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Design of the ocular coil, a new device for non-invasive drug delivery

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\begin{abstract}
Eye drops and ointments are the most prescribed methods for ocular drug delivery. However, due to low drug bioavailability, rapid drug elimination, and low patient compliance there is a need for improved ophthalmic drug delivery systems. This study provides insights into the design of a new drug delivery device that consists of an ocular coil filled with ketorolac loaded PMMA microspheres.

Nine different ocular coils were created, ranging in wire diameter and coiled outer diameter. Based on its microsphere holding capacity and flexibility, one type of ocular coil was selected and used for further experiments. No escape of microspheres was observed after bending the ocular coil at curvature which reflect the in vivo situation in human upon positioning in the lower conjunctival sac.

Shape behavior and tissue contact were investigated by computed tomography imaging after inserting the ocular coil in the lower conjunctival fornix of a human cadaver. Thanks to its high flexibility, the ocular coil bends along the circumference of the eye. Because of its location deep in the fornix, it appears unlikely that in vivo, the ocular coil will interfere with eye movements.

In vitro drug release experiments demonstrate the potential of the ocular coil as sustained drug delivery device for the eye. We developed PMMA microspheres with a 26.5 ± 0.3 wt% ketorolac encapsulation efficiency. After 28 days, 69.9% ± 5.6% of the loaded ketorolac was released from the ocular coil when tested in an in vitro lacrimal system. In the first three days high released dose (48.7% ± 5.4%) was observed, followed by a more gradually release of ketorolac. Hence, the ocular coil seems a promising carrier for ophthalmic drugs delivery in the early postoperative time period.

\end{abstract}

\section{Introduction}
The world market for ophthalmological products is valued at approximately USD 50 billion, of which pharmaceuticals take up 44% \cite{1}. Most pharmaceutical agents (drugs) are administered via eye drops or ointments. However, these drug delivery methods have several drawbacks such as low drug bioavailability \cite{2-5}, rapid drug elimination \cite{6,7}, side effects (such as allergies) \cite{8} and low patient compliance \cite{9-13}. Therefore, new drug delivery methods have been developed, such as intravitreal injectable inserts (e.g. Ozurdex\textsuperscript{®} (Allergan, Dublin, IE) or Iluvien\textsuperscript{®} (Alimera Sciences, Alpharetta, GA, US)) and conjunctival inserts (e.g. Mydriastin\textsuperscript{®} (Thea Pharmaceutical, Clermont-
The coil code is composed of the length, wire diameter and outer diameter (all expressed in mm).

### Specifications of the different types of ocular coils.

**Table 1**

| Coil code (L/Dₒ) | Coil length (L) ± tolerance | Wire diameter (Dᵢ) ± tolerance | Outer coil diameter (Dₒ) |
|------------------|-----------------------------|--------------------------------|--------------------------|
| 16/0.054/0.60    | 16.00 mm ± 0.05 mm           | 0.054 mm ± 0.005 mm            | 0.60 mm                  |
| 16/0.084/0.60    | 16.00 mm ± 0.05 mm           | 0.084 mm ± 0.005 mm            | 0.60 mm                  |
| 16/0.111/0.60    | 16.00 mm ± 0.05 mm           | 0.111 mm ± 0.010 mm            | 0.60 mm                  |
| 16/0.054/0.90    | 16.00 mm ± 0.05 mm           | 0.054 mm ± 0.005 mm            | 0.90 mm                  |
| 16/0.084/0.90    | 16.00 mm ± 0.05 mm           | 0.084 mm ± 0.005 mm            | 0.90 mm                  |
| 16/0.111/0.90    | 16.00 mm ± 0.05 mm           | 0.111 mm ± 0.010 mm            | 0.90 mm                  |
| 16/0.054/1.20    | 16.00 mm ± 0.05 mm           | 0.054 mm                        | 1.20 mm                  |
| 16/0.084/1.20    | 16.00 mm ± 0.05 mm           | 0.084 mm ± 0.005 mm            | 1.20 mm                  |
| 16/0.111/1.20    | 16.00 mm ± 0.05 mm           | 0.111 mm ± 0.010 mm            | 1.20 mm                  |

The coil code is composed of the length, wire diameter and outer diameter (all expressed in mm).

The aim of this study is to provide insights into the physical characteristics of the microsphere-filled ocular coil. We investigated the effect of several different diameters of stainless steel wire on the flexibility of the ocular coil under the assumption that higher flexibility might correlate to higher comfort for the patient. In addition, we studied the effect of variable outer diameters of the ocular coil on its flexibility. Furthermore, the effect on flexibility after filling the inner lumen with polymethyl methacrylate (PMMA) microspheres was tested. PMMA was used since it has proven not to degrade or decompose in the human body; besides, it does not provoke an immune response when used for ocular purposes [23]. However, in order to ensure that the microspheres remain inside the ocular coil, we calculated the gap-space between the turn-wwindings to prevent escape. We measured in vitro escape of microspheres from the ocular coil. Based on the these experiments, one type of ocular coil was selected and was inserted in the lower conjunctival fornix of a formalin fixed human cadaver in order to visualize the interaction between the anatomical boundaries of the eye and shape behavior of the ocular coil for further clinical applications.

Finally, with potential application in a post-operative setting, we investigated the use of ketorolac, a non-steroidal anti-inflammatory drug (NSAID) for drug release. Ketaorolac tromethamine is used for the prevention of Cystoid Macular Edema, a common complication of cataract surgery. The in vitro drug release profile of the ocular coil filled with ketorolac-loaded microspheres was tested and compared to the release profile of eye drops.

