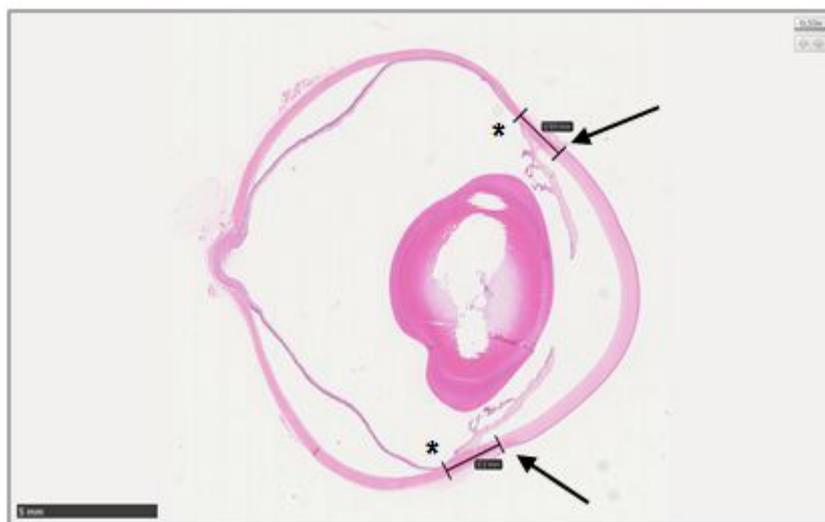


RESEARCH ARTICLE

Supplementary Information

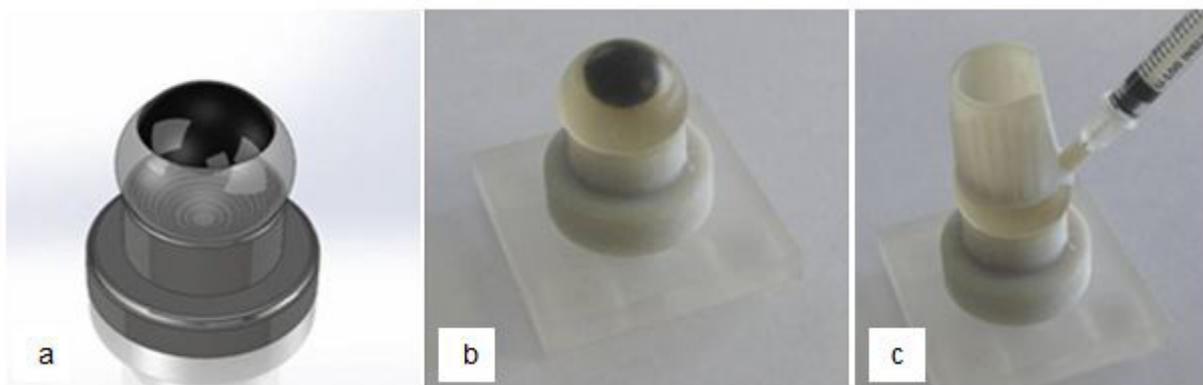
Animals

Male and female Chinchilla Bastard (CrI:CHB), CB rabbits (*Oryctolagus cuniculus*) with pigmented eyes and a weight of 3.54 ± 0.64 kg and male and female New Zealand White (HsdJf:NZW) rabbits (*Oryctolagus cuniculus*) with a weight of 3.25 ± 0.54 kg were used. All rabbits were tested in accordance with FELASA guidelines and were negative for all pathogens. Female NZW rabbits were housed in groups of 3 either in floor pens or in two linked Arrow might Rabbit Cages. Male NZW and female and male CB rabbits which are prone to display aggression towards conspecifics were singly housed in Arrow might Rabbit Cages with visual contact. They were fed Purina Mills International (PMI) 5322 Rabbit diet ad libitum and were provided reverse osmosis treated drinking water from water bottles ad libitum. Environmental enrichment was provided daily (Cardboard Tunnel and Box; Plastic balls; Hay; Wood Chews; Bunny Blocks). The light cycle was 12/12 h with lights on at 06:00 a.m. The room temperature was 18 ± 3 °C and the relative humidity was 40 – 70%. All animals were euthanized by intravenous injection of Pentobarbital (150 mg/kg). Death was confirmed by exsanguination.



