Faculty of Postgraduate Studies and Scientific Research  
German University in Cairo  

Clearing of Blood Clots Using Helical Robots  

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Computer Science and Engineering  

By  

Dalia Samir Mahdy  

Supervised by  

Dr. Islam Khalil  
Assistant Professor  
Faculty of Media Engineering and Technology  
German University in Cairo  

Dr. Slim Abdelnader  
Professor  
Faculty of Material Science and Engineering  
German University in Cairo  

2019
Declaration of Authorship

I, Dalia Mahdy, declare that this thesis titled, “ and the work presented in it are my own. I confirm that:

• This work was done wholly or mainly while in candidature for a research degree at this University.

• Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated.

• Where I have consulted the published work of others, this is always clearly attributed.

• Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work.

• I have acknowledged all main sources of help.

• Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself.

Signed: ________________________________

Date: ________________________________
Abstract

The risk of side-effects from thrombolytic agents can be mitigated by using relatively small doses, assisted by mechanical rubbing against blood clots using helical robots. This work studies the influence of rubbing against clots on their removal rate in vitro. A hydrodynamic model is developed based on the resistive-force theory to investigate the rubbing behavior of the helical robot driven by two rotating dipole fields. In addition, the shear modulus and ultimate shear strength of the blood clots is experimentally characterized and entered into the model to predict the dissolution rate of blood clots. The influence of mechanical rubbing on the dissolution of blood clots is experimentally evaluated by image processing of camera feedback, blood clot weight analysis, and cell count and spectrophotometric analysis post 40 minutes of mechanical rubbing. Not only do the results show that the removal rate of mechanical rubbing (-0.56 ± 0.27 mm$^3$/min) is approximately three times greater than the dissolution rate of chemical lysis using streptokinase (-0.17 ± 0.032 mm$^3$/min), but it also shows that this removal rate can be controlled via the rubbing speed of the robot. Analysis of the pre- and post conditions of the blood clots following 40 minutes of mechanical rubbing, under the influence of a rotating magnetic field in the frequency range of 20 Hz to 45 Hz. Measurements show that the weight of the blood clot is decreased by 74.4±11.1% at 25 Hz, cell count and concentration of the samples past the robot and the blood clot are calculated as $654\pm108 \times 10^4$ cells/ml at 40 Hz and $4.35\times10^{-6}$ mol at 35 Hz, respectively. Compared to 51.9±3.1%, $54\pm12\times10^4$ cells/ml, and $1.05\times10^{-6}$ mol in the absence of mechanical rubbing, respectively. Further, mechanical rubbing of blood clots is achieved under ultrasound guidance. Position of the helical robot is determined using ultrasound feedback and used to control its motion towards the clot. The robot is navigated controllably towards blood clots using ultrasound feedback with maximum position error of 2.15 mm. Furthermore, the propulsion of the helical robot is experimentally characterized against the flowing streams of phosphate buffered saline (PBS) at flow rate of 90 ml/hr. Averaged speed is measured at actuation frequency of 8 Hz as $11.3 \pm 0.52 (n = 5)$
Acknowledgements

I would like to express my sincere gratitude to my supervisor and godfather Dr. Islam Khalil for his sincere guidance, irreplaceable support, inspiration and motivation, patience and immense knowledge and experience. I could not have imagined having a better advisor and mentor for my research work.

I would also like to thank Dr. Nabila Hamdi, Dr. Mohamed Elwi and Dr. Anke Klininger for all the help and support.

Besides my advisor, I would like to thank my friends and colleagues in the MNR lab, Barbara Adel, Mina Maged, Alaa Adel, Sara Hesham, Ibrahim Basla, Loaa Zahar, Mostafa Yasser and Mahmoud Abdel-fattah. For all the long days and nights we have spent together working and enjoying each others company.

Last but not the least, I would like to thank my lovely family for this accomplishment would not have been possible without them.
# Contents

<table>
<thead>
<tr>
<th>Declaration of Authorship</th>
<th>i</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>iii</td>
</tr>
<tr>
<td>1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Motivation</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Literature Review</td>
<td>2</td>
</tr>
<tr>
<td>1.2.1 Microrobotic systems</td>
<td>2</td>
</tr>
<tr>
<td>1.2.2 Biologically inspired microrobotics</td>
<td>4</td>
</tr>
<tr>
<td>1.2.3 Helical propulsion</td>
<td>6</td>
</tr>
<tr>
<td>1.2.4 Removal of blood clots</td>
<td>7</td>
</tr>
<tr>
<td>1.3 Objectives</td>
<td>9</td>
</tr>
<tr>
<td>1.4 Thesis organization</td>
<td>9</td>
</tr>
<tr>
<td>2 Modelling and characterization of mechanical rubbing</td>
<td>10</td>
</tr>
<tr>
<td>2.1 Helical robot</td>
<td>11</td>
</tr>
<tr>
<td>2.2 Permanent magnet-based robotic system</td>
<td>12</td>
</tr>
<tr>
<td>2.3 Resistive-Force Theory model</td>
<td>14</td>
</tr>
<tr>
<td>2.4 Characterization of blood clots</td>
<td>20</td>
</tr>
<tr>
<td>2.4.1 Preparation of blood clots</td>
<td>20</td>
</tr>
<tr>
<td>2.4.2 Morphology of blood clots</td>
<td>21</td>
</tr>
<tr>
<td>2.4.3 Rheology of blood clots</td>
<td>22</td>
</tr>
<tr>
<td>3 Mechanical rubbing of blood clots</td>
<td>25</td>
</tr>
<tr>
<td>3.1 Volume detection using camera feedback</td>
<td>26</td>
</tr>
<tr>
<td>3.2 Dissolution of blood clots</td>
<td>26</td>
</tr>
<tr>
<td>3.2.1 Group 1: Dissolution using Full Dose of Streptokinase</td>
<td>27</td>
</tr>
<tr>
<td>3.2.2 Group 2: Mechanical Rubbing Against Blood Clots</td>
<td>28</td>
</tr>
<tr>
<td>3.3 Blood clot weight</td>
<td>33</td>
</tr>
<tr>
<td>3.4 Cell count</td>
<td>34</td>
</tr>
<tr>
<td>3.5 Spectrophotometric analysis</td>
<td>36</td>
</tr>
<tr>
<td>4 Localization and control of helical robots</td>
<td>38</td>
</tr>
<tr>
<td>4.1 Diagnostic Ultrasound and its deployment in microrobtics</td>
<td>39</td>
</tr>
<tr>
<td>4.2 Ultrasound guided localization of helical robots</td>
<td>40</td>
</tr>
</tbody>
</table>
4.3 Closed-Loop Control ................................................. 42
4.4 Mechanical rubbing of blood clots under Ultrasound guidance ............................. 43
4.5 Characterization in rabbit aorta .................................. 45

5 Conclusions and future work 47
  5.1 Conclusions .......................................................... 48
  5.2 Future work .......................................................... 49

A Blood clot volume detection code (implemented using Matlab) 50

Bibliography 51
# List of Figures

1.1 A schematic representation of the interaction of the tip of the helical robot with the fibrin network of the blood clot .......................................................... 2

1.2 Biological, hybrid micro-bio-robot and biologically inspired robots. (a) A schematic representation of the propulsion of Magnetotactic bacteria (MTB) towards a cancer cell [33]. (b) A sperm-driven microrobot consisting of a micro-tube and a bovine sperm cell [34]. (c) A robotic sperm fabricated using polymer solution of polystyrene in dimethyl formamide and iron-oxide nanoparticles [35] .......................................................... 4

1.3 Biologically inspired helical robots [45] and [46] ............................................. 6

1.4 Mechanical devices for the removal blood clots. (a) MERCI thrombectomy device [59] (b) The Solitaire FR Revascularization Device [60] ....................... 8

2.1 Scanning electron microscopy image of a helical robot. It consists of a cylindrical magnet with a magnetization vector oriented perpendicular to the axis of its helical body, where \( R \) is the radius of the helical body and \( r \) is the radius of the filament of the helical body. \( \lambda \) and \( \alpha \) are the pitch of the helical body and the helix angle, respectively ................................................. 11

2.2 Helical robot can be inserted \emph{in vivo} using a cannula ........................................ 11

2.3 A permanent magnet-based robotic system is used to actuate the helical robot. The catheter segment accommodating the helical robot, blood clot, and the swimming medium is positioned between two rotating permanent magnets .......................... 12

2.4 Simulations of the magnetic field exerted on the robot are presented for single rotating dipole field (top) and two rotating dipole fields (bottom) at orientations of 0°, 90°, 180° and 270°(from Hosney \emph{et al.} 2015) .......................................................... 13

2.5 The helical robot (inset) is contained inside a catheter (diameter \( D_{ch} \)) segment between two rotating permanent magnets (magnetization \( \mathbf{M} \)). Rotation of the permanent magnets generates a rotating field (\( \mathbf{B} \)) that exerts a magnetic torque on the dipole of the robot (\( \mathbf{m} \)). The robot consists of a cylindrical Nd-FeB magnet with diameter \( r_x \), height \( r_yz \) and tail length \( l \) .................................................................. 15

2.6 Simulation results of the drag forces and torques (using (6)) exerted on the head and the helical tail of the robot, at frequency of 35 Hz ........................................ 16
2.7 Rubbing behavior of a helical robot against a blood clot for \( t \in [0,8] \). (a) The evolution of the position of the robot is plotted at equal time intervals over 75 cycles. (b) The robot drifts towards the channel wall while rubbing against the clot. The blue circle represents the inner-diameter of the catheter segment, whereas the red circle represents a position limit on the robot. (c) Motion of the robot along \( x \)-axis is constrained by the clot during rubbing, whereas oscillations are observed along \( y \) - and \( z \)-axis. The removal rate of clot in this simulation is -0.834 mm\(^3\)/min, at frequency of 35 Hz.

2.8 (a) Fresh venous blood is drawn from healthy donors between the age of 25 and 28, and inserted into a vacutainer without anticoagulant. Then, (b) A blood clot is extracted from the vacutainer and (c) a small slice is cut and inserted inside the catheter segment.

2.9 Morphology and mechanical properties of the blood clots are characterized using scanning electron microscopy (SEM) imaging.

2.10 Rheology test is conducted to characterize the shear modulus of the blood clots. The test is done using a Bohlin Gemini instrument (Malvern Instruments, U.K.).

2.11 Rheology test is conducted to characterize the shear modulus of the blood clots. The test is done using a Bohlin Gemini instrument (Malvern Instruments, U.K.).

3.1 Volume detection of gelatin thrombus model.

3.2 A representative experiment of dissolution of a blood clot using full dose of streptokinase (1,500,000 I.U.). The blue arrow indicates the direction of the flow. In this trial, the dissolution rate of the clot is -0.19 mm\(^3\)/min and at time, \( t \approx 20 \) minutes, formation of fluid channels (white dashed lines) inside the clot is observed.

3.3 A representative experiment of mechanical rubbing against a blood clot using a helical robot. In this trial, the removal rate of the clot is -0.885 mm\(^3\)/min at frequency of 35 Hz.

3.4 Chemical lysis and mechanical rubbing of blood clots are tested on a blood clot with initial volume of \( (v_0) \) of 94.24 mm\(^3\). The zero-input response indicates that the clot does not undergo any change in its size. The lysis is done using streptokinase, at flow rate of 10 ml/hr.

3.5 The influence of rubbing frequency on the removal rate of the blood clot is investigated experimentally between 20 Hz to 45 Hz.

3.6 The rubbing frequency influences the removal rate of the blood clot. Mechanical rubbing is effective within a frequency range of 20 Hz to 45 Hz. Maximum removal rate is achieved at 35 Hz (inset). The magnetic field at the position of the helical robot is 5.5 mT, and the flow rate is 10 ml/hr.

3.7 Mixture past the robot and the blood clot is collected every 5 minutes into a small tube.
3.8 Hemocytometer chamber is observed under the microscope. The frame of the counting chamber consists of 9 large squares. The large central square is divided into 25 medium squares (marked in red) each with 16 small squares (marked in blue).

3.9 Samples past the robot and blood clot are collected every 5 minutes during the experiments for analysis.

3.10 Spectrophotometric analysis is performed to study the influence of mechanical rubbing on the concentration of blood clots. First, Baseline is selected as \( \lambda = 416 \text{ nm} \), then absorbance is measured under the influence of a rotating magnetic field with varying frequency in the range of 20 Hz to 45 Hz.

4.1 The helical robot is localized using ultrasound feedback (a) On-line localization of the helical robot (white arrow) is conducted using ultrasound feedback. The insets show representative localization trials at depths of 2 cm, 3 cm, and 4 cm. (b) A container with catheter segments fixed at varying depths from the gelatin surface is used for the localization.

4.2 Closed-loop control of a helical robot is achieved using ultrasound guidance. (a) The robot swims in phosphate buffered saline inside a catheter segment with inner diameter of 4 mm. (b) The average speed of the robot is \( 5.32 \pm 1.17 \mu\text{m/s} \) and the average and maximum steady-state errors are \( 0.84 \pm 0.41 \text{ mm} \) and 2.15 mm, respectively.

4.3 Filtering algorithm for the localization of helical robot using ultrasound feedback.

4.4 Mechanical rubbing is achieved under ultrasounds guidance. The volume of the blood clot is determined with two cameras oriented with 45° with respect to the horizontal plane and have 90° with respect to each other.

4.5 Influence of the mechanical rubbing on the removal rate of clots is characterized at room temperature of 25°C and body temperature of 37°C, with rotating magnetic field at \( \omega = 35 \text{ Hz} \). (a) Mechanical rubbing achieves removal rate of \( -0.614 \pm 0.303 \text{ mm}^3/\text{min} \) (\( n = 6 \)) at 25°C, (b) Mechanical rubbing achieves removal rate of \( -0.482 \pm 0.23 \text{ mm}^3/\text{min} \) (\( n = 6 \)) at 37°C.

