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Release Rates of Prednisolone-21-Hydrogen-Succinate from 3D-Printed Silicone as Material for Patient-Individualized Drug Releasing Implants

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Abstract: For treating idiopathic sudden sensorineural hearing loss, prednisolone is commonly used. However, systemic or middle ear injections often lead to insufficient drug delivery to the inner ear, causing ineffective treatment and systemic side effects. An implant inserted into the middle ear and delivering the drug directly to the inner ear offers a promising solution, providing controlled, long-term drug release with potentially better efficacy and fewer side effects. Individualized implants made of prednisolone-containing silicone can optimize inner ear treatment by fitting the patient's middle ear anatomy.

To gauge the properties of prednisolone-21-hydrogen succinate containing silicone, samples with different geometries and drug concentrations have been 3D-printed. The shore hardness of samples with three different drug concentrations was assessed. Three different shapes with four different drug concentrations were incubated in artificial perilymph for up to 56 days to evaluate the release rates. The resulting eluates were analyzed via Ultra high precision liquid chromatography coupled with a time-of-flight micro-mass spectrometer.

Samples were softer when a higher drug concentration was used. A high burst release of prednisolone after one hour was measured. Afterward, the release rates decreased and reached a relatively constant rate after ten days and stayed there for at least another 46 days. The release rates were multiple times higher when the samples had a higher surface-to-volume ratio. The softer the sample, the higher the release rate, unproportional to the concentration increase.

Keywords: 3D printing, release kinetics, release rates, inner ear therapy, individualized implant, prednisolone, silicone, drug delivery

1 Introduction

For the treatment of idiopathic sudden sensorineural hearing loss prednisolone is routinely used [1]. Today, the drug is applied systemically or uncontrolled via injection into the middle ear, resulting in insufficient bioavailability in the inner ear, i.e., not treating the hearing loss effectively, and often causing systemic side effects [2]. Application of the drug via an implant positioned in the round window niche (RWN) within the middle ear is preferable. Such an implant would enable a controlled local long-term drug delivery, possibly resulting in a more effective therapy with reduced side effects compared to today's therapy options. To optimize the result, such a drug-eluting implant can be of a patient-specific shape and size. A possible approach would be to use silicone containing prednisolone and precisely adapted it to the individual anatomy of the respective patient RWN using additive manufacturing.

We investigated if prednisolone-silicone is form stable printable and evaluated how geometry, drug load, and hardness affect the release kinetics.

2. Materials and Methods

2.1 3D printing of Samples

The printing material consisted of silicone and the respective catalyst (Momentive, Niskayuna, USA) [3] mixed with no or

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one of four different prednisolone-21-hydrogen succinate (mibe, Sandersdorf-Brehna, Germany) concentrations (1, 5, 10 and 20 % (w/w)). A homogenous mixture was achieved using a speedmixer (Hausschild, Hamm, Germany). Afterward, the mixture was loaded into a cartridge (25cc, Nordson, Lüneburg, Germany) tipped with a conic needle (gauge 400 µm, EnvisionTec, Gladbeck, Germany). The cartridge was loaded into a Desktop Health 3D Bioplotter (Manufacturer Series) and the samples were 3D-printed. For the release kinetics experiment four different mixtures in three different cuboid shapes with the same basis (60x60 mm) but different heights (1, 3, or 6 mm, Figure 1) were printed. For the shore hardness experiment, 6 mm thick 36x26 mm samples (in accordance with the ISO standard 48-4) consisting of pure silicone or silicone with a prednisolone concentration of 10 or 20% (w/w)

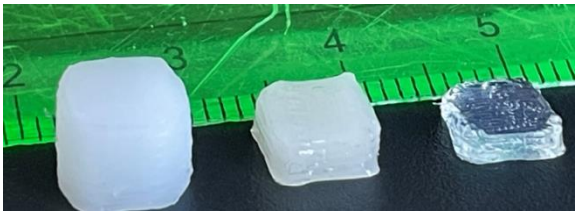

Figure 1: Samples with 6x6 mm basis but different height and drug load. From left to right - A 6 mm high 5 % prednisolone, a 3 mm high 1% prednisolone, and a 1 mm high pure silicone sample as used for release kinetics.

were printed. Each layer was 320 µm thick and separately cured via ultraviolet (UV) light (365 nm wavelength) for 30 minutes. The finished samples were additionally post-cured (UV light, 120 mJ/cm²; Spectrolinker XL-1000 UV Crosslinker, Farmingdale, USA).

