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Additive manufacturing of hydrogel-based materials for next-generation implantable medical devices

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Abstract

Implantable microdevices often have static components rather than moving parts, and exhibit limited biocompatibility. This paper demonstrates a fast manufacturing method which can produce features in biocompatible materials down to tens of microns in scale, with intricate and composite patterns in each layer. By exploiting unique mechanical properties of hydrogels, we developed a “locking mechanism” for precise actuation and movement of freely moving parts, which can provide functions such as valves, manifolds, rotors, pumps, and delivery of payloads. Hydrogel components could be tuned within a wide range of mechanical and diffusive properties, and can be controlled after implantation without a sustained power supply. In a mouse model of osteosarcoma, triggering of release of doxorubicin from the device over ten days showed high treatment efficacy and low toxicity, at one-tenth of a standard systemic chemotherapy dose. Overall, this platform, called “iMEMS”, enables development of biocompatible implantable microdevices with a wide range of intricate moving components that can be wirelessly controlled on demand, in a manner that solves issues of device powering and biocompatibility.

Current additive manufacturing methods are enabling rapid production of static three-dimensional structures (1). However, they exhibit some limitations which restrict their utility. First, they cannot easily generate structures with freely moving parts. Moving components, such as valves and pumps, require control in fabrication in the z-axis, followed by the alignment and integration of unattached components. Second, current methods have limitations in constructing entirely biocompatible medical devices. Microelectromechanical (MEMS) devices (2–5) feature moving parts manufactured with methods developed for silicon and metals (such as thin-film deposition, photolithography, and etching (6)), but silicon-based materials present challenges as implantable devices due to their low

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biocompatibility (7–9). This limitation extends to implantable electronic devices that use silicon-based transistor circuits, which further require a reliable power supply via either a toxic battery or electronic interconnects between the interior and exterior of the body.

We sought to demonstrate that additive manufacturing can be developed to build biomaterial-based devices with moving parts that can be wirelessly and precisely controlled. Although there are various wireless modes of activation of implantable MEMS-based devices (such as electromagnetic methods, body-energy harvesting techniques, and ultrasonic powering), most reported methods involve the incorporation of electronic components that are non-biocompatible (10–12). We chose PEG-based hydrogels as building materials because they exhibit high biocompatibility and non-fouling properties, are flexible and allow conformal contact to surrounding tissues, and some have demonstrated safety in humans including approval by the Food and Drug Administration (FDA) (13–15). These properties of hydrogels could impart new functionalities compared to other materials used in soft robotics, such as poly(dimethylsiloxane)(16–18). For example, in addition to their biocompatibility, some PEG-based hydrogels are biodegradable or can be synthetically modified to be biodegradable. PEG-based hydrogels can also be chemically modified or grafted with proteins, forming bioactive sites(19–21). Previously, photolithography and micromolding (9) have been used to form micropatterns of hydrogels, but the final structures tend to be of a single layer and single material, typically to be used as scaffolds, (22–24) or devoid of moving parts(25–28). It has yet to be demonstrated that actively moving parts, inside multiple aligned and spatially complex 3D components, can be fabricated solely using hydrogels and function *in vivo*. In addition to drug delivery, such techniques for processing biomaterials could, in the future, serve as an enabling platform for developing mechanically active hydrogel-based micromachines and soft robots which can interact with living systems (29, 30).

RESULTS

Fabrication strategy

We developed a simple yet flexible and rapid setup for assembling layers of polymerized hydrogels (Fig. 1a; Supplementary Fig. 1). Layers could contain different biomaterial compositions and device components, including ones that can be controlled with an external magnet (Fig. 1b; Supplementary Fig. 2). Preformed hydrogel components that were fabricated separately using the same setup, including those that are doped with iron-oxide nanoparticles, can also be easily aligned and incorporated to form a final integrated device. Magnetic actuation allows for wireless control and has been used clinically as a modality for controlling implantable medical devices (31). We devised this set of fabrication strategies for building implantable micromachines or “iMEMS” (implantable MEMS).

Specifically, our micromachining procedure consists of three steps: controlled movement in the z-axis to determine thickness of a layer, microfabrication of individual hydrogel layers, and selective release and bonding of adjacent layers. We used poly(ethylene glycol) diacrylate (PEGDA) to form PEG-based hydrogels, which are widely used, non-toxic, and non-fouling, some of which are FDA-approved (32) (specific compositions shown in Supplementary Table 1). For controlled z-axis movement, we used a PDMS chamber with a