4.6 The speed of the helical robot is characterized in rabbit aorta under the influence of rotating magnetic field with varying frequency.
List of Tables

3.1 Comparison between lysis and rubbing at 35 Hz. The final volume ($v(t_f)$) is measured after 40 minutes. The initial volume ($v_0$) of the clot is 94.24 mm$^3$. 30

3.2 Measurements of weight reduction percentage ($w_{d}$) under the influence of rotating magnetic fields with varying frequency in the range of 20 Hz to 45 Hz. 33
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABF</td>
<td>Artificial Bacterial Flagella</td>
</tr>
<tr>
<td>CAM</td>
<td>Chorioallantoic Allantoic Membrane</td>
</tr>
<tr>
<td>E. Coli</td>
<td>Escherichia Coli</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetraacetic Acid</td>
</tr>
<tr>
<td>HEK</td>
<td>Embryonic Kidney Cells</td>
</tr>
<tr>
<td>PBS</td>
<td>Magneto Tactic Bacteria</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>RFT</td>
<td>Resistive Force Theory</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Micro and nano robots have shown promise in diverse biomedical applications. This chapter explains the motivation for the research and presents a review of the related work in the literature. The background knowledge of the design, modelling, and control of micro and nano robots are presented here with a review of the existing approaches.
1.1 Motivation

A potential minimally invasive biomedical application of microrobots is clearing of clogged blood vessels via direct interaction with the clot using a microrobot towards its dissolution as illustrated in Fig. 1.1. Traditional fibrinolytic therapy for ischemic disease includes catheter-directed localized thrombolysis or systemic administration of thrombolytic agents such as streptokinase like are traditionally used for thrombolytic therapy. However, medical precautions and tests are necessary to avoid their several side-effects such as excessive bleeding. The deployment of microrobotics in the mechanical removal of blood clots would minimize the risks associated with traditional thrombolytic therapy.

![Figure 1.1: A schematic representation of the interaction of the tip of the helical robot with the fibrin network of the blood clot](image)

1.2 Literature Review

1.2.1 Microrobotic systems

Over the past decade, micro and nano robots have shown potential to revolutionize medicine and nanotechnology. These robots can be externally actuated and navigated inside the human body owing to their small size without the need for onboard power supply or control system [1]. Potential biomedical applications of microrobotic systems include targeted therapeutic interventions [2], [3], cargo delivery [4], localized hyperthermia [5], [6], micro–scale biopsy [7], tissue–engineering [8], bio–sensing [9], detoxification of biological fluids [10], and neurosurgery [11]. In micro and nano scale, the domination of the viscous forces suppresses the hydrodynamic inertial forces which necessitates nonreciprocal periodic changes in the shape of the robot to break time–reversal symmetry [12]. Various propulsion mechanisms have been proposed to provide locomotion in low Reynolds number. Locomotion
have been achieved using electric fields generated by miniature diodes [13], chemical energy by using an internal catalytic chemical engine [14] or by ejecting micro–bubbles via the decomposition of hydrogen–peroxide [15], monochromatic light [16], acoustic levitation [17], thermal stimulus [18], resonant ultrasound [19], and magnetic fields [20]. Magnetic fields have long been employed for the control and steering of micro and nano robots, as the use of magnetic fields for actuation enables wide variety of swimming mechanisms and functionalities.

The research effort devoted for the fabrication of magnetically actuated micro and nano robots has been remarkably increased in recent years. Ghosh et al. have described a method that is known as glancing angle deposition for the fabrication of large numbers of nano–structured propellers, and reported high–precision navigation of nano propellers of 200–300 nm in width and 1–2 μm in length using rotating magnetic fields [21]. Kim et al have fabricated and characterized non cyto toxic three–dimensional porous structures with SU-8 photoresist for targeted cell delivery using laser lithography. These microrobots were coated with Ni and Ti layers for control with external magnetic fields and used for three–dimensional cell culture of human embryonic kidney cells (HEK 293 cells) [22]. Carols et al have presented fabrication of iron microrobots using template-assisted electrodeposition in 3D-printed micromolds, the microrobots are biocompatible and exhibit high magnetic torque for overcoming flow and gravity [23]. Schuerle et al. have reported the fabrication of phospholipidic tubular and helical magnetic microrobots via biotemplating synthesis. The microrobots were coated with multi–alloy magnetic coating and controlled by a coil setup implemented in an inverted microscope [24]. Ullrich et al. have characterized the swimming performance of helical microrobots in fibrous environments, and have investigated the effect of solutions with varying collagen concentrations. In agreement with theory, results show that the velocity of the microrobot is increased in the presence of medium concentration collagen fibers in gelatin swimming medium [25]. Chen et al. have developed piezoelectric magnetic micro–swimmers for targeted cell therapy applications. The micro–robots are made of helical bodies consisting of a piezoelectric polymer matrix dispersed with magnetic nano particles, the helix can rotate along its short axis when it is actuated with a rotating magnetic field actuated by the nano particles, while the piezoelectric polymer acts as a steerable frame and an acoustically responsive cell electrical stimulation platform [26].

Robust systems have been designed and experimentally used to generate magnetic fields for the wireless control of micro and nano robots. Kummer et al. have designed an electromagnetic system for the control of microrobots with the non uniform magnetic field. They have demonstrated wireless magnetic control of a microrobot with five–degree–of–freedom to perform vessel puncture of the chorioallantoic membrane (CAM) of a developing chicken embryo [27]. Thomas et al. have proposed a rotating permanent magnet manipulator for the wireless control of magnetic helical microrobots, and have experimentally verified the ability of propulsion towards and away from the manipulator [28]. Hosney et al. have presented a permanent magnet–based robotic system for the control of helical microrobots via the synchronized rotation of two dipole fields. The utilization of the two rotating dipole fields increase the magnetic torque applied on the dipole moment of the microrobot and
eliminated the magnetic field gradients along the lateral direction of propulsion [29].

Researchers have been working on in vivo navigation and imaging of micro and nano robots for future biomedical applications. Mathieu et al. have achieved controlled navigation of a 1.5 mm chrome steel magnetic spherical beads inside the carotid artery of a living swine. The sphere was propelled with velocity of 13 cm/s using a Using a clinical Magnetic Resonance Imaging (MRI) system. They developed preliminary models based on the obtained experimental data to calculate the magnetic field gradient required for steering microrobots in vessels of various size [30]. Ullrich et al. have developed an electromagnetic system for the precise control of a microrobot for minimally invasive intraocular surgery. They have demonstrated the rotation and translation of the microrobot in ex vivo and in vivo experiments inside rabbit eye [31].

1.2.2 Biologically inspired microrobotics

Nature has always been an inspiration for novel designs of robotic motion, from swimming and wiggling motions, to walking or flying [32]. Biological organisms have evolved to adapt and survive in the environment. Thus, concepts from biologically inspired robots will sooner or later allow scientists to design and produce real systems that will maintain some of the desired properties of biological organisms, such as robustness, adaptability and quick response. Microrobots can be categorized as; Biological microrobots, bio-hybrid microrobots and biologically inspired microrobots as shown in Fig. 1.2.

![Figure 1.2](image)

**Figure 1.2:** Biological, hybrid micro-bio-robot and biologically inspired robots. (a) A schematic representation of the propulsion of Magnetotactic bacteria (MTB) towards a cancer cell [33]. (b) A sperm-driven microrobot consisting of a micro-tube and a bovine sperm cell [34]. (c) A robotic sperm fabricated using polymer solution of polystyrene in dimethyl formamide and iron-oxide nanoparticles [35].

The recognition of biological microrobots have been achieved in mainly two ways; the coupling between a microorganism and a magnetic component providing a hybrid micro-bio-robot and magnetotactic bacteria (MTB). Magdanz et al. have developed hybrid bio-micro-robot consisting of a motile sperm cell and a magnetic microtube. The locomotion of the robot is solely based on the falgellar propulsion of the motile cell. They have demonstrated the selective separation of the sperm and tube via remote magnetic guidance. The
application of cell release mechanism could be helpful for transportation of a single sperm cell to a required location, which could be a promising alternative fertilization method in vivo where the selected sperm cells are remotely guided to the egg cell [36]. Behkam et al. have proposed the use of the flagellar motors inside the intact cell of *Serratia marcescens* bacteria to provide controlled propulsion for swimming robotic bodies. They show the propulsion of 10 μm polystyrene beads by several bacteria randomly attached on their surface. On and off motion control activated by introducing copper ions to stop the bacteria flagellar motors and ethylenediaminetetraacetic acid (EDTA) to resume their motion [37]. Unlike hybrid micro-bio-robots, MTBs develop magnetite nanocrystals inside their cells, and accordingly their magnetic dipole moment enables directional control using external low range magnetic fields. Martel et al. have demonstrated controlled manipulation of 3 μm beads by MTB along pre-planned paths via directional magnetic fields generated from a small programmed electrical current [38]. Further, Serag El Din et al. have investigated the sensing capabilities of MTB to breast cancer cells. Experimental analysis and count of motile MTBs indicate that they tend to migrate towards less-oxygenated regions within the region of cancer cells [33].

Biologically inspired microrobots strive to achieve the versatility of micro–organisms and their ability to acquire locomotion in complex media and respond to the surrounding environment. Khalil et al. investigated the swimming behavior of microrobotic sperms fabricated from polystyrene dissolved in Dimethylformamide and iron–oxide nanoparticles, and have achieved propulsion in low Reynolds number with velocity of 1 body length per second. The study shows that the morphology of the proposed sperm microrobots is similar to that of biological sperm cells, and can be controlled and navigated with magnetic fields in the range of mill–tesla [35]. Li et al. have demonstrated a magnetically propelled fish–like nano robot. The deformable body of the swimmer is fabricated from nanowire segments that are connected through flexible nanoporous silver joints, and aquatic swimming behavior is achieved by passing a wave of bending along the length of the flexible body [39]. Zhang et al. have reported a microrobot exhibiting motion characteristics similar to a water strider. The microrobot is supported by ten hydrophobic artificial legs and actuated by two legs connected to DC motors, potential applications of the microrobot may include water quality surveillance and water pollution monitoring [40]. Dreyfus et al. have presented the propulsion of a flexible flagellum inspired by the locomotion of bacteria and eukaryotic cells, consisting of a chain of colloidal magnetic particles linked by DNA and actuated with an external magnetic field. The filament generated controlled deformations owing to the coupling between magnetic forces, filament flexibility and the viscous drag from the solvent that acts on the filament [41]. One biologically inspired locomotion mechanism that has proven success in diverse potential biomedical applications is helical propulsion. Helical micro and nano robots can be propelled using external magnetic fields. Rotation of such structures around their long axes generates a translational motion similar to that of cork–screw movement.
1.2.3 Helical propulsion

Helical propulsion is inspired by the movement of Escherichia coli (E. coli) bacteria which swims by rotating their helical flagellar filaments. In 1975, Berg have worked on the characterization and tracking of E. coli bacteria. He developed a tracking microscope continuously moves a small chamber containing bacteria to keep a particular cell fixed in space and its image fixed in the optical eyepiece and on the detector, and observed the three-dimensional locomotion of bacteria in translational and rotational movement proportional to the viscous drag [42]. Later, E. M. Purcell discussed and analyzed how Swimming in the micro and nano scale is very dissimilar to swimming in macro scale, where the domination of the viscous forces suppresses the hydrodynamic inertial forces [12]. The ratio of the inertial forces to the viscous forces is characterized by Reynolds number, and is calculated as

\[
Re = \frac{\rho v L}{\mu},
\]

where \(\rho\) is the density of fluid, \(v\) is the maximum velocity of the swimmer and \(L\) is its length, \(\mu\) is the dynamic viscosity of fluid. At low Reynolds numbers (<10^{-4}), inertia does not influence the locomotion and the propulsion is governed by the viscous forces exerted on the swimmer. Helical propulsion is inspired from the locomotion of bacteria. It is suitable for propulsion generation at low Reynolds numbers and has remained a topic of research for decades [43]. In 1973, a leading study discussing the swimming paradigm of bacteria showed that E. coli bacteria use molecular motors to rotate their helical flagella [44].

![Image](image1.png)

**Figure 1.3:** Biologically inspired helical robots [45] and [46]

The first micro robot resembling the helical bacterial propulsion (Fig. 1.3 (a)) was reported as artificial bacterial flagella (ABF) in 2007 by Belle et al. [45]. It has been later controlled by three orthogonal electromagnetic coil pairs under the influence of weak magnetic field [47]. Researchers have developed various fabrication platforms and designs of helical robots in the micro and nano scale. Huang et al. have demonstrated an origami–based platform for the fabrication of microrobots with varying body plans for kinematics analysis of the mobility of flagellated microrobots. They present and analysis of the geometry
of the helical propeller on the locomotion of externally actuated microrobots [48]. Barbot et al. have characterized a helical microrobot with three different propulsion mechanisms; rolling, spin–top motion and corkscrew swimming. The microrobot can switch between the three mechanisms by adjusting the direction and the frequency of the rotating magnetic field used for actuation [49]. Medina–Sánchez et al. have implemented magnetically actuated polymer-metal composite helical structures as micro-carriers. Experimental results in a micro–fluidic channel show that the micro–structures can actively capture, transport, and release single live sperm cells with motion deficiencies to help them carry out their natural function [1]. Helical propulsion has been characterized for various potential biomedical applications. Zhang et al. have characterized the locomotion of ABFs (Fig. 1.3 (b)) under the influence of rotating magnetic fields in milliTesla range. Swimming tests show the influence of the frequency of applied magnetic fields and the head size on the translational velocity of ABFs [46]. Ullrich et al. have investigated the velocity profile of helical microrobots in fibrous environments. Results show increased swimming velocity for a 280\mu m–long helical microrobot in a fibrous solution with collagen concentration [25]. Considerable progress have been achieved towards the transfer of helical propulsion into in vivo. Servant et al. have achieved in vivo controlled navigation of a swarm of ABFs using external magnetic fields. The magnetic controlled motion of the helical micro swimmers in the intra peritoneal cavity of a mouse was tracked in real–time using IVIS optical imaging system [50]. Yan et al. have fabricated biohybrid helical microswimmer made from Spirulina microalgae dip–coated in magnetite (Fe$_3$O$_4$) suspensions. They have achieved in vivo fluorescence imaging and tracking of a swarm of the micro–swimmers inside a rodent stomach via magnetic resonance imaging. Also, biocompounds contained in spirulina allowed the microrobots to experimentally degrade and exhibit selective cytotoxicity to cancer cell [51].

