Robotics and Automation in Cardiovascular-Inspired Platforms for Bioengineering

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Haley Caitlin Fuller, Ph.D. University of Pittsburgh, 2022

Targeted drug delivery systems are an emerging technological focus for precision medicine. Ideal drug delivery systems would employ strategies to enhance treatment efficacy and safety by localizing therapeutic release and minimizing toxic side effects. Towards this end, micro-scale robotics, or microrobots, have been under development as untethered tools to noninvasively navigate fluidic environments and perform medical tasks. However, continued microrobot development is stunted by the lack of a standardized empirical model of physiological relevance in which microrobot functionality for targeted drug delivery can be benchmarked.

To demonstrate targeted drug delivery potential, a standardized, *in vitro* tool to model essential *in vivo* functionalities of microrobots, such as locomotion, actuation, and on-target drug release, is needed. On-a-chip technologies have gained traction as modular, fluidics-based physiological systems for bioengineering. Capitalizing on the compatibility of on-a-chip systems for microrobot development and testing, a preclinical on-a-chip platform for testing novel drug delivery microrobots and indicating translational potential at an early stage could be realized.

Here, a biomimetic, on-a-chip platform for standardized testing of biomedical microrobot functionality is described. This platform features a cardiovascular-inspired arena integrated with an open-source control system for magnetic microrobot actuation. The semi-automated control system uses common laboratory hardware to magnetically guide a swimming microrobot to a target location within the on-a-chip arena using user-defined commands that are informed by real-

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time visualization. This platform models a promising control scheme for *in vivo* deployment to demonstrate robust locomotion and actuation of a swimming microrobot. The on-a-chip platform was then enhanced as a biologically active, on-a-chip (biochip) platform and employed as a model system for microrobot-mediated targeted drug delivery. The spatially discretized delivery of antibiotics by a magnetic microrobot in a minimal disease model on-a-chip is presented as a tool to benchmark and ultimately fast-track technological advances in microrobotic drug delivery systems and automation in bioengineering.

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Preface

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1.0 Introduction

Precision medicine describes an approach to tailor treatment to an individual's lifestyle, environmental factors, and genomic similarity to other patients [1, 2]. While the concept of precision medicine has existed in clinical blood typing practices for the past century, the US Precision Medicine Initiative, envisioned in President Obama's 2015 State of the Union address, inspired a new era of precision therapy [3, 4]. Under this initiative, personalized medical practices for informed drug design are envisioned for patients with genomic similarities. Even with genomeoptimized drug design, however, potential therapeutics still face pharmacologically related issues such as efficacy and toxicity. Thus, the field of precision medicine has shifted focus from optimizing drug formulation and *de novo* synthesis to designing delivery systems that release drugs at the target site, in turn enhancing pharmaceutical effects and offsetting potential side effects [5, 6]. The application of targeted drug delivery systems could be revolutionary for high-dose and/or long-term therapies, such as treatment with antibiotics, where off-target toxicity and acquired resistance are widespread and perpetual issues [7-9]. While genome sequencing, analysis algorithms, and disease understanding is improving in throughput and accuracy [1], continued research focus on systems that can modulate drug pharmacokinetics are needed for precision treatment.

Robotics and their afforded automated behaviors have improved accessibility and efficiency across divergent disciplines of bioengineering. Liquid handling robotics alleviate bioprocessing workflow bottlenecks by automating routine laboratory tasks, in turn increasing laboratory throughput and minimizing error [10, 11]. In the hospital, robotic-assisted surgery, pioneered by the da Vinci® Surgical System, enables seven degrees of freedom and improved

surgical ergonomics compared to the four degrees a surgeon has access to during laparoscopic surgery without robotic assistance [12, 13], alleviating surgical fatigue and expanding the applications for minimally invasive procedures. Robotics are even being used to teach expert daVinci® surgical techniques not captured through traditional training methods [14]. While these examples describe the biomedical potential of macroscale robotics, the potential global applications of small-scale robotics are better highlighted in science fiction stories such as the nanobots in the 2021 James Bond film that traverse the bloodstream in search of a specific DNA marker to perform a biomedical task.

Microrobots are an emerging class of biomedical microrobotics with the potential to revolutionize minimally invasive, targeted drug delivery by providing access to hard-to-reach locations *in vivo* [15]. Consistent with microrobotics research jargon, "microrobot" is used to identify small-scale mobile agents with dimensions larger than 1 µm that give the operator access to cellular-level environments [16]. The sub-millimeter form factor of microrobots, while allowing non-invasive access to remote regions in the body, makes them too small to house traditional onboard electronic circuity [17]. This means that actuation must occur by controlled energy waves (e.g. magnetic fields [18, 19], acoustic waves [20], or light triggers [21, 22]) or preprogrammed responses exploiting the endogenous milieu (e.g. chemical engine [23] or conjugation to kinetic biological components [24]). Their size and need for specific control schemes add a layer of difficulty for envisioned automated behaviors not encountered in their macroscale counterparts.

Precise control of microrobotic actuation is critical as a loss of function *in vivo* could result in off-target drug dosing or physical blockage of critical blood supplies during translation from injection or administration site to the target site [15]. For noninvasive access to remote regions in the body, a microbiorobot would have to navigate the circulatory system following intravenous injection or be able to penetrate mucosal layers following oral administration. While research investments over the past decade have led to the development of cargo-towing flexible swimmers [25], cell-harnessing biohybrid machines [24], and maneuvering biofluids and tissue constructs in microfluidic channels [24-28], further evaluation of biomedical functionality needs to be tested in a representative model system before translation to clinical use will be possible [17, 29].

Animal models have been traditionally considered an experimental *in vivo* steppingstone in the drug development process, however, their correlation to and predictive power of human pathogenesis is poor [30-32]. An evaluation of 7,300 drugs seeking clinical approval between 2003-2011 suggests that ~89.6% of drug candidates entering the clinical approval process will be unsuccessful mostly due to inadequate efficacy considerations [33]. Furthermore, of the drugs that do make it to the clinical trial pipeline, over 90% of clinical trial fails are contributed to cardiovascular toxicity [34, 35]. These statistics reveal a substantial gap in the extrapolation of safety and efficacy animal trial data to human trial success and identify the need for an empirical model system for a more complete evaluation in preclinical stages, enhance the predictability of clinical trial success, and improve translational potential. Without the development of a predictive tool, the translational potential of novel drug delivery technologies will remain bottlenecked in developmental and preclinical stages.

Organ-on-a-chip technologies offer a biomimetic platform in which organ-level physiology and function can be reproduced, probed, and utilized to predict biological responses to novel therapeutics [36, 37]. These microfluidic environments provide a three-dimensional (3D) microenvironment that promotes native-like cellular behavior and functionality over a traditional culture dish [38]. Organ-on-a-chip technologies are enticing as experimental models because they can account for complex physiology, offer fine control over physical and biological variables, and

can predict biological responses to novel therapeutics [36, 37]. As a demonstration of their physiological relevance, organ-on-a-chip systems have been utilized as drug dosing and toxicity tools. In 2012, Huh, Ingber, and colleagues reproduced a cancer drug dosing regimen in a mechanically active lung-on-a-chip model to study off-target pulmonary toxicities [39]. This system was able to recapitulate features of pulmonary edema and offer mechanistic insight into drug action that was not captured by other *in vitro* environments, animal models, or human patients. Additionally, the ability of organ-on-a-chip technologies to incorporate primary or human-derived cells renders these devices more ethically viable and relatable than animal models of disease [40]. As a first step in achieving exact physiological replicas of disease, on-a-chip systems could be the key to unlocking a universal testbed for novel drug delivery systems.

This dissertation speaks to the current limitations of microrobots as drug delivery systems by offering a perspective and demonstration of the coalescence of robotics and automation with on-a-chip empirical models for precision medicine (Figure 1.1). The objective of this research is to develop a proof-of-concept model system of physiological relevance in which drug-laden microrobots can be tested for locomotion, actuation, and targeted drug release. These measures of microrobot functionality in a physiologically relevant, standardized testing tool will allow experimental data to be benchmarked against state-of-the-art developments in the field, will add predictive power early in the drug development cycle, and optimize translational potential. While this research speaks to proving grounds of microbiorobots with intentional biomedical tasks including actuation control and drug dissemination, this same methodology is envisioned to apply to all microrobots. For this reason, the term "microrobots" is used to describe biomedical, biomimetic microrobots in this research but is meant to be broadly applicable to all microrobot systems.



Figure 1.1. A proof-of-concept model system is envisioned for the testing and development of biomedical microrobots as targeted drug delivery systems.

1.1 Dissertation Contributions

Chapter 2 presents a detailed overview of organ-on-a-chip systems and bioinspired and/or biocompatible microrobots and offers a perspective on the future applications of organ-on-a-chip technologies as proving grounds for microbiorobots.

Chapter 3 presents a platform composed of a physiologically relevant, on-a-chip system integrated with open-source magnetic control and real-time visualization for standardized

magnetic microrobot actuation and biomedical function. This platform enables the characterization of robust locomotion and actuation of magnetic microrobots within a cardiovascular-inspired, ona-chip system, as well as the permeation of a representative biological barrier.

Chapter 4 reports the demonstration of a biologically active, on-a-chip (biochip) platform for microrobot-mediated, targeted drug delivery. Drug-laden magnetic microrobots can be actuated towards a target location and be assessed on treatment capacity and off-target release. Ontarget treatment capacity is the first step in realizing targeted drug delivery by microrobots.

Chapter 5 discusses the conclusion and outlook for cardiovascular-inspired, on-a-chip platforms for the continued development and testing of biomedical microrobots. By integrating robotics and automation with on-a-chip technologies, we anticipate that the work presented in this dissertation will have a positive impact by unlocking the design, test, and build potential for a novel targeted drug delivery technology.

2.0 Background

An evolving understanding of disease pathogenesis has compelled the development of new drug delivery approaches. Recently, bio-inspired microrobots have gained traction as drug delivery systems. By leveraging the microscale phenomena found in physiological systems, these microrobots can be designed with greater maneuverability, which enables more precise, controlled drug release. Their function could be further improved by testing their efficacy in physiologically relevant model systems as part of their development. In parallel with the emergence of microscale robots, organ-on-a-chip technologies have become important in drug discovery and physiological modeling. These systems reproduced organ-level functions in microfluidic devices, and can also incorporate specific biological, chemical, and physical aspects of a disease.

Organ-on-a-chip technologies combine microfluidics with tissue engineering to create robust disease models that mimic *in vivo* environments. These systems allow for fine control of experimental variables and real-time visualization of cellular responses [41]. As a result, they offer an opportunity for testing microrobots designed to deliver drugs (Figure 2.1). Many current drug delivery technologies are based on micro- and nanoscale particles that leach drugs into the local tissue environment [42-44]. These particles are injected into specific locations or into the body's blood vessels. In the case of the latter, particles can be designed to home to specific areas by chemically modifying the particle's surface [45, 46]. Drug-laden microrobots can improve upon this functionality by incorporating robotic control of navigation [47], remote control [25, 48-50], and specific location targeting [25, 51, 52], potentially offering a new direction for precision medicine. When loaded with drugs, they have the potential to form new treatments. However,

testing these robots in environments representing human physiology will be essential. Here, the utility of organs-on-a-chip is explored for this purpose.

In these systems, organ functions are partially reproduced in microfluidic devices. Cells cultured from a subset of the different tissues that make up an organ can be grown in close proximity (Figure 2.1B), in structures that mimic the body's endogenous morphology, which enables cell-cell communication, coordination, and, subsequently, the emergence of some organ functions. Researchers can deconstruct disease complexity by introducing environmental perturbations to these systems and observing their effect on disease pathogenesis. Organ-on-a-chip devices have previously been used to investigate blood clot formation in patient samples, and have been integrated into animal extracorporeal circuits to provide real-time monitoring of antithrombotic therapies [53]. As organ-on-a-chip devices are based in microfluidics, it is straightforward to couple these systems to other microscale-engineered technologies that have been developed in liquid. As a result, microrobots, often developed in aqueous solutions, can be easily deployed within organ-on-a-chip devices.

One microrobotics application is drug delivery (Figure 2.1C) which is particularly relevant to a class of microrobots known as microbiorobots (MBRs). These microrobots are partially composed of living or non-living biological components. In some cases, bio-inspired microrobots that do incorporate biological components can also be considered MBRs. In the past decade, MBRs have evolved from drug-laden, flexible, magnetic swimmers [25] to self-folding, multifunctional microrobots capable of untethered (and thus, noninvasive) manipulation for targeted drug delivery [54]. MBRs have uses in nanomedicine; for example, porous nanocapsules can respond to different physical conditions such as temperature increases [55] or the presence of microwaves [56, 57]. MBR motion has been actuated and controlled using technologies such as customized permanent magnets [18] and programmable motility algorithms [58], as well as strategies that exploit the helical translational action or magnetotactic effect of systems found in nature [52, 59]. These different actuation and control strategies are compatible with standard microfluidic and organ-on-a-chip device form factors. As a result, deploying and testing MBR-based drug delivery approaches in organ-on-a-chip devices is the next significant step in assessing the potential of MBRs as delivery systems.



Figure 2.1. Organ-on-a-chip systems as proving grounds for microbiorobots. A) Organ-on-a-chip technologies have evolved from microfluidics to be able to model physiological conditions and could be used as proving grounds for microrobots before clinical use. B) These systems are 3D models that recapitulate complex tissue anatomy and physiology. C) Drug-carrying microbiorobots could be deployed in these systems to develop precise, targeted therapeutics.

The rest of this chapter, which is published in Fuller *et al.* "The Future Application of Organ-on-a-Chip Technologies as Proving Grounds for MicroBioRobots," *Micromachines*, 2020 [60], highlights recent developments in microbiorobotics and organ-on-a-chip technologies and envisions their conjoined use as a necessity for future drug delivery development.

2.1 Organ-on-a-Chip Devices Mimic Physiological Systems

Organ-on-a-chip systems are engineered, biohybrid systems that dynamically integrate mechanical, chemical, and physical inputs to create functionally coupled tissue-like structures in microdevices. They allow fine control over experimental conditions, such as the biochemical and mechanical cues that serve to coordinate tissue development and maintenance. Of particular interest, organ-on-a-chip systems have been shown to provide the individual physical inputs delivered in standard cell and tissue culture yet are also sufficiently adaptable to recapitulate tissue and organ function. Ideally, organs-on-a-chip reproduce organ function so well that they can be considered as alternatives to engineered, three-dimensional *in vitro* tissue models or live animal models of disease. In some cases, live animals are insufficient for understanding human disease. For example, Seok *et al.* showed how mice are poor at mimicking human inflammatory diseases [30].

Organ-on-a-chip development is currently an especially active area of significant research investment. Even prior to this recent focus, researchers had already demonstrated *in vitro* tissue growth and development in systems partially similar to recent organ-on-a-chip systems. For example, semipermeable membranes that allow different types of cells to be cultured in close physical proximity have existed for decades. In 1997, Fillinger *et al.* reported the use of a 13 µm-

thick semipermeable membrane to investigate the collaborative effect of culturing endothelial cells (EC) and smooth muscle cells (SMC) together [61]. In this study, SMCs were cultured on one side of a semipermeable membrane and ECs were grown on the opposite side, which allowed for physical contact between SMCs and ECs. Alternatively, each cell type was cultured separately, but connected in a manner that allowed for culture solution exchange between the two cell types. Both types of coculture demonstrated increased SMC proliferation and density, indicators of cell growth and viability, in comparison to cultures of only SMCs. In the case where ECs and SMCs were growth on opposite sides of the membrane, SMCs exhibited spontaneous growth through the membrane's pores, allowing for contact with the EC layer, which is similar to the actual orientation of these cell types *in vivo*. A similar semipermeable membrane was later used in the important development of a lung-on-a-chip system [62].

