Whole organ culture in rotating bioreactor: the rat embryonic inner ear.

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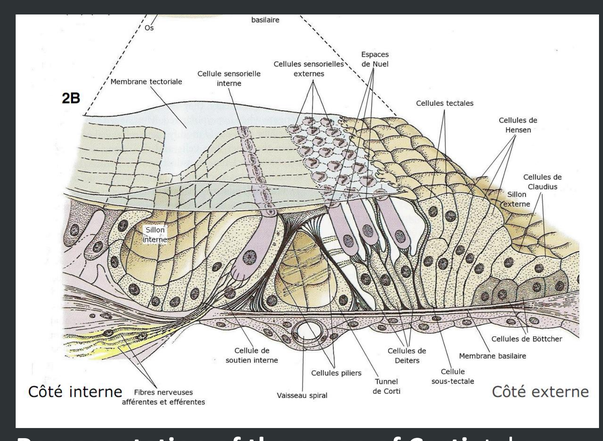
Introduction

In eutherian mammals, the organ responsible of the transduction of sound waves into nerve impulses is called the organ of Corti. This structure located within the cochlea, a portion of the inner ear, is composed by two types of cells: sensory hair cells that are characterized by stereocilia and non-sensory supporting cells. All these cells are distributed according to a specific arrangement along the whole length of the cochlea.

The rotating bioreactor is a suspension culture vessel optimized to produce laminar flow and minimize the mechanical stresses on cell aggregates in culture (Hammond and Hammond, 2001). These physical properties support the culture of the entire inner ear organ with preservation of its structural tissue integrity (Hahn et al., 2008).

So far, the mammalian inner ear is very sensitive to damage, with no hair cell replacement or cell proliferation occurring in the cochlea. That is why understanding the mechanisms regulating the mammalian cochlear development is important to pursue strategies inducing sensory hair cells regeneration. Here, we present a technique of whole embryonic inner ear culture in rotating bioreactors.

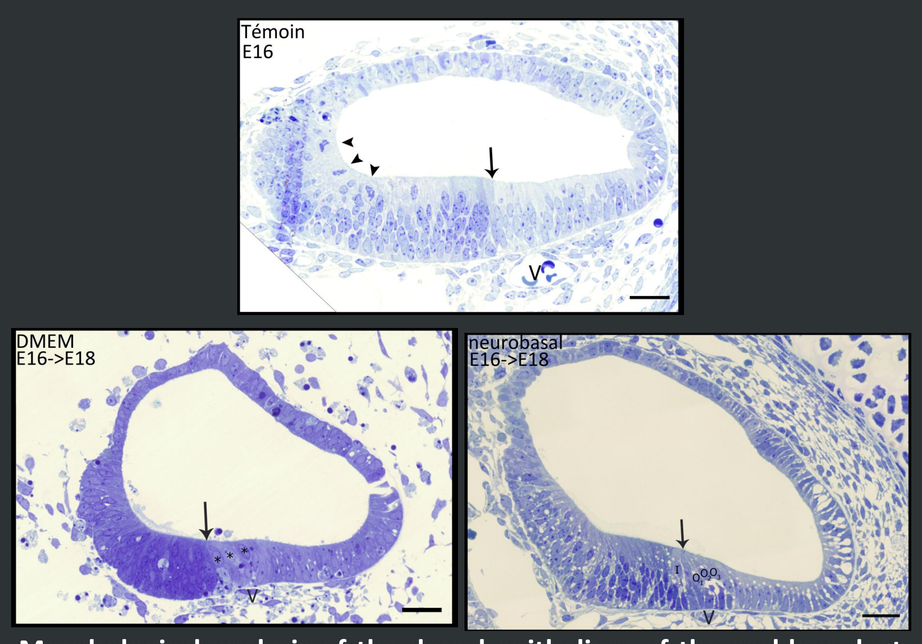


Representation of the organ of Corti, taken and modified from Randall et al. 1999.

