Microfluidic device for controlled ocular drug delivery

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Abstract

Proper drug concentration during treatment is a challenge faced in ocular diseases. The need for repeated administration of the drug over the course of days, weeks and even years, sometimes with 1- or 2-hours regularity, changes peoples' lives, and is more problematic when it is dependent on a third party, as is the case of domestic animals, elderly, children and disabled people. To mitigate this issue, in this work a microfluidic device composed of hydrogel and a monolayer of graphene with 320 μm is researched as an alternative, attempting to provide a controlled ocular delivery of drug, replacing the repeated administrations by its occasional substitution. The work focuses on the chronic dry eye disease in animals, for which there is not a viable cure and may require treatment for life. The device has a rectangular shape of 10 x 5 mm and is inserted in the cul-de-sac of the eye, with the graphene layer anterior facing and the hydrogel in contact with the conjunctiva and portion of cornea. The hydrogel composition includes hyaluronic acid, due to its lubricant and wound healing properties, as well as being a common element in the body, making it biocompatible and safe. The active component is present at 0.5 % w/v, 1 % w/v and 2 % w/v. Graphene growth conditions in CVD are studied and the resulting samples are characterized by Raman spectroscopy. *In-vivo* testing in the eye of a small dog was conducted as a proof of concept to test the device's biocompatibility and biofunctionality, with good results regarding the former and improvement needed in the latter, since the device was expelled in three different trials after a few hours. It is expected that such a device can positively impact the lives of animals suffering from the disease, alleviate worry of the animals' owners and be mass produced, eventually also crossing the barrier for human treatment as well.

Keywords: Microfluidics; ocular drug delivery; graphene; hydrogel; hyaluronic acid.

1. Introduction

When faced with a disease needing treatment, proper drug administration is key for a fast and good recovery, or even to properly manage the symptoms in case of chronic diseases. In ocular treatment, due to the particularities of the eye, drug administration faces some challenges with keeping proper concentration and thus, ensure a good treatment. Trying to reduce the number of administrations, the concentration tends to be higher and shoot over the maximum safety concentration, then stay for a while in the desired range and finally fall bellow the minimum effective concentration. Using less concentration and more frequent administration improves the time when the drug is between these concentration limits, but requires more availability of the patient, and when dealing with third party dependency, many times the treatment is not properly given. By researching and developing new treatments and new devices that take away a significant burden in the caretaker, helps increasing the quality of life, not only for the patient, but also for the caretakers.

Dry eye disease (DED) is a condition affecting the proper lubrication of the eyes. It can be caused by a decrease in tear production or increase in tear evaporation, which can lead to damage of the corneal surface and declining vision. The decrease in tear production can be caused by autoimmune or inflammatory systemic conditions, that damage the tear glands and the increase in evaporation can be caused by production of low-quality tears, that have less constituents to hold the water in the eye and prevent it from evaporating.

Usual treatment for DED include artificial tears, warm compresses and changing some habits, re-

ducing stresses in the eye, for example using less aggressive detergents [10]. Many of the common artificial tears include hyaluronic acid in its constituents since it is already distributed widely throughout connective, epithelial, and neural tissues and serves the purpose of lubrication and also wound healing [9]. Contact lenses soaked in drug solutions or more engineered to have incorporation of the drug during its fabrication are also being explored for prolonged use, serving as a means of alternative delivery [3, 6, 4]. Fabricating hydrogel lens or lens like materials is an enormous world of possibilities, that spawns from the range of available polymers; the formula of components in a recipe; different polymerization techniques (e.g. radical or catalytic polymerization); and polymerization conditions (e.g. temperature, initiator, vessel). HEMA hydrogels for one, are inexpensive, biocompatible and have a high number of copolymer possibilities, but have a low oxygen permeability and face protein adsorption issues, causing severe visual impairment, inflammation, dryness and eye discomfort. The copolymer possibilities may in fact solve these negative aspects, by contributing with their own set of advantages [7]. Due to the ubiquitous presence of HA in the body and its water retention properties, HA has many ophthalmology related applications. Among these are the treatment of DED and as an additive in contact lens. HA can also be included in the production material; as surface coating; and as a multipurpose solution. It can be used in several formats: *in situ* gels, nanoparticles, intravitreal injection or in tissue engineering. More specifically, in its applications on contact lenses, modifies the surface roughness of the lens, enhances surface water retention, reduces protein adsorption onto the lens, and consequently improves biocompatibility [2]. HA also assists in drug release, by promoting a more complete release of another drug that is incorporated in conjunction with it in the lens [8]. Since contact lens are widely used, are convenient, and inexpensive to produce, using them as delivery vehicles is a natural step. Entrapping HA in contact lenses to prolong the duration of its release, has shown satisfactory results of up to 15 consecutive days of release [5]. Deriving from the knowledge above, the present work focuses on the fabrication and testing of a HA laden hydrogel with a layer of graphene to treat DED.

