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Original Research Article

Digital light processing and drug stability of Dexamethasone-loaded implant prototypes for medical treatment of the inner ear

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Abstract: 3D printed, patient-individualized drug-eluting implants for the round window niche (RWN) are an innovative, minimally invasive concept for the medical treatment of inner ear disorders. In this study, we investigate the 3D printing via digital light processing (DLP) and the long-term drug stability of Dexamethasone(DEX)-loaded implant prototypes (storage of 12 months at 25 °C/60 % relative humidity and 40 °C/75 % relative humidity ("accelerated"). A PEGDA-based, DEX-loaded (1 % w/v) photopolymer composition was used. We are able to 3D print 3 x 2.5 x2 mm implant prototypes without the need for a supporting structure. The drug was in accordance with the theoretical drug load as detected via HPLC in fresh 3D printed implant prototypes, so there was no significant drug degradation of DEX during the 3D printing process (duration of time ~ 11 mins). Under the long-term storage conditions approx. 15 % of DEX degradation were detected during 12 months (recovery of DEX ~ 90 % after three months and ~ 85 % after 12 months with 25 °C/60 % relative humidity). Drug degradation increased with "accelerated" storage conditions (recovery of DEX ~ 20 % after 12 months). Where there was a post-curing process (t = 30 mins) via UV light curing oven, it led to a pronounced degradation of DEX (recovery of DEX ~ 80 % recently after manufacturing of implant prototypes with post-curing).

I. Introduction

There is a growing interest in minimally invasive treatment of inner ear disorders as idiopathic sudden sensorineural hearing loss (ISSHL) and Ménière's disease (MD). An innovative concept is drug-eluting implants, which are implanted in the round window niche (RWN, see Figure 1) and enable an active pharmaceutical ingredient (API) to pass from the middle to the inner ear via diffusion through the semipermeable round window membrane (tympanic membrane). We assume this idea of precisely in the RWN fitting implants, which meet the individual anatomical and pharmaceutical needs of a patient, provide the potential for more controlled drug delivery in the inner ear as it's possible with current-state methods as drug delivery via microcatheter or injected gels. [1]

Modern medical imaging methods such as cone beam computed tomography (CBCT) enable precise digital models for implant manufacturing that meet the anatomical needs of a patient [1]. For the manufacturing of small-sized, complex-shaped RWN implants, there is the need for high-precision manufacturing to ensure safe and adequate fitting of the 3D printed implants that meet the anatomical needs of the patients. Further to this requirement, 3D-printing methods such as digital light processing (DLP) are very promising because of their high 3D-printing

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resolution [2]. Moreover, these methods enable a relatively easy drug loading just by mixing the API in the liquid photopolymer before 3D-printing [3][4]. Nevertheless, photopolymerizing 3D-printing methods such as DLP use UV light to cure the photopolymer. This may degrade the contained API.

In this study, we investigated the manufacturing process and the drug stability of Dexamethasone (DEX) of DLP-3D-printed, DEX-laden RWN implant prototypes. As biocompatibility is essential for implant applications, the prototypes are manufactured using Polyethylene glycol diacrylate (PEGDA) as a base material. PEGDA is well established in the research of photopolymerizing 3D printing for (bio)medical applications [5][6]. The manufacturing process of the prototypes was done with a post-curing process via UV light as well as without a post-curing process.

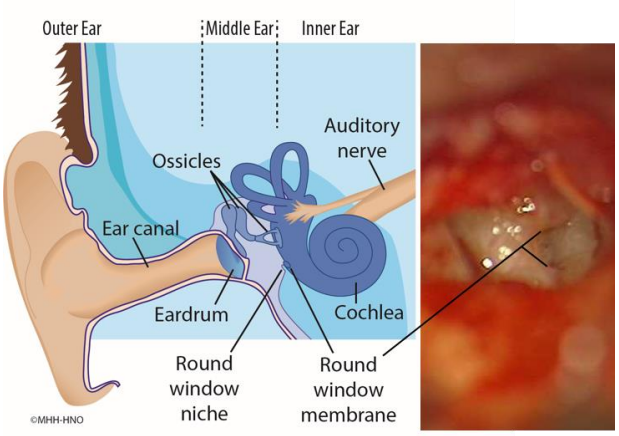

Figure 1: Left: Illustration of healthy ear anatomy containing the RWN (round window niche). Right: Intraoperative microscopic photo of the round window region with focus on the RWN and the round window membrane.

