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Prolonged stability and biocompatibility testing of an alginate hydrogel cochlear implant coating under simulated inner ear conditions

https://doi.org/10.1515/cdbme-2025-0113

Abstract: An atraumatic implantation of the Cochlear Implant (CI) to prevent, inter alia, loss of spiral ganglion neurons (SGN) and residual hearing, might be achieved by a flexible, smooth, and hydrophilic coating of the electrode. Such a coating needs to be long-term stable to avoid swelling or shrinking, which cause micro-movements of the electrode. In parallel, it has to be harmless to hair cells (HC) and SGN. Due to its inert and biocompatible character, barium cross-linked ultra-high viscosity alginate is a promising candidate for this propose and was tested for stability and biocompatibility under simulated inner ear conditions.

CI dummies were coated with alginate by using a cone-shaped semipermeable membrane. Alginate sol was injected in these membrane cones, dummies inserted, and all covered with barium cross-linking solution for ionic gelation. Coated dummies were incubated in artificial perilymph and observed for one year with weekly medium change and photo documentation for stability assessment. Additionally, beads of alginate hydrogel were co-cultivated with murine inner ear tissue for one week to test biocompatibility with SGN and HC.

Alginate hydrogel coating stayed attached over the one-year observation period. Small variations in the diameter of coated dummies were detectable, likely due to rotation of the floating dummies, but no clear shrinking or swelling was seen. Neither number of SGN nor number of HC was significantly reduced by co-culture with alginate beads.

In simulated inner ear conditions, alginate hydrogel was stable as coating for one year without greater changes of shape and had no neuro- or ototoxic effect. These promising results support testing of alginate as lubricant-coating *in vivo* as a further step towards translation to clinical use for CI improvement.

Keywords: spiral ganglion neurons, hair cells, neurotoxicity, ototoxicity, artificial perilymph, barium cross-linking

1 Introduction

Severe to moderate sensorineural hearing loss can be treated with the cochlear implant (CI), whose electrode stimulates electrically the spiral ganglion neurons (SGN) [1]. The electrode array is inserted in the perilymph-filled Scala tympani of the inner ear in a surgical procedure. Due to progress in implant technology and surgical techniques, also patients with residual hearing in the apical, low-frequency region are treated with CI to benefit from electro-acoustic stimulation for a better speech perception [2,3]. Despite best efforts, about 10-30% of these patients lose, directly or over time, partially or completely, their residual hearing as a consequence of the implantation. Beside several biological mechanisms, following main factors were detected to influence the postoperative performance of CI-patients: mechanical electrode characteristics, surgical technique, insertion trauma, subsequent foreign body reaction, electrode-neuron interface and long-term stability of the electrode position and function [4]. A further softening of the implantation procedure mediated by a flexible, smooth, and hydrophilic coating may help to increase the hearing preservation rate by a reduction of inner ear trauma and foreign body reaction. Such a coating has to be long-term stable without swelling or shrinking to avoid electrode movement and needs to be biocompatible with the inner ear tissue, including hair cells (HC) and SGN. Barium cross-linked ultra-high viscosity alginate hydrogel is a promising candidate for such a CI coating. It is described as overall inert

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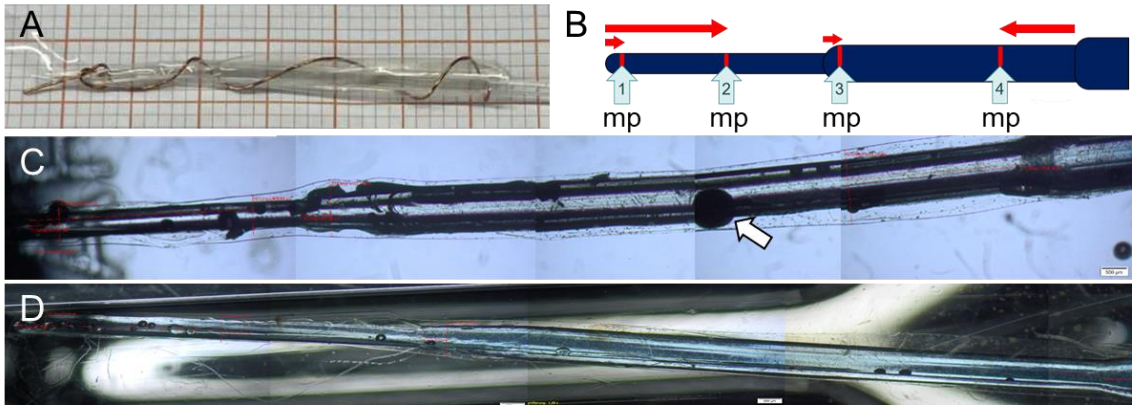

