Male and female Chinchilla Bastard (Crl:CHB), CB rabbits (Oryctolagus cuniculus) with pigmented eyes and a weight of 3.54 ± 0.64 kg and male and female New Zealand White (HsdI: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.

Animals

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
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).

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.

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

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 Sakura 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. Microtomes 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.
<table>
<thead>
<tr>
<th>strain</th>
<th>body weight (kg)</th>
<th>cornea Ø (mm)</th>
<th>orbit Ø (cm)</th>
<th>pars plana (µm)</th>
<th>limbus to pars plana (mm)</th>
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<tr>
<td></td>
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</tbody>
</table>
| NZW    | 3.25 ± 0.54     | 12.0 ± 1.9¹    | 12.1 ± 1.9¹ | 2.03 ± 0.13¹    | 2.28 ± 0.16¹            | own data, ⁷
|        |                 | vertical       | horizontal  | 904.92 ± 289.44³| 2.25±0.45    |            |
| 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 |
|        |                 | -              |            | -               | -                       |            |
| NZW    | -               | 13.8           | 15.6        | 2.0             | 2.1                     | [5]        |
|        |                 | -              |            | -               | -                       | [6]        |

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