A resistance-sensing mechanical injector for the precise delivery of liquids to target tissue

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The precision of the delivery of therapeutics to the desired injection site by syringes and hollow needles typically depends on the operator. Here, we introduce a highly sensitive, completely mechanical and cost-effective injector for targeting tissue reliably and precisely. As the operator pushes the syringe plunger, the injector senses the loss-of-resistance on encountering a softer tissue or a cavity, stops advancing the needle and delivers the payload. We demonstrate that the injector can reliably deliver liquids to the suprachoroidal space—a challenging injection site that provides access to the back of the eye—for a wide range of eye sizes, scleral thicknesses and intraocular pressures, and target sites relevant for epidural injections, subcutaneous injections and intraperitoneal access. The design of this simple and effective injector can be adapted for a broad variety of clinical applications.

Misdirected injection of therapeutic and diagnostic agents can severely compromise efficacy and lead to serious complications1–5. The century-old method of infusing fluids into the body with a syringe and a hollow needle can be improved by using a system that is less reliant on the operator’s skills and enables automated access to the target site despite anatomical variations. The insertion of sharp tools into a target space is also common for biopsy, aspirating fluids and laparoscopic surgery, which face the same challenge. While these procedures are ubiquitous and have enabled multiple life-saving treatments, improper insertion or positioning of needles, catheters and trocars can cause mechanical injury to adjacent tissues, or misdirected drug infusion, and often require repeated attempts to achieve correct placement. Live imaging/sensing systems, such as fluoroscopy, computed tomography scan or ultrasound, are available with added cost, time and resources; however, they often do not have sufficient resolution to perfect needle placement, are not preferred by physicians or simply cannot be used (for example in emergent situations). Current techniques for accessing specific regions of the body with needles typically involve blind insertion, where the physician or nurse relies entirely on superficial anatomical landmarks, experience, fluid return through the instrument and tactile feedback from the change in resistance to pierce through tissue layers. Current approaches require the operator to quickly sense and stop advancing the needle once in the targeted tissue, cavity or potential space. Depending on the application, such feedback may be absent or not sensitive enough to be detected by the operator. Where feedback is detectable, final placement of the needle is highly sensitive to the speed of insertion and the reaction time of the operator.

Devices such as the Veress needle, spring-loaded trocars and mechanisms to stop, retract or sense the position of the needle attempt to limit the injury caused to neighbouring tissue during pneumoperitoneum or epidural injection. Data supporting the effectiveness of these devices in avoiding damage remain weak6–11. Furthermore, these mechanisms cannot be miniaturized cost-effectively to adapt for smaller needle sizes. A force-sensing microneedle has been developed for retinal vein cannulation that demands complex sensing devices and relies on the operator to arrest the needle motion. Here, we introduce a highly sensitive, completely mechanical and cost-effective device, an intelligent injector for tissue targeting (I²T²), which senses the loss-of-resistance, stops advancing the needle automatically and delivers the payload, while the operator simply pushes the syringe plunger in one continuous motion to enable all three actions. Since the device is designed based on the physics underlying the general process of needle insertion, it can be adapted for a large range of medical applications. An analytical model developed for the design of this device enabled us to customize parameters for the I²T² to ensure successful tissue-targeted injection to access multiple cavities and tissues ranging from the large peritoneal cavity under layers of skin, fat and muscles to the micro-thin suprachoroidal space (SCS) below the sclera. Amongst multiple targets, the SCS is one of the most unforgiving sites. The SCS is a relatively new site for drug delivery that has the potential to allow instantaneous delivery to the back of the eye20–30. Direct injection near the diseased site minimizes the dosage necessary for therapeutic effect and minimizes systemic toxicity. SCS injections involve the insertion of a needle through the sclera to access the SCS and deliver drugs to the back of the eye, without penetrating the underlying choroid and retina. The thin tissue layers (healthy human sclera: 0.7–1.1 mm; choroid: 0.18–0.24 mm; retina: 0.22–0.34 mm) make it difficult to consistently access the SCS without overshooting into the vitreous space. Moreover, the sclera is 10 times stiffer than...
the choroid and 200 times stiffer than the retina\textsuperscript{11}, making it even more challenging to pierce the sclera without puncturing through the choroid and retina. This application demands engineering precision and miniaturization with limited room for error. Although it is simpler to implement I2T2 for applications involving thicker tissue structures and larger cavities, we selected drug delivery into the SCS as a gateway model to first validate functionality and safety of the I2T2. Specifically, our thinking was that success of the I2T2 in SCS delivery would open the door to many other applications. We recognize that multiple specialized techniques and devices\textsuperscript{22,32,33} have been developed to access this space. However, these techniques cannot be translated to other medical applications. Following a robust proof of concept with SCS delivery, we demonstrated that the I2T2 can target the site of interest for epidural injections, subcutaneous injections and intraperitoneal access. Overall, the I2T2 overcomes the limitations of standard fixed-length needles by automating the loss-of-resistance technique to enable safer and easier access to target sites with applications ranging from SCS delivery to epidural injection.

