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In vivo performance of a microfabricated catheter for intraparenchymal delivery

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HIGHLIGHTS

• We tested a new type of catheter made with microfabrication techniques.
• Small molecule to virus-sized particles were infused into living porcine brain.
• No backflow was observed along the shaft at flow rates up to 30 μL/min.
• The design principles allow future construction of smart catheters with biosensors.

ABSTRACT

Background: Convection-enhanced delivery (CED) is currently the only effective clinical technique to deliver biological therapeutic agents that would otherwise not cross the blood–brain barrier. Despite the promise of CED, several technical problems have limited its effectiveness.

New method: Brain infusions into a large mammal (pig) were performed with a catheter that was fabricated using micro-electro-mechanical systems (MEMS) technology. The performance of the catheter was evaluated for infusions at increasing infusion rates. Magnetic resonance (MR) images were acquired in real time to examine the distribution of infused tracers in the parenchyma. The results were quantified. Concentration profiles were determined for several MR contrast reagents as well as a fluorescent dye that are the sizes of small molecules, therapeutic proteins and an adeno-associated virus (AAV). The reagents can serve as surrogates for assessing the convective distribution of active molecules. Infusion rates up to 20 μL/min were attained without evidence of backflow along the catheter.

Comparison with existing methods: The device performed well in terms of both backflow and infusion, superior to that of many studies reported in the literature on other catheters. All infused molecules had comparable ratios of distribution to infusion volumes.

Conclusions: The catheter described in this report appears able to target tissue structures with precision, deliver therapeutics at high infusion rates, and resist backflow that can compromise the efficacy of CED therapy. The technology allows development of “smart” catheters for future applications.

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1. Introduction

Convection enhanced delivery (CED) is an advanced technique used to distribute therapeutic agents into targeted regions of the brain (Bobo et al., 1994; Prabhhu et al., 1998). CED involves the direct infusion of compounds into brain parenchyma through a
catheter implanted in the brain through a hole in the skull. The technique shows promise for treating a variety of neurological disorders, including glioblastoma, epilepsy, and Parkinson’s disease. However, practitioners have been continually plagued with unreliable delivery of infused therapeutics, which may have resulted in the failure of pivotal clinical trials (Sampson et al., 2010; Morrison et al., 2007; Salvatore et al., 2006).

In recent years, CED practitioners have focused on the design of new catheters that mitigate or eliminate backflow. Backflow, or reflux, refers to the tendency of infused fluid to flow up the external surface of the catheter instead of flowing into the parenchyma surrounding the catheter. Several innovative designs have been tested, such as catheters consisting of multiple cylindrical tubes in series with stepwise reductions in tube diameter toward the distal tip of the catheter, which have been shown to reduce backflow (Krauze et al., 2005). Another design involves a tube whose wall is porous at the end of the catheter so that fluid is released into the parenchyma along the length of the porous segment instead of release at a single outlet (Oh et al., 2007). These examples and similar designs are fabricated using traditional machining techniques.

An alternative strategy is to use micro-electro-mechanical systems (MEMS) technology to fabricate catheters that contain microfluidic circuits to deliver fluid into tissue. Microfabricated catheters not only can contain the desirable step reductions in diameter to reduce backflow, but also can contain features that are difficult to incorporate using tubes and standard mechanical fabrication. For example, a microfabricated catheter containing multiple microfluidic channels on an insertable silicon shank was used to deliver multiple therapeutics sequentially in rodent studies (Olbricht et al., 2010). The outlets of the channels were recessed from the leading edge of the shank to prevent clogging of the channels by soft tissue when the device was inserted into the brain. Similar microfabrication methods have been used to build a flexible catheter made completely from parylene, a soft polymer (Olbricht et al., 2010). The flexible catheter was coupled to a rigid biodegradable shank for insertion into the rodent brain. Beyond the conformal features demonstrated in these devices, electronic features could be included in a microfabricated device, such as integrated sensors that could provide information about the efficacy of the infusion and measure biochemical or electrical conditions in the surrounding tissue to assess the effectiveness of therapy.

