Hybrid ear cubes for local controlled dexamethasone delivery to the inner ear

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Research article

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1. Introduction

Treatments of disorders and diseases of the inner ear are extremely challenging because of the *blood cochlear barrier*, which effectively protects this organ against potentially toxic substances (similar to the *blood brain barrier*, which protects the brain) (Swan et al., 2008). Thus, using classical administration routes (e.g., orally, i.v., i.m.), elevated drug concentrations might be achieved in the rest of the human body, but the drug amount reaching the target site (the inner ear) remains low. This leads to low (or no) therapeutic efficacy and potentially severe side effects. To overcome this crucial hurdle, different types of *local* controlled drug delivery systems have been proposed (El Kechai et al., 2015a; Sircoglou et al., 2015). They can roughly be divided into systems which are administered *into the middle ear* (Salt and Plontke, 2005; El Kechai et al., 2015b, 2016, 2017; Salt et al., 2016) or *directly into the inner ear* (Krenzlin et al., 2012; Takumi et al., 2014; Liu et al., 2015, 2016; Douchement et al., 2015; Alstolfi et al., 2016): intratympanic versus intracochlear administration. The advantage of intratympanic drug delivery systems is the lower invasiveness of their administration compared to intracochlear systems. However, their residence time in the middle ear is generally uncertain: Often, semi-solid formulations are more or less rapidly eliminated, depending for instance on the presence or absence of liquids in the middle ear. Furthermore, drug that has been released from intratympanic delivery systems must subsequently diffuse through the round or oval window into the perilymph. On the other hand, the placement of drug delivery systems into the inner ear might cause damage, infections and/or inflammation, potentially leading to various degrees of hearing loss.

Recently, a new type of miniaturized implants aiming at controlled local drug delivery to the inner ear has been proposed: so-called *Ear Cubes* (Gehrke et al., 2016a). They essentially consist of two parts: (i) a cylinder, which is placed into a tiny hole (< 4 mm) drilled into (or close to) the oval or round window, and (ii) a cuboid, which serves as “larger” drug reservoir,

which is placed into the middle ear. The advantage of these devices is the possibility to provide reliable residence times at the site of administration (in contrast to most intratympanic systems), while minimizing the invasiveness of the surgery compared to intracochlear implants. Furthermore, the "larger" drug reservoir does not expulse any perilymph (since it is located outside of the cochlea). This is important, since only about 70 µL of this liquid are present in a human inner ear (Sterkers et al., 1988). Please note that *Ear Cubes* should not become too porous and permeable over time, leading to potential perilymph leakage (e.g., the initial drug loading should not be too high). Also, it should be possible to remove them upon drug exhaust, if desired. Drug release from an *Ear Cube* occurs via the cylindrical part of the implant, which is partially in direct contact with the perilymph, and via diffusion from the parts of the cuboid, which are in contact with the oval or round window. These miniaturized implants can for instance be based on silicone [cross-linked poly(dimethyl siloxane)]. Importantly, the variation of the type of silicone, of the initial drug loading as well as the addition of certain excipients [e.g., polyethylene glycol (PEG)] can be used to adjust desired drug release kinetics from dosage forms based on this polymer (Krenzlin et al., 2012, Gehrke et al., 2016b).

Silicones are widely used for controlled drug delivery, e.g. in intravaginal rings (Malcolm et al., 2003; Fetherston et al., 2013), in thin films for scar treatment (Mojsiewicz-Pienkowska et al., 2015), in films for transdermal drug delivery (Snorradottir et al., 2011), in film coatings for tablets (Schulze Nahrup et al., 2004), in constructs for islet transplantation (Weaver et al., 2015) and in tiny rings for pacemakers (Herrlich et al., 2012). Different types of device structures (e.g., three layer matrices) (Soulas et al., 2012), chemical modifications (Soulas et al., 2013) or certain additives (e.g., linoleic acid) (Brook et al., 2008) have been reported to alter the resulting drug release kinetics. Various types of physico-chemical characterization techniques have been applied to better understand these systems, for instance scanning electron microscopy to monitor the distribution of the drug within the silicone matrices (Karami et al.,

2014). Also, mathematical modeling was used to elucidate the underlying drug release mechanisms (Krenzlin et al., 2012; Snorradottir et al., 2013).

Dexamethasone is a particularly interesting drug to be incorporated into local drug delivery systems, which are implanted in the human body for long time periods. For example, if metal electrodes are implanted into the heart tissue to transmit electrical signals as part of a pacemaker, one of the associated challenges is to prevent the formation of fibrous connective tissue: Such tissue can be a resistance for the electric current, resulting in less efficient electrical signal transmission to the target tissue and increased stimulation threshold values for the muscle cells. For this reason, tiny dexamethasone-loaded, silicone-based rings have been proposed to provide local controlled corticoid release to the vicinity of pacemakers lead electrodes (Herrlich et al., 2012; Mond and Stokes, 2014). Importantly, such steroid eluting electrodes can remain for many years in the human body, keeping the simulation threshold values low. This is because the resulting dexamethasone release rates can be very low. For instance, about 20 % of the drug was still present in leads which were explanted from patients after 10 years. The same rational of local, controlled, slow dexamethasone release to minimize fibrosis can be applied to metal electrodes, which are placed into the inner ear to transmit electrical signals to improve hearing (Krenzlin et al., 2012). The fact that dexamethasone release from such silicone-based implants can be very slow, can probably at least partially be attributed to slow drug diffusion through the polymeric systems, combined with limited drug solubility effects and limited amounts of water available within the devices (Siepmann and Siepmann, 2008, 2012, 2013).

The aim of this study was to develop a novel type of controlled release implants, allowing to effectively release one (and potentially more drugs) at pre-programmed rates into the inner ear. The idea was to modify the recently proposed *Ear Cubes* (Gehrke et al., 2016a) in such a way that two (or potentially more drugs) can be *independently* released. Instead of simply blending different types of drugs within the same silicone matrix, the new miniaturized implants

are composed of two halves, which can differ in composition. These *Hybrid Ear Cubes* might for instance contain a first drug in one half of the system, and a second drug in the other half. Importantly, the two halves can be based on the same type of silicone, or different types of silicone, and optionally differ in the initial drug loading, or presence of certain additives. This advanced implant design allows for a substantial formulation flexibility and the adjustment of desired drug release kinetics can be expected to be facilitated: Since the polymeric matrices in the two implant halves can be different, also the system porosity and drug mobility can be fundamentally different.

2. Materials and methods

2.1. Materials

Kits for the preparation of silicone elastomers: LSR 5 (Applied Silicone, Santa Paula, USA); Kwik-Sil (World Precision Instruments, Sarasota, USA); dexamethasone (Discovery Fine Chemicals, Dorset, UK); calcium chloride dihydrate, magnesium sulfate tetrahydrate, potassium chloride, sodium chloride and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES Pufferan) (Carl Roth, Lauterbourg, France); acetonitrile (HPLC grade; Fisher Scientific, Illkirch, France).

2.2. Preparation of thin hybrid films

Ten grams of “Part A” of the silicone preparation kit LSR 5 were manually blended for 5 min with appropriate amounts of dexamethasone powder (as received) in an ice-cooled mortar. Subsequently, 1 g of “Part B” of the preparation kit LSR 5 was added, and the mixture was further manually blended for 10 min in an ice-cooled mortar. The mixing of the two parts

of the silicone kits initiated cross-linking, resulting in solidification of the silicone. The cooling intentionally slowed down this crosslinking process (to facilitate matrix shaping). The obtained blends were transferred into 5 mL polypropylene luer lock syringes (Terumo Europe, Leuven, Belgium) and degassed under vacuum during 60 min to remove air bubbles. Drug-free mixtures were prepared accordingly, without dexamethasone.