II. Material and methods

II.I. Preparation of drug-laden photopolymer

A drug-laden photopolymer composition to be DLP-3D-printed with a wavelength of $\lambda = 405$ nm was prepared pre-3D-printing. There is a need for $\lambda = 405$ nm because of the utilized DLP 3D printing device as it will be mentioned in chapter II.II. Nevertheless, a wavelength of $\lambda = 405$ nm is quite common for DLP 3D printing devices.

PEGDA with an average molecular weight of $M_n = 700$ g/mol was used as base material. Diphenyl(2,4,6-trimethyl-benzoyl)phosphinoxid (TPO, c = 1 % w/v) was used as the photoinitiator and the stain Orange G (c = 0.05 % w/v) was used as a light-absorbing agent. Both these substances are promising candidates for biocompatible photopolymers [3][7]. After stirring the photopolymer composition for 12 h, the API Dexamethasone (CAS: 50-02-2; Pharmaceutical Secondary Standard, Certified Reference Material; powder) was added (c = 1 % w/v) and the drug-laden composition was stirred for another 12 h until the API had dissolved in the photopolymer. All substances were purchased from Merck KGaA, Darmstadt, Germany.

II.II. 3D printing via digital light processing method

For additive manufacturing of drug-laden RWN implant prototypes, a digital model (Figure 2) in standard triangle language (STL) format and based on digital medical imaging data sets were used. The digital model was established by the reconstruction of 3D volumes from cone beam computed tomography (CBCT) images of patients, as presented before in [1].

There was no history of oto-surgical manipulation of the patients before imaging. The reconstruction was done via 3D SlicerTM software version 4.11. The study was done in accordance with the Declaration of Helsinki. Only patients who agreed to use their data were selected.

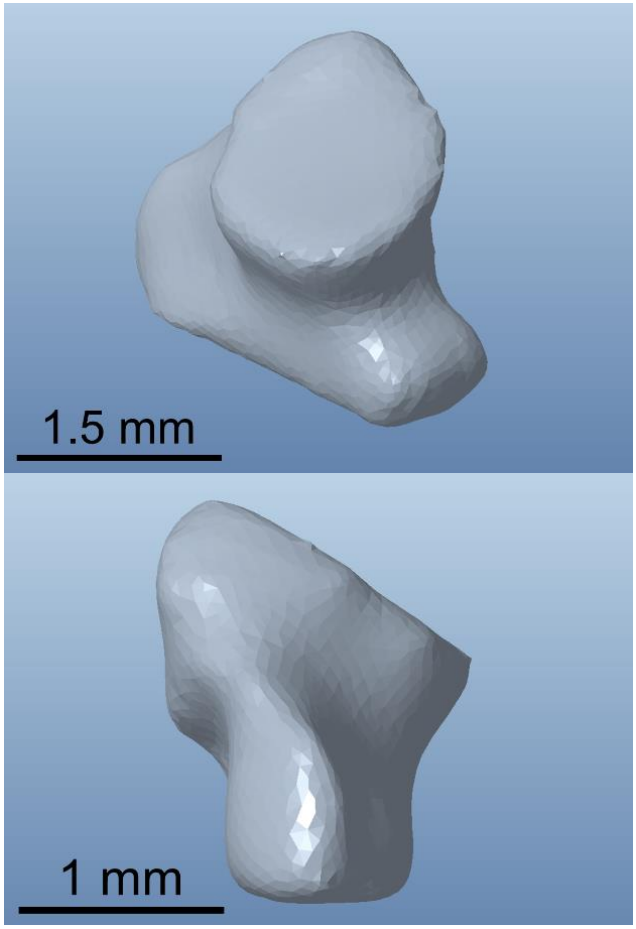

Figure 2: Top: Perspective view of digital model (STL-format) of patient-individualized RWN implant prototype. Bottom: Side view of digital model of implant prototype with build angles

Additive manufacturing was performed using a VIDA 3D printer (envisiontec GmbH, Gladbeck, Germany), which uses a wavelength of $\lambda = 405$ nm and a full-HD DLP projector with an irradiance of about 225 mW/cm². There is a XY-resolution of about 73 µm. The prepared drug-laden photopolymer was 3D-printed using an exposure time per layer of $t_{layer} = 9$ s and a layer height of $z_{layer} = 100$ µm. Because of that relative high layer height

z_{layer} there is a relatively low built time of 11 mins. With the foresight to a possible further application of that manufacturing method in clinical practice, a short built times is very promising. A short manufacturing time may enable implant manufacturing shortly after medical imaging and right before the implantation process, which is promising for a time-saving, patient-friendly intervention.

