The Influence of Mechanical Rubbing on the Dissolution of Blood Clots
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Abstract—Mechanical rubbing of blood clots is a potential minimally-invasive method for clearing clogged blood vessels. In this work, we investigate the influence of the interaction of the tip of a helical robot with blood clots. This interaction enables the dissolution of the blood clot and the release of the entrapped red blood cells and platelets from its three-dimensional fibrin fiber network. We analyze 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. Our measurements show that the weight of the blood clots is decreased by 22.5±11.1% at frequency of 25 Hz. We also validate the influence of mechanical rubbing using cell count and spectrophotometric analysis on phosphate buffered saline samples past the robot and the clot. The maximum cell count is measured as 654 ± 108 × 10^4 cells/ml and 54 ± 12 × 10^4 cells/ml, whereas the absorbance is measured as 4.35 × 10^-6 mol and 1.05 × 10^-6 mol under the influence of mechanical rubbing and without mechanical rubbing, respectively.

I. INTRODUCTION

The early concepts of the formation of thrombus have been described by the German Physician Rudolph Virchow in 1856. The major categories of risk factors of thrombosis have been grouped later on under the name of Virchow’s triad which includes injury to the endothelium, abnormal blood flow, and abnormal blood consistency (hypercoagulability) [1]. Thrombosis can form in arteries and veins, leading to their obstruction which in many cases results in serious cardiovascular diseases. According to the American Heart Association, cardiovascular diseases are listed as the leading cause of death in USA, with coronary heart diseases being the leading cause followed by stroke [2]. The gold standard therapy of thrombosis is the administration of anti-coagulant and thrombolytic drugs. However, this systemic approach might be associated with risks of serious hemorrhagic complications. Targeted drug delivery of anticoagulant drugs to the blood clot using micro and nano robots could prevent the occurrence of such complications [3]. Researchers have demonstrated various methods to fabricate and control micro and nano robots towards potential biological in vitro applications. For instance, Qiu et al. have characterized the swimming performance of non-cytotoxic artificial bacterial flagella (ABF) to create highly biocompatible helical swimmers [4]. Servant et al. have also demonstrated optical imaging for in vivo tracking of magnetically controlled swarm of ABF in the peritoneal cavity of a mouse [5]. Power et al. have used biocompatible hydrogels in the fabrication of hybrid microrobots for tumor targeting and treatment [6].

Recently, Adams et al. have also achieved wireless control of microrobots in a rabbit bladder for endoscopic applications in urology [7].

In this work, we focus on the removal of blood clots using magnetically actuated helical robots, as shown in Fig. 1(a). Mechanical removal of blood clots represents an attractive therapeutic modality that could prevent the complications related to systemic therapy as presented in our previous work [8], [9]. Thus, we resume to investigate the influence of mechanical rubbing using helical robots on the dissolution of blood clots and achieve the following:

- measure the influence of rubbing frequency on the weight of the blood clots;
- determine the influence of the rubbing frequency on the red blood cell (RBCs) and platelets count;
- spectrophotometric analysis on samples past the clot to measure the concentration of released RBCs and platelets from the fibrin network of the clot.

Experimentally, mechanical rubbing is achieved using a helical robot [Fig. 1(b)]. As blood clots are formed of a fibrin network where blood cells are entrapped, mechanical rubbing of the fibrin network results in the release of RBCs and platelets. Samples past the robot and the blood clot are...
collected at different time intervals during the experiments for analysis. We calculate the change in the weight of the blood clot after mechanical rubbing, as well as cell count and absorbance of the samples. The remainder of this paper is organized as follows: Section II provides descriptions pertaining to the interaction between the tip of the helical robot with the fibrin network of the blood clot. Characterization of the influence of rubbing frequency on the dissolution of blood clots is discussed in Section III. Finally, Section IV concludes and provides directions for future work.

II. CHARACTERIZATION OF THE INFLUENCE OF MECHANICAL RUBBING ON THE PROPERTIES OF CLOTS

Following our previous work [9], the helical robot is directed inside a catheter segment towards a blood clot against the flowing streams of phosphate buffered saline (PBS). Mechanical rubbing is achieved once the robot comes into contact with the clot.

