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Microparticle manipulation technologies: advancing cancer research and clinical treatment through novel device design

Doctoral Dissertation Proposal

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EXAMPLE

I. Motivation

The development of medical technology throughout the past century has fueled advancements in cancer screening, detection, and treatment. Even so, cancer continues to be a major global health burden. While reports indicate that cancer incidence and mortality rates are declining in developed countries, such as the United States, there are still increasing trends in some cancer sites including liver, pancreas, and brain [1, 2]. This continued prevalence necessitates ongoing efforts to improve cancer research and treatment. Two distinct areas this research aims to address are: 1) radioembolization (RE) cancer treatments and 2) 3D cancer culture processing techniques.

RE is an advanced treatment for intermediate stage hepatocellular carcinoma (HCC, primary liver tumors) for patients who are not eligible for transplant or surgical resection. This treatment involves catheterizing the hepatic artery (HA) and injecting radioactive microparticles into the hepatic vasculature. These particles embolize the blood vessels which supply oxygenated blood to the tumor's periphery while also delivering therapeutic doses of β^- radiation. This combination of embolization and radiotherapy aims to reduce tumor size allowing for other treatments such as ablation and surgical resection [3]. While this is a promising treatment, complications [4] often arising from non-target deposition of RE particles limit its overall effectiveness. This research attempts to provide a solution by **developing an electromagnetically actuated catheter to enable targeted deposition of RE particles**. Furthermore, this work aims to **develop a multi-scale microvasculature model to study the embolic properties of various microparticle materials and sizes.**

Concomitant to cancer treatment, cancer research provides not only an in depth understanding of cancer biology but actively investigates more effective treatments. While two-dimensional (2D) tissue culturing is a key tool in these investigations, several factors limit the ability for these cultures to model *in vivo* tissues [5, 6]. To address this issue, three-dimensional (3D) culture techniques have been developed to better predict the *in vivo* efficacy of potential treatments in drug screening studies [7]. Even with the benefits of 3D culture techniques, 2D cultures remain the standard in pharmacological and pathobiological studies for their compatibility with high-throughput and automated methods. To address the need for high-throughput 3D culture processing methods, this research also aims to **develop a centrifugal microfunnel array device enabling rapid histological processing of cancer derived 3D spheroid cultures.**

II. Methodology

Mechanical targeting of RE particles in the liver has been established by Richards et al. who showed that a particle's trajectory through the hepatic vasculature could be accurately predicted by its injection location within the common hepatic artery (CHA) cross section [8]. Figure 1 illustrates the simulation results of this study which were also experimentally verified using a 4.1:1 scale model of the depicted artery system. These results show that a patient

derived vascular model could be used to generate particle injection maps like the one shown in Figure 1. Given sufficient ability to control the injection location, these release maps would enable targeting of RE particles thereby mitigating the negative health effects of non-targeted deposition.

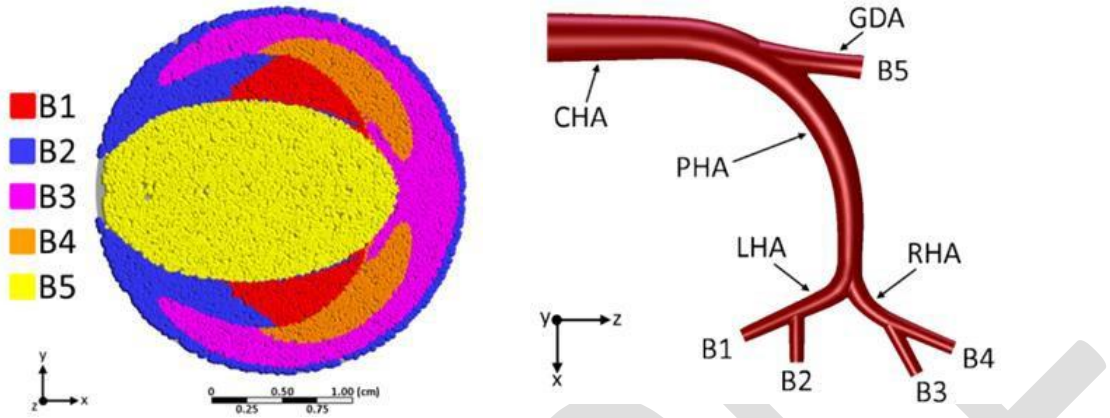

Figure 1. Simulation results from Richards et al. [8] showing the relationship between the injection location within the CHA cross section (left) and the vessel outcome (right). CHA: common hepatic artery, GDA: gastroduodenal artery, PHA: proper hepatic artery, LHA: left hepatic artery, RHA: right hepatic artery.