1.2.4 Removal of blood clots

Traditional treatment of clogged blood vessels includes chemical lysis therapy with thrombolytic agents. Food and Drug Administration have approved anticoagulants acting as plasminogen activators for clearing of blood clots such as streptokinase and urokinase. Traditionally, treatment of blood clots and obstructed blood vessels have been limited to administration of a thrombolytic drug infused over around 1–hour into the systemic circulation. It is required that the treatment is initiated within 3 hours of the onset of symptoms [52]. Unluckily, not all patients arrive at the hospital for treatment of a developed blood clot within 3 hours. In order to extend the treatment window of conventional therapy, clinical studies investigated localized therapy with thrombolytic drugs administered intravenously or via catheter. Localized treatment of occluded blood vessel started with intra-arterial delivery of thrombolytic drugs few decades ago. In 1983, Zeumer et al. have described the successful vascular recanalization of three out of five reported cases with arterial thrombosis, by Selective perfusion of streptokinase using a coaxial catheter system [53]. Semba et al. have demonstrated the efficiency and safety of catheter-directed thrombolytic therapy with urokinase for the treatment of deep venous thrombosis (DVT). Patients with iliofemoral DVT have undergone treatment with a urokinase dose infused over an average of 30 hours,
lysis was complete in 18 (72%), partial in five (20%), and not achieved in two (8%) of 25 treated limbs [54]. Further, Alexandrov et al. have shown that ultrasound energy, used in combination with tissue plasminogen activator has a beneficial effect in patients with acute ischemic stroke, the treated stroke mainly formed due to thromboembolism. The mechanism of action of ultrasound on dissolution of thrombus is completely clear but it works clinically [55]. Another approach for clearing clogged blood vessels is by using Laser and localized hyperthermia. In 1985, Abela et al. have evaluated a metal-capped fiber heated by laser radiation for arterial recanalization. Angiography Results from in vivo experiments show successful treatment of occluded arteries using the proposed approach [56]. Schwarzenberg et al. have investigated the influence of hyperthermia up to 45°C on thrombolytic therapy in combination with a fibrinolytic agent. Experimental results on in vitro fibrin clots have shown the distinct rise of the activity of the fibrinolytic agent with the rise in temperature [57]. Photo–thermal thrombus ablation with gold nano–rods exposed to near-infrared irradiation have been demonstrated by Singh et al.. A clot was induced in the femoral veins of a mouse and its photo–thermal ablation was tracked with color Doppler and the blood flow velocity showed restored blood flow at the end of the experiment. However, they suggest that the proposed approach is combined with a sub-therapeutic dose of streptokinase to avoid possible complications [58].

![Figure 1.4: Mechanical devices for the removal blood clots. (a) MERCI thrombectomy device [59] (b) The Solitaire FR Revascularization Device [60]](image)

Thrombectomy is the interventional procedure of removing a blood clot from a blood vessel. Recent studies have proposed and tested various mechanical devices for retrieval and removal of blood clots. The efficacy and safety of a catheter device (Merci, Concentric Medical, Inc.) for the removal of emboli have been investigated by Pierre et al.. The system consists of a tapered wire with 5 helical loops of decreasing diameter (from 2.8 mm to 1.1 mm) at its distal end, and is advanced through a micro-catheter in a straight configuration and proceeds a helical shape once it is delivered into the occluded artery in order to entrap the thrombus [59]. Monsky et al. have proposed a mechanical device for clot retrieval similar in its general design to a vascular guide wire with detachable coil. Actuated coils form loops to capture, encapsulate, and retrieve a blood clot or other obstruction.
1.3 Objectives

Tests on animal models were carried out to demonstrate the safety and efficiency of the proposed device [61]. Recanalization of occluded blood vessels in an animal model have been demonstrated by Jahan using Solitaire Flow-Restoration device, designed to be delivered by a micro catheter. Results from in vivo experiments on animals shows efficient removal of blood clots with acceptable safety [60].

1.3 Objectives

In this research, I aim to model and experimentally characterize mechanical rubbing of blood clots using helical robots. Towards future in vivo experimentation, helical robots are localized and controlled under ultrasound guidance as a medical image modality and locomotion is characterized in ex vivo model of animal blood vessel. Detailed objectives of this thesis includes:

- Modelling of mechanical rubbing of blood clots.
- Experimental characterization of the morphology of blood clots.
- Experimental characterization of the mechanical properties of blood clots.
- In vitro rubbing of human blood clots using helical robots in comparison to chemical lysis using a streptokinase thrombolytic agent.
- Measurement of the weight of blood clots pre–and post–mechanical rubbing.
- Cell count and spectrophotometric analysis on the mixture past the helical robot and blood clot throughout 40 minutes of mechanical rubbing.
- Localization and control of helical robot in one–dimensional channel guided by ultrasound feedback, as a medical imaging modality towards future translation of the application into in vivo experimentation.
- Mechanical rubbing of blood clots under ultrasound guidance. The helical robot is externally controlled towards the blood clot.
- Characterization of helical propulsion in rabbit aorta as an ex vivo model.

1.4 Thesis organization

The remainder of this thesis is organized as follows; Chapter 2 provides modelling and characterization of helical propulsion, a resistive force theory-based model of mechanical rubbing and characterization of blood clots. Ultrasound guided closed loop control of helical robot in one-dimensional channel and localization in ex-vivo model is experimentally investigated in chapter 3. Chapter 4 presents experimental in-vitro validation of mechanical rubbing of blood clots using helical robots. Finally Chapter 6 concludes the work and provides future work.
Chapter 2

Modelling and characterization of mechanical rubbing

This chapter discusses the modelling and characterization of helical propulsion and mechanical rubbing. A resistive force theory based model is presented for the modelling of the locomotion of helical robot inside the channel. In addition to experimental characterization of the mechanical properties of the blood clots.
2.1 Helical robot

The helical robot used in this consists of a magnetic head and helical tail. The head of helical robot contains a permanent magnet (Neodymium grade N40, Amazing Magnets LLC, California, USA), with an edge length of 500 µm. It is rigidly attached to one end of the helical tail to provide magnetic dipole moment, and an axial magnetization oriented perpendicular to the spring axis and magnetic flux density ($B_r$) of 1420 mT. A scanning electron microscopy image of the helical robot is shown in Fig. 2.1, Where $R$ is the radius of the helical body and $r$ is the radius of the filament of the helical body. $\lambda$ and $\alpha$ are the pitch of the helical body and the helix angle, respectively. The screw-like tail of the robot is designed and fabricated using aluminum spring with length and diameter of 4 mm and 100 µm, respectively. The pitch of the helical body is 500 µm and the helix angle is approximately 35°.

![Figure 2.1: Scanning electron microscopy image of a helical robot.](image)

Although the length of the helical robot is 4 mm, low Reynolds number ($<10^{-4}$) is achieved by using mediums with relatively high viscosity. Reynolds number is calculated as $Re = \frac{\rho v L}{\mu} = 0.089$, where $\rho$ is the density of the fluid (995 kg.m$^{-3}$), $v$ is the velocity of the robot before rubbing ($20 \times 10^{-3}$ m.s$^{-1}$) and $L$ is its length ($4 \times 10^{-3}$ m), and $\mu$ is the dynamic viscosity of the fluid (0.8882 cP). In future biomedical application, the helical robot could be inserted inside the human body via a cannula as shown in Fig. 2.2.

![Figure 2.2: Helical robot can be inserted in vivo using a cannula.](image)

Parameters of the helical robot such as: length, helix outer diameter, pitch, number of turns and etc., contributes to the locomotion speed and behavior of the helical robot [62]. Analysis and optimization of such parameters is required for enhanced performance of the robots of each particular robot design and application.
2.2 Permanent magnet-based robotic system

Open-configuration systems have two main advantages over closed-configuration systems; they can be scaled up to the size of in vivo devices, and are easier for incorporation of a medical imaging modality. Unlike closed-loop configuration systems that have enclosed and relatively tiny work space. Mahoney et al. have demonstrated the actuation of magnetically actuated tools using a rotating permanent magnet positioned using a robotic manipulator. They have shown that the rotation of a permanent magnet around a fixed axis such that the dipole is perpendicular to the axis of rotation, results also in the rotation of magnetic field vector at every point in the space around a fixed-axis [63]. Applying such property allows the rotating microrobots to be operated in any position, which is a great progress towards clinical feasibility of actuation of microrobots using single rotating permanent magnet. Ryan et al. have presented an actuation system using an array of rotating permanent magnets for the control of microrobots, capable of producing magnetic field and magnetic field gradient in all directions. The performance of the proposed system is similar to that of electromagnetic actuation systems, with potential for stronger field generation and minimal heat. They have shown the effectiveness of the proposed system through characterization and feedback control of a micro-magnet in a path following task [64].

A permanent magnet-based robotic system with open-configuration is used for the control and steering of helical robot. The system consists of two linear motion stages. Each motion stage carries a robotic base (Cyton Gamma 300, Robai, Cambridge, USA) with 3 degrees-of-freedom. The end-effector of each robotic base holds a DC motor (Maxon 47.022.022-00.19-189 DC Motor, Maxon Motors, Sachseln, Switzerland) that rotates a permanent magnet (N40 Neodymium, Amazing Magnets LLC, California, U.S.A) with outer diameter and thickness of 38 mm and 20 mm, respectively. This system is mounted on a tuned damped optical table (M-ST-UT2-58-12, Newport, California, U.S.A) to minimize vibration. The two rotating dipole fields to exert magnetic torque on the magnetic dipole of the helical robot.
The resultant magnetic field at the position of the helical robot is 5.5 mT. Rotating magnetic field is generated by the two rotating permanent magnets to actuate and navigate the helical robot in a three-dimensional, two-dimensional or one-dimensional work-space. The two rotating permanent magnets allow the microrobot to rotate and move forward. The robotic bases change the orientation of the permanent magnets to direct the helical microrobot towards the reference positions. This control is achieved by maintaining the axis of the permanent magnets parallel to the longitudinal axis of the microrobot. The utilization of two rotating dipole fields as presented and analyzed in [29], allows an increased magnetic torque to be exerted on the magnetic dipole of the helical robot. In addition, it compensates for the forces that causes undesirable drifting and instability in the locomotion of the robot, i.e., forces due to gravity and gradient forces along the lateral directions of the robot. This strategy allows the locomotion control of the helical robot with almost uniform field in the center of the work space, and decreased magnetic force along the lateral direction of the helical robot. The movement of the two dipole fields along the long axis of the robot will provide the mitigated gradient effect while the robot swims along the z-axis. Simulations of the magnetic field exerted on the helical robot are presented for single rotating dipole field and two rotating dipole fields (Fig. 2.4).

Magnetic field magnitude is calculated at 4 representative orientations of the the dipole fields (0°, 90°, 180° and 270°). The magnetic field \( \mathbf{B}(\mathbf{r}) \) exerts a magnetic torque on the dipole \( \mathbf{m} \) of the helical robot (gray circle). At the center of the workspace \( (x = 0) \), the magnetic field generated using the two rotating dipole fields is 100% greater than the magnitude of the magnetic field generated using single dipole field, and the magnetic field gradient almost vanished at \( x = 0 \) for the two rotating dipole fields.
2.3 Resistive-Force Theory model

The actuation mechanism of the helical robots is based on two rotating permanent magnets placed parallel to a cylindrical catheter segment (Fig. 2.5) \[29\]. This segment contains the robot, and is filled with a medium (phosphate buffered saline) with higher viscosity than blood to approach the low Reynolds numbers (\(< 10^{-2}\)) achieved by microrobots. The calculations of the corresponding field and field gradient are based on the analysis presented in \[65\] and \[66\], and formulate scalar potential for the rotating cylindrical magnets, \(\Phi_m(r, \theta, z)\). Using the assumptions of azimuthal symmetry, i.e., \(\theta=0\), and uniform polarization with respect to radial position, the potential is approximated at the robot position \(r\) as follows:

\[
\Phi_m(r, \theta, z) = \frac{1}{4\pi} \int \frac{\nabla \cdot M}{|r - \rho|} dV,
\]

where \(M\) is the magnetization vector of the permanent magnet, \(\rho\) is a position vector to be integrated over the volume of the permanent magnet, and \(dV\) signifies the infinitesimal increment in the volume. The gradient of \(\Phi_m(r, \theta, z)\) provides the following magnetic field density \((B)\):

\[
B = -\mu_0 \nabla \Phi_m(r, \theta, z),
\]

where \(\mu_0\) is the permeability of free space. Once the magnetic field \((B)\) is calculated for the two permanent magnets, the magnetic force \((F_m)\) and magnetic torque \((T_m)\) exerted on the robot are calculated using

\[
\begin{pmatrix}
F_m \\
T_m
\end{pmatrix} = \begin{pmatrix}
(m \cdot \nabla)(R_1 B_1 + R_2 B_2) \\
(m \times (R_1 B_1 + R_2 B_2))
\end{pmatrix}.
\]