2.2 Shore Hardness

To determine the hardness of the printed objects, a digital shore durometer (Figure 2) was used. All samples were probed for three times at four measurement points on each side per object (thus n = 24 per object) with a minimum distance of 12 mm from each other and from the edge of the object.

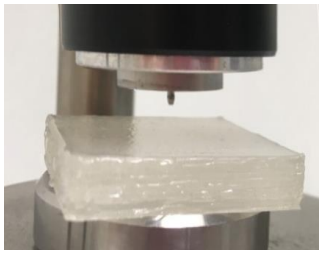

Figure 2 Illustration of the shore durometer probing tip and the 6 mm thick silicone sample.

2.3 Release Kinetics

The printed cuboids were incubated inside a 2 ml Eppendorf tube with 1 ml artificial perilymph (AP) [4] (sample volume and medium ratio according to ISO 10993-5) for up to 56 days (n = 3 per condition). The supernatant was collected and replaced by fresh AP at various time points (1 hour and 1, 2, 3, 7, 14, 21, 28, and 56 days after incubation start) and frozen at -20°C until further use.

2.4 Analysis of the eluates via Chromatography

The prednisolone concentrations of the harvested eluates were quantified using an Ultra High Precision Liquid Chromatograph (Xevo Qtof MS, Waters, Milford, USA) coupled with a time-of-flight micro-mass spectrometer (Q-TOF, Waters).

2.5 Data Analysis

The mean shore A values of the different sample groups were compared (analysis of variance, ANOVA) using OriginLab 2021 (Version 9.8, Northampton, USA).
The release rate results are reported descriptively due to the small sample size (n=3). The measured amounts of released prednisolone over time were added up to show the overall cumulative released amount within 56 days. Additionally, the measured amounts were converted to show the release rates of 1 mg implant per day to correlate the effects of drug load and surface-to-volume ratio (A/V ratio) of the implant to its release rates. As the mean release rate of any given incubation time interval was calculated, the mean release values were visualized in a graph were the data points lay in the middle between the AP changing time points.

3. Results and Discussion

3.1 Shore Hardness

The shore hardness within one test specimen was the same at every point measured. However, based on the included drug amount, the shore hardness of the three sample groups differed highly significantly (ANOVA, $p < 0.001$). The hardness of the test specimens with 10% drug content was with 44.9 ± 3.9 Shore A on average 80% of the hardness of the pure silicone with 52.7 ± 4.2 Shore A (Figure 3). A significantly reduced shore hardness of 2.8 ± 0.6 Shore A was observed in samples loaded with 20% prednisolone, which is only 5% of the hardness of the pure silicone samples. The hardness, which is a measure of the silicone crosslinking, is therefore significantly affected by drug loading. The fact that increased drug load reduces the hardness and therefore the silicone crosslinking should be taken into account for further

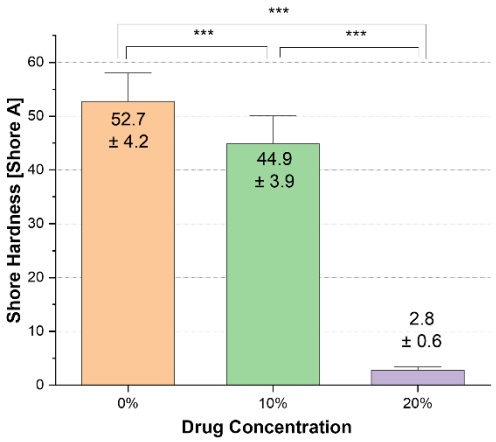

Figure 3: Results of the shore hardness measurements from 3D-printed samples. The samples with 10 or 20% prednisolone content were 10 or 95% softer, respectively, than the pure silicone samples. The mean +/- SD are given; N = 24 per group.

development of the drug loaded silicone implants given possible effects on the biocompatibility.