Thin hybrid films (schematically shown on the left hand side of Figure 1) were prepared using a customized mold. The latter was built by covering a microscope slide with 2 layers of a Teflon sheet (Bytac, Sigma Aldrich, St. Louis, USA), and cutting a rectangular piece (6 x 1.5 cm) out of the upper Teflon sheet. The bottom halves of the films were prepared by placing an appropriate amount of a "silicone kit - drug" mixture into the mold. Using a casting knife (Multicator, Erichsen, Hemer, Germany) variations in the film thickness were minimized. Silicone crosslinking was completed by a thermal treatment in an oven at 60 °C for 20 h. After cooling to room temperature, the top halves of the hybrid films were added as follows: A third Teflon sheet with a hole (6 x 1.5 cm) was added to the mold (the holes of the second and third layer were superimposed). Subsequently, an appropriate amount of a "silicone kit - drug" mixture was added. Again, a casting knife (Multicator) was used to minimize film thickness heterogeneity, and curing in an oven at 60 °C for 20 h finalized silicone crosslinking. If indicated, the bottom halves of the films were turned around in the mold before adding the top halves. The thickness of the films was measured with a micrometer gauge (Digimatic Micrometer, Mitutoyo, Tokyo, Japan).

2.3. Preparation of Hybrid Ear Cubes

"Smaller" and "larger" *Hybrid Ear Cubes* as illustrated in Figure 1 were prepared using customized high precision molds. Blends of dexamethasone and "Parts A and B" of the silicone kit LSR 5 were prepared as described in section *"2.2. Preparation of thin hybrid films"*. The

bottom half of a *Hybrid Ear Cube* was prepared by spreading such a blend into one half of a high precision mold, careful removal of the excess and curing at 60 °C for 20 h in an oven. After cooling to room temperature, the other half of the mold was added, followed by injecting an appropriate second drug-silicone blend (using a 5 mL syringe and a texture analyzer: TAXT plus, Stable Micro Systems, Surrey, UK). The *Hybrid Ear Cube* implants formed during curing at 60 °C for 20 h in an oven. They were carefully removed from the mold under a microscope.

2.4. Drug release measurements

From hybrid films: Film pieces (10 x 10 x 0.4 mm) were placed into amber glass flasks containing 10 mL artificial perilymph: an aqueous solution of 1.2 mmol calcium chloride dihydrate, 2 mmol magnesium sulfate tetrahydrate, 2.7 mmol potassium chloride, 145 mmol sodium chloride and 5 mmol HEPES Pufferan. The flasks were horizontally shaken (80 rpm) in an incubator (GFL 3033, Gesellschaft fuer Labortechnik, Burgwedel, Germany) at 37 °C. At predetermined time points, 1 mL samples were withdrawn and replaced with fresh artificial perilymph. The drug concentrations in the withdrawn samples were determined by HPLC analysis (Thermo Fisher Scientific Ultimate 3000 Series, equipped with a LPG 3400 SD/RS pump, a WPS-3000 SL autosampler, a TCC 3000 D/RS column compartment and a VWD-3400RS UV-Vis detector; Thermo Fisher Scientific, Waltham, USA). One hundred µL samples were injected into a C18 RP column (Gemini 3 µm C18 110 A, 100 mm x 4.6 mm; Phenomenex, Le Pecq, France). The mobile phase was an acetonitrile:water 33:67 V:V blend, the flow rate was set at 1.5 mL/min. Dexamethasone was detected at λ = 254 nm. Each release experiment was performed in triplicate, mean values +/- standard deviations are reported.

From *Hybrid Ear Cubes*: To measure dexamethasone release from the miniaturized implants, the in vitro release set-up illustrated in Figure 2a was used. Briefly, a hole (0.4 mm diameter) was drilled into the bottom of a 0.2 mL Eppendorf vial, which was cut at half height.

The cylindrical part of a *Hybrid Ear Cube* was placed into this hole. The cuboid part of the *Hybrid Ear Cube* was fixed in the Eppendorf vial with some Kwik-Sil silicone. This Eppendorf vial was then placed into a second 0.2 mL Eppendorf vial, filled with 0.1 mL artificial perilymph. The orifice at the bottom of the first Eppendorf vial was always immersed in the release medium. The system was protected from light and placed in a horizontal shaker at 37 °C (80 rpm, GFL 3033). At predetermined time points, the release medium was completely renewed. The drug concentrations of the withdrawn artificial perilymph were determined by HPLC analysis as described above (but injecting only 40 µL samples into the HPLC column). Each release experiment was conducted six times, mean values +/- standard deviations are reported.

2.5. Swelling kinetics of Hybrid Ear Cubes

To monitor the swelling behavior of *Hybrid Ear Cubes* upon exposure to artificial perilymph, the experimental set-up illustrated in Figure 2b was used. Briefly, *Hybrid Ear Cubes* were fixed using stainless steel wire and a drop of Kwik-Sil silicone at the caps of 2 mL Eppendorf vials. The implants were immersed into 2 mL artificial perilymph, and the systems were placed in a horizontal shaker at 37 °C (80 rpm, GFL 3033). At predetermined time points, the implants were withdrawn from the medium and photos were taken with a Zeiss camera (AxioCam ICc 1, Zeiss, Jena, Germany; optical image analysis system: Nikon SMZ-U, Nikon, Tokyo, Japan). The medium was completely renewed at each time point.

2.6. Scanning electron microscopy

The morphology of cross-sections of *Hybrid Ear Cubes* (before and after 200 d exposure to the release medium) was studied using a scanning electron microscope (S 4000, Hitachi High-Technologies Europe, Krefeld, Germany). Samples were fixed with a ribbon carbon

double-sided adhesive on the sample holder and covered with a fine carbon layer. Cross-sections were obtained upon embedding the implants in Kwik-Sil silicone, freezing in liquid nitrogen and manual breaking.

3. Results and discussion

The aim of this study was to prepare miniaturized implants, which can be placed into tiny holes (less than 0.4 mm in diameter) drilled into (or close to) the oval (or round) window, able to deliver one or more drugs at a desired rate into the inner ear. To achieve this goal, previously proposed *Ear Cubes* (Gehrke et al., 2016b) were modified: In contrast to *Ear Cubes*, which are mono-block implants, *Hybrid Ear Cubes* are composed of two halves. Importantly, the latter might: (i) be loaded with different drugs, (ii) be loaded with the same drug at different concentrations, and/or (iii) be based on two different matrix formers. This hybrid design offers considerable formulation flexibility. For example, it allows to *independently* optimize the drug release rates from the two *Hybrid Ear Cubes* halves, e.g. due to differences in the permeability of the matrix formers and/or porosity of the systems upon drug exhaust. Initially, drug is released from surface-near regions. The latter become more or less porous, depending on the drug loading (e.g., drug crystals disappear and leave water-filled cavities behind). Thus, the mobility of remaining drug to be released through these drug-depleted zones can be fundamentally different. Also, water-soluble excipients might be added to only one half of the miniaturized implants, allowing for differences in the water uptake kinetics and drug permeability. This formulation flexibility is not given if two drugs are simply mixed in a mono-block system: The matrix former would be the same for both drugs, and the porosity of the matrix, the drugs have to diffuse through, would also be the same. For these reasons, a *hybrid*

system can be expected to be much more powerful with respect to the adjustment of desired drug release kinetics compared to a mono-block system.