2. Methodology

2.1. Materials and methods

The materials used to fabricate and test the device are Hydroxylethylmethacrylate (HEMA, CAS 868-77-9, Sigma-Aldrich), ethylene glycol dimethacrylate (EGDMA, CAS 97-90-5, Sigma-Aldrich), methacrylic acid (MAA, CAS 79-41-4, Sigma-Aldrich), and Darocur (2, 4, 6-trimethyl benzoyl-biphenyl-phosphinoxide, CAS 75980-60-8, Tokyo Chemical Industry), Cetyltrimethylammonium bromide (CTAB, CAS 57-09-0, VWR Chemicals), hyaluronic acid (1.8–2.2x10^6 Da, Shandong Topscience Biotech Co., Ltd), and copper foil (99.999% purity, CAS 7440-50-8, Alfa-Aesar).

2.2. Fabrication of HA laden hydrogel

The conditions used for hydrogel fabrication are presented in 1 using spin-coating, in 2 using wetting and in 3 using rigid molds for casting.

The prepolymer composition used to fabricate the hydrogel was 46.7% w/w HEMA, 0.8% w/w MAA, 0.975% w/w EGDMA (cross linking agent) and 52% w/w water [4]. The hydrogel was fabricated by free radical polymerization using prepolymer mixture and incorporates 0.5 % w/w Darocur (photoinitiator). Part of the samples where added with 0.5 % w/v, 1 % w/v or 2 % w/v HA. After mixture of the reagents, sonication for 15 min is done to remove air bubbles. The mixture was then either pre-cured for 1 min 5 s prior to be used in spin-coating (without HA in formulation), or was cast in a mold (with and without HA in formulation) with 320 μm spacers and then polymerized in a UV-KUB 2 machine (365 nm $\pm$ 5 nm wavelenght). The precursors solution was irradiated at 10.5 mW/cm^2 $\pm 10\%$ for 20 min in 8 cycles of 2 min ON and 30 s OFF, totalling 20 min (16 min of effective exposure). After polymerization the hydrogels were peeled from the substrate and stored dehydrated until further use.

2.2.1 Thickness definition

The first method tested was spin-coating. The centrifugal forces acting on the liquid, drive the liquid outwards. The rotation speed and time will define the film thickness. A given volume of solution is poured on top of a flat substrate and rotated for a given time and velocity. In the second method used for thickness definition, a given volume of precursor solution was pipetted directly onto the substrate and let flow and spread on the surface by the action of cohesive and adhesive forces only. In the third method the precursors solution is casted

Table 1: Conditions for hydrogel fabrication using **spin-coating**, in different substrates.