The devices mentioned above were designed for use in rodents, so the microfabricated component was only a few millimeters in length. Scale-up of a microfabricated device to carry out CED in humans is a significant challenge. Although it may be possible to microfabricate a device of sufficient length for use in humans, it may not be necessary, cost-effective, or otherwise advantageous to do so. Instead, the advantageous features described above could be realized by fitting a small microfabricated device to the distal end of a “standard” cannula that is modified to accommodate the microfabricated tip. This is the strategy used in this study to assess the performance of a microfabricated device suitable for use in humans. Here, results are reported for intraparenchymal infusions into the brains of live, anesthetized pigs. The distributions of infused molecules of various sizes are characterized using real-time magnetic resonance imaging (MRI). The primary outcomes of the study are backflow and infuse distribution measurements for CED into a variety of cytoarchitectures.

2. Equipment and materials

The design characteristics of the class of microfabricated devices have been described elsewhere (Olbricht et al., 2010). Fig. 1 shows the microcatheter (Alcyone Lifesciences, Inc., Concord, MA) that is used in this study. The device consists of four parts. The most proximal part (far left in Fig. 1) is the catheter shaft, which is a 1.6-mm diameter tube. A custom-designed transition called the “bullet nose” connects the catheter shaft to a fused silica tube with a length of 4 mm and a diameter of 350 μm. The bullet nose is designed to prevent backflow, and the narrow tube provides additional strength and a backflow stopping step to the microfabricated tip. The tip, which is 7 mm long, is fitted into the fused silica tube so that 3 mm of the tip remains exposed (far right in Fig. 1). The silicon tip narrows to a point at its distal end to ease insertion of the device into the brain. Two parallel microfluidic channels, each having a cross-section of 30 μm × 30 μm (a few of the experiments used a 30 μm × 53 μm section, but the outer dimensions which determine backflow were the same in all cases), are etched into the top of silicon tip. A thin layer of silicon is bonded to the top surface of the tip to cover the microfluidic channels over most of their length. The inset of Fig. 1 shows a magnified view of the tip. The uncovered distal ends of the microfluidic channels, which are visible in the inset, are the channel outlets. The proximal end of each microfluidic channel is connected to a microtube (not visible in the photograph) that resides inside the narrow tube and catheter body. Each microtube is connected to a syringe pump through a standard Luer fitting. Thus, the fluid delivery systems for the two microfluidic channels are completely independent of each other.

The small cross-sectional dimensions of the tip may reduce tissue trauma during insertion and allow more precise therapeutic delivery in smaller cytoarchitectural targets. The independent fluidic channels in the device can be used to deliver two therapeutic compounds or a therapeutic compound and an imaging agent in programmed sequences. The side-facing outlets of the microfluidic channels are intended to diminish the possibility of occlusion by tissue during insertion of the device into the brain. The bullet nose transition between the catheter shaft and the narrow tube and the change in size from narrow tube to the microfabricated tip are designed to help reduce backflow at higher infusion rates.

3. Methods

Infusion experiments were performed at the University of Virginia. The animal use committee at the University of Virginia approved all protocols. The general methods for catheter placement and navigation to target have been described in Brady et al. (2011) and Emborg et al. (2010).

3.1. Animal preparation and catheter navigation and device insertion

On the day of the infusion, each animal had free access to water for 12 h before the experiment. Initial sedation was by intramuscular injection of ketamine (25 mg/kg) and xylazine (1–2 mg/kg). Anesthesia was maintained by inhalation of isoflurane (~1%) using a ventilator.

Pre-infusion imaging on the anesthetized animal was then performed in a 3 Tesla Clinical MR scanner (Trio, Siemens, Malvern, PA) to characterize the normal brain and plan for the infusions. Among other sequences detailed below, these included a high-resolution 3D T1-weighted FLASH and a 3D MP-RAGE to distinguish anatomy. The images were used to plan trajectories on the MRI console workstation based on the targets planned for the animal. The x–y coordinates for skull burr hole placement were measured relative to the inion.