3.1. Morphology of Hybrid Ear Cubes and hybrid films

Two types of *Hybrid Ear Cubes* were prepared by injection molding: "Smaller" and "larger" ones. The geometries and dimensions (in mm) are illustrated in Figure 1. Differently sized implants have been prepared to demonstrate the possibility to adapt the size of the system to the animal species or to take patient variability (e.g. children vs. adults) into account, if desired. The systems essentially consist of two main parts: (i) a cuboid, which serves as a "drug reservoir" which is located in the middle ear, and (ii) a small cylinder, which is placed into a hole drilled into (or close to) the oval (or round) window. The scheme on the right hand side of Figure 1 shows how a *Hybrid Ear Cube* can be securely fixed in vivo. The drug is released through the cylindrical part, which is in direct contact with the perilymph, and via diffusion from the cuboid into and through the oval (or round) window (since the cuboid is partially in direct contact with the surface of one of these windows). It has to be pointed out that the placement of the cylindrical part of the implant into the hole drilled into (or close to) the oval/round window assures a reliable fixation, which is of crucial importance: The major disadvantage of various other drug delivery systems, which are placed into the middle ear, is the considerable uncertainty of how long they will remain at the site of administration. The design and placement of the *Hybrid Ear Cubes* assure this reliable fixation, while the invasiveness of the required surgery is minimized compared to intracochlear implants (which are directly placed into the inner ear): Only a tiny hole is drilled into (or close to) the oval (or round) window. Importantly, the biggest part of the implant is located in the middle ear, and not in the inner ear. This allows providing a "larger" drug reservoir, while only a small amount of the perilymph is expulsed due to the presence of the implant. Note that the cochlea is a very

small and highly sensitive organ, containing only very minor amounts of perilymph: about 70 µL in humans (Sterkers et al., 1988). Placing the cuboid drug reservoir into the inner ear would be much more invasive.

Figure 3 shows a macroscopic picture of a "larger" *Hybrid Ear Cube.* The upper half is free of drug, whereas the bottom half is loaded with 10 % dexamethasone. As it can be seen, the upper half of the implant is transparent, whereas the bottom half is white. This can be explained by the fact that the dexamethasone is likely to be distributed in the form of tiny crystals throughout the silicone matrix (Krenzlin et al., 2012; Gehrke et al., 2016b). Importantly, both implants halves have a homogeneous appearance. Thus, the drug concentration within the bottom half is likely uniform.

Figure 4 shows macroscopic pictures of a series of "smaller" and "larger" *Hybrid Ear Cubes* which are loaded with 10 % dexamethasone in their bottom halves, while the drug concentration in their upper halves was varied from 0 to 60 %. In addition, on the left hand side of Figure 4, macroscopic pictures of a series of thin hybrid films are shown, the bottom halves were loaded with 10 % dexamethasone, the upper halves with 0-60 % drug. These films were prepared using customized molds. Importantly, in all cases (hybrid films and *Hybrid Ear Cubes*) the respective film/implant halves had a homogeneous appearance with respect to the drug distribution, irrespective of the dexamethasone loading. The "whiteness" of the upper layers became more and more pronounced with increasing dexamethasone loading. Thus, *Hybrid Ear Cubes* with a broad range of drug loadings, differently composed upper and bottom halves, homogeneous drug distributions and dimensions appropriate for use in humans can be prepared. However, note that systems containing 60 % dexamethasone were mechanically fragile, which can cause difficulties during implant placement and removal. In the latter case, the porosity created due to drug leaching can be expected to further impact system stability.

SEM pictures of cross-sections of *Hybrid Ear Cubes* are shown in Figures 5 and 6. The schemes on the left hand side illustrate where the cross-sections were made: either through the cuboid, or through the cylindrical part of the miniaturized implants. In these examples systems loaded with 10 % dexamethasone in one half and 30 % dexamethasone in the other half have been studied. Figure 5 shows an implant before exposure to the release medium, Figure 6 a *Hybrid Ear Cube* after 200 d exposure to artificial perilymph. Different degrees of magnification were used to obtain the pictures. Clearly, the drug was homogeneously distributed in the form of tiny crystals throughout the silicone matrices, irrespective of the drug loading and part of the implant (cuboid or cylinder). Furthermore, the difference in drug loading in the two system halves can be clearly seen. The matrix parts loaded with 30 % dexamethasone contain many more drug crystals than those loaded with 10 % drug. Importantly, various tiny cavities are visible after exposure to the release medium in Figure 6. It can easily be imagined how porous the 30 % drug loaded halves of the *Hybrid Ear Cubes* become once the drug is completely released from these parts of the implants. This is in clear contrast to the system halves containing only 10 % dexamethasone. Consequently, the mobility of the drug can be expected to significantly increase with increasing initial drug loading.

Figure 7 shows another SEM picture of a cross-section through the cylindrical part of a *Hybrid Ear Cube* after 200 d exposure to artificial perilymph. Again, the drug loading in one half was 10 %, and in the other half 30 %. Note that on the left hand side of the SEM picture also a part of the surface of the cylinder (which is not a cross-section) can be seen (initially loaded with 30 % drug). The scheme on the left hand side of Figure 7 illustrates this view point. The SEM picture shows that numerous tiny cavities are also located directly at the implant's surface. This part of the drug has probably been relatively rapidly released once the implant got into contact with the release medium.

3.2. Drug release from thin hybrid films

Figure 8 shows the experimentally determined dexamethasone release kinetics from different types of hybrid films into artificial perilymph. One half of the films was loaded with 10 % drug, the other half with 0, 10, 20, 30, 40, 50 or 60 % drug. Both film surfaces were exposed to the release medium. The absolute and relative drug release rates are shown. As it can be seen, the absolute dexamethasone release rate monotonically increased with increasing drug loading. This can be attributed to the fact that the silicone matrices become more and more porous upon drug exhaust (as discussed above), leading to an increased mobility of the remaining dexamethasone. In contrast to the *absolute* drug release rate, the *relative* drug release rate first decreased when increasing the dexamethasone loading, and then increased. This can probably be explained as follows: Upon water penetration into the system, not all of the drug is immediately dissolved (= in the form of individual molecules), because the amount of water is not sufficient to dissolve all drug. Thus, dissolved and non-dissolved drug co-exist. Importantly, only dissolved drug is available for diffusion. Thus, when increasing the initial drug loading, the concentration of *dissolved* drug in the matrices does not change (saturated dexamethasone solutions can be expected in all cases). Consequently, the concentration gradients of *dissolved* drug (the driving forces for diffusion) remain unaltered. However, the 100 % reference value for the relative drug release rate increases. This leads to decreasing relative dexamethasone release rates from the systems. At high initial drug loadings (in this case higher than 30 % drug), the above described increased porosity effect upon drug exhaust dominates, leading to increasing relative drug release rates with further increasing initial dexamethasone loadings. Note that this increased porosity effect also leads to increasing absolute drug release rates at constant concentration gradients of dissolved drug (left hand side of Figure 8). On the right hand side of Figure 8 the degrees of saturation of the withdrawn bulk fluids are shown, as a function of time and system composition. Please note that these are not the degrees of saturation

of the aqueous medium located *within* the silicone films (discussed above). Importantly, sink conditions were provided throughout the entire observation periods in all cases. Thus, the observed effects of the initial drug loading on drug release cannot be attributed to potential drug saturation effects in the *surrounding* bulk fluid.

A hybrid film was manufactured in two stages: (i) First, one half of the film was prepared in a customized mold: The drug was blended with Parts A and B of the silicone kit, the blend was placed into a mold. Using a casting knife variations in the film thickness were minimized. The system was solidified upon cross-linking of the silicone at 60 °C for 20 h. (ii) The second half of the film was added by introducing an appropriate "drug – silicone preparation kit blend" into the mold (the "walls" of which were increased in height), application of a casting knife and subsequent silicone cross-linking. Note that once the first film half is cross-linked, it might optionally be turned around in the mold before adding the second film half. To evaluate whether such a "turning around" of the first film half affects the resulting drug release kinetics from the final hybrid film, the release rates of dexamethasone from films prepared using the standard manufacturing procedure (no "turning around") were compared with the release rates from films prepared with a "turning around" of the first film half. If the inner structure of the first film half is not homogeneous (e.g., exhibits a different density of drug crystals towards its two main surfaces), a "turning around" could lead to altered drug release rates. As it can be seen in Figure 9, the dexamethasone release kinetics from thin hybrid films prepared in one or the other way were similar (the systems were loaded with 10 % drug in the "first" half, and 50 % drug in the "second" half). This is consistent with the SEM pictures of cross-sections of the investigated hybrid films (data not shown) and *Hybrid Ear Cubes* (Figures 5-7), showing no evidence for inhomogeneous structures in the respective film/implant halves. Also, the optical microscopy pictures of films and implants showed homogeneous system halves (Figures 3 and 4).