The digital models were positioned on the build platform without any supporting structure, as shown in Figure 3. That orientation of each single implant on the build platform is preferable, as the upper half of the implant prototype is designed for contact with round window membrane tissue (at potential clinical use) and should not be altered, even if supporting structures would be necessary. Moreover, the lower half has a relatively large footprint, which is promising for adequate adhesion on the build platform.

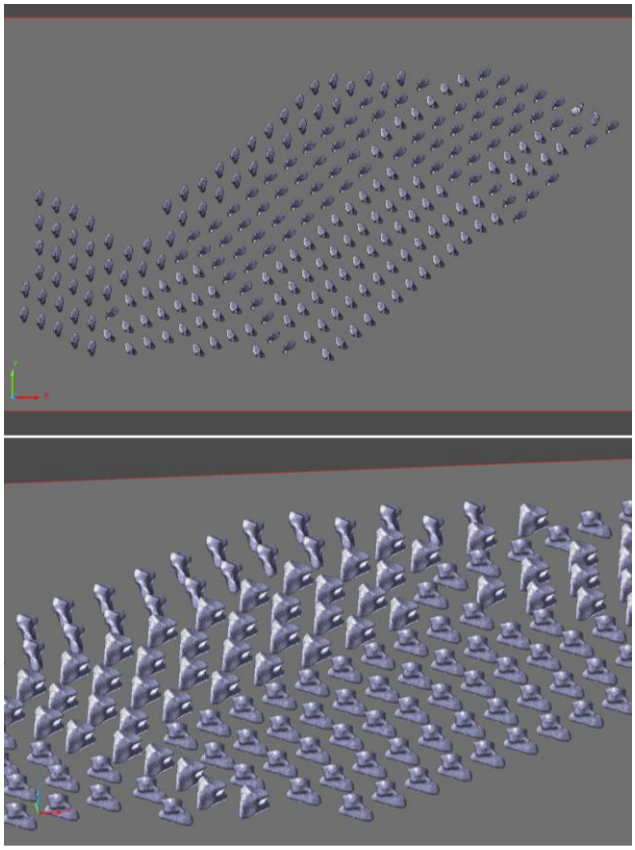

Figure 3: Preparation of DLP-3D-printing. Top: The positioning of n = 250 RWN implant prototypes on the digital build platform (top view). Bottom: Detailed view of RWN implant prototypes on digital build platform. Positioning of prototypes without any supporting structures (perspective view).

For a sufficient sample number for long-term drug-stability investigations, a number of n = 250 implant prototypes were 3D-printed simultaneously.

The digital models of the implant prototypes were arranged randomly all over the build platform by the software Perfactory RP (version number 3.2.3530, envisiontec GmbH, Gladbeck, Germany) as shown in Figure 3. Because of the small size of the implant prototypes, there is a very little material consumption of just about m = 1.5 g for the whole 3D print job of n = 250 of the solid implant prototypes. Notably, the DLP method is suitable for printing multiple parts simultaneously without increasing build time.

II.III. Post-processing

After the 3D printing process, the implant prototypes were unpacked from the build platform and cleaned from unpolymerized photopolymer with a dry wipe. In this cleaning process, the utilization of any alcoholic solvents, which are common in photopolymerizing 3D printing, must be avoided. Otherwise, there is a risk that the drug-load could be washed out.

Half of the 3D printed prototypes underwent post-curing in a UV light curing oven (type ACC-04-1000, envisiontec GmbH, Gladbeck, Germany) at room temperature for 30 mins. This UV light curing oven is equipped with three UV light bulbs model PL-L 18W/52/4P (Signify GmbH, Hamburg, Germany), each operating with a power of 18 watts and emitting light within $\lambda = 400$ to $\lambda = 500$ nm. Post-curing is common in photopolymerizing 3D printing and is often necessary to complete the polymerization process [3].

II.IV. Analytics

To determine the stability of the DEX in the implant prototypes, a Shimadzu Nexera XR HPLC system (Shimadzu Corporation, Kyoto, Japan) was used. It consists of a DGU-$20A_{5R}$ degassing unit, two $LC20AD_{XR}$ solvent delivery modules, a Sil-$20AC_{XR}$ auto sampler, a CTO-20AC column oven and a SPD-M20A photodiode array detector.