In (2.3), \(m\) is the total magnetization vector of the robot, and \(R_1\) and \(R_2\) are the rotation matrices from frames of the first and second permanent magnets to the frame of the robot, respectively. Further, \(B_1\) and \(B_2\) are the magnetic fields of the first and second permanent magnets, respectively. The configuration of the rotating permanent magnets (Fig. 2.5) enables us to apply pure magnetic torque on the robot (when the robot is located at the common centers of the rotating permanent magnets along \(x\)-axis) \[29\]. Any deviation in the position of the robot along \(x\)-axis will result in a magnetic force that will contribute to the propulsion force. We calculate the Reynolds number as \(Re = \frac{\rho v L}{\mu} = 0.089\). The calculated Reynolds number represents an upper-limit since the speed of the robot during rubbing is on the order of \(O(10^{-4})\) m.s\(^{-1}\). This speed results in a Reynolds number of 0.004. Therefore, motion of the robot is governed by

\[
\begin{pmatrix}
F_m + F_g + F_d + F_c + F_f \\
T_m + T_g + T_d + T_c + T_f
\end{pmatrix} = 0,
\]

(2.4)
2.3. Resistive-Force Theory model

Figure 2.5: The helical robot (inset) is contained inside a catheter (diameter $D_{ch}$) segment between two rotating permanent magnets (magnetization $M$). Rotation of the permanent magnets generates a rotating field ($B$) that exerts a magnetic torque on the dipole of the robot ($m$). The robot consists of a cylindrical NdFeB magnet with diameter $r_x$, height $r_y$, and tail length $l$.

where $\mathbf{F}_d$ and $\mathbf{T}_d$ denote the viscous drag force and torque vectors, respectively. $\mathbf{F}_f$ and $\mathbf{T}_f$ are the fretting force and fretting torque due to the rubbing action between the robot and the clot (as shown in Fig. ??(c)), respectively. $\mathbf{F}_c$ and $\mathbf{T}_c$ denote the reaction force and torque when the head or the tail of the robot are in contact with the channel boundary. Further, $\mathbf{F}_g$ and $\mathbf{T}_g$ are the force and torque exerted on the robot due to gravity, and are given by

$$
\begin{bmatrix}
\mathbf{F}_g \\
\mathbf{T}_g
\end{bmatrix} =
\begin{bmatrix}
V(\rho_t - \rho_f)\mathbf{R}_{Lab}^T \mathbf{g} \\
(\mathbf{r}_{cov} - \mathbf{r}_{com}) \times \mathbf{F}_g
\end{bmatrix},
$$

(2.5)

where $\mathbf{g}$ is a vector signifying gravitational attraction and $\mathbf{R}_{Lab}$ is the time-dependent rotation matrix between the robot and a fixed frame of reference. $\rho_t$ and $\rho_f$ are the density of the robot and density of the medium, respectively. Further, $\mathbf{r}_{cov}$ and $\mathbf{r}_{com}$ are the center of volume and center of mass of the robot, respectively. The viscous drag on the magnetic robot is calculated based on RFT, by incorporating the wall effects on the tail and head [67, 68], and the complex hydrodynamic interactions [69] as:

$$
\begin{bmatrix}
\mathbf{F}_d \\
\mathbf{T}_d
\end{bmatrix} =
\left(\int l \begin{bmatrix}
\mathbf{R} \mathbf{C} \mathbf{R}^T & -\mathbf{R} \mathbf{C} \mathbf{R}^T \mathbf{S} \\
\mathbf{S} \mathbf{R} \mathbf{C} \mathbf{R}^T & -\mathbf{S} \mathbf{R} \mathbf{C} \mathbf{R}^T \mathbf{S}
\end{bmatrix} dl + \begin{bmatrix}
\mathbf{R}_{ch} \mathbf{D} \mathbf{R}_{ch}^T \\
\mathbf{S} \mathbf{R}_{ch} \mathbf{D} \mathbf{R}_{ch}^T \mathbf{E}
\end{bmatrix}
\right)
\left(\begin{bmatrix}
\mathbf{U} + \mathbf{U}_{ch}
\end{bmatrix}
\right),
$$

where $l$ is the length of the tail. $\mathbf{U}$ and $\mathbf{\Omega}$ are the rigid-body linear and angular swimming velocity vectors of the robot, respectively. $\mathbf{U}_{ch}$ is the flow vector inside the channel and $\mathbf{R}$ is the rotation matrix between local Frenet-Serret frames and the reference frame of the
robot. In addition, $\mathbf{R}_{\text{ch}}$ is a projection matrix of the cylindrical coordinates of the channel to the Cartesian coordinates residing on the center of mass of the robot, and $\mathbf{S}$ signifies local cross products. The hydrodynamic forces, i.e., propulsive and resistive drag acting on the moving surfaces, are given by the resistance matrices, $\mathbf{C}$, $\mathbf{D}$, and $\mathbf{E}$. Matrix $\mathbf{C}$ is calculated for the tail as, $\mathbf{C} = \text{diag}(c_t, c_n, c_b)$, where $c_t$, $c_n$, and $c_b$ are the local resistance coefficients along the Frenet-Serret frames of the tail and are articulated in [67]. Matrix $\mathbf{D}$ provides the fluid resistance coefficients of the magnetic head for the translational rigid-body motion as follows:

$$\mathbf{D} = \begin{pmatrix}
\Upsilon_x D_x & 0 & 0 \\
0 & \Upsilon_\theta D_\theta \cos \phi & \Upsilon_\theta D_\theta \sin \phi \\
0 & \Upsilon_\phi D_\phi \sin \phi & \Upsilon_\phi D_\phi \cos \phi
\end{pmatrix}$$

(2.6)
2.3. Resistive-Force Theory model

Figure 2.7: Rubbing behavior of a helical robot against a blood clot for $t \in [0, 8]$. (a) The evolution of the position of the robot is plotted at equal time intervals over 75 cycles. (b) The robot drifts towards the channel wall while rubbing against the clot. The blue circle represents the inner-diameter of the catheter segment, whereas the red circle represents a position limit on the robot. (c) Motion of the robot along $x$-axis is constrained by the clot during rubbing, whereas oscillations are observed along $y$- and $z$-axis. The removal rate of clot in this simulation is -0.834 mm$^3$/min, at frequency of 35 Hz.

Here $D_x$, $D_r$, and $D_0$ are provided in [68] using lubrication theory, and the complex hydrodynamic interaction terms, i.e., $\Upsilon_{x\alpha}$, $\Upsilon_{r\alpha}$, and $\phi_\alpha$ are articulated in [69]. These coefficients are used to predict the hydrodynamic interaction between moving and stationary surfaces. Finally, $E$ provides the fluid resistance coefficients of the head for the rotational rigid-body motion as follows:

$$E = -\text{diag} \left( 8\zeta_x \mu r_x r_y^2, 8\zeta_y \pi \mu r_y r_z^2, 8\zeta_z \pi \mu r_z r_y^2 \right),$$

(2.7)

where $\mu$ is the dynamic viscosity of the medium, and $r_x$ and $r_y$ are the radius of the magnetic head and its height, respectively. Further, $\zeta_i$, for $i = x, y, z$, are numerical tuning coefficients to compensate for geometric aberrations. Fig. 2.6 provides the drag forces and torques exerted on the head and tail of the robot. The total drag force on head and tail, along $x$-axis, cancel each other during free swimming as there is no other significant force along $x$-axis. On the other hand, the drag torque on the head and tail, along $x$-axis, do not balance each other because of the presence of magnetic torque. Similarly, one can deduce the importance of other physical stimuli by inspecting the pure drag force and drag torque balance on all axes. The wall contact is given by a series of equations based on a penalty method for the following hypothetical scenario [70]:

$$\begin{align*}
F_c & = \begin{cases} 
\delta, & \text{if } \delta \leq 0 \\
0, & \text{if } \delta > 0 
\end{cases} \left( \frac{\delta}{\delta}, \frac{\delta}{|\delta|} \right) n_c \\
t_c & = r_c \times F_c
\end{align*},$$

(2.8)

where $\delta$ is the penetration depth along the radial direction, $w$ is a weighing function to enable the realization of an over-damped system condition to prevent oscillations ($0 \leq w \leq 1$). Further, $n_c$ is the surface normal of the channel wall at the point of contact, and $r_c$ is the position vector of the contact point on the surface of the magnetic robot. In (2.8), $k$ and $b$ are given by

$$k = \frac{w |F_c|}{\delta} \quad \text{and} \quad b = \left( 1 - \frac{w}{|\delta|} \right) |F_c|.$$

(2.9)
Furthermore, (10) includes the reaction force of the channel walls using a spring-damper model, and allows damping effect only for positive velocities, while avoiding sticky surface condition for negative velocities and non-zero $\delta$ larger than a predefined value of $\delta \geq 10^{-6} r_{yz}$. $| F_c |$ is calculated by adding the hydrodynamic, magnetic, and forces due to gravity along the surface normal on the wall at the point of contact [70], and by solving (4). In order to obtain the velocities and the position vector relative to a fixed frame of reference we use, 

$$
\begin{bmatrix}
U_{Lab} \\
\Omega_{Lab}
\end{bmatrix}^T = R_{Lab} \begin{bmatrix}
U \\
\Omega
\end{bmatrix}^T
$$

The rotation matrix $R_{Lab}$ is obtained by quaternion calculations [71] as the robot experiences complex rigid-body rotations. Position vector along the cylindrical channel and rigid-body rotation matrix ($R_{Lab}$) are obtained by simple Forward-Euler integration over time.

From this point onwards we focus on the fretting force and torque using conservation of energy and momentum. The rubbing against (Fig. ??(e)) the blood clot, $\delta_{bc}$, is calculated based on torque equilibrium between the fretting torque and the difference between external magnetic torque and drag torque exerted on the helical robot using dimensional analysis. The fretting of material in volume requires certain amount of power which is harnessed from the rotation of the tail with a net torque that is approximated by

$$
(| T_{m,x} | - | T_{d,x} |) \Omega_x \approx 0.25 \tau \pi \Omega_x \delta_{bc}^2,
$$

where $T_{m,x}$ and $T_{d,x}$ are the magnetic and efficient drag torque of the rest of the physical stimuli acting on the robot with respect to $x$-axis of the robot, respectively. $\tau$ is the ultimate-tensile strength of the blood clot [72]. It is possible to approximate $\delta_{bc}$ based on the assumption that the removal rate is relatively small, and the helix removes the material in small fragments during its rotation with respect to $x$-axis of the robot. Based on the principle of conservation of momentum, the work done by the tip of the rotating tail is equal to the work done by the removed material. Under the assumptions that the removed material with infinitesimal volume is cylindrical in shape, and the fracture strength is the maximum measured ultimate tensile strength, we can approximate $\delta_{bc}$ using

$$
\delta_{bc} = \begin{cases} 
0, & \text{if } x_{bc} < r_{xtip} \\
\max \left( \frac{2 \sqrt{r_{x bc} x_{bc}}}{\sqrt{x_{bc} + r_t}}, 0.5 r_h \right), & \text{if } x_{bc} \geq r_{xtip}
\end{cases}
$$

where $r_h$ and $r_t$ are the radii of the helix and tail, respectively. Further, and $x_{bc}$ and $r_{xtip}$ denote the position of the blood clot and position of the tip of the tail, respectively, along $x$-axis of the channel ($x_{ch}$). The conservation of energy in terms of work done on small volumes of soft-tissue is used for modeling and characterization of local cutting and puncturing as presented in [73], as opposed to finite element analysis used to compute soft-tissue behavior under fracture conditions [74]. This penetration depth prediction leads to the following
fretting force and torque on the robot:

\[
\begin{align*}
    f_{t,x} &= \begin{cases} 
        k_{bc} \delta_{bc} + b_{bc} \left( \frac{d\delta_{bc}}{dt}, \text{ if } \frac{d\delta_{bc}}{dt} > 0 \right) n_{bc}, \\
        0, & \text{if } \frac{d\delta_{bc}}{dt} \leq 0
    \end{cases} \\
    f_{t,y} &= -|T_{t,x}| \left( \frac{m_{c,x}(t)}{r_n} \right) \\
    f_{t,z} &= -|T_{t,x}| \left( \frac{m_{c,z}(t)}{r_n} \right)
\end{align*}
\]  

(2.12)

Here \( f_{t,x}, f_{t,y}, \) and \( f_{t,z} \) are the fretting force components along \( x-, y-, \) and \( z- \) axis of the robot, respectively. Further, \( T_{t,x} \) is the fretting torque exerted on the robot with respect to \( x- \) axis, and is given by

\[
\begin{align*}
    T_{t,x} &= -\text{sgn}(\Omega_x) \pi r_{hi}^2 \\
    T_{t,y} &= r_{c,z} f_{t,x} - r_{c,x} f_{t,y} \\
    T_{t,z} &= r_{c,x} f_{t,y} - r_{c,y} f_{t,x}
\end{align*}
\]

(2.13)

where \( n_{bc} \) is the surface normal of the blood clot. \( F_f \) and \( T_f \) vectors (similar to \( F_c \) and \( T_c \)) represent force constraints to prevent the robot from moving beyond the point of contact. In (2.11), \( n_{t,y}(t) \) and \( n_{t,z}(t) \) are the components of the surface normal of the blood-clot contact at the rotating tip of the tail in the robot frame of reference denoting the fretting direction, whereas \( n_{t,x}(t) \) is assumed to be -1 in the robot frame of reference denoting the direction of penetration into the blood clot. The surface normal components \( n_{t,y}(t) \) and \( n_{t,z}(t) \) of the tail are also computed as follows:

\[
\begin{align*}
    n_{t,y}(t) &= -\cos \left( \frac{\pi}{2} - \arctan \left( r_{c,z}(t), r_{c,y}(t) \right) \right) \\
    n_{t,z}(t) &= -\sin \left( \frac{\pi}{2} - \arctan \left( r_{c,z}(t), r_{c,y}(t) \right) \right)
\end{align*}
\]