The anesthetized animal was then placed on a surgical table and the scalp in the midline incised and retracted widely. Burr holes were placed bilaterally in the skull at locations determined by the pre-operative planning. A disposable MR-compatible
trajectory guide (Navigus, Medtronic) for the Alcyone microcatheter was installed at each burr hole and secured to the skull using the screws provided. The wound was covered steriley for repeat imaging and infusion procedures. The animal was then transferred to the MR scanner for targeting, placement of the Alcyone microcatheters and infusions.

To obtain initial trajectory alignment, machined plastic tubular adapters were filled with diluted Magnevist™ (final concentration 2.5 mM) and placed in each trajectory guide. The animal was scanned in MRI, and the trajectory guides were then adjusted to the appropriate angles for the intended trajectories and targets. The distances from the skull surface to the target for each guide were also measured on the MRI console. Then, the Alcyone microcatheters filled with infusate were inserted through the trajectory guides to the measured depths for the anatomical targets chosen for the infusion (white matter/internal capsule, thalamus or putamen). The infusion rates and durations varied among experiments as described below.

3.2. MR contrast agent and dye marker preparation

For the backflow studies, the infusate was Magnevist™, which consists of gadopentetate dimeglumine, with a molecular weight of 938 Daltons. Magnevist™ serves as an MR-visible surrogate for a low-molecular weight therapeutic. It is used here to monitor convection in brain parenchyma and to detect backflow along the outside of the catheter.

For the spatial distribution studies, several compounds were used as infusates. Galbumin™ (Biopal, Inc., Worcester, MA), a chelated gadolinium conjugated to albumin with a molecular weight of 66 kDa, served as a surrogate for an assortment of proteins used in therapies for brain cancer and Parkinson’s disease. IVIS dye (Lumiprobe, Hallandale Beach, FL), a near-infrared fluorescent dye (Cy7 NHS ester) of molecular weight 828 Da, is, like Magnevist™, a representative small-molecular weight molecule. The IVIS dye was co-infused with the Galbumin™, and its spatial distribution was measured by adapting an IVIS in vivo measuring system to detect the dye post mortem in brain slices. CellTrack™ (Biopal), a polydisperse suspension of gadolinium oxide nanoparticles, was used as a surrogate for adeno-associated viral (AAV) particles and synthetic nanoparticles that have been used to deliver genetic material and other therapeutics. The mean particle size in the suspension was about 30 nm, which is slightly larger than the 24-nm AAV particles. The gadolinium oxide molecules inside the CellTrack™ nanoparticles are highly paramagnetic, which means that the polarization of electrons due to the applied magnetic field results in a susceptibility of protons in surrounding water molecules that is much larger than the corresponding susceptibility induced in water by the other reagents used in this study. This difference in susceptibility induced in surrounding water must be taken into account in obtaining concentrations from the MR signal due to T1 dynamics.

The molarity (or concentration) of the active tracer is not known for the Biopal reagents. In these cases, a linear relationship was found between the administered amount of reagent and the measured dose. Therefore, concentrations can be reported as fractions of the infused concentration. For the case of Galbumin™, concentrations can be determined by calibrating the slope of measured values versus administered dose, because both the mass density of the reagent and its molecular weight are known.

3.3. In vivo animal infusions

The following pre-infusion imaging studies were performed on the anesthetized animal to characterize the normal brain and plan for the infusions: high-resolution 3D T1-weighted FLASH sequence and a 3D MPRAGE sequence to distinguish anatomy, diffusion tensor imaging to estimate diffusivity and hydraulic conductivity, non-contrast MR angiography to define perivascular spaces, T2/PD-weighted imaging to locate CSF-containing regions and to obtain baseline proton density for estimation of tissue expansion, and SPGR sequences with two different flip angles to establish the baseline T1 of the tissue. A specialized scan to obtain the actual flip angle map was performed to correct for field distortions in obtaining the concentration maps during infusion. All imaging was conducted at 3T Siemens scanner at the University of Virginia. High-resolution brain scans were obtained using a large flexible surface coil.

Three regions of the porcine brain were selected for studying backflow: (i) the white matter of the internal capsule, (ii) the putamen, and (iii) the thalamus. Each region presents its own challenge for backflow.