The 2010 report of a lung-on-a-chip by the group of Donald Ingber was a seminal study and a primary driver of the past decade of organ-on-a-chip research activity. In this system, epithelial cells and endothelial cells were cultured on two sides of an elastomeric, semi-permeable membrane (Figure 2.2A). Epithelial cells formed an epithelium, a barrier between an organ and its environment (e.g., the lining of the lung or gastrointestinal tract), and endothelial cells formed an endothelium, the lining of blood vessels. Here, the researchers combined these tissues to mimic the alveolar-capillary interface found in the lung where blood is oxygenated. The two cell layers formed tubular lumens in device channels to mimic the flow of blood in the microvasculature and the air transport in the alveolus. Parallel to these channels were two vacuum chambers. When suction was applied to mimic respiratory mechanics, the epithelial-endothelium construct stretched. This mechanical coupling increased the tissue's response to toxic and inflammatory silica nanoparticles, and stimulated their uptake and transport into the microvasculature, similar to the effect observed in a whole mouse lung. In addition to showing how tissues could be coupled to recapitulate organ function, and how biomechanics can play a critical role, this study also highlighted how laboratory animal use could potentially be minimized by creating organ-on-a-chip devices as replacements.



Figure 2.2. Organ-on-a-chip technologies as engineered biohybrid machines. A lung-on-a-chip system was constructed based on i) integrated epithelium and endothelium and integrated respiratory mechanics to mimic ii) the alveolar-capillary interface [62]. B) The i) AngioChip is a porous, biodegradable scaffold based on a branched, fluid channel that mimics vascularized cardiac and hepatic tissue. This biohybrid system mechanically supported perfusion and promoted angiogenesis (i.e, new vessel growth). It also performed *in vivo after* ii) surgical implantation into rat femoral vessels [63]. Images reproduced with permission from Huh *et al. Science*, 2010 and Zhang *et al. Nature Materials*, 2016.

The integration of biological and mechanical components within organ-on-a-chip systems has allowed for the functional and mechanistic reproduction of physiological structures, including components of the cardiovascular, gastrointestinal, and hepatic systems. In 2015, Yasotharan et al. reported an artery-on-a-chip microfluidic platform to support automated and quantitative immunohistochemical analysis of olfactory artery segments explanted from mice [64]. To recreate the olfactory microenvironment, this research group integrated valves and regulators with a microfluidic device to generate physiological pressure conditions. In another format, Marsano et al. created a beating heart-on-a-chip using micro-engineered cardiac tissues from both murine- and human-derived sources. This system incorporated uniaxial, cyclic strain, representative of the mechanical environment of the myocardium [65]. Rhythmic stretch and contraction were also utilized by Lee *et al.* to direct flow through a 3D gastric organoid in order to model gastrointestinal functions in a stomach-on-a-chip [66]. In a vascularized human liver acinus microphysiological system (vLAMPS), Li et al. characterized oxygen zonation and uninterrupted delivery of circulatory support in a microphysiological system by leveraging the hydrophobicity of glass in their device [67]. These various physiological structures provide real-time optical monitoring of complex physiology, demonstrate superior functionality with respect to other *in vitro* systems, and provide a platform for mechanical and biochemical co-stimulation.

In some instances, organ-on-a-chip systems have been successfully integrated in living organisms, demonstrating their biocompatibility and their physiological similarity to the endogenous environment. The AngioChip (Figure 2.2B), developed by Zhang *et al.* in 2016, features a porous microchannel scaffold and a mechanically tunable matrix resembling a microvascular network [63]. Upon endothelialization (i.e., after a surface becomes covered by a continuous layer of endothelial cells), the scaffold supports a permeable, open-vessel lumen

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capable of surgical implantation. In addition to enabling remodeling, extravasation, and intercellular crosstalk in an experimental setup, the AngioChip has can immediately establish blood perfusion when connected to femoral vessels of murine hindlimbs. It also promotes angiogenesis (i.e., the growth of new blood vessels) within a week following the procedure. More recently, Lee *et al.* have engineered an implantable poly(lactide-co-glycolide) (PLG) scaffold to aid in the investigation of tumor progression and metastasis in hypoxic microenvironments [68]. These microporous PLG scaffolds enable the creation of a readily accessible, subcutaneous site that can be chemically modified to investigate hypoxic regulation, blood vessel formation, and tumor cell migration.

Organ-on-a-chip systems also have demonstrated potential for *in vitro* drug toxicity screening. Healy and colleagues have improved drug discovery safety and efficacy testing using *in vitro* systems with the development of their cardiac microphysiological system [69]. In this system, human cardiac tissue, derived from induced pluripotent stem cells (iPSCs), was cultured within the boundaries of a 2 µm-thick, endothelium-like semipermeable barrier that allows for diffusion of nutrients and drug candidates, which ultimately provided results that were more consistent than cardiotoxicity data produced from human cardiac tissue samples. A series of interconnected tissue culture chambers can allow for interactions between separate cultures to form a single system. As an example, a system developed by Shuler and colleagues used microfluidics and mathematical modeling to predict the pharmacokinetic-pharmacodynamic (PK-PD) effect of drug candidates in interconnected, multi-organ systems [70]. These systems featured multiple cell culture chambers representing liver, tumor, and marrow tissues, connected with fluidic channels to mimic blood circulation. The researchers used this system to screen toxicity responses to a novel anticancer drug. In 2017, Skardal *et al.* studied systemic, nonspecific effects of a drug using a

system that integrated individual tissue constructs with a closed circulatory perfusion loop [71]. Multiple efforts have demonstrated the efficacy of organ-on-a-chip systems for drug safety testing, and thus they offer a clear opportunity for testing the safety of drug delivery by MBRs.

Organ-on-a-chip systems have been used to recapitulate human physiology in microdevices by incorporating mechanical, chemical, and physical features of native tissue. Highlighted in this section are seminal studies of organ-on-a-chip systems that either demonstrated basic organ function, served as implantable scaffolds for tissue regeneration, or were used as drug toxicity screening tools. Moving forward, organs-on-a-chip that reproduce the native physiological environment could provide an arena in which MBRs can be deployed to assess their drug delivery potential.

2.2 Microbiorobots: Motility in Fluid Environments and Penetrating Tissue Barriers for Drug Delivery

Ensuring that MBRs can operate in an organ-on-a-chip's fluid environment will be critical if these devices are to be used to demonstrate drug delivery efficacy. Fortunately, MBRs are compatible with the fluid flow regimes found in organs-on-a-chip. These devices often aim to reproduce the hemodynamic flow found in the microvasculature. As the circulatory system transports biologics throughout the human body, molecular transport to and from the microvasculature enables oxygen and nutrient exchange. This hemodynamic flow is driven primarily by the heart's pumping action and regulated by complex cardiovascular mechanisms. When the goal is to precisely target a microscale payload, such as an MBR, to a specific tissue location in the body (Figure 2.3A), the cardiovascular fluid dynamics can pose a significant hurdle

[15]. In particular, as the characteristic diameter of the flow reduces in the transition from large arteries to, ultimately, microscale capillaries, the flow becomes strictly laminar. Organ-on-a-chip systems can reproduce microvascular structures while allowing for fine experimental control over volumetric flow rate, pressure gradients, and flow direction. A range of fluid actuators enables this control, and current examples include syringe pumps [72], peristaltic pumps [73], and gravity-driven flow generators [74]. Microroboticists can use these systems with organs-on-a-chip to create developmental testbeds for studying motility and maneuverability within physiologically relevant conditions.

For their eventual deployment within the body, microrobots must be remotely actuated and their motion controlled in order to deliver payloads to the target site of interest. Current approaches for the actuation and motion control of MBRs use light or magnetic fields [48]. Researchers have also harnessed the motility of living organisms to actuate MBRs. One example is the use of magnetotactic bacteria - microbes that naturally synthesize magnetic particles - to deliver drug-laden nanocarriers [59]. As another example of using the capabilities of bacteria to create biohybrid microrobots, bacterial-polymer MBRs embedded with magnetic nanoparticles showed unidirectional chemotaxis under magnetic guidance [49]. Among these different actuation and motion control approaches, magnetic fields are particularly promising for controlling MBRs in organ-on-a-chip systems. Although both light-based and magnetics-based actuation can be used for *in vitro* systems like organs-on-a-chip, once MBRs demonstrate their utility in these organ-mimicking proving grounds, they will need to be deployed *in vivo*. Actuation and motion control in the body require the use of energy fields that can penetrate greater depths, through optically opaque tissue. For these applications, magnetic actuation and control will be the optimal systems.



Figure 2.3. Microbiorobot motility and its relevance to organ-on-a-chip systems. MBRs will need to navigate within the laminar fluid regime generated in microvessels in order to precisely deliver drugs to a target. B) Multifunctional microrollers have been engineered with i) antibodies to target and bind cancer cells present in endothelialized surfaces. The microrollers were able to resist ii) disturbances from physiological fluid flow (scale bar, 100 μm) [51]. Images reproduced with permission from Alapan *et al. Science Robotics*, 2020. C) In another approach, i) biologically inspired microswimmers were maneuvered through ii) tortuous glass microchannels, demonstrating the feasibility of MBR delivery in the vasculature [47]. Images reproduced with permission from Huang *et al. Science Advances*, 2019.

Several medically relevant MBRs have been tested in microdevices with fluid environments inspired by those found *in vivo*. Sitti and colleagues developed MBRs inspired by the rolling and tumbling motion of immune cells in the blood stream. These immune cells attach to the cells lining the wall of vessels (i.e., endothelial cells) with just enough adhesion to remain in contact with the wall while also allowing the force of the blood flow to cause them to roll along the vessel surface. In their study, Sitti and colleagues reported surface-functionalized, magnetic microrollers (Figure 2.3B) that achieved translational velocities of up to $600 \,\mu\text{m/s}$ across layers of endothelial cells cultured in microchannels [51]. When the team applied a magnetic field, these microrollers demonstrated propulsion opposite the direction of flow, overcoming shear forces of up to 2.5 dynes/cm². When the team tested the microrollers in culture vessels, they were able to precisely localize the microrollers near targeted cancer cells. In another study, Yu et al. investigated magnetic MBR swarm formation and swimming behavior in biologically inspired fluids of various ionic strength and viscosity. After their surfaces were functionalized to achieve hydrophobicity, the nanoparticle swarms maintained their swim pattern and trajectories in an ex vivo bovine eye tissue sample [75]. Other researchers have reported 3D models of tissue-fluids with potential for integration of organ-on-a-chip techniques and microrobotic interactions. For example, Sitti and colleagues directed stem cell-carrying MBRs through microchannels using an external magnetic field [50]. Upon reaching the damaged tissue site, the contained stem cells migrated out of the MBRs for localized tissue repair. Bylis et al. demonstrated that chemically propelled microparticles can actively transport functional protein cargo through microfluidic channels perfused with whole blood via lateral propulsion, buoyancy, and convection [26]. This system, which was then deployed in vivo, provided evidence that organs-on-a-chip can be physiologically relevant systems for MBR directed drug delivery development.

Other microrobots have navigated the fluidic environment using helical, flagella-like tails similar to those found in some bacterial species [47, 52]. Nelson and colleagues created "adaptive locomotion designs" (Figure 2.3C) that can be incorporated in microrobots to allow for flexible maneuvering through complex channels that mimic the vessel heterogeneity found within the body [47, 76]. Sitti and colleagues studied the interaction of magnetic helical microswimmers in a microenvironment from an immunogenicity perspective. The response of murine macrophages to these microrobots suggests an opportunity for morphology-dependent design optimization [77]. Wu *et al.* magnetically actuated helical micropropellers through porcine eyes to deliver drugs to the retina [78]. Xu *et al.* combined a synthetic microstructure with sperm cells for magnetically guided cargo transport [24]. Bioinspired MBRs capitalize upon the flexibility, propulsion, and biocompatibility of biologically evolved swimming behaviors, which allow for future development to be focused on the merging of precise navigation with efficient and effective drug delivery.

Organ-on-a-chip systems can also replicate the tissue barriers that will need to be crossed by MBR-delivered drugs in future clinical applications. This review envisions MBRs as drugladen delivery vehicles (Figure 2.4A). For these drugs to move from the MBR and enter a tissue, they must cross the hemorheological, fluidic barriers found in the vessel, penetrate the endothelium, and diffuse into the surrounding tissue [15]. Fortuitously, several organ-on-a-chip systems have been engineered to understand drug transport across these different barriers at the microscale. For example, Mair *et al.* used a crawl-based approach in the development of magnetically aligned nanorods in alginate capsules (MANiACs) to form a tumbling robot capable of translational motion across a tissue surface with diffusion-based cargo delivery [79]. Schuerle *et al.* used helical swimming microrobots to enhance convective transport of fluorescent nanoparticles across a vessel-matrix interface representative of blood vessel extravasation (Figure 2.4B) [28]. Alternately, Esteban-Fernandez de Avila *et al.* created an active transport-based system, and incorporated a propulsive motor in a multicompartment microstructure to aid in physically penetrating a tissue barrier (Figure 2.4C) [23]. In this case, the drug of interest was encapsulated in a pH-responsive cap, which only began to dissolve upon penetration of the tissue lining, which exposed the cap to an increased pH. Sufficient propulsion was generated by a zinc-propellant engine. The engine was placed in a microcompartment opposite the payload compartment in a tubular robot chassis. This MBR could then be driven into the targeted area of interest to deliver the payload.

Here, the critical requirement for MBRs to operate in an organ-on-a-chip's dynamic fluid environment has been described. For future drug delivery applications, microrobots will need to be remotely actuated and precisely controlled to deliver drug payloads. Already, medically relevant MBRs have been tested physiologically similar fluid environments, and new propulsion systems, inspired by biology, have been developed. These systems for operating in the fluid environment will be critical for exiting blood vessels and penetrating tissue to deliver drugs. Hemorheological barrier Endothelial barrier Tissue barrier

В

Α

Figure 2.4. Microbiorobots can penetrate physical barriers in organ-on-a-chip systems. Tissue surfaces ary in their diffusive permeability and must be penetrated for drugs to reach their targets. B) Artificial bacterial flagellum (ABF) microbiorobots could enhance i) drug uptake across an artificial vessel-matrix interface in an organ-on-a-chip [28]. ii) Bright-field and iii) fluorescent images revealed an ABF localized along a vessel-matrix interface in the organ-on-a-chip device. Actuation of the ABF facilitated increased nanoparticle transport (scale bar = 50 μm) [28]. Images reproduced with permission from Schuerle *et al. Science Advances*, 2019. C) In another example of enhancing barrier permeability, i) multi-compartment microbiorobots demonstrated localized, permeable cargo delivery by utilizing a micromotor as a propulsion system to penetrate tissue surfaces [23]. ii) Polymer capped-cargo was visualized via SEM imaging and fluorescent imaging to depict iii) dissolution and iv) cargo release [23]. Images reproduced with permission from Esteban-Fernandez de Avila et al. Advanced Materials, 2020.

2.3 Organ-on-a-Chip Disease Models for Microbiorobot-Assisted Drug Delivery & A Potential Application

Organs-on-a-chip will also be ideal environments for testing drug-laden MBRs because they can reproduce the environment found in diseased tissues. Thus far, this review has explored both the capacity of organs-on-a-chip for replicating the fluid environment found in tissue as well as their ability to reproduce tissue barriers that must be penetrated by drugs. Yet, their capacity to reproduce specific diseases will be especially important for understanding how MBRs might ultimately be deployed in the body to treat disease.

Following the initial progress in developing organ-on-a-chip systems that replicated different aspects of healthy organ environments, several organ-on-a-chip disease models have been developed. For example, Agrawal *et al.* developed a musculoskeletal organ-on-a-chip to characterize mechanical strain generated by multinucleated, skeletal muscle bundles [80]. This

system was then used to evaluate the effect of dose-dependent muscle injury in response to cardiotoxin. Griffith, Wells, and colleagues probed dormant and metastatic tumor states using hydrogel scaffolds of varied stiffness, representative of healthy and diseased states, in a hepatic microphysiological model [81, 82]. While endothelialization of microchannels has often been used to demonstrate the construction of a minimal vasculature for organs-on-a-chip, [39, 51, 63], Jain et al. expanded upon this approach to study thrombus formation in response to increased physiological shear stress in small volumes of whole blood [83]. The previously mentioned lungon-a-chip developed by Huh et al. combined bilayer tissue coculture with mechanical stimulation [62], and has been used as a disease model for pulmonary edema by demonstrating vascular leakage in response to cytokines [39, 84]. Similar to lung-on-a-chip models, gut-on-a-chip systems have incorporated mechanical forces, in these cases to mimic the peristaltic motion of the gut. In one example, Ingber, Collins, and colleagues recapitulated the structure of intestinal villi in an organ-on-a-chip to study inflammatory bowel disease [85]. In an effort to investigate cellular crosstalk between the gut and liver, Chen *et al.* studied bile acid metabolism in a coculture system with both healthy and inflammatory states. Their results were more consistent with in vivo observations when compared to standard *in vitro* models [27].

