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Original Research Article

Development and validation of a 3D-printed artificial round window niche for use in release kinetics analysis of round window niche implants

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Abstract: The regular way to determine the in vitro release rates of drugs from implantable drug delivery systems consists of the complete immersion of the implant into a medium. The medium surrounds the implant, and the diffusion of the drugs occurs across the whole implant surface directly into the medium. This method does not accurately model the release rates if the real diffusion only happens across only one part of the surface of the implant, through a membrane, and into a small volume of medium. It also does not address the anatomical shape of the studied structure. One example for this is the insertion of an implant at the round window niche (RWN) in the middle ear, which enables the diffusion of drugs through the round window membrane into the inner ear. To solve this problem, we designed, three dimensionally (3D) printed and validated an artificial RWN (aRWN) to analyze the diffusion of dexamethasone from round window niche implants (RNI) into artificial perilymph over time. The aRWN consists of a human sized scala tympani with an opening at the apex and round window, respectively. We adjusted the model to be able to position an artificial membrane over the round window area and place a round window niche implant (RNI) on top of it. The scala can be filled with artificial perilymph which can be sampled via the opening at the apex. The established aRWN incorporates small dimensions, small liquid volumes, geometry, and the diffusion through a membrane into the results of release kinetics experiments. It allows better understanding of the diffusion rates of drug delivery implants for the treatment of inner ear disorders through the RWN and RWM.

I. Introduction

The success of pharmacological therapy is dependent on the amount of drug that reaches its destined target. Local drug delivery increases the amount of drug that reaches its goal, improving the desired effect, while decreasing the overall amount of drug that has to be applied, reducing the likelihood of adverse effects [1].

For the development of drug delivery implants and the quantification of their release rates, an in vitro system that models the target structure is advantageous. One such structure is the round window (RW) niche (RWN) and membrane (RWM), at the border between middle and inner ear, that can serve as the access point to the cochlea to treat inner ear disorders which affect approximately 4 per 1,000 people annually [2].

The RWN is a small anatomical structure that resembles a cavity with a membrane on the back wall. The use of high-resolution 3D printing methods such as stereolithography (SLA) may enable the rapid prototyping of an artificial round window niche (aRWN) that allows the in vitro analysis of the release kinetics in an anatomically correct shape. The purpose of this study was to construct an aRWN with a hollow space mimicking the scala tympani in anatomically relevant size, allowing the addition of a membrane and the sampling of fluid off the scala to enable in vitro drug release kinetics studies.

II. Material and methods

The basis of the aRWN was the open access scalable model for human scala tympani phantoms from the University of Utah [3] (Fig. 1).

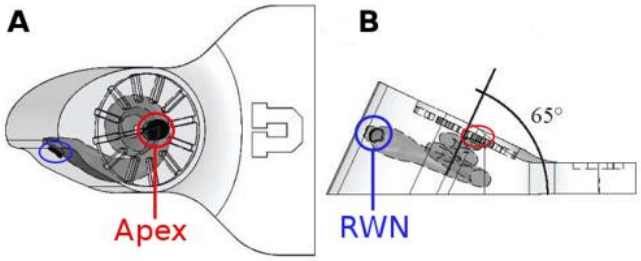

Figure 1: Scala tympani *phantom model. A shows the top view. B shows the side view. The spiral like structure is the* scala tympani. *It has an opening at the apex and RWN [3].*

The model has a RW opening at the side and an apical opening at the top. To enable drug release and diffusion analyses the model had to be adjusted using a computer aided design software Autodesk Inventor Pro 2021 (Autodesk, San Rafael, USA). The model was rotated to position the RW at the top to allow a membrane and implant to be placed above it. Additionally, the surface was adjusted to be flat at the top to enable an easy fit of a polyethylenterephthalat (PE) membrane "ThinCert" (Greiner Bio-One, Kremsmünster, Austria). Furthermore, a ⌀ 10.5 mm ring was added to hold the membrane inlay in place. To stabilize the model more material was added to build a cuboid (Fig. 2).

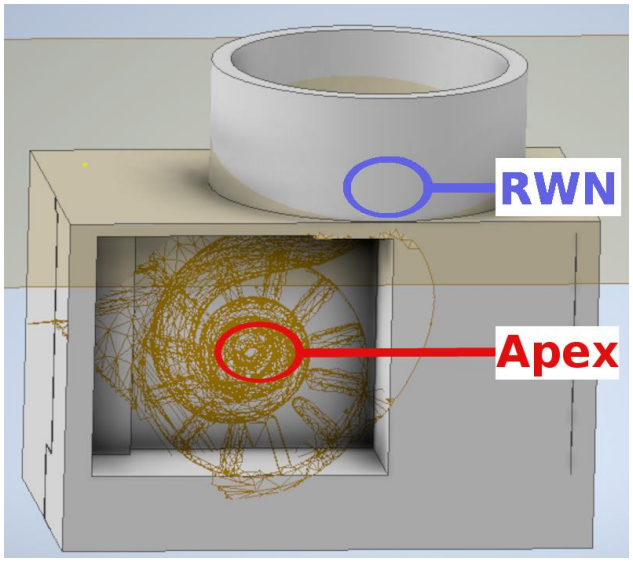

Figure 2: Scala tympani *model adapted to be an aRWN model. The orange structure is the* scala tympani *phantom basis. The cuboid around and the ring above they were added for ensuring stable positioning of the aRWN and securing a membrane inlay respectively.*