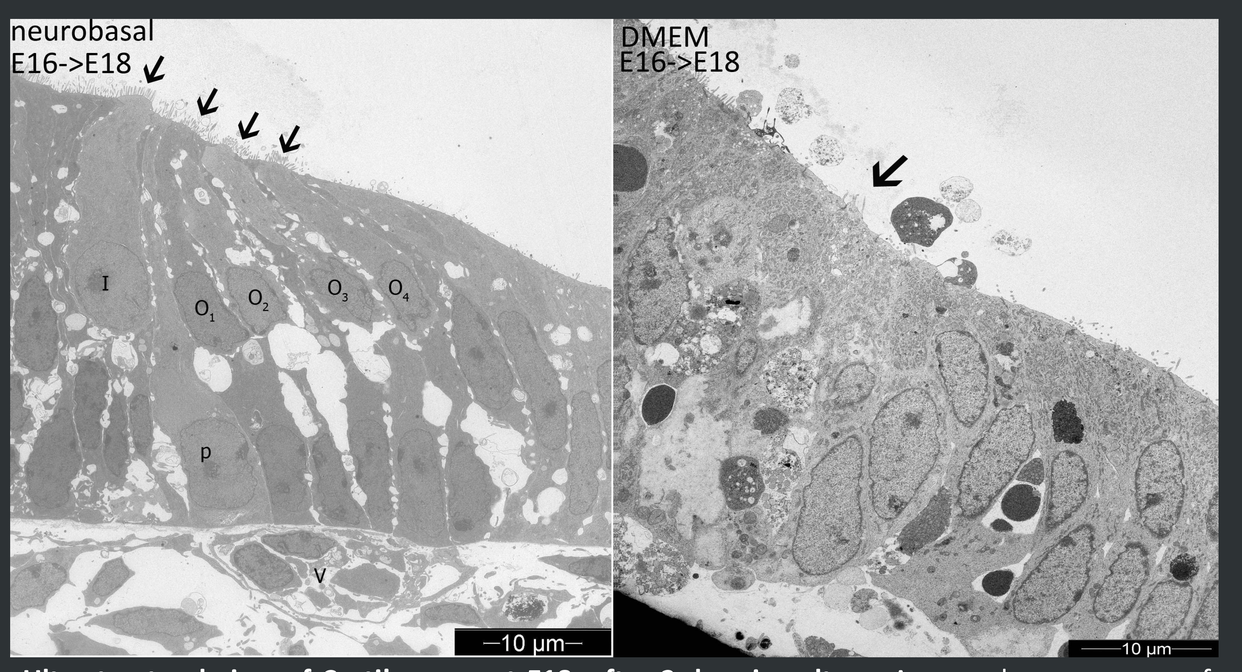
Rotary Cell Culture Systems. Synthecon.

Results

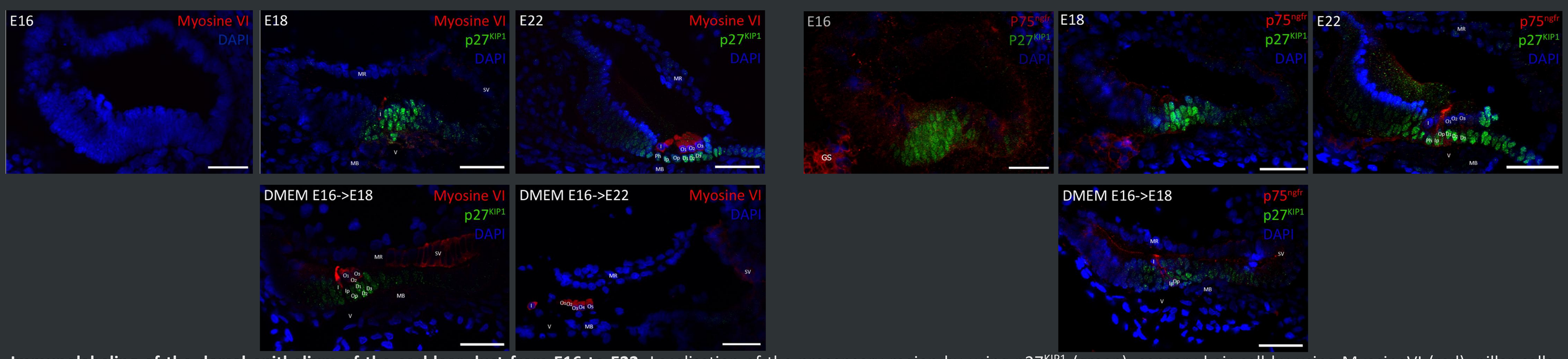
To determine the development of the cochlea in culture, the morphology of the organ of Corti was analyzed by semithin and ultrathin sections. Immunohistological labeling also confirmed the differentiation of sensory hair cells and supporting cells.



Morphological analysis of the dorsal epithelium of the cochlear duct before and after culture. Asterisks show sensory hair cells; Arrows show depression of the dorsal epithelium; Arrowheads show mitosis. $O_{1,2,3}$ Outer hair $cell_{1,2,3}$. Bar: 25µm.



Ultrastructural view of Corti's organ at E18, after 2 days in culture. Arrows show presence of stereocilia. I Inner hair cell; $O_{1,2,3,4}$ Outer hair $cell_{1,2,3,4}$.



Immunolabeling of the dorsal epithelium of the cochlear duct from E16 to E22. Localisation of the prosensory region by using $p27^{KIP1}$ (green), sensory hair cell by using MyosineVI (red), pillar cells (supporting cells) using $p75^{ngfr}$ (red) and nucleus by using DAPI (blue). $D_{1,2,3}$ Deiters cell $_{1,2\ 3}$; DMEM Dulbecco's Modified Eagle Medium; GS Spiral Ganglion; I Inner hair cell; Ip Inner pillar cell; MB Basilar Membrane; MR Reisner Membrane; $O_{1,2,3}$ Outer hair cell $_{1,2,3}$; Op Outer pillar cell; Ph Phalangeal cell; SV Stria Vascularis; V Spiral Vessel. Bar: 20µm.

Conclusion

Our preliminary results demonstrate that organ culture of the embryonic inner ear in rotating bioreactor is possible. Such a method provides an *in vitro* model for the investigation of developmental, regulatory, and differentiation processes that could be helpful in the understanding of the mechanisms underlying the development of the mammalian cochlea.

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