Pulmonary hypertension (PH) is an example of a disease where current treatments are limited, and therefore new therapeutics, such as the proposed drug-laden MBRs, should be developed for its treatment. Here, this potential application will be explored to illustrate the key steps in developing an organ-on-a-chip as a proving ground for MBR-based therapies. PH is characterized by narrowed, hardened, and obstructed pulmonary arterioles resulting in an increased mean arteriole pressure [86]. This persistent, increased pressure causes the heart to grow larger and become weak, resulting in decreased perfusion of the lungs and ultimately leads to heart failure [86, 87]. Symptoms include chest pain, fatigue, shortness of breath, and swelling in the abdomen or lower extremities. Pulmonary hypertension (PH) will likely remain incurable and fatal without improved experimental model systems that reproduce the cellular, tissue, and organ environment of pathogenesis [88].

Several steps would be needed to create PH-on-a-chip and test MBRs within it. First, a system similar to the Huh *et al.* [62] device previously described in Figure 2.2A could be developed to incorporate the key cell types involved in PH [88-90]. Because their initial lung-on-a-chip system incorporated a vessel lumen, its form provides a starting point for developing a PH arteriole-on-a-chip. In place of the semipermeable membrane used in the Huh *et al.* system, an extracellular matrix (ECM) layer embedded with SMCs and fibroblasts would be incorporated to recapitulate the medial layer of the vascular wall. During PH pathogenesis, this wall is anatomically remodeled by cellular processes. This improved, living membrane would be held between the epithelialized airway lumen and the endothelialized vessel lumen using a micropillar cage. An example of such a caging system was developed by Kamm, Asada, and colleagues [91] and used by Schuerle *et al.* [28] as previously mentioned (Figure 2.4Bii). In such devices, the micropillars create surface tension at the air-liquid interface to cage the ECM contents within its boundaries.
Pulmonary Hypertension-on-a-Chip Drug-carrying Microbiorobots Endothelial Cells Diseased Smooth Muscle Cell Healthy Smooth Muscle Cell

Figure 2.5. A potential application of MBRs in organs-on-a-chip: treating pulmonary hypertension. The PHon-a-chip model would incorporate the key cell types (e.g., smooth muscle cells, SMCs) involved in disease progression in an anatomically similar format. For examples, SMCs embedded in extracellular matrix (yellow) could be caged between micropillars (white triangles). This device is inspired by a previously published device used by Schuerle *et al.* [28]. Next, a magnetically actuated microbiorobot carrying an Cpd22, an inhibitor of SMC-driven remodeling [92], could be engineered to maneuver within an endothelialized lumen representing the vasculature. By employing a SMC-sensitive polymer cap [23], the robot would release the inhibitor upon contact with SMCs.

In order to initiate pulmonary hypertension, the system would be probed with different stimuli suspected of triggering pathogenesis (e.g., mechanical and biochemical stimuli) [93]. The PH-on-a-chip model would be expected to withstand mechanical stimulation, such as increased shear stress or intravascular pressure, due to the caging effect imposed by the micropillar supports [91]. Biochemical stimuli could be introduced via a peripheral inlet. MBRs, carrying pharmaceuticals expected to alter disease pathogenesis, would then be introduced to the system (Figure 2.5). Once candidate drug for the MBRs is Cpd22, a signaling inhibitor that has shown promise as a potential therapeutic for reducing vascular remodeling in existing PH experimental models [92]. As this inhibitor has only been shown to have an effect on smooth muscle cells, the organ-on-a-chip's ECM would need to be penetrated. Here, a compartmentalized MBR would be used. It would contain a cargo-carrying cap, similar to the previously described work of Esteban-Fernandez de Avila et al. (Figure 2.4C) [23]. For in vivo applications, the MBR's cargo-carrying cap could even be functionalized to target the endothelial lining of the pulmonary vasculature, and would only release the contained Cpd22 upon detection of chemical markers indicating penetration of the extracellular matrix and localization with the fusion with the contained SMCs [28]. The proposed integration of a multifunctional microbiorobot with a customized PH disease-model-ona-chip would be useful both for greater understanding of PH pathogenesis and in developing new, MBR-based treatments for PH.

2.4 The Future Application of Organ-on-a-Chip Technologies as Proving Grounds for MicroBioRobots

Both the organ-on-a-chip and MBR fields have experienced exciting progress over the past decade, and combining these technologies presents a significant opportunity for developing new medical technologies (Figure 2.6). The current state of the art of integrating microrobots within organs-on-a-chip is demonstrated by the previously discussed work of Schuerle *et al.* (Figure 2.4B) [28]. Here, the future of each field is discussed along with their potential for future integration.

The future of organ-on-a-chip research will be in the development of human-on-a-chip systems. To that end, Zhang et al. reported the creation of a compartmentalized, microfluidic cell culture system that mimicked the interaction of organ systems within the body [94]. This system was used to culture liver, lung, kidney, and adipose tissue in individual microenvironments under a common cell culture media. Griffith and colleagues have created 4-, 7-, and 10-organ-like fluidic systems connected via a mixing chamber [95]. In an attempt to increase the throughput of these microphysiological systems, parallel multi-organ experimentation using array formats are gaining traction [96, 97], such as the PREDICT-96 platform developed by Tan et al. [98] and the microwell-based, liver-on-a-chip developed by Khademhosseini and colleagues for modeling nonalcoholic fatty liver disease [99]. Along these lines, Lee *et al.* utilized a pumpless, multiorgan approach combined with mathematical modeling to illuminate the systemic mechanism of action of different drug candidates [100]. Ingber and colleagues utilized liquid-handling robotics to individually culture and analyze eight-organ-on-a-chip systems connected by endothelialized microfluidic channels [101]. Their completely automated system was able to monitor, supplement media, and collect samples in a perfused multiorgan system. Healy and colleagues developed a plug-and-play system, the μ Organo system [102], which allowed independent organ-on-a-chip

systems to be integrated. These types of human-on-a-chip systems will allow for disease-specific understanding of system responses.



Figure 2.6. Future directions for microbiorobot drug delivery in organ-on-a-chip systems. A) Plug-and-play human-on-a-chip systems could be customized to monitor systemic effects of drug-laden MBRs, such as changes in the brain, lungs, and gut. B) Using these systems, next generation microbiorobots could be developed to optimize motility, precision, and drug efficacy. Examples of features for customizing microbiorobot would include functionalization with biological components [103], specific recognition and binding components for targeted delivery [51], and automated, multi-axis magnetic actuation [104].

As human-on-a-chip systems are realized, next-generation MBRs will be developed for deployment in these devices. These new, drug-laden MBRs will incorporate advanced mechanisms for propulsion and mechanical flexibility to navigate in dynamic fluid environments. Biocompatibility of material components and MBR shape and motility will be required to achieve reliable and noninvasive drug delivery at target sites. Autonomous and automated control will be essential in clinical applications for local sensing and reorientation within optically opaque tissue environments. Automated, multi-axis stability and actuation systems will enable high-precision motion control [104, 105]. User-friendly, optics-based motility algorithms that steer robotic systems to within 10 µm of their targets [58] will be employed within organ-on-a-chip systems. Additionally, biohybrid systems that can engage and interact with biological systems for niche treatment options, such as the sperm-driven micromotor for gynecological health developed by Xu *et al.*, will increase biocompatibility and minimize pathogenic side effects, to speed drug development and encapsulation [103]. In the future, we expect to see the scientific advancement of automated, biohybrid MBRs acting within integrated organ-on-a-chip devices. These systems will serve as proving grounds for new multifunctional, robotic drug delivery systems for the prevention and treatment of disease.

3.0 On-a-Chip Platform for Standardized Biomedical Microrobot Functionality Testing

We have discussed how biomedical microrobots are next-generation drug delivery or surgical tools that can be guided to a site of interest to perform a medical function. The translational potential of these tools, however, is hindered by a lack of standardized testing tools. In Chapter 2.0, we presented our perspective on how organ-on-a-chip systems could serve as proving grounds for biomimetic microrobots, or microbiorobots, for targeted drug delivery. The research advancements presented in support of this perspective, however, describe microrobot actuation and/or function in experiment-specific designs. As a first step towards realizing the synergetic potential of robotics and bioinspired on-a-chip systems, we propose that the generation of a standard testing platform for the evaluation of novel microrobot designs will allow for field-wide comparison and eventual advancement of microrobots for targeted drug delivery.

This work presents an on-a-chip platform with an integrated, open-source magnetic control system and real-time visualization for standardized testing and development of magnetic microrobots. Magnetic microrobots were used as the first microrobot model design to evaluate in this platform due to magnetic actuation showing significant promise for *in vivo* deployment. This platform is used to demonstrate semi-automated locomotion and actuation of a helical, magnetic microrobot within a U-channel on-a-chip testbed, as well as biomedical function via microrobot interaction with a biophysical barrier. This platform enjoys a simple, yet robust design engineered from common laboratory hardware and techniques that are amenable to adaptation for an array of microrobot control schemes and enhancement with additional physiological characteristics. This work is in line with our previous motivations for creating open-source technologies for improved access to previously inaccessible laboratory techniques, which is published in Behrens, Fuller *et*

al. "Open-source, 3D-printed Peristaltic Pumps for Small Volume Point-of-Care Liquid Handling," *Scientific Reports*, 2020 [73]. We present this platform as a tool with the potential to advance modern medicine through improved lab-to-lab accessibility for the standardization of microrobot testing on-a-chip.

3.1 Introduction

New medical products must undergo evaluation by standardized assays as a critical step in the development pipeline. These standardized tests, identified and validated by organizations such as the National Institute of Standards and Technology and the U.S. Food and Drug Administration [106], are used to benchmark the efficacy of new drugs and drug delivery systems against the current standard in the field. In the case of biomedical microrobots, actuation control schemes, as well as biomedical functionality, are essential criteria for translation *in vivo* and are currently evaluated in experiment-specific models [15, 107]. While the experimental proving ground in each research pursuit is selected to highlight the specific control scheme and/or biomedical function of the microrobot, the lack of standardized testing tools remains a pertinent challenge inhibiting microrobot translation. For example, how can a magnetically guided microrobot capable of affixing to a microtissue in a fluidic microchannel [104] be compared to a chemically driven, penetrating micromotor evaluated in a mouse model [23]? A body-on-a-chip model elucidates actuation and control schemes in physiological replicas, whereas demonstration in vivo prompts biomedical function in an endogenous microenvironment. Could each of these microrobots demonstrate success in the other's experimental testbed? A standardized platform could benchmark microrobot capabilities early in development.

In an effort toward implementing a standardized testbed for biomedical microrobots, we have developed a platform for microrobot actuation and biomedical function that addresses current challenges associated with the translation of microrobots (Figure 3.2). This platform is designed to actuate microrobots in on-a-chip fluidic environments, which is consistent with our previously published perspective that on-a-chip systems could be key for novel drug delivery system testing [60]. Instead of focusing on organ function, a key feature of organ-on-a-chip systems [108], a minimal on-a-chip model would capitalize on the benefits of organ-on-a-chip systems to recapitulate physiological function in a modular format. These features could include but are not limited to, maintaining fine control over experimental variables, having visual access to biological function through a glass focal plane, maintaining biological closeness, and having a 3D structure to support physiologically relevant cell culture. A cardiovascular-inspired design is adopted for the dual purpose of modeling intravenous microrobot drug delivery and could be developed as a preclinical testbed to combat the high rate of cardiovascular toxicity observed in clinical trial fails [34, 35]. This research development is anticipated to have a positive impact on the on-a-chip and microrobotics communities by streamlining developmental progress and allowing for a standardized field-wide comparison between research advances.



Figure 3.1. An on-a-chip platform for microrobot actuation and biomedical function testing.

3.2 Materials and Methods

3.2.1 Materials

Z-ABS (Zortrax). Translucent photopolymer resin (Elegoo). Polydimethylsiloxane (PDMS) Slygard 184 (Sigma Aldrich, 761036). Agar (Fisher BioReagents, BP1423-500). LB Broth, Miller (Fisher BioReagents, BP1426-500). Food coloring (Red Food Color, McCormick Culinary). PBS, pH 7.4, 10X (Life Technologies, 70011-044). Iron (II, III) oxide (Sigma-Aldrich, 637106-100G).

3.2.2 Open-source Platform for Microrobot Actuation and Visualization

Our platform featured an integrated open-source magnetic control system and real-time visualization for user-defined, semi-automated microrobot control (Figure 3.2A). Magnetic

actuation operated by common laboratory hardware was selected as the first demonstration of microrobot control due to its promise for *in vivo* deployment [48]. This platform utilized an opensource microcontroller (Arduino) to control two NEMA17 specified stepper motors (SparkFun, ROB-09238); one to rotate an axially magnetized, neodymium, iron, and boron (NdFeB) permanent magnet (grade N42, 1.32 T Gauss rating) (K&J Magnetics, Inc, RX033CS-N), and one to translate the magnet along a track. The stepper motors were held in place by a 3D-printed cart. The components of the cart (Appendix A.1) were 3D-printed by extruding acrylonitrile butadiene styrene (ABS) from a fused deposition modeling printer (Zortrax M200). A mount was also 3D-printed to position a 22 x 40 mm glass cover slip-bound device within the focal plane of the camera and central to the magnet on the track.

The 3D-printed cart and mount were secured to an acrylic stage for system integration and visualization (Figure 3.2C). The 3-sided stage housed an Avium 1800 U-500c camera with a 6 mm fixed focal length lens (Edmund Optics) for real-time visualization using camera-compatible software (Vimba Viewer, Version 2.4.0). The incorporation of a camera addresses current obstacles in microrobot development due to biomedical imaging technologies lacking in frame rate and resolution limitations [107], inhibiting the integration of control systems with imaging modalities [109].



Figure 3.2. On-a-chip platform as a testbed for magnetic microrobot actuation. A) The platform consists of an open-source, magnetic control system integrated with a camera for real-time visualization of a magnetic microrobot in an on-a-chip device. The platform uses image-informed, user-defined controls to rotate and translate a permanent magnet along a track. B) Picture of the assembled platform atop an acrylic stage.

The user entered commands for cart destination position, cart translational speed, magnet rotational speed, and magnet spin direction into the Arduino integrated development environment (IDE) program (Appendix A.2), which communicated with an onboard Arduino UNO microcontroller via USB to process user commands. The Arduino microcontroller powered an easy driver stepper motor driver (SparkFun, ROB-12779) that translated user commands into the movement of the permanent magnet and resultant actuation of a microrobot enclosed in the on-a-chip device. Using real-time visual feedback from the camera, the user can then input subsequent commands for semi-automated microrobot actuation.

3.2.3 U-channel Device Fabrication

The U-channel device designed for use with this platform featured a branched microfluidic channel in the shape of a U with multiple ports for experiment-specific customization (Figure 3.3A). The U shape of the device architecture featured straight and curved channels that model the tortuous luminal topography of the cardiovascular system. The device was designed with millimeter-scale features for visualization without the use of a high-powered microscope (Figure 3.3B). The device also retained airholes that assisted with complete channel filling.