### 2. Materials and methods

#### 2.1. Materials

Different sizes of ocular coils were ordered from EPflex (Dettingen an der Erms, DE). A lens folding forceps for handling of the ocular coil was bought at Malosa medical (#1131, Malosa Medical, Eiland, UK). Polymethylmethacrylate (PMMA, Mn ≈ 43 kg/mol, Diakon MG102) and PMMA microspheres of 155 µm ± 15 µm in diameter (#009011-14-7) were purchased from Lucite International (Cumberland Place, UK). Polyvinyl alcohol (PVOH, Mn ≈ 80 kg/mol, Mowiol 8–88), potassium dihydrogen phosphate (KH₂PO₄), and dichloromethane (CH₂Cl₂) were used as received, and were bought at Sigma-Aldrich (Saint Louis, Missouri, USA). The water used for the synthesis of the particles was filtered on a Millipore Milli-Q plus system (R = 18.2 mΩ).

Toluene (99.8% HiPerSolv) and micro-sieves (450, 160, 140 and 70 µm) were purchased from VWR (VWR international, Oud-Heverlee, BE). Keterolac tromethamine was purchased from MSN laboratories (Telangana, IN).

#### 2.2. Ocular coil size and shape

Ocular coils, made of coated stainless steel (SS304) with a total length (L) of 16 mm, and an outer diameter (Dₒ) of 0.6 mm, 0.9 mm or 1.2 mm and a wire diameter (Dᵢ) of 0.054 mm, 0.084 mm or 0.111 mm...
were purchased from EPflex with tolerances as mentioned in Table 1 and Fig. 2.

The ocular coils were filled using a proprietary funnel-volume based technology that allowed filling of the lumen of ocular coils with PMMA microspheres. Filling of 103 ocular coils with 3.0 mg PMMA microspheres was analyzed and gave a Gaussian curve according to the D’Agostino’s K² test.

The dimensions of the ocular coils were manually measured with a caliper (#1150MI, IHM, Seynod, FR). Subsequent, scanning electron microscopic (SEM) pictures were taken with a JSM 6010 Plus/LV (JEOL, Tokyo, JP).

2.3. Flexibility tests

Flexibility tests were performed to determine whether filling the inner lumen of the ocular coil with microspheres affects their flexibility. The flexibility of the ocular coils was measured through a three-point bending test [19] using a Rheometric solids analyser (RSA3, TA instruments, Lukens Drive New Castle, DE, US), equipped with RSI-Orchestra software (version 6.5) (TA instruments, Lukens Drive New Castle, DE, US). The ends of each ocular coil were placed onto two solid points and a force was applied to the center of the ocular coil. The displacement was set at 2.5 mm and the force as a function of the displacement was measured at the center of the ocular coil (Fig. 5a).

To compare flexibility between the different ocular coils, empty and microsphere filled ocular coils were compared using unpaired t-test.

2.4. Gap size between the turn-windings

Analysis of the gap size between the turn-windings was performed in order to assure that microspheres do not escape from the ocular coil. Ocular coils were bent on curved glass discs with a diameter of 24 mm (average diameter of the ocular globe [24]). Bend and stretch forces were measured on the ocular coil using proprietary equipment. Images of bent coils were taken using an optical microscope (BX51, Olympus, JP) equipped with 4X and 10X objectives and a digital camera (SC50, Olympus, JP). The images were analyzed using the ruler tool in Adobe Photoshop CC 2015 (Adobe Systems Inc., San Jose, CA, US).

The measured gap sizes between the turn-windings were compared to a Gaussian curve, with good fit, using the D’Agostino’s K² test. The mean values of the measured gap size between the turn-windings was compared to the calculated mean gap size between the turn-windings when bent along the circumference of a disk with a diameter of 24 mm, using the student t-test. Furthermore, the measured mean gap size between the turn-windings was also compared to the calculated mean maximum gap size between turn-windings (see Section 3.1 Estimation of the gap size between the turn-windings of the ocular coil) using an unpaired student t-test.

2.5. Post mortem ocular coil insertion and computed tomography imaging

For this study the head of an intact human cadaver specimen from the Maastricht University body donation program was used. A hand-written and signed codicil from the donor is kept at the Department of Anatomy and Embryology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, the Netherlands.

Comfort of the ocular coil in vivo is related to the outer surface of the ocular coil, flexibility of the ocular coil and its capacity to follow the anatomical boundaries of the adnexa of the orbita (e.g. muscles and other soft tissues). To gain more insight in shape behavior of the ocular coil, the ocular coil was inserted in the lower conjunctival fornix of the head of a formalin fixed human cadaver. The ocular coil (16/0.084/0.90, see Table 1) was inserted in the fornix using a lens folding forceps.

A computed tomography (CT) scan was made with a multi-detector helical scanner (SOMATOM force, Siemens Healthcare, Forchheim, DE). The head was scanned in the supine position using the following technical parameters: 192 × 0.6 mm collimation, 100–120 kV, 50–461 mA, 0.5–1 s scan time, and 0.6 mm section thickness. The scan
was analyzed with Versalis 3D v1.0 (ps-medtech B.V., Amsterdam, NL) and Photoshop CC 2015 (Adobe Systems inc., San Jose, CA, US).