Prior to our proposed research, there were no established ways of controlling a clinical microcatheter to allow for this type of targeted RE particle deposition. To provide actuation without penetrating or damaging the vessel walls, this research developed a novel active magnetic bearing (AMB) device for microcatheter position control (Figure 2 top right). Due to the inherent instability in these types of electromechanical systems, a model based non-linear sliding mode controller was developed to control the AMB electromagnets thus accurately regulating the position of the microcatheter within a simplified HA model (Figure 2 top left) [9]. This work presents new contributions to field of AMBs by designing and controlling a large air-gap AMB (Figure 2 top left) where the object to be suspended by the electromagnets is significantly smaller in diameter than the diameter of the magnetic bearing. This is in contrast to typical AMBs in which the bearing and the rotor are separated by a comparatively thin air-gap [10, 11]. Additionally, this research presents new contributions to the RE field by showing that an electromagnetically controlled microcatheter can successfully effect targeted particle deposition within a model artery system [12].

While this research addresses the need for microcatheter position technologies, there is still a gap in the literature regarding the properties and effectiveness of different types of embolizing particles currently on the market. Clinical comparisons of commercially available microspheres have been done for procedures such as prostatic artery embolization [13–15], however no research has been conducted on the use of rigid glass microparticles. Furthermore, the literature currently available is aimed at understanding the clinical outcomes associated with the use of different particles, while little has been published about the mechanical behavior of various microparticle sizes and materials with respect to embolization characteristics.

Our research in this area will focus on the development of a multi-scale microvascular model (µVM) which captures the anatomical features of human vasculature on the order of small arteries, arterioles, and capillaries. This microfluidic model will be fabricated from silicon cast upon a multi-scale mold, part of which is machined from aluminum with the other part being constructed using cleanroom photolithography techniques. Figure 3 depicts a preliminary µVM we constructed showing a clinical microcatheter positioned within the macro-scale portion of the model (left) and embolizing glass microspheres (right). This µVM will then be used to conduct a series of embolization experiments on glass, polyvinyl alcohol, and gel microparticles of sizes ranging from 50 to 500µm in diameter.

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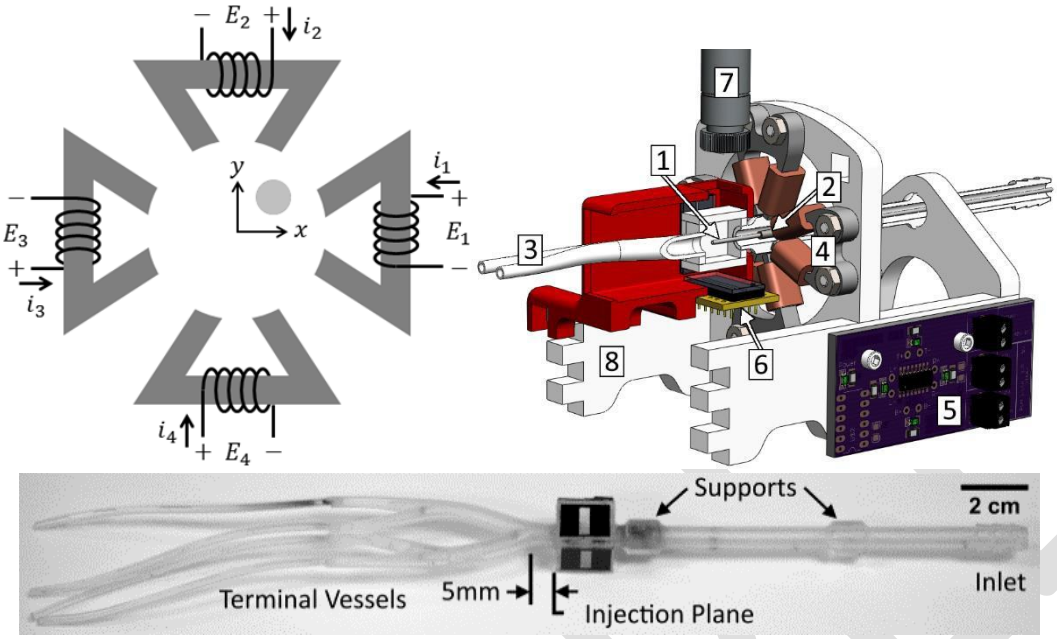