Considering the PE membrane was bigger than the RW opening, the round window niche implant (RNI) had to be positioned to lay directly above the RW opening on the membrane. For this purpose, a positioner (Fig. 3) was constructed. The positioner consisted of a plunger like object that had four half-ring-shaped recesses on its edges. The recesses had different sizes to allow a stable hold of differently sized implants.

Figure 3: Positioner. The plunger like object holds the RNI in place above the RW opening between its round recesses and the inner membrane inlay wall.

The 3D models of the aRWN and positioner were saved (data format: Standard Tessellation Language, STL) and sliced using Ultimaker CURA 5.3.1 (Ultimaker, Utrecht, Netherlands), to enable 3D printing.

II.I. 3D printing of the aRWN and positioner

For the manufacturing of the aRWN and positioner an SLA 3D printer Form 2 (Formlabs Somerville, USA) was used. Form 2 prints with a layer thickness of 25 µm and a resolution of 25 µm. The material was resin (Clear Resin V4, Formlabs), that hardened using ultraviolet light. After printing the aRWN was rinsed and flushed with 95% ethanol. Finally, the objects were cured for 30 minutes under ultraviolet light ($\lambda = 450$ nm).

II.II. 3D printing of the implants

In order to ascertain the appropriateness of utilizing the aRWN for the purpose of drug release analysis, a RNI incorporating dexamethasone was employed. The RNI was 3D printed using a 3D Bioplotter Manufacturer Series (ETEC, Gladbeck, Germany). The STL file was created using a mean model of human RWNs.

The RNI (Fig. 4) was produced using a mixture of 80% (w/w) medical grade UV-silicone 60A MG (ETEC) and 20% (w/w) dexamethasone (50-02-2, caelo, Hilden, Germany). The details for printing silicone implants were previously published [4][5].

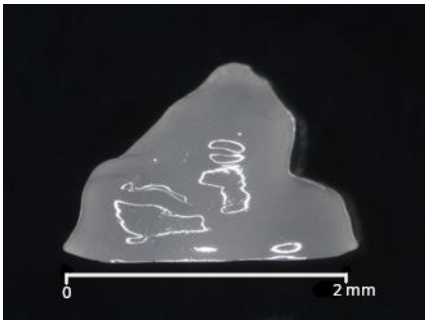

Figure 4: Example of an RNI based on a mean model of RWN made of medical grade silicone.

II.III. Release Kinetics

To prepare the release kinetics experiment, first the artificial perilymph (AP) (made of sodium chloride, potassium chloride, magnesium sulphate heptahydrate, calcium chloride dihydrate and 2-(4-(2-Hydroxyethyl)-1-

piperazinyl)-ethane sulfonic acid) was injected into to the hollow *scala tympani* of the aRWN using a syringe and a needle (gauge 0.5 mm). Due to the capillary effect, it is possible to fill the hollow space without it leaking through the apex opening. Afterwards, the membrane inlay was placed on top of the model above the RW opening inside the ring (Fig. 5) and fixed with

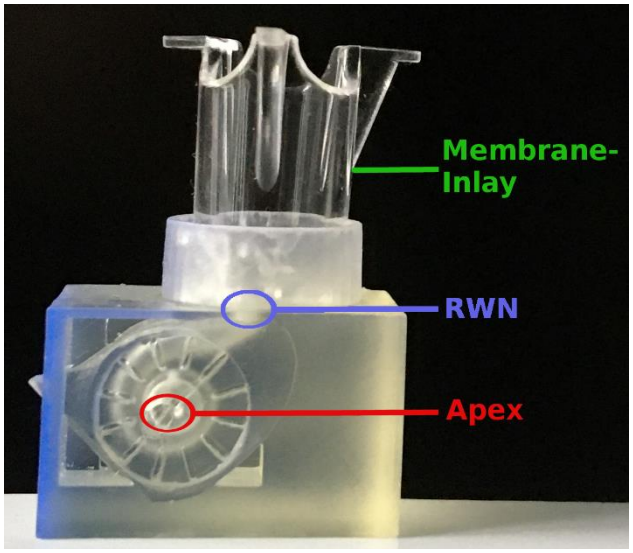

Figure 3: Frontal view of the aRWN. The spiral is the scala tympani *cavity with an apical opening at the side. The round window opening is on top surrounded by a ring for fixating a membrane inlay.*

parafilm and the RNI placed inside the inlay above the RW opening. Afterwards, the positioner was used to keep the RNI between the inlay wall and the positioner above the RW opening (Fig. 6).

