BDNF overexpressing human stem cells in alginate for cochlear implant optimization

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Rational

Brain-derived neurotrophic factor (BDNF) has a protective effect on spiral ganglion neurons (SGN) and can potentially help to increase the success of a cochlear implant (CI). A long-term supply with BDNF is desirable to establish a clinically relevant chronic growth factor therapy. Long-term administration of BDNF can be achieved by genetically modified human mesenchymal stem cells (hMSCs). This concept requires protection of the hMSCs from immune responses of the recipient organism and prevention of uncontrolled proliferation and migration of these cells. Alginate may serve as a matrix for the BDNF-producing MSCs, so that these requirements can be fulfilled. We investigated the feasibility of BDNF-over-expressing hMSCs with a proven SGN-protective effect *in vitro* [1] embedded in ultra-high viscous alginate as local drug delivery system to the inner ear.

Methods

Alginate gel stability, survival of alginate encapsulated hMSC and BDNF bioactivity after electrical stimulation *in vitro* (Fig. 1) were evaluated. Insertion forces and coating stability on custom-made electrode arrays were analyzed, impedances were measured *ex* and *in vivo* and the effects on neuronal protection and fibrosis were investigated *in vivo*.

Electrical stimulation (ES) *in vitro*:
- in petri dish, central stimulating electrode, peripheral ground electrode [2]
- 1 kHz, bi-phasic 800 µs pulses, 400 s per phase, 120 µs interpulse gap
- 2, 1, 0.88, 0.66 or 0.33 mA, 24 hours
- visual inspection of alginate, hMSC counting

Insertion forces and coating stability *in vitro*:
- alginate-hMSC as viscous solution or electrode with or without dip-coating
- custom-made electrode arrays
- artificial human sized cochlea model filled with saline
- automated insertion test bench

Electrode impedances *in vitro and in vivo*:
- Maestro-system (MEDEL)
- before coating in PBS, before coating in medium, coated in medium, coated after CI insertion *in vivo* and weekly thereafter

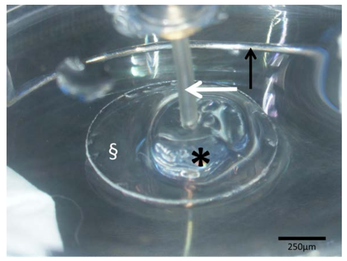


Fig. 1: Cover glass (§) with polymerized alginate/MSC-mixture (asterisk) in a petri dish. The active electrode (white arrow) was placed in the alginate/hMSC-mix and an annular electrode served as ground (black arrow). The petri dish was filled up with hMSC-culture-medium.

***In vivo*:**
- guinea pigs, normal hearing and systemically deafened (kanamycin & furosemide)
- experimental groups and animal numbers per group: see Fig. 2
- ABR measurements: day -7 before deafening (in deafened groups), day 0 before implantation and day 35 before temporal bone harvesting
- electrodes provided by MEDEL
- CLSM of cleared cochleae
- semiautomatic SGN counting per area of Rosenthals canal
- fibrosis determination by score:
  0: no fibrosis (never detected)
  1: thin film on electrode surface
  2: thin fibrous cloudy structures
  3: more prominent cloudy structures
  4: almost the entire Scala tympani is filled (never detected)

Fig: 2

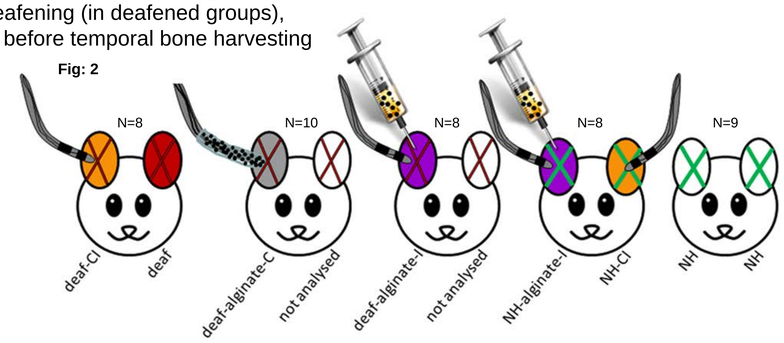
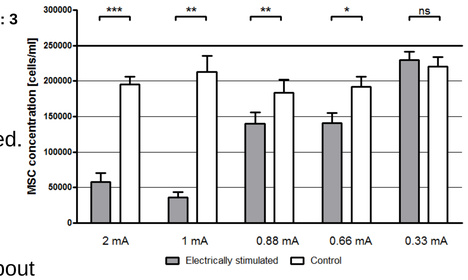