Figure 2. Novel AMB microcatheter positioning device schematic (top left), device rendering (top right), and HA model (bottom). Microcatheter (1), ferromagnetic ring (2), HA model (3), stator with windings (4), amplifier board (5), linear optical array (6), laser diode (7), and acrylic chassis (8).

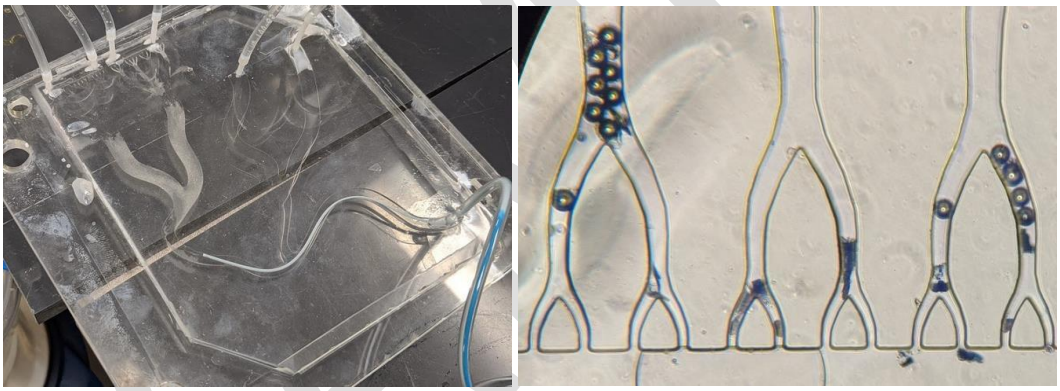

Figure 3. Overview of preliminary µVM with a microcatheter (left) and embolizing glass microparticles (right).

With the two research topics presented above, the last area our research considers is enabling high-throughput processing of 3D cancer cultures. Currently, 3D spheroid cultures, where cancer cells are grown in ~500µm diameter clusters, have gained popularity as they are simple to grow while preserving the 3D structure of *in vivo* tissues [16]. Microscopic analysis of spheroid cultures is often conducted to assay the distribution of biochemical targets in a way that preserves the spheroid structure. This is done by employing typical tissue histology techniques which involve embedding preserved tissue samples in paraffin, sectioning on a microtome, mounting the sections on microscope slides, and finally staining the sections for biomarkers. Longitudinal and cross-sectional drug discovery studies potentially involve microscopic analysis of hundreds of spheroid cultures, making these histological techniques prohibitively time consuming. Previous work by Ivanov et al. has established a method for manually constructing arrays of spheroids which can then be processed as a single histological specimen [17]. While this method improves histological processing, the spheroid array must still be constructed by hand-pipetting individual spheroids into a prefabricated agarose array. Our work aims to address the issue of rapidly and precisely transferring spheroids from a standard 96-well culture plate to a smaller array which can be processed using standard histology techniques. This work resulted in the development of a novel microfunnel array device which deposits spheroids into micro-wells within an agarose block using a standard cell culture centrifuge (Figure 4) [18].