The U-channel device was 3D-printed by selective light exposure of a transparent resin using a masked stereolithographic printer (Elegoo Mars) (Figure 3.3C). The design was conceptualized in OpenSCAD (Version 2015.03-3) 3D rendering software (Appendix A Figure 1) and printed with a 0.05 mm layer height of transparent resin that was cured for 15 seconds. For added printing stability, the bottom layer was exposed for 75 seconds, and anti-aliasing exposure was selected to ensure smooth surfaces. After printing, support structures were manually removed and the device skeleton was rinsed in isopropyl alcohol for 30 seconds, then rinsed in deionized water for 1 minute. After drying, the device was cured by ultraviolet light (Life Technologies, TFX-20M).

Polydimethylsiloxane, abbreviated as PDMS, was used to bind the device to a glass cover slide through a transfer bonding process [110] (Figure 3.3D). First, the curing agent and elastomer base were mixed in a 1:10 ratio. Then 1 mL of uncured PDMS was added to the surface of a glass cover slide secured to a spin coater by vacuum pressure (Laurell Technologies, Inc.). The glass cover slide was spin-coated with uncured PDMS at 1000 RPM for 1 minute with an acceleration of 500 RPM. A U-channel device was then set on top of spin-coated PDMS for 1 second, removed, and transferred to a clean glass cover slide. The freshly bound device was then cured for 1 hour at

65 °C (VWR INCU-Line Digital Incubator 115V). After 1 hour, the remaining uncured PDMS was distributed around the edge of the device using a syringe and blunt tip needle to ensure a watertight seal was formed between the device and glass. The device was then cured for 12 hours at 65 °C and then rinsed with 70% ethanol before media is introduced.



Figure 3.3. Fabrication of the U-channel device. A) U-channel device (scale bar = 10 mm). B) Technical drawing of device dimensions (bottom-up). C) Scheme of device skeleton printed by masked stereolithographic 3D-printing. D) Device skeleton is bound to glass by a transfer bonding process using uncured PDMS.

3.2.4 Formation of a Physical Biological Barrier

Biological barriers describe the chemical, biological, or physical obstacles that a circulatory microrobot could encounter *in vivo* during translation from deployment to the target site [15, 107]. Such obstacles could include plaque buildup, a biofilm layer, or the lining of a capillary bed. As a demonstration of these endogenous or diseased barriers, a multiphase, liquid-gel physical interface was reproduced in the U-channel device.

The open chamber device was subdivided into distinct liquid and gel components by the formation of a liquid-gel interface (Figure 3.4A). This approach was inspired by a previously published approach that used surface tension and capillary pressure to form a multiphase interface [111]. To do so, a semisolid agar gel was first cast into the end ports of the U-channel. The semisolid agar gel solution was formulated by mixing Luria broth (LB) culture media with 0.25% (weight/volume) agar. LB broth was used as the solution base in place of deionized water to maintain nutrient and pH conditions of physiological relevance. The semisolid gel was sterilized by autoclave (Steris Steam Sterilizer Model 20VS) and once cooled to the touch, 250 µL of semisolid gel was pipetted into the end ports of the U-channel. The semisolid agar gel was allowed to cure in place with the U-channel set at a slight incline. Once cooled, 500 μ L of deionized water or food dye was dispensed into the center port of the U-channel to form a liquid-gel interface in each channel. Correct channel filling was observed when the agar gel seeped ~4.4 mm into the channel so that the surface of the gel layer was adjacent to the airhole (Appendix A Figure 2). Devices that experienced agar infusion past or significantly shy of the position of the air hole were unable to form intact liquid-gel interfaces, apparent by the formation of an air bubble.



Figure 3.4. U-channel device with a physical liquid-gel barrier. A) Schematic describing physical barrier formation between semisolid gel and liquid media. B) Bottom-up images depicting the semisolid gel set in place before the formation of the liquid-gel interface (prefill), and after liquid media is introduced (postfill). C) Schematic describing the expected diffusion of dye across the liquid-gel interface over time. D) Images depicting dye diffusion from liquid, through the liquid-gel interface, and into the gel over 90 minutes.

Images were acquired immediately before and following the addition of red food coloring to visualize the formation of the liquid-gel interface (Figure 3.4B). Hydrogels such as agar have a mesh structure and high-water retention, allowing for solute diffusion to be a function of crosslinking density [112, 113]. Because of this, it was expected that food dye would passively diffuse from the liquid solution and across the liquid-gel interface over time (Figure 3.4C). To confirm contact of the liquid-gel interface and permeability of the semisolid agar gel, the diffusion of food dye across the liquid-gel interface was visualized over 90 minutes (Figure 3.4D).

3.2.5 Magnetic Microrobot Fabrication

Microrobots were fabricated by molding a molten hydrogel composite into a 1.0 x 4.4 mm helical structure [114]. Molding was accomplished using a 3D-printed mold. The two-part, negative mold for the microrobot structure was designed in a 3D computer-aided design software (Autodesk Inventor), converted to a printable .stl file via 3D printing preprocessing software (CHITUBOX, China), and photocured with UV light on an Elegoo Mars stereolithography printer (Elegoo Mars, ELEGOO). Once printed, the two-part mold was removed from the printing platform and support structures, rinsed with isopropyl alcohol for 30 seconds, rinsed with deionized water, and allowed to air dry at room temperature for 1 hour. The two-part negative mold was cured by a 5-minute exposure to UV light (UV light imager, Life Technologies, TFX-20M).

The hydrogel composite material was mixed by adding phosphate buffered saline (PBS) to a beaker and heated to 90 °C under constant stirring (Fisherbrand, SP88857290). Next, agar was added to 2% (w/v). Once the agar was visibly liquefied, the temperature was reduced to 50 °C to prevent evaporation. Iron oxide was then added to the solution to a final concentration of 10% (w/v) and mixed thoroughly. The agar mixture was then transferred to one-half of the negative mold using a 10 mL syringe outfitted with a blunt tip needle to fill the 5 microrobot negative impressions. The remaining half of the mold was replaced and manually held in place for 10 seconds until the agar solution cooled. The two-part mold was outfitted with alignment posts to ensure consistent alignment during the molding process. The top of the negative mold was removed to reveal the microrobots. Using a fine metal dental pick, microrobots were transferred from the mold to a Petri dish containing PBS and stored at room temperature. The shelf life for the microrobots was not characterized, however, microrobots were fabricated and used for experiments within 1 week. Microrobots were also individually selected for experimental use based on morphology and appearance of homogenous composition since the consistency of fabricated microrobots varied amongst fabrication batches.

3.2.6 Microrobot Movement Characterization

Images for microrobot locomotion and actuation characterization were acquired using an Alvium 1800 U-500c camera with a 6 mm fixed focal length lens (Edmunds Optics, 8.5 mm/F1.3, 86599). Bottom-up images were taken to monitor movement in the x-direction and along the length of the channel. Side-view images were acquired to monitor microrobot rotational movement. Images were acquired using Vimba Viewer (Version 2.4.0) with image settings set at Exposure: 938544.38 μ s, Gain: 2.4 dB, and White Balance: 1.27_{RED}, 2.18_{BLUE}. Video editing software was used (VN video editing software, version 0.13.1) to characterize microrobot motion as a function of frame rate. Data analysis was performed in Matlab (version R2022a).

3.3 Results and Discussion

3.3.1 Magnetic Microrobot Locomotion

Magnetic actuation is promising for *in vivo* deployment because magnetic fields can harmlessly penetrate opaque tissue with precision [15, 48]. For the same reasons, magnetic fields are commonly used in routine medical procedures such as magnetic resonance imaging (MRI). Magnetic forces are typically applied as a magnetic gradient or a rotating magnetic field [48]. Motion due to a magnetic gradient is the result of a pulling force, whereas the application of a rotating magnetic field produces a magnetic torque on a magnetized body. These magnetic forces typically result in microrobot mobility by either a swimming [25, 47, 52, 115] or a tumbling motion [51, 79]. As the motion of magnetic microrobots is largely influenced by magnetic field strength, rotational frequency, and direction, manipulating these parameters can help realize control schemes for microrobot actuation [116]. The platform presented here demonstrates reproducible magnetic microrobot actuation by magnetic gradient and rotating magnetic field using a permanent magnet to be broadly applicable to various magnetic microrobot motility mechanisms. This is a simpler, more straightforward approach to magnetic actuation than magnetic actuation by other modalities such as Helmholz coils [48, 117] or electromagnets [114] which typically require bulky hardware for operational maintenance.

The platform was first validated for magnetic microrobot actuation by the application of a magnetic gradient. A magnetic gradient was induced by the movement of the magnet master containing the magnetic field source. Locomotion due to magnetic dragging was then observed of the microrobot, or slave magnet [118], contained within the device. A magnetic gradient was manipulated by magnet master movement along the platform track at a user-specified rate,

distance, and direction (Figure 3.5A). The distance between the volume of the magnetic microrobot, V, with a magnetic moment, m, and the magnetic field strength, B, causes a pulling magnetic force, F_m that can be described as $F_m = V(m * \nabla)B$ [48]. To isolate the pulling force to two dimensions, the permanent magnet was secured to a mount that was positioned at a set yand z position relative to the centerline of the straight channels of the U-channel device where the microrobot was anticipated to reside. For the microrobot to move by magnetic pull, the magnetic field source would need to be positioned at a distance to impart a pull on the microrobot, but not be at too great of a distance that this force cannot overcome drag forces resisting microrobot movement [119]. The position of the axially magnetized, NdFeB permanent magnet used in this setup relative to a microrobot in the straight arm segment of the U-channel device resulted in a maximum field strength of 2.4 mT (Appendix A Figure 3). This field strength was sufficient to actuate a microrobot between locations within the U-channel device and was visualized by bottomup, time-lapse imaging from the platform's camera (Figure 3.5B). Microrobot translation from the center port to a target end port was recorded by time-lapse imaging. Microrobot velocity was quantified within a predetermined 10 mm section of a straight channel and measured by microrobot position with time.

Bidirectional magnet movement along the track was afforded by a user-defined stepper motor control. This process is described in detail in Section 3.2.2. Briefly, a step speed and destination position were input into the Arduino IDE interface, processed by an Arduino UNO microcontroller, and converted to a stepper motor speed setting that was imparted to a motor driver to power the step motor. This workflow converted a digital input into the mechanical rotation of a stepper motor that moved the magnet along the cart at a defined rate (Figure 3.5C). A preliminary

characterization of magnet translational velocity, v_{magnet} , as a function of step speed revealed that step speeds of 25, 50, and 100 correspond to $v_{magnet} = 0.36$, 0.72, and 1.37 mm/s, respectively.



Figure 3.5. Platform for magnetic microrobot translation by magnetic gradient. A) Schematic describing bidirectional translation afforded by the platform. B) Still images of microrobot position during translation from center port to target end port (scale bar = 5 mm). C) Magnet translational velocity by cart stepper motor speed. D) Microrobot translational velocity by magnet translational velocity by direction of travel. Trials traveling from straight to curved channel topography are identified as "straight" and trials traveling from curved to straight channel topography are identified as "curved". All error bars are representative of 1 standard deviation about the mean (n = 3).

The platform was then used to characterize microrobot translational velocity, $v_{microrobot}$, by magnet translation velocity in each direction along the U-channel device track (Figure 3.5D). Microrobot velocity as a function of magnet translational velocity was characterized for 3 individual microrobots in 3 individual U-channel devices to verify the robustness of the platform. During the translation along a path composed of mostly straight channel topography, the microrobot exhibited a translational velocity that linearly corresponded to the linear translation of the magnet for cart speeds < 50, or $v_{magnet} = 0.72$ mm/s, but appeared to fall behind the pace of the cart at a cart speed of 100, or $v_{magnet} = 1.37$ mm/s. The increased distance between the microrobot and the magnetic field caused the microrobot to experience a lower magnetic field strength, ultimately resulting in a slower translational velocity. Translation in the opposite direction and along a path composed of mostly curved topography appeared to be more resilient to faster cart paces, however, the overall translational speeds attained by the microrobot were lower in the curved direction than in the straight direction. This is likely due to additional friction between the microrobot and channel walls that impede forward microrobot translation through the curved topography.

The on-a-chip platform can also be used to generate a rotating magnetic field for microrobot actuation by magnetic torque. Under a rotating magnetic field, the magnetic particles in a microrobot will rotate to align the microrobot with the changing magnetic field direction [120]. A rotating magnetic field was generated by a microcontroller accepting user commands to rotate a permanent magnet. Magnetic actuation of the helical microrobots was achieved by the magnetic moment, m, of the microrobot aligning with the magnetic field, B. The nonuniform magnetic field, induced by the rotation of the permanent magnet, caused the microrobot to experience torque, τ_m according to $\tau_m = V(m \times B)$ resulting in microrobot spin (Figure 3.6A).

The microrobot rotation is expected to align with the frequency of the rotating magnetic field up until the step-out frequency [116], which is the rotational frequency at which the microrobot is no longer rotating in synchrony with the magnet. Time-lapse images were recorded from an additional mounted side view camera and used to quantify magnet (Figure 3.6B) and resultant microrobot rotational motion (Figure 3.6C). The countersink side of the magnet and the wobbly motion of the microrobot were identified as visual markers to track rotational motion with time (VN video editing software, version 0.13.1). The observed wobble motion has been previously reported for helical microrobots of similar length to radius ratio that were operated at low rotational frequencies of < 6 Hz [115]. The angular frequency of the microrobot, $\omega_{microrobot}$, in response to the angular frequency of the magnet, ω_{magnet} , was characterized in a deionized water-filled U-channel device that was aligned to the axial magnetic force of the permanent magnet.

Measurements for 10 magnet rotations per step speed, ranging from 0 - 6000, were recorded and used to characterize magnet angular frequency, ω_{magnet} , as a function of stepper motor step speed (Figure 3.6D). The results of the magnet angular frequency characterization reveal that step motor speeds 0 – 6000 correspond to magnet angular frequencies of 0 – 3 Hz for each spin direction; either with the direction of the helix, defined as positive spin, or against the direction of the helix, defined as negative spin. Next, the microrobot angular frequency, $\omega_{microrobot}$, in response to magnet angular frequency was characterized (Figure 3.6E). Characterization experiments were also performed for 3 individual microrobots in 3 individual U-channel devices. A regression of the measured data revealed a linear relationship between magnet and microrobot angular frequencies from 0 – 3 Hz, validating the robustness of the platform. The plotted data reveals that spin direction does not appear to influence the resultant angular frequency of the microrobot.



Figure 3.6. Platform for magnetic microrobot actuation by a rotating magnetic field. A) Schematic describing bidirectional angular frequency afforded by the platform. B) Side-view image of U-channel alignment with rotating magnet, C) used to visualize magnet rotation. D) Magnet angular frequency, ω_{magnet} , by stepper motor speed. E) Microrobot angular frequency, $\omega_{microrobot}$, by magnet angular frequency, ω_{magnet} , measured by spin direction. Spin direction was identified as with helical shape (positive) or anti-helical shape (negative). All error bars are representative of 1 standard deviation about the mean (n=3).

3.3.2 Magnetic Microrobot Actuation

Microrobot actuation control schemes are utilized to achieve microrobot functions such as locomotion and localization. Drug-delivering microrobots require evaluation of actuation control within physiologically relevant microenvironments to assess localization capabilities [15]. Microrobot linear velocity was characterized during translation towards a target position in the Uchannel to model these functionalities. Helical microrobots have an added feature that enables them to maneuver more efficiently in seemingly more viscous environments [47]. The helical shape is biomimetic and resembles that of a bacterial flagellum. Motile bacteria have evolved with a flagellum as an optimized means of overcoming the hydrodynamic drag forces present in low Reynolds number environments. Helical microrobots model this behavior and show efficient swimming in response to low-strength magnetic fields and operation in low Reynolds environments [116]. Therefore, it was expected that helical magnetic microrobot actuation.