	DI water (ml)	HEMA	EGDMA	MAA	PI (g)	Mix	Pre-curing (30 %)	Substrate	O_2 Plasma	Spin-coat	UV (30 %)
HT1	5.1757	4.673g	0.095g	0.094g	0.04857	15 min	1 min 1min10s 1min20s	-	-	-	-
HT2	5.1757	4.5788g	0.0788ml	0.0877ml	0.0505	5 min	1min10s	-	-	-	-
HT3	5. 176	4.3686g	0.0788ml	0.0877ml	0.0547	6 min	1min5s	Glass	-	P4	
HT4	5. 176	4.3686g	0.0788ml	0.0877ml	0.0547	-	-	-	-	-	
HT5	5.176	4.369ml	0.079ml	0.088ml	0.055	5 min	1min5s	Glass	-	P4 P5 P4	2min 4min 5min
HT6	5.1757	4.3686ml	0.0788ml	0.0877ml	0.058	5 min	1min5s	Glass	- -	P5 PH1 P5	10min 15min 20min
HT7	5.1757	4.3686ml	0.0788ml	0.0877ml	0.0507	5 min	1min5s	Glass	-	PH2	15min
HT8	3.1054	2.6212ml	0.0473ml	0.0526ml	0.0312	5 min	1min5s	Glass	-	PH2	15min
HT9	3.105	2.6212ml	0.0473ml	0.0526ml	0.03	5 min	1min5s	Glass PMMA	-	PH3	8min -
HT10	3.105	2.6212ml	0.0473ml	0.0526ml	0.03	5 min	1min	Glass PMMA	1min MED	PH4	- -
HT11	2.5879	2.1843ml	0.0394ml	0.0438ml	0.0256	1min30s	1min	PMMA	-	S1	200 cycles 2s ON 1s OFF
HT12	2.588	2.184ml	0.039ml	0.044ml	0.030	1min30s	1min5s	Acetate sheet	1min MED	S1	200 cycles 2s ON 1s OFF
HT13	2.588	2.184ml	0.039ml	0.044ml	0.028	3 min	1min10s	Acetate sheet	1min MED	S2	200 cycles 2s ON 1s OFF

Table 2: Conditions for hydrogel fabrication in different substrates by directly **pipetting** the precursors solution onto the substrate.

	DI water (ml)	HEMA	EGDMA	MAA	PI (g)	Mix	Pre-curing (30 %)	Substrate	Pipetted	UV (30 %)
HT14	2.588	2.184	0.039	0.044	0.025	4 min	1min5s	Acetate sheet	2 ml	200 cycles 2s ON 1s OFF
HT15	2.588	2.184	0.039	0.044	0.025	5 min	1min5s	-	-	-
HT16	3.1054	2.6212	0.0474	0.0526	0.033	3 min	1min	Glass PMMA Acetate sheet	2 ml	200 cycles 2s ON 1s OFF

Table 3: Conditions for hydrogel fabrication trials using different **mold** configurations and HA concentrations.

	DI water (ml)	HEMA	EGDMA	MAA	PI (g)	HA	Mix	Sonication	Substrate	Mold	UV (30 %)
HT17	5.1757	4.3686	0.0788	0.0877	0.05	-	5 min	15 min	PMMA	V1	200 cycles 2s ON 1s OFF Oven 70 °C 1h30
HT18	5.1757	4.3686	0.0788	0.0877	0.05	-	4 min	10 min	PMMA	V1	5 min 10% 8 min 10%
HT19	2.588	2.184	0.039	0.044	0.030	-	3 min	-	PMMA	V1	8 cycles 2min ON 30s OFF
HT20	2.588	2.184	0.039	0.044	0.030	-	3 min	-	PMMA	V2	8 cycles 2min ON 30s OFF
HT21	1.294	1.092	0.0195	0.022	0.015	-	3 min	-	Graphene	V2	8 cycles 2min ON 30s OFF
HT22	2.588	2.184	0.039	0.044	0.031	0.0756g	10 min	-	-	-	8 cycles 2min ON 30s OFF
HT23	2.588	2.184	0.039	0.044	0.031	0.052g	20 min	-	-	-	-
HT24	2.588	2.184	0.039	0.044	0.030	0.01294g	10 min	15 min	PMMA	V3	8 cycles 2min ON 30s OFF
HT25	2.588	2.184	0.039	0.044	0.031	0.053g	30 min	30 min	PMMA	V4	8 cycles 2min ON 30s OFF
HT26	2.588	2.184	0.039	0.044	0.031	0.0516g	10 min	-	PMMA	V4	8 cycles 2min ON 30s OFF
HT27	2	1.712	0.031	0.036	0.0195	2.2% w/v	10 min	-	-	-	-
HT28	2.588	2.184	0.039	0.044	0.025	0.01294g	5 min	15 min	PMMA	V4	8 cycles 2min ON 30s OFF
HT29	2.588	2.184	0.039	0.044	0.031	0.02588g	5 min	-	PMMA	V4	8 cycles 2min ON 30s OFF

onto a PMMA mold, sealed with a PMMA cover, and cured.

