ABSTRACT

Objectives: In vitro models of the inner ear are an effective method of studying inner ear disease. Several models have been described, but no comparison between different approaches has previously been attempted. We aimed to summarize current knowledge and techniques in inner ear cell culture, compare relative yields and viability, and determine the optimal culture method with regards to tissue source, harvesting technique, growth conditions, and culture characterization.

Study Design: A systematic literature review.

Methods: A search was conducted in MEDLINE (1946-2014) and EMBASE (1976-2014). Only English language articles reporting the results of cell culture of non-neuronal tissue derived from inner ear sites were included. Studies reporting hair cell cultures from non-inner ear stem cells were included for cell characterization.

Results: 32 studies met the inclusion criteria. These studies exclusively used fresh tissue, primarily harvested from mice (50%). There was little variation in tissue harvesting techniques. The cell type targeted for culture correlated with the culture media used. Immunocytochemistry was the predominant method of phenotypic characterization. There was variation in growth, culture techniques, and use of functional assays. Culture duration was limited primarily by fibroblast overgrowth.

Conclusions: Characteristic inner ear cells can be cultured by several methods. There is insufficient data regarding yield and cell function for comparative analysis. Long-term culture growth remains a challenge.

INTRODUCTION

The prevalence of sensorineural hearing loss is increasing as our population ages. Age-related hearing loss, or presbycusis, refers to the development of progressive sensorineural hearing loss with age and is associated with degeneration of the inner ear sensory, neural, and metabolic cells. Several factors are believed to predispose to this cellular damage including noise exposure, genetic predisposition, and small blood vessel disease. Currently, the main available treatments are electromechanical stimulation devices such as hearing aids. Despite the high prevalence of hearing loss, the use of hearing aids among those with presbycusis is low. In the elderly this can result in social isolation, which has been associated with cognitive decline and an increased risk of dementia.

The age-related reduction in cochlear function parallels a concomitant reduction in the function of the saccule, part of the inner ear balance system that is developmentally related to the cochlea. The sensory cells in the balance, or vestibular, structures are similar to those seen in the cochlea. Additionally, vestibular structures, have been implicated in the pathogenesis of some human ear diseases characterized by hearing loss e.g. Meniere’s disease.

Importance of in vitro work

It will be advantageous to obtain a better understanding of the mechanisms leading to human cochlear and vestibular cell dysfunction. In vitro models are commonly used to study cells in isolation. The ideal would be to study human inner ear cells, however human inner ear tissue is not easily obtained because surgical procedures on the inner ear are uncommon and even when undertaken, such as in cochlear implantation, seek to preserve the inner ear. Animal models have therefore been adopted to study inner ear cells. Successful inner ear cell cultures have been described, but no consensus on optimal culture technique exists. To our knowledge, no prior review of inner ear cell culture techniques has been undertaken. We undertook a systematic review of the literature to determine optimal culture conditions with regard to tissue source, harvest technique, growth conditions, culture duration, purity, yield, and cell characterization.

STUDY DESIGN

Study Type: Systematic Literature Review

Source: MEDLINE and EMBASE

Search Terms: A search for “inner ear”, “cochlea”, “vestibule” and related cellular structures was conducted and cross-referenced with “primary cell culture” and “cell culture techniques”.

Analysis: 30 methods were analyzed for all criteria. 6 non-inner ear stem cell transformations and 1 immortalized cell line were analyzed for cell characterization techniques only.

REFERENCES


RESULTS

Tissue Source and Types of Inner Ear Cells Cultured

Table 1: The majority of studies used mouse models (n=16). Other notable species used to obtain cell cultures included: rat1,4,15,16,21-23 (n=5), guinea pig1,24,26,28,34 (n=4), humans5,14,17,20 (n=3), and frogs19-22 (n=2) and 2 reports of cell cultures using bovine31 and embryonic chicken ear cells19,23.

Harvest Technique

Methods of target organ removal were similar across studies, but not reported in detail by all studies. In postnatal animals, cell retrieval generally involved isolating the temporal bone or whole inner ear, followed by microdissection to isolate the target organ and remove undesired tissue. In some models where the inner ear was not fully mature the lesser and greater epithelial ridges were isolated. Human samples were obtained during translabyrinthine acoustic neurumom resection.4,12,17 Harvesting was performed immediately after animal sacrifice.

Mechanical and chemical disaggregation (41.7%) and mechanical disaggregation only (25%) were performed prior to plating in some studies.