(2.14)

where the negative sign is due to chirality. The material properties \( k_{bc} \) and \( b_{bc} \) are approximated by considering that the overall system must be over-damped to eliminate undesirable oscillations

\[
k_{bc} = \frac{|F_m| - |F_d|}{\delta_{bc}} \quad \text{and} \quad b_{bc} = 2\chi \sqrt{m_r k_{bc}}
\]

(2.15)

where \( m_r \) is the mass of the robot, and \( \chi \) denotes the damping tuning for the blood clot contact. \( \chi \) is modeled by approximating the non-Newtonian behavior of the clot with a nonlinear velocity relation, i.e., \( \chi = \chi_0 + \exp(\chi_1 \Omega_x) \), similitude to the variation of viscosity of fluids under high pressure [75]. According to RFT, impact power of the tip of the tail increases with the square of the actuation frequency, which increases the local viscosity non-linearly. Finally, the location of the clot is updated at each time-step using position dependent normal distribution function, \( \psi(r) \), to simulate the material removal as follows:

\[
x_{bc}(t + dt) = x_{bc}(0) + \text{sgn}(u_x) \int_0^{\psi(r)} \frac{\psi(r)}{D_{ch}} \int_0^{\psi(r)} r n_{bc} | \Omega_r | \ dt,
\]

(2.16)

where \( x_{bc}(t) \) and \( x_{bc}(0) \) are the positions of the blood clot at time \( t \) and the initial position.
of the clot, respectively. \( u_x \) is the velocity of the robot along \( x \)-axis in its frame of reference. Further, we map the one-dimensional prediction of material removal into three-dimensional prediction based on the preliminary simulation results as the robot does not remove material from a single location (Fig. 2.7). In (2.16), \( \psi(r) \) is a normal distribution function and is given by

\[
\psi(r) = \frac{1}{\sqrt{2\pi}\sigma} \exp\left(-\frac{(r-r_0-\eta)^2}{2\sigma^2}\right), \tag{2.17}
\]

where \( \sigma \) is the standard deviation and \( \eta \) is the location, i.e., center of the channel, where the maximum cumulative material removal is expected, and \( r \) is the radial position of the robot in the channel. \( \kappa \) is the weighing function, which is based on the location of the tip of the rotating tail, handling each of the four quadrants of the channel cross-section separately to obtain accurate distribution. We begin by simulating the rubbing behavior by solving the equation of motion (2.4), and obtain the rigid-body velocity vectors of the robot. Initially, the robot is assumed to be parallel to the longitudinal axis of the cylindrical channel and is concentric with the cross-section. Fig. 2.7 shows the rubbing behaviour of the robot against the blood clot. We allow the applied magnetic field of the two rotating permanent magnets to exert magnetic force and torque based on (2.3). This magnetic actuation is responsible for defining the swimming direction of the robot along \( x \)-axis. As one would expect, the contact between the robot and the clot constrains the motion of the robot along \( x \)-axis (Fig. 2.7 (a)), and causes the robot to drift towards the wall with time, as shown in Fig. 2.7 (b). Fig. 2.7 (c) shows the components of the velocity of the robot during rubbing. The robot exhibits oscillatory motion along \( y \)- and \( z \)-axis, whereas the net displacement along \( x \)-axis is negligible. Our simulation also allows for the calculation of the removal rate of the clot based on (2.11), (2.12), (2.13), and (2.16). The removal rate is calculated at 26 frequencies of the rotating dipole fields, and maximum dissolution rate of \(-0.834 \text{ mm}^3/\text{min}\) is observed at frequency of 35 Hz. The existence of an optimal rubbing frequency before the step-out frequency of the robot could be attributed to the presence of flow against the motion of the robot and the increased damping during rubbing at relatively high frequencies.

### 2.4 Characterization of blood clots

Modelling and experimental work to achieve dissolution of blood clots requires knowledge of their mechanical and rheological properties. To achieve efficient mechanical rubbing of blood clots, it is important to characterize the blood clot samples to gain knowledge on their morphology and structure and also their mechanical properties for relevant modelling.

#### 2.4.1 Preparation of blood clots

Blood clots are prepared based on the protocol proposed by Hoffmann and Gill [76], 5 ml of fresh venous blood is drawn from two blood donors between the age of 25 and 28, neither having records of blood coagulation disorders or acute illness, and without a recent history of oral contraceptive use or anticoagulant therapy. (Local Institutional Ethical Board
approval is obtained for the preparation protocol of the blood clots, and donors gave written informed consent). Also donors have similar health conditions and daily habits for the aimed consistency of the results. Since diet, smoking, and other daily habits could influence the properties of human blood \cite{77, 78}. Blood sample is inserted into a red top vacutainer without anticoagulant (Fig. 2.8 (a)), held upright and kept inside a water bath at 37°C for one hour to coagulate forming a long columnar “mother” blood clot.

**Figure 2.8:** (a) Fresh venous blood is drawn from healthy donors between the age of 25 and 28, and inserted into a vacutainer without anticoagulant. Then, (b) A blood clot is extracted from the vacutainer and (c) a small slice is cut and inserted inside the catheter segment.

Then, the vacutainer is removed from the water bath and the clot is extracted on a dry plastic parafilm sheet (Fig. 2.8 (b)). The clot is then immediately roll dried for 20 cm, allowed to air dry for 5 minutes, and then further roll dried for another 20 cm to rid the clot of gross amounts of adherent serum. (c) A small piece of the clot "daughter" clot is cut for the insertion into the catheter segment(Fig. 2.8 (c)).

### 2.4.2 Morphology of blood clots

Characterization of the morphology of blood clots supports better understanding of the clotting mechanism and the composition and structure of the clot. Red blood cells and platelets forms a big portion of thrombus mass specially in venous thrombosis \cite{79}. Cines \textit{et al.} have discussed the process of clot formation and contraction, in which platelets aggregate to form a temporary sealant and fibrinogen is converted into a network of fibrin polymers, causing contraction of blood clot by the cytoplasmic proteins inside platelets. Such contractions helps the retention of Red blood cells inside the fibrin network of the clot. They have observed the morphology of entrapped blood cells inside a blood clot \cite{80}. 


A similar morphology is observed in the scanning electron microscopy (SEM) image (Fig. 2.9) of a dried fragment of 1-hour-old blood clot. The SEM image indicates the entrapped blood cells inside the three-dimensional fibrin network of the clot, which by turn validates that it has similar structure to the blood clots forming inside the human circulatory system.

### 2.4.3 Rheology of blood clots

Quantification of the mechanical properties of blood clots is fundamental to understanding many aspects of cardiovascular disease and its treatment. In addition, it is essential for the modelling of mechanical rubbing and its influence on the dissolution of clots. Epidemiological studies have shown a relationship between the mechanical properties of blood clots and myocardial infarction, as the formation of *in vitro* blood clots with patients with myocardial infarction show rigid fibrin network structure compared to control [81], [82]. Mechanical properties of fibrin have been characterized by John W. Weisel. He stated that viscoelastic properties of fibrin are notable and unique among polymers, and that these properties are essential to the physiology of blood clotting and crucial for preventing and treating thrombosis [83]. Further, studies of mechanical properties of blood clots has been conducted for better understanding of clot composition and structure from its formation to dissolution. Rheology tests have been long used to assess the deformation and flow behavior for various kinds of materials. In 1964, Rozenberg *et al.* have studied blood coagulation and the mechanical properties of thrombosis by means of viscometric analysis. They have found that the degree of platelet aggregation during thrombosis process is a function of the measured sheer, and they found that the morphology of the formed clots *in vitro* are similar to *in vivo* clots [84]. Modern rheometers can be used for shear tests, they operate with continuous
2.4. Characterization of blood clots

Figure 2.10: Rheology test is conducted to characterize the shear modulus of the blood clots. The test is done using a Bohlin Gemini instrument (Malvern Instruments, U.K.).

Rotation and rotational oscillation. Rheology test is done on 1-hour-old blood clot samples using a Bohlin Gemini instrument (Malvern Instruments, U.K.), equipped with a parallel plates measuring system (shown in Fig. 2.10). Blood clots are prepared using the same protocol explained earlier in section 2.4.1. But instead of the vacutainer the sample is inserted into a beaker with a round base to allow the blood to coagulate in a round structure required for the rheology test. Samples are placed between a lower plate with diameter of 40 mm and an upper plate with diameter of 25 mm. The clots are surrounded by oil with viscosity of 0.06 Pas to avoid drying and denaturation of the samples. The gap between the plates is 1.569 mm and oscillatory shear with maximum shear stress of 0.1 Pa is applied at frequency of 1 Hz. At room temperature (25°C) and body temperature (37°C), shear modulus is measured as 40.5 ± 0.6 Pa and 41.4 ± 0.5 Pa, respectively. A representative trial is shown in Fig. 2.11(a).

Figure 2.11: Rheology test is conducted to characterize the shear modulus of the blood clots. The test is done using a Bohlin Gemini instrument (Malvern Instruments, U.K.).

The sample is heated up from 25°C to 37°C by the rheometer while enclosed inside a temperature control unit, and shear modulus is measured against increasing temperature
during the heating up process. In addition, the stress-strain relation of the blood clots for two representative trials is shown in Fig. 2.11 (b). This characterization experiment indicates that the ultimate shear strength of 1-hour-old blood clots is approximately 1 kPa. This value represents the force under which the blood clot breaks or fractures. Ultimate shear strength value is used in the resistive force theory model to predict the theoretical removal rate of the clot during rubbing.
Chapter 3

Mechanical rubbing of blood clots

The dissolution of blood clots is experimentally investigated using two distinct groups, i.e., dissolution using full dose of streptokinase (Group 1) and mechanical rubbing (Group 2). In each trail, the clots are prepared and inserted into catheter segments, that are mounted between two rotating permanent magnets to exert magnetic torque on the dipole of the helical robot.
3.1 Volume detection using camera feedback

The volume of blood clot is calculated from camera feedback (TavA1000-120kc, Basler Area Scan Camera, Basler AG, Ahrensburg, Germany) using a morphological filtering algorithm implemented on Matlab (MathWorks Inc.) during the experiment. The camera provides orthogonal top view of the blood clot. The volume detection algorithm (included in Appendix A) is based on morphological filtering with the following steps; (1) Frames are acquired from camera feedback as a sequence of images, morphological operations are performed on a frame by frame. (2) The acquired frame is manually cropped around the blood clot to reduce the processing time. (3) The cropped RGB image is converted into a gray image. (4) Threshold is applied on the obtained gray image resulting in a black and white image where the white pixels represents the blood clot. (5) Finally dimensionless area of the white pixels is calculated and stored for each frame.

![Figure 3.1: Volume detection of gelatin thrombus model](image)

Prior to the volume dissolution detection of blood clots with camera feedback, the volume detection algorithm is tested on a gelatin thrombus model under the same settings for validation. Gelatin is dyed with Methylene blue (dye) and inserted inside the catheter segment taking an almost cylindrical shape, and the catheter segment is filled with phosphate buffered saline with flow rate of 10 ml/hr. The volume of gelatin thrombus model as shown in (Fig 3.1(a)) was calculated to be almost constant throughout the experiment (Fig 3.1(b)).

3.2 Dissolution of blood clots

The dissolution of blood clots is experimentally investigated using two distinct groups, i.e., dissolution using full dose of streptokinase (Group 1) and mechanical rubbing using helical robots (Group 2). Blood clots are prepared (as explained in section 2.4.1) and inserted into a polyvinyl chloride catheter segment. All experiments are done in the presence of a flow rate of 10 ml/hr. This flow rate is devised based on the administration and infusion rates for adult patients (maximum flow rates in small arteriole, capillaries, and venule are approximately 0.25 ml/hr, 0.045 ml/hr, and 0.00324 ml/hr). For instance, 10 ml of Streptokinase is usually given intravenously in a 1-hour infusion. The flow rate is provided and
3.2. Dissolution of blood clots

controlled using a syringe pump (Genie Plus, GT-4201D-12, Kent Scientific, Connecticut, USA). The pump is fixed to one end of the catheter segment while the other end is inserted into a beaker for the collection of samples when needed.

3.2.1 Group 1: Dissolution using Full Dose of Streptokinase

The physiology of the fibrin—clot formation is relatively known [86]. A blood clot or thrombus consists of blood cells entrapped in a matrix of the protein fibrin. Enzyme-mediated dissolution of the fibrin clot is known as fibrinolysis. There are three major classes of fibrinolytic drugs: tissue plasminogen activator (tPA), streptokinase (SK), and urokinase (UK). Drugs in these three classes all have the ability to effectively dissolve blood clots, but they differ in their precise mechanisms in ways that alter their selectivity for blood clots. Streptokinase has been of choice for treatment of myocardial infarction for more than 40 years [87]. It is an enzyme secreted by several species of *streptococci* bacteria that work by converting fibrin-bound plasminogen to plasmin, a natural fibrinolytic agent which breaks down fibrin contained in a blood clot leading to its lysis. Characterization of streptokinase through biophysical techniques such as magnetic resonance spectroscopy [88], Fourier transform infrared spectroscopy [89] have provided useful information on its structure. The efficacy of streptokinase-based thrombolytic therapy and methods to improve it has been an interesting matter for investigation by researchers. Prasad *et al.* have studied clot lysis using streptokinase. Blood samples were obtained from volunteers without history of anticoagulant therapy and incubated to form blood clots. They observed significant percentage of clot lysis when streptokinase was used, calculated based on weight measurements of clots before and after applying the thrombolytic agent [90]. Sameni *et al.* have demonstrated *in vivo* characterization of the bio—activity of a mutated streptokinase with enhanced immunogenicity. [91]. In this group, the dissolution of blood clots is achieved under the influence of a streptokinase-based thrombolytic agent at flow rate of 10 ml/hr. The medium inside the catheter segment is a mixture of one vial of the drug (Sedonase, SEDICO Co., Egypt) where each vial contains: streptokinase 1,500,000 I. U. and phosphate buffered saline, the drug vial is reconstituted with 5 ml of phosphate buffered saline and rolled gently forming the mixture. The volume of the clot is calculated throughout a lysis period of 40 minutes. The average dissolution rate calculated from 6 trials is -0.17±0.032 mm$^3$/min. In the representative trial shown in Fig. 3.2, the initial and final volumes are calculated to be 94.24 mm$^3$ and 91.99 mm$^3$, respectively. The measured dissolution rate is -0.19 mm$^3$/min. Furthermore, formation of fluid channels inside the clot is observed after approximately 20 minutes of streptokinase injection.
Figure 3.2: A representative experiment of dissolution of a blood clot using full dose of streptokinase (1,500,000 I.U.). The blue arrow indicates the direction of the flow. In this trial, the dissolution rate of the clot is \(-0.19\, \text{mm}^3/\text{min}\) and at time, \(t \approx 20\) minutes, formation of fluid channels (white dashed lines) inside the clot is observed.