3.3. Drug release from Hybrid Ear Cubes

The experimental set-up illustrated in Figure 2a was used to monitor dexamethasone release from the investigated *Hybrid Ear Cubes*. Briefly, a small hole was drilled into the bottom of an Eppendorf tube and the cylindrical part of an implant was placed into this hole. The cuboid was fixed with some Kwik-Sil silicone in the Eppendorf vial. The latter was placed into a second Eppendorf tube, filled with 100 µL artificial perilymph. The system was shaken at 80 rpm at 37 °C. At predetermined time points the release medium was completely renewed and the drug concentration in the withdrawn samples measured by HPLC-UV. Figure 10 shows the resulting dexamethasone release kinetics from "smaller" and "larger" *Hybrid Ear Cubes*. Again, the relative and absolute drug release rates are illustrated, the drug loading was 10 % in one half of the systems, and varied from 0 to 50 % in the other halves (60 % drug loading was not studied, due to the considerable mechanical fragility of the respective miniaturized implants). Importantly, the observed drug release rates were extremely small: Less than 0.5 % of the drug was released after 2 months. This corresponds to less than about 0.6 µg after 60 d (in the average: less than 10 ng/d). These very low drug release rates can be explained by the very limited surface area of the miniaturized implants, which is exposed to the artificial perilymph, and the type of silicone that was used (Gehrke et al., 2016a). Comparing drug release from hybrid films (Figure 8) and drug release from *Hybrid Ear Cubes*, very roughly, the same tendencies were observed with respect to the impact of the initial drug loading (discussed above). However, the standard deviations were rather high in the case of the *Hybrid Ear Cubes* (due to the very low absolute release rates, resulting in very low dexamethasone concentrations to be measured). This leads to quite some overlap between the different systems and less clear tendencies (at least in the investigated time period). The diagrams at the bottom of Figure 10 show the degrees of dexamethasone saturation of the withdrawn samples. Clearly, in all cases,

the surrounding bulk fluid was far from being saturated, irrespective of the exposure time and initial drug loading.

In the case of more hydrophilic drugs, much faster drug release rates can be expected (Siepmann and Siepmann, 2008). This is because more water would penetrate into the system, leading to higher drug permeability in the release rate controlling matrix. Also, limited drug solubility effects *within* the polymeric system would be much less important. In order to increase the release rate of drugs with limited water solubility, different types of matrix formers might be used and/or water-soluble excipients be added, providing higher drug permeability (Krenzlin et al., 2012, Gehrke et al., 2016b). Also, please note that the observed very slow release rates might be desirable in the case of highly potent drugs [e.g., Zhang et al., 2011].

3.4. Hybrid Ear Cube swelling and inner structure

From a practical point of view, potential implant swelling upon contact with aqueous media can be crucial: Especially the cylindrical parts of the *Hybrid Ear Cubes*, which are placed into the tiny holes drilled into (or close to) the round (or oval) window, should not swell to a noteworthy extend. To monitor potential changes in the dimensions of the miniaturized implants upon exposure to artificial perilymph, the experimental set-up illustrated in Figure 2b was used. Briefly, a *Hybrid Ear Cube* was fixed at the cap of an Eppendorf tube, filled with 2 mL release medium. Thus, almost the entire surface of the implant was exposed to the aqueous bulk fluid. In practice, often only a portion of the cylindrical part would be exposed to the perilymph. However, in case the middle ear would be filled with a liquid, also the cuboid would be exposed to aqueous medium. So, the experimental set-up used simulates some kind of "worst case scenario" for system swelling in vivo. At pre-determined time points pictures were taken and the implants' dimensions measured. On the left hand side of Figure 11, some examples for macroscopic pictures are illustrated. On the right hand side of Figure 11, the dimensions of

"smaller" and "larger" *Hybrid Ear Cubes* are plotted as a function of the exposure time to artificial perilymph. Cleary, no noteworthy implant swelling (or shrinkage) was observed. This is very important from a practical point of view.

4. Conclusion

The newly proposed *Hybrid Ear Cubes* offer an interesting potential to adjust desired release kinetics of one or more drugs from miniaturized implants, which can be securely fixed at or close to the round or oval window to allow prolonged release into the inner ear with minimized surgery compared to intracochlear implants. Importantly, the hybrid design allows for considerable formulation flexibility, since the two system halves might: (i) be loaded with different drugs, (ii) be loaded with the same drug at different concentrations, and/or (iii) be based on two different matrix formers. This flexibility can be crucial, especially for innovative long term treatments of the inner ear. The observed very low drug release rates from the investigated dexamethasone loaded prototypes (e.g., < 0.5 % after 2 months) can be increased by altering the type of matrix former and/or the addition of appropriate additives, increasing drug permeability. Also, for more water-soluble drugs, much higher drug release rates can be expected.

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Figure captions

Fig. 1: Schematic presentation of the geometries and dimensions (in mm) of a hybrid film as well as of a "smaller" and "larger" *Hybrid Ear Cube*. The drawing on the right hand side illustrates how a *Hybrid Ear Cube* can be placed into the oval window (of humans or animals).

Fig. 2: Schematic presentations (not up to scale) of the experimental set-ups used: a) to measure drug release from *Hybrid Ear Cubes*, and b) to monitor the potential swelling of *Hybrid Ear Cubes*. Details are given in the *Materials and Methods* section.

Fig. 3: Macroscopic picture of a "larger" *Hybrid Ear Cube*, loaded with 10 % dexamethasone in its bottom half, and free of drug in its top half.

Fig. 4: Macroscopic pictures of hybrid films as well as of "smaller" and "larger" *Hybrid Ear Cubes*, loaded with 10 % dexamethasone in their bottom halves, and 0-60 % drug in their top halves. Note that systems containing silicone layers loaded with 60 % drug were fragile.

Fig. 5: SEM picture of a cross-section of the cuboid of a *Hybrid Ear Cube* (before exposure to the release medium): The scheme on the left hand side illustrates where the cross-section was made. On the right hand side, zooms are shown. The arrows mark drug crystals. The dexamethasone loading was 10 % in one half and 30 % in the other half.

Fig. 6: SEM picture of a cross-section of the cylindrical part of a *Hybrid Ear Cube* after 200 d exposure to artificial perilymph: The scheme on the left hand side illustrates where the cross-section was made. The dotted circle marks the limit between the implant and surrounding silicone (used to obtain the cross-section). On the right hand side, zooms are shown. White arrows mark drug crystals. The dexamethasone loading was 10 % in one half and 30 % in the other half.

Fig. 7: SEM picture of a cross-section and parts of the surface of the cylindrical part of a *Hybrid Ear Cube* after 200 d exposure to artificial perilymph: The scheme on the left hand side illustrates where the cross-section was made. Holes mark the places where drug crystals have been released from the matrix. The dexamethasone loading was 10 % in one half and 30 % in the other half.

Fig. 8: Dexamethasone release from thin hybrid films into artificial perilymph: Impact of the initial drug loading: 10 % dexamethasone in the bottom half, and 0-60 % in the top half (as indicated). The *absolute* and *relative* drug release rates are shown, as well as the degree of sample saturation.