thin flexible membrane coupled with a micrometer screw gauge via vacuum ports (Fig. 1a; Supplementary Fig. 1). This setup allowed us to create complex composite structures within one layer of the hydrogel, as well as control the thickness of each layer throughout the fabrication process. To fabricate devices with multiple layers, we: 1) polymerized and released a layer of hydrogel from PFOTS (trichloro perfluorooctyl silane)-treated glass (Fig 1a, step 1); 2) build “upwards” layers of hydrogel as support structures (Fig 1a, step 2), 3) aligned prefabricated iron-oxide doped hydrogel structures (eg. gears) onto the support structures (Fig 1a, step 3(i)), layered the polymerized hydrogel layer from step 1 ensuring proper alignment with the support structures (Fig 1a, step 3(ii)), and 4) photopolymerized it to seal the device and released it from untreated glass (Fig. 1a, step 4). We built a construct consisting of 15 aligned 50- μm thick layers of PEGDA (8 of which contained fluorescently-doped disks arranged to form a spiral pattern) (Fig. 1c). We also built structures from more complex designs, such as a Geneva drive (Fig. 1d), which is an intricate mechanism used in the mechanical watchmaking industry to implement precise intermittent movement. This complex structure was built within 30 minutes (Supplementary Table 2).

Design, fabrication, and actuation of versatile structures

We demonstrate that our fabrication strategy can be used to generate components and structures typically found in mechanical systems, such as a simple gate valve component (Fig. 2a), a gated linear manifold device (Fig. 2b), a spinning toothed rotor (Fig. 2c) and a spinning gear with multiple reservoirs (Fig. 2d). These structures represent typical components that are used in mechanical movement systems, including both linear (Fig. 2a and Fig. 2b) and rotational movement (Fig. 2c and Fig. 2d). For linear movement, placement of a magnet at one end generates movement of the iron-doped component; this mechanism is simple and works *in vivo* for a simple gate valve (Fig. 2a, right panel and Supplementary Fig. 4). This mechanism can also be applied in a manifold which contains multiple reservoirs for releasing payloads (Fig. 2b, Supplementary Movie 1). However, linear movement also requires dead spaces (i.e. spaces into where the iron-doped components move) which add to a larger footprint of the device.

Alternatively, our fabrication platform can also produce robust rotational movements, where the iron-doped component traverses through a cyclical path. In one design, a spinning toothed rotor (Fig. 2c, left) was fabricated in which the speed of rotation of the rotor can be controlled reliably (from 4 to 18 rpm) by changing the voltage supplied to the motor of an external actuator (Fig. 2c, right). Mimicking a simple pump, we observed significantly higher velocities of beads in the solution around the gear when we increased the rotation frequency of the rotor (Supplementary Fig. 5). Moreover, this spinning toothed rotor can also be reliably actuated when implanted inside a euthanized mouse (Supplementary Fig. 6). In another design of rotational movement, payloads can move in concert with the iron-doped component (Fig. 2d). These designs feature minimal dead spaces and decreased footprint of the device. We also developed two different methods for including superparamagnetic iron-oxide nanoparticles into components (Fig. 2e). Bulk doping yields components that contain uniform but only moderate concentrations of nanoparticles; mixing iron nanoparticles uniformly in prepolymer increases its opacity and hence decreases its ability to be photopolymerized, hence limiting the amount of nanoparticles that can be incorporated

(~0.6% v/v). Depot doping (Fig. 2f), on the other hand, results in densely packed reservoirs of iron oxide nanoparticles (~25% v/v) which render components highly responsive to magnetism.

Leveraging hydrogel properties for versatile actuation

To support a versatile set of static and moving components to be used in complex devices, we investigated a range of PEG-based materials that can be used in this fabrication method (Supplementary Table 1). PEG-based materials have easily tunable mechanical and diffusive properties (Fig. 3a) (14, 33–35). By varying the length of the PEG chain as well as the concentration (w/v%) of PEG used (Fig. 3b), the Young's moduli range from the order of 10kPa (soft; similar to soft tissues) to 10MPa (stiff, similar to rubber) with sustained mechanical strength with repeated testing over a 2-week period. In the Geneva drive design, we used a stiff PEG gel as a structural material for the exterior, support structures and moving gears. We exploited the range of mechanical strength in another manner, by devising a robust “locking” mechanism. This design features PEG components of complementary shapes but of differing mechanical strengths. As an initial example, a soft star-shaped axle holds a stiff gear in place, but actuation generates movement of the gear, which deforms the axle. Figure 3c and Supplementary Movie 2 show the operation of a star-shaped axle constructed out of softer PEG gels and a stiff PEG gear with a star-shaped bore.