Arrows (\Rightarrow) point to the externally visible corneoscleral limbus and stars (*) to the internal pars plana/retina junction. Measurements were taken using Hamamatsu NPD software in two positions of 9 NZW rabbit eyes.

Figure 1: Representative histological image of a rabbit's eye hemisphere



(a) Computer generated 3-D CAD simulated eye model using Solidworks design software (b) 3-D printed model of the rabbit eye incorporating 3 different hardness polyurethane materials (c) Trial of 3-D printed intravitreal injection device prototype using the 3-D printed rabbit eye model

Figure 2: Design and Development tools for a novel intravitreal injection device

Histology Protocol

After the injection, the needle was cut off below the hub, ensuring the needle remained in place, which made it easier for the histopathology's to select the appropriate area to visualize the needle penetration point. A suture was placed through the sclera to

mark the top of the eye. The eye was enucleated and rinsed in phosphate buffered saline (PBS). The suture was used to suspend the eye in a pot containing 1% Formaldehyde, 1.25% Glutaraldehyde as a fixative [1]. The fixed eyes were carefully cut into hemispheres cutting close to the injection site. The remains of the needle were removed. Samples were placed in pre-labelled "Mega" cassettes. The hemispheres containing the injection sites were processed into paraffin wax using a Sukura VIP6 tissue processor, overnight program. Following completion of processing the cassettes containing the eye, hemispheres were transferred to a Tissue Tek tissue embedding centre. The hemispheres were removed from the cassettes and placed in embedding molds containing molten paraffin wax. The hemispheres were orientated so that the cut surface of the globe was positioned on the base of the mold. The molds were topped up with molten paraffin wax and allowed to solidify on a cold plate. As part of the embedding process standard sized pre-labelled processing cassettes were used for identification. Paraffin-embedded hemispheres were cooled/hardened on wet ice to facilitate microtomy using a Leica rotary microtome. The hemisphere blocks were trimmed down on the microtome to expose the cut surface, and ribbons of 4 μm thick sections were cut and floated out on warm water. Individual sections were picked up from the water surface using pre-labelled clean microscope slides. Slides were labelled with rabbit ID and section number. Initially, approximately twenty 4 μm sections were taken from the hemisphere blocks to locate the injection site. Microtome sections could dry overnight at 37.0 °C followed by Haematoxylin and Eosin staining using a Leica Autostainer, cover-slipped and allowed to dry. As the needle penetration location was likely to be present in several of the 4 μm thick sections, batches of stained slides from each hemisphere were digitally scanned using a Hamamatsu Nanozoomer scanner and examined using Hamamatsu's NDP.view software. If following examination of the twenty H&E stained hemisphere sections, the injection site was not located, further batches of sections were cut and examined.

Perceived required force to pierce the sclera during device-guided and freehand intravitreal injections

During the *ex vivo* study in the development phase operators fed back that they perceived the required force to pierce the sclera to be lower using the device compared to freehand injections. We therefore asked each operator to record the force they perceived was required to pierce the sclera on a 10 cm Numeric Rating Scale (NRS) after each injection. Operators had been instructed to give a score of 0 if the needle just glided in without perceivable force and a score of 10 if the perceived pressure felt impenetrable. The perceived injection pressure scored on the NRS was analysed with a Wilcoxon/Kruskal-Wallis test and these data are presented as medians plus minimum and maximum. A perceived reduction which is likely to correlate with an actual reduction of force required to pierce the sclera would be beneficial as the resulting distortion of the globe would be minimized. This, in turn, could lead to a more accurate needle penetration point and a reduced risk of inadvertently damaging structures of the eye. Additionally, as reported by patients, the pain perceived during intravitreal injection with a similar device for humans was statistically reduced and this could potentially be a result of a reduction in the force required to pierce the sclera. We used a numeric rating scale (NRS) to analyze this effect as NRS's are widely accepted as an instrument to measure subjective characteristics that cannot otherwise be measured directly [2-4]. Contrary to our hypothesis our results do not show a difference in the perceived pressure (Figure 3).