2.6. Oil/water emulsification for encapsulation of ketorolac tromethamine into PMMA microspheres

Ketorolac tromethamine loaded PMMA microspheres were prepared according to an oil/water emulsification method, modified from Govender et al. [25]. The organic phase was prepared by adding 5 mL (corresponding to 746 mg PMMA) of a 10% weight PMMA-CH2Cl2 (10 g PMMA in 67 mL CH2Cl2) solution to 600 mg freshly grounded ketorolac tromethamine. The suspension was homogenized by gentle shaking and ultrasonic bath treatment for 15 min. The aqueous phase consisted of a 100 mL PVOH solution (1% weight) in MilliQ water supplemented with 6 g of KH2PO4. The organic phase was then poured in one shot to the aqueous phase at room temperature and stirred for 1 h at 500 RPM using a mechanical stirrer (Heidolph RZR 2021, Heidolph instruments, Schwabach, DE). After stirring, the microspheres were washed three times with 100 mL MilliQ water and recovered on filter paper. In order to collect microspheres of 150 ± 10 µm diameter, the microspheres were transferred onto a stack of sieves (from 450, 160, 140 to 70 µm) under a continuous tap water flow for 10 min. After sieving, the microspheres were collected by washing off the 140 µm sieve with water, filtrated on a paper filter and freeze-dried before being stored at 23 °C.

To determine the remaining ketorolac content in the microspheres after the in vitro drug release experiment, the microspheres within the ocular coils were dissolved in 10 mL toluene, and ketorolac was extracted from the liquid using water (liquid–liquid extraction). One ocular coil was placed in a beaker with 10 mL toluene, the windings were gently pulled open using tweezers, and stirred overnight (at least 12 h) using a magnetic stirrer. Afterwards, two fractions of MilliQ water (10 mL and 40 mL) were added while stirring and the solution was stirred for 2 h per fraction. Finally, the aqueous phase was collected by decantation and measured at 313 nm with a Nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 313 nm. All data are expressed as mean ± SD.

2.7. In vitro drug release

Simulated tear fluid (STF) was prepared according to the description of Zhang et al. [26] and was used with a pH of 7.4. Ocular coils were submerged in 2.5 mL STF in one well of a 12-well-plate. In order to simulate physiological tear flow conditions, we designed an in vitro lacrimal system. One syringe pump created a continuous inflow of 2 µL/min STF whereas another syringe pump provided a continuous outflow of 2 µL/min STF (Fig. S1). The experiment was performed in the dark at room temperature for 28 days. Parafilm and a silicon inlay were used to prevent evaporation. At specific time intervals during the first day (10 min, 30 min, 1 h, 4 h, 8 h, 24 h, and 48 h), samples of 60 µL were drawn from the solution in the well. During the following days (day 3, 6, 7, 9, 10, 13, 15, 17, 20, 22, 24, 27, and 28), samples were drawn by collection the solution from the outflow syringes. A similar experimental set-up was used to measure drug release from ketorolac eye drops (0.5% Acular®, Allergan, Dublin, IE). A drop (50 µL) of ketorolac ophthalmic solution was added to the well at 0, 4, 8, 24, 28 and 32 h. Samples were taken right before and after addition of the eye drop. The concentration of ketorolac was measured using a Nanodrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 313 nm. All data are expressed as mean ± SD.

3. Calculations

3.1. Estimation of the gap size between the turn-windings of the ocular coil

To determine the optimal size of microspheres which prevent an escape from the ocular coil, the gap size between the turn-windings was predicted. The ocular coil is made of a coiled wire characterized by a total length (L), wire diameter (Dw), and outer diameter (Do) (Fig. 3a).
When the ocular coil is bent (not considering stretching or torsions) we assume that the maximum gap size between the turn-windings is realized if both extremities of the ocular coil would touch each other ($A = A'$ and $B = B'$), implies an applied rotation of $\alpha = 360^\circ$, as shown in Fig. 3b. In such a case, $r_i$ and $r_o$ (Fig. 3b) represent the inner and outer radius of the bent coil respectively, whereas the internal and external circumferences are indicated with $c_i$ and $c_o$. For $\alpha = 360^\circ$, the inner circumference ($c_i$) equals $L$ (Eq. (1)), $c_i = L = 2\pi r_i$ whereas, because the stainless steel wire cannot be compressed, the outer circumference ($c_o$) equals (Eq. (2)). The circumference follows from (Eq. (3)). The quantity $\Delta L$ is calculated from combining Eqs. (1)–(3): (Eq. (4)).

$$c_o = c_i + \Delta L$$

$$c_o = 2\pi r_o = 2\pi \left( r_i + D_w - \frac{D_w}{2} \right)$$

$$\Delta L = 2\pi \left( r_i + D_o - \frac{D_o}{2} - 2\pi (r_i) \right) = 2\pi \left( r_o - \frac{D_w}{2} \right)$$

The gap size between the turn-windings of the ocular coil (defined as: $\lambda$) is obtained from $\lambda = \frac{\Delta L}{N}$. Combining this with the calculation for the number of windings, $N = \frac{L}{\lambda}$, the gap size for each ocular coil can be calculated from $L$, $D_i$, and $D_w$ (Eq. (5)). Interestingly, Eq. (6) can be generalized and the gap size between the turn-windings can be predicted for any given rotation $\alpha$ ($\lambda = \frac{\alpha c_{0(eye)}}{N}$).