\textbf{Results}

\textbf{System design and approach.} The device consists of a standard hypodermic needle that senses the loss-of-resistance to fluid flow as it advances through the tissue and arrests the motion automatically as the needle tip enters the tissue that offers lower resistance. Specifically, a sliding hypodermic needle penetrates through the outer tissue layer, automatically stops at the interface of two tissue layers and delivers the contents of the syringe into the targeted space as the user pushes the plunger in a single continuous motion (Fig. 1a–e). The needle is mounted on a sliding support (the ‘needle-plunger’) to enable movement of the needle along the axis of the syringe barrel. The first step of delivery with the I2T2 is insertion of the needle tip into the tissue at a shallow depth, just enough to block fluid flow through the needle (stage I: pre-insertion). The syringe barrel prevents excessive penetration of the needle into the tissue and the needle-plunger-seat prevents the backward motion of the needle (Fig. 1b). As the internal fluid is pressurized by pushing the plunger, the driving forces acting on the needle overcome the opposing forces and advance the needle deeper into the tissue, while the barrel remains stationary (stage II: tissue penetration; Fig. 1c). When the needle tip enters the target space (Fig. 1d), fluid starts to exit immediately (Fig. 1e) to reduce the internal pressure and effectively lower the driving force to below the opposing force and arrest the needle at the interface. Hence, fluid is delivered into the targeted tissue of lower resistance as the operator continues to move the pushing-plunger (stage III: targeted delivery). This automatic stop is accompanied by tactile feedback due to a sudden drop in pushing force (Fig. 1f) and visual feedback through the now motionless plunger (Fig. 1g), indicating that the I2T2 will function as designed for SCS injections. We picked a 30 G needle for the device because of its widespread use for intravitreal injections.

The syringe size and speed of injection are determined with the help of an experimentally verified analytical model (Supplementary Section 1) that predicts maximum allowable fluid flow rate (speed of injection) for a given set of parameters (fluid viscosity, plunger friction, syringe barrel diameter and needle size). Due to the lack of approved formulations for SCS delivery, the design is based on formulations used for intravitreal injections, which typically have a viscosity similar to water (1 cP). Figure 2c plots the maximum flow rates that allow an automatic stop in stage III for aqueous formulations (viscosity = 1 cP) for multiple needle sizes and syringe sizes based on the analytical model. Clearly, a 1-ml syringe allows for a large range of fluid flow rates (0.18 ml min\textsuperscript{−1} to 2.2 ml min\textsuperscript{−1}) and it can comfortably deliver 50–200 \(\mu\)l of fluid, a typical volume for SCS injections. In other words, this allows a clinician 33 s to inject 100 \(\mu\)l fluid into the SCS. A typical intravitreal injection (time of injection: \(~15 s, excluding injection site preparation time) fits well within this window of injection speed with sufficient room for error.

\textbf{The I2T2 enables targeted delivery into the SCS.} The I2T2 was tested for its ability to deliver a drug into the SCS by injecting a model dye formulation (histology marking dye or X-ray contrast agent) into ex vivo bovine eyes (Fig. 2d). Histology slides clearly show the green dye spread out across the SCS (Fig. 2e,f). The angle of the needle track visible in the sclera is representative of the initial insertion angle. The three-dimensional (3D) images obtained with X-ray micro-computed tomography (microCT) (Fig. 2g) show that a single injection spreads the dose along the periphery to cover a large portion of the SCS immediately after injection. These observations confirm targeted delivery into the SCS with the I2T2. As a negative control, a standard needle was pierced through all the tissue layers to reach the vitreous. We did not observe the presence of contrast agent in the SCS and all the payload was delivered into the vitreous, which is observed as a bright ‘spot’ within the vitreous at a distance from the retina (Supplementary Fig. 3). We also performed serial sections along the path of the needle to verify that the needle travelled through the sclera and did not pierce the retinal pigment epithelium or other inner tissue layers (Supplementary Fig. 4).

\textbf{Variation in eye anatomy does not affect delivery with the I2T2.} We performed SCS injections with the I2T2 in multiple eyes (ex vivo) with a wide range of scleral thicknesses, intraocular pressures and eye sizes. The location of the delivery (observed with microCT, Supplementary Fig. 3) and total coverage of SCS with a single injection (Fig. 3a,b) are not affected by scleral thickness at a given species, one can ensure that the needle is well positioned to enter the SCS.