Magnevist™ was diluted 1:40 with saline solution to a concentration of 12.5 mmol/L. For the backflow studies, both channels of the device contained the same fluid. The channels were pre-filled with the Magnevist™ solution, and the device was placed in the chosen tissue target under MR guidance. The infusion rate started at 1 µL/min and was then increased in discrete steps. The sequence of infusion rates was 1 µL/min for 8 min, 3 µL/min for 5 min, 5 µL/min for 5 min, 7 µL/min for 5 min, 10 µL/min for 5 min, and either 15 or 20 µL/min for 5 min (Table 1). The duration for each infusion rate was sufficiently long to observe infusate and identify backflow, if any, at that infusion rate. However, the duration at each rate was short enough so that the infusion volume did not obscure backflow, if any, at the next higher infusion rate. T1 weighted MR scans for contrast enhancement imaging were continuously performed to record the infusate distribution and to determine backflow distances by post processing analysis.

Distribution studies were designed to simulate delivery of therapeutics into various cytoarchitectures. For studies in white matter, CellTrack™ was infused through both channels of the microcatheter to simulate AAV infusions into brain tumors, which often occur in regions surrounded by white matter. For studies in the thalamus, Galbumin™, which is similar in size to many therapeutic proteins, was infused through one channel of the microcatheter while IVIS-visible dye, which is a small-molecular tracer, was infused simultaneously through the other channel. The thalamus is often the target of therapeutics in neurodegenerative diseases.
Table 1
Backflow studies summary. Top row #s: infusion rates in μL/min; other rows: backflow in mm. All distances to be read “−” as in first row entries.

<table>
<thead>
<tr>
<th>Anatomic target</th>
<th>Pg#, run#</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>7</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal capsule LH</td>
<td>1, 1</td>
<td>≤3</td>
<td>≤3.5</td>
<td>≤4</td>
<td>≤4</td>
<td>≤4.5</td>
<td>≤5</td>
<td>≤6</td>
<td>≤7</td>
<td>No flow past bullet nose; past step at 2 μL/min; no flow past bullet nose; past step at 2 μL/min; forward flow along preferred path to CSF</td>
<td></td>
</tr>
<tr>
<td>Internal capsule RH</td>
<td>1, 2</td>
<td>3</td>
<td>5</td>
<td>5.5</td>
<td>6.5</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>No flow past bullet nose; past step at 2 μL/min; no flow past bullet nose; past step at 2 μL/min; forward flow along preferred path to CSF</td>
<td></td>
</tr>
<tr>
<td>Thalamus RH</td>
<td>1, 3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Forward flow along preferred path to CSF</td>
<td></td>
</tr>
<tr>
<td>Midbrain posterior to thalamus</td>
<td>1, 4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4</td>
<td>6.5</td>
<td>7</td>
<td>7</td>
<td>Flow past bullet nose at 3 μL/min; ventricle; flow past bullet nose at 15 μL/min</td>
<td></td>
</tr>
<tr>
<td>Central thalamus RH</td>
<td>4, 1</td>
<td>7</td>
<td>–</td>
<td>9</td>
<td>–</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>Flow past bullet nose at 3 μL/min; ventricle; flow past bullet nose at 15 μL/min</td>
<td></td>
</tr>
<tr>
<td>Posterior thalamus LH</td>
<td>4, 2</td>
<td>7</td>
<td>–</td>
<td>7</td>
<td>–</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>Flow past bullet nose at 15 μL/min</td>
<td></td>
</tr>
<tr>
<td>Medial edge of putamen RH</td>
<td>4, 3</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>No flow past bullet nose; past step at 8 μL/min</td>
<td></td>
</tr>
<tr>
<td>Interior capsule (approx.) LH</td>
<td>4, 4</td>
<td>3</td>
<td>–</td>
<td>3</td>
<td>–</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>No flow past bullet nose</td>
<td></td>
</tr>
<tr>
<td>Putamen LH</td>
<td>6, 1</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>No flow past bullet nose</td>
<td></td>
</tr>
<tr>
<td>Putamen RH</td>
<td>6, 2</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>Reaches WM at 5 μL/min</td>
<td></td>
</tr>
<tr>
<td>Thalamus RH</td>
<td>6, 3</td>
<td>2</td>
<td>–</td>
<td>4</td>
<td>–</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>No flow past bullet nose</td>
<td></td>
</tr>
<tr>
<td>Corona radiata LH</td>
<td>7, 1</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>–</td>
<td>6</td>
<td>No flow past bullet nose</td>
</tr>
<tr>
<td>Thalamus midline RH (1 mm from CSF)</td>
<td>7, 2</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>No flow past bullet nose</td>
<td></td>
</tr>
<tr>
<td>Thalamus lateral edge LH</td>
<td>8, 1</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>–</td>
<td>7</td>
<td>9</td>
<td>10</td>
<td>11.5</td>
<td>12.5</td>
<td>No CSF loss, infuse within thalamus, internal capsule</td>
</tr>
<tr>
<td>Thalamus anterior edge RH</td>
<td>8, 2</td>
<td>–</td>
<td>–</td>
<td>4</td>
<td>–</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>10</td>
<td>Loss into CSF but continues to infuse tissue</td>
<td></td>
</tr>
</tbody>
</table>