Which implant hardness is preferable is dependent on the needed release rate (see Section 3.2). For the implantability into the RWN, a moderately soft implant allows an easy insertion into the small cavity with a good firmness for handling. The optimal hardness needs to be assessed in the future in cooperation with physicians.

3.2 Release Kinetic

The total dose of prednisolone released within 56 days varied greatly based on sample geometry and drug loading (Figure 4). For example, below 3% (≈ 32 µg) of the total prednisolone pre-load (= 1 mg) was released from the 1% 6x6x3 mm samples and over 36% (≈ 19,7 mg), more than 12 times more, from the 20% 6x6x6 mm samples (pre-load: 55 mg). Similar percentage distributions were found for the 1 and 5% samples of the same geometry. For example, all samples with a height of 3 mm released a share of around 3% and samples with a height of 1 mm released a share of over 16%. However, contrary to expectations, the released share of the larger relative surface area of the 3 mm high samples was around 3% smaller than that of the 6 mm high samples, which had a smaller relative surface but released 3.3 to 4.1% of their prednisolone. It is possible that the larger samples were better able to maintain the concentration gradient. The 1 mm high samples had a lower concentration gradient, but the much larger surface area (260% compared to the 6 mm high sample (=100%)) possibly compensated for this. The 3 mm high sample may have had a poorer concentration gradient and an only slightly larger surface area (130%).

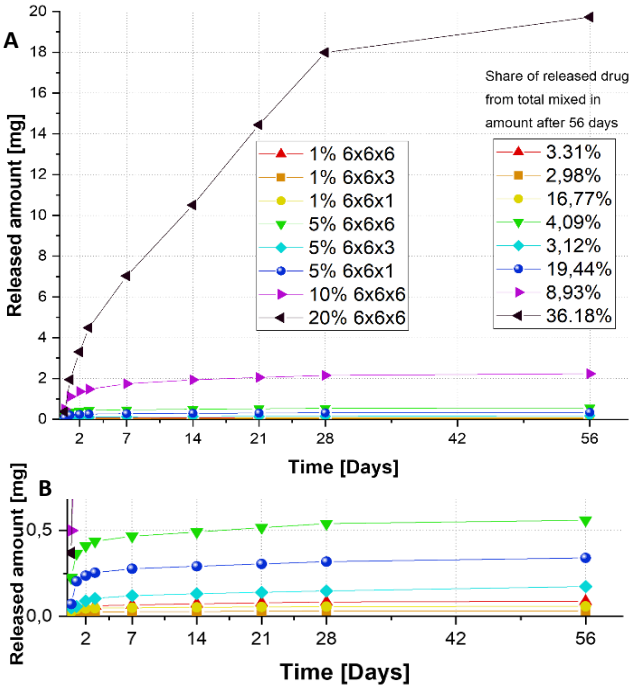

Figure 4: A) Cumulative amount of released prednisolone from 3D-printed silicone samples with 1, 5, 10, or 20% prednisolone and 1, 3, or 6 mm height within 56 days. The released amount is higher for the samples with a higher concentration or bigger relative surface area. Between 3 and 36% of the incorporated prednisolone was released. B) Close up of the lower concentration samples.

To compare the release rates solely based on their drug loading and A/V ratio, the measured values were converted to show the release rates of 1 g of sample per day. The drug release of all samples started at a high rate within the first 30 minutes (Figure 5), corresponding to the known burst release of passive diffusion drug delivery systems [5]. It was observed that higher loading concentrations resulted in higher drug release and that the burst release was higher for objects of the same concentration but larger A/V ratio.

After the first 30 minutes of cultivation (0.021 days), the release rates of all samples dropped sharply, thus flattening the graph, and then remained relatively constant over time. For example, the 10% sample started with a daily release rate of 48000 µg/g which decreased at time point 10.5 days to 112.6 µg/g and at time point 42 days to 12 µg/g. Only the 20% sample did not achieve a relatively constant release rate within 42 days. It needs to be checked in the future whether these values would be more constant with longer incubation time.