Streptokinase is commonly used for myocardial infarction, arterial and venous thrombosis, and pulmonary embolism. Dotter et al. have demonstrated the treatment of 17 patients with arterial thromboembolism by selective doses of streptokinase delivered by catheter near or directly into blood clots. They have shown that such method offers improved lysis with the targeted delivery of the drug, and enables the treatment with lower doses of streptokinase to reduce possible bleeding and other side effects associated with fibronolytic agents [92]. Azdaki et al. have presented successful intravenous streptokinase therapy for Deep Vein Thrombosis (DVT) associated with failure of inferior vena cava in a 40 year old patient. Final examination using ultrasound reported that the blood flow is normally restored where the DVT has formed [93]. Although streptokinase have been long used in thrombolytic therapy, side effects of the drug such as excessive bleeding, chemical–driven liver damage, thrombocytopenia (low blood platelets count), and Osteoporosis (thinning of the bones, with reduction in bone mass, due to depletion of calcium and bone protein) [94]. Removal of blood clots by mechanical rubbing with helical robots can mitigate such risks.

3.2.2 Group 2: Mechanical Rubbing Against Blood Clots

The helical robot is propelled along \(x\)-axis towards the clot and against similar flow rate to that used in the chemical lysis experiments (10 ml/hr). The permanent magnet-based robotic system (explained in section 2.2) is used to actuate the robot, as shown in Fig. 2.5.
Each permanent magnet generates magnetic field of 0.552 T on its surface. The two permanent magnets are rotated and the linear speed of the robot is measured to be 15 mm/s, at frequency of 35 Hz. The step-out frequency of the robot is experimentally measured to be 67.3 Hz, and a linear increase of the swimming speed is observed versus the angular frequency of the rotating permanent magnets within this range. Therefore, the particular choice of the actuation frequency affects the swimming speed, i.e., the approaching speed to the clot, before the rubbing behaviour. The resultant magnetic field at the position of the robot is measured as 5.5 mT using a Tesla meter (3MH3A teslameter, Senis, Switzerland). The rubbing behaviour is observed once the tip of the tail comes into contact with the clot. In the representative trial shown in Fig. 3.3, the initial and final volumes of the clot are calculated to be 94.24 mm$^3$ and 60.65 mm$^3$, respectively, after 40 minutes of rubbing against the clot. The dissolution rate at frequency of 35 Hz is -0.885 mm$^3$/min.

![Figure 3.3: A representative experiment of mechanical rubbing against a blood clot using a helical robot. In this trial, the removal rate of the clot is -0.885 mm$^3$/min at frequency of 35 Hz.](image)

The efficiency of mechanical rubbing is compared to chemical lysis under similar experimental settings. Mechanical rubbing achieves higher removal rate than chemical lysis, as shown in Fig. 3.4. The ratio between the measured volume and the initial volume ($v_0/v$) of the clot is measured at every time instant during the lysis and rubbing. Further, the volume ratio ($v_0/v$) is measured in the absence of lysis and rubbing (zero-input response) to evaluate the volume dissolution due to experimental settings that does not include the effect of the robot or the drug. This response shows that the blood clot does not undergo any change in its volume (Fig. 3.4). Nevertheless, the lysis and rubbing have constant rates of change, as shown in Fig. 3.4. The dissolution rate of the lysis is -0.19 mm$^3$/min in this trial. The rubbing is done at 35 Hz and 40 Hz, and flow rate of 10 ml/hr. The dissolution rate of the rubbing is -0.885 mm$^3$/min and -0.315 mm$^3$/min at 35 Hz and 40 Hz, respectively.
Chapter 3. Mechanical rubbing of blood clots

Figure 3.4: Chemical lysis and mechanical rubbing of blood clots are tested on a blood clot with initial volume of \( v_0 \) of 94.24 mm\(^3\). The zero-input response indicates that the clot does not undergo any change in its size. The lysis is done using streptokinase, at flow rate of 10 ml/hr.

In contrast to chemical lysis, the removal rate of the clot can be controlled via rubbing using the frequency of the rotating dipole fields. The influence of the rubbing frequency on the removal rate of the blood clot between 20 Hz and 45 Hz is investigated. The removal rate by the robot is almost negligible at frequencies below 20 Hz (Figs. 3.5(a) and (b)). Removal rates of -0.230 mm\(^3\)/min, -0.885 mm\(^3\)/min, and -0.315 mm\(^3\)/min are measured at rubbing frequencies of 30 Hz, 35 Hz, and 40 Hz, respectively (Figs. 3.5(c), (d), and (e)). Maximum removal rate is observed at 35 Hz in agreement with simulation results generated from the hydrodynamic model (presented in section 2.4). At and above 45 Hz, negligible removal of the clot is observed (Fig. 3.5(f)).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>( \nu(t_f)/v_0 )</th>
<th>Dissolution rate [mm(^3)/min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical lysis</td>
<td>0.94±0.011</td>
<td>-0.17±0.032</td>
</tr>
<tr>
<td>Rubbing at 35 Hz</td>
<td>0.85±0.042</td>
<td>-0.56±0.27</td>
</tr>
</tbody>
</table>

Table 3.1: Comparison between lysis and rubbing at 35 Hz. The final volume \( \nu(t_f) \) is measured after 40 minutes. The initial volume \( v_0 \) of the clot is 94.24 mm\(^3\).

Fig. 3.5(a and b) Mechanical rubbing is not effective below 20 Hz and this could be attributed to the presence of a flow against the helical robot. At a result, the robot does not
generate sufficient thrust to come into contact with the clot and achieve effective rubbing at these frequencies. Fig. 3.5(c, d, and e) Removal rates of -0.230 mm$^3$/min, -0.885 mm$^3$/min, and -0.315 mm$^3$/min are measured at 30 Hz, 35 Hz, and 40 Hz, respectively. Fig. 3.5(f) The rubbing is not effective at and above frequency of 45 Hz. Material removal (fretting) phenomenon is affected by at least two physical stimuli. First, the flow inside the catheter segment exerts a drag force (6) against the robot. This flow is essential to provide fair comparison between chemical lysis and mechanical rubbing. Therefore, the drag force decreases the material removal at low frequencies.

![Figure 3.5](image)

**Figure 3.5:** The influence of rubbing frequency on the removal rate of the blood clot is investigated experimentally between 20 Hz to 45 Hz.

The penetration depth and fretting is not effective at low frequencies, as shown in Fig. 3.6. Second, the non-Newtonian nature of the blood clot affects the removal rate. Resistive force dictates that the velocity is in a linear relationship with the rotation rate, and power of the penetration increases with the square of the velocity. However, the effective viscosity of the blood clot under sudden impact should increase exponentially (or in an equivalent manner). Therefore, the damping effect becomes dominant as the frequency increases. This means that penetration decreases as spring behavior becomes more negligible. Hence, once again penetration depth and fretting becomes ineffective at relatively higher frequencies (Fig. 3.6).
Chapter 3. Mechanical rubbing of blood clots

Figure 3.6: The rubbing frequency influences the removal rate of the blood clot. Mechanical rubbing is effective within a frequency range of 20 Hz to 45 Hz. Maximum removal rate is achieved at 35 Hz (inset). The magnetic field at the position of the helical robot is 5.5 mT, and the flow rate is 10 ml/hr.

The deviation between the theoretical and experimental removal rates is due to several aspects. First, it is observed that mechanical rubbing causes small angular rotations of the clot in the direction of rubbing. Initially, the cylindrical area of the blood clot is completely in contact with the inner-surface of the segment, and hence the resulting friction force does not allow for rotation or any mobilization of the clot. However, the size of the clot decreases after rubbing for several minutes. Therefore, the contact between the clot and the inner-surface of the segment is decreased and friction is reduced. Second, the natural clot-to-clot variability in properties and original shape (during insertion of the clots inside the segments) results in a deviation between the theoretical and experimental results.

The scatter in the experimental data (evident at 30 Hz and 35 Hz) is attributed to the clot-to-clot variability, the irregularity of the shape of each blood clot (Fig. ?? affects the volume calculations. Nevertheless, the optimal rubbing frequency of the experimental removal rate is in agreement with the results of the mathematical model. The experimental results do not show significant difference within the measurement error between chemical lysis and rubbing at 20 Hz, 25 Hz, 30 Hz, and 45 Hz. However, the rubbing results at 35 Hz and 40 Hz show a significant increase in the removal rate of the clot compared to lysis under similar conditions. In conclusion, it is experimentally shown that mechanical rubbing against blood clots using helical robots results in higher removal rates than chemical lysis using a thrombolytic agent (Table ??). Streptokinase achieves dissolution rate of \(-0.17 \pm 0.032 \text{ mm}^3/\text{min} \) \((n=6)\), whereas rubbing achieves removal rate of \(-0.56 \pm 0.27 \text{ mm}^3/\text{min} \) \((n=6)\), at frequency of 35 Hz.
3.3 Blood clot weight

As discussed earlier, mechanical rubbing of blood clots results in their volume dissolution. It is expected that the decrease in volume of blood clots implies a loss in their weight according to law of conservation of matter. Hence, Weight of blood clots is measured before and after applying mechanical rubbing. Measurements are done via an electronic balance (ABS 220-4 Analytical Balance, KERN & SOHN GmbH, Balingen, Germany). The blood clot is measured before insertion into the catheter segment, and then is extracted from the catheter segment and weighed again after 40 minutes of mechanical rubbing. The percentage of decrease in the weight of the blood clot $w_d$ is calculated by

$$w_d = \left( \frac{w(t_f) - w(t_0)}{w(t_0)} \right) \times 100.$$  \hspace{1cm} (3.1)

Weight reduction percentage measurements ($w_d$) under the influence of rotating magnetic fields with varying frequency in the range of 20 Hz to 45 Hz are shown in Table 3.2 below.

<table>
<thead>
<tr>
<th>$\omega$ [Hz]</th>
<th>0</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>$w_d$ [%]</td>
<td>0</td>
<td>51.9±3.1</td>
<td>71.4±5.1</td>
<td>74.4±11.1</td>
<td>67.8±4.5</td>
<td>72.6±5</td>
<td>73.5±8.8</td>
</tr>
</tbody>
</table>

Table 3.2: Measurements of weight reduction percentage ($w_d$) under the influence of rotating magnetic fields with varying frequency in the range of 20 Hz to 45 Hz

In (3.1), $w(t_f)$ is the weight of blood clot after 40 minutes of mechanical rubbing, $w(t_0)$ is the initial weight of the blood clot. In the absence of mechanical rubbing (zero-input-response), $w(t_0)$ is measured as 51.9±3.1% ($n=3$) and is attributed to the effect of the flowing solution in the catheter segment at a rate of 10 ml/hr on the freshly formed blood clot (one-hour old). Also the loss due to insertion and extraction of the blood clot from the catheter segment. An increase in $w_d$ is observed for all values of $\omega$ in comparison to 0 Hz. This increase validates the dissolution of blood clots under the influence of mechanical rubbing. Related work have also used weight analysis to assess the dissolution of blood clot. Prasad et al. have developed a new model system to study lysis of blood clots using streptokinase in a simplified way. They have shown in vitro dissolution of clots by four different dilutions of streptokinase and lysis percentages were calculated using weight measurement of clot before and after lysis [90]. However, the weight decrease does not highlight all the changes related to the effect of the mechanical rubbing on the fibrin network of the blood clot. Hence, cell count of the samples past the robot and blood clot is calculated, which reflects the breakdown of the fibrin network causing the release of red blood cells (RBCs) and platelets previously entrapped within the blood clot.
3.4 Cell count

Venous blood clots are typically rich in fibrin and RBCs. Fibrin cross linking by Factor XIII (fibrin stabilizing factor) and elastic properties of the blood clot play an important role in the retention of RBCs inside the fibrin network of the clot. After clot formation, active platelets pull on the fibrin fibers and reduces the size of the blood clot in a physiological mechanism called clot retraction. This retraction mechanism augments the retention of RBCs inside the blood clot [95]. The interaction between the rotating tip of the helical robot and the blood clot allows the RBCs and platelets to break free from the fibrin network of the blood clot. The influence of mechanical rubbing frequency on cell count is investigated. Mixture past the robot and the blood clot is collected every 5 minutes into a small tube (Fig. 3.7) for analysis.

