A Stem Cell-Alginate Coated Cochlear Implant for Chronic BDNF-Delivery is Stable and Neuroprotective *In Vitro*

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Objective

The nerve-electrode-interface of a cochlear implant (CI) might be improved by a growth factor induced protection of the spiral ganglion neurons (SGN). An application of exogenous BDNF (brain-derived neurotrophic factor) has a great potential for this implementation, with a chronic application being a prerequisite for a longer lasting neuroprotection. One possibility for a chronic drug delivery to the cochlea is the implantation of genetically modified cells as a CI-coating. To avoid an uncontrolled migration of these cells or an enhanced immune reaction in the inner ear, an encapsulation in bioinert ultra high viscosity (UHV-) alginate is a possible way to protect patients. Mesenchymal stem cells (MSC) are promising candidates for encapsulation in alginate. An additional advantage of this drug delivery system is the possibility to apply the cell-alginate-matrix as a CI-coating.

Methods

MSCs:
- isolated from human bone marrow of one donor
- genetically modified for production of BDNF and tomato red
- expansion, concentration by centrifugation and resuspension in alginate

CI-coating & stability testing:
- manual dip coating
- applied to human-sized CI-electrode models (Fig. 1)
- measurement of the coating thickness (Fig. 3)
- triple insertion in a human cochlea model (Fig. 4, A)
- microscopic documentation after the 1. and 3. insertion (Fig. 4, B,C)
- classification in grades of abrasion (Tab. 1, Fig. 5).

Neuroprotective effect (Fig. 2)**:**
- beads of encapsulated MSCs
- co-cultivation for 48 h with dissociated rat spiral ganglion cells

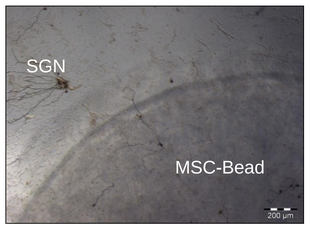

Fig. 2: Beads were formed out of 10 µl MSC-alginate-suspension (2500 MSCs/Bead) by cross-linking ($BaCl_2$, 20 minutes, 37˚C). Co-cultivation for 48 h with dissociated SGC. All beads were macroscopically intact afterwards. SGN were stained for anti-neurofilament with DAB (dark brown).
Scale bar: 200 µm

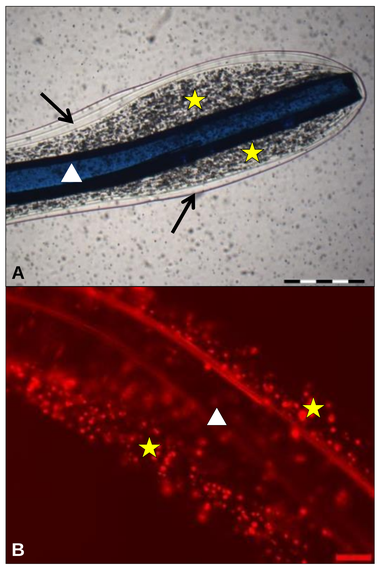

Fig. 1: Apical (A, brightfield) and medial (B, fluorescence) part of a coated electrode (triangle). Inner cell containing alginate layers (asterisks, ~800000 MSC/ml), outer pure UHV-alginate layers (arrows).
Scale bar: 1mm (A), 200 µm (B)

Cell culture conditions:
- Medium: SGN-medium (no serum) 1:1 MSC-medium (10% FCS)
- negative control (NC): MSC-medium; positive control (PC): MSC-medium + 50 ng/ml BDNF
- seeding control (seeded number of neurons): fixed after 4 h
- 48 h: macroscopic control of bead-stability, quantification of BDNF content in the bead-supernatant by ELISA (Fig. 6), SGC fixation, immunocytochemical staining and SGN-counting, calculation of the neuronal survival rate (Fig. 7).

Results

CI-coating & stability testing:

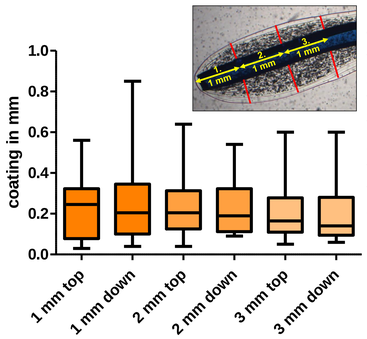

Fig. 3 Coating thickness measured at the apical 3 mm, above and below the electrode. The average of the thickness is ~ 0.2 mm and apical slightly thicker due to droplet formation. Overall the coating thickness varies between 0.03-0.85 mm. N=4, n=5

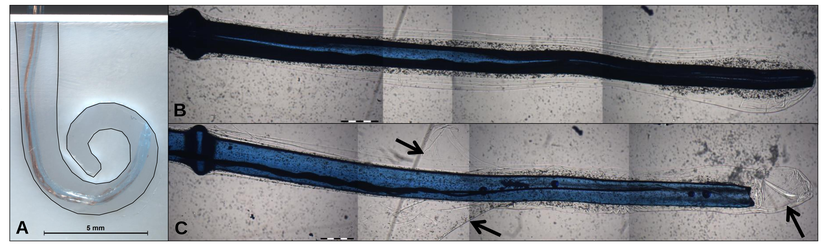

Fig. 4: **Documentation of abrasion** after insertion into the cochlea model (**A**). For correct positioning at the beginning of the insertion, the coated electrodes have to be straightened and positioned at the model's round window with forceps. **B** depicts an intact coating (grade 0) and **C** an extensive, moderate abrasion (grade 4, marked by arrows) after 1. insertion.
Scale bar: 5mm (A),1mm (B,C)