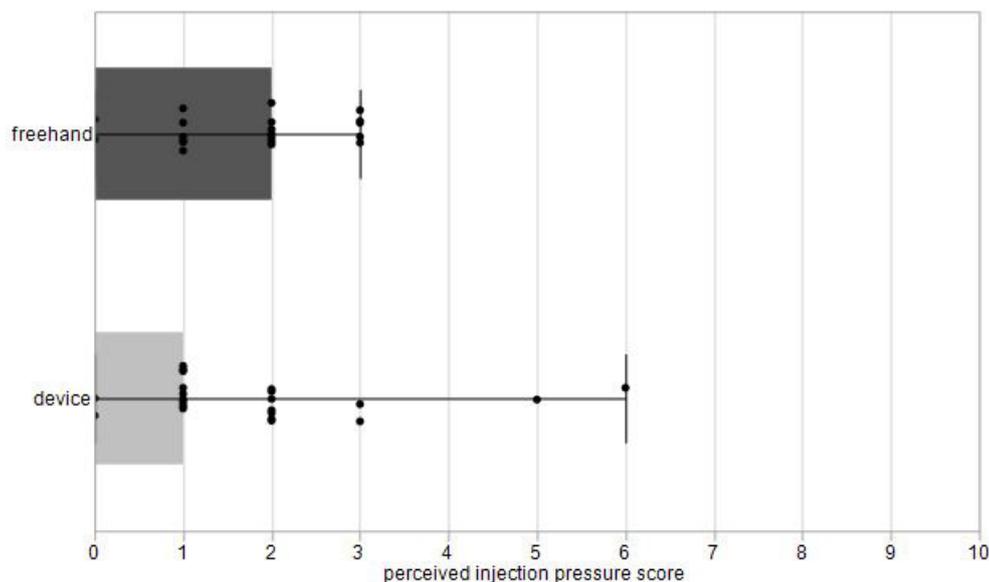


Figure 3: Numeric Rating Scale score for the perceived force necessary to pierce the sclera

Each dot represents the score of an individual injection. Each error bar is constructed using the min and max of the data. Differences in the median are not statistically significant (Wilcoxon/Kruskal-Wallis test, $p = 0.3692$, $n = 46$).

It is likely that the NRS was not sensitive enough to pick up a subtle difference and ideally the actual pressure and or distortion of the globe would have to be measured. This was not technically feasible in this study. Two high scores recorded on the NRS were most likely attributed to the needle getting caught within the injection guide which contained the air bubble.

strain	body weight (kg)	cornea Ø (mm)		orbit Ø (cm)		pars plana (µm)	limbus to pars plana (mm)	references
		vertical	horizontal	vertical	horizontal			
NZW	3.25 ± 0.54	12.0 ± 1.9 ¹	12.1 ± 1.9 ¹	2.05 ± 0.13 ¹	2.28 ± 0.16 ¹	904.92 ± 285.44 ²	2.25±0.45 ³	own data, ¹ n=90, ² n= 46, ³ n=18
CB	3.54 ± 0.64	13.6 ± 0.9	14.1 ± 1.1	2.20 ± 0.19	2.27 ± 0.16	-	-	own data, n=156
-	-	13.5	15.0	-	-	-	-	[5]
-	-	13.8	15.6	2.0	2.1	-	-	[6]
NZW	-	-	-	-	-	~1	-	[7]

Own data are presented as Mean ± Standard Deviation. - = no data. NZW = New Zealand White Rabbits. CB = Chinchilla Bastard Rabbits

Table 1: Anatomical data of rabbit eyes

References

- Margo CE, Lee A (1995) Fixation of whole eyes: the role of fixative osmolarity in the production of tissue artifact. *Clinic Experiment Ophthalmol* 233: 366-70.
- Ratnarajan G, Nath R, Appaswamy S, Watson SL (2013) Intravitreal injections using a novel conjunctival mould: a comparison with a conventional technique. *Br J Ophthalmol* 97: 395-7.
- Hawker GA, Mian S, Kendzerska T, French M (2011) Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care Res* 63: 240-52.
- Haefeli M, Elfering A (2006) Pain assessment. *Eur Spine J* 15: 17-24.
- Krause W (1868) The anatomy of the rabbit in topographical and operative review. Leipzig: Verlag von Wilhelm Engleman.
- Davis FA (1929) The Anatomy and Histology of the Eye and Orbit of the Rabbit. *Trans Am Ophthalmol Soc* 27: 400-41.
- Short BG (2008) Safety evaluation of ocular drug delivery formulations: techniques and practical considerations. *Toxicol Pathol* 36: 49-62.