$$\lambda = 2\pi \left( \frac{D_w - \frac{D_o}{2}}{L} \right)$$

However, in clinical practice it is more convenient to use the diameter of the eye to estimate the bending of the ocular coil. Therefore, we translated the equations to a clinical situation. When the eye is observed from a frontal plane, the globe can be represented by a circle (Fig. 3c). Since the location of the ocular coil is expected to be at the lower side of the eye, according to the curve of the globe, the outer circumference of the eye ($c_{0(eye)}$) equals the length of the ocular coil ($L$) plus a variable ($\xi$). Since the gap size between the turn-windings is dependent on the outer circumference of the eye ($c_{0(eye)}$), $D_o$, and the $D_w$, these variables can be used to calculate the gap size between the turn-windings: (Eq. (6)). Thereby assuming the length of the ocular coil equals the length of $c_{0(eye)}$.

$$\lambda = 2\pi \left( \frac{D_o - \frac{D_w}{2}}{c_{0(eye)}} \right)$$

3.2. Calculation of microsphere mass of the inner lumen of the ocular coil

To estimate the available volume of the ocular coil, the inner volume of the ocular coil was calculated. The inner lumen, i.e. the central cavity, of the ocular coil has a cylindrical shape ($V = \pi r^2 L$). When calculating the accessible inner volume of the ocular coil ($V_{occ}$), the total accessible length ($L_{occ}$) equals the length ($L$) of the ocular coil subtracted with the length of the two cap heads ($L_h$) and cap plugs ($L_p$) ($L_h + L_p$ is the total length of the cap ($L_c$)) create (Fig. 2). Besides the caps, also the diameter of the wire ($D_w$) is subtracted twice from the outer diameter ($D_o$) to estimate the inner diameter ($D_i$) (see Fig. 2).

We estimate that the maximum concentration of microspheres correspond to the random close packing (rcp) volume fraction. The rcp volume fraction $\eta$ of identical (monodisperse) spheres results in a packing density of 0.64 [27–29]. Therefore, we used $\eta = 0.64$ as packing density inside $V_{occ}$ of the ocular coil.

The inner volume ($V_{occ}$) can be split in two compartments: the occupied volume ($V_{occ}$) which represents the volume occupied by the microspheres and the empty or free volume ($V_{void}$). The ratio of $V_{occ}$ over $V_{occ}$ is expressed as % filling. The total mass of the microspheres ($m_{sphere}$) inside the ocular coil is computed from (Eq. (7)). The density ($\rho$) of PMMA is: 1.18 g/cm$^3$ [MSDS Poly(methyl methacrylate) #182230, Sigma-Aldrich].

$$m_{sphere} = \left( \% filling \times V_{occ} \right) \times \rho \times \eta = V_{occ} \times \rho \times \eta$$

3.3. Process variation by a Monte Carlo simulation

To include a simulated process variation, a Monte Carlo simulation was used with 2000 samples. Table 2 gives an overview of the average values, set tolerances (if applicable) and standard deviations which were used.

### 4. Results

#### 4.1. Ocular coils

To design an optimal ocular coil for drug delivery purposes, ocular coils with a total length $L = 16.0$ mm ± 0.5 mm (including caps) and different wire thicknesses and outer diameters were used to calculate the inner volume of each type of ocular coil. The measured inner volume was obtained by subtracting the average cap lengths (provided by the manufacturer) from the measured lengths of the ocular coil. The calculated inner volume was computed using experimentally derived and Monte Carlo simulated values for the $L$, $D_w$, $I_p$, and $I_c$ of ten ocular coils. When the measured values were compared to the calculated values, small differences in the accessible volume were observed for ocular coils 16/0.111/1.20, 16/0.084/1.20, and 16/0.111/0.90 (Fig. 4a). Ocular coil 16/0.054/1.20 could not be produced, due to the large $D_o$ and the thin $D_w$ the ocular coil was unstable and could not hold its shape. Because of the Monte Carlo simulation no tests were executed to test significance of the differences. Due to large samples in the calculated groups all differences would show significance.

Based on these inner volume calculations, the filling volume of the ocular coil was estimated by considering a packing density of 0.64 and 70% filling with monodisperse PMMA microspheres. From Fig. 4b it follows that filling volume increases with increasing outer diameter and decreasing wire diameter.

#### 4.2. Flexibility of ocular coils

Flexibility of the ocular coil is an important factor that can affect comfort. Previous results indicate that filling the ocular coil with drug-loaded wire-filaments affect the ocular coil’s flexibility [19]. Therefore, the ocular coils in this study were filled with microspheres. Flexibility of the ocular coils (empty and filled) was measured with a three point bending test, see Fig. 5a. The required forces measured to bend the ocular coils with wire diameter of 0.054 mm were below the detection limit (0.0005 N/mm). The ocular coils with an outer-diameter of 0.60 mm, 0.90 mm, and 1.20 mm, and a wire thickness of 0.084 mm, and ocular coils with an outer diameter of 0.60 mm, and 0.90 mm with a wire thickness of 0.111 mm do not show difference in stiffness when filled with microspheres during displacement in the three point bending test. Only for ocular coil 16/0.111/1.20 a significant difference

| Table 2: Values for the Monte Carlo simulation | Average | Tolerance | Stdev |
|-----------------------------------------------|--------|-----------|-------|
| $L$ (mm)                                        | 16.0   | 0.5       | 0.17  |
| $L_o$ (mm)                                      | 0.76   | N.A.      | 0.10  |
| Microspheres (mg)                              | 3.00   | 0.30      | 0.10  |
| $D_o$ (mm)                                      | 0.90   | 0.02      | 0.007 |
| $D_w$ (mm)                                      | 0.084  | 0.005     | 0.002 |
in stiffness between the filled and empty condition was detected, as shown in Fig. 5b.