Different materials were studied as substrates for the definition of the thickness of the hydrogel: glass (24x50 mm, 0.13-0.17 mm thick, Hirschmann Deckglases), PMMA (20x20 mm, 1 mm thick, Perspex) and acetate sheet (210x297x0.1 mm, PP2410, 3M). Surface treatment with O_2 plasma before dispensing the precursors solution onto the substrate was also performed and its effects studied. The methodology to extract the polymerized hydrogel from the substrate was also addressed. Test trials were done for the hydrogel in hydrated or dehydrated form and using different solvents (acetone, ethanol and IPA).

2.2.2 Spin-coating

The testing and optimization of the insert thickness was done for the mixture of pHEMA without HA. As the solution presents very low viscosity for spin-coating, an intermediate step of pre-curing by UV was added, (UV-KUB 2 365 nm $\pm$ 5 nm, maximum exposure energy 35 mW/cm^2 $\pm 10\%$). The UV-KUB 2 is further used for the polymerization of the hydrogel.

The first trials were done using a Laurell spin-coater model WS-650MZ-23NPP/LITE, that has already some settings in memory for the most common uses of the machine, namely with PDMS and SU-8. Two programs were used, that were optimized for SU-8 spreading, P4 and P5. Then, new conditions were introduced, PH1, PH2, PH3 and PH4, to test for different conditions both in the spreading and in the thickness definition steps. The detailing of the programs used can be seen in Table 4.

In the trials that used the Laurell spin-coater, the substrate was secured with kapton tape onto a waffer and the waffer is mounted on the spin-coater's vacuum nozzle.

Table 4: Spin speeds set for spin-coating the precursors solution. A first step (initial spreading) spreads the liquid evenly across substrate. A second step follows (thickness definition) to define the thickness. Programs with letter P are performed in a Laurell spin-coater model WS-650MZ-23NPP/LITE and programs with letter S in a in-house setup.

	Initial spreading		Thickness definition	
Program	**Time (s)**	**RPM**	**Time (s)**	**RPM**
P4	5	500	20	2200
P5	5	500	20	1200
PH1	5	500	20	1700
PH2	5	500	20	800
PH3	5	100	20	800
PH4	5	100	20	300
S1	5	100	20	300
S2	5	500	10	300

A different setup was mounted afterwards, consisting in a stepper motor controlled by an Arduino, using a driver and G-code commands to set the spin coating speeds (range) to obtain the required flow thicknesses. The commands used were as follows:

- G0 - tells the motor to move.
- X - tells the motor how much to move. A full revolution is X1.6, with each full revolution being a multiple of 1.6.
- F - sets the feed rate of the motor, that translates into RPM. In this setup F136.8 is equivalent to 100 RPM.

2.2.3 Casting

The hydrogel was also casted onto a mold designed and fabricated in PMMA (100x100x5 mm) using a milling machine. The design was made using FreeCAD 0.20 and finalized in AutoCAD. The design translated into G-Code and run in the milling machine with the help of MACH2 sofware. The mold was then engraved using a laser cutter (Speed = 150 mm/s, Power = 25 %). The bottom and top lids were designed in Beam Studio v1.8.3 and cut also in the laser cutter (Speed = 8 mm/s, Power = 60 %). Improved versions of the PMMA mold designs were done in the Beam Studio v1.8.3, cut and engraved using the laser cutter (Cut: Speed = 8 mm/s, Power = 60 % || Engrave: Speed = 150 mm/s, Power = 25 %). A spacer (CROMOlab chromatography fibers with 320 μm diameter) is used to define the thickness of the hydrogel.