Successful delivery with the I2T2 requires the injector to perform as intended in all three stages. With a minimal human scleral thickness\textsuperscript{31} in mind, we limited the pre-insertion depth to 500 \(\mu\)m. If inserted at an angle other than perpendicular, one can bury the needle deeper without piercing through the sclera. For example, a 30 G needle with the standard bevel (angle: 12º; length: 1.45 mm) inserted at an angle <20º to the surface satisfies these criteria. The driving force (which enables forward motion of the needle) and opposing force (which opposes needle motion) play a critical role in stages II and III to control the needle’s motion and its automatic stop at the loss-of-resistance (Supplementary Section 1). Commonly used ocular needles (27 G, 30 G and 33 G) are able to overcome the opposing force with a sufficient driving force in stage II (Fig. 2a) as the needle moves through sclera at a range of speeds (10–50 mm min\textsuperscript{−1}), indicating successful tissue insertion. Similarly, with water as the fluid, the driving force for the same needles is lower than the opposing friction at multiple speeds (10, 30 and 50 mm min\textsuperscript{−1}) of the pushing-plunger (Fig. 2b), indicating that the I2T2 will function as designed for SCS injections.
the site of injection or by intraocular pressure. While the eye size does not affect the location of delivery (Fig. 3c–e) it does increase relative SCS coverage for smaller eyes (bovine, porcine and rabbit) when the same fluid volume is administered (Fig. 3f). Similarly, increasing the volume increases the SCS coverage (Fig. 3g).

The I2T2 can deliver a payload to the retina and nearby inner tissue layers through diffusion. We used Ultravist (active molecule: iopromide; molecular mass: 791 Da) contrast agent as a surrogate for a small-molecule drug to visualize diffusion through multiple tissue layers (choroid, retinal pigment epithelium and retina) by microCT. A bovine eye imaged at multiple time points after SCS injection (Fig. 4a) showed that while SCS coverage was maintained, the agent started to diffuse within minutes across both inner and outer tissue layers. Immediately after injection, the dye had spread along the periphery yet was confined to a thin layer indicated by a sharp spike in brightness (Fig. 4b). This confined dye diffused to the surrounding tissue (Fig. 4c), increasing in the vitreous near the retina, as indicated by the increasing area under the curve (AUC; Fig. 4d).

The I2T2 can deliver microparticles and live cells throughout the entire SCS for cell therapy. Drug delivery to the posterior segment of the eye through the SCS is limited by the clearance rate of drugs through the choroidal vasculature. Sustained drug delivery using polymeric particles or cells can have more lasting effects. The trophic effect of certain cell types, such as mesenchymal stem cells (MSCs) and human umbilical cord cells, has been shown to be effective in limiting ocular tissue degeneration for multiple diseases. With the I2T2, microparticles and cells can be distributed within the SCS to be in close proximity to the retina and retinal pigment epithelium. We delivered polymer particles (Polybeads;
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0.20 µm) and tagged (DiD) MSCs into the SCS of bovine eyes (ex vivo). Particle delivery into the SCS was confirmed using histology (Fig. 5b) compared to a control injected with PBS (Fig. 5a). The presence of tagged cells below the sclera was confirmed with intravital microscopy (Fig. 5c−e). In a separate experiment, tagged (CellTrace Violet) MSCs were injected into the SCS and recovered after a few minutes through a recovery port placed diametrically opposite the injection site (Fig. 5f). An excess amount of saline was

Fig. 2 | The I2T2 can inject into the SCS to deliver a drug to the back of the eye. a, b, Driving and opposing forces measured in sclera for stage II (a) and stage III (b). Red dashed line is a constant force line to facilitate comparison of opposing and driving forces. Blue and green horizontal lines represent the mean value of the corresponding data set. c, Model predicting the flow rates that allow an automatic stop for a range of needles and syringes, which helped with the design of the I2T2 for SCS injection. Red shaded region indicates non-functional fluid-flow rate for an I2T2 (with 1 ml syringe and 30G needle) based on the analytical model. d, SCS injection with the I2T2 showing the angle of insertion and positions of the plungers in different stages. e, f, Histology images of the injected eyes show the presence of green dye in the SCS, in the sagittal plane (e) and the coronal plane (f). g, 3D reconstructions of microCT-imaged eyes following injection with contrast agent. For e−g, the experiments were repeated independently (n > 10) with similar results shown.
pushed through the first needle and effluent containing the cells was collected through the recovery port. The tagged cells from the collected samples show 99% viability, indicating that the force of injection and transit through the SCS does not appreciably affect cell survival (Fig. 5g−i).