(A) Additional backflow studies in white matter at high infusion rates

<table>
<thead>
<tr>
<th>Anatomic target</th>
<th>Pg#, run#</th>
<th>5</th>
<th>10</th>
<th>25</th>
<th>30</th>
<th>40</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal capsule LH</td>
<td>9, 1</td>
<td>5</td>
<td>6.5</td>
<td>8</td>
<td>11</td>
<td>13.5</td>
<td>Past bullet nose after 25 μL/min; no flow past CSF</td>
</tr>
<tr>
<td>Internal capsule RH</td>
<td>9, 2</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>13</td>
<td>14</td>
<td>Ditto</td>
</tr>
</tbody>
</table>

Both MR reagents were infused at a concentration of 25% of the supplied stock concentration, diluted 1:3 with saline solution, which resulted in an infused Galbumin™ concentration of 0.0845 mmol/L. In both cases, the infusion rate through each channel was 2.5 μL/min (5 μL/min total), resulting in a total of 150 μL (75 μL of Galbumin™ solution and 75 μL of IVIS dye) infused at this rate over 30 min. Because the white matter volume is larger than the thalamic volume, the 30-min infusion into white matter was followed by a 5.0 μL/min per channel burst for 2 min for an additional 20 μL.

Following completion of each infusion study, the animal was euthanized while under general anesthesia by intravenous administration of a combination of pentobarbital sodium and phenytoin sodium (Euthasol, Virbac AH, Inc., Ft. Worth TX). For animals receiving infusions of IVIS dye, the brain was harvested and sliced parasagittally. Brain slices were then imaged using a high-sensitivity pre-clinical in vivo fluorescence imaging system (IVIS 200, PerkinElmer, Waltham, MA).

3.4 Line pressure measurements

The line pressures were measured using in-line single-use PendoTech™ pressure sensors (P/N PRESS-5-000). These sensors read pressure, relative to atmospheric, immediately distal to the pump syringe, and thus included the pressure drop along the 20-ft long 0.01-in fused silica extension line in addition to the pressure drop along the microfabricated catheter and the pressure required to drive the infusate into the tissue.

3.5 Data analysis

Backflow distances were measured as described in prior studies (Raghavan et al., 2010; Sillay et al., 2013) using T1-weighted MR images acquired immediately after each change of flow rate. Backflow at the initial flow rate can usually be measured directly as the distance from the catheter tip to the most proximal point of observed tracer along the catheter. However, if the backflow distance is not large compared to the tracer penetration into tissue, then the lateral penetration is subtracted from the proximal distance along the catheter. (A spherical infusion centered on the catheter tip would have zero backflow by this method.) As the flow rate is increased, the backflow tends to increase along the catheter shaft. If the increase in backflow brings it beyond the main infusion mass, it is visible as a thin band along the catheter shaft, and can be measured. If no such band is visible at the new infusion rate, then the backflow is obscured by the infusion cloud. In this case, the distance that the infusate extends up the catheter is defined as an upper bound to the backflow distance. In this case, the backflow distance is reported as less than or equal to this upper bound.