When comparing different concentrations with the same sample size, clear jumps were observed at 10 and 20%. For example, on day 10.5 the daily release rate of 1% samples was 3.1 µg/g, and for 5% samples 13.3 µg/g. This was an increase of almost fivefold and was in line with the expectation for a fivefold increase in pre-load drug concentration. On the

same day, however, 112.6 µg/g was measured for the 10% sample. This was more than eight times that of the 5% sample and therefore well above the expected doubling of the release rate. With 1.85 mg/g (=16 times higher), the daily release rate from the 20% samples at day 10.5 was also much higher than

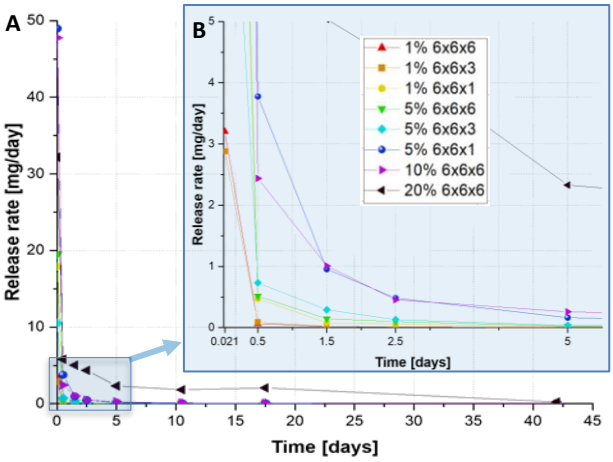

Figure 5: A) Mean daily release rates of prednisolone per 1 g of implant from 3D-printed samples within 42 days. As the mean release rate of any given incubation time interval was calculated, the data was visualized in the middle of the AP changing time points. An initial high release rate followed by a sharp decrease after 0.5h (=0.021 days) was observed. Subsequently, the release rate stabilized to a near constant release rate after 10.5 days. B) Close up of the lower concentration samples marked in blue in A).

the expected doubling of the release rate between 10 and 20%. This was presumably due to the hardness of the objects (see section 3.1). Thus, the 10% sample was slightly softer than the pure sample and the 20% sample was significantly softer than the 10% sample. As the material was softer, i.e. less linked, it could be speculated that the prednisolone could diffuse out of the sample more easily.

In the context of clinically relevant dosages/release rates, most studies worked with four intratympanic injections of 40 mg prednisolone within two weeks, achieving mixed results [6]. The inconsistency of clinical results regarding the effect of prednisolone may be because it is unknown how much of the in the middle ear injected drugs are actually reaching the cochlea and not draining via the Eustachian tube. RWN implants (RNI) may facilitate better diffusion, as they are positioned in the niche, separated from the cochlea only by the round window membrane. Therefore, even though the RNI are smaller and the released amount of prednisolone from the 20% samples were with ~ 20 mg (within 56 days) well below the referred dosages of up to 160 mg, RNI may achieve higher drug levels within the cochlea. This theory would have to be further investigated, as well as which dosage is needed to achieve a therapeutic effect.

4. Conclusion

The release rates of 3D-printed prednisolone containing silicone is influenced by drug loading, geometry, and hardness. The higher the drug loading and the bigger the surface area in relation to its volume, the higher the released drug amount. Similarly, the higher the drug concentration the softer the 3D-printed sample and the higher the release rate.

The drug-material combination can be used in future development of 3D-printed, drug releasing, patient-individualized implants to locally treat inner ear pathologies by pharmacotherapy. Additionally, the hardness of the implant can be used to adjust the drug release rates. For example, by including an additional matrix material that has no physiological effect on the patient but affects the object's hardness.

In the future, we may test the release rates of RWN shaped samples in a more anatomical similar setting (diffusion through a membrane into a small volume) by using our artificial round window niche [7].

Author Statement

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Conflict of interest: Authors state no conflict of interest.

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