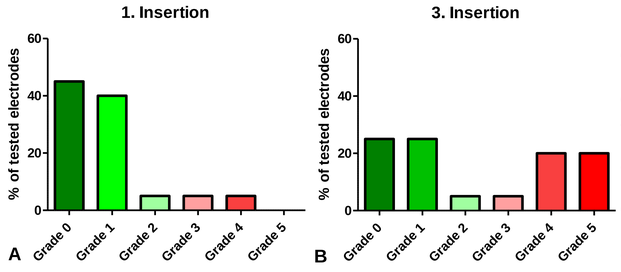

Fig. 5: After 1. insertion (**A**) 85 % of the tested electrodes showed no or only minimal signs of **coating abrasion** (grade 0-1). After repeated insertions into the model, the impact on the coating and therefore the abrasion was increased. Nearly 50 % of the electrodes were classified to higher grades (3-5) of abrasion after 3. insertion (**B**). N: 4, n: 5

Tab. 1: Grade of coating abrasion

grade	Abrasion of coating
0	no abrasion
1	minimal abrasion on max. ½ of electrode
2	minimal abrasion on > ½ of electrode
3	moderate abrasion on max. ½ of electrode
4	moderate abrasion on > ½ of electrode
5	severe/complete abrasion

Neuroprotective effect:

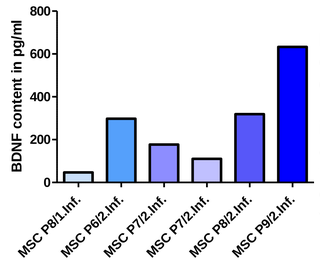

Fig. 6: BDNF-concentration in culture supernatant. MSCs of different passages (P6-9) and genetic modifications schemes(1. and 2. infection) were encapsulated in UHV-alginate. BDNF was detectable in all tested supernatants after 48 hours of cultivation. The BDNF-concentration varied widely between 50-600 pg/ml.

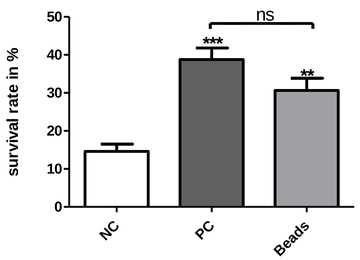

Fig. 7: Neuronal survival of SGN is significantly increased by the addition of 50 ng/ml BDNF(positive control, PC) and co-cultivation with MSC-Beads. There is no significant difference between the neuroprotective effect of MSC-Beads and PC.
Data given as mean and standard error of mean (SEM). Asterisks above error bars indicate significance of PC and Beads compared to NC (**p<0,01, ***p<0.001; ns = not significant); N = 8, n = 3

Conclusion

We conclude that it is possible to **encapsulate genetically modified MSCs in UHV-Alginate.** An application as **coating, injection or beads** seems to be a feasible way for **drug delivery** to the inner ear. CI-coating is possible by a **simple manual dip-coating** procedure. However, there is a wide variety of coating thickness especially due to droplet formation at the tip of the electrode. The **stability of the CI-coating** is good after the first insertion (cf. CI-implantation), but declines with intense stress inter alia due to handling with forceps for correct positioning. The amount of **detectable BDNF** in culture is in pg range and very variable. Nevertheless the encapsulated MSCs have a significant **neuroprotective effect on SGN**.

Literature:

Zimmermann, H. et al. 2007: Alginate-based encapsulation of cells: Past, present, and future, *Curr. Diab. Rep.*, vol. 7, no. 4, pp. 314–320.
Zimmermann, H. et al. 2005: Towards a medically approved technology for alginate-based microcapsules allowing long-term immunoisolated transplantation, *J. Mater. Sci. Mater. Med.*, vol. 16, no. 6, pp. 491–501.
Hütten, M.et al. 2013: UHV-Alginate as Matrix for Neurotrophic Factor Producing Cells-A Novel Biomaterial for Cochlear Implant Optimization to Preserve Inner Ear Neurons From Degeneration. Otol Neurotol 34: 1127-1133.
Gillespie, L.N. et al. 2015: Cell-based neurotrophin treatment supports long-term auditory neuron survival in the deaf guinea pig, *J. Control. Release*, vol. 198, pp. 26–34.
Goren, A et al. 2010: Encapsulated human mesenchymal stem cells: a unique hypoimmunogenic platform for long-term cellular therapy, *FASEB J.*, vol. 24, no. 1, pp. 22–31.
Hügl S. et al. 2017; *Impact of insertion velocity on insertion forces in cochlear implantation surgery*; [Poster] Biomedical Engineering Bd. 62 (2017), S1, Seite S167, 10.-13.09

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For *in vivo* results see:
Presentation #: PS 677
Abstract Title: „BDNF-Producing Stem Cells as Drug Delivery System in Cochlear Implanted Guinea Pigs"
presented by Verena Scheper