4.3. Filling of the ocular coils with PMMA microspheres

Based on the results of the filling (Table 3) and flexibility (Fig. 5b) of the ocular coils, we decided to narrow down the different types of ocular coils and continued with ocular coil 16/0.084/0.90 for further experimentation. This ocular coil was selected because it is rigid enough for manual handling and has a holding capacity for microspheres which appears sufficient for future drug loading. The ocular coil 16/0.084/0.90 was filled with PMMA microspheres (155 µm ± 15 µm) using our proprietary funnel based technology, which was set to a filling of 3.0 mg. The distribution of filling is plotted in a histogram in Fig. 6 where after the histogram was analyzed for its goodness of fit towards a Gaussian distribution using the D’Agostino’s K2 test. Filling of 103 ocular coils with PMMA microspheres was not normally distributed since a large amount of ocular coils (n = 68/103) were under- or overfilled (P < 0.0001). Filling of 103 ocular coils with PMMA microspheres was significantly different from the Gaussian distribution (P < 0.0001). The average filling was 3.01 mg ± 0.238 mg which is within our defined tolerances (2.9–3.1 mg).

4.4. Microsphere escape from the ocular coil

To investigate potential microsphere escape, ocular coil 16/0.084/0.90 was bent along the outer circumference of a glass disc with a diameter of 24 mm. Fig. 7a shows the distribution of the gap size between the turn-windings. The majority of gap sizes between turn-windings are between 4 µm and 10 µm. The gap-size between turn-windings is not normally distributed (D’Agostino & Pearson omnibus normality test, K2 = 4.671 with a P value of 0.0968). The mean calculated gap size between turn-windings was 7.93 µm with a standard deviation of ± 3.66 µm when bent along a 24 mm disc. The maximum measured gap size between turn-windings was 21.51 µm which was much larger than calculated from Eq. (7).

When comparing measured to calculated values for the gap size between turn-windings, the measured gap size between the turn-windings is significantly larger than the calculated value (P value below 0.0001) as shown in Fig. 7b. The measured gap-size between the turn-windings was also significantly larger than the calculated maximum gap size between the turn-windings (P value equals 0.0045), calculated from Eq. (5).

To experimentally test microsphere escape, a platform was designed to apply stretching forces simultaneously to the ocular coil while bending it. An increase in gap size between turn-windings was observed after applying both forces to the ocular coil; however, no escape of
Drug release studies revealed a high release of 48.7 ± 5.4% in the first three days followed by a slow and sustained drug release period for up to 28 days (Fig. 9b). The highest drug release was observed between 1 and 4 h after initiation of the study (Fig. 9a). During the first day, the ocular coil releases 274.7 ± 43.5 µg. In the second day, 126.9 ± 11.3 µg was released. After one, two, three, and four weeks the release lowered to 14.6 ± 1.7 µg, 5.1 ± 2 µg, 3.1 ± 1.1, and 1.2 ± 0.18 µg per day, respectively (Fig. 9c).

Extraction of the microspheres from the ocular coil after 28 days of release showed that a total of 69.9 ± 5.6% ketorolac was released from the ocular coil (Fig. 9b).

In contrast to the sustained release profile of ketorolac from the ocular coil, eye drops delivered high concentrations of ketorolac per drop but decayed quickly, which results in the typical stair-like pattern of eye drops (Fig. S2). 1 h after application, the drug concentration from the ocular coil and eye drop are equally in our system. The concentration of ketorolac rises fast with each applied eye drop, whereas the ocular coil releases more gradually.

5. Discussion

Several characteristics of a novel ocular drug delivery system have been investigated in this study. By tuning the geometrical characteristics wire thickness and outer diameter, the holding capacity and flexibility of the ocular coil can be varied. A larger outer diameter of the ocular coil in combination with a smaller wire diameter results in a larger central cavity with a greater holding capacity the ocular coil. Differences in the accessible inner volume were observed between the measured and the calculated volume of the ocular coils 16/0.111/0.90 and 16/0.111/1.20, where the measured accessible inner volume was slightly lower compared to the theoretically predicted accessible inner volume. This difference was probably caused by a slightly smaller outer diameter of the ocular coils in the received batch.

Next, we focused on ocular coil 16/0.084/0.90 for the following reasons. The ocular coils with an outer diameter of 0.60 mm might not hold enough microspheres for future drug release purposes. Ocular coils 16/0.084/1.20 and 16/0.054/0.90 were too flexible for proper handling. Ocular coils 16/0.111/1.20 and 16/0.111/0.90 showed lowered flexibility due to microsphere filling. To prevent the risk of an ocular coil which is too rigid; therefore, uncomfortable [19], we decided not to select these ocular coils for further investigations.

The ocular coil was filled with a volume based manual method, whereas mass is used to control whether the ocular coil is properly filled. In addition, there is some uncertainty in the volume fraction of particles in the filling, which might explain the deviation. Another important factor related to the variability in filling is that current batches of the ocular coil are filled manually. Eliminating the human interaction and replacing it by an automated system might lower the standard deviation.

