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Stability and Biocompatibility Tests of Alginate as Cochlear Implant Coating Material

Abstract: Inner ear trauma caused by cochlear implantation is a severe clinical problem. It was shown that an electrode alginate coating can reduce the insertion forces in vitro. The grade of the alginate viscosity can be adjusted by using different metal ions for cross-linking the salts of the alginic acid. The aim was to investigate the stability and inner ear biocompatibility of alginate using different in vitro established cross-linkers.

Alginate beads were cross-linked in either calcium chloride (CaCl_2) or barium chloride (BaCl_2) solution. The beads were cultivated in artificial perilymph and stability and swelling were observed for 13 months. Ototoxicity was tested on cochlear whole mount explants from neonatal rats. Neomycin served as positive control to induce hair cell damage and explants without any addition served as negative control. The beads and explants were co-cultured for 48 hours and the hair cell survival was analysed microscopically.

Neomycin treatment induced an extensive inner and outer hair cell loss. Neither CaCl_2 nor BaCl_2 cross-linked alginate beads caused any damage to the hair cells. Even though the same volume of alginate and cross-linkers were used, in CaCl_2 cross-linked beads were initially almost double the size of in BaCl_2 cross-linked beads. None of the cross-linked alginate beads had a significant volume change within 3 months being cultured in artificial perilymph. After 3 months the CaCl_2 cross-linked beads swelled massively and dissolved within one week whereas BaCl_2 cross-linked alginate beads remained unchanged until month 13 after culture start.

Alginate beads gelled with both cross-linkers are biocompatible with the inner ear sensory epithelium. Both cross-linkers ensure a stable gelation of alginate but a swelling

followed by degradation of the in CaCl_2 cross-linked beads occurred after 3 months. For coatings, which need to be long term stable, BaCl_2 should be chosen whereas CaCl_2 may be more suitable for applications where limited stability is needed and the swelling is not affecting the surrounding tissue. Therefore, BaCl_2 cross-linking of alginate may be the best choice for cochlear implant coating.

Keywords: Alginate, BaCl_2 , CaCl_2 , stability, ototoxicity, cochlear implant coating, lubricant, implant functionalization, insertion forces

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1 Introduction

Every cochlear implantation bears the risks of traumatizing the inner ear structures. To avoid intracochlear trauma and to optimize the cochlear implant (CI) outcome, the surgical approach, the insertion technique, and the electrode design are in the focus of research and development. An atraumatic insertion protecting the structural integrity of soft and bony cochlear tissue may reduce the risk for inflammatory reactions and may preserve the residual hearing. A CI coating with alginate showed a significant reduction of insertion forces in vitro [1].

Alginate consists of the salts of the alginic acid: guluronic and mannuronic acid. They can be cross-linked by divalent cations, which form a gel, when hydrated. The gels characteristics can be modified e.g. by the chosen ratio of guluronic and mannuronic acid or the alginate concentration, resulting in a viscous to firm gel. Additionally, the choice of

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Figure 1: Size differences $\pm$ mean range between CaCl_2 and BaCl_2 cross-linked alginate beads. Diameter of CaCl_2 cross-linked beads increased over time until they dissolved completely after 3 months. BaCl_2 cross-linked beads remained stable for the observed period of time.

the divalent cation for cross-linking the alginic acid and the pH of the surrounding medium influences the properties of the gel [2,3,4]. Suitable cross-linker solutions are CaCl_2 and BaCl_2 . They contain the necessary divalent cation Ca^{2+} or Ba^{2+} . Ba^{2+} cross-linking of alginate in contrast to CaCl_2 cross-linking results in a better long-term mechanical stability [4,5,6] and the inflammatory response against coated cells or implants is reduced [2,3,6,7].

The aim of the study was to investigate the inner ear biocompatibility and stability of alginate cross-linked with either CaCl_2 , known for its general biocompatibility, or BaCl_2 , increasing the gel stability.

2 Methods

20 mM BaCl_2 and CaCl_2 cross-linker solutions were prepared by solving 115 mM NaCl, 20 mM BaCl_2 / 20 mM CaCl_2 (SIGMA-ALDRICH GmbH, Germany), and 5 mM L-Histidin (GENAXXON bioscience, Ulm, Germany) in 100 ml ddH₂O and adjusting to pH 7.0. Both cross-linker solutions were sterile filtered with a DuallexTM – 0.22µm filter (Merck Millipore Ltd., Irland).

Ultra-pure alginate (alginattec GmbH, Riedenheim, Germany) with a mixing ratio of 1:1 *Lessonia trabeculata* and *Lessonia nigrescens* and a 0.65% weight volume (w/v) ratio solved in 0.9 % NaCl was used. Drops of 10 µl volume were cross-linked in either 20 mM CaCl_2 or 20 mM BaCl_2 solution for 30 minutes at 37 °C, forming beads in the solution, followed by three washing steps in 0.9 % saline (B. Braun Melsungen AG, Germany) for 10 minutes at room temperature each.

The beads stability and swelling behaviour were observed by placing beads formed as described above into artificial perilymph (145 mM NaCl, 2.7 mM KCl, 2.0 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 5.0 mM HEPES in 1L HPLC H₂O, pH set to 7.4), storing them in an airtight well-plate at 37°C and monitoring them microscopically once a week for the first 7 months and afterwards quarterly.