Figure 1: **A** Semipermeable membrane was wrapped in cone-shape around a scale-increased dummy to leave space for a hydrogel layer around the CI-dummy. Shape was fixed with a wire. **B** Scheme of measurement point (mp) 1-4 for diameter measurement of the coated dummy. Red arrows reflect length and direction of point definition (mp1: 600µm from tip, mp2: 4500µm from tip, mp3: 600µm from first step, all measured towards base; mp4: 3500µm from second step, measured towards tip). **C** Alginate coated CI-dummy directly after cross-linking and **D** in the cuvette after 365 days in artificial perilymph (arrow pointing on air bubble in the hydrogel; scale bar: 500µm).

and biocompatible, it showed a reduction of insertion forces when used as coating matrix for cell-based drug delivery with the CI, and in short-term it is stable *in vitro* in artificial perilymph and has no ototoxic effect [5,6,7]. This study presents the results of the extended stability and biocompatibility testing of a pure alginate CI coating for inner ear application.

2 Methods

2.1 Preparation and observation of alginate coated CI dummies

To test the stability of an alginate hydrogel coating, custom-made silicone dummies (Sylgard 184; Dow, Barry, UK) [6] were used to mimic the CI electrode. The viscous alginate sol was hold in place around the dummy by a cone-shaped membrane (see Figure 1A). This membrane (MWCO 8kDa, # 12757486; ThermoFisher Scientific, Schwerte, Germany) supports a controlled and solid gelation by slow permeation of the barium ions [8]. For sterility reason, dummies and membranes were UV-radiated before use. The negatively charged alginate was linked to the silicone surfaces by precoating with positively charged poly-L-lysine (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Next, the cone-shaped membrane was filled with alginate sol (*Lessonia negrescens* and *trabeculata* 1:1, 0.65 % in saline; Alginatec®, Riedenheim, Germany), the dummy was inserted in the sol, and all was placed in barium cross-linking solution (20 mM $BaCl_2$, 115 mM NaCl, (both Sigma Aldrich), 5 mM L-Histidin (GENAXXON bioscience, Ulm, Germany) in 100 ml ddH_2O, pH 7.0) for 10 minutes at room temperature (RT). Afterwards, the membrane was carefully opened, the coated dummy removed, and washed in saline to rinse off residual, unbound $BaCl_2$. The coated dummies were placed in cuvettes (no. 67.742, Sarstedt, Nümbrecht, Germany) filled with 1 ml artificial perilymph (see [7], including 1 mg/ml bovine serum albumin (Sigma Aldrich)) and placed in an incubator at 37°C. Coating was observed for one year with weekly (except for two weeks) solution change, microscopic photo documentation, and measurement of the diameter at four defined locations on the dummy (see Figure 1B).

2.2 Biocompatibility with inner ear tissue

For co-culture with inner ear tissue, beads were formed by dropping 10 µl alginate sol in barium cross-linking solution (see above), followed by incubation at RT for 10 minutes and subsequent washing in saline. Tissue was harvested from three to five days old rats (Sprague Dawley, both sexes). After decapitation, temporal bones were harvested and spiral ganglia (SG; neurotoxicity testing) or cochlear whole mounts (Organ of Corti and SG; ototoxicity testing) were prepared with fine forceps and under microscopic control. SG were dissociated enzymatically (0.01% DNase, 0.1% Trypsin in Panserin 401) and mechanically while whole mounts were cut in apical, medial, and basal turns. Both were seeded in ornithine-/laminin-coated wells of 48 well plates: dissociated cells in a density of 1 x 10^4 cells/well (20 x 10^4 cells/ml), cochlear whole mounts (2/well) on coated glass slides. Panserin complemented with penicillin, glucose, insulin, N2-supplement and 10 % serum was added as cell culture medium.