2.2.4 Graphene Growth

The graphene layer that will protect the hydrogel insert was grown in an Aixtron Black Magic 2" CVD furnace. For the machine and parameters control, programmable recipes in the machine's language can be created by the user and are divided into the following main steps:

- Initial cleaning of the substrate surface with an Ar flow (Fig. 1 - A)
- Setting of the temperature ramp to heat the substrate up to the process temperature (Fig. 1 - B)
- Annealing of the substrate after reaching and maintaining the process temperature. Both Ar and H_2 gases flow one imposed to the chamber (Fig. 1 - C)
- Growth of graphene with the introduction of the precursor gas, CH_4, at the same temperature as the annealing (Fig. 1 - D)
- Cooling ramp to room temperature (Fig. 1 - E)

2.2.5 Graphene Extraction

The graphene grown in the previous section is transferred to the hydrogel, by a series of steps. First there is a protective layer of PMMA spin-coated on top of the graphene, then the copper substrate is etched in a solution of 0.5 M Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$) until complete Cu etching. After, a series of cleaning steps are done to remove impurities from the graphene's surface. After fabricating the hydrogel and preparing

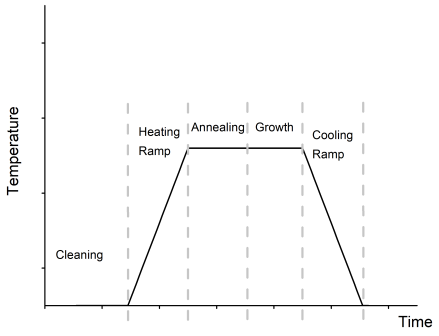

Figure 1: Schematic detailing the main steps to CVD graphene growth as a function of time and temperature.

the graphene thin film, the graphene is fished onto the hydrogel surface, left to rest for at least 12h in a clean Petri dish, during which the hydrogel slowly dehydrates and the adhesion between the two layers is promoted.

2.2.6 Graphene characterization

The characterization of graphene was performed on a Cu graphene stack, by using a LabRAM HR 800 Evolution Raman spectrophotometer (wavelength 200 nm - 1600 nm). The process starts with a calibration step done with a crystalline silicon piece, a material with a very sharp and defined peak at λ = 532 nm, and with very low backscattering noise. The peak obtained is used as a reference in the machine, before each measurement. The samples of graphene are analyzed as follows: the sample is loaded in the machine optical microscope to inspect the surface. Regions and spots to analyze are chosen after usual inspection. With a magnification of 100x resolution, the spot is amplified and a measurement is taken. Three or four measurements are taken for each sample in different regions.

2.2.7 *In-vivo* testing of the insert

When the patient is first assessed there is an initial examination and before each of the three trials a quantitative assessment of conjunctivitis clinical signs was performed, in conjunction with baseline tests. It is then decided if the patient is healthy enough and within parameters to participate and continue with the trial. Before placement of the insert, a drop of anesthetic is placed in the eye that will receive it and the patient is left to rest until starting to take effect. While the patient is secured by the head, the insert is taken from the sterilized eppendorf with a sterile tweezer and placed in the *culde-sac*.

2.2.8 Sterilization protocol

To ensure the safety of the animal used in the *invivo* study, it was necessary to reduce the amounts of contaminants and to sterilize the inserts that were going to be used in the animal's eyes and also of the material used in their fabrication and manipulation. After hydrogel polymerization, it is placed in boiling DI water for 15 min to remove unwanted non reacted monomers, that are toxic. The transfer process of graphene onto the hydrogel was conducted in class 10 and class 100 clean room areas, ensuring a low amount of contaminants between the layers. The inserts were placed individually in eppendorfs with 2 ml STF solution that was made with ultra-pure water filtered with a 0.2 μm nylon mesh, then the eppendorfs were subjected to 2h sterilization by radiation under UV light (velleman, 15 W) at close range 15 cm and in an enclosed setup.

