In situ-gelling hydrogels for ophthalmic drug delivery using a microinjection device

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ABSTRACT SUMMARY
In recent years, several injectable in situ crosslinking hydrogels have been developed to deliver drugs or cells to the back of the eye; however there is currently no device capable of injecting these materials for in vivo testing. This work describes the development of a novel microinjection device that is capable of controllably injecting small volumes of in situ crosslinking hydrogels.

INTRODUCTION
Diseases associated with the posterior eye, such as age-related macular degeneration, diabetic retinopathy, posterior uveitis, and retinitis due to glaucoma, are the primary causes of vision loss in developed nations.\(^1,2\) The posterior region of the eye is a particularly difficult target tissue due to anatomic and physiologic limitations, but these diseases are now routinely treated via intraocular injections. Unfortunately, the required frequent injections greatly increases the risk of complications over time.\(^2\)

Hydrogels present as a potential solution to this issue, as they can facilitate prolonged delivery of a drug to the eye and limit the number of required injections for effective treatment.\(^3\) Furthermore, unlike most drug release vehicles, hydrogels can be designed to effectively match the refractive index of the vitreous humour, have desirable degradation kinetics, and be injectable in situ, exploiting the rapid chemical reaction of complementary groups grafted to two polymers (such as aldehyde-mediated crosslinking of alcohol-, amine-, or hydrazide-functionalized polymers) to form the crosslink structure.\(^4\)

Several in situ-gelling injectable hydrogels have been developed in our lab using aldehyde-hydrazide chemistry to produce hydrolytically degradable hydrazone crosslinks.\(^5,6\) When polymers of these two respective functional groups come into contact they rapidly form a hydrogel, entrapping the desired drug to be released in the process. These hydrogel materials have promising tunable drug delivery characteristics for a variety of drugs, can have refractive indices similar to that of the vitreous, and slowly degrade in physiological conditions.\(^5\)

However, to assess the in vivo capabilities of these hydrogels in mouse, rabbit, and ultimately human eyes, very small amounts of each reactive material (1–10 μL) must be injected. While this is facilely done with single component systems, no suitable system exists for the administration of in situ-gelling hydrogels at such low volumes.

This work describes the fabrication of a novel microinjector that: (1) effectively mixes the two precursor polymers from separate microchannels upon injection; (2) controllably and precisely injects volumes in the 1-10 μL range through a narrow gauge needle suitable for ophthalmic applications; and (3) rapidly injects these materials to prevent gelation, and blockage within the needle. The developed dual-barrel microinjection system is characterized in terms of the precision of the injected volumes and its use in initial in vivo studies, and the properties of the ejected hydrogels after injection will be characterized in terms of their physical properties and release kinetics of model drugs.

EXPERIMENTAL METHODS
Our in situ-injectable hydrogels are typically prepared by the mixing a hydrazide-functionalized polymer (A-Polymer) and an aldehyde-functionalized polymer (B-Polymer) upon injection using a double barrel syringe (Figure 1). The double-barrel syringe has two partitions: one with hydrazide-functionalized polymer and the other with aldehyde-functionalized polymer, both in PBS solutions. Upon injection, these two solutions interact in the mixing channel and needle, forming the hydrazone bonds that crosslink the polymer to form a hydrogel.

The microinjector was designed to operate analogously to this macroscale system, but using a microfluidic method. The device design (Figure 2) consists of two separate inlets to which a double barrel syringe can be connected, a serpentine mixing channel with herringbone grooves (developed by Strook et al.\(^7\)) to promote mixing, and a volume control reservoir with a one-way valve coupled with second syringe input port that can be used to eject the mixed polymer solutions out the needle (into the eye). The device...
is operated by placing the two reactive polymer solutions in separate barrels of a double barrel syringe and attaching that syringe to the two polymer solution inlets, pushing the mixed fluids through to the needle. The needle can then be inserted into the eye and an ejection syringe can be compressed to only eject the volume of mixed polymer solution in the volume control region, due to the one-way valve that is present. The volume control region can be designed be of any volume from 1 μL – 1 mL.

Figure 2: Microinjector device design, highlighting the volume control reservoir with the associated one-way valve.

RESULTS AND DISCUSSION
Mixing fluids within microfluidic channels is inhibited by the fact that fluid flows at microfluidic scales are laminar in nature. The addition of herringbone grooves was found to dramatically improve the degree of mixing within the channels of preliminary device prototypes, creating microvortexes and allowing for nearly complete mixing only a short distance (1.75 cm) from the point where the two separate solutions first interact within the channels.

With the volume control chamber and the one-way flow valve, the device is able to eject droplets with controlled volumes (~±10%) in the volume range of interest (1–10 μL) via an entirely handheld operation, requiring no additional equipment. The entire volume of the material can be ejected from the device, as confirmed by using fluorescently labelled polymers and observing the volume control reservoir before and after the ejection of a 2 μL sample of gel. Figure 3 shows the greatest variation in droplet sizes from a device intended to deliver 2 μL hydrogel droplets observed thus far, within the ~±10% range.

Figure 3: Variation in droplet sizes from a 2 μL microinjection system.

The microinjectors described can produce hydrogel droplets from multiple combinations of reactive polymer solutions. These hydrogels can be ejected from devices with 33G needle outlets and even smaller capillary outlets into various materials, including, importantly, bovine vitreous humour at 37°C, still forming hydrogels (Figure 4).

Figure 4: Gelled hydrogel droplet (dyed blue) injected into bovine vitreous humour at 37°C.

The devices have been designed for ease of use for in vivo mouse eye injections (experiments are now underway). The release kinetics of hydrogel materials that could be used for ophthalmic purposes have already been studied on a bulk scale, but release studies from micron-scale hydrogels produced by these devices will also be studied and compared to that of the bulk scale.

CONCLUSION
A microinjection device has been developed that is capable of injecting precise small volumes (1 - 10 μL) of in-situ gelling hydrogel precursors, allowing for the in vivo assessment of injectable hydrogels as ocular drug delivery materials. The hydrogels that this device can deliver to the eye (or other organs, including for embolic plugs or cell encapsulation matrices) could provide significant improvements over current therapies.

REFERENCES

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