostaining indicated survival of ciliated cells, and long connections resembling axons, likely related to the spiral ganglion neurons.

The current study demonstrates the ability to successfully isolate and culture adult human cochlear-derived cells in vitro in a novel 3-D culture preparation.

INTRODUCTION

Sensorineural hearing loss is a result of irreversible loss of sensory hair cells in the cochlea and is the cause of a significant proportion of hearing impairment in the world. The causes of hair cell loss and damage is multifactorial and irreversible in mammals.6 Utilizing animal models, much research has been dedicated to regenerate hair cells through gene therapy as well as stem cell regeneration and transplantation techniques.1,6 Although successful attempts to isolate hair cells from the human cochlea and vestibular structures have been reported, no studies to date have attempted to isolate and culture these cells in vitro.7 This report will focus on the isolation of inner ear hair cells and their supporting cells with attempts to culture the hair cells in vitro.

METHODS

Patient Population. Brain dead donors who consented to tissue and organ donation through the Center for Donation and Transplant at Albany Medical Center.

Surgical Technique. Tissue was removed through a post-auricular incision in which a radical mastoidectomy was performed. In one patient on one ear, two cochleostomies were performed and the inner contents of the cochlea were flushed with saline and collected in a suction container. On the remaining 9 ears, a radical mastoidectomy was performed. The cochlea was harvested utilizing a combination of osteotomies, a #1 cutting bur and pituitary forceps.

Hair Cell Collection and Preparation. All tissue samples were placed in a sterile, saline filled container and transported to the Neural Stem Cell Institute (NSCI) in an ice-filled cooler within 12 hours of recovery. The NSCI plated the human cochlea-derived cells in vitro using newly-established isolation and culturing protocols (Fig 1). Studies of surface and intracellular markers, including cilia markers, and growth characteristics were performed.

RESULTS

Six patients were consented and tissue from 10 cochleae has been recovered. Tissue was plated as dissociated cells or as organotypic explants.

Dissociated cells were plated in a variety of culture media shown to be successful for the growth of other types of human neural cells. More cells survived and grew in a uncoated 24 well plate in 500ul DMEM plus 10% fetal bovine serum (FBS) than other tested conditions. However, these dissociated cultures grew into fibroblast-like monolayers, indicating that mesenchymal cells, rather than neural cells, had survived and grown. It is possible that such cochlear tissue-derived mesenchymal cultures will have some value as a feeder layer support for neural cells in a co-culture design. (Fig 2)

Organotypic cultures were placed in a 24 transwell insert that fits inside the culture well and provides access to upper and lower surfaces, which tends to increase cell survival. The micro-dissected explants were placed in DMEM culture medium with 10% FBS with feeding biweekly. They were grown for 14-21 days then fixed and immunostained. The organotypic cultures maintained a 3D resemblance of the inner cochlear structure, with evident cell layers, even after this considerable time in culture. After 14 or 21 days, staining for alpha-tubulin revealed groups of puncta typical of en face views of cilia in regular rows. In addition, this immunostaining revealed long connections resembling axons, likely related to the spiral ganglion neurons. (Figs 3 and 4.)

DISCUSSION

The current study demonstrates the ability to successfully isolate and culture adult human cochlear-derived hair cells in vitro in a novel 3-D culture preparation. Future studies will involve continued recovery of cochlea from brain-dead donors for further study of the cells remaining after several days of organotypic culture. We will test whether added growth factors will aid in stimulating cell division in these explants. Such a 3-D arrangement of cells has advantage over dissociated cell culture as it provides a more authentic model of the cell-cell interactions of the neural cells and their supporting cells.

CONCLUSIONS

This 3-D model is valuable to study how added exogenous factors, including drugs, impact the survival and potentially the growth of the human inner ear neural cells. In this manner, we plan to ascertain whether the adult human cochlea contains cells with regenerative potential for use in hearing restoration.

REFERENCES


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