As a demonstration of how this platform can be used to elucidate control schemes, optimized microrobot actuation as a function of both magnet angular frequency, ω_{magnet} , and translational velocity, v_{magnet} , was characterized (Figure 3.7A). The rationale is that magnetic torque is more resilient to separation distance than magnetic force [120], giving more freedom for maneuvering than just drag with immediate proximity to the magnetic source. This would be optimal for translation through heterogeneous topography as the added rotational motion of the microrobot would also result in a smaller cross-sectional area [115], hopefully alleviating the additional friction experienced by the microrobot along curved topography (Figure 3.5D). Microrobot translational velocity was improved with added rotational frequency across both the

straight (Figure 3.7B) and curved (Figure 3.7C) paths. A direct comparison of microrobot translation at 2 Hz along these trajectories revealed that microrobot translational velocity was still optimized along the straight path (Figure 3.7D). This is likely due to the low rotational frequency of < 6 Hz of the platform that is capable of resolving drag forces, but not high enough to result in corkscrew motion of the microrobot motion [115]. At higher angular frequencies of 2 and 3 Hz, propulsion was observed and characterized (Figure 3.7E). As a result of their morphology, the rotation of helical microrobots in response to magnetic torque results in forward translation at higher angular frequencies [116]. A propulsion matrix, $\begin{bmatrix} F_m \\ T_m \end{bmatrix} = \begin{bmatrix} a & b \\ b & c \end{bmatrix} \begin{bmatrix} v \\ \omega \end{bmatrix}$, can be used to relate force and torque to translational and angular velocity. Forward velocity is proportional to angular velocity until the step-out frequency of the microrobot which is dependent on microrobot geometry and fluid interface properties.



Figure 3.7. Magnetic microrobot actuation. A) Schematic describingmicrorobot actuation as a function of magnet angular frequency and magnet translational velocity. B) Microrobot translational velocity along straight topography as a function of rotational frequency. C) Microrobot translational velocity along curved topography as a function of rotational frequency. D) Comparison of microrobot translational velocity across mostly straight and curved topography as a function of magnet translation of magnet translational velocity and rotational frequency (2 Hz). E) Propulsion observed at higher angular frequencies of 2 and 3 Hz. F) Microrobot translational velocity can be optimized by magnet translational velocity and rotational frequency. Reported data is from trials conducted in the straight channel topography and with a positive spin direction kept constant. All error bars are representative of 1 standard deviation about the mean (n=3).

A summary of the degree of custom microrobot locomotion and actuation potential afforded by this platform is summarized in Figure 3.7F. These results summarize the degree of customization afforded by the platform on microrobot translational velocity by manipulation of the magnetic gradient and rotational magnetic field in the local environment of the microrobot. These operating parameters are comparable to other characterizations of magnetic microrobot actuation at low rotational frequencies [115, 117]. Other variables that could be considered for further characterization could include the effect of spin direction (negative or positive spin) on microrobot translational velocity, microrobot orientation (head or tail first), and the topography of the trajectory. This platform's modularity also affords future magnetic force customization by incorporating an alternative magnetic source or adjusting the ratio of magnetic particles in the microrobot composition. In the case of helical microrobots, these experimental results can be expanded to include microrobot locomotion at higher rotational frequencies at which the rotation of the microrobot is expected to take on a cork-screw motion. This will also allow for the characterization of microrobot step-out frequencies and velocities by angular frequency (ν/ω) . v/ω is a measure of the sensitivity of the microrobot to magnetic fields and can be used as a metric

for comparison amongst helical microrobots [116]. Additionally, this platform leaves room for reverse engineering other natural systems that have evolved to solve problems associated with motility about the body [121], as well as serve as a testbed to explore microrobot maneuverability unlocked by materials and precision microfabrication techniques [122, 123].

3.3.3 Microrobot-mediated Permeation of a Physical Biological Barrier

As a preliminary demonstration of biomedical functionality, the platform modeled microrobot-mediated permeation of a representative biological barrier that would be encountered by a microrobot *in vivo* (Figure 3.8A). Biological barriers include, but are not limited to the dynamic, physical, chemical, and biological milieu that exists as natural protective strategies to eliminate intruders [5, 107]. Previous organ-on-a-chip devices have been designed to reproduce physical barriers such as tissue layers *in vitro*, however, one major limitation of these designs is the use of either a nondegradable, polymer-based membrane barrier or an external microfluidic channel to distinguish individual chambers [41, 65, 67]. The physical separation between chambers allows for the transfer of molecules through microfabricated pores in the membrane or external fluid network, however, this limits the insight into endogenous, uninterrupted contact between components. In addition to limited interaction, the parallel orientation of another minimal tissue barrier-on-a-chip published by Nelson, Bhatia, and colleagues limits the potential to trial tissue barrier penetration by the microrobot itself [28].

Here, this system employs simple user interfaces to control and process information regarding microrobot actuation and proof-of-concept testing [17]. A physical endogenous or disease barrier, such as a tissue barrier, cardiovascular plaque, or a disease biofilm, is represented by the formation of a fully intact gel-liquid interface formed by the sequential casting of a

semisolid agar gel into the device, followed by the incorporation of liquid media (Section 3.2.4). Agar, a bioderived hydrogel, was chosen as the gel component due to its physical similarity to a thrombus [124, 125]. This design relies on a change in capillary pressure to control the formation of a liquid-air interface in an open chamber format [111].

The magnetic microrobot was loaded with red food coloring to visualize the permeation of the liquid-gel barrier over time. A preliminary experiment of magnetic microrobot penetration of semisolid agar gel was conducted to benchmark the results of these soft-bodied microrobots compared to the drilling behavior observed in other hard-bodied or macroscale counterparts [117, 124]. The results of this experiment lead to the selection of a semisolid gel with 0.25 % agar to ensure an adequate barrier would be formed for permeation with the possibility of penetration (Appendix A Figure 4). Since hydrogel microrobots have been previously shown to locally release chemical cargo in fluidic environments [19, 79], the hydrogel-based microrobot was expected to locally release food dye to aid in visualizing the effect of the microrobot motion near the liquid-gel interface.



Figure 3.8. Microrobot permeation of a physical biological barrier. A) Schematic describing actuation of a dyeloaded microrobot towards the liquid-gel interface. B) Still images of dye-loaded microrobot encountering and permeating the liquid-gel interface, evidenced by the visual diffusion of red food dye from liquid media into the gel. The microrobot was operated by a magnet angular frequency of 1 Hz and magnet translational velocity of 0.72 mm/s. C) Quantification of dye diffusion into the target gel interface compared to off-target diffusion.

Time-lapse imaging revealed that the microrobot was actuated from the center port to the liquid-gel interface within 90 seconds, locally releasing the food dye cargo along the way (Figure 3.8B). With continued microrobot motion at the interface, the food dye began to permeate the target liquid-gel interface proximal to the microrobot at a higher concentration compared to the off-target interface. Quantification of the intensity of the dye across the liquid-gel interface by computer vision was used to represent relative dye concentration as a function of saturation. The results reveal that dye intensity at the off-target interface was limited to 58.1 % of the intensity at the target interface over the measured period (Figure 3.8C). These results suggest that the presence

of the microrobot at a target interface localizes molecular permeation at the target location. While this rudimentary demonstration lacks the biological complexity apparent in previous demonstrations of biophysical barrier permeation [23, 115], this platform provides an accessible and baseline demonstration by which microrobot to microrobot biomedical functionality can be assessed at an early stage. These results can be expanded to measure the rate of permeation at the target interface due to the microrobot presence compared to the rate of permeation from a homogenous solution of dye or be adapted as a specific tissue or disease model by the incorporation of a disease or tissue-specific hydrogel solution. A tissue barrier, for example, could be modeled by including a collagen solution in place of the semisolid agar. Using this platform as a standardized starting point, biophysical barrier complexity, composition, and analyses can be incorporated and used as the new criteria for success.

3.4 Conclusion

This chapter presents an integrated magnetic actuation and visualization system with an on-a-chip arena as a steppingstone towards standardized microrobot testing and development. Our work demonstrates the actuation of a helical, magnetic microrobot within a U-channel device using common laboratory equipment and techniques. Utilizing an open-source microcontroller to manipulate a rotating magnetic field and visualization from a fixed focal length camera, magnetic microrobot actuation is achieved by both the spatial magnetic gradient and rotating magnetic field; each of which can be customized by the user. Additionally, this platform can be used to evaluate biomedical function through the interaction with a physical biological barrier formed by a completely intact liquid-gel interface utilizing an open chamber format. While the data presented in this paper show promise for this platform for standardized actuation and biomedical function testing, the characterization of other microrobot designs, operational parameters, and a thorough comparison to other microrobot research advances will be essential during field-wide adoption.

For biomedical microrobots to reach their full potential, standardized testing platforms need to be implemented to track and benchmark progress in pursuit of clinical translation. As the driving principle behind the creation of this platform is to standardize microrobot testing and development, an open-source system will expand accessibility, enable broader applications, and allow for user-specified modifications, in turn, driving development and furthering scientific discovery. For example, other means of actuating microrobots can include acoustics, light, and chemical gradients [15], to name a few. Actuation by ultrasound acoustic waves, an imaging modality also well characterized in the medical field, faces limitations in precision and omittance of background signals which could affect microrobot control and visualization *in vivo* [107]. The U-channel device could be encompassed by a tissue-like material and used as a testbed for ultrasound-guided microrobot actuation and visualization development. This type of a setup could be simultaneously advantageous for microrobot actuation and design improvement as well as continued enhancement of ultrasound for clinical use.

The on-a-chip platform serves as a bottom-up approach for future developments for biomedical microrobots, such as for multiagent and autonomous control or on-site triggered drug release. The cooperation of multiple microrobots will allow small-scale robots to make an impact on a macro-scale system [17]. Multiagent control could begin to be modeled in this platform by the addition of a second magnetic field source, the combination of multimodal control, or by predetermining differential microrobot responses by manipulating microrobot material composition. For example, microrobots fabricated with varying concentrations of iron oxide nanopowder would be affected by the same magnetic field forces at magnitudes corresponding to their composition. This would result in distinct microrobot control schemes within a swarm of magnetic, hydrogel-based microrobots. Further microrobot research advances will require well-documented and established fabrication and operation procedures for anticipated technology transfer to other research groups [17, 107]. Moving into the future, accessibility of standardized equipment and protocols will unite the biomedical research efforts of on-a-chip developers, microrobotics, and pioneers of precision medicine.

4.0 Microrobot-mediated Targeted Drug Delivery in a Biochip Platform

Targeted drug delivery could greatly benefit antibiotic treatment where overuse and offtarget delivery are recognized to lead to resistance and systemic toxicity. Drug delivery systems such as drug-laden microrobots could help localize antibiotics to a hard-to-reach site needing treatment by remote actuation and on-site drug release. Before *in vivo* deployment, these systems need to be evaluated in representative model systems. Chapter 2.0 discusses a perspective of organon-a-chip systems as fluidically based, physiological systems for microrobot development. In Chapter 3.0, we expand on this perspective and discuss the development of a cardiovascularinspired on-a-chip platform for magnetic microrobot locomotion, actuation, and permeation of a representative biological barrier. These parameters are essential to a microrobot control scheme to ensure precise actuation towards the target location.

In this chapter, a demonstration of targeted antibiotic delivery by hydrogel microrobots is described. Agar-based, hydrogel microrobots are first characterized for their ability to carry and release tetracycline; an antibiotic used clinically to treat acne and pulmonary infections. Then, tetracycline-laden microrobots are used to deliver bacteriostatic concentrations to gel encapsulated bacteria as a model of infection. Ultimately, tetracycline-laden microrobots are deployed in a 3D, biologically active on-a-chip (biochip) platform to both prevent and treat bacterial growth. This research potentiates the future use of on-a-chip systems to model hydrogel microrobots as targeted drug delivery systems.

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4.1 Introduction

Conventional forms of drug delivery rely on systemic administration of high drug concentrations in recurring doses [112]. The rationale for this approach is that a high circulating drug concentration will eventually affect the intended site. This approach is problematic for treatment with antibiotics as overuse contributes to high rates of resistance and off-target toxicity [7-9]. For example, it has been shown that each additional day of treatment past clinical cure increases the risk for antibiotic resistance by 5% [9]. On average, infection is resolved within 3-5 days yet antibiotic treatments are typically prescribed for 7-14 days. This is especially problematic for biofilm-related infections as conventional treatment with antibiotics more often leads to recalcitrance and infection recurrence than biofilm eradication due to the excreted biofilm layer affording bacterial protection [126, 127]. With biofilm-related infections accounting for 80% of clinical cases [128], it is increasingly evident that antibiotic treatment could be better served by localizing antibiotic effects to the site of infection.

Future targeted drug delivery is envisioned to be materials-based systems that enhance drug effect and modulate drug faults [5]. Drug-laden microrobots enable noninvasive and remote access to hard-to-reach locations [15], which could eliminate the need for systemic administration of therapeutics (Figure 4.1A). Future microrobots for targeted drug delivery should be soft, controllable vehicles with the capacity to overcome biological barriers and effectively deliver therapeutic cargo. Hydrogel-based microrobots are promising as drug delivery materials due to their tunable physical and mechanical properties that afford structural stability and cargo retention [112]. Hydrogels are porous, biocompatible, polymeric structures that can be tailored by polymer architecture to control drug release.



Figure 4.1. Biochip platform for microrobot-mediated targeted drug delivery. A) Drug-loaded microrobots show promise over conventional drug delivery by localizing drug release to the disease target, enhancing drug effect, and minimizing off-target side effects. B) A biochip platform serves as a minimal model of disease for *in vitro* targeted drug delivery by hydrogel-based microrobots.

In this research, we demonstrate that existing hydrogel microrobots can carry clinically relevant therapeutics and permeate representative biological barriers for proof-of-concept targeted drug delivery (Figure 4.1B). First, we demonstrated that agar hydrogel-based microrobots are capable of absorbing and releasing antibiotic cargo into the local environment. Next, we identified the minimum inhibitory concentration (MIC) of tetracycline necessary for bacterial growth inhibition and confirm that the hydrogel microrobots can deliver bacteriostatic concentrations. Wild-type *E. coli* was selected as a model microbe due to its association with biofilm formation [128]. The culmination of these findings led to a demonstration of hydrogel-based, magnetic microrobots as drug delivery biomaterials for targeted antibiotic delivery in a biologically active,
on-a-chip (biochip) platform. This research suggests the translational potential of hydrogel-based, magnetic microrobots can be realized through the marriage of microrobotics with on-a-chip technologies.

4.2 Materials and Methods

4.2.1 Materials

Agar (Fisher BioReagents, BP1423-500). Iron (II, III) oxide (Sigma-Aldrich, 637106-100G). Translucent photopolymer resin (Elegoo). Tetracycline hydrochloride (Sigma Aldrich, 31741-250MG). LB Broth, Miller (Fisher BioReagents, BP1426-500). PBS, pH 7.4, 10X (Life Technologies, 70011-044). Tween ® 80 (Fisher BioReagents, BP338-500).

4.2.2 Antibiotic Loading of Hydrogel Microrobots

Drug loading of hydrogel-based microrobots has been previously accomplished through incubation in a concentrated drug solution [19, 115, 129]. In these experiments, hydrogel-based, magnetic microrobots were individually submerged in concentrated tetracycline solution to allow the antibiotic to infuse the mesh hydrogel structure. Microrobot loading was accomplished by a 1-hour static incubation at room temperature in a 200 μ L aliquot of 5 μ g/ μ L tetracycline-HCl unless otherwise specified. A fine metal dental pick was used to transfer the microrobot between storage and loading solutions. In between transfers, a pipette was used to manually remove excess liquid from the microrobot.

4.2.3 Antibiotic Concentration Quantification

Tetracycline has been previously quantified by UV spectroscopy [130]. Tetracycline concentration in solution was therefore measured by absorbance at a 343 nm wavelength (Abs₃₄₃) and interpreted as a concentration by a standard curve (Figure 4.2). A standard curve was generated by pipetting an 11-step 1:2 serial dilution from 1000 μ g/mL tetracycline-HCl across a 96 flat bottom microwell plate to a final volume of 100 μ L per step. Solutions were immediately mixed after each transfer by manual pipetting 3x. The 12th step of 1x PBS was included for endpoint analysis. The serial dilution was performed in triplicate and measured for Abs₃₄₃ using a BioTek Synergy HT plate reader (Agilent Technologies, Inc). Data points were fit with a linear regression using Matlab (version R2022a).