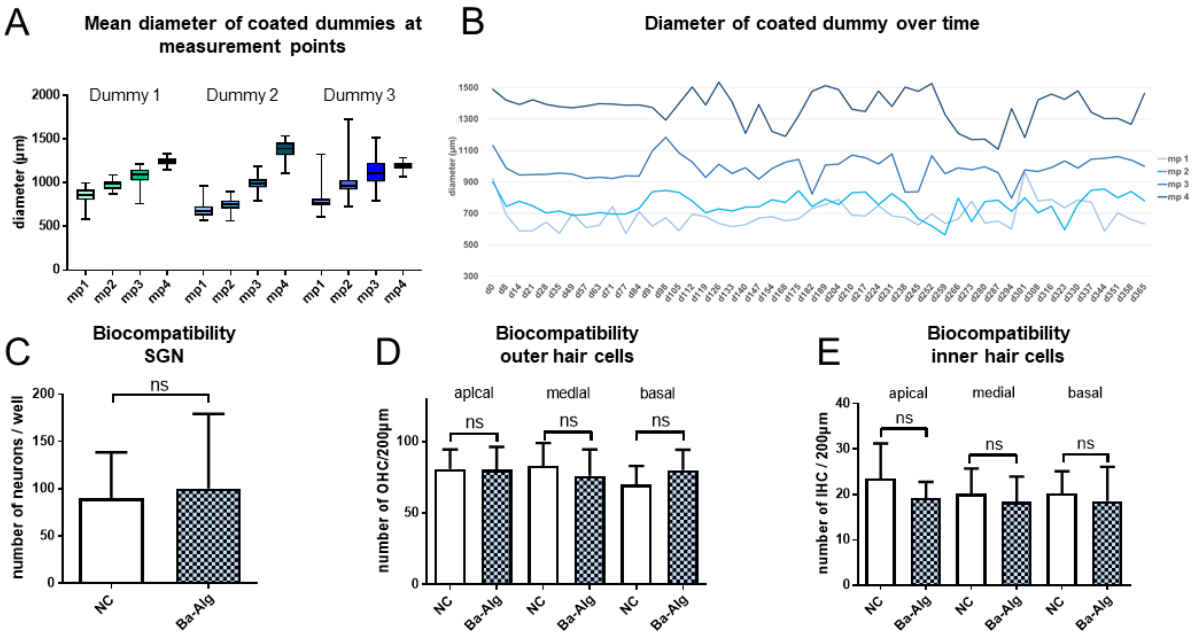

Figure 2: Mean with minimum and maximum of diameters of coated dummies, measured at the four defined points (mp 1-4 from tip to base) over time (**A**) and weekly measured diameters over one year exemplarily shown for dummy 2 (**B**). Measured diameters display the cone shape of the coating and stay in a relatively stable range but vary between time points without time dependent changes. Co-culture of alginate beads with SGN (**C**, N=3, n=3) or cochlear whole mounts (**D**, **E**, N=4-12) showed no significant (ns) difference when compared with the negative control (NC).

For detailed solution compositions and manufacturer specification see [9]. Negative control (NC) was performed without beads, while each of the co-culture wells included one alginate bead. Culture was performed for one week. Due to great cell growth in the dissociated cell culture, medium change was performed after two and three days. Cells were fixed and stained by immunocytochemistry for neurofilament to label the SGN and phalloidin to highlight the HC (SGN: see [9]; whole mounts: see table 1). To identify toxic effects, number of SGN and inner and outer HC was counted and compared between NC and alginate bead co-culture.

Table 1: Immunocytochemical staining procedure for neurons (primary and secondary AB) and hair cells (phalloidin). (AB: antibody, h: hour, min: minute)

step	solution	time/temp.
permeabilization	1 % Triton X in PBS	1,5 h, RT
blocking	5% normal horse serum and 1% Triton X in PBS (BS)	overnight, 4°C
primary AB	anti-neurofilament chicken (ab 4680, Abcam) (1:10,000) in BS	48h, 4°C
washing	PBS	3x, RT
secondary AB/phalloidin	goat anti chicken (ab 96947, Abcam, DyLight 488) Phalloidin (ab176759, Abcam, iFluor 647 Conjugate) both 1:200 in BS	24h, 4°C 3h, RT
washing	PBS	3x, RT

3 Results

Alginate coating of the silicone dummies mediated by the cone-shaped membrane was possible. The applied coating displayed the shape of the individual membrane cone prepared for each dummy (n=3) (see Figure 1C, 2A). Overall, the diameter increased from apex (mp1) to base (mp4) corresponded to the cone shape (see Figure 2A). Alginate hydrogel coating of all dummies stayed attached for the observed period of one year (see Figure 1D). Over time, the measured diameters varied with differences between a minimum of 184 µm (dummy 1, mp4) and a maximum of 1000 µm (dummy 3, mp2) (see Figure 2A) but no consistent sign of shrinking or swelling, only fluctuation of measured diameters, was detectable (see Figure 2B).

In the biocompatibility testing with inner ear tissue, neither the number of SGN nor the number of outer or inner HC was significantly reduced by a one-week co-culture with alginate beads compared to NC (see Figure 2C, D, E). Therefore, no neurotoxic or ototoxic effect was detectable for the barium cross-linked alginate in the timely increased *in vitro* experiments.

4 Discussion

The reported results support the suitability of alginate coating for combination with the CI electrode and a safe application in the inner ear e.g. as lubricant-coating for trauma reduction.