![Figure 3.7: Mixture past the robot and the blood clot is collected every 5 minutes into a small tube](image)

A hemocytometer (Neubauer Improved, Germany), a device originally designed for counting blood cells, is used to calculate the number of RBCs and platelets for each sample under microscope (MF Series 176- Measuring Microscope, Mitutoyo America Corporation). Samples are diluted and a droplet of the sample is introduced to the counting chamber of the hemocytometer and observed under the microscope. The frame of the counting chamber consists of 9 large squares. The large central square is divided into 25 medium squares (marked in red (Fig. 3.8)) each with 16 small squares (marked in blue (Fig. 3.8)).
3.4. Cell count

The cells inside a medium square are counted for each sample from the four corner squares of the chamber. Pictures are captured by a camera ((TavA1000-120kc, Basler Area Scan Camera, Basler AG, Ahrensburg, Germany)) mounted on the microscope, and a simple Matlab code is implemented to detect and count circular objects. The number of RBCs and platelets after 40 mins of mechanical rubbing under the influence of varying frequency in the range of 20 to 45 Hz is shown in Fig. 3.9(b). Further,

![Hemocytometer chamber observed under microscope.](image)

**Figure 3.8:** Hemocytometer chamber is observed under under microscope. The frame of the counting chamber consists of 9 large squares. The large central square is divided into 25 medium squares (marked in red) each with 16 small squares (marked in blue).

![Samples past the robot and blood clot collected every 5 minutes during experiments.](image)

**Figure 3.9:** Samples past the robot and blood clot are collected every 5 minutes during the experiments for analysis.

First, cell count for the mixture past the robot and blood every 5 minutes during the experiments is shown. Averaged sum of cell count after 40 minutes of rubbing provides
a maximum value of $654 \pm 108 \times 10^4$ cells/ml as calculated at 40 Hz, compared to $54 \pm 12 \times 10^4$ cells/ml in the absence of mechanical rubbing (Fig. 3.9(b)). Averages and standard deviations are calculated from 3 trials. Cell count at all frequencies in the range of 20 to 45 Hz is increased in comparison to cell count at 0 Hz. The increased cell count indicates increased number of released RBCs and platelets from the fibrin network of the blood clot, validating the mechanical breakdown of fibrin by the rotating tip of the helical robot.

### 3.5 Spectrophotometric analysis

Spectrophotometry is the method of measuring the amount of light absorbed by a substance by measuring the attenuation of light after travelling through this substance. It is widely used for quantitative analysis in a variety of applications in biochemistry, chemical engineering and clinical applications. Spectrophotometric analysis is carried for further validation of the efficiency of mechanical rubbing. Samples past the robot and blood clot are collected every 5 minutes during the experiments. Absorbance of the collected samples is measured using a spectrophotometer (V-730 UV-Visible Spectrophotometer, Oklahoma city, USA). The spectrophotometer generates a beam of light that interacts with the tested sample and measures the amount of light absorbed by the sample over a certain range of wavelength. This allows the calculation of the total amount of chemical substance in the tested sample using Beer-Lambert law, which relates the attenuation of light to the properties of the material which the light travels through as follows:

$$ A = \epsilon bc. $$

In (3.2), $A$ is the absorbance measured by spectrophotometer, $\epsilon$ is the wavelength-dependent molar absorptivity coefficient, $b$ is the path length of the cuvette in which the sample is contained and $c$ is the compound concentration. Beer-Lambert law states that the proportion of incident light absorbed by a transparent medium is independent of the intensity of light, provided that there is no other physical or chemical changes in the medium. The absorption of light is directly proportional to both the concentration of the absorbing medium and the thickness of the medium in the light path. The procedure of spectrophotometric analysis is carried out as follows. First, a base line measurement of the samples is done for the optimum wavelength selection within the range of visible light (400 nm to 800 nm). Maximum absorbance was measured at wave length ($\lambda = 416$ nm). Second, absorbance of each sample is measured at the selected wave length ($\lambda$). Finally, Beer-Lambert Law is used to calculate the concentration where $\epsilon = 521880$ as reported in [96] and $b = 1$ cm. Absorbance is linearly proportional to the concentration of the substance in the same sample. Thus, increased absorbance implies increased concentration and correspondingly increased number of blood cells released from the blood clot.
3.5. Spectrophotometric analysis

Figure 3.10: Spectrophotometric analysis is performed to study the influence of mechanical rubbing on the concentration of blood clots. First, a baseline is selected as $\lambda = 416$ nm, then absorbance is measured under the influence of a rotating magnetic field with varying frequency in the range of 20 Hz to 45 Hz.

Concentration of samples calculated past the robot and blood clot, under the influence of varying frequency of rotating magnetic fields in the range of 20 Hz to 45 Hz is shown in Fig. 3.10. Maximum total concentration was measured as $4.35 \times 10^{-6}$ mol at a frequency of 35 Hz, compared to $1.05 \times 10^{-6}$ mol in the absence of mechanical rubbing. The values of absorbance measured at all frequencies is higher than absorbance measured at 0 Hz, which by turn shows that the concentration of RBCs and platelets is also increased. The increased concentration of RBCs and platelets in the samples collected post mechanical rubbing validates its efficiency. As explained in section 4.3, the interaction of the tail of the helical robot results in the breakdown of the fibrin network of the blood clot and the release of entrapped cells.
Chapter 4

Localization and control of helical robots

An imaging modality is required for the visualization and control of the helical robot in ex-vivo and in-vivo models. The development and spread of such devices utilizes their use in clinical validation trials. Imaging modalities include conventional film/screen x-ray, computed tomography(CT), MRI, Ultrasound, Doppler ultrasound, and various imaging techniques based on nuclear emission(PET, SPECT, and etc). In this section, ultrasound guided localization and control of helical robot in-vitro is achieved.
4.1 Diagnostic Ultrasound and its deployment in microrobtics

Towards potential in vivo application of the proposed approach, a medical imaging modality is required to localize and visualize the helical robot rather than cameras used in in vitro experiments. The increasing availability of inexpensive computational technology and resources with time, has been encouraging the evolution and commercialization of new imaging technologies. The development and spread of such devices utilizes their use in clinical validation trials for qualification, efficiency, and safety. An important addition to medical imaging devices in recent years, is the development and deployment of methods for archiving and transmitting digital images with high quality for offline analysis. Medical Imaging modalities include conventional film/screen x-ray, computed tomography(CT), MRI, Ultrasound, Doppler ultrasound, and various imaging techniques based on nuclear emission(PET, SPECT, and etc) [97]. Research studies have demonstrated the control of micro and nano robot with the feedback of medical imaging modalities. For example, Martel et. al have achieved in vivo controlled navigation and tracking of a 1.5 mm ferromagnetic bead inside the carotid artery of a living swine, using a clinical magnetic resonance imaging (MRI) platform. The performed method performs robust tracking and propulsion that allows the navigation through pre-planned paths along the circulatory system [98]. Also, Park et. al have presented a paddling-based microrobot for capsule endoscopy. The motion of the capsular microrobot is monitored in vivo in the intestinal tract of a pig by a C arm mobile X-ray system [99]. Oh et. al have describe and image-based guidance system for an intravascular microrobot. They used Computed tomography angiography (CTA) feedback images to extract preoperative data of the vessel, and integrated two pairs of X-ray sources to electromagnetic actuation system in perpendicular direction to the extract the position information of the microrobot [100]. In specific, diagnostic ultrasound travels in way similar to audible sound waves. It consists of mechanical vibrations that propagates through the human body. The speed at which the waves travel changes based on the acoustic impedence of the material. The point at which the acoustic impedence changes, a part of the pulse will be reflected back in the form of an echo and the remainder will resume travelling through the body. These returning echoes are converted into a visual display and used to form a sectional image. Although diagnostic ultrasound is a form of radiation as it uses energy emitted from a source, but it does not cause tissue ionization since sound waves are not related to the electromagnetic spectrum. Therefore, the technique is free from the risks associated with X-ray [101]. Advantages of Ultrasound diagnostic imaging includes: (1) Small and easy-to-use transducer, which allows the generation of real-time tomographic images at positions and orientations that can be manipulated by the user. (2) The ultrasound imaging system is relatively inexpensive. (3) Modern ultrasound systems are compact and mobile. (4) Procedure is safer than other diagnostic techniques such as X-rays and CT scans as patients are not exposed to ionizing radiation. (5) Ultrasound imaging can provide real-time images and information on blood velocity and flow. Ultrasound imaging and Ultrasound guided control have been employed with microrobotic systems. Sanchez et. al have demonstrated motion control of self propelled microjets using feedback of B-Mode ultrasound images. The
motion of microjets is directed towards a pre-defined target by steering them with magnetic torque, and binary image analysis is performed to estimate the position of the microjets from ultrasound feedback [102]. Khalil et. al have achieved control of paramagnetic microparticles using ultrasound feedback. The adaptation of the magnetic system used for the motion control to provide feedback from an ultrasound system and a microscopic system allows point-to-point motion control of the microparticles [103].

4.2 Ultrasound guided localization of helical robot

An ultrasound system (HD 5 Diagnostic Ultrasound System, Philips and Neusoft Medical Systems, Amsterdam, The Netherlands) with maximum depth of field of 16 cm is integrated to the permanent magnet-based robotic system to visualize and localize the helical robot. The transducer of the system (NOCTN340, Philips and Neusoft Medical Systems, Amsterdam, The Netherlands) is mounted on the surface of the gelatin at height of 7.5 mm from the catheter segment, as shown in Fig. 4.1. The ultrasound system is set to motion mode (M-mode) to display a sequence of rapidly acquired scans during helical propulsion inside the catheter segment. The thermal index score and the mechanical index are 0.2 and 0.9, respectively. All scans are acquired for gain setting, frequency, and depth of 43, 10 MHz, and 3 cm, respectively. The wavelength and frequency of the propagating ultrasound waves are inversely proportional. Therefore, high-frequency ultrasound waves generate images with higher resolution and can only be used to localize superficial structures. The high-frequency ultrasound waves are attenuated as the depth increases and therefore low-frequency ultrasound waves are more suitable for relatively deep clots. Frequency of the probe used in the experiments is in the range of 3 to 12 MHz. Given the equation, $\lambda = \frac{f \cdot V}{c}$. Where $V$ is the velocity of sound (1480 m/s in PBS solution), and $f$ is the frequency. Wavelength is calculated as 0.12 to 0.49 mm. The smallest-sized objects that can be detected with ultrasound waves are on the order of the calculated wavelength.

Further, it is important to investigate the localization of the helical robot at varying depths from the gelatin surface. In future potential biomedical application of mechanical rubbing of blood clots, the depth of the targeted blood vessel under the skin would vary according to the location and depth of the blood vessel in the circulatory system. The depth of a blood vessel varies from one individual to another depending on the thickness of the subcutaneous adipose tissue. Depth of superficial veins of the lower limbs ranges from 1.5 mm to 31.6 mm [104]. For further investigation of the visibility of the helical robot under ultrasound feedback at varying depths from gelatin surface 4.1(a), a container is prepared with five catheter segments placed at varying depth from the gelatin surface 4.1(b). The helical robot is localized with ultrasound feedback at depth of 2 cm and 3 cm with magnetic index of 0.4 and thermal index of 0.1 and gain of 44, and at 4 cm with magnetic index of 0.8 and thermal index of 0.3 and gain of 44.
The position of the helical robot obtained from online localization using ultrasound feedback enables the controlled navigation of the robot inside the channel. It is required to propel the helical robot inside the channel towards the location of the blood clot, then change the actuation frequency to the desired frequency for applying mechanical rubbing. Further, \( k_1 \) is a positive proportional gain. This control input is supplied to the first DC motor of the rotating dipole fields as follows:

\[
\frac{d}{dt} \begin{pmatrix} \omega_i \\ I_i \end{pmatrix} = \begin{pmatrix} -\frac{b}{J} & \frac{k}{J} \\ -\frac{k}{J} & -\frac{R}{L} \end{pmatrix} \begin{pmatrix} \omega_i \\ I_i \end{pmatrix} + \begin{pmatrix} 0 \\ \frac{1}{L} \end{pmatrix} u_i \text{ for } i = 1, 2, \tag{4.1}
\]

where \( \omega_i \) and \( I_i \) are the angular velocity and input current of the \( i \)th DC motor, respectively. Further, \( b, J, \) and \( k \) are the motor viscous friction constant, moment of inertia of the rotating dipole field and the rotor of the motor, and torque constant, respectively. \( L \) and \( R \) are the electric inductance and resistance of the motor, respectively. To ensure that the second rotating dipole field is synchronized with the first, we calculate \( u_2 \) based on the angular positions of the two motors

\[
u_2 = k_2 (\theta_1 - \theta_2) + k_3 (\omega_1 - \omega_2), \tag{4.2}\]

where \( k_2 \) and \( k_3 \) are the proportional and derivative positive gains, respectively. Control law (4.3) provides zero output for zero position tracking error. Therefore, the angular velocity of the rotating dipole fields decreases as the helical robot approaches the reference position. The angular velocity of the rotating dipole fields will ultimately be zero since \( A \) is a Hurwitz matrix. The low Reynolds number characteristic and the control input (4.3) enable the helical robot to approach the reference position without overshoot. The angular velocity of the
rotating dipole fields is the output of the state-space equation (4.1). This angular velocity is equal to that of the helical robot below its step-out frequency in the absence of interaction with the clot and contact with the channel wall. Therefore, the angular velocity of the motors decrease as the input to the state-space representation (4.1) decreases with error, and the linear velocity of the robot decreases as it approaches the reference position. A representative closed-loop motion control trial of a helical robot towards a reference position is shown in Fig. 4.2(a) (red dashed line). The average speed of the robot is $5.32 \pm 1.17 \, \mu m/s$ and the average and maximum steady-state errors are $0.84 \pm 0.41 \, mm$ and $2.15 \, mm$, respectively.

### 4.3 Closed-Loop Control

A proportional control input is employed to move the helical robot towards the clot owing to the over-damped characteristic of our system based on its low Reynolds number. The robot position ($x_r$) is detected using the ultrasound system and image processing presented previously in the previous section, and used to calculate the control input

$$u_1 = k_1 (x_c - x_r),$$

where $u_1$ is the control input to the first rotating dipole field and $x_c$ is the position of the edge of the clot from the side of the helical robot and represents the reference position.