Figure 4.2. Standard curve of absorbance at 343 nm versus tetracycline concentration.

4.2.4 Bacterial Culture of Wild-type E. Coli (MG1655)

In this research, a laboratory-evolved strain of wild-type K12 *E. Coli* MG1655 (Coli Genetic Stock Center, 8237) was grown from ice and cultured in liquid culture media. An ice chip was removed from a -80 °C freezer and added to a 17 x 100 mm culture tube (VWR, 60818-725) containing sterilized Luria broth (LB). The culture tube was incubated at 37 °C for \geq 12 hours and constant shaking at 200 rpm in a shaking incubator (Barnstead Lab-Line, MaxQ 5000). The culture tube was incubated until visible bacterial growth was observed, indicated by culture opacity. Once culture growth was observed, 5 µL of the culture was transferred to a new culture tube containing 5 mL of sterilized LB. Bacteria were grown to an exponential growth phase, confirmed by UV spectroscopy (Appendix B Figure 1), before experimentation.

Bacteria grown in semisolid agar gel were first grown in liquid culture media as described above prior to transfer to the semisolid agar gel. Semisolid agar gel was prepared by mixing LB culture media with 0 - 1 % (w/v) agar and sterilizing by autoclave. Once cooled to the touch, a serological pipette was used to dispense 20 mL sterilized semisolid agar gel into 60 x 15 mm polystyrene Petri dishes (Fisher Scientific, AS4052). Petri dishes were allowed to cool for 6 hours before bacterial inoculation with 2 µL of MG1655. Before inoculation, the mid-log growth phase of the bacterial culture was confirmed by OD₆₀₀. Culture stabs of MG1655 were either centered or spaced equidistant from the center of the Petri dish and were dispersed throughout the depth of the semisolid agar gel. Bacterial growth was confirmed by image processing (Section 4.2.6).

4.2.5 Minimum Inhibitory Concentration (MIC) of Antibiotic

Wild-type *E. coli* growth in the presence of tetracycline was quantified by a micro-dilution assay [131](Figure 4.3A). A 100 μ g/mL tetracycline-HCl stock solution was prepared and used to perform a 1:2 serial dilution in LB across a 96 flat bottom microwell plate. The serial dilution generated tetracycline concentrations from 0 – 40 μ g/mL at a total volume of 150 μ L per well. The 12th step of 1x PBS was included to represent bacterial growth without antibiotic treatment (Appendix B Figure 1). Next, 1.5 μ L of wild-type *E. coli* culture was transferred from a liquid culture in the mid-log growth phase into each well of the microwell plate. Mid-log growth phase was confirmed by OD₆₀₀ before transfer into the microwell plate containing tetracycline. The microwell plate was measured for OD₆₀₀ every 10 minutes over 24 hours (Figure 4.3B).



Figure 4.3. Minimum inhibitory concentration (MIC) of tetracycline on MG1655 growth. A) Characterization of MIC of tetracycline for wild-type *E coli* strain, MG1655. B) MG1655 bacterial growth in the presence of tetracycline concentrations from $0 - 40 \mu g/mL$ over time.

4.2.6 Imaging and Image Processing

Images for bacterial growth quantification were acquired using an Alvium 1800 U-500c camera with a 6 mm fixed focal length lens (Edmunds Optics, 8.5 mm/F1.3, 86599). Top-down images were taken of growth in 60 x 15 mm Petri dishes (Appendix B.2) and bottom-up images

were taken of growth in the U-channel on-a-chip device (Appendix B.4). Images were acquired using Vimba Viewer (Version 2.4.0) with image settings set at Exposure: 938544.38 μ s, Gain: 2.4 dB and White Balance: 1.27_{RED}, 2.18_{BLUE}. Culture growth was represented as bacterial plume opacity. Time-lapse images were binarized and then quantified by the number of true pixels, corresponding to opacity, and expressed as a percent of the number of total pixels in the image. Inhibition of growth was quantified by the number of false pixels, corresponding to transparency, and expressed as a percent of total pixels. All images were quantified by Matlab (version R2022a) computer vision. Binarized images for the experiments described in this chapter are included in Appendix B.2.

4.3 Results and Discussion

4.3.1 Hydrogel-based Microrobots Retain Liquid Cargo

Hydrogels have been exploited as drug delivery materials in the form of injectables [132-134], untethered mobile systems [115, 135], and antibiotic-loaded scaffolds [136, 137]. In a wave of recent hydrogel material developments [138-143], agar has been added to hydrogel composites to enhance mechanical stability and drug carrying capabilities. Agar is a non-toxic hydrogel derived from algae that has a high absorption capacity, reversible gelling behavior, and has been approved by the FDA as a medical material [139, 141, 144]. Agar-based, hydrogel microrobots embedded with magnetic microparticles have been recently manipulated by external magnetic fields to transport cell and chemical cargo [19] and navigate tortuous microfluidic paths [114]. As a demonstration of their mechanical stability, agar-based microrobots were employed as mechanical tools capable of drilling and navigation within extracted root canals to eradicate biofilms [117]. While this recent effort demonstrated mechanical stability and potential catalytic action of agar-based microrobots, their ability to transport therapeutics has not yet been defined.

To anticipate the therapeutic loading capacity of agar microrobots, the volume of liquid cargo retained by each microrobot was first defined. The agar-based, magnetic microrobots used in these experiments were composed of 2% (w/v) agar and 10% (w/v) iron oxide nanopowder, measured 1.0 x 4.4 mm overall, and featured a bioinspired helical shape for swimming [114] (Figure 4.4A). Microrobot fabrication by a molding process is described earlier (Section 3.2.5). Once cast, the microrobots maintained their shape while hydrated but were susceptible to dehydration out of solution (Figure 4.4B). The mass of hydrated versus dehydrated microrobots was used to quantify the liquid cargo carrying capacity of these hydrogel structures (Figure 4.4C). To measure these values, microrobots were transferred from PBS to an analytical balance (OHAUS Adventurer, AX124) using a metal dental pick and allowed to dehydrate for 1 hour. A pipette was used to remove excess liquid from the microrobots and the net mass lost was assumed to be equal amongst the microrobots. The dehydration experiment was performed in triplicate using 6 microrobots from 3 different fabrication batches.



Figure 4.4. Hydrogel-based microrobots retain liquid cargo.A) Hydrogel microrobots in solution. B) Brightfield images reveal hydrogel microrobots i) retain helical shape when hydrated but ii) are susceptible to dehydration out of solution (scale bars = 500 μ m). C) Mass difference between hydrated and dehydrated microrobot states exposes an average liquid cargo carrying capacity of 1.083 μ L per hydrogel microrobot. Error bars are representative of 1 standard deviation about the mean (n = 3).

4.3.2 Microrobots Locally Release Antibiotics

Molecular movement into and out of hydrogels in aqueous environments is largely dominated by diffusion [113]. This means that the porous nature of the hydrogel-based microrobot allows for spatial and time-dependent dissemination of infused substances. Molecular diffusion of chemical cargo by agar-based microrobots has been previously demonstrated [19]. In this work, an agar-based microrobot was loaded with dye and the local release of retained dye into an aqueous environment was resolved over time. Using this same approach, the therapeutic carrying capacity of agar-based microrobots was investigated.

Tetracycline, an antibiotic used clinically in the treatment of acne and pulmonary infections [145-149], was selected as a model drug in this work due to clinical relevance and welldocumented resistance. Microrobot loading and release capacity of tetracycline was quantified by measuring released tetracycline into solution over time (Figure 4.5A). First, an agar-based microrobot was incubated in a concentrated tetracycline solution as described in Section 4.2.2. Once loaded, the tetracycline-loaded microrobot (tet-microrobot) was transferred to a 300 μ L aliquot of deionized (dI) water. Tetracycline release was tracked by measuring the absorbance every 15 minutes over the course of 2 hours. At each time point, a 100 μ L aliquot was removed from the solution for measurement and replaced with fresh dI to model sink conditions [150]. This volume was selected to achieve a significant sample to be detectable by UV spectroscopy while not disturbing the microrobot. After all time points were collected, Abs₃₄₃ was measured on a BioTek Synergy HT plate reader (Agilent Technologies, Inc). An absorbance versus concentration curve was generated (Figure 4.2) to interpret the measured absorbance of tetracycline in solution as a concentration of tetracycline. Tetracycline total loading and estimated release kinetics are interpreted from a plot of the cumulative concentration released into solution over time (Figure 4.5B). This plot reveals that tetracycline is released at a steady rate over 90 minutes, with the total cargo load being released into solution within 120 minutes. Additionally, the cumulative concentration of 45.7 μ g/mL of tetracycline released into the solution at 120 minutes corresponds to a total mass of 4.57 μ g of tetracycline. This value is only a 15.7 % deviation from the expected mass of 5.42 μ g tetracycline to be retained by a microrobot loaded by incubation in 5 μ g/ μ L tetracycline (Section 4.2.2) with a liquid carrying capacity of 1.083 μ L (Figure 4.4C). These discrepancies are likely due to composition inconsistencies amongst microrobots due to an imprecise fabrication procedure that is not considered to affect the scope of this research (Section 3.2.5).



Figure 4.5. Antibiotic cargo is released from hydrogel-based microrobots in solution.A) Hydrogel microrobots retain and release tetracycline into solution over time. B) Measured tetracycline released into solution over time. Data is background corrected by subtracting the absorbance of 100 μ L of dI from each measured value. Error bars are representative of 1 standard deviation about the mean (n = 3).

The antimicrobial action of tetracycline is accomplished by blocking protein synthesis and thus inhibiting cellular proliferation and viability [151]. Therefore, the lack of cell growth can confirm the presence of tetracycline in a bacterial environment. Bacterial growth inhibition proximal to the location of a polymer, termed the zone of inhibition (ZOI), is an empirical practice of assessing the antimicrobial activity of therapeutic polymers [152]. Previously described methods of determining the ZOI are limited, however, by only considering antimicrobial activity across a 2D surface. To resolve both the spatial and temporal distribution of antibiotics from tet-microrobots, a ZOI in 3D semisolid gel-encapsulated bacteria was investigated (Figure 4.6A).

Gel-encapsulated bacteria were used as a culture condition to measure both cell growth and the spatial and temporal distribution of antibiotics from a tet-microrobot. Semisolid agar gel has been used in motility assays as a constrained, gelatinous environment through which motile bacteria can migrate [153, 154]. The composition of semisolid agar gel containing 0.35 % agar was selected to resolve gradual culture growth with visual resolution over 25 hours. This condition was informed by a preliminary experiment of wild-type *E. coli* (MG1655) growth in 0-1% agar over time (Appendix B Figure 2 and Appendix B Figure 3). Culture growth and the ZOI surrounding a tet-microrobot can be compared to an untreated culture to assess antibacterial activity over time (Figure 4.6B).



Figure 4.6. Local antibiotic delivery by tet-microrobots.A) Resulting zones of inhibition (ZOI) are measured to assess antimicrobial activity. B) ZOI observed surrounding tetracycline-laden microrobot compared to an untreated culture in 0.35 % semisolid agar media over time. The visible separation between culture plumes is expected to be compressed agar gel resulting from the expansion of rapidly growing bacterial cultures. C) MG1655 growth of treated and untreated cultures are expressed as a percent of total culture area over time. D) Differential culture growth of treated culture is further explained by the region of no growth, or ZOI, that is apparent in the vicinity of the tet-microrobot.

The distribution of tetracycline was assessed radially and throughout the depth of the semisolid agar gel by culture inoculation by 3 culture stabs that were uniformly distributed across each Petri dish. The Petri dishes of semisolid agar gel-encapsulated bacteria were imaged at 5hour intervals over 25 hours and quantified by computer vision (Appendix B Figure 4). Overall culture growth at each time point was quantified by the percent of culture area coverage of the bacterial plumes and expressed as a percent of total culture area (Figure 4.6C). The growth observed in the untreated culture demonstrated exponential growth between 5 - 15 hours that visibly saturated the total area of the Petri dish by 25 hours. This condition was used as a control to compare the growth rate observed in the treated condition which was exposed to a tetmicrorobot. The treated condition seemed to experience the same growth rate, however, was limited to < 80 % of the total culture area by the tet-microrobot. The inhibited growth quantified by the bacterial culture plume area was corroborated by the percent of inhibited growth corresponding to the ZOI surrounding the tet-microrobot in the treated condition (Figure 4.6D). Quantification of the ZOI over time revealed that the tet-microrobot was capable of inhibiting growth to 26.9 % of the total culture area in 0.35 % semisolid agar gel. These results corresponded to a ZOI diameter of 13.93 mm and ultimately reveal that the 1.0 x 4.4 mm tet-microrobot distributed 1.083 µL of therapeutic cargo to 0.97 mL of the local environment.

4.3.3 Microrobots Deliver Bacteriostatic Concentrations of Antibiotics

Prior to clinical use, microrobots need to demonstrate that they can load and unload effective concentrations of therapeutic cargo [17]. Antibiotic treatment of biofilm infection requires 10-1000 times the concentration of antibiotic as is needed for treatment of the planktonic bacteria [127]. Such concentrations are difficult to achieve *in vivo* by conventional treatment

schemes. To model antibiotic treatment of biofilm infection, bacterial tolerance to the specific antibiotic must first be identified and biofilm penetration must be demonstrated.

Effective treatment concentrations for gel-encapsulated bacteria were identified through an experiment that measured the minimum inhibitory concentration (MIC) of tetracycline for bacteriostatic activity (Figure 4.7A). The MIC of tetracycline on wild-type *E. coli* growth was determined through a micro-dilution assay (Section 4.2.5). Bacterial growth in the presence of tetracycline was then normalized by total growth at 8 hours to reveal overall growth with tetracycline concentration (Figure 4.7B). The similarity between bacterial growth in tetracycline concentrations of $\leq 0.1 \,\mu$ g/mL to the growth of untreated bacterial culture suggested the bacteria were tolerative of these concentrations of tetracycline. This response was different from the growth observed under $0.1 < 5 \,\mu$ g/mL tetracycline concentrations where bacteria appeared to be susceptible to increased concentrations of tetracycline. And ultimately, no growth was observed in tetracycline concentrations of $\geq 5 \,\mu$ g/mL meaning that 5 μ g/mL was identified as the MIC of tetracycline for wild-type *E. coli* growth.

The results of the MIC experiment were expected to apply to the treatment of gelencapsulated bacteria and were confirmed by differential treatment of gel-encapsulated bacteria by microrobots loaded with tolerative, susceptible, and inhibitive concentrations of tetracycline. These results were visualized by hydrogel microrobot loading with concentrations of tetracycline that corresponded to tolerated ($\leq 0.1 \ \mu g/mL$), susceptible ($0.1 < 5 \ \mu g/mL$), and inhibited ($\geq 5 \ \mu g/mL$) bacterial growth. To achieve tetracycline treatment conditions corresponding to the ranges, hydrogel microrobots were incubated in concentrated tetracycline solutions informed by the microrobot loading capacity (Figure 4.4C), and the resultant ZOI in 0.35 % semisolid agar gel (Figure 4.6D). The effective loading concentrations were determined to be 0.13, 3.34, and 6.68 $\mu g/\mu L$ and were calculated by:

$$Concentration_{load} \left[\frac{\mu g}{\mu L} \right] = \frac{Concentration_{released \ tetracycline} \left[\frac{\mu g}{mL} \right] * ZOI_{microrobot} \ [mL]}{Load \ capacity_{microrobot} \ [uL]}$$
(4-1)

The results with the gel-encapsulated bacteria replicate the results seen in the MIC experiment in that the bacteria exposed to tolerative levels of tetracycline are tolerated while the inhibitive levels of tetracycline inhibit bacterial growth (Figure 4.7C). Bacteria cultures treated with tolerated ($\leq 0.1 \ \mu g/mL$) concentrations were expected to not show altered growth from an untreated group while cultures treated with susceptive ($0.1 < 5 \ \mu g/mL$) and inhibitive ($\geq 5 \ \mu g/mL$) concentrations of tetracycline were expected to each affect a resultant ZOI, with bacterial growth restricted to a larger area in the cultures treated with inhibitive levels of tetracycline. The bacteria treated with susceptive concentrations demonstrated stressed growth, however, were less restricted than the bacteria treated with inhibitive tetracycline concentrations (Figure 4.7D – E). These results were compared to control conditions (Appendix B Figure 9) to confirm that the resulting ZOIs from the tet-microrobots are a result of the tetracycline in the microrobot and not the microrobot composite material or its components. These results showcase tunable loading of hydrogel-based microrobots to affect regional bacterial growth and highlight the flexibility afforded by hydrogel materials as tailored drug delivery materials.