A Phenomenex Kinetex C8 column (150 × 2.1 mm, 2.6 µm, equipped with an according precolumn (Phenomenex Inc., Torrance, CA, USA) was used to separate the DEX and the rising degradation products.

A gradient method employing water with 0,1 % formic acid as mobile Phase A and acetonitrile as mobile phase B was used. A detailed overview of the used gradient can be found in Table 1.

The total run time of one sample was 32 mins. The temperature of the column oven was set to 40 °C at a flow rate of 0.5 mL/min. 5 µL were injected for analysis at 240 nm. The retention time of DEX was at 10 mins.

The analytical method was validated following the ICH guideline “Validation of Analytical procedures Q2(R1)” [8] concerning linearity, specificity, accuracy (within-day and between-day), precision (within-day and between-day), and robustness (freeze-thaw and rack stability). It was also checked whether the extraction of DEX from the implant samples was quantitative.

Table 1: Overview of gradient method (phase A: water with 0.1 % formic acid; phase B: acetonitrile).

time (min)	mobile phase A/B (%)
0	75/25
12	75/25
20	30/70
21	30/70
21.5	75/25
32	75/25

II.V. Long-term drug stability

The stability of DEX in implant prototypes was checked following the ICH guideline "Stability Testing of new Drug Substances and Products Q1A(R2)" [9] under long-term (25 °C/60 % relative humidity) and accelerated (40 °C/75 % relative humidity) storage conditions in climatic chambers (Binder KBF 115, Binder, Tuttlingen, Germany and Memmert HPP 110, Memmert, Schwabach, Germany).

Drug implants were stored in SureMed blister cards (Omnicell GmbH, Bochum, Germany) and analyzed at a frequency consistent with the guideline. Implant prototypes were extracted in 1.5 mL of a mixture of acetonitrile and water (1:1) for at least 4 h hours under continuous shaking. After this, the supernatant was directly measured by HPLC.

III. Results and discussion

III.I. 3D printing and post-processing

All of the n = 250 implant prototypes were successfully printed without any supporting structure (Figure 4), despite the relatively complex geometry with overhanging structures (see Figure 2 bottom and Figure 5 bottom). Supporting structures are commonly needed in DLP 3D printing [4].

Here, we assume that there is no need for supporting structures, because of the small size of the implant prototype and a high adhesion of the drug-laden PEGDA composition on the aluminum build platform of the DLP device. With respect to the visual assessment of the photographs of the implant prototypes, the precision of contour of the DLP 3D printed implant prototypes seems to be acceptable.

Nevertheless, further research with suitable 3D scanning methods would be needed to compare digital models and 3D printed models adequately and to define the exact need for precision for a adequate fit in the RWN, respectively a adequate tissue-implant interface. Because of the relatively high layer height of z_{layer} = 100 µm, there is a significant staircase effect (Figure 5 bottom). The utilized DLP-3D-printing device VIDA enables a z_{layer} = 25 µm.

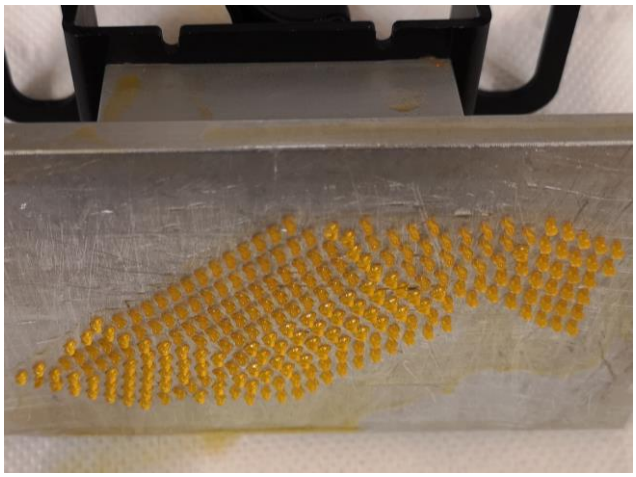

Figure 4: 250 RWN implant prototypes were successfully DLP-3D-printed (t = 11 mins).

Further research is needed, to investigate (1) if the build platform adhesion of the prototypes is suitable to process with even lower layer heights and (2) if there is the need for more a precise contour of 3D printed implants, e.g. via lowered layer height, for a sufficient tissue-implant interface. Nevertheless, a lower layer height would result in a significantly longer duration of the 3D printing process.