Figure 2: experimental groups: red X: deafened ears; green X: normal-hearing ears; color code for ears: orange: deafened (red X) or normal-hearing (green X) ears with CI: *deaf-CI and NH-CI*; red: deafened ear without further treatment, included in group *deaf*; gray: deaf and CI-implantation with alginate-hMSC coating: *deaf-alginate-C*; white with red x: contralateral ears of those treated with factor-releasing cells. Since it cannot be ruled out that the factor has an effect on contralateral neurons, theses ears were not included in the analysis. Violet: animals first received an alginate-hMSC injection using a microcatheter system (provided by MED-EL Corp.) inserted 3 mm deep into the scala tympani. After injection the catheter was removed, normal CI was inserted and the polymerization solution for alginate crosslinking was applied for 30 min. at the round window niche. Violet with red X: *deaf-alginate-I*; violet with green X: normal hearing with CI and alginate-hMSC injection: *NH-alginate-I*; white with green X: normal hearing: *NH.*

Results

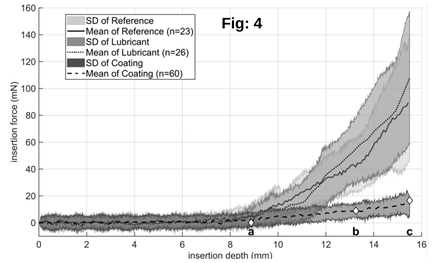
Electrical stimulation (ES) *in vitro*:
Electrical stimulation with 0.33 mA did not affect the MSC survival (Fig. 3). High current levels decreased the hMSC number significantly. In some cases of max. stimulation (2 mA) the alginate directly located at the active electrode was charred.

Fig: 3



Insertion forces and coating stability *in vitro* :
Coated arrays significantly reduced the insertion forces. Comparing the maximal insertion forces of the reference group to those of the alginate coated array a significant reduction of about 75 % was shown (Fig. 4). Stability of coating was good after first insertion. 85% of the tested arrays had no or only minimal signs of coating abrasion (grade 0-1). When insertion into the cochlea model was repeated, the impact on the coating and therefore the abrasion was increased [3].

Fig: 4



***In vivo*:**
No difference in fibrosis was detectable between experimental groups (Fig. 6). Coating of the electrode array with BDNF-producing hMSCs embedded in alginate resulted in neuronal protection whereas injection of the alginate hMSC-mixture with subsequent CI insertion did not affect the SGN density (Fig. 7, [4])

Electrode impedance:
Impedance was not affected by alginate gel coating (Fig. 5). The impedance development *in vivo* over time did not differ between experimental groups (data not shown).

Fig: 5

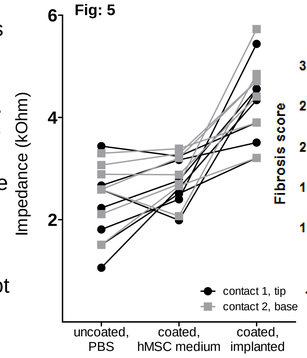


Fig: 6

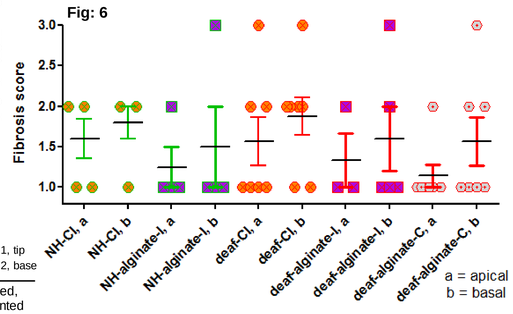
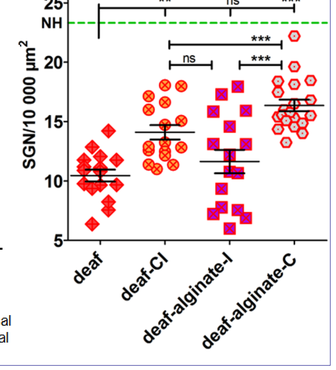


Fig: 7



Conclusion and Discussion

The hMSCs survive moderate ES. Coating of the electrode array with BDNF-overexpressing hMSCs embedded in alginate is stable and significantly reduces the insertion forces *in vitro*. Electrode impedances are not affected by the alginate-hMSC-coating. The alginate-hMSC-coating of electrode arrays protects SGN from degeneration *in vivo* whereas an injection of alginate-hMSCs has no effect on SGN. The coating of electrode arrays with alginate incapsulating BDNF-overexpressing hMSCs is a very promissing approach for future functionalisation of CI to reduce insertion forces and to protect neurons from degeneration. Future studies have to investigate the long term stability of the coating and hMSC survival *in vivo*. Additionally, the effect of the delivery strategy on residual hearing should be determined.

References

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