The I2T2 can target SCS in vivo with minimal tissue trauma. Three Dutch belted rabbits injected with I2T2 in both eyes were killed immediately following the SCS injection to evaluate device performance and its acute impact. Each injection lasted less than 20 s and a 15-s delay in removing the needle avoided reflux along the needle track. None of the injections showed any visible signs of haemorrhage during the procedure. Three eyes, one from each animal, that were cut open immediately showed a clear vitreous (absence of green dye or blood) indicating that the dye was delivered within the tissue layers as expected and the injection did not

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**Fig. 3** The I2T2 can inject into the SCS and achieve broad coverage despite anatomical variations. **a,** SCS coverage for injections performed with the I2T2 (bovine eyes, n = 9) is not influenced by variations in scleral thickness (the dotted red line indicates linear regression fit and the shaded region shows 95% confidence interval; Pearson’s r test indicates non-significant correlation with P = 0.76). **b,** SCS coverage for injections performed with I2T2 (bovine eyes, n = 3) is not influenced by variations in intraocular pressure (test: ordinary one-way analysis of variance (ANOVA), error bars indicate s.d., filled bars indicate mean value of the corresponding data set). **c−e,** MicroCT images of bovine (c), porcine (d) and rabbit (e) eyes injected with contrast agent. The arrow indicates the location of injection. The experiments were repeated independently (n = 3) with similar results. **f,** For a given volume of injection (120 µl), ocular coverage increases for smaller eye sizes (n = 3; error bars indicate s.d., filled bars indicate mean value of the corresponding data set, Φ indicates diameter). **g,** SCS coverage can be improved by increasing the amount of fluid injected into the SCS (bovine eyes, n = 3; the red dotted line connects the mean values for each volume).
cause vitreous haemorrhage. Photographs of frozen section (Fig. 5j) and microCT images (Fig. 5k) of the other three frozen eyes confirm these results. All three of the imaged eyes showed the contrast agent distributed through the SCS and one of the three eyes showed partial leakage of the dye into the vitreous. The largest volume proven safe in our ex vivo experiments, 100 µl, was used for these in vivo injections to maximize the sensitivity of visualization, which is significantly higher than previously reported safe injection volumes. Such large volumes (≥ 100 µl) have been known to cause adverse events, such as backflow from needle entry, localized serous retinal elevation or choroidal haemorrhage away from the needle entry. We did not see these adverse events with the I2T2, and the injected dye travelled through the SCS to reach the back of the eye. The SCS coverage observed in vivo (up to 100%, Fig. 5l) immediately after injection shows that therapeutic agents can be delivered to the entire posterior section with a single minimally invasive local injection.

Macroscopic applications of the I2T2. The basic principle of the I2T2 can be used for multiple applications where one needs to blindly access a target tissue/cavity hiding beneath a denser tissue (Supplementary Table 1).

Epidural injections are widely used to deliver medication into the outermost space around the spinal cord (the epidural space) for temporary or prolonged relief from pain or inflammation. Every injection involves a risk of injury to the dura protecting the spinal cord (and the leakage of cerebrospinal fluid). Figure 6a–d shows the injection into the spinal canal (porcine tissue, ex vivo) with the I2T2. The spinal cord was removed to enable direct visualization of the potential needle overshoot and the spinal canal (Fig. 6a). The I2T2 design depicted in Fig. 1g, with an 18G x 9 cm needle is used for this application. As the plunger was pushed forwards by the operator, the needle travelled through the dense tissue between two vertebrae and delivered the dye into the spinal canal, evident by the stream of fluid and overflowing blue dye (Fig. 6b). The needle overshoot was too small to be detected (Fig. 6c) during fluid delivery. The needle position was subsequently confirmed by pushing the needle forwards by 5 mm (Fig. 6d).

Subcutaneous injections are frequently administered for medications that require a slow, sustained rate of absorption. Misplaced delivery to intradermal or intramuscular locations directly affects the absorption rate, which is critical for life-sustaining medications, such as insulin for diabetic patients. The difference in resistance to fluid flow offered by the cutis and the hypodermis can be leveraged by the I2T2 to deliver the medication subcutaneously. The operator does not need to control the needle depth to ensure delivery to the hypodermis. Figure 6e–h shows subcutaneous injection (porcine tissue, ex vivo) with an I2T2 (design depicted in Fig. 1g; 25 G needle). Multiple tissue layers (dermis, hypodermis, muscle) are visible in the cross-section (Fig. 6e). The needle is pre-inserted into the dermis (Fig. 6f) and the plunger is pushed forwards. Initially, the needle squeezes the tissue as it advances through the dermis and delivers the fluid immediately, as it senses reduction in resistance to fluid flow into the subcutaneous space (Fig. 6g,h).