Our previously described CI-coating for cell-based drug delivery was applied manually using a layer-by-layer dip coating procedure [6]. In the present study, alginate sol was hold in place around the CI-dummy by a cone-shaped semipermeable membrane, which is impermeable for the viscous sol but permits a slow permeation of the barium ions for cross-linking [8]. Compared to manual dip coating, this new technique allows a more precise application of the alginate coating as one layer in a defined diameter and shape around the dummy determined by the pre-formed membrane. However, since the membrane cones were formed manually, they caused a slightly variable coating for each dummy. Additionally, a central positioning of the dummy in the sol could not be ensured, resulting in a variability in coating thickness within one dummies measurement point. Since the Scala tympani, where the CI electrode is inserted, is a narrow space surrounded by vulnerable tissue, a more precise molding of the membrane is required for safe translation into clinical practice. However, using membrane-mediated alginate cross-linking for CI-coating is a promising technique and the coating applied stayed attached and stabile for one year under simulated inner ear conditions.

Overall, the diameter of the coating remained relatively constant but showed fluctuation over time. Most of this was probably due to rotation of the free-floating dummies. The used cuvettes allowed microscopy of the coated dummy in the artificial perilymph without removal and transfer to another container. This reduces the mechanical stress of the coating but the used cuvettes were 1 cm wide and a fixation of the dummy was not feasible. Therefore, measurement of the diameter at the exact same orientation of the dummy could not be ensured. A narrower container allowing less movement and fixation could overcome this problem in future experiments. Occasional air bubbles were present in some coatings, which disappeared within the first weeks. They could be another reason for changes in diameter. Such air bubbles would be of clinical relevance. Although, air defuses out of the alginate gel and is absorbed in the cochlear, it would hinder the electrical stimulation [10] and a change in diameter could lead to electrode movement. Therefore, an air bubble-free filling of the membrane with the alginate sol has to be ensured for clinical application.

Barium cross-linked alginate hydrogel showed no reduction in the number of SGN or HC in a one-week co-culture with inner ear tissue. However, functionality of the cells was not tested and an impact on hearing and an interaction with the immune system needs to be examined in the entire organism.

One-year stability and one-week biocompatibility of the alginate hydrogel was proven for the inner ear *in vitro*. These results have to be confirmed *in vivo* as a next step before translation. The membrane-mediated application and gelation of the coating is a promising method but needs to be further improved regarding precise molding and air bubble prevention before being suitable for clinical use.

Author Statement

Research funding: Research was funded by the DFG (German research foundation). Conflict of interest: Authors state no conflict of interest. Informed consent: not applicable. Ethical approval: All animal involving procedures are in accordance with international and national guidelines and approved by the local authorities.

References

[1] Lenarz, T. Cochlear Implant – State of the Art. *Laryngo-Rhino-Otologie* 2017;96, S123–S151

[2] Jurawitz, M.-C., et al. Hearing Preservation Outcomes with Different Cochlear Implant Electrodes: Nucleus® Hybrid™-L24 and Nucleus Freedom™ CI422. *Audiology and Neurotology* 2014;19, 293–309

[3] von Ilberg, C. A., et al. Electric-Acoustic Stimulation of the Auditory System: A Review of the First Decade. *Audiology and Neurotology* 2011;16, 1–30.

[4] Lenarz, T., and Scheper, V. Preserving Residual Hearing in Cochlear Implant Patients in (Springer), 2015;423–442

[5] Zimmermann, H., et al. Towards a medically approved technology for alginate-based microcapsules allowing long-term immunoisolated transplantation. *J Mater Sci Mater Med* 2005;16, 491–501

[6] Hügl, S., et al. Coating stability and insertion forces of an alginate-cell-based drug delivery implant system for the inner ear. *J Mech Behav Biomed Mater* 2019;97, 90–98.

[7] Peck, C.; et al. Stability and Biocompatibility Tests of Alginate as Cochlear Implant Coating. Material *Current Directions in Biomedical Engineering* 2021;7(2) 775-778

[8] Schulz, A., et al. Alginate- and Hyaluronic Acid–Based Hydrogels as Vitreous Substitutes: An In Vitro Evaluation. *Translational Vision Science & Technology* 2020;*9*(13), 34

[9] Schwieger, J., et al. Alginate-encapsulated brain-derived neurotrophic factor–overexpressing mesenchymal stem cells are a promising drug delivery system for protection of auditory neurons. *Journal of Tissue Engineering* 2020;11, 204173142091131

[10] Goehring, J. L., et al. How Well Do Cochlear Implant Intraoperative Impedance Measures Predict Postoperative Electrode Function? *Otology & Neurotology* 2013;*34*(2), 239–244