**Figure 4.2:** Closed-loop control of a helical robot is achieved using ultrasound guidance. (a) The robot swims in phosphate buffered saline inside a catheter segment with inner diameter of 4 mm. (b) The average speed of the robot is $5.32 \pm 1.17 \, \mu m/s$ and the average and maximum steady-state errors are $0.84 \pm 0.41 \, mm$ and $2.15 \, mm$, respectively.

Closed-loop control of helical robot inside the channel is achieved with total of 30 trials to calculate the control characteristics, some representative trials are shown in Fig. 4.2(b). The average rise-time and maximum steady-state error are $4 \pm 0.2$ seconds and $2.15 \, mm$, respectively. The average steady-state error of all trials is $0.84 \pm 0.41 \, mm$ ($n=30$). A LabVIEW interface is developed for the control of the robotic system and localization of helical robot. Image acquisition and processing on ultrasound feedback using morphological operations provides the position of the helical robot. The filtering algorithm (Fig. 4.3) used to provide position of the helical robot from ultrasound feedback is based on morphological operations on color images and is described as follows: (a) Online image acquisition is done using motion mode on LabVIEW and the ultrasound feedback video is opened as a sequence of
RGB images (frames) one by one in real-time. The following steps are implemented on each extracted frame. (b) Color subtraction: A color image can be represented as a wide band component resembling brightness, and two narrow band color components. So, The unit RGB color cube is transformed from R,G,B primary components which are Red, Green and Blue color components into luminance Y and two color difference components B - Y and R -Y. (c) Color plane extraction: Green color plane mainly resembling brightness of each pixel is extracted from RGB components. (d) Proper opening: Erosion followed by dilation are implemented for noise removal. Erosion removes pixels on object boundaries, while dilation adds pixels to the boundaries of an object in an image. (e) Thresholding: Applying threshold is a simple method of image segmentation and is set to remove the background objects and noise, and emphasize gray objects. (f) Convex hull: a given set of points in plane is constructed by a polygonal capsule called convex polygon, which is used to characterize the shape of the robot. (g) Remove small objects: removing objects with area smaller than a specified number of pixels. (h) Circle detection: detection of helical robot by detecting circular objects in the image.

![Images]

**Figure 4.3:** Filtering algorithm for the localization of helical robot using ultrasound feedback

### 4.4 Mechanical rubbing of blood clots under Ultrasound guidance

Closed-loop motion control of the helical robot is followed by mechanical rubbing of the clots. Rubbing is achieved against flow rate of 10 ml/hr. This flow is devised based on the infusion rates of anti-coagulation agents. The helical robot is allowed to rotate at $\omega = 35$ Hz during the mechanical rubbing. Mechanical rubbing is not efficient below and above this frequency owing to the relatively large drag force due to the induced flow rate and the increased damping at relatively high frequency, respectively. Once the helical robot approaches the clot using control law (4.3), the dipole fields rotate at $\omega = 35$ Hz and the volume dissolution of the blood clot is calculated from camera feedback. The feedback provided by the ultrasound system is used to track the helical robot. However, the level of echogenicity of the blood clot samples does not enable visualization using ultrasound feedback. We attribute the low echogenicity of the clots to their age. 1-hour-old blood clot samples are used in our trials as it is essential to achieve early intervention in the beginning of the clot formation. The mechanical rubbing time is limited to 40 minutes as it is essential to remove the clot within a biologically meaningful time.
Chapter 4. Localization and control of helical robots

The volume of the clot \( v \) is determined and used to calculate the non-dimensional ratio \( \left( \frac{v}{v_0} \right) \), where \( v_0 \) is the initial volume of the clot, as shown in Fig. 4.5. Although our rheology test (Section 2.5.3) indicates that there exist a slight difference in the shear modulus of blood clots at 25°C and 37°C, we calculate the removal rate of the clot at each temperature to study its influence. At 25°C and 37°C, mechanical rubbing achieves average removal rate of \(-0.614 \pm 0.303 \text{ mm}^3/\text{min} \) \((n = 6)\) and \(-0.482 \pm 0.23 \text{ mm}^3/\text{min} \) \((n = 6)\), respectively.

The hydrodynamic model (explained in section 2.3) predicts removal rate of \(-0.5992 \text{ mm}^3/\text{min} \). This removal rate is in agreement with the trials conducted at temperature of 25°C. We attribute this observation to the characterized parameters entered to the model. Characterization of the clots is done at 25°C. Therefore, mechanical rubbing results at 25°C are in agreement with our theoretical prediction. This experimental result also show that mechanical rubbing of blood clots remains efficient under 37°C. Therefore, our in vitro model (Section 2.4) mimics essential in vivo conditions.
4.5 Characterization in rabbit aorta

In order to investigate the behavior of the helical robot in a real blood vessel, locomotion of the helical robot is characterized in rabbit aorta. The aorta is an elastic artery with an expandable media for circulating blood, it is the main artery that originates in the heart and delivers oxygenated blood to the organs. The use of arteries in experiments is clinically relevant to the potential biomedical application of clearing clogged blood vessels, since the major cause of ischemic diseases such as stroke and myocardial infarction is the obstruction of the corresponding artery by blood clots.

**Figure 4.6:** The speed of the helical robot is characterized in rabbit aorta under the influence of rotating magnetic field with varying frequency.

The aorta consists of three main layers from inside to outside, i.e., *tunica intima*, *tunica media*, and *tunica adventitia*. *Tunica media* is the muscular layer of arteries and veins, it provides elasticity and controls the diameter of a blood vessel. In arteries, the *tunica adventitia* is supported by external elastic lamina that increases the elasticity needed for greater expansion in case of relatively higher flow rate [95]. A rabbit weighting 1.5 kg is dissected and its aorta is isolated. The ends of the aorta are connected to a catheter segment of 3 mm inner-diameter for fluid circulation, and to provide a stationary locomotion model for the helical
robot during experiments. The aorta is connected to a syringe pump (Genie Plus, GT-4201D-12, Kent Scientific, Connecticut, USA) and flow of 90 ml/hr is induced against the direction of propulsion, which maintains the viability of the aorta during the experiments. The diameter of the aorta is measured as $4 \pm 0.3$ mm, this variation in the diameter could be attributed to the elasticity of the aorta. The speed of the helical robot is characterized inside rabbit aorta (Fig. 4.6(a)). Maximum speed in rabbit aorta is measured as $11.3 \pm 0.52$ mm/s versus $14.8 \pm 0.37$ mm/s in catheter segment at actuation frequency of 8 Hz. Averaged speed and standard deviations are calculated from 5 trials ($n = 5$). A representative trial at actuation frequency of 7 Hz is shown in Fig. 4.6(b). The speed reduction inside the rabbit aorta in comparison to catheter segment could be attributed to the elastic properties of the aorta, and the interaction between the robot and the inner layer of the aorta compared to the interaction with the channel wall of the catheter segment. A statistical test is conducted using analysis of variance (ANOVA) to investigate the influence of the actuation frequency and the host model (rabbit aorta and catheter segment) on the swimming speed of helical robot. Results show statistical significance ($F_0 > F_\alpha$), where $F_0$ is calculated as 3.25 and 14.94 and $F_\alpha$ is calculated as 2.48 and 4.12 for the actuation frequency and the host model, respectively.

Related research studies have investigated the locomotion of microrobots inside rabbit aorta. Jeong et al. have demonstrated the penetration of an induced arterial thrombembolism in the aorta of a live pig using an intravascular therapeutic microrobot system, the path of the robot in vivo was tracked and controlled with X-ray [105]. Choi et al. have achieved position control of a microrobot in a pulsating flow of blood vessel using an electromagnetic actuation system. They have performed in vivo experiments in the aorta of a 4-month-old female Micro pig, to evaluate the locomotion of the microrobot in a real blood vessel under similar conditions to coronary interventions [106].
Chapter 5

Conclusions and future work
5.1 Conclusions

Mechanical rubbing results in higher removal rates than chemical lysis using a thrombolytic agent under the same settings. Experimental results show that mechanical rubbing at achieves average removal rate of $0.17 \pm 0.032 \text{ mm}^3/\text{min}$ at $\omega = 35 \text{ Hz}$ in comparison to $0.85 \pm 0.042 \text{ mm}^3/\text{min}$ with chemical lysis using streptokinase-bases thrombolytic agent. The influence of the rubbing frequency on the dissolution rate of the clots is experimentally investigated, an optimal rubbing frequency that achieves maximum removal rate is found at frequency of $35 \text{ Hz}$. The experiments are in agreement with the RFT-based model that describes the locomotion of the robot in low-Reynolds number and the rubbing behaviour against blood clots. This model suggests the presence of an optimal rubbing frequency that enables the robot to achieve maximum removal rate. In particular, the model predicts an optimal frequency as both low and high frequencies produce negligible removal rates. The first is ineffective owing to the excessive drag due to flow in the segment, and the second owing to the increased damping at relatively high frequencies.

Closed-loop motion control of helical robots is demonstrated towards blood clots and mechanical rubbing under ultrasound guidance. The movement of helical robot is controlled towards the clot at an average speed of $5.32 \pm 1.17 \mu\text{m/s}$ using a proportional control input without overshoot. This control input is designed using ultrasound feedback and enables the helical robot to achieve average and maximum steady-state errors of $0.84 \pm 0.41 \text{ mm}$ and $2.15 \text{ mm}$, respectively, and average settling time of $4.5 \pm 0.1 \text{ seconds}$. The closed-loop motion control of the helical robot is followed by mechanical rubbing to decrease the size of the clots. The closed-loop motion control of the helical robot is followed by mechanical rubbing of blood clots under ultrasound guidance. Mechanical rubbing achieves average removal rate of $-0.614 \pm 0.303 \text{ mm}^3/\text{min}$ and $-0.482 \pm 0.23 \text{ mm}^3/\text{min}$ at temperature of $25^\circ\text{C}$ and $37^\circ\text{C}$, respectively.

The influence of mechanical rubbing on the weight of the blood clot is experimentally studied, and cell count and absorbance is calculated for the collected samples past the helical robot and the blood clot. During mechanical rubbing, the interaction of the tip of the rotating helical robot with the three-dimensional fibrin network of the blood clot results in tearing of the fibrin and its decomposition. The effect of this interaction is validated by the count of released RBCs and platelets and concentration of the samples collected past the robot and the blood clot. Maximum decreased weight percentage was measured as $74.4\pm11.1\%$ at $25 \text{ Hz}$, cell count as $654\pm108\times10^4 \text{ cells/ml (n=3)}$ at $40 \text{ Hz}$ and concentration as $4.35\times10^{-6} \text{ mol (n=2)}$ at $35 \text{ Hz}$. Compared to $51.9\pm3.1\%$, $54\pm12\times10^4 \text{ cells/ml (n=3)}$ and concentration as $1.05\times10^{-6} \text{ mol (n=2)}$ in the absence of mechanical rubbing.

The conditions that will be encountered by the helical robot during swimming inside the human body are challenging. To investigate similar conditions, the swimming speed of the helical robot is characterized inside a segment of a dissected rabbit aorta, under the influence of rotating magnetic fields with varying frequency in the range of $3 \text{ Hz}$ to $8 \text{ Hz}$. The robot swims against a flow of PBS at $90 \text{ ml/hr}$ with a maximum speed of $14.8 \pm 0.37 \text{ mm/s}$ inside the aorta compared to $14.8 \pm 0.37 \text{ mm/s}$ in catheter segment at actuation frequency of $8$
The outer-diameter of the aorta is measured as $4 \pm 0.3$ mm varying according to the expansion by the applied flow rate. We attribute the reduction of swimming speed to the elastic properties of the aorta in comparison to a rigid catheter segment.

5.2 Future work

As part of future studies, the blood clots echogenicity will be increased to enable position and size detection using ultrasound feedback. The clots will be functionalized using echogenic liposome compositions to enhance the ultrasound detection. We will also study the influence of rubbing in combination with chemical lysis at different doses of a fibrinolytic agent. The comparative study between mechanical rubbing, rubbing in combination with different percentages of fibrinolytic agent, and pure chemical lysis is essential to optimize the integration between mechanical rubbing and chemical lysis. Our experimental results are conducted against flow rate of 10 ml/hr. This flow rate is greater than blood flow in small arteriole and capillaries only. Therefore, it is essential to modify our system to enable mechanical rubbing against greater flow rates comparable to medium arteries and veins. In addition, the influence of mechanical rubbing of blood clots in combination with chemical lysis at different doses of thrombolytic drugs will be studied. The comparative study between mechanical rubbing, rubbing in combination with different percentages of thrombolytic agents and pure chemical lysis is essential to optimize the integration between mechanical rubbing and chemical lysis of blood clots. In addition, we will characterize the locomotion behavior of helical robot inside animal blood vessels against increased flow rates to mimic the physiological environment and blood flow rates.
Appendix A

Blood clot volume detection code
(implemented using Matlab)

```matlab
% read video as video object
vidIn = videoreader('control.mp4');
% get total number of frames
NOF = vidIn.NumberOfFrames;
% initialize a matrix to store the volume at each frame
v = zeros(NOF,1);
%initialize countdown counter
a = NOF;
%Parameters of crop rectangle
rect = [187.5 79.5 98 55];
%loop over video frame by frame
for ii = 1: NOF
%read incoming frame
pic = read(vidIn, ii);
%crop frame p = imcrop(pic,rect);
% convert RGB image to gray
p = rgb2gray(p);
% black and white thershold value determined by try and error
thresh = 0.8;
% convert gray image to black and white at chosen threshold
p = 1 - im2bw(p, thres);
%view final frame
imshow(p)
%calculate the area of white pixels in frame (blood clot pixels)
area1(ii,1) = bwarea(p);
%count down remaining frames
a = a - 1
end
```
Bibliography