Blind insertion of a sharp needle or a trocar into the intraperitoneal space through the abdominal wall is the first step performed in every laparoscopic surgery, with an inherent risk of injury to internal organs. To enable the travel of an I2T2 through the multiple tissue layers of the abdominal wall (skin, fat, muscle and connective tissue), we used a hydrogel plug (placed at the end of the needle tip) to avoid fluid leakage into porous tissue such as muscle and fat (Fig. 6i). This increased the driving force in stage II while maintaining the

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**Fig. 4** | Drug injected into the SCS with the I2T2 can reach the inner tissue layers of the retina and choroid through diffusion shortly after injection. a, MicroCT images show that the contrast agent injected in the SCS diffuses into surrounding tissue over time (scale bar, 10 mm). The experiments were repeated independently (n = 2) with similar results (T indicates time post-injection). b, Normalized grey value, indicative of the concentration of contrast agent, is high in the SCS where the contrast agent was injected. The inset is a representative cross-section showing the line where the mean grey value was measured. c, A closer look at the tissues of interest (vitreous near retina, retina, choroid and posterior sclera) shows the contrast agent diffusing in surrounding tissue layers over time (not all data points are shown, for clarity). d, Increasing amount of drug (as represented by AUC) in the vitreous region near the retina (highlighted by the red shading in c) indicates diffusion of the injected molecule into the vitreous through multiple tissue layers, including the retina and Bruch’s membrane. The red dotted line indicates linear fit and the shaded region shows the 95% confidence interval. Pearson’s r test indicates significant correlation with r = 0.95 and P = 0.001. R², coefficient of multiple correlation.
**Fig. 5 | The i2T2 can be used to deliver microparticles and cells throughout the SCS.**

a, PBS injected into the SCS as a control. b, Polymer microparticles delivered into the SCS. c–e, DiD (1,1-dioctadecyl-3,3,3,3-tetramethylindodicarbocyanine)-tagged cells (red) delivered via SCS injection (c) can be visualized (d) with intravital imaging (e). Collagen fibres were detected as second harmonic generations shown in blue. f–l, Immediate retrieval of the injected MSCs (f); recovered from the eye (g); pushed through the needle (h); and unprocessed (i). Dot plots from fluorescence-activated cell sorting (FACS) analysis indicate 99% viability of the retrieved cells in samples after delivery through the needle and recovery from the SCS, which was similar to control cells that were not passed through the SCS (~99%) (cell staining dyes used: CellTrace Violet and 7-AAD (7-aminoactinomycin D)). Lower quadrants show unstained cells. Samples from multiple (n = 3) independent experiments were combined together to reach the minimum cell number threshold for the cytometer. j–l, In vivo SCS injections in rabbits show that a large area is covered with a single injection containing a mixture of green dye and microCT contrast agent. j, Photograph of a rabbit eye embedded in optimal cutting temperature compound (OCT) and sectioned after in vivo SCS injection. Injected green dye diffuses from the periphery towards the centre indicating that the retina and choroid were exposed to the dye. Red colour indicates blood that is observed only outside the sclera due to enucleation of eyes. Lack of blood inside the globe indicates absence of internal haemorrhage. k, MicroCT image of the same sample showing contrast agent (white) dispersed along the ocular periphery in the SCS. l, SCS coverage observed in vivo in comparison to ex vivo coverage in rabbit eyes. All the experiments were repeated independently (n = 3, horizontal line indicates mean value of the corresponding data set) with similar results.
force structure in stage III (Fig. 6j). Figure 6k–n and Supplementary Video 1 show the delivery of fluid through the abdominal wall (porcine tissue, ex vivo) into a cavity below. The small hydrogel plug (2 mm long) in the needle escapes into the cavity (Fig. 6m) to clear the path for the fluid as soon as the needle enters the cavity and the drop in internal pressure arrests the needle motion (Fig. 6n). Similarly,
hydrogel-enhanced I2T2 can be used to access the ventricular cavity through the heart wall (Supplementary Video 2).

**Discussion**

The century-old method of injection with a hypodermic needle and a syringe is simple and effective, but limited by its dependence on the operator to target the injection site. The manual feedback-based injection has prevailed despite the availability of live imaging methods, largely because of its simplicity and cost-effectiveness. The I2T2 introduced here can deliver the same simplicity and cost-effectiveness with the precision to target the potential space of the SCS between the thin tissue layers of the sclera and the choroid. This completely mechanical system, designed with simplicity as the modus operandi, can sense when the needle reaches a cavity, stop advancing and start ejecting its payload in the target space.

Ex vivo and in vivo studies prove that the I2T2 can reliably inject in the SCS, providing extensive coverage. Interestingly, in vivo injections show a better SCS coverage (up to 100%) than ex vivo rabbit eyes (up to 80%) for the same dosage. Porosity of the outer choroid may have allowed for the fluid to spread along the periphery more easily compared to an ex vivo sample that may have compromised porosity due to the loss of interstitial pressure. Minor serious choroidal detachment was observed in one of the eyes, possibly due to the large volume of injected fluid. Such small choroidal detachments often heal on their own in a few days and their occurrence can be minimized by optimizing the injected fluid volume. Unaffected by anatomical variations, the I2T2 can be used as a universal device for SCS injection across the entire patient population. The ability to deliver and distribute live cells to the entire SCS can accelerate efforts to implement cell therapy for curing ocular diseases.