We measured the concentration of MR reagents by the method described for in vivo infusions by (Brady et al., 2011). T1 maps were computed from pairs of 3D FLASH MR scans at flip angles of 6 and 34 degrees using the variable nutation method (Deoni et al., 2003, 2004). B1 field inhomogeneities were corrected using the method for measuring the actual flip angles (Wang et al., 2005). The 3D pairs were acquired before infusion, at 7- to 8-min intervals during the infusion, and once after the infusion. Gadolinium concentration, C, was computed from the T1 maps using the equation 1/T1 = 1/T1o + R0C, where the tracer relaxivity R0 was assumed to be 3.81/mmols. Here, T1o, T1 are the values before and after the introduction of tracer, respectively. In the case of co-infusion of Galbumin™ with IVIS dye, it was assumed that the two infuses mix at the tip and that the effective gadolinium concentration is reduced by half, i.e., a single flow of 0.0422 mmol/L Galbumin™ at 5 μL/min.

The volume of distribution Vg was estimated as the volume of the voxels containing a measurable concentration of gadolinium tracer, and was used to compute the ratio Vg/Vi, where Vi is the volume of fluid infused. Since the resting interstitial volume fraction is about 0.2 in most parts of the brain, the ratio Vg/Vi would be about 5 if the interstitium does not change as a result of the infusion and if there are no losses of infusate from the parenchyma (e.g., into
the vasculature or into the cerebrospinal fluid spaces). However, the interstitial spaces can change and may vary intrinsically, and so the ratio can vary. In calculating \( V_g \), we apply a threshold to the concentration instead of applying a threshold to the T1-enhanced image of the contrast reagent. For GdDTPAM, the threshold was 0.002 mmol/L or 4.8% of the infusate concentration (this was the minimum computable concentration). In the case of CellTracker\textsuperscript{TM}, the latter was 0.8% of the infusate concentration. Thus, \( V_g \) is the total volume of all voxels that contain at least this concentration of reagent. Increasing or decreasing this threshold would tend to decrease or increase \( V_g \), respectively.

4. Results and discussion

A few representative examples of the backflow studies are discussed here. Table 1 provides a comprehensive list of results for all experiments.

Fig. 2 shows MR images for an infusion into white matter. The catheter was placed deep and posterior to the thalamus. The shaft crossed the lateral ventricle 14 mm proximal to the tip. The images in the figure show that the infusion cloud for low infusion rates is close to spherical. There is no backflow visible at the junction between the microfabricated tip and the narrow tube, which is 3 mm proximal to the microwcatheter tip. The infusion cloud remains close to spherical at higher infusion rates. The infusate does not pass the bullet nose step, which is 7 mm proximal to the tip, for infusion rates up to 20 \( \mu \text{L/min} \).

Fig. 3 shows MR images for an infusion into the thalamus. The catheter was placed deep and posterior to the thalamus. The shaft crossed the lateral ventricle 14 mm proximal to the tip. In contrast to the infusate in white matter, the infusate in the thalamus exhibits backflow beyond the junction between the microcatheter tip and the narrow tube at an infusion rate of 1 \( \mu \text{L/min} \). The infusate continues to flow up to the bullet nose. However, no further increase in backflow is observed for infusion rates between 3 and 10 \( \mu \text{L/min} \); in this case, the bullet nose appears to prohibit further backflow. At an infusion rate of 15 \( \mu \text{L/min} \), infusate enters the lateral ventricle, which is 14 mm from the tip. Flow of the infusate into the ventricle affects the spatial distribution of infusate, which makes it difficult to assess the possibility of further backflow at high infusion rates in this case.