Figure 4.7. Microrobots deliver bacteriostatic concentrations of tetracycline. A) Microrobots loaded with tolerative, susceptive, and inhibitive levels of tetracycline are expected to have a differential effect on bacterial growth. B) Normalized bacterial growth by tetracycline concentration results observed in the previous MIC experiment (Figure 4.3). Error bars are representative of 1 standard deviation about the mean (n = 3). C) Microrobots deliver tolerative, susceptive, and inhibitive levels of tetracycline to MG1655 in semisolid agar gel over time. D) Quantification of bacterial growth and E) resultant ZOI in the presence of differential tetracycline concentrations.

4.3.4 Targeted Antibiotic Delivery by a Microrobot in a Biochip Platform

Future *in vivo* deployment of microrobots for targeted drug delivery is dependent on proofof-concept testing *in vitro* [17]. Towards this end, we developed a biochip platform in which drugladen microrobots could be actuated towards a biofilm infection to locally release antibiotics as a model for targeted drug delivery (Figure 4.8A). Biofilm-related bacterial infections in the body are composed of a bacterial colony contained within an excreted polymeric biofilm layer that provides protection for infection perpetuation [127, 128]. A biofilm infection was recapitulated by the inoculation of a semisolid agar gel with wild-type *E. coli* within our previously described Uchannel device (Section 3.2.3). Briefly, this device serves as an arena for standardized testing of magnetic microrobot actuation and biomedical functionality (Chapter 3.0). Once inoculated, this device acted as a minimal cardiovascular disease model for microrobot-mediated targeted tetracycline delivery.

To effect treatment of the model of infection, a tet-microrobot was loaded with 5 μ g/mL tetracycline and expected to locally release an inhibitive concentration of tetracycline at the target biofilm site. This loading concentration was informed by the 1 mL volume of the device (Section 3.2.4) and the tetracycline concentration required for bacterial growth inhibition (Figure 4.7C).

Once loaded with tetracycline, the microrobot was introduced to the cardiovascular disease model and magnetically actuated toward the target location. Microrobot-mediated targeted drug delivery for both prophylactic and therapeutic treatment of bacteria was quantified over time. In the prophylactic condition (Figure 4.8B), a tet-microrobot was deployed at the time of culture inoculation to model preventative treatment. As a model of therapeutic intervention (Figure 4.8C), the tet-microrobot was deployed at 5 hours after inoculation. In each treatment condition, the U-channel devices were incubated at 37 °C and repositioned on the stage for imaging every 5 hours to track bacterial growth.

The results depict a visual proof-of-concept demonstration of targeted drug delivery with minimized off-target effects by hydrogel-based, tet-microrobots. This conclusion was determined from computer vision-based analyses of gel-encapsulated bacterial culture in the treated and untreated models of infection over time (Appendix B.4). The results of the prophylactic treatment condition revealed mitigated bacterial growth in the target, tet-microrobot treated infection compared to the uninhibited growth observed in the off-target model of infection (Figure 4.8D). These results represent a clinical case of early diagnosis and treatment to prevent infection. This case justifies the use of targeted drug delivery of antibiotics to prevent downstream infection, such as the risk of cardiac infection during oral surgery [155, 156]. Moreover, therapeutic treatment was also demonstrated by the deployment of a tet-microrobot after initial culture development (Figure 4.8E). This treatment condition represents targeted therapeutic treatment of an ongoing infection which is consistent with the conventional prescription of antibiotics [6]. In both cases, the bacteriostatic effect in the target culture is significantly improved over the growth observed in the off-target culture, which appears to be unaffected by the presence of antibiotics in the fluidicallyconnected system.



Figure 4.8. Microrobot-mediated targeted tetracycline delivery to semisolid gel-encapsulated bacteria over time. A) Schematic describing the experimental procedure. i) Wild-type *E. coli* was first inoculated into the semisolid gel retained within the U-channel biochip. ii) A tet-microrobot was then introduced to the inlet at the apex of the U channel and magnetically actuated towards the target culture and iii) time-lapse imaging was used to quantify culture growth over time. B) Bottom-up images of prophylactic antibiotic delivery over time. C) Bottom-up images of therapeutic antibiotic delivery over time. Computer vision was used to quantify culture growth over time at the apex of the D prophylactic and E) therapeutic treatment conditions.

Further analysis of these results advocates a need for targeted drug delivery of antibiotics by targeted drug delivery systems. While rudimentary in complexity and physiological representation, the comparison of bacterial growth in the off-target culture of the therapeutic condition compared to the off-target culture in the prophylactic condition resolves off-target side effects observed in typical antibiotic treatment [7-9]. The slight delay in the growth of the offtarget prophylactic culture could be indicative of the off-target delivery of tetracycline to that culture prior to exponential culture growth. The recovery of that culture by the next time point compared to the target prophylactic culture proves that most of the loaded tetracycline still had the intended effect on the target culture. This is confirmed by the unaffected growth observed in the target therapeutic off-target colony. Furthermore, the steep decline in growth observed in the target therapeutic delivered in the target there may be sufficient tetracycline delivered in this system to enact a bactericidal effect in addition to the bacteriostatic effect observed in previous experiments. This is a promising result in support of the use of targeted drug delivery systems for antibiotics for treating biofilm infections.

4.4 Conclusion

In conclusion, microrobot-mediated targeted drug delivery in an on-a-chip platform is the next step towards the translation of hydrogel-based, biomedical microrobots. Agar-based hydrogel microrobots have previously shown promise as untethered microrobots and we envision the possibility for them to serve a dual role as targeted antibiotic delivery materials. In this research, the agar-based microrobots were shown to be capable of carrying and delivering antibiotics into

the local environment. Furthermore, they could be loaded with tolerative, susceptive, and inhibitive concentrations of tetracycline for the differential treatment of gel-encapsulated bacteria as a model of infection. Using our previously developed platform for standardized microrobot testing, tet-microrobots were able to deliver effective concentrations of antibiotic prophylactically and therapeutically to prevent bacterial growth in a target location while mitigating off-target effects in fluidically connected models of infection.

Upon further development, this research can be expanded upon to reveal the targeted drug delivery potential of microrobots. Using the described actuation platform, hydrogel-based, magnetic microrobots can be engineered with targeting moieties and controlled release of drugs in response to specific stimuli [5, 157]. Additionally, this platform could be used to model cooperative microrobot behaviors for consistent target engagement of resilient models of infection [5, 158]. This characterization is an effort towards the long-term goal of enabling therapeutic access and effective drug delivery to otherwise unreachable disease sites of interest using untethered, mobile, drug delivery materials.

5.0 Conclusion and Outlook

The research proposed here is intended as the next step toward developing a physiologically relevant model system in which microrobots for targeted drug delivery can be tested. An ideal model system would enable testing and development of precise control for actuation in 3D environments [116], be able to overcome biological and disease barriers [107], and demonstrate local delivery of therapeutic cargo [5]. This platform incorporates an on-a-chip system that can simultaneously provide a physiologically similar microenvironment and swimming arena in which microrobots can interact with relevant biological components and optimize locomotion, actuation, and biomedical functionality. As discussed in Chapter 3.0, our platform addresses current obstacles in microrobotic research by creating user interfaces that are enabled by real-time visualization. This system, while simple, is one step away from achieving closed-loop, machine learning-based microrobot control for precise positioning [109]. Chapter 4.0 explores the platform and U-channel device as an *in vitro* model for microrobot-mediated targeted drug delivery. By localizing drug diffusion to a target site in a multisegmented fluidic arena, hydrogel-based magnetic microrobots can deliver bacteriostatic, and possible bactericidal concentrations of tetracycline to gel-encapsulated bacteria as a model of infection. The adaption of this platform can elucidate microrobot control schemes and treatment schemes in an effort towards personalized, precision medicine.

Before incorporation as a steppingstone in the clinical development pipeline, an *in vitro* model that mimics and predicts biomedical microrobot performance *in vivo* need to be identified and validated against traditional empirical methods [108]. This process of validation will rely on the interdisciplinary cooperation amongst bioengineers, data scientists, and physicians to identify

essential design criteria to guide iterations of this standardized testing platform. We envision the first criterion for success in the design-test-build cycle is the ability for this platform to serve as a standard swimming arena for existing advances in microrobot design, composition, and actuation techniques for locomotion. To accomplish this, existing microrobot design and actuation advances, such as the needle-like scaffold [104] that was described in Section 3.1, should be actuated in the U-channel platform. The resulting microrobot translational velocities of the needle-like scaffold in the U-channel platform can be compared to the velocities generated in the original microfluidic testbed for this microrobot design. These results will inform a database of microrobot performance that could be directly compared between trials and against other experimental data. A performance database informed by state-of-the-art advances across research groups can then be used to inform the next design criterion for the U-channel platform.

To be predictive of *in vivo* performance, a sequential iteration of the U-channel platform would also need to be proven to maintain sufficient biological complexity to be truly considered a minimal model of disease [108]. An example of the biological complexity not currently captured by the proposed biochip platform is the dynamics of the body. For actuation through the circulatory system, microrobots would need to be able to withstand hemodynamics, peristalsis, and arterial pulsation [15]. A possible option to capture the dynamics of cardiovascular fluid flow in the biochip platform could include integration with low-volume liquid handlers that could be adapted to generate hemodynamic-like flow patterns in microfluidic systems [72, 73]. A process for identification and prioritization of essential biological components will need to be established through collaborative efforts and used to inform iterations of a standardized platform.

5.1 Integration with Multi-scale Robotics for Automated Precision Medicine

We share anticipation with pioneers in the fields of organ-on-a-chip systems that highthroughput bioengineering made possible by modularity and robotics will ignite new potential for eradicating disease. Following in the steps of Don Ingber and colleagues [101], we envision the next iteration of a fully automated, robotic system for biochip monitoring and support. A highthroughput screen for precision medicine could be realized by creating a closed-loop feedback system between robotic tools for observation, manipulation, and processing. Using real-time visualization of a biochip, or array of interconnected biochips, an on-a-chip platform could be monitored for biological indicators of disease and treatment. Our vision for an array of organ-ona-chip devices to create a human-on-a-chip system is described in Chapter 2.4. A machine learning algorithm could then process images by computer vision and feed information into a liquid handling robot for culture support or procedural action.

This sort of system could be enhanced by integration with the efforts that resulted from the US Precision Medicine Initiative to develop a genomic knowledge network to inform drug design and clinical treatment of subcategories of disease [3, 4]. Since then, a large thrust in precision medicine has been genome sequencing for DNA-based therapeutic targeting, already showing particular promise for cystic fibrosis, precision oncology, and pharmacogenomics [1]. Capitalizing on the genome sequencing, analysis algorithms, and disease understanding that has improved in throughput and accuracy over the past decade, these two systems could be synced to update conditions in real time. In the future, an iteration of this platform can be used as a building block toward a fully automated, predictive screening system for patient and disease-specific testing, bringing new opportunities to the field of precision medicine.

5.2 Platform for Automated Microrobot Positioning and Triggered Release

This platform could also be used as a baseline to elucidate more specific targeted drug delivery mechanisms for microrobots such as triggered release or the delivery of engineered cell therapies. Ongoing efforts in microrobotics emphasize adaptive locomotion [47], functionalization for target recognition [51, 52], and algorithm-based control [58, 105] for precision medicine, however, these focuses lack an adequate platform to assess swimming and biomedical function performance. The presented platform was designed as a simple, user-friendly interface, but to be amenable to vision-based, machine-learning algorithm development. Our group has prior experience magnetically manipulating helical magnetic microrobots through simple, curved microfluidic paths [114]. In this setup, a machine learning algorithm is employed to optimize helical, magnetic microrobot swimming along an unfamiliar trajectory within a circular microfluidic arena. This work could be enhanced by merging it with the capabilities of this on-achip platform. By providing a similar machine learning algorithm with microrobot position information informed by real-time visualization, the machine could optimize control schemes that would control the magnetic field as an output. This would be the next step towards realizing fully automated microrobot actuation through physiologically relevant systems.

The emphasis on real-time visualization afforded by this platform is also amenable to optics-based experimentation which is beneficial for biological development. For example, synthetic biology shows promise for microrobot functionalization and triggered release and is well-known to rely heavily on fluorescence-based outputs. The field of synthetic biology has evolved to be able to engineer cellular behaviors such as sense and response [159, 160], motility [161], environmental sensing [162], and the formation of 3D structures [160], that can be modeled in 3D, on-a-chip cultures. Synthetic biology can also be employed as a tool for drug-microrobot

conjugation to explore triggered drug release options, such as a light-cleavable linker, [163], or be delivered as a therapeutic tool itself [164]. In the case of biofilm infections, CRISPR gene editing techniques have persevered as treatment options over the conventional delivery of antibiotics [128]. These suggestions are by no means an exhaustive list, but instead a conversation starter for the potential afforded by interdisciplinary research efforts.

Appendix A Supplement for Standardized Platform

This section contains supplementary material and explanatory detail to enhance the research presented in Chapter 3.0 On-a-Chip Platform for Standardized Biomedical Microrobot Functionality Testing.

Appendix A.1 Supplementary 3D Parts Files

The platform magnet and cart were 3D printed with the following 3D parts files.

- 1. track cart.stl
- 2. track cart clamp.stl
- 3. <u>90 degree track.stl</u>
- 4. <u>90 degree track pinion.stl</u>
- 5. <u>6mm shaft clip.stl</u>
- 6. <u>magnet clamp.stl</u>
- 7. rotating magnet bracket.stl
- 8. <u>sample holder.stl</u>

Appendix A.2 Arduino program for actuation of magnetic cart

```
//add required libraries
   #include <AccelStepper.h>
   #include <Timer.h>
```

```
//initialize constructs
     Timer t1;
     AccelStepper stepper1(1, 8, 9);
     AccelStepper stepper2(1,10,11);
     int printTiming = 200;
     int tPos = 0;
     int d2q = 0;
     int cp = 0;
     char rawData[100] = "";
     char keyword[] = "";
     char theText[20];
     float velocity = 0.0;
     float rotation = 0.0;
     float cartPosition = 0.0;
     int startRun = 0;
//initialize global variables
     int stepperSpeed = 50;
//initial setup
     void setup()
     {
       stepper1.setMaxSpeed(20000);
       stepper1.setCurrentPosition(0);
       stepper1.setSpeed(6000);
       stepper2.setMaxSpeed(6000);
       stepper2.setSpeed(6000);
       Serial.begin(9600);
       while (!Serial) {
         ;
       }
       t1.every(printTiming, printReading,0);
     }
//main loop
     void loop()
     {
       t1.update();
       getCommand();
       if (d2g == 0) {
          stepper2.setSpeed(0);
       }
       else {
         stepper2.setSpeed(rotation);
       }
```

```
stepper2.setSpeed(rotation);
       stepper2.runSpeed();
       stepper1.setSpeed(velocity);
       stepper1.moveTo(cartPosition);
       stepper1.runSpeedToPosition();
     }
     void printReading()
     {
       d2g = stepper1.distanceToGo();
       cp = stepper1.currentPosition();
       String Output = String("v:" + String(velocity) + ", w:" +
String(rotation) + ", pos:" + String(cartPosition) + ", cp:" +
String(cp) + ", d2g:" + String(d2g));
       Serial.println(Output);
     }
     void getCommand()
     {
       if (Serial.available() > 0)
       { //new data in
         size t byteCount = Serial.readBytesUntil('\n', rawData,
     sizeof(rawData) - 1); //read in data to buffer
         rawData[byteCount] = NULL; //put an end character on the
     data
//Serial.print("Raw Data = ");
//Serial.println(rawData);
//now find keyword and parse
         char *keywordPointer = strstr(rawData, keyword);
         if (keywordPointer != NULL)
           int dataPosition = (keywordPointer - rawData) +
     strlen(keyword);
           const char delimiter[] = ",";
           char parsedStrings[3][50];
           int dataCount = 0;
//Serial.print("data position = ");
//Serial.println(dataPosition);
           char *token = strtok(&rawData[dataPosition],
           delimiter); //look for the first piece of data after
           keyword until comma
           if (token != NULL && strlen(token) <
```

```
sizeof(parsedStrings[0]))
           {
             strncpy(parsedStrings[0], token,
     sizeof(parsedStrings[0]));
             dataCount++;
           }
           else
           {
             Serial.println("token too big");
             strcpy(parsedStrings[0], NULL);
           }
           for (int i = 1; i < 3; i++)
           {
             token = strtok(NULL, delimiter);
             if (token != NULL && strlen(token) <
     sizeof(parsedStrings[i]))
             {
               strncpy(parsedStrings[i], token,
     sizeof(parsedStrings[i]));
               dataCount++;
             }
             else
             {
               Serial.println("token 2too big");
               strcpy(parsedStrings[i], NULL);
             }
           }
//convert to usable variables
           if (dataCount == 3) {
             strncpy(theText, parsedStrings[0], sizeof(theText));
             velocity = atof(parsedStrings[0]);
             rotation = atof(parsedStrings[1]);
             cartPosition = atof(parsedStrings[2]);
             startRun = 1;
             Serial.println("Data Received");
             //printReading();
           }
           else
           {
             Serial.println("Arduino fault: data no good");
           }
         }
         else
```

```
{
    Serial.println("Arduino fault: sorry no keyword");
  }
}
```

Appendix A.3 U-channel Device



Appendix A Figure 1. 3D render of U-channel device. The device is shown from A) perspective, B) side, and C) bottom-up views.

