

## Methods

Twenty-four temporal bone labyrinth capsules were harvested from 12 adult pigs, age range 15-19 weeks (*Sus scrofa*). The cochlea duct was extracted piecemeal and macerated under microscopic control. Two-thirds of the resulting tissues were collected in 15ml sterile tubes containing 10ml Dulbecco's phosphate-buffered saline (DPBS) to which 2ml of 0.25% trypsin-EDTA was added prior to incubation at 37°C for 20-25 minute. The remaining tissue was used for RNA extraction. The cells were then placed in a growth medium consisting of Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% Fetal Bovine Serum (FBS) on a cell culture plate in an incubator at 37°C and 5% CO<sub>2</sub> for 3-4 days. When cell growth reached greater than 80% confluence, the cells were washed with DPBS, then trypsinized using 250µl 0.25% trypsin-EDTA per well for 5 minutes. One-third portion of the passage 1 (P1) cell pellet was re-suspended in fresh growth medium and placed in T<sub>25</sub> vented culture flasks for the generation of the next passaged cells (P2 cells). Phase-contrast microscopy, Scanning electron microscopy (SEM), fluorescence immunocytochemistry and quantitative reverse transcription polymerase chain reaction (RT-qPCR) were used to characterize the cells.

## Results

Primary cultures were heterogeneous and formed monolayers. Sphere forming cells were identified on phase-contrast microscopy. SEM demonstrated that the sphere-forming cells displayed stemcell ultrastructural features. Hair cell markers myosin VIIa and prestin; and stem –cell markers nestin and Sox2 were identified on fluorescence immunocytochemistry across all passages. Nestin gene expression level was high in the harvested cochlear tissue and persisted through passages tested up to P6. Gene expression levels of supporting cell marker cyokeratin 18 and hair cell markers myosin VIIa and prestin were significantly positively correlated [ $p=0.005$ , Spearman correlation ( $\tau$ ) =0.943].

## Conclusion

Inner ear hair cell and supporting cell characteristics persist across passages up to P6. Innate otic stem/progenitor cells exist in the mature porcine cochlea.

**Keywords:** Porcine, inner-ear, multi-potent, stem/progenitor cell

## C08

### How the computational methods can contribute to better knowledge of the ear

Fernanda Gentil <sup>(1)</sup> - Marco Parente <sup>(2)</sup> - Carla Santos <sup>(2)</sup> - Bruno Areias <sup>(2)</sup> - Jorge Belinha <sup>(3)</sup> - Renato Natal Jorge <sup>(2)</sup>

*Escola Superior de Saúde - ESS, IPP, Porto, Portugal* <sup>(1)</sup> - *FEUP, INEGI, Porto, Portugal* <sup>(2)</sup> - *IPP, ISEP, Porto, Portugal* <sup>(3)</sup>

The biomechanical behavior of the human ear can be studied using mathematical approaches, such as the finite element method. This methodology allows to perform simulations to evaluate the influence of sound transmission through all way of the ear, since the sound is selected by the outer ear to the inner ear.

The finite element method is currently the preferred method for the numerical solution of partial differential equations, and it is used in several fields of the biological sciences. Due to its large applicability and efficiency, there are several works where this methodology is applied in different specialties, to analyze loads, strains or displacements, as in the case of the biomechanical study of the ear. From CT images, three-dimensional accurate computational models are created. The technique employs a set of calculations of numerical analysis for solutions of kinematic (displacements and rotations) and mechanical aspects (strength, stress, pressure, deformation). The use of these computer models enables the motion analysis of all ossicular chain points at any moment.

More specifically, these are the following steps performed in this work:

1. From CT images, it was taken the geometry of the tympanic membrane and ossicles.
2. The respective discretization into finite elements, was performed.
3. The appropriate material properties were assigned.
4. The boundary conditions were defined.
5. Several static and dynamic studies of the behavior of the model were made.
6. Calculation of the umbo and stapes footplate displacements.

CAD software was used to treat each of the parts that compose the human ear. The model was composed of

the external auditory canal, eardrum, ossicles, two muscles, six ligaments, two joints, the temporal bone and the cochlea.

The model was simulated numerically with the Abaqus software.

The frequency band used in this study was between 100 Hz and 10 kHz.

After the construction of the model representative of the normal ear, the results can be compared with other models illustrating some ear diseases, such as eardrum perforations, tympanosclerosis, otosclerosis, tumors, as well as inner ear disorders. Other application can be related with prosthesis replacement in the middle ear (stapes prosthesis or all ossicular chain), as well as a simulation of electrode insertion in cases of cochlear implant. These models are created according to the specification of the surgical techniques described by various authors.

The aim of this study is to analyse different situations to improve the understanding of ear pathologies and its rehabilitation.

In cases of eardrum perforations, we can conclude, for example, that different sizes of eardrum perforations result in different displacements, being smaller with bigger perforations. When simulating otosclerosis, it can be concluded that there was a decrease of the displacements for all frequencies, with the increase of stiffness of annular ligament.

With this technique it is possible to promote the best possible performance of prostheses in the ear, in order to know the best results for different diameter and weight of these prosthesis.

The present study allows to conclude that computational methods can help the audiological evaluation/rehabilitation of the ear.

**Keywords:** Biomechanics, Ear, Computational, Finite Element Method