Fig. 4 shows MR images for an infusion into the putamen. The catheter tip was centered 3–4 mm deep in the putamen, just beyond halfway along the depth of the putamen on the trajectory chosen. Negligible backflow was observed in the putamen at 1 \( \mu \text{L/min} \). At 3 \( \mu \text{L/min} \), backflow is observed past the first 3-mm step, which is located outside the top of the putamen. At 4 \( \mu \text{L/min} \), the backflow reaches the bullet nose. However, there is no backflow beyond the bullet nose for infusion rates up to 15 \( \mu \text{L/min} \). The infusion rate was not increased beyond 15 \( \mu \text{L/min} \) in this case because boundaries of the putamen were breached at this infusion rate.

The list of results in Table 1 indicates that there is no discernible effect of the cytoarchitecture of the target location on the extent of backflow in these studies. The infusion rate that could be attained before the infusate reached a sink, such as perivascular space or
ventricles, varied among the three targets in these studies. Nevertheless, there is no observable trend for backflow with tissue type in the studies. Moreover, the bullet nose of the catheter seems to act as an effective bulwark or seal against backflow at flow rates as high as 20 μL/min. As Table 1 indicates, while higher flow rates seem to drive infusates past the first step at 3 mm from the tip, the flow almost never passed the bullet nose. In fact, the large diameter of the nose would facilitate greater backflow (Raghavan et al., 2010); thus, we infer that it serves other purposes such as imposing pre-stress on the tissue that would act to prevent backflow. Detailed theoretical and experimental studies to elucidate the precise mechanism for backflow reduction remain for the future.

**Fig. 5A** for an infusion of Galbumin™ into the thalamus shows the T1-enhancement of Galbumin™ on the left and the Galbumin™ concentration profile on the right. The concentration is color-coded in mmol/L. **Fig. 5B** shows similar results for the concentration of CellTrack™ expressed in units of fraction of the infused concentration for reasons explained in Section 3.2. Although the diameter of the CellTrack™ particles is about four times the diameter of Galbumin™ molecules, the spatial concentration distributions for the two infusates are similar. The similarity in distributions is borne out in **Fig. 6**, which shows the volume of distribution $V_d$ versus the volume of infusion $V_i$ for all infusions with Galbumin™ and CellTrack™. The significance of the graph is that there is no evidence of retardation of the spread of the reagents, despite the large difference in their sizes. Perhaps even more importantly, it shows that large constructs the size of AAV are readily convected through the parenchyma. Of course, biological agents can encounter binding sites that limit their spread, but the convective properties of such molecules are likely adequate for CED. Other investigators have often reported significantly less spread of albumin than small molecule tracers (Neeves et al., 2006; Olbracht et al., 2010); however, all such studies have been based on image intensity-based thresholds, which may be misleading.

The Galbumin™ distribution was compared with that of IVIS dye, a molecule comparable in size to a small chemotherapeutic, in one brain slice from the animal. The comparison of the
real-time MR image of Galbumin™ and the post mortem image of the IVIS dye is shown in Fig. 7. The figure shows that the distributions are sufficiently similar to indicate that Galbumin™ is not retarded in its motion through the parenchyma relative to the motion of a small molecule. The spread of dye along the catheter track superior and frontal to the ventricle is leakback along the track upon withdrawal, which is ubiquitous, as noted in Brady et al. (2013), and has nothing to do with the quality of the infusion. This also indicates that both channels of the device functioned well, since the MR and the IVIS tracer were infused simultaneously through separate channels at the same infusion rate. There is also no interference evident between the two infusions, but rather from the overlap between the dye and the MR tracer, the two infusates are essentially miscible.

The volumes of distribution of the protein-size tracer (Galbumin™) and the virus-size tracer (CellTrack™) both indicated good advection of the tracers with the infused fluid. The distributions were consistent with the expected distributions of infusates consisting of small molecules or labeled water molecules. The cytoarchitectures (white matter and thalamus) did not affect the distributions. In fact the white matter which expands easily should have worked to reduce the distribution volume, which is the same tendency that large particles are expected to display. No such effects were noticeable, and all the particles that were tracked displayed good convective properties.