Similarly, we employed a “locking mechanism” for the Geneva drive device by incorporating soft PEGDA posts which act as bendable posts (Fig. 3d); the posts fit between the spokes of the driven gear to keep it aligned in the absence of actuation, but deform and bend to allow for rotation of the gear from external force. From beam-bending equations, about 0.0039N would be needed to bend all four posts (Fig. 3d), much less than the calculated magnetic force acting on the driving gear of 0.73 N (COMSOL simulations shown in Fig. 3e). In this manner, we were able to achieve precise actuation and movement of the Geneva drive (Fig. 3f), which consists of two engaged gears: a driving gear that is doped with iron oxide nanoparticles, and a driven gear with 6 spokes. Each spoke of the Geneva drive contains a reservoir and an aperture that aligns with another aperture on the topmost layer of the assembled device with each 60° rotation. The smallest features in this design were 100 µm in the x-y plane. These feature sizes enabled us to fabricate small reservoirs holding 200 to 600 nL of fluids. After alignment of the gears we sealed the entire construct with a final top layer to form the final assembled device (Fig. 1d, inset). The assembled Geneva gear device consisted of 6 layers (base layer, support for driven gear, axles for both gears, soft PEG posts, and two top layers), was about 5 mm tall and 15 mm in its longest dimension, and took a total of 30 minutes to manufacture (see Supplementary Table 2 for breakdown of time). By comparison, current commercial 3D methods (with print rates of millimeters to centimeters per hour) for printing a 6-layer static polymeric structure would take several hours and would still be incompatible for use with soft hydrogel materials (36).

By rotating a magnet ~1 cm below the assembled device, one full rotation of the iron-doped driving gear results in a 60° rotation of the driven gear, and a corresponding alignment of each reservoir with the aperture on the top layer (Fig. 3f, Supplementary Movie 3). When

the driving gear was actuated with a permanent magnet, it engaged and moved a larger gear synchronously. This device was mechanically robust and could be actuated *in vitro* without mechanical failure for a period of at least a month with dozens of rotations per day.

We also investigated the permeability of the materials as a design consideration for the device. For example, the hydrogel that lines a drug reservoir must be impermeable to the drug, whereas a different composition that is permeable to the drug may be used for other components. We performed fluorescence recovery after photobleaching (FRAP) experiments to determine the diffusion coefficients of the hydrogels; these experiments confirmed the permeability of the hydrogel decreased with decreasing PEG chain length, as predicted (Fig. 3g; Supplementary Fig. 11, Supplementary Table 3). For example, the PEG400 hydrogels would be appropriate as the walls of reservoirs to contain the drugs as they were impermeable to small molecules such as FITC (389 Da); incubation with FITC solutions yielded no uptake after 2 weeks as evidence by fluorescence imaging (data not shown). Hence, a palette of mechanical strengths and diffusion coefficients could be obtained for the PEGDA components in the iMEMS device.

In vivo actuation and localized low doses of chemotherapy in an osteosarcoma model

To investigate the functionality of the device *in vivo*, we implanted the Geneva drive device filled with two dyes (FITC-dextran and AF680-dextran) into the subcutaneous space of nude mice (Fig. 4a). We incorporated fluorescent references into the device so that the movement of the gears can be tracked via *in vivo* imaging (Fig. 4b). Over two days, for every full rotation of the driving gear, the driven gear reliably rotated 60° inside the mice (Fig. 4c), as intended by the intricate gear mechanism. This device also successfully delivered two different types of payloads (Supplementary Fig. 12).

Next, we tested the effectiveness of an iMEMS device in a mouse model of osteosarcoma. Clinically, patients who have undergone surgery to remove bone tumors are administered follow-up treatments with chemotherapy such as doxorubicin (37–39). This drug is toxic when administered systemically at high concentrations (40). We hypothesized that a localized delivery of chemotherapy at low doses, supplemented by release of additional doses of drugs at any chosen time after implantation (e.g. in response to abnormal tumor growth and without further harming the patient), could be an efficacious form of treatment. Compared to constant release from simple polymer-based systems, such a pulsatile release of cancer drugs could better mimic the current regimens of chemotherapy, which is typically given in cycles (with periods of days to weeks) in order to best balance the targeting of rapidly proliferating cells in tumors while providing extra time for host cells to recover (41, 42). An actively controlled system could also enable a clinician to adjust dosage dynamically to achieve this balance.

To this end, we used a single-gear design that was loaded with doxorubicin as its size is better suited for small-animal models. As a baseline, our device steadily releases doxorubicin at a low sub-lethal level (similar to most current drug-delivery devices), and magnetic actuation was performed to provide supplemental doses on demand after implantation. In an *in vitro* model, we found that total doxorubicin released could be controlled by changing the actuation frequency (Fig. 5a). We demonstrated control from

20% to 90% release over 10 days (e.g. 45% by actuating the device every 4 days, and 87% by actuating every 2 days); we anticipate that further changing actuation frequencies would expand this range of control even further. Moreover, in passive-release systems, the release profiles are determined during the fabrication of the microspheres or scaffolds; once implanted, the release profiles cannot be changed or personalized. In the iMEMS device, the release profile can be controlled after the device is implanted by adjusting the actuation frequency.