The current design of the device leaves a small amount of dead volume that depends on the final positions of the needle-plunger and pushing plunger. This variation in dosage due to the unknown travel distance of the needle can be minimized by reducing the diameter of the needle-plunger. In particular, for SCS injections in rabbit eyes, we reduced the dead volume and its variation, from 11 ± 8 μl to 6 ± 4 μl, by reducing the plunger diameter from 4.78 mm to 3.25 mm. For larger dosage volumes, the relative error can be reduced from 6 to 1.67% (Supplementary Section 5). These error values are within the acceptable range for intraocular injections. Alternatively, one could control the delivered dose by continuously monitoring the positions of the two plungers and stopping injecting at a predetermined relative position of plungers, to inject a known amount of dose.

The simplicity of the I2T2 mechanism enables its adaptation for multiple clinical indications, each with distinct design criteria. Here we focused on the ocular applications of the I2T2 and demonstrated its ability to target a micrometre-thin cavity below a millimetre-thin tissue. Preliminary ex vivo experiments for epidual injection, subcutaneous delivery and peritoneal access demonstrated the versatility and adaptability of the I2T2. At present, such cavities are accessed either blindly with prior knowledge of the anatomy through imaging, or with the help of live imaging systems such as fluoroscopy, computed tomography scanning or ultrasound. Performing a blind injection is simplest, but carries the risk of missing the target and damaging vital organs in the vicinity. Procedures to access these cavities could benefit from the I2T2 mechanism. For instance, in the case of venipuncture for intravenous injection or fluid administration, one could use I2T2 to target the right depth. Combined with vein-illumination technology such as VeinViewer, near-perfect venous access could be achievable, reducing complications and ensuring vein preservation. A spring-loaded/hydraulic-powered pushing-plunger can automate the I2T2 for more complex procedures and robotic systems. The design process and the analytical model enable quick iterations for new applications.

In summary, we have introduced a generalized I2T2 mechanism for targeted needle insertion and shown its application for targeting the SCS. This mechanical system senses when the needle reaches the target cavity and immediately stops advancing, while injecting its payload of therapeutic agent or live cells. The I2T2 enables drug delivery to the entire posterior eye through the SCS despite anatomical variation within the patient population, which could help address multiple debilitating ocular diseases including uveitis, diabetic retinopathy and macular degeneration. The simplicity and versatility of the I2T2 allows one to adapt the design for multiple clinical applications and offers an automated approach to access the targeted tissue or cavity through an overlying tissue layer. This cost-effective, sensitive and user-friendly technology enables the next generation of precisely targeted safer injections.

**Methods**

**Device fabrication.** Standard hypodermic needles and components of commercially available plastic syringes were used to fabricate functional prototypes. Two standard syringes are required to make one I2T2. First, the barrel of one syringe is cut to remove the front end that mounts the needle. Next, the plunger from the second syringe is machined to create a needle-plunger and inserted into the machined barrel. A needle-plunger seat (to prevent backward motion during pre-insertion) is created by inserting a metal wire (stainless steel 316L; diameter: 300 μm) across the barrel such that the wire is affixed to the barrel wall. Finally, a commercially available standard hypodermic needle is mounted on the Luer lock connector of the needle-plunger. These process steps can be applied to any syringe to make an I2T2 device and any commercially available hypodermic needle with a Luer lock connector can be mounted on it. Other design variations were also prototyped similarly using off-the-shelf components to create an I2T2. The design used for ocular drug delivery uses a fluidic connector that acts as the needle-plunger seat, eliminating the need for metal wire, and the needle (30G) is mounted directly on the needle-plunger using epoxy adhesive.

**Force measurements.** All the measurements were performed using a universal testing machine by ADMET. To measure the driving force in stage II, a needle was inserted into the tissue to bury the tip and fluidic pressure was applied using a pushing-plunger. Maximum force applied before there was backflow along the shaft of the needle or the fluid started infusing into the tissue was noted. Frictional force was measured separately and subtracted from the maximum applied force to calculate the driving force for each needle in stage II. To measure the stage III driving force, the needle tip was kept in the open air to mimic its presence in a cavity and the plunger was pushed at a given rate while monitoring the maximum applied force. Similar to stage II, pushing-plunger friction was subtracted to calculate the stage III driving force. The opposing force for stage II was calculated by addition of needle-plunger friction and needle penetration force through the tissue. For stage III, the opposing force is the same as the needle-plunger friction (and therefore it is also independent of the needle size and plunger speed). See Supplementary Section 1 for more details.