Size and shape of the beads were analyzed with an Olympus CKX41 and XC30 camera. Photos were captured with the Olympus Software cellSense Standard and the digital images were analyzed by the free license software ImageJ V1.53C (<http://rsbweb.nih.gov/ij/>). The correct scale was set (pixels/µm) by assigning the information of the scale bar and Feret's diameter was added to the measurements set. Oval and freehand tool were used to define the border of the beads. Measurements showed the exact Feret's diameter in µm to determine the beads diameter. Since the beads were not fixated to the underground and could move freely, the diameter was never measured between the same two points of a bead. The method described above just helps to determine the largest diameter of a bead from the actual point of view.

Cochlear whole mount explants from neonatal rats (postnatal day 3 to 4) were carefully prepared and dissected into their apical, medial, and basal parts. The different parts represent different frequency regions of the hearing organ with the apical part being responsible for the low frequencies and the basal part enabling high frequency hearing.

The cochlear explants were co-cultivated with BaCl_2 or CaCl_2 cross-linked beads, with Neomycin (100 µM; as positive control to induce hair cell damage; SIGMA-

Figure 2: Apical, medial and basal parts of the cochlear explants. Hair cells were dyed with Phalloidin (Alexa Fluor 488, green). The negative control hair cell status was set as 100%. Neomycin caused severe damage to the hair cells in the positive control. All cochlear explant parts treated with alginate beads cross-linked in BaCl_2 or CaCl_2 solution were comparable to the negative control. No ototoxicity could be detected. Scale bar: $155\mu\text{m}$.

ALDRICH, Germany) and without any additional treatment (negative control) in Panserin 401 (PAN Biotech, Germany) with supplements [8] and 10% serum. After 48 hours, the explants were fixed with 4% Paraformaldehyde (PFA; Merck Millipore, Germany) and hair cells were stained for Phalloidin conjugated with Alexa Fluor 488 (dilution 1:40; A12379; Invitrogen, Germany) and their integrity was analysed microscopically.

3 Results

The measured difference in size between CaCl_2 and BaCl_2 cross-linked alginate beads was factor 1.66 ($3516\mu\text{m}$ vs. $2113\mu\text{m}$) due to 0.9% NaCl uptake of the CaCl_2 cross-linked beads during the previous washing steps after 1 day (see Table 1, month 0). During the first 3 months CaCl_2 cross-linked alginate showed several fluctuations in the size compared to the more stable BaCl_2 cross-linked alginate. CaCl_2 cross-linked alginate beads underlay a slight but continuous swelling

Table 1: Mean starting bead diameter of the different cross-linker groups with SD error and subsequent diameters over time.

Diameter (μm) at month	CaCl_2 alginate	BaCl_2 alginate
0	3516 ± 171.47	2113 ± 47.31
1	3695 ± 89.79	2098 ± 88.32
2	3869 ± 186.55	2093 ± 47.31
3	4278 ± 377.66	2117 ± 103.75
6	dissolved	1948 ± 100.45
7	dissolved	1902 ± 65.86
10	dissolved	2098 ± 387.83
13	dissolved	1833 ± 77.94

and dissolved completely after 3 months (see Figure 1). BaCl_2 cross-linked alginate beads remained consistent in diameter and stable within the observation period (13 months).

In the ototoxicity test, neomycin treatment induced an extensive inner and outer hair cell loss. Neither alginate beads cross-linked with CaCl_2 nor BaCl_2 solution caused any damage to the inner or outer hair cells (Fig. 2).

4 Discussion and Conclusion

CaCl_2 cross-linked beads had initially the 1.6-fold size of BaCl_2 cross-linked beads, which is due to a higher amount of 0.9% NaCl being bound in the beads during the washing steps and storage in artificial perilymph. Mørch et al. and Bajpai et al. 2004 described, that divalent cations in combination with different concentrations of guluronic and mannuronic acid lead to swelling of the alginate beads. Especially CaCl_2 cross-linked alginate beads underlie an ion exchange process, which can lead to a degradation of the bead [3,9]. This is in accordance with our findings. CaCl_2 cross-linked alginate beads underlay a slight but continuous swelling with a complete degradation after 3 months. This factor has to be considered. A swelling of a coated cochlear implant electrode could cause tissue damage by lifting the basilar membrane or setting the stria vascularis under pressure. The SD error could be a result due to the point of view depending diameter determination of the free moving beads.

Both CaCl_2 and BaCl_2 cross-linked alginate beads are compatible with the inner ear sensory epithelium using our experimental setup. No hair cell loss could be detected in the different frequency regions from the low frequencies in the apical part to the high frequencies in the basal part. Noteworthy, that this experiment was not repeated thrice.

We co-cultured the beads for 48 hours with inner ear explants. For patients' safety, a prolonged diffusion time of Ba^{2+} and Ca^{2+} off the beads should be considered in follow up experiments. By adding eluates of long-term diffusion experiments to inner ear tissue, biocompatibility of chronic implantation could be tested. Additionally, that upcoming experiment should include analysis of inner ear synapses to elucidate if the cations may have an impact on subcellular

levels. For coatings which need to be long-term stable BaCl_2 should be chosen for alginate cross-linkage whereas CaCl_2 is more suitable for applications where limited stability is required.

Author Statement

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