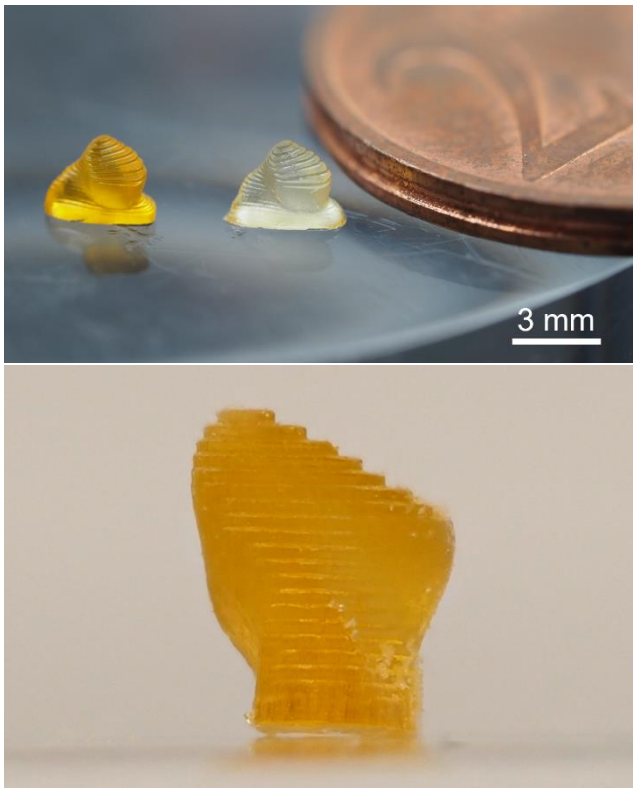

Figure 5: Top: DLP 3D printed RWN implant prototype manufactured without a post-curing process (left) and manufactured with a post-curing process (t = 30 mins, right). The stain Orange G is faded as a result of the post-curing because of prolonged UV light exposure. Bottom: Side view of DLP 3D printed RWN implant prototype. There is staircase effect (3D print with layer height z_{layer} = 100 µm).

Figure 5 (top) shows two DLP-3D-printed implant prototypes, one manufactured without post-curing (left) and the other with the described post-curing process with a duration time of t = 30 mins (right). There is no deforming of the implant prototypes after post-curing. However, the Orange G stain of the post-cured implant prototype has faded due to prolonged exposure to UV light.

III.II. Analysis of drug stability

In Figure 6 the DEX recovery over a period of 12 months is depicted. As can be seen, the implant prototypes that were not post-cured by UV light showed 100 % recovery at the initial analysis point. In contrast, only about 80 % of the declared DEX content was found in implant prototypes, which were manufactured with a post-curing process. During storage in climatic chambers, all implants showed a decrease of recovered drug over time. However, the decrease under accelerated conditions was more pronounced compared to long-term conditions. This was within the range of expectations since the accelerated storage conditions are more stressful for the implant and the contained DEX.

As indicated by the results, the post-curing process already led to a pronounced degradation of DEX. In literature, UV photopolymerization 3D printing processes are not necessarily associated with significant drug degradation [3][10][11]. A reason for the increased drug degradation might be the duration of time of the post-curing process. Compared to the 3D printing time of about 11 min, a post-curing of 30 min results in a significant plus of UV light exposure. Additionally, there is a light sensitivity of DEX, which is already described in the literature [12]. However, the post-curing process could be necessary for full conversion of the photoreactive substances [3]. This may be beneficial for biocompatibility and mechanical properties [13]. This will be the subject of further research.

Another well-known factor strongly affecting the stability of DEX is temperature [12]. The manufacturing process used in this work has the major advantage that no heating process is needed. Especially the degradation product 17-oxo-Dexamethasone increases sharply when manufacturing processes are used that are based on thermoforming technologies. However, the oxidative degradation of DEX is, of course, a function of temperature and also of time. Nonetheless, since DLP does not apply thermal stress to the drug and product, this technique could be a well suitable alternative, especially for substances, which are degraded during thermal printing processes such as the frequently used fused deposition modeling [3][4].

In addition to DEX degradation during the post-curing process, continuous drug degradation was also observed during the storage period. During the initial three months, no significant differences were observed during the storage conditions (25 °C/60 % relative humidity and 40 °C/75 % relative humidity (accelerated)). However, at later time points DEX degradation was more pronounced under accelerated conditions. After 6 months, several degradation products could be detected (Figure 7).