**Ex vivo SCS injections.** Fresh bovine and porcine eyes were procured on the day of sacrifice from a local supplier (Research 87 Inc.). Fresh rabbit eyes were obtained from Pel-Freeze biologicals and were received within 48h of death. Eyes were stored in a refrigerator and used within 2–3 days of procurement. Eyes were secured with one hand and injections were performed with the other. The needle was orientated at a 30° angle to the sclera, with the open side of the bevel pointing towards the eye for easier pre-insertion. The needle was inserted into the sclera at a set distance from the limbus depending on the species (bovine: 8 mm; porcine: 5 mm; rabbit: 3 mm). Although the needle insertion depth could exceed the scleral thickness (measured normal to the eye surface), this angled approach ensured that the needle tip would insert completely and not leak, yet not overshoot the SCS. Multiple payloads including PBS, green histology dye (Davidson marking system), polymeric particles (Polybeads, 0.20 μm, Polysciences Inc) and MSCs (tagged with DiD, ThermoFisher) were injected using this injection method.

**Ex vivo epidural injection.** Fresh porcine back tissue with an intact spinal canal with skin was procured from a local supplier (Research 87 Inc.) and used within 12h from death. Blue food grade dye was used to colour the tissues. The tissue was placed in a tray on the experimental bench with the spinal canal parallel to the table and the back skin facing the operator. The needle (18G; 9 cm long) was pre-inserted (5 mm deep) at an angle in between two vertebrae with the needle tip pointing towards the intervertebral space. The plunger was pushed by the operator in one continuous motion to drive the needle through the tissue and deliver the fluid into the canal without any measurable overshoot.

**Ex vivo subcutaneous injection.** Fresh porcine skin with connected muscular layer was procured from a local supplier (Research 87 Inc.) and used within 12h from death. Blue food grade dye was used to colour the tissues. The tissue was placed in a tray on the experimental bench with the spinal canal parallel to the table and the back skin facing the operator. The needle (18G; 9 cm long) was pre-inserted (5 mm deep) at an angle in between two vertebrae with the needle tip pointing towards the intervertebral space. The plunger was pushed by the operator in one continuous motion to drive the needle through the tissue and deliver the fluid into the canal without any measurable overshoot.
of death. Blue food grade dye was used to colour the injected PBS. The tissue was placed in a tray on the experimental bench with the skin facing the operator. The needle (25 G, 2.5 cm long) was pre-inserted into the tissue to block the needle tip. The plunger was pushed by the operator in one continuous motion to drive the needle through the skin layer and deliver the fluid subcutaneously.

Ex vivo peritoneal injection. Fresh porcine abdominal wall (full thickness) was procured from a local supplier (Research 87 Inc.) and used within 12 h of death. Green histology dye (Davidson marking system) was used to color the injected PBS. The tissue was placed on a four-well plate to create a cavity under the tissue with the skin facing the operator. The I2T2 was filled with the fluid and the needle was impregnated with a hydrogel plug (pre-moulded to shape; agarose; 1.5%). The needle (18 G, 9 cm long) was pre-inserted (5 mm deep) into the skin tissue to block the needle tip. The plunger was pushed by the operator in one continuous motion to drive the needle through the multiple tissue layers and deliver the fluid into the cavity under the abdominal wall.

Histology. The eyes injected with dye were snap frozen immediately after injection by dipping the eyes into acetone maintained at ~80 °C on dry ice for 15 min to keep the dye from diffusing into surrounding tissue. These frozen samples were cut into smaller sections, embedded in OCT and sectioned using a cryotome (8-µm-thick sections). The eyes injected with particles followed a separate protocol. Since polymeric particles do not diffuse through the tissue, we fixed the whole eye by immersing it in Davidson’s solution (30 ml 95% ethyl alcohol; 20 ml 10% neutral buffered formalin; 10 ml Vistal V liquid; 30 ml distilled water) for 12 h. Then the samples were transferred into graded ethanol series (70 to 80 to 90 to 100%) for 1 d each. Finally, paraffin blocks were prepared from fixed tissue and sectioned at 5-µm thickness. This protocol was also effective for serial sectioning. All the sections were stained with haematoxylin and eosin.

MicroCT. We used the Nikon Metrology (X Tek) HMXT2225 MicroCT system to perform 3D imaging of the injected contrast agent. The eyes were frozen immediately after injection and imaged in the frozen condition. Scan time was limited to 15 min to avoid melting of the sample during imaging. The raw image data was processed using VGStudio MAX software® and 40–60-µm-thick sections were obtained. For the experiment with diffusion of the contrast agent, the eye was maintained at room temperature.

SCS coverage measurement. CAD (computer-aided design) files were generated for the injected contrast agent and the posterior section of the eye based on the 3D image constructed by imaging using the microCT system. Intensity cut-off was manually adjusted to extract dye or eye portion. Total available SCS was assumed to be the same as the inner surface area of the sclera in the posterior section. The area of the 3D model was quantified with a CAD software (CATIA®). Relative SCS coverage was calculated by dividing the dye area by total available SCS area.