Fig. 9: Dexamethasone release from thin hybrid films in artificial perilymph: Impact of an optional "turning around" of the bottom layer (in the mold) during preparation, before applying the top layer. The drug loading was 10 % in the bottom layer and 50 % in the top layer.

Fig. 10: Drug release from "smaller" and "larger" *Hybrid Ear Cubes* in artificial perilymph: Impact of the initial dexamethasone loading (10 % in one half, 0-50 % in the other half). From the top to the bottom, the *absolute* release rates, *relative* release rates and degrees of saturation of the withdrawn samples are shown.

Fig. 11: Absence of implant swelling upon exposure to the release medium: Macroscopic pictures of "smaller" and "larger" *Hybrid Ear Cubes* after different time intervals (as indicated), and "dimensions vs. time" plots. The dexamethasone loading was 10 % in the left halves and 30 % in the right halves.

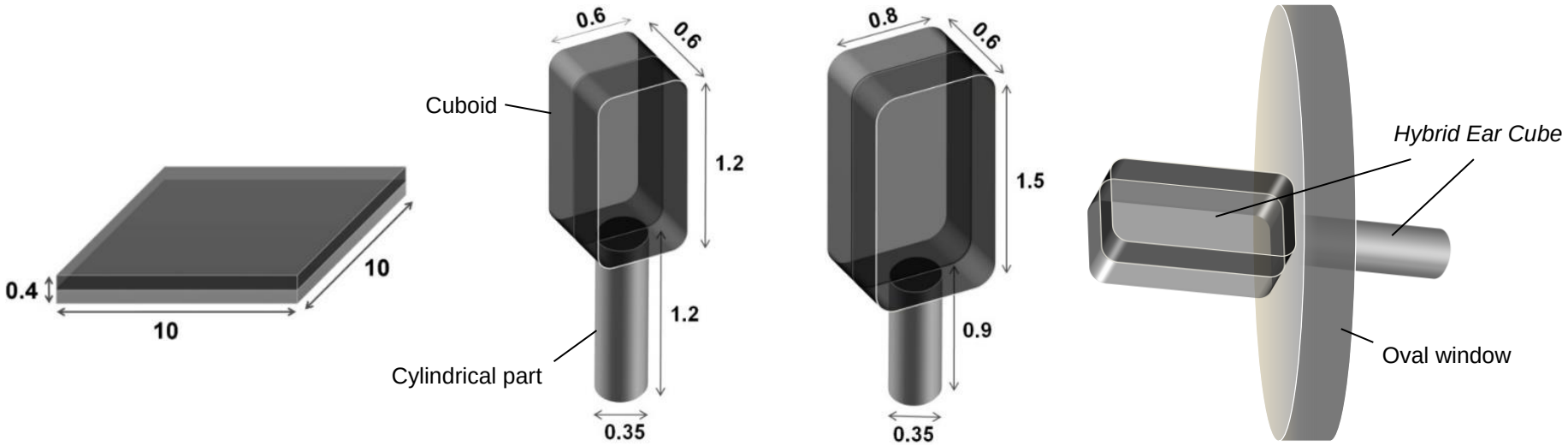

0.4
10
10
0.6
0.6
Cuboid
1.2
1.2
Cylindrical part
0.35
0.8
0.6
1.5
0.9
0.35
Hybrid Ear Cube
Oval window

Figure 1

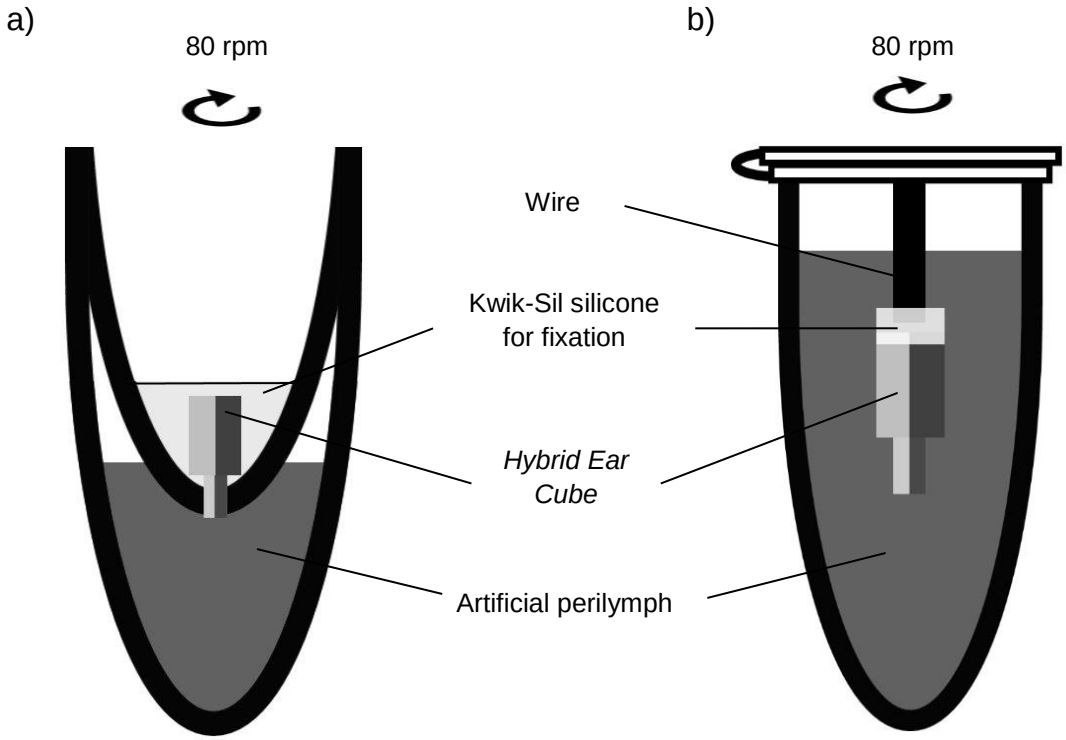
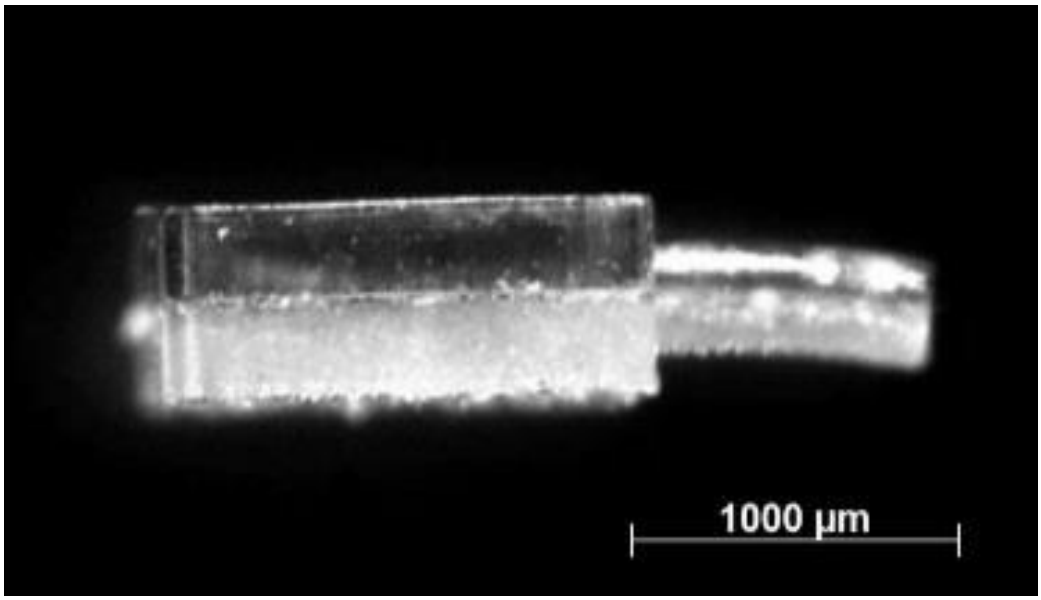

a)
80 rpm
b)
80 rpm
Wire
Kwik-Sil silicone
for fixation
Hybrid Ear
Cube
Artificial perilymph