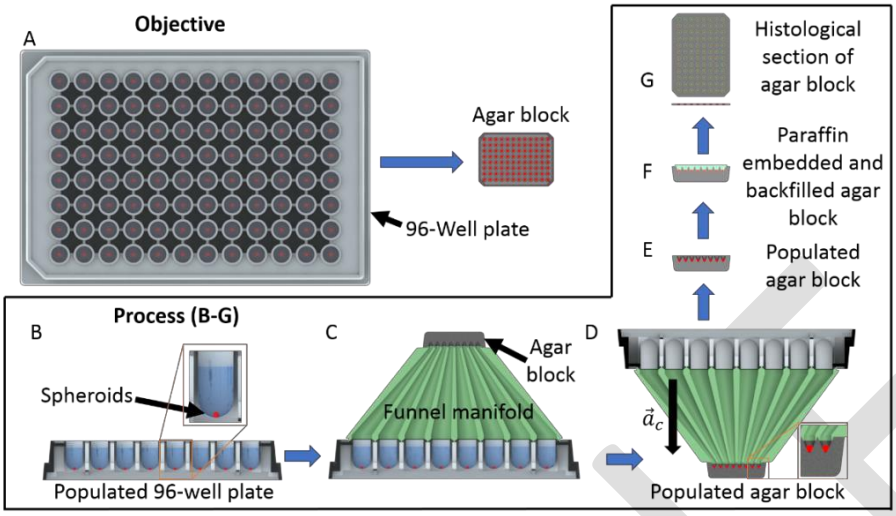

Figure 4. Microfunnel array device concept and operation. Objective of device (A), spheroids (red) grown in a 96-well plate (light grey) with liquid medium (blue) (B), funnel array (green) with attached agar block (dark grey) mounted on a 96-well plate (C), centrifuge process (D), populated agar block removed from funnel manifold (E), agar block encapsulated (light green) and paraffin embedded (F), top and side view of a histological section (G).

III. Research Objectives

Two key objectives were achieved for the AMB device: 1) development of a model based sliding model controller for regulating the catheter position and 2) experimental confirmation that vessel targeting can be achieved using this device. Figure 5 illustrates the regulation performance of the control system showing multiple steps in the horizontal and vertical positions of the microcatheter (left). Figure 5 also shows the outcome of microsphere injection experiments (right) which provided statistically significant evidence that different vessels were indeed targeted between the three catheter locations tested.

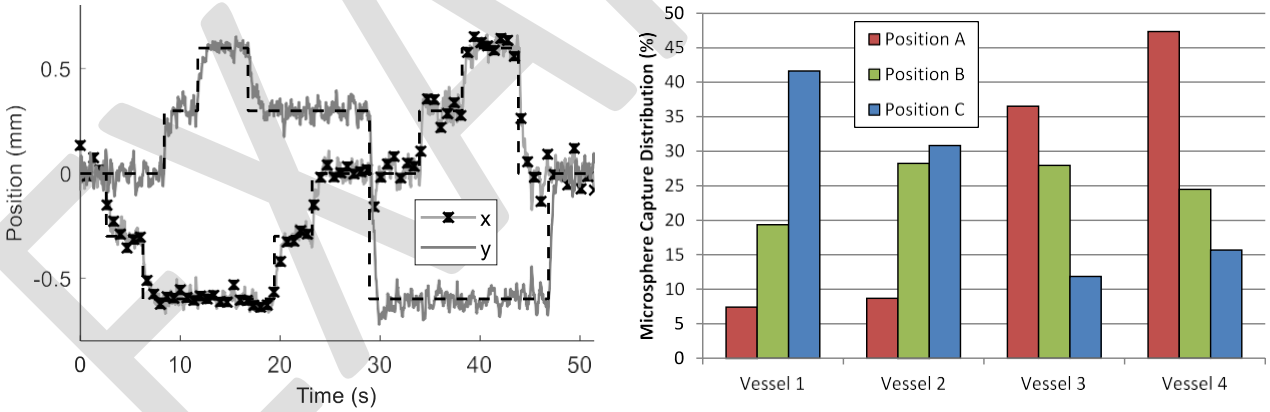

Figure 5. Closed-loop (x, y) positioning performance of the model-based sliding mode controller (left) and results of particle injection experiments (right). Position A: (0.6mm, 0mm), Position B (0mm, 0mm), Position C (-0.6mm, 0mm) [12].

The objective of the µVM work is to conduct a series of embolization experiments on glass, polyvinyl alcohol, and gel microparticles of sizes ranging from 50 to 500µm in diameter. The tests will be administered under physiological pulsatile flow conditions. The resulting embolized µVMs will be microscopically analyzed for particle deformation, agglomeration, and distal penetration (Figure 6). The goal of this research is to identify differences in the embolization characteristics between particles of different size and material.