Since microspheres might escape after filling the central coil cavity, the gap size between the turn-windings of the ocular coil was theoretically predicted and measured. The experiments showed that the calculated predictions are an under-prediction of the gap size between the turn-windings. The underestimation of the calculated gap size between the turn-windings can be explained by two reasons. Firstly, the calculations do not take into account, that ocular coils have caps of about 1 mm on both ends which are not capable of bending; due to the caps the ocular coil can only bend in the center. Secondly, an ocular coil with

| Table 3 | Overview of the measured values of L, Lc, Lh, and Lp and the calculated accessible value of V_acc obtained by knowing Do and Dw (provided by the manufacturer) and using Eq. (7). |
| --- | --- |
| Coil code (L/Dw/Do) | Measured lengths (mm) | Calculated volume (µL) | Calculated mass of microspheres (mg) |
| | L | Lc | Lh | Lp | V_acc | mspheres |
| 16/0.054/0.60 | 15.12 ± 0.28 | 1.62 ± 0.21 | 0.69 ± 0.06 | 0.83 ± 0.19 | 2.75 ± 0.08 | 1.45 ± 0.04 |
| 16/0.084/0.60 | 16.40 ± 0.06 | 1.00 ± 0.12 | 0.70 ± 0.09 | 0.31 ± 0.10 | 2.13 ± 0.08 | 1.13 ± 0.04 |
| 16/0.111/0.60 | 14.83 ± 0.19 | 1.05 ± 0.09 | 0.54 ± 0.04 | 0.51 ± 0.07 | 1.57 ± 0.08 | 0.83 ± 0.04 |
| 16/0.054/0.90 | 15.98 ± 0.08 | 0.76 ± 0.10 | 0.41 ± 0.10 | 0.34 ± 0.04 | 7.04 ± 0.19 | 3.72 ± 0.10 |
| 16/0.084/0.90 | 15.95 ± 0.16 | 0.82 ± 0.13 | 0.46 ± 0.04 | 0.36 ± 0.10 | 6.09 ± 0.16 | 3.22 ± 0.08 |
| 16/0.111/0.90 | 15.59 ± 0.24 | 0.80 ± 0.09 | 0.35 ± 0.04 | 0.44 ± 0.08 | 4.60 ± 0.16 | 2.43 ± 0.08 |
| 16/0.054/1.20 | – | – | – | – | – | – |
| 16/0.084/1.20 | 15.85 ± 0.17 | 0.66 ± 0.07 | 0.31 ± 0.06 | 0.35 ± 0.06 | 12.03 ± 0.27 | 6.36 ± 0.14 |
| 16/0.111/1.20 | 15.76 ± 0.07 | 0.66 ± 0.05 | 0.34 ± 0.06 | 0.32 ± 0.06 | 9.07 ± 0.42 | 4.79 ± 0.22 |

Data is expressed in Mean + SD, ocular coil 16/0.054/16, could not be produced. n = 10, Do = outer diameter, Dw = wire diameter, L = length, Lc = cap length, Lh = cap head length, Lp = cap plug length, V_acc = accessible volume, mspheres = mass of microspheres.
a smaller wire diameter in combination with a larger outer diameter stretches more easily than predicted. Due to the high flexibility of the ocular coil, a small extension occurred during the bending experiments, which could also explain the difference between the calculated and the measured values. However, no escape of the microsphere filling was observed, even when the ocular coil was extended to 179.9% of its original length (about 44.7 mm in length), thereby creating gaps between the turn-windings larger than 150 µm. Because the ocular coil was filled for 70% with microspheres, the microspheres were able to freely move in the ocular coil. We observed that the microspheres clumped together in the ocular coil and assume that the interaction of the coating with the PMMA microspheres induced a static interaction resulting in this effect; this hypothesis however, needs further investigation.

Eventually, the ocular coil was placed in the lower conjunctival fornix of a head of a human cadaver to investigate shape behavior and tissue interaction of the ocular coil. Bending of the ocular coil according to the anatomical configuration of the adnexa was visualized to gain insight in the contact between the ocular coil and the anatomical boundaries of the eye and shape behavior of the ocular coil. It was found that the ocular coil is located deep in the fornix, ventral from the muscles rectus inferior. The coil lies caudal from the lens in the fornix, where the bulbar conjunctiva transfers into the palpebral conjunctiva. Due to the position in the lower fornix, it seems unlikely that the ocular coil interferes with muscle movements and sensitive ocular tissues such as the cornea. CT imaging showed that the ocular coil fits well in the fornix and seems not to interfere with any critical ocular parts such as the cornea or muscles. However, we need to realize that the used human cadaver was formalin fixed, thus the tissue was much stiffer compared to a living human. We should also keep in mind that living humans have softer tissues so the ocular coil could be located even deeper into the fornix. As shown in prior studies by Pijls et al., use of an even more rigid ocular coil, although for a short period, was safe and did not cause irritation or discomfort [20,21]. In this study, two ocular coils were placed in the conjunctival fornix, without affecting comfort. However, an in-man study should confirm safety, comfort, and tolerability of the ocular coil in the lower conjunctival fornix for a prolonged time up to 28 days. Interestingly, a study conducted by Katz et al. showed that a rod shape ocular device is beneficial for fitting in the lower conjunctival fornix, compared to for instance oval or oblong shaped devices [30].

In our study, we compared 0.5% ophthalmic solution containing ketorolac (Acular®) to a ketorolac loaded ocular coil in vitro. In this proof-of-concept study, loading of the ocular coil depended on the drug loading capacity of the microspheres and the volume of the inner lumen of the ocular coil. Drug release of the ketorolac-loaded microsphere filled ocular coil was tested in an in vitro lacrimal system.