Figure 2



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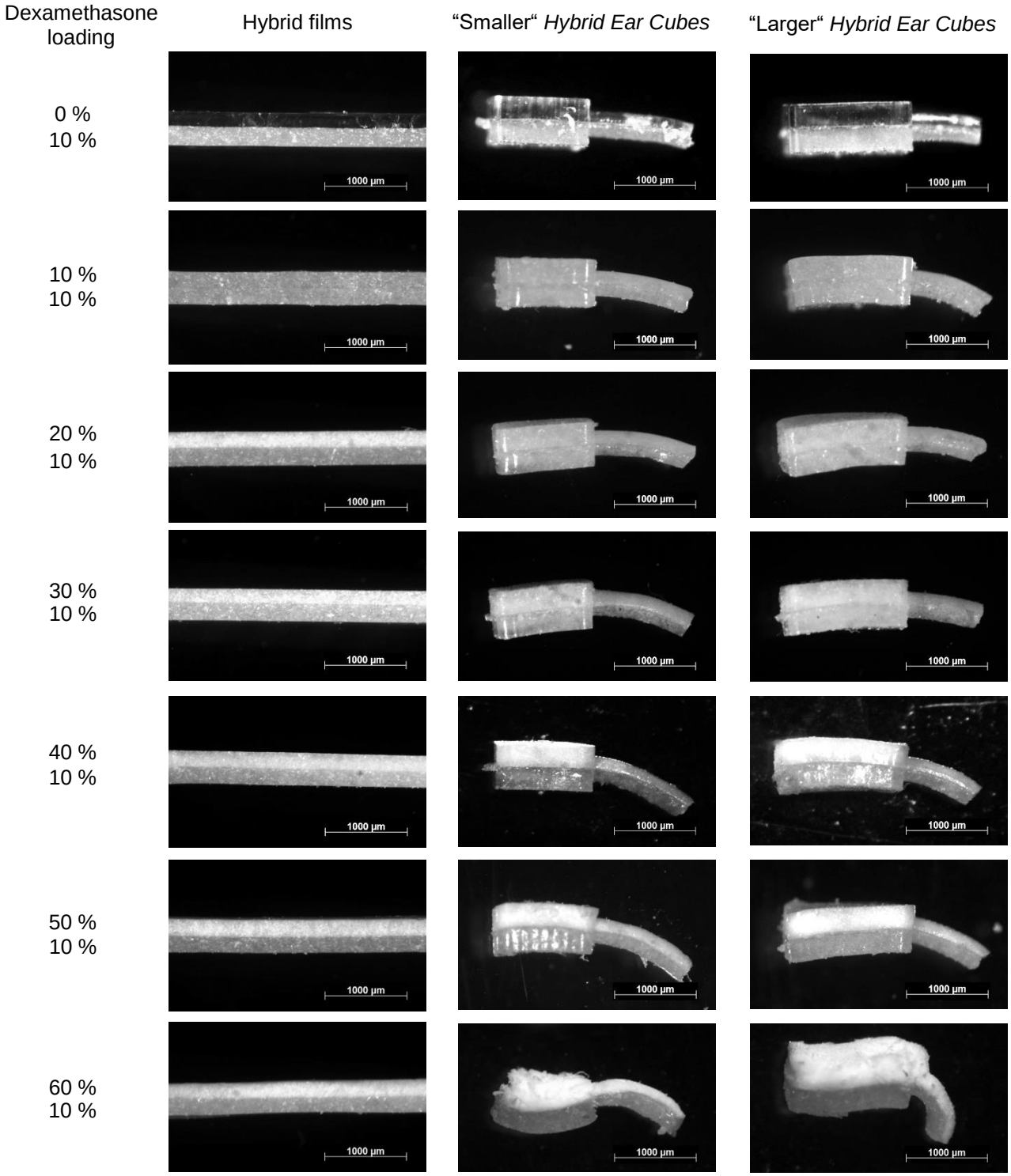
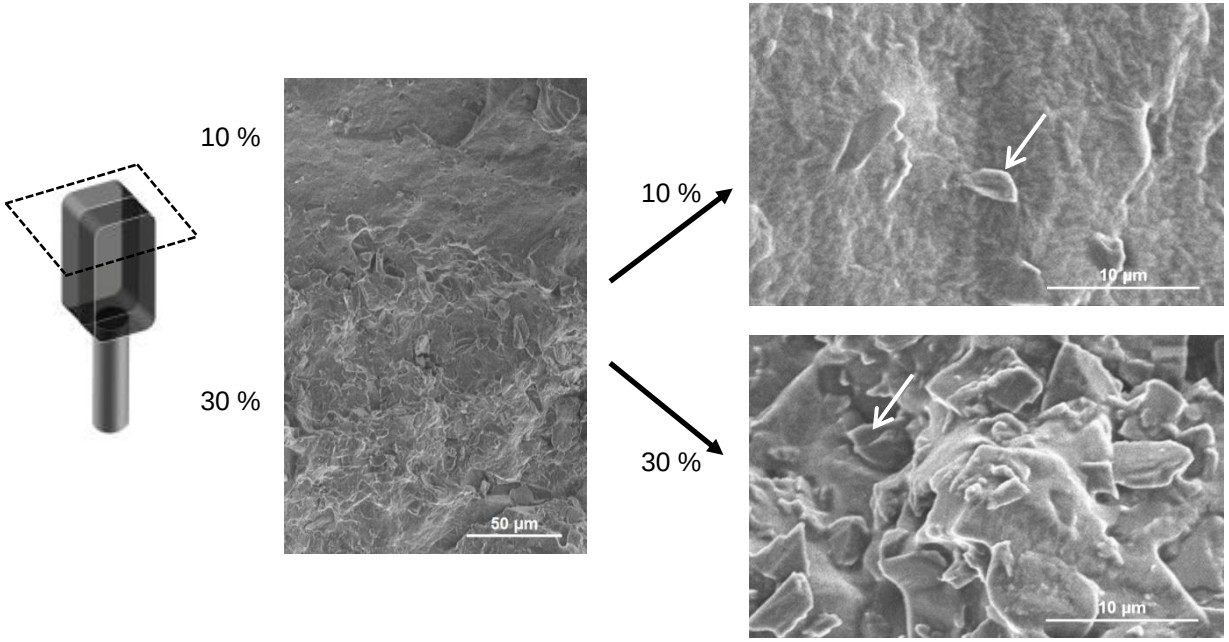

Dexamethasone loading
Hybrid films
"Smaller" Hybrid Ear Cubes
"Larger" Hybrid Ear Cubes
0 %
10 %
10 %
10 %
20 %
10 %
30 %
10 %
40 %
10 %
50 %
10 %
60 %
10 %
1000 µm