Next, we tested the efficacy of this device in an *in vivo* osteosarcoma model, in which nude mice injected with human osteosarcoma cells (43, 44) developed palpable tumors. We implanted the iMEMS device (carrying payloads of doxorubicin) subcutaneously, and tracked in real time the growth of tumors by bioluminescent imaging (Fig. 5b) and movement of gears by small-animal fluorescence imaging (Supplementary Fig. 23). We selected a local dose of doxorubicin at 10% of standard systemic dose by estimating the mass of tumor compared to body weight (37). We compared bioluminescent intensities (normalized to the intensity before treatment) for 4 different treatment groups: no treatment, high systemic dose (administered every 4 days), low systemic dose (administered every 2 days), and low local doses administered using the implanted iMEMS device (actuated every 2 days) (Supplementary Table 4). Compared to no treatment (45), treatment with high systemic doses resulted in a 44% inhibitory rate (comparable to literature values), whereas iMEMS local delivery resulted in a 60% inhibitory rate, the highest rate among all treatment groups (Fig. 5b). Moreover, we analyzed hematoxylin and eosin histological staining of the tumors at the distal end, middle, and proximal end of the femur, to analyze tumor necrosis (46, 47). Tumors from mice treated with high systemic doses showed necrosis of $39 \pm 7\%$, those with low systemic doses showed $19 \pm 6\%$, and those treated with iMEMS showed $56 \pm 15\%$ (Fig. 5c). A major side effect of doxorubicin is cardiotoxicity resulting from apoptosis of cardiomyocytes. We anticipated low side effects as iMEMS used only one-tenth the conventional high systemic dose. We analyzed basal, mid-ventricular, and apical sections of heart ventricles by TUNEL-staining (48), for at least 10 fields of view for each sample (Fig. 5d). Mice treated with high and low systemic doses had 0.23% and 0.099% apoptotic cardiomyocytes, respectively, whereas those treated with iMEMS showed 0.045% apoptotic cardiomyocytes, which was comparable to mice that did not receive any doxorubicin (0.046%). Overall, compared to a large dose of systemic chemotherapy, treatment of tumor-bearing mice with iMEMS-delivered local doses of doxorubicin resulted in the least tumor growth, greatest tumor necrosis, and lowest apoptosis of cardiomyocytes, and analysis of the excised device and surrounding tissue showed little to no inflammatory cells, no giant body cells (indicating the lack of chronic inflammation at the site of implantation), and presence of a thin ($>500\mu\text{m}$) fibrous capsule (indicating normal wound healing) (Supplementary Fig. 24).

DISCUSSION

The iMEMS method extends additive manufacturing to make a variety of moving parts (valves, manifolds, rotors, pumps) in biocompatible microdevices, which forms the basis of many machines with advanced functions such as delivery of payloads. The method is based on simple and repeatable steps, enabled by a stepper mechanism which allows for

fabrication, alignment, and bonding of multiple intricately-designed layers. Importantly, building on the potential of additive manufacturing for human health applications (49), the technology works with biomaterials well-tolerated in the human body. Current methods for 3D printing of hydrogels (50) are limited to spatial resolutions above 200 microns at print speeds no more than millimeters per second for simple geometries (51), and cannot easily assemble structures with separate individual components which may be needed for moving parts. The construction and demonstration of *in vivo* function of a hydrogel-based Geneva drive, which features an intricate design where a driving gear must correctly engage a driven gear, strongly suggests that hydrogel materials, while often soft and compliant, can be tuned such that they make suitable materials for constructing complex MEMS-like devices. This data, especially the successful demonstration of force transmission within such devices, support the notion of hydrogels as a versatile basis material for soft robotics, alongside poly(dimethylsiloxane) and other soft polymers (16, 52, 53). Previously, progress has also been made on producing polymeric microstructures that respond to stimuli (54, 55), but most focused on individual components rather than integrated devices. In future studies where biodegradation of the hydrogel devices is desired, biodegradable hydrogels (56, 57) could be used, with the biodegradability of the device being triggered by enzyme or heat.