3. Results & discussion

3.1. Hydrogel fabrication and HA quantification

In Tables 1, 2, and 3 the resumed information of the trials that were performed are presented. Table 1 has the information from the trials done with spin-coating, that required an initial optimization of the process of fabrication of the hydrogels, implementation and optimization of a pre-curing step, and different substrates experimentation. The main results from the spin-coat trial are the order for which to mix the reagents and timings for waiting between pre-curin and spin-coating; the need for an increase in viscosity of the prepolymer solution to be able to spin-coat, that was done in a pre-curing step for 1 min 5 s at 30 % power; that either PMMA or acetate sheet are the better substrates, and that a surface functionalization with O_2 plasma, helps in the spread and minimization of the receding of the prepolymer solution on the substrates. The parameters of speed and time for the pin-coating were also high, since upon characterization of the thickness of some samples, it was observed the thickness was too low at first, and then when passing for the stepper motor, that the set-up was not created in a fashion that would allow proper definition, varying substancially from trial to trial. The main results from the directly pipetting trials are the inconsistencies in the pre-curing step, with big variations in the

outcome and the issue with setting a comparable test between substrates, and the information can be viewe in Table 2. For the casting trials, Table 3 presents the resumed information of the casting trials. The main results are the improvement of the solution after sonication, the whiteness of the hydrogel is most likely a product of the thickness, the optimization of the mixing of HA into the prepolymer solution and the first fabrications of hydrogels with HA, at a concentration of 0.5 (HT25) and 1 % w/v (HT29), that had structural integrity, good homogeneity and water retention. Trying to fabricate with 2 % w/v HA is not feasible, since the solution forms lumps, doesn't dissolve properly and entraps too much air during the process. New iterations of the casting mold helped the process, and also improved the reproducibility.

A calibration curve (Fig. 2) was made with basis on the turbidity assay, and a trial for HA diffusion was tested. Two pieces of hydrogel with HA 1% w/v were cut to size and placed in small laboratory bottles during 5 days, in simulated tear fluid, with replacement of the fluid every 24h. It was not possible to properly read the concentration of HA in the diffusion trial made with HT29, making it inconclusive if there is HA diffusion happening from the hydrogel into the medium, since the expected diffusion rate will give a concentration of HA at the end of the 24 h close to the minimum capacity of the spectrophotometer to read.

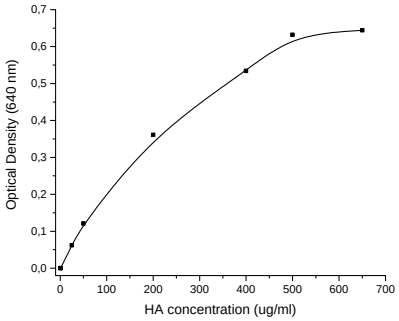

Figure 2: Relation between the turbidity as measured at $\lambda = 640$ nm and the hyaluronic acid concentration.

3.2. Graphene growth, extraction and characterization

The majority of the trials done in the Aixtron BlackMagic CVD were done in temperature testing, to figure out what was happening with the machine that many times wouldn't reach the set temperature. Eventually it was found that the culprit was the TUNE HTTC commands that limited the machine's max power output to the heater to a defined percentage, which many times was insufficient to allow the temperature to reach the target. This, combined with the variations of temperature that the thermocouple is subjected depending on the position on the heater it is placed, made the outcome of a recipe for the machine difficult to reproduce. 3 trials were successful in growing graphene on top of the copper foil, the machine reached the target temperature, the copper substrate was subjected to the annealing step and managed to grow graphene on its surface. This graphene, based on the results of Raman spectrometry are indeed quite good, with an I(2D/G) ratio higher than 1.6 for all, and with an I(D/G) ratio that was under 0.5 in trial 2 and 3, but significantly higher on sample 3, althougn still below 1. This means that there was presence of graphene found, that the graphene has quality and that the defects are relatively low.