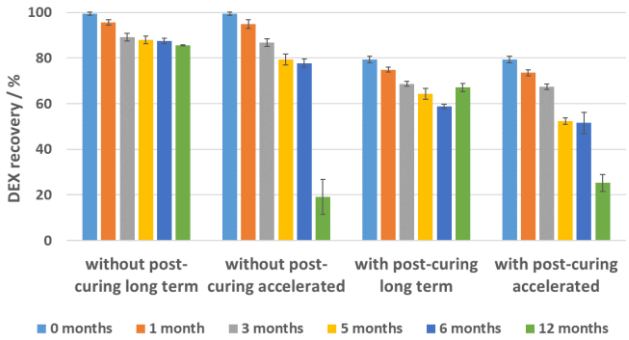

Figure 6: Recovery of DEX in % based on the declared content of the implant prototypes over a period of 12 months. Means ± SD, n = 3.

After 12 months under accelerated conditions a strong decrease of DEX was observed for both the post-cured and the untreated implant prototypes. Only one of the detected degradation products could be identified. The structure and thus the pharmacological profile of the other substances remains unclear.

It must be mentioned that only one packing material was used during these investigations. However, since the FDA certified this material for oral solid dosage forms we assumed it would be well suited for storing the implants. In the future, it might also be interesting to test other packing materials.

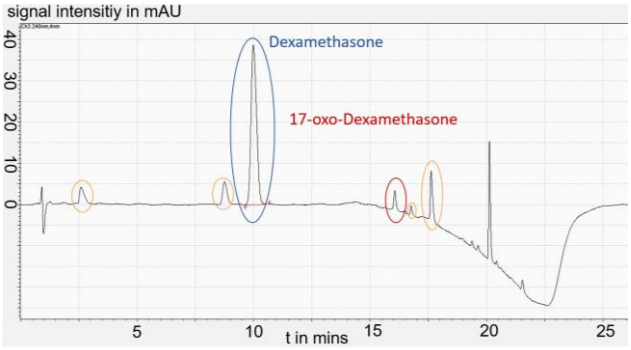

Figure 7: Section of an exemplary chromatogram of a post-cured implant stored for 6 months under accelerated conditions. Different unknown degradation products (yellow) formed over time, as well as the well-known degradation product 17-oxo-Dexamethasone (red).

IV. Conclusions

In this study, we demonstrated the manufacturing of drug-laden, patient-individualized RWN implant prototypes (dimensions about 3 x 2.5 x 2 mm) via the photopolymerizing 3D printing method DLP. These prototypes were manufactured with and without a post-curing process of 30 mins in a UV light curing oven. For drug-loading, the API DEX was incorporated in the liquid photopolymer material before 3D printing (1 % w/v). After manufacturing

the long-term drug stability of DEX was analyzed to investigate the influence of UV light exposure from the 3D printing and the post-curing process.

We found that there is no need for supporting structures while 3D printing. Despite the fact that there are overhanging structures, there is no need for a supporting structure because of the small dimensions of the prototypes and high adhesion on the build platform.

Drug-loading of the implant prototypes was successful. Analysis of stability followed the ICH guidelines. In the fresh 3D-printed implant prototypes, which were processed without post-curing, the theoretical drug load of incorporated DEX was detected by HPLC.

During the initial three months, no major differences could be observed during the different storage conditions. At later time points DEX degradation was more pronounced under accelerated conditions. Where there was a post-curing process, it led to a pronounced degradation of DEX recent after manufacturing process.

Further research is focused on implantation studies (anonymized formalin-fixed human temporal bone) to investigate the handling of the implant prototypes and to ensure adequate mechanics for implantation.

Moreover, there is a need for investigations of the drug release behavior of such implant prototypes. Further drug release studies need to be performed with regard to implantation into the RWN and the adequate diffusion of the drug through the round window membrane in the inner ear. We're aiming for a duration of drug release of about one month.

Another focus is on the biocompatibility of the photopolymerized materials. Probably, post-processing as washing procedures are needed for sufficient bio-compatibility, as demonstrated in literature [5].

It should be noted, such post-processing methods needs to be suitable with the realization a proper drug-load. In addition, other alternate manufacturing processes such as micro injection molding using rapid tooling and rapid manufacturing methods for implant manufacturing are being investigated [14]. These methods are promising for the high-precision manufacturing of implants with further (medical grade) materials.

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AUTHOR'S STATEMENT

Conflict of interest: D. Alcacer Labrador and S. John are employees of HörSys GmbH; F. Repp is an employee of OtoJig GmbH; T. Lenarz is shareholder of HörSys GmbH and OtoJig GmbH. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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