The iMEMS strategy addresses several fundamental considerations in building biocompatible microdevices, micromachines, and microrobots: how to power small robotic devices (as batteries are toxic), how to make small biocompatible moveable components (as silicon has limited compatibility), and how to communicate wirelessly once implanted (as RF microelectronics require power, are relatively large and are not biocompatible) (58, 59). The entire iMEMS device consisted of nontoxic components and requires no toxic battery. Previous work on hydrogel drug-release focused on sustained release of one compound whose release profile must be determined prior to implantation (60–62) and often have more complicated (63, 64) and invasive mechanisms for triggering delivery (65), with some concepts not having yet shown *in vivo* implementation (63, 65). The iMEMS device can be triggered to release additional payloads over days to weeks after implantation (62). We achieved precise actuation by using magnetic forces to induce gear movements, which in turn bend structural beams made of hydrogels with highly tunable properties. Magnetic iron particles are commonly used and FDA-approved for human use as contrast agents (66, 67). The ability to manufacture spatially complex biocompatible components, including freely moving parts, which can respond magnetically, enables continuous control and powering of the implanted microdevices without the need for on-board electronics or battery. In the future, such microscale components can be used for MEMS, larger devices (ranging from drug delivery to cardiac pacemakers) (6), and soft robotics (29, 30).

While passive-release systems would still be useful for many disease scenarios, in cases of extensive solid tumors which fail to respond to low-dose chemotherapy and in the broader paradigm of personalized medicine where treatments are individualized according to the patient's dynamic health status, it would be critical to be able to tailor the therapy in an "on-demand" fashion, such that the therapy for each individual can be adjusted in real time depending on growth of tumor and condition of patient. This study takes a step towards that vision. For controlling cancer growth, a surgeon could insert this delivery device after surgical resection (i.e. near the margin where cancer frequently recurs), and thereafter in

outpatient settings, the physician or even patient could adjust the start of treatment and dosing schedule painlessly, after implantation of the device, depending on the growth of the tumor as revealed by real-time imaging. Should there be large tumors or multiple tumor sites, more than one device can be implanted at the time of biopsy. The surgeon could remove the devices or leave the device at the surgical field as long as it causes no inflammation. By effectively delivering low doses of drugs at the site of a tumor, the efficacy of standard chemotherapy can be increased while circumventing detrimental side effects such as cardiotoxicity. Our envisioned approach with an iMEMS device could either negate or supplement systemic chemotherapy, as shown in other cancers (68)(69). In addition, our device would not require repeated injections or physical ablations (as it could be implanted under the currently scheduled biopsy under image guidance before chemotherapy and surgical resection), which may cause further spreading of cancer cells to surrounding healthy tissues. Compared to constant-release systems, the iMEMS approach could also prove particularly useful in clinical applications where pulsatile release is necessary (for example, in osteoporosis, constant delivery of the hormone would result bone loss due to increased osteoclast activity (59)).

To achieve clinical impact, multiple improvements can be made. First, designs that allow for payloads to be stored in solid or lyophilized phases could offer advantages in stability and decreased background release over time by decreasing convective leakage. Second, to further decrease the amount of background release from the device, designs with minimal spaces between moving layers could be implemented. Third, robustness of the actuation mechanism could be further increased: while magnetic actuation of some implantable devices (such as vagus nerve stimulation) are in clinical use and normally robust (31), and our calculations confirm the forces needed to move the gears are not felt in every day conditions, future designs could increase the size or stiffness of support posts to enhance the locking mechanism. Moreover, a wearable device could sense the current spatial state of the device (via capacitive sensing of iron oxide nanoparticles or low-field electromagnetic sensing) and feedback to the actuator to ensure correct movement of device components.

Actuation of implanted components by magnetism could be particularly useful for interventions close to the skin surface, such as subcutaneous delivery (such as delivery of therapies for melanomas, wound healing and scar prevention) or control over other superficially implanted systems (such as cerebral shunt systems). In the future, the iMEMS fabrication strategy could incorporate small implantable components responsive to other stimuli (such as temperature, pH and light), including ultrasound which could penetrate deep into the body(70).

In summary, we have demonstrated a strategy to manufacture biomaterials-based micromachines composed of moving components. Through designs which feature a mixture of mechanical stiffnesses, we can wirelessly impart force transmission to components with mechanical stops, with end results of precise and robust movements. Additionally, these devices can be implanted safely into living host to effect environmental and physiological changes. These capabilities could find applications in drug delivery(59), in applications which require control of microenvironments around delivered cells or engineered tissues, and implantable medical devices (such as stents), and could bring the field a step closer in

developing soft miniaturized robots that can safely interact with humans and other living systems.

MATERIALS AND METHODS

Materials.