Figure 4



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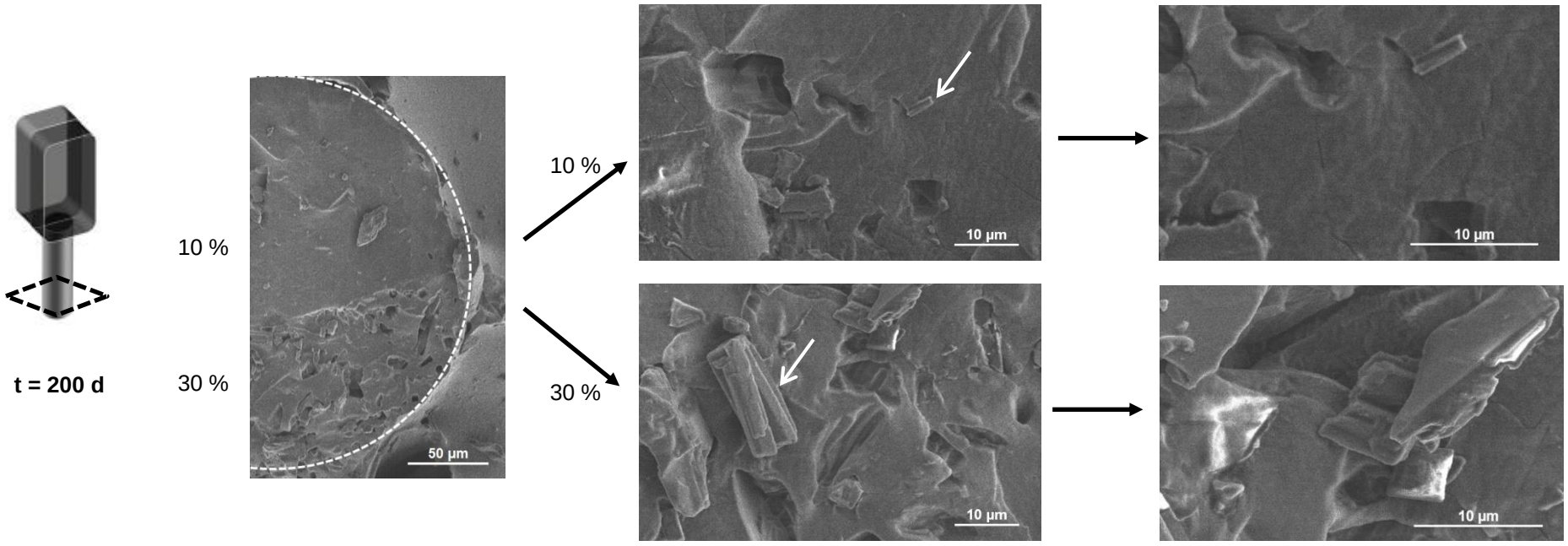

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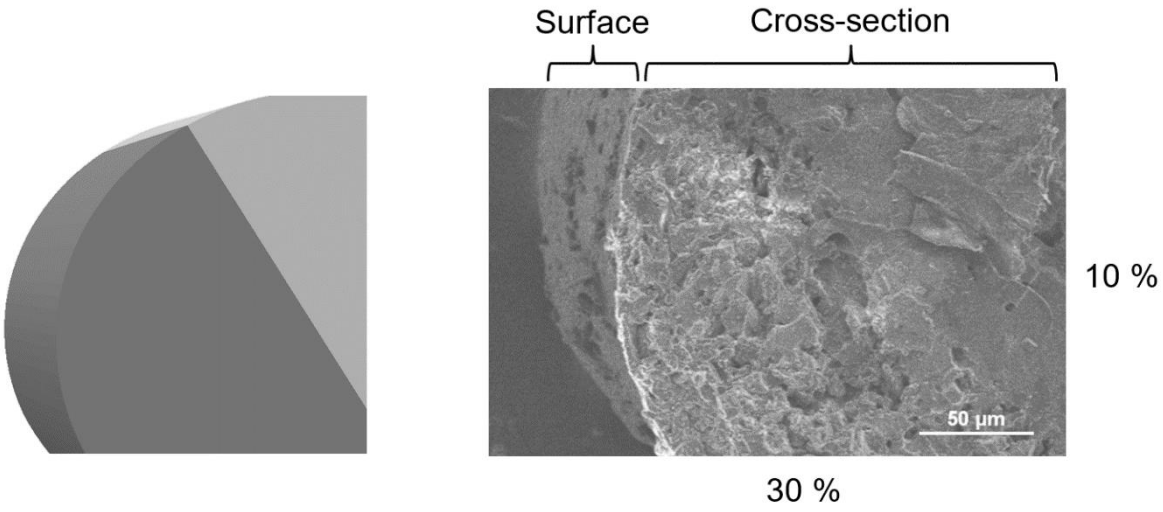

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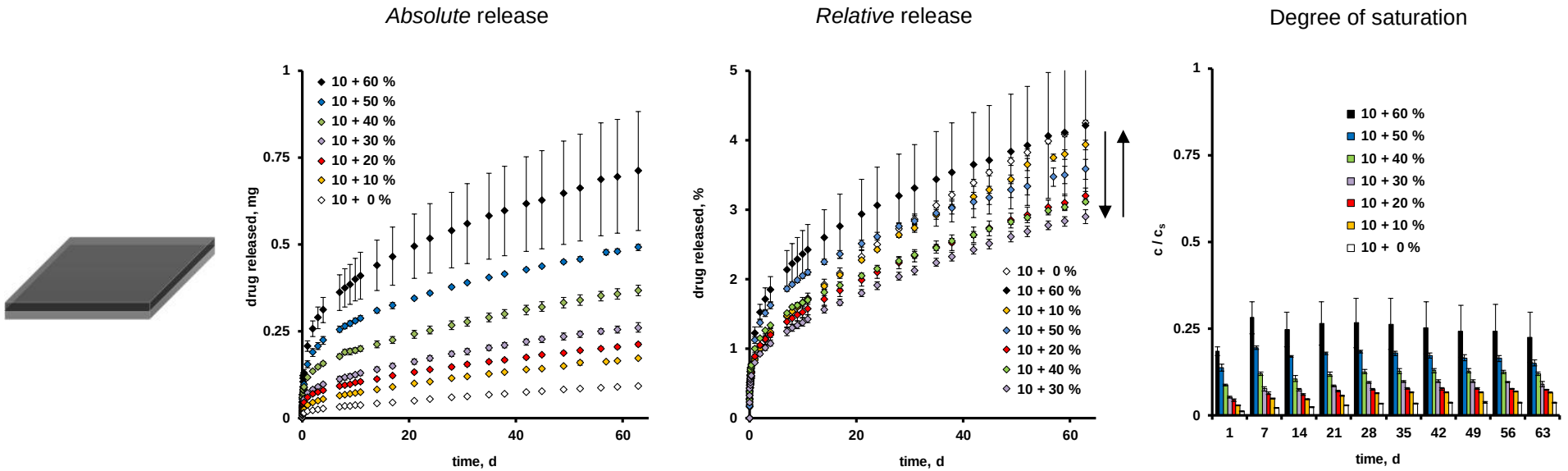

Absolute release
Relative release
Degree of saturation
drug released, mg
drug released, %
c / c_s
time, d
10 + 60 %
10 + 50 %
10 + 40 %
10 + 30 %
10 + 20 %
10 + 10 %
10 + 0 %

Figure 8

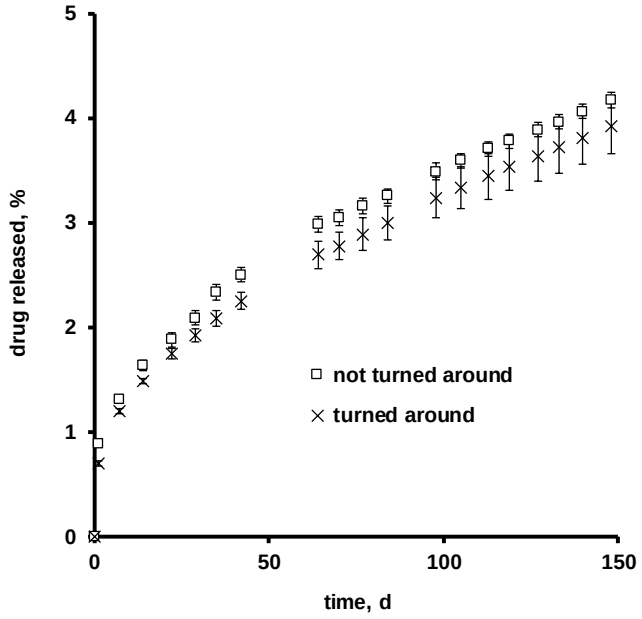

drug released, %
time, d
not turned around
turned around
0
1
2
3
4
5
0
50
100
150