Cell injection and sample recovery. MSCs were cultured under standard media conditions. Before injection, cells were stained with a cell dye (DiD or CellTrace Violet). Stained cells (1 million) were injected into the SCS using an I2T2 and recovered after a few minutes through a recovery port placed diametrically opposite the injection site (Fig. 5d). An excess amount of saline was pushed through the first needle and effluent containing the cells was collected through the recovery port. Collected samples were pooled together and analysed with a flow cytometer.

In vivo studies. Ethical approval was given by the Institutional Animal Care and Use Committee of Schepens Eye Research Institute (Boston, MA, USA). All animals were treated according to the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Three Dutch belted rabbits (2–3 kg body weight) were anaesthetized with an intramuscular injection of ketamine hydrochloride (35 mg kg−1) and xylazine (1 mg kg−1) and xylazine (1 mg kg−1): 1, 2, 3

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Data availability

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information.
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Author contributions
G.D.C. M.K.S.V. and J.M.K. conceptualized and iterated the principle and design of the I2T2. G.D.C., M.K.S.V. and J.L. were responsible for study design, guided by J.M.K. G.D.C. and J.L. developed the analytical model, improved the design and performed detailed experimental studies with focus on ocular applications. G.D.C., M.G.-A., A.C. and J.L. designed and performed the in vivo experiments. B.E.M., A.S., N.L.-B., Z.T., K.M. and K.Y. provided experimental support for the experiments with cell injections in the SCS. G.D.C., M.K.S.V. and A.D. tested the device for applications other than eye. G.D.C. and J.L. were responsible for conceptual drawings and data representation. B.E.M. captured all the photographs under the supervision of G.D.C. and J.L. G.D.C. wrote the manuscript with constructive feedback and editing from J.M.K., J.L., B.E.M. and P.A.J.

Competing interests
The authors declare no competing interests.

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- n/a | Confirmed
- The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. $F$, $t$, $r$) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted. Give $P$ values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's $d$, Pearson's $r$), indicating how they were calculated
- Clearly defined error bars

State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection: VGStudio MAX 2.2

Data analysis: CATIA V5R21, VGStudio MAX 2.2, GraphPad Prism 6, and Wolfram Mathematica 9 (for analytical modeling).

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- A description of any restrictions on data availability

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size      | Experiments were performed with a sample size of 3. The experiments were repeated independently to enlarge the sample size when the preliminary experiment showed an effect, but this was limited by the sample size. |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions  | None                                                                                                                                                                                             |
| Replication      | All the experiments have been repeated in multiples independently. The number of replicates are mentioned in the paper.                                                                          |
| Randomization    | Device samples were prepared in advance and selected randomly from the pool of devices during experiments (force measurements, ex vivo, and in vivo).                                           |
| Blinding         | In all the experiments performed, a cavity was targeted using the device, and the operator was blinded to the depth of the target cavity. For the in vivo experiments, there was a single group of animals to test the feasibility of the device; hence, blinding was not needed. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
|     | - Unique biological materials |
|     | - Antibodies |
|     | - Eukaryotic cell lines |
|     | - Palaeontology |
|     | - Animals and other organisms |
|     | - Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
|     | - ChIP-seq |
|     | - Flow cytometry |
|     | - MRI-based neuroimaging |

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) | Primary MSCs we purchased from Lonza  
http://www.lonza.com/products-services/bio-research/stem-cells/adult-stem-cells-and-media/human-mesenchymal-stem-cells-media/hmsc-human-mesenchymal-stem-cells.aspx
Authentication | Not applicable.
Mycoplasma contamination | Yes. They were tested for negative mycoplasma contamination, both by Lonza and lab tests.
Commonly misidentified lines (See FLAC register) | Not applicable.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals | Dutch Belted rabbits (2–3 kg body weight).
Wild animals | The study did not involve wild animals.
**Flow Cytometry**

**Plots**

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

- **Sample preparation**: MSCs were cultured under standard media conditions. Prior to injection, cells were stained with a cell dye (DiD or CellTrace Violet). Stained cells (1 million) were injected into the SCS using i2T2, and recovered after few minutes through a recovery port placed diametrically opposite to the injection site (Fig. 5d in the paper). An excess amount of saline was pushed through the first needle, and effluent containing the cells was collected through the recovery port. Collected samples were pooled together and analyzed with a flow cytometer.

- **Instrument**: BD LSR II

- **Software**: FACSDiva for data acquisition and FlowJo 6 for data analysis

- **Cell population abundance**: There were ~10K cells in the final gate (FSC vs. 7AAD). The purity of the cells was confirmed by CellTrace Violet positive staining before cell injection and after cell retrieval.

- **Gating strategy**: FSC vs. SSC was used to gate out doublets, cell aggregates or dead/dying cells. Live cells (i.e. 7AAD-negative) were defined by Y-axis with value below 400.

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.