Fig. 8 shows results of some high flow rate infusions, into the thalamus (left image) and into the internal capsule white matter (right image). The volumes infused were quite large (as noted in the figure) and so the infusate has started to cover over the bullet nose, but in no case is there any reflux up the catheter shaft indicating large backflow. The infusion cloud has covered any resultant backflow so that the latter cannot be observed, and the quoted figures of backflow are more indicative of large volume of infusion and distribution than of backflow.

Finally, Fig. 9 shows representative plots of the line pressure measured near the proximal end of the catheter. The abscissa gives the flow rate per channel; the total infusion rate was twice the abscissa value. Two different designs of the channel cross section were used in these measurements. The measured pressure per unit infusion rate is about 0.8 psi/μL/min (≈40 mm Hg/μL/min). This is much higher than the pressures required for conventional endpoint catheters e.g. (Brady et al., 2013), which is attributed principally to the very narrow fused silica lines and the micron-sized channels of the catheter tip. Calculations for laminar flow through tubes with an inner diameter of the fused silica lines and rectangular channels with dimensions of the microcatheter tip are in good agreement with the observed pressures.
5. Conclusions and future work

In vivo infusions into large mammal (porcine) brain were carried out for the first time with a microfabricated catheter meant for intraparenchymal delivery of therapeutics. The catheter has a rectangular cross section at the tip, a step in diameter 3 mm proximal from the tip, and a novel “bullet-nose” transition commencing a further 4 mm proximal from the step. The catheter has two independent fluidic channels with lumens substantially narrower than those in existing intraparenchymal catheters. There were no instances of catheter or catheter tip breakage during insertion, infusion, or removal. Backflow did not extend beyond the bullet nose junction with the catheter shaft except in one out of ten cases, even at flow rates of 20 μL/min. The ability to infuse at such high flow rates without backflow is unprecedented for catheter infusions in mammalian brain. The cytoarchitectural regions chosen were the thalamus, the white matter of the internal capsule, and the putamen. There was no discernible pattern to the onset of backflow with cytoarchitecture, in part because there was little discernible backflow in any case. In these studies, we did not directly compare the backflow obtained with those of other catheter designs. Generally, lower infusion rates are used in other studies, although there are some anecdotal reports of flow rates in the range of the higher flow rates reported here. Direct comparisons with a state of the art catheter will be reported in the near future.

Distribution studies were conducted for particles spanning a wide range of size, the largest about 30 nm (CellTrackTM), an intermediate about 7 nm (GalbuminTM), and the smallest about 3 nm in diameter, and for dyes comparable in size to typical chemotherapeutic molecules (OmniscanTM and IVIS dye). There was no discernible decrease in the ratio of distribution to infusion volume with increased particle size in this range. All particles were convected readily through the interstitial spaces. Further, the two channels successfully infused two very different sized molecules (GalbuminTM and IVIS dye), which distributed equally as far as could be ascertained in a comparison between fluorescent stain and MR contrast. The transmittance of the MR contrast agent (albumin conjugated with Gadolinium – GalbuminTM) was complete in that the measured dose kept pace with infused dose as measured by the input concentration of reagent and volume infused. The same was true of the largest agent used (gadolinium oxide nanoparticle – CellTrackTM). Thus despite the small size of the channels the transmission of virus-sized particles was not hindered.

The mechanical strength of the microfabricated tip has been tested and found sufficient for most applicable conditions, such as deep tissue insertion, worst-case lateral shifts in bovine and porcine tissue, compressive break strength, and high pressure testing. The catheter design is also being optimized for chronic infusions, which will require a flexible body and anchoring system suitable for long term implantation. In addition, preliminary tests indicate that the device is capable of aspirating fluids from the brain, which has utility for procedures involving biopsies, drainage, or flushing. The high flow rates that were achieved without significant backflow offer the potential for infusing larger volumes in shorter times, which is likely to enhance the efficacy of CED in humans. Microfabricated catheters offer the potential to improve efficiency of target engagement, with precise, predictable and reliable delivery to focal brain regions. They can be optimized for a multitude of specific applications and requirements, such as multiple outlets, sensors, sizes, and profiles. This flexibility may encourage the development of novel therapeutic strategies and improve current CED treatments for a variety of neurological diseases.

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