In terms of drug release, two materials can influence drug release, the coils itself or the microencapsulating polymer. Previous studies suggest that the ocular coil itself does not hinder drug release, when fluorescein sodium and ciprofloxacin were used [19]. Ketorolac tromethamine is a highly soluble compound with a logP of 1.9 [31]. We believe that the mechanism of drug release from the microspheres relies on delayed ketorolac dissolution that is the result of microencapsulation in hydrophobic PMMA. After increased release the first three days, the ocular coil has released about 50% of the drug (with an average release of 150 µg per day). Drug release of the ocular coil slowly reduces to a more consistent dose over the following week (a total of 60% is released after 10 days). In the following 18 days, the ocular coil releases up to 69% of its drug content with an average
Fig. 8. Location of the ocular coil in the conjunctival fornix of a formalin fixed human cadaver. (a) Sagittal section of the head with an ocular coil located in the fornix of the eye. (b and c) Bending of the ocular coil along the radius of the eye with calculated angle and size with Versalius 3D (b) and applied schematic drawing (c). (d) Overview photo of the ocular coil in the lower conjunctiva of the eye with all soft tissues removed (ocular coil is colored green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 9. Drug release from the ocular coil. (a) Concentration of ketorolac (µg/mL), released by the ocular coil in the lacrimal system. (b) Cumulative release of ketorolac (%) from the ocular coil over 28 days. (c) Release of ketorolac (µg/day) from the ocular coil over 28 days. All data are reported as mean ± standard deviation, n = 4.
release rate of 4.5 ± 0.8 µg per day. Release of the last 18 days tempered from 10.1 ± 1.9 µg per day on day 10, to 2.2 ± 0.3 µg per day on day 28. The drug-release profile with current microspheres suggests optimum usage for 14 days instead of 28 days. Furthermore, release experiments were performed at room temperature whereas the temperature of a human *cul-de-sac* lies between the 35 °C and 36 °C [32]. Based on the glass transition temperature (T_g) (> 85 °C) and the temperature of a human release experiments were performed at room temperature whereas the temperature of a human *cul-de-sac* lies between the 35 °C and 36 °C [32]. Based on the glass transition temperature (T_g) (> 85 °C) and the temperature of a human *cul-de-sac* lies between the 35 °C and 36 °C [32]. Based on the glass transition temperature (T_g) (> 85 °C) and the heat distortion temperature (HDT) (99 °C-102 °C) of PMMA, provided by the supplier, no temperature related release effect was anticipated, therefore, the experiments were performed at room temperature.

This system was also used to evaluate drug release from eye drops. After 30 min, the same amount of drug was released from ocular coil compared to one eye drop (Fig. S2). Afterwards, drug release from the eye drop increased gradually. However, this system does not consider pre-conneal loss of eye drops due to blinking or spillage during application. Hence, the entire eye drop (50 µL, ~2.5 mg of ketorolac) can be found in the system and results in an over-estimation of the drug release. Since less than 5% of an eye drop actually penetrates into the anterior chamber [14]. The ocular coil does not affect the drug properties, such as solubility, permeability, metabolic stability, and transporter effects, since it is just a carrier. However, we created microspheres without additives, hence, the pH is not optimized, nor have we used penetration-enhancing stabilizers such as EDTA or benzalkonium chloride. This could affect ocular penetration as previously shown by Bertens et al. in post-mortem pig eyes [33]. To investigate the pharmacokinetics and the effectiveness of the released drug doses from the ocular coil, in vivo animal experiments are planned.

6. Conclusion

In this study we demonstrated an optimization pathway of an ocular coil for drug delivery purposes. Due to the coil design drug loaded microspheres can be filled into the inner lumen of the ocular coil without affecting its flexibility. The ocular coil seems a promising carrier for ophthalmic drugs delivery in the early postoperative time period. To investigate safety and comfort of the ocular coil, a first-in-man study is planned (NCT03488017). In addition, experimental animal drug delivery studies will be performed.

**CRediT authorship contribution statement**

Christian J.F. Bertens: Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing.
Chiara Martino: Methodology, Formal analysis, Investigation.
Martyn O. Osch: Formal analysis, Investigation.
Arno Lataster: Methodology, Resources.
Aylvin J.A.A. Dias: Validation, Resources, Writing - review & editing, Funding acquisition.
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**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A. Supplementary material**