3.2.1 *In vivo* testing of the insert

With a fabricated sample of hydrogel from trial HT25 and a sample of graphene from the work of [1], an assembly of the whole device was possible. Starting with the graphene extraction process, the Cu was etched and the graphene/PMMA stack was cleaned from impurities and residues of etching solution. Then the stack was transferred as a final step onto the hydrogel and left overnight to improve the bonding between the layers. The bonding was successful and the hydrogel was manipulated without detachment of the graphene/PMMA stack. The device was cut to size with the help of some fabricated cutters that do a rectangular shape of 10 x 5 mm and a corner cut for identification of correct positioning in the eye. The pieces that were cut had a stack of hydrogel/graphene/PMMA, and also just hydrogel, because the graphene piece wasn't big enough to cover the whole surface of the hydrogel. Two of the cut pieces were placed in an acetone bath to remove the PMMA layer and leave just hydrogel/graphene. With this pieces, it was possible to perform three different trials in a patient, a little female dog of the Podengo breed. The goal was to assess the biocompatibility and biofunctionality of the insert, in its various configurations. An examination of the patient (Fig. 3, 4, and 5) is done before and after the insertion and removal or expelling of the insert, and the various tests gave results in line with normality, except after the second trial, where the patient exhibited some increased

conjunctivitis clinical signs. None of the trials manage to reach the 6 h mark without the insert being expelled, but there weren't major clinical sign of aggravation of the condition of the patient, meaning that the insert, the device, seems to be biocompatible, but its biofunctionality is still poor. The last trial there was an attempt at improving the shape to conform better to the bottom of the *cul-de-sac*, but the insert was still expelled.

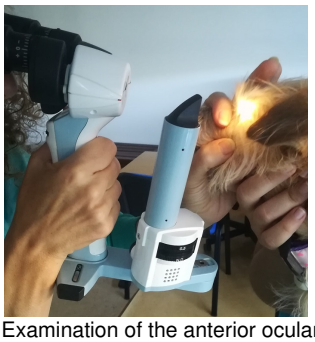

Figure 3: Examination of the anterior ocular segment

Figure 4: Schirmer tear test performed with a STT strip with blue band

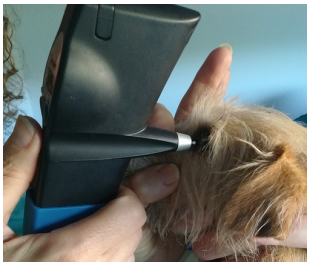

Figure 5: Measurement of the intraocular pressure with a TonoVet tonometer

4. Conclusions

The main objective of the work was to fabricate a device to treat the chronic dry eye syndrome, by placing it non invasively in the eye, allowing for a slow and sustained release of HA in time at an effective dose. The majority of the work focused the fabrication of the hydrogel, in the optimization of the conditions for which the result would be an hydrogel film of ideal dimensions and drug loading. Different methods of achieving the hydrogel thickness were studied namely: spin-coating, direct pipetting and casting, as were different amounts of HA concentration and different approaches for being able to mix the components. The best results were obtained for casting spreading technique, concentration 0.5% w/v and by mixing the HEMA, MAA, EGDMA, and PI separately from the HA with DI water, joining them after. The testing of the release profiles of the device were insufficient to draw conclusions and more testings needs to be performed in the future. Cutters were designed and fabricated to define the shape of the device, allowing for better uniformity between different samples. Better blade stability will help preventing wobbling of the blades during cutting in the future.

A novel approach of using graphene as an added layer for water and drug retention was a specific objective, involving the graphene growth, extraction and characterization. Tests for process temperature, cleanings and thermocouple replacement were conducted to enhance the yield of graphene growth. In particular it was found that the parameters that control the looping feedback of the heater, were restricting the power output at which the heater could reach for an increase of process temperature. Also, the temperature of the heater surface was seen to vary significantly depending on the positioning of the sample relative to its center and to the connector of the electrodes who delivers the current and voltage to the heater. The inconsistencies in temperature readings and set points made more difficult to control the sublimation processes of the copper.

The insert was tested *in-vivo*, to conclude on its biocompatibility and biofunctionality. A volunteer patient was found and three trials conducted. The devices remained in the eye for at least 1 h 15 min in trial 3 up to an indeterminate amount out time below 6 h, for trials 1 and 2. Although none reached the programmed 6 h hour evaluation after placement, the development showed promising results. The patient responded well to the insert in terms of bio compatibility.

5. Acknowledgement

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