A survey of 15 U-channel devices revealed that devices exhibiting an intact liquid-gel interface were the result of the correct filling of the semisolid agar gel. Upon introduction into the U-channel device, the lukewarm semisolid agar gel is restricted to the port in which it was dispensed by the capillary pressure of the air-filled channel. At a point during this process, the gravitational force exerted by the 250 μ L volume of semisolid agar gel overcomes the force of the capillary pressure, causing the liquid-air interface to infuse the channel. To achieve correct filling,

the semisolid agar gel is dispensed slowly so that the infused liquid-air interface will align with the pressure induced by the airhole. When dispensed too slowly or too quickly, the liquid-gel interface will either be retained to the dispense port or will surpass the location of the airhole, trapping air in the channel. In some cases the airhole was used to extract semisolid agar gel that had surpassed the airhole, however, this practice resulted in visible disturbance of the liquid-gel interface, and these devices were not selected for further experimentation. This survey revealed a 66.7 % correct fill average, with 6.6 % of devices experiencing under fill and 26.7 experiencing over fill.



Appendix A Figure 2. U-channel device channel filling. A) Scheme depicting the technical details of the port and channel dimensions of the U-channel device. B) U-channel devices with an intact liquid-gel interface formed the interface at a mean distance of 4.4 mm along the channel. U-channel devices with an obvious air bubble and an intact liquid-gel interface were either under filled or over filled past the air hole, observed at mean distances of 2.7 and 7.2 mm along the channel, respectively.

Appendix A.4 Magnetic Field Characterization

The magnetic field strength generated by the permanent magnet (K&J Magnetics, Inc, RX033CS-N) was characterized using a 9DOF Sensor Stick (Sparkfun, Sen-13944 ROHS) (Appendix A Figure 3A). To do so, the permanent magnet was centered on a 5 mm grid on a laboratory benchtop. The sensor stick was mounted on an Arduino breadboard and stand to keep the position of the magnet in the z-direction constant relative to the position of the center of the magnet. The sensor was then used to record the magnet. The closest measurements were restricted by the physical contact between the magnet and the sensor. At each point of measurement, the orientation of the sensor stick was kept constant relative to the magnet. For each position of measurement, the distance, *d*, was calculated by the Pythagorean theorem, $d = \sqrt{(d_x^2 + d_y^2)}$, and the total magnetic field strength, *B*, was calculated by $B = \sqrt{M_x^2 + M_y^2 + M_z^2}$. A plot of the magnetic field strength by distance (Appendix A Figure 3Appendix A.2B) follows an inverse square relationship that was defined by a power fit in Matlab (version R2022a).



Appendix A Figure 3. Magnetic field characterization. A) The magnetic field of an axially magnetized permanent magnet was characterized by measuring the magnitude of the magnetic field in the x (M_x) , y (M_y) , and z (M_z) direction within a 150 mm radius of the magnet axis. B) The total magnetic field strength, B, by distance, d, is related by B = 96.05 $d^{-1.08}$.

Appendix A.5 Microrobot Penetration of Semisolid Agar Gel

Previous studies have demonstrated that hard-bodies, helical microrobots are capable of drilling behavior in lumen-shaped environments [124, 125]. To determine if soft, magnetic microrobots are capable of penetrating semisolid gel, a preliminary experiment was conducted to measure microrobot translational velocity from liquid media and into semisolid agar gel (Appendix A Figure 4). A liquid-gel interface was formed between deionized water and semisolid agar gel by first filling a glass Pasteur pipette (Fisher Scientific, 2-183632) with deionized water. Then, the pipette was held horizontally with thumb-applied vacuum pressure to keep the water in the pipette while a micropipette was used to dispense agar solution into the narrow end of the capillary. Agar gel solutions were prepared, sterilized by autoclave, and cooled to the touch before dispensing into

the capillary. ~ 1 mL of semisolid gel solutions of 0 - 0.4 % agar were added to each glass capillary to form a liquid-gel interface halfway along the length of the capillary. The process of adding the agar gel to a water-filled capillary prevented the formation of air bubbles. The narrow end of the capillary was then capped with a small piece of PDMS to prevent leaking. The capillary rested vertically at room temperature for 1 hour to ensure the semisolid gel solution was fully cooled before microrobot penetration experiments.

Microrobot translational velocity was characterized by time-lapse imaging and Video editing software was used to report the frame time and location of the microrobot. Microrobot translational velocity starting 10 mm before the liquid-gel interface, and then along the length of the capillary was reported. Microrobot velocity was expressed as a percent of the starting velocity due to inconsistent starting translational velocities, likely due to the variable location of the liquid-gel interface along the capillary. The results show that the hydrogel-based, magnetic microrobots are capable of penetrating 0.2 % semisolid agar gel, but incapable of penetrating 0.4 % semisolid agar gel solution. These results are consistent with the drilling capabilities seen previously [124].



Appendix A Figure 4. Microrobot penetration of agar gel in glass capillaries. A) Microrobots were actuated along a glass capillary containing a liquid-gel interface at a constant angular frequency of 2 Hz and a constant magnet translational velocity of 0.72 mm/s. B) Microrobot translational velocity along liquid media, across the liquid-gel interface, and into the gel was calculated and expressed as a percent of the original translational velocity. Data points are representative of n = 3 trials for each agar solution.
Appendix B Supplement for Targeted Drug Delivery

This section contains supplementary material and explanatory detail to enhance the research presented in Chapter 4.0 Microrobot-mediated Targeted Drug Delivery in a Biochip Platform.

Appendix B.1 Wild-type E. coli (MG1655) growth

To characterize the growth phase of wild-type K12 *E. coli*, an ice chip of a laboratory evolved strain of motile MG1655 (Coli Genetic Stock Center, 8237) was cultured in a 17 x 100 mm culture tube (VWR, 60818-725) containing sterilized Luria broth (LB) culture media with constant shaking at 200 rpm and 37 °C incubation overnight (Barnstead Lab-Line, MaxQ 5000). After 12 hours, 5 μ L of overnight culture was transferred to a new culture tube containing 5 mL LB and cultured at 37 °C with constant shaking. After 6 hours, 5 μ L of the shaking culture was transferred to 5 mL LB in a new culture tube, briefly vortexed (Fisher Scientific, 15050639), and then 150 μ L was immediately transferred to a 96 flat bottom microwell plate. This was repeated in triplicate (3 x 5 μ L aliquots into 3 new culture tubes, each one aliquoted in 150 μ L volume into the same 96 microwell plate). The OD₆₀₀ of the microwell cultures was measured every 10 mins for 24 hours by a plate reader (Appendix B Figure 1). To prevent evaporation, the microwell plate lid was pretreated with 20% ethanol and 0.01% Tween 80 (Fisher BioReagents, BP338-500) and allowed to air dry under laminar airflow.



Appendix B Figure 1. Growth curve of MG1655 in liquid media over 24 hours. Measurements at each time point were background corrected by subtracting OD_{600} of LB at each time point. Error bars are representative of 1 standard deviation about the mean (n = 3).

Wild-type *E. coli* growth in semisolid agar gel (LB and agar) was characterized in 60 x 15 mm polystyrene Petri dishes (Fisher Scientific, AS4052) containing 20 mL of sterile semisolid agar gel. Briefly, LB with 0 - 1 % (w/v) agar was sterilized by autoclave. Once cooled to the touch, a serological pipette was used to dispense sterilized semisolid agar gel into Petri dishes. Petri dishes were allowed to cool for 6 hours prior to bacterial inoculation with 2 µL of MG1655 bacterial culture in the mid-log growth phase and confirmed by OD₆₀₀. Culture stabs of MG1655 were centered and dispersed throughout the depth of the semisolid agar gel. Petri dish cultures were incubated at 37 °C (VWR INCU-Line Digital Incubator 115V) and removed for imaging using a fixed focal length camera (Edmunds Optics, 8.5 mm/F1.3, 86599) at time points of 0, 5, 10, 15, 20, and 25 hours (Appendix B Figure 2).



Appendix B Figure 2. MG1655 growth in semisolid agar gel (0-1% agar) over 24 hours. Each semisolid agar gel culture condition was repeated in triplicate.

Culture growth over time was quantified by ImageJ (Version 1.52q). For each time point, the ImageJ scale was calibrated to a 10 mm segment of known length and then the diameter of the culture plume was measured at each time point (Appendix B Figure 3). Cultures grown in ≤ 0.4 % agar covered the entire culture area within 25 hours, whereas bacteria inoculated in agar concentrations of > 0.4 % were observed to collect on the surface of the gel immediately after culture inoculation and not permeate the depth of the gel. This is due to the increased viscosity of the gel conditions that prevented the addition of the culture. These culture conditions were observed to grow on the surface of the gel and not result in the formation of a culture plume.



Appendix B Figure 3. MG1655 growth versus time by agar concentration. Error bars are representative of 1 standard deviation about the mean (n = 3).

Appendix B.2 Binarized Image Processing of MG1655 Growth in Semisolid Agar Gel

Appendix B.2.1 Matlab Image Processing

Bacterial growth in semisolid agar gel was quantified over time by computer vision image processing. Still images were captured at each time point according to the description provided in Section 4.2.6. Native Matlab (version R2022a) functions were then used to convert the still images to a readable file to be quantified. Time-lapse images of a single experimental condition (e.g., Petri dish replicate) over time are referred to as series images in this description.

Series images for all experimental conditions were imported to a custom Matlab program with the function 'imread'. Excess background graphic detail was excluded from each image using 'imcrop'. Series images were cropped to the same region at each time point for time point comparison. Images were then converted to grayscale by 'rgb2gray' to eliminate hue and saturation detail that may mask luminosity pixel detail. Grayscale images were then converted to binary by 'imbinarize' by a user-defined threshold. A threshold value producing binary images that most closely resembled the visible differential opacity in the original images was selected for each experimental dataset. 'numel', 'nnz', and 'nnz(~)' functions were then utilized to count the number of true (white) and false (black) pixels in each image. True (white) pixel counts correspond to the luminous graphic information indicative of bacterial growth while false (black) pixel counts correspond to an inhibition of growth. Series image pixel counts were normalized by subtracting out the true negative and false positive values. The bright circumference of the Petri dish was considered a false positive as this detail was captured as true pixels but is not indicative of culture growth. The background surrounding the Petri dish was considered a true negative as these pixel numbers are false, however, are not in the region of interest and do not correspond to inhibition of growth. Normalized values were then averaged across experimental replicates using 'mean' and expressed as an averaged percent of the total culture area. Culture growth total area was identified by computer vision of the total true (white) pixel count of a normalized control condition, in which the total true (white) pixel count corresponded to the complete culture permeation of the Petri dish. ZOI or inhibition of growth total area was identified by computer vision of the false (black) pixel count of a normalized time 0 Petri dish, in which the total false (black) pixel count corresponded to no observable bacterial growth.

Appendix B.2.2 Binarized Image Datasets

Local antibiotic delivery by a tet-microrobot was evaluated by time-lapse imaging of the resultant ZOI over time and compared to an untreated culture (Appendix B Figure 4).



Appendix B Figure 4. Binarized images of MG1655 growth over time in the presence of a tet-microrobot compared to untreated culture.

Bacteriostatic delivery of tetracycline into the local environment was quantified by the delivery of none (Appendix B Figure 5), tolerative (Appendix B Figure 6), susceptive (Appendix B Figure 7), and inhibitive (Appendix B Figure 8) concentrations of tetracycline.



Appendix B Figure 5. Binarized images of MG1655 growth over time without treatment with tetracycline. These conditions are used as a control for MG1655 growth in the presence of a tet-microrobot loaded with tolerative, susceptive, and inhibitive concentrations of tetracycline.



Appendix B Figure 6. Binarized images of MG1655 growth over time in the presence of a tet-microrobot loaded with a tolerative concentration of tetracycline.



Appendix B Figure 7. Binarized images of MG1655 growth over time in the presence of a tet-microrobot loaded with a susceptive concentration of tetracycline.



Appendix B Figure 8. Binarized images of MG1655 growth over time in the presence of a tet-microrobot loaded with an inhibitive concentration of tetracycline.

Appendix B.3 Tetracycline-Loaded Microrobot Treatment Control Conditions

The antimicrobial potential of the individual components of a tet-microrobot were tested on gel-encapsulated bacteria to confirm that the bacteriostatic effects are due to the local delivery of tetracycline from the microrobot (Appendix B Figure 9). Conditions that did not contain agar were prepared as a slurry and pipetted on the surface of a bacteria-inoculated Petri dish in place of a microrobot. Petri dishes were incubated at 37 °C and removed for imaging at each time point.



Appendix B Figure 9. Control conditions of local delivery of tetracycline from microrobots over time.

Appendix B.4 Image processing to quantify targeted drug delivery in U-channel on-a-chip device

In each treatment condition, gel-encapsulated bacteria growth in the target culture proximal to the tet-microrobot was compared to the growth of the gel-encapsulated bacteria in the distal, off-target infection. Image acquisition and processing for culture growth quantification in the biochip platform mirror the procedures described in Section 4.2.6 and Appendix B.2.1, with the exception that the 'imcrop' function was customized for each image. This was done to capture the central region of culture growth while excluding confounding graphic detail such as the microrobot or bubbles captured on the surface of the semisolid gel (Appendix B Figure 10). Each region was resized or reoriented to capture the most descriptive detail in each image. These sections were further processed for the prophylactic condition (Appendix B Figure 11) and therapeutic condition (Appendix B Figure 12).



Appendix B Figure 10. Regions identified for image processing of microrobot-mediated targeted drug delivery in-a-chip modeling prophylactic and therapeutic conditions. Regions were converted to binary images with a threshold of 0.65, and then culture growth was expressed as a ratio of pixels corresponding to opacity compared to the total pixel count.



Appendix B Figure 11. Binarized regions to characterize MG1655 growth in target and off-target colonies in the prophylactic treatment condition using a tet-microrobot.



Appendix B Figure 12. Binarized regions to characterize MG1655 growth in target and off-target colonies in the therapeutic treatment condition using a tet-microrobot.

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