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**References**

[1] Stratagem IP Management Limited. The ophthalmology market online (18-Sep-2018), http://www.evaluationsonline.org.uk/evaluations/Documents/doaction=download&id=775ku=basict Stratagem Intelligent Property Management Limited. 2015:46.
[2] R. Gaudana, H.K. Ananthula, A. Parenky, A.K. Mitra, Ocular drug delivery, AAPS J 12 (2010) 348–360.
[3] P.M. Hughes, O. Olejnik, J.E. Chang-Lin, C.G. Wilson, Topical and systemic drug delivery to the posterior segments, Adv Drug Deliv Rev 57 (2005) 2010–2032.
[4] A. Urtti, L. Salminen, Concentration-dependent preconjunctional loss of pilocarpine in rabbit eyes, Acta Ophthalmol (Copenh) 63 (1985) 502–506.
[5] A. Urtti, L. Salminen, Minimizing systemic absorption of topically administered ophthalmic drugs, Surv Ophthalmol 37 (1993) 435–456.
[6] E.M. Del Amo, A.K. Rimpela, E. Heikkinen, et al., Pharmacokinetic aspects of retinal drug delivery, Prog Retin Eye Res 57 (2017) 134–185.
[7] L. Pelkonen, U. Tengwall-Uadilike, M. Ruponen, et al., Melanin binding study of clinical drugs with cassette dosing and rapid equilibrium dialysis islets, Eur J Pharm Sci 109 (2017) 162–168.
[8] A. Farkouh, P. Frigo, M. Czekaj, Systemic side effects of eye drops: a pharmacokinetic perspective, Clin Ophthalmol 10 (2016) 2433–2441.
[9] A.M. Eaton, G.M. Gordon, A. Konовал, et al., A novel eye drop application monitor to assess patient compliance with a prescribed regimen: a pilot study, Eye (Lond) 29 (2015) 1363–1391.
[10] C. Mohindroo, P. Ichpichijani, S. Kumar, How ‘drug aware’ are our glaucoma patients? J Curr Glaucoma Pract 9 (2015) 33–37.
[11] P.A. Newman-Casey, A.L. Robin, T. Blachley, et al., The most common barriers to glaucoma medication adherence: a cross-sectional survey, Ophthalmology 122 (2015) 1308–1316.
[12] J.P. Nordmann, C. Baudouin, J.P. Renard, et al., Measurement of treatment compliance using a medical device for glaucoma patients associated with intraocular pressure control: a survey, Clin Ophthalmol 4 (2010) 731–739.
[13] J.A. An, O. Kanner, D.A. Samek, V. Levesque, Evaluation of eyedrop administration by inexperienced patients after cataract surgery, J Cataract Refract Surg 40 (2014) 1857–1861.
[14] C.J.F. Bertens, M. Gijs, V. den Biggelaar, R. Nuijts, Topical drug delivery devices: A review, Exp Eye Res 168 (2018) 149–160.
[15] J.D. Brandt, H.B. Dubliner, R. Benza, et al., Long-term safety and efficacy of a sustained release bimatoprost ocular ring, Ophthalmology 124 (2017) 1565–1566.
[16] J.D. Brandt, K. Sall, H. Dubliner, et al., Six-month intraocular pressure reduction with a topical bimatoprost ocular insert: results of a phase II randomized controlled study, Ophthalmology 123 (2016) 1685–1694.
[17] T. Walters, M. Endl, T.R. Elmer, J. Levenson, P. Majmundar, S. Market, Sustained-release dexamethasone for the treatment of ocular inflammation and pain after cataract surgery, J Cataract Refract Surg 41 (2015) 2049–2059.
[18] G. Torkildsen, M.B. Abelson, P.J. Gomes, E. McLaurin, S.L. Potts, F.S. Mah, Vehicle-controlled, phase 2 clinical trial of a sustained-release dexamethasone in tracanalicular insert in a chronic allergen challenge model, J Ocul Pharmacol Ther 33 (2017) 79–90.
[19] R.T. Pijls, L.P. Crueysberg, R.M. Nuijts, A.A. Dias, L.H. Koole, Capacity and tolerance of a new device for ocular drug delivery, Int J Pharm 341 (2007) 152–161.
[20] R.T. Pijls, H.H. Hanssen, R.M. Nuijts, G.W. Daube, L.H. Koole, In vivo tolerance and kinetics of a novel ocular drug delivery device, J Control Release 116 (2006) 467–49.
[21] R.T. Pijls, H.H. Hanssen, R.M. Nuijts, L.H. Koole, Flexible coils with a drug-releasing hydrophilic coating: a new platform for controlled delivery of drugs to the eye? Biomed Mater Eng 14 (2004) 383–393.
[22] R.T. Pijls, T. Sonderkamp, G.W. Daube, et al., Studies on a new device for drug
delivery to the eye, Eur J Pharm Biopharm 59 (2005) 283–288.

[23] B.A. Holden, A. The Glenn, Fry award lecture 1988: the ocular response to contact lens wear, Optom Vis Sci 1989 (66) (1988) 717–733.

[24] American academy of ophthalmology. Fundamentals and principles of ophthalmology. Basic and clinical science course. San Francisco, CA: American Academy of Ophthalmology; 2014, 37.

[25] T. Govender, S. Stolnik, M.C. Garnett, L. Illum, S.S. Davis, PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug, J Control Release 57 (1999) 171–185.

[26] P. Zhang, X. Liu, W. Hu, Y. Bai, L. Zhang, Preparation and evaluation of naringenin-loaded sulfobutylether-beta-cyclodextrin/chitosan nanoparticles for ocular drug delivery, Carbohydr Polym 149 (2016) 224–230.

[27] H.M. Jaeger, S.R. Nagel, Physics of the granular state, Science 255 (1992) 1523–1531.

[28] S. Torquato, T.M. Truskett, P.G. Debenedetti, Is random close packing of spheres well defined? Phys Rev Lett 84 (2000) 2064–2067.

[29] Z. Zhou, A.C. Anselmo, S. Mitragotri, Synthesis of protein-based, rod-shaped particles from spherical templates using layer-by-layer assembly, Adv Mater 25 (2013) 2723–2727.

[30] I.M. Katz, W.M. Blackman, A soluble sustained-release ophthalmic delivery unit, Am J Ophthalmol 83 (1977) 728–734.

[31] PubChem. Ketorolac (Compound): Online (accessed: 12-feb-2020), https://pubchem.ncbi.nlm.nih.gov/compound/Ketorolac#section=Canonical-SMILES.

[32] N. García-Porta, F.J. Gantes-Nuñez, J. Tabernero, S. Pardhan, Characterization of the ocular surface temperature dynamics in glaucoma subjects using long-wave infrared thermal imaging, J Opt Soc Am A 36 (2019) 1015–1021.

[33] C.J.F. Bertens, S. Zhang, R.J. Erckens, et al., Confocal Raman spectroscopy: Evaluation of a non-invasive technique for the detection of topically applied ketorolac tromethamine in vitro and in vivo, Int J Pharm 570 (2019) 118641.