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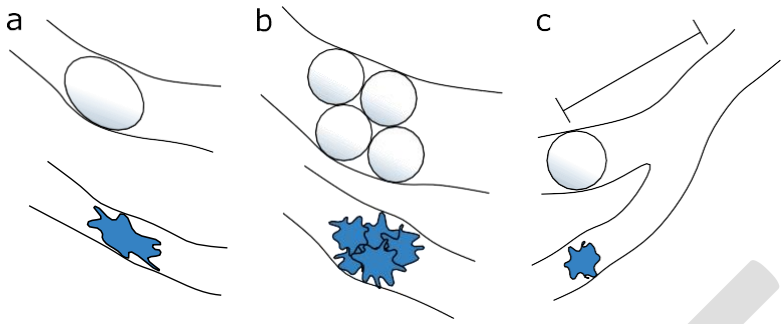

Figure 6. Microscopic analysis of particle deformation (a), agglomeration (b), and distal penetration(c). Some commercially available particles are spherical while others are irregularly shaped (blue).

Finally, the objective of the microfunnel array device was to demonstrate that the device could effectively deposit microspheres in an array that can be processed, sectioned, and imaged in a single slide. The device we developed reliably transferred 80% of spheroids into the agar array. Optical sections of the arrays showed that all spheroids that were deposited could be imaged in a single section (Figure 7).

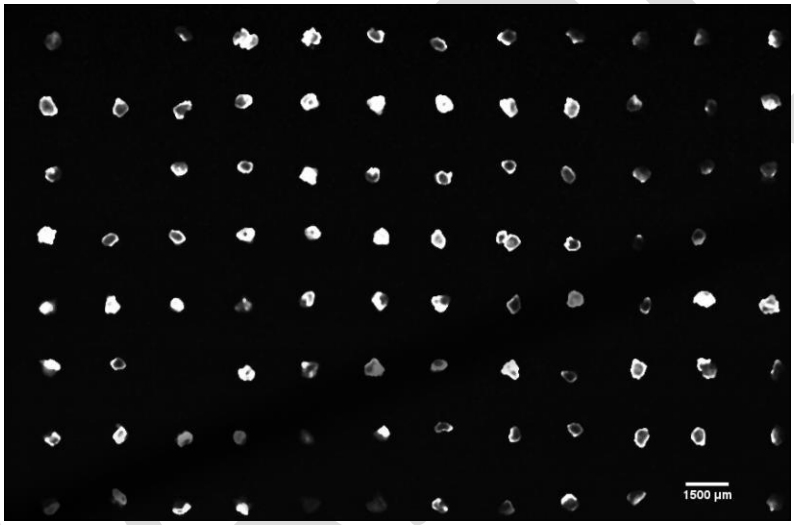

Figure 7. Optical section of a representative spheroid agar array obtained using confocal laser scanning microscopy showing cross sections of all captured spheroids [18].

IV. Proposed Work

The remaining work proposed for this dissertation involves finalizing the µVM design and conducting microparticle embolization experiments as outlined previously. The µVM must match expected systolic and diastolic pressure levels for small arteries, arterioles, and capillaries to ensure the validity of the experimental results. Commercially available microparticles will be obtained and tested by injecting them into the final µVM using a computer controlled syringe pump. Injections will be made incrementally to record changes in pressure and flow rate as the embolic state develops for each combination of microparticle size and material. In addition, experiments will be conducted to analyze particle distributions in µVM HCC models using computed tomography (CT) to provide validity for clinical use of this technique with radiopaque microparticles during interventional procedures.

V. Timeline

The timeline for completing the proposed remaining work is summarized in the Gantt chart in Table 1 below.

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Table 1: Gantt chart of proposed work timeline for fall 2020

Task	Jul	Aug	Sep	Oct	Nov
Finalize µVM design	X				
Validate µVM pressures and flow rates	X	X			
Fabricate µVMs needed for trials		X			
Conduct embolization experiments		X	X		
Analyze µVM experiment micrographs			X		
Collect/analyze µVM HCC model CT images			X	X	
Preparation of µVM publication				X	X
Preparation of final manuscript				X	X

VI. References

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