Polyethylene glycol diacrylate (PEGDA, M.W: 4kDa and 400Da), FITC- labeled dextran (150kDa), Fluoresbrite® YG and YO Carboxylate Microspheres (dia: 0.5µm), and ProMag™ Superparamagnetic Microspheres (dia: 3µm) were purchased from Polysciences (Warrington, PA). Polyethylene glycol diacrylate (PEGDA, MW: 10kDa and 20kDa) was purchased from Laysan Bio, Inc. (Arab, AL). 2-methyl-1-(4-(hydroxyethoxy)phenyl)-2-methyl-1-propanone (Irgacure 2959 or I2959) was obtained from Ciba (Tarrytown, NY). Darocur 1173 was obtained from Octochem, Inc. (Vandalia, IL). 3-(Trimethoxysilyl)propyl methacrylate (TMSM), Trichloro(1H, 1H, 2H, 2H - Perfluorooctyl)Silane (PFOTS), and 1-vinyl-2-pyrrolidone (NVP) were purchased from Sigma (St. Louis, MO). OmniCure® S2000 UV curing system and adjustable collimating adaptor were purchased from EXFO (Canada). NdFeB disc magnets (dia: 9.5mm; H: 3mm) were purchased from ForceField (Fort Collins, CO).

Preparation of hydrogel prepolymer.

All hydrogel prepolymer used in this study consist of 10–90w/v% PEGDA (400, 4k, 10kDa or 20kDa), 1w/v% photoinitiator (I2959 or Darocur 1173), and 0.2v/v% NVP in PBS. The specific compositions are listed in Table S1. They were stored at 4°C and shielded from light until use.

Fabrication of hydrogel structure.

To perform microfluidics-based lithography and have precise control over the thickness of the microstructures, we used a PDMS fluidic device (19 × 19 × 1.3 mm) featuring a flexible bottom membrane (~250µm thick) together with a custom-made z-axis stage that changes the height of the center chamber in precise micrometer movement (left, in Supplementary Fig. 1). The fluidic device was secured onto the stage and the center chuck by vacuum. As the center chuck moved up and down, the bottom membrane flexed upward and downward accordingly, resulting in changes of height in the center chamber (Supplementary Fig. 1, right). Achievable heights ranged from 0 to 1.5mm ± 2.5µm. We bonded a piece of 1×1cm cover glass (#2, ~190–230µm thick) onto the bottom of the center chamber to ensure the bottom remains flat at all times.

Surface treatment of glass substrate.

All cover glasses were cleaned, bake dried, and treated in oxygen plasma for 1 minute prior to the application of silane. To promote hydrogel adhesion, cover glasses were immersed in 10w/v% TMSM (in 70% ethanol) for an hour. The cover glasses were rinsed three times in 100% ethanol and baked at 120°C for at least 10mins. To prevent hydrogel adhesion, cover glasses were treated with PFOTS vapor in a desiccator for 15mins. All treated cover glasses were stored in desiccator before use. Alternatively, a thin layer of PDMS (<100µm) was spun onto the cover glass to provide a non-adherent surface to the hydrogel.

Fabrication of hydrogel MEMS devices.

The schematic of the fabrication steps are shown in Figure 1a. Prior to fabrication, a clean PDMS fluidic device was placed on top of the fabrication platform (channel side faced up, as shown in Supplementary Fig. 1) where it was secured onto the stage via vacuum. A 50 × 35mm, #2 cover glass was placed on top of the PDMS fluidic device to form a closed channel. The micrometer was brought upwards until the small cover glass (10 × 10 mm) attached to the bottom of the fluidic chamber (hereafter known as the bottom substrate) was in complete contact with the top cover glass. The respective z-position was used as a reference zero point. This step was done whenever a new PDMS fluidic device was used to account for any thickness variations. At this point, the bottom substrate was lowered and the hydrogel prepolymer was drawn into the PDMS fluidic device using a syringe at the outlet. Then, the bottom substrate was raised until the fluidic chamber reached the desired height. A mask was placed directly on top of the top cover glass and the hydrogel was exposed using a collimated UV light source (Omnicure S2000, EXFO) for 10–20s. After this, excess prepolymer was removed using a pipette or wicked away with filter paper and a new hydrogel prepolymer was introduced for the subsequent cycle. The bottom substrate was adjusted to a new z-position. A new mask was aligned with respect to the hydrogel microstructures formed in the previous cycle and a new cycle of photopolymerization began. After the supporting structures were formed (which remained attached to the bottom substrate), the top cover glass was removed to allow the transfer of free-standing, prefabricated components (e.g. microgear) onto the supporting structures (Figure 1a, step 3). Once the free-standing component was successfully transferred onto the supporting structures, the PDMS fluidic device was closed by a new cover glass to which a piece of preformed hydrogel attached (Figure 1a, step 4). As the bottom substrate was being adjusted to the new z-position, an external magnet was used to guide the free-standing component to the desired position. Finally, a new mask was used to photopolymerize selected regions on the hydrogel device which joined the top hydrogel piece to the underlying supporting features. We removed uncrosslinked precursors within voids to prevent swelling of hydrogel structures (especially for long-chain PEGs which could swell about 10%) either by diffusion in the presence of a vacuum (for hydrogel devices with apertures that open to the surrounding medium) or by using fine-gauge needles to replace uncrosslinked materials with buffer such that the puncture sites self-closed (in cases where the device was sealed from the surroundings). All fabricated devices are left to osmotically stabilize in saline or water prior to introduction of payload and use *in vitro* or *in vivo*.

