Molecular Optical Imaging Enables Visualization of Exogenous Stem Cells in the Intact Guinea Pig Cochlea

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Introduction

It is generally accepted that the preservation of a critical number of functional auditory neurons is crucial with regard to cochlear implant performance. A growing amount of evidence suggests that stem cell therapy may be applied to replace degenerated auditory neurons in the cochlear nerve of patients suffering from severe or profound hearing impairment. However, to achieve functional repair, survival of stem cells is a prerequisite. This calls for *in vivo* visualization of grafted stem cells and long-term monitoring of their survival and fate in deafened cochleas. We have investigated if molecular optical imaging enables visualization of exogenous stem cells within the intact guinea pig cochlea.

Experimental Setup

Human embryonic kidney (HEK293) cells and epidermal neural crest stem cells (EPI-NCSCs) were transduced with a lentiviral construct, resulting in a stable dual expression of the fluorescent (copepod green fluorescent protein; copGFP) and bioluminescent (click beetle green luciferase; CBG99) reporters at an equimolar ratio. Transduced cells were injected into the cochleas of guinea pig cadaver heads, either via the internal auditory meatus or through the round window membrane following a retro-auricular approach. Guinea pigs (Dunkin Hartley; N=30 females) were obtained from non-related experiments. All imaging experiments were performed with the IVIS® *Spectrum* multimodal imaging system (Xenogen, Caliper Life Sciences, Hopkinton, MA, USA). Image acquisition and analysis were done with *Living Image* 3.1 software.

Molecular Optical Imaging

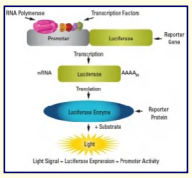

Figure 1. Schematic of the luciferase reporter assay (© Piercenet)

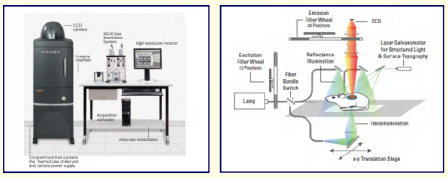

Figure 2. The IVIS® *Spectrum* (Xenogen, Caliper Life Sciences) multi-modal imaging system combines laser scan surface topography with fluorescence and bioluminescence imaging capabilities. It enables non-invasive visualization and tracking of cellular and genetic activity within a living organism in real time (© Xenogen, Alameda, CA, USA).

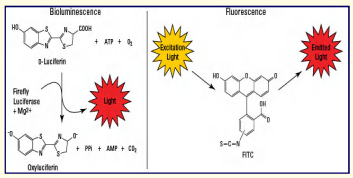

Figure 3. Bioluminescence imaging is based upon the enzymatic conversion of D-luciferin into oxyluciferin, which is ATP and oxygen dependent (***left panel***). Fluorescence imaging is based upon the phenomenon that a fluorophore absorbs energy from a light source and emits light at a different wavelength (***right panel***) (© Piercenet). The high sensitivity of bioluminescence imaging allows monitoring of the ATP-dependent signal as a quantitative measure of cell viability. The *Living Image* 3.1 software provides in addition spectral unmixing algorithms that allow detection and separation of multiple reporters (GFP emits at λ_{max}=502 nm and CBG99 emits at λ_{max}=537 nm).

Results

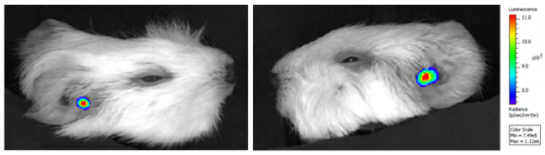

Figure 4. Following injection of copGFP/luciferase-expressing HEK293 cells or EPI-NCSCs (~5x10⁴ cells in 5 µl buffer containing 0.2 M D-luciferin) into the internal auditory meatus of both ears, a bright bioluminescent signal was invariably located near the concha of the auricle, indicating that the light emitted by the cochlea is passing through the tympanic membrane and the external auditory meatus. Fluorescence due to copGFP could not be detected. Similar results were obtained after direct injection into the scala tympani or the modiolus of the basal cochlear turn through the round window membrane following a retro-auricular approach.

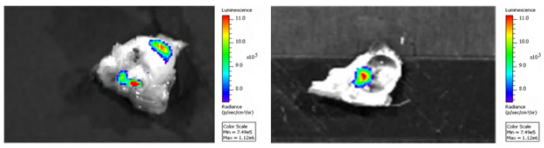

Figure 5. Imaging of the bulla after its removal from the skull demonstrates that the bioluminescent signal is passing through the bony wall of the bulla (***left image***). After removing part of the bony wall of the bulla, the cochlea could be discerned and a bright bioluminescent signal was observed at the basal cochlear turn, localized near the round window (***right image***).

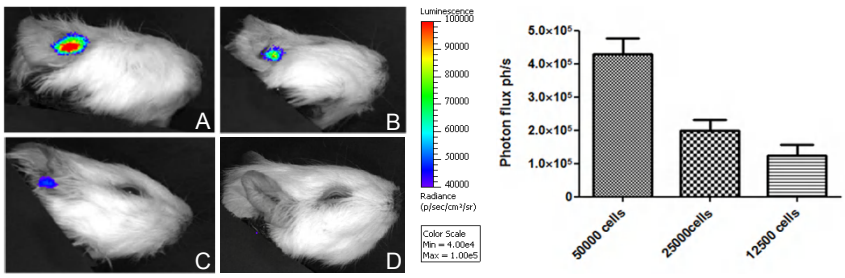

Figure 6. A dilution series was performed to determine the amount of HEK293 cells or EPI-NCSCs needed to reach signal threshold for bioluminescence imaging. Cells were suspended in buffer at a concentration of ~10⁶ cells per 100 µl. After appropriate dilution, 0.2 M D-luciferin (Synchem, Felsberg, Germany) was added and a volume of 5 µl containing either ~5x10⁴ (***A***), ~2.5x10⁴ (***B***), ~1.25x10⁴ (***C***) or ~0.5x10⁴ cells (***D***) was injected directly into the modiolus of the basal cochlear turn using a 20 µl Hamilton syringe with a 25G needle. Signal threshold for bioluminescence imaging lies between 5,000 and 12,500 cells.

Acknowledgements

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Guinea pig cadaver heads were provided by Dr. Harry Blom (Animal Welfare Officer) and Hans Sturkenboom (Department of Pharmacological Sciences) from Utrecht University, the Netherlands.

Conclusions

This feasibility study demonstrates that bioluminescence imaging enables visualization of exogenously applied luciferase-expressing stem cells in the intact guinea pig cochlea. Studies are currently in progress to reproduce these results in living animals.