Figure 9

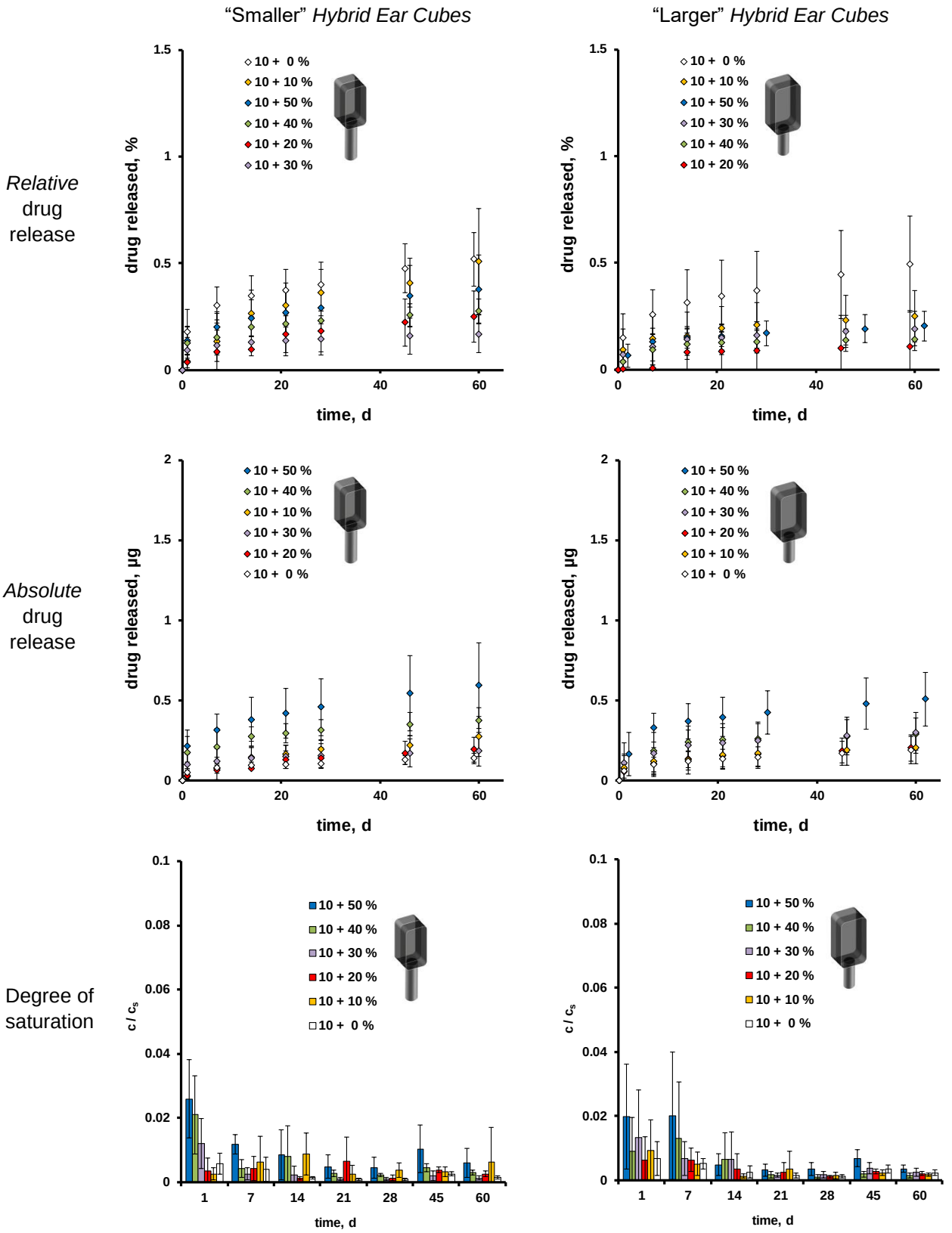
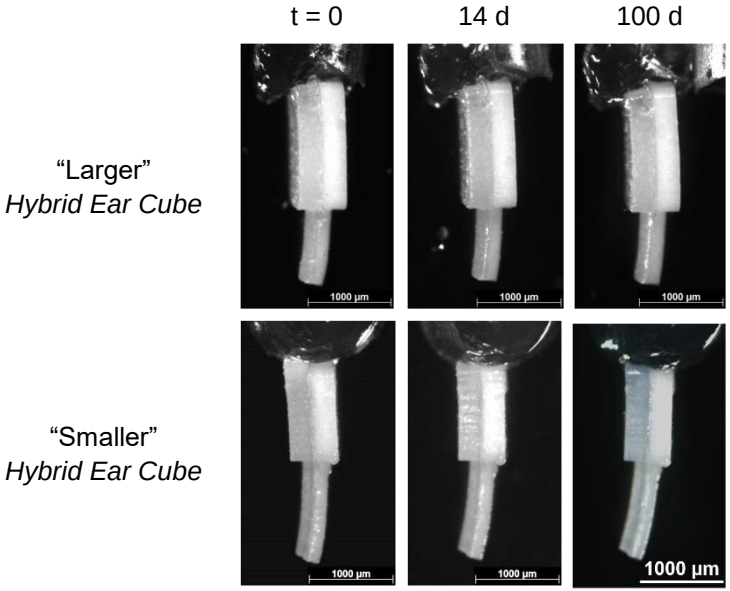

"Smaller" Hybrid Ear Cubes
"Larger" Hybrid Ear Cubes
Relative drug release
Absolute drug release
Degree of saturation
drug released, %
drug released, µg
c / cs
time, d
10 + 0 %
10 + 10 %
10 + 50 %
10 + 40 %
10 + 20 %
10 + 30 %

Figure 10


t = 0
14 d
100 d
"Larger"
Hybrid Ear Cube
"Smaller"
Hybrid Ear Cube
1000 µm
1000 µm
1000 µm
1000 µm
1000 µm
1000 µm

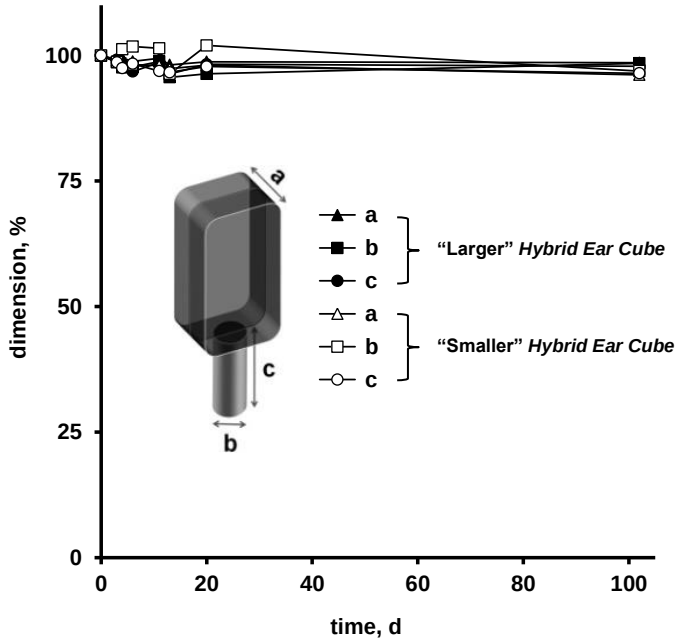

100
75
50
25
0
dimension, %
0
20
40
60
80
100
time, d
a
b
c
"Larger" *Hybrid Ear Cube*
a
b
c
"Smaller" *Hybrid Ear Cube*
a
c
b

Figure 11