All other methods (material characterizations, design and fabrication of specific designs, *in vitro* and *in vivo* actuation and delivery of payloads) are included in Supplementary Materials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. 3D fabrication of implantable micromachines.

(A) Multilayer hydrogel fabrication. Photolithography and precise z-axis control via a stepper enables fabrication of hydrogel structures in the micron-scale. (B) Noninvasive, wireless actuation of implantable MEMS device. Upon subcutaneous implantation, the device can be noninvasively and wirelessly actuated using an external motorized rotating magnet. (C) Precision and resolution of microfabricated PEGDA structures. Inset shows a series of PEGDA discs fabricated in a spiral pattern, demonstrating the resolution of our technique. (D) Implantable hydrogel MEMS devices. Panel shows schematic diagram of a MEMS device made entirely of biocompatible PEGDA (a Geneva drive mechanism) and an image of the fabricated device. The device contains iron nanoparticle-doped components

which respond to magnetic actuation. Actuation of device triggers release of payloads from reservoirs.

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Figure 2. Fabrication and actuation of versatile designs of components for hydrogel-based micromachines.

(A) Gate valve. Picture of component (left) and schematic diagram and corresponding *in vivo* imaging of operation (right). This design consists of an iron nanoparticle (NP)-doped T-shaped plug, and a reservoir with a hypothetical drug loaded in hydrogel; upon magnetic actuation, the T-shaped plug moves into the recess, allowing the drug to diffuse out of the construct via the outlet. For *in vivo* visualization, the T-shaped plug was doped with green fluorescent beads and the drug-loaded hydrogel was doped with red fluorescent beads. The gate-valve component was implanted subcutaneously in a euthanized nude mouse, and imaged by a small-animal fluorescence imaging system within an hour after implantation. (B) Gated linear manifold. Picture of component (left), and schematic diagrams and corresponding pictures showing operation. The manifold features six reservoirs; with each

magnetic actuation, the plug moves in step-wise fashion to the right and exposes a new pathway for diffusion of a hypothetical drug out of the device. **(C)** Spinning toothed rotor. The design features rotational movement of a multi-layer device constructed entirely out of PEG hydrogel, with a freely spinning toothed rotor in the center of a closed-loop microchannel. The hydrogel-based microgear was doped with iron-oxide NPs. Graph (right) shows that the speed of rotation of the gear (revolutions per minute, RPM) can be controlled by changing the voltage supplied to the actuator (see Supplementary Fig. 2 and 6). Experimentally observed rotational speeds were based on $n=4$ (multiple trials on the same device). Rotation of gear generated fluid flow within the surrounding microchannels (Supplementary Fig. 5), performing the function of a simple mixer and agitator for surrounding fluids. **(D)** Multi-reservoir single gear. Picture (left) and schematic diagram (right) showing the operation. This rotational-movement device contains a single gear, on which each of the six spokes contains a drug-loaded reservoir. Here, one reservoir is loaded with iron-oxide NPs, and five reservoirs loaded with different dye-infused gels. Each drug-containing reservoir has a small aperture which aligns to a larger aperture on the topmost layer of the device upon actuation. The iron-doped component and the reservoirs move in concert with each other during actuation and release, hence reducing the footprint of the device. **(E)** Two doping methods for incorporating iron oxide NPs into moving components. Bulk doping (top) works by polymerizing a suspension of iron oxide NPs in PEGDA prepolymer; this method features components with uniformly distributed iron-oxide NPs. Depot doping (bottom) works by creating a well in PEG structures, followed by packing dense iron-oxide NPs into the well as aided by a magnet, and sealing of well by aligning and UV polymerizing a preformed PEG hydrogel layer over the well.

Figure 3. Material characterization and device performance *in vitro*.

(A) Tuning of and selection of material properties of PEGDA. Mechanical and diffusive properties of PEGDA hydrogels can be tuned by simply changing the molecular weight of PEGDA used (B) Young's modulus of PEGDA hydrogels. A large range of mechanical stiffnesses can be obtained by changing the PEGDA composition. These hydrogels are also mechanically stable over 2 weeks as there is no appreciable decrease in Young's modulus over time. (C) Use of stiffness properties to achieve precise actuation. (i) A star-shaped axle (outlined) constructed from softer gel is fitted with a gear constructed from stiffer gel of a complementary shape. (ii) In the absence of magnetic actuation, the gear does not move and the star-shaped axle holds it in place. (iii) When the gear is magnetically actuated it rotates and compresses the softer star-shaped axle. This deformation allows for further rotation of the device. (iv) When the gear has reached the desired position and magnetic actuation is removed, the star-shaped axle returns to its original shape and holds the gear in place. (D) Leveraging the stiffness properties to achieve precise actuation in the Geneva drive device. The Geneva drive device is composed of two different types of hydrogels; a stiffer gel (PEG400) that is used for exterior of the device and gears, and a softer gel (PEG10k) that is