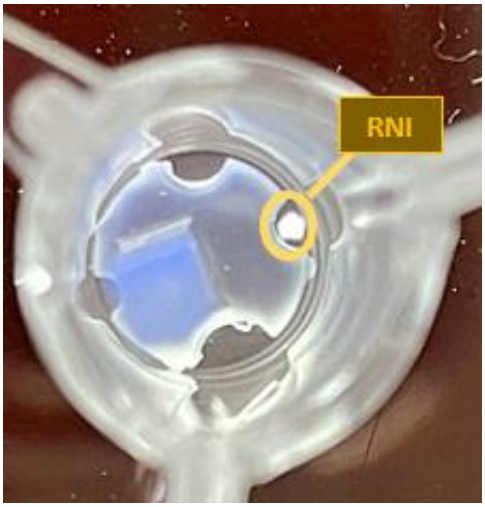

Figure 6: Top view inside the filled membrane inlay. An RNI is placed between positioner and inner wall of the membrane inlay to secure it above the RW opening.

The placement of the positioner was secured with some parafilm and the whole model placed within a 25cc airtight mixing cup (Hausschild, Hamm, Germany) with 5 ml AP at its bottom to minimize evaporation of the AP within the scala tympani (Fig. 7).

Figure 7: AP filled scala tympani of an aRWN with membrane inlay on top inside an airtight cup, filled with AP at its bottom and sealed with parafilm.

To start the release kinetics experiment, the cup containing the prepared aRWN was placed inside an incubator at 37°C for 72h.

After incubation, the container was opened and the membrane inlay was removed. The eluate (~ 50 µl) was sucked out using a syringe, the scala was flushed with an additional 50 µl to rinse out potential remaining dexamethasone and the total 100 µl eluate was placed inside an Eppendorf tube and frozen at -20°C until analysis.

II.IV. Chromatography

To ascertain, that dexamethasone from the RNI diffused through the PE membrane into the AP within the scala tympani, four independent experiments with four dexamethasone loaden RNIs placed in four aRWNs were performed. The eluates were harvested and the dexamethasone was quantified using an Ultra High Performance Liquid Chromatograph Acquity UPLC (Waters, Milford, USA) coupled with a time-of-flight micro-mass spectrometer Xevo Q-TOF (Waters).

III. Results and discussion

It was possible to change the open access data set to construct an aRWN, which can be used for release kinetics analyses of RNI. The aRWN and positioner could be printed using stereolithography and resin. The hollow structure of the scala tympani could be filled with AP with the help of a syringe. A stable hold of the RNI above the RW was achieved with the positioner.

A concentration between 0.3 and 2.4 µg / mL could be detected within the eluates, confirming that dexamethasone

is diffusing through the PE membrane into the fluid of the *scala tympani*. It is notable that within the four performed experiments a high variability of dexamethasone concentration in the AP was determined. This may be explained by evaporation of the fluid which we tried to avoid by positioning the whole construct in an airtight closed cup and wrapping all in parafilm, which may not totally avoid evaporation.

An important factor influencing the release of active substances off the RNI and diffusion into the scala is the presence of a fluid film between the membrane and the *scala tympani*. The absence of air between the fluid and the membrane is crucial, as significant diffusion can only occur when the RNI establishes contact with the medium through the membrane. Any measurable diffusion ceases when the medium undergoes evaporation. Subsequent experimental investigations are necessary to identify and mitigate potential evaporation, thereby ensuring reliable determination of drug diffusion rates.

IV. Conclusions

A testing system to study the *in vitro* diffusion of pharmacological substances through a membrane into the *scala tympani* was successfully designed and manufactured. The established aRWN incorporates small dimensions, small liquid volumes, geometry, and the diffusion through a membrane into the results of release kinetics experiments. It allows better understanding of the diffusion rates of drug delivery implants for the treatment of inner ear disorders through the RWN and RWM. The system is cheap and easily replicable with an SLA printer.

In the future, a more reliable determination of diffusion rates could be achieved by changing the position of the RW, lowering it from the highest point of the *scala tympani* and thereby enabling gravity to push the medium against the membrane and RNI. Alternatively, creating a narrow opening of the niche such as present in the physiological condition or better waterproofing may achieve the desired results. Next to this using natural membranes is one focus of our future work for improving the aRWN to mimic the physiological conditions in our hearing-impaired patients.

Acknowledgments

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Author's statement

Authors state no conflict of interest.

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