Growth Conditions

Growth Factors

53.3% of cultures used growth factors. Specific growth factors included EGF, FGF2, IGF-1, TGFα, hereregulin, PDEG, and ECGF. Non-specific growth factors included bovine pituitary extract and mouse embryo extract. Heparan sulfate was used in the majority of endogenous stem cell cultures.

Antibiotics and Antimycotics

63.3% of cultures used antibiotics. Of these, 46.7% added only antibiotics. Penicillin and streptomycin was the most common combination. 13.3% used antibiotics and antmycotics. 1 study used antibiotics, antmyfungs, RNase, and DNAase. 36.6% of studies did not report the use of antimicrobials.

Supplements

66.7% of studies added supplements to cell culture method. The most common supplements were glutamine (36.7%), HEPES (16.7%), transferrin (16.7%), and hydrocortisone (16.7%). 33.3% of studies did not report addition of any supplements.

Serum

Bovine serum was used in 60% of studies, primarily at 10% concentration. Human and horse serum were used in one study, respectively. In addition, 33.3% of studies used serum-free conditions. Of these 33.3% added B27 and N2 (proprietary supplements), 40% used B27 alone, and 30% had neither. All hair stem cell cultures used serum-free media.

Media

DMEM-F12 (30%), Dulbecco’s Modified Eagle’s Medium i.e. DMEM (26.7%), and Minimal Essential Medium i.e. MEM (23.3%) were most common. Other media included CS-C media12, GIBCO keratinocyte medium28, self-designed external solution22, media 199, medium 254CF23, and Neumann & Tytell’s. Each of these were reported in single studies.

DISCUSSION AND CONCLUSIONS

Characteristic inner ear cells can be cultured by several methods. We report 32 papers using 37 culture methods. Further studies reporting yield and cell function are needed to determine optimal culture conditions.

Tissue Source

• The majority of cultures have been reported in mice. Auditory tissue is the most common source.
• The majority of studies grew hair cells or hair stem cells.
• A smaller number of studies grew support cells.

Harvest Technique

• Studies exclusively used fresh tissue.
• Methods of cell harvest were generally similar and involved isolating the inner ear then microdissection.
• Mechanical and chemical mechanical and chemical disaggregation were utilized in the majority of studies.

Growth conditions

• DMEM-F12, DMEM, and MEM were the most commonly used media.
• Cultures primarily used bovine serum. 33% used serum free media, including all hair stem cell cultures.
• Media supplements and antmycotics were used variably.
• The majority of cultures reported addition of antibiotics to culture media.
• Growth factors were used in 53% of cultures.

Culture Duration, Purity, and Viability

• The median culture duration was 14 days.
• There was limited quantitative calculation of purity & viability.

Cell Characterization

• Immunocytochemistry was most commonly used.
• Many hair cell specific markers exist. Myosin VIIa is used most commonly.
• There are fewer specific markers for support cells.

Cell Characterization

Immunocytochemistry was the primary method used to characterize cells. Several specific and non-specific markers were used. Myosin VIIa was used as a hair cell specific marker in 72.7% of studies that grew hair cells or hair stem cells. Brn3c, Math1, and calcium-binding proteins were also used. Transgenic mice and viral vectors containing GFP tagged to Math1 were used by 3 studies. 53% used GFP tagged to Brn3c. Non-specific epithelial markers included cadherin, cytokeratin, and ZO-1. Vimentin was used to identify fibroblasts in cell culture. Actin arrangement was also used to characterize cells.

RNA and protein expression was also assessed by RT-PCR and Western blot, respectively. These were primarily adjuncts to immunocytochemistry. Other characterization methods included microscopic morphology assessment by light and electron microscopy. In studies that did not perform immunocytochemistry this was the primary method used to characterize cells. In a few studies, cell function was assessed through hair cell activity. Calcium concentration, sodium-potassium ATPase activity, mechanosensitivity, and measurement of voltage-dependent membrane depolarization have been described.

maximum cell culture duration was reported as the number of passages or the number of days cells could be cultured. Some studies did not passage cells, thus the maximum duration was converted into days. When this was not reported, the average number of days between passages was used to calculate the duration. The duration of culture ranged from 5 hours20 to 252 days, with a median of 14 days.

Viability was generally not quantified, only 12.5% used Trypan blue exclusion assay to determine viability. 21.9% of methods used a BrdU proliferation assay to assess the proportion of proliferating cells. Apoptosis assays were used in 2 studies. More recent studies utilized fluorescence-activated cell sorting (FACS) to either determine culture purity26 or to delineate cell populations within culture.14,16