used for posts that hold the driven gear in place during the absence of magnetic actuation. Schematic diagram shows an individual spoke/reservoir of the driven gear and its interaction with a neighboring soft post when viewed from top (top) and cross-section (bottom). The posts hold the gear in place, but bend during actuation to allow for passage of the gear. The force required to bend the softer posts was calculated using simple beam-bending equations. **(E)** COMSOL magnetic field simulation of the magnetic force acting on the driving gear. The geometries and material properties of neodymium magnets (N52), and driving gear with iron oxide depot were used to set-up the simulation. A calculated force of 0.7342N was obtained via this simulation. **(F)** Operation of Geneva drive device. With each full rotation of the driving gear, the pin on the driving gear engages with one of the slots on the driven gear. In this manner, the driven gear, which contains reservoirs for payloads is rotated by one position (60°), thereby aligning the next reservoir with an aperture that is located on the top of the device. Time-lapse images show the operation of the device through all 6 “states” of the device. **(G)** Table of diffusion coefficients as obtained via FRAP experiments. The permeability of the hydrogels can be tuned to achieve a wide range of diffusion coefficients.

Figure 4. In vivo movement of implantable MEMS device.

(A) Schematic diagram of Geneva drive device with payloads before implantation. Geneva drive devices are fabricated with fluorescent references (red and green fluorescent beads) to aid *in vivo* imaging to track the movement of the gears within the device. The reservoirs are filled with AF680-dextran and FITC-dextran solutions, similar to previous *in vitro* experiments (not shown). (B) In vivo imaging of mice implanted with Geneva drive device. Image shows the implanted device in the dorsum of the mouse. Fluorescent references are visible post-implantation. The device is in the “off” position. Inset shows the device as imaged by the Maestro Cri Imager prior to implantation. (C) Operation of Geneva drive device in an *in vivo* environment. Fluorescent images show the position of the references within the device and hence the state of the Geneva drive device after each actuation.

Figure 5. iMEMS as a means for localized, low dose chemotherapy for osteosarcoma.

(A) In vitro release of doxorubicin from single-gear iMEMS device. Schematic diagram is shown in the inset. The device delivered 5 doses of doxorubicin, with each dose containing ~10% of a single conventional doxorubicin dose that would be administered systemically. We compared the release of doxorubicin from devices that were not actuated (n=3), actuated once every four days (q4d, n=3), and actuated once every other day (q2d, n=3). The error bars show the standard deviation for each averaged data point. (B) Change in tumor bioluminescent signal over time in a mouse osteosarcoma model treated with doxorubicin. Nude mice injected with luc-2 transfected osteosarcoma cells developed tumors that produced a bioluminescent signal in the presence of luciferin (inset). Doxorubicin-loaded devices were implanted close to the tumor site without interfering with the bioluminescent signal, and the growth of the tumor tracked over time via bioluminescence imaging (n=3). Tumor bioluminescent signals were tracked over 10 days for all treatment groups (n=3 for each group). The graph shows the ratio of the average bioluminescent signal (photons/cm²/s) at days 0 and 10 to the signal at the start of treatment. Mice treated with device showed the smallest increase in bioluminescent signal with a p-value of 0.035. A one-tailed t-test was used for statistical comparison. Error bars show the standard deviation for each averaged

data point. A single mouse (n=1) was also implanted with a doxorubicin-loaded single gear device but was not magnetically actuated (■) (C) Histological assessment of tumor necrosis after treatment. Bar chart shows the percentage necrotic area of the tumors for all treatment groups (n=3 for each treatment group, 3 slices for each sample). Mice that were treated with local device dosing showed the highest percentage tumor necrosis compared to all different treatment groups. (D) Histological assessment of cardiotoxicity after treatment. Bar chart shows the percentage apoptosis of cardiomyocytes in mouse hearts following treatment with doxorubicin (n=3 for each treatment group). Inset shows examples of apoptotic cardiomyocytes as detected via TUNEL staining. 10 sections per heart (at 40x magnification) were analyzed for apoptotic cells and the percentage apoptotic to healthy cardiomyocytes were calculated. Mice that were treated with local device dosing showed lower levels of cardiotoxicity compared to other treatment groups (*p<0.05, one-tailed t-test) and the percentage apoptosis of cardiomyocytes in this group were similar to that of mice that were not treated with doxorubicin.