A. Helical Propulsion and System Description

The helical robot consists of a magnetic head and a screw like tail. The tail is fabricated using an aluminum spring (length, diameter, and pitch of 2.2 mm, 346 mm, and 580 mm, respectively). This spring is rigidly attached to a permanent magnet (Neodymium grade N40) with an edge length of 500 μm and a magnetization vector perpendicular to the spring axis. Helical propulsion is achieved using two rotating permanent magnets [10]. Each permanent magnet generates magnetic field of 0.552 T on its surface. The permanent magnets are placed parallel to the catheter segment [11]. Blood clots are prepared [12], with length of 7.5 mm and diameter of 4 mm, and inserted inside the segment. We calculate Reynolds number as $Re = \frac{\rho U L}{\mu}$, where $\rho$ is the density of the PBS (995 kg.m$^{-3}$), $U$ is the velocity of the helical robot before rubbing, $L$ is its length ($4 \times 10^{-3}$ m), and $\mu$ is the dynamic viscosity of the PBS (0.8882 Pa.s). The hydrodynamic drag experienced by the helical robot in low Reynolds numbers can be simplified by employing the resistive-force theory [13], in which the drag force and torque are related with the linear and angular velocities via an anisotropic operator as follows:

$$
\begin{pmatrix}
F_d \\
T_d
\end{pmatrix}
= Z_r
\begin{pmatrix}
U + U_{ch} \\
\Omega
\end{pmatrix}
,$$

where $F_d$ and $T_d$ denote the viscous drag force and torque vectors, respectively. Further, $\Omega$ is the rigid-body angular velocity of the helical robot. $U_{ch}$ represents the flow-fields inside the channel, and $Z_r$ is a matrix of the resistive coefficients of the head and tail [9]. The drag torque $T_d$ exerted on the helical robot is linearly proportional to the rigid-body angular velocity of the helical robot ($\Omega$). Hence, we deduce that the exerted drag torque is linearly proportional to the actuation frequency of mechanical rubbing below the step out frequency of the helical robot. Therefore, we achieve mechanical rubbing at different actuation frequencies and analyze the pre- and post-conditions (weight, cell count, and absorbance) of the clots.

B. Analysis of the Blood Clot Weight

Mechanical rubbing of blood clots results in dissolution and a volume decrease, which implies a loss in weight. Hence, we measure the weight of blood clots before and after 40 minutes of mechanical rubbing using an electronic balance (ABS 220-4 Analytical Balance, KERN & SOHN GmbH, Balingen, Germany). The percentage of decrease in the weight of the blood clot ($w_d$) is calculated using

$$w_d = \frac{w(t_f) - w(t_0)}{w(t_0)} \times 100.$$  (2)

In (2), $w(t_f)$ is the weight of blood clot after 40 minutes of mechanical rubbing and $w(t_0)$ is the initial weight of the blood clot. Measurements of $w_d$ under the influence of rotating magnetic fields with varying frequency in the range of 20 Hz to 45 Hz are shown in Table 1. An increase in $w_d$ is observed for all actuation frequencies $f$, as opposed to $f = 0$ Hz (zero-input response). This increase validates the dissolution of blood clots under the influence of mechanical rubbing. However, the weight decrease does not describe other changes related to the effect of the mechanical rubbing on the fibrin network of the blood clot. Hence, we calculate the cell count of the PBS samples past the robot and blood clot, which indicates the dissolution of the fibrin network causing the release of red blood cells (RBCs) and platelets previously entrapped within the blood clot.

C. Analysis of the Red Blood Cells and Platelets Count

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. We experimentally study the influence of rubbing frequency on the cell count. Mixture past the robot and the blood clot is collected every 5 minutes inside small tubes [Fig. 2(a)]. 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 [Fig. 2(b)] under microscope (MF Series 176 Measuring Microscopes, Mitutoyo, Kawasaki, Japan). Averaged sum of cell count after 40 minutes of rubbing provides a maximum value of $654 \pm 108 \times 10^4$ cells/ml at $f = 40$ Hz, compared to $54 \pm 12 \times 10^4$ cells/ml in the absence of mechanical rubbing [Fig. 3(c)]. Averages and standard deviations are calculated from 3 trials. The
Fig. 2. Number of red blood cells (RBCs) and platelets is analyzed to study the influence of mechanical rubbing on blood clots. Mixture past the robot and the blood clot is collected every 5 minutes and analyzed using a hemocytometer to calculate the cell count. (a) RBCs and platelets are counted during mechanical rubbing with frequency in the range of 20 Hz to 45 Hz, and against flow rate of 10 ml.hr\(^{-1}\). (b) Total sum of RBCs and platelets provides maximum of \(646 \pm 206 \times 10^4\) cells/ml at \(f = 40\) Hz compared to \(54 \pm 12 \times 10^4\) cells/ml at \(f = 0\) Hz (zero-input response). Averages and standard deviations are calculated using 3 trials.

D. Spectrophotometric Analysis of Blood Clots

Spectrophotometric analysis is conducted to measure the absorbance to indicate the concentration of the blood clot and validate the efficiency of mechanical rubbing. PBS samples past the robot and blood clot are collected every 5 minutes during the experiments [Fig. 3(a)]. The absorbance of the collected samples is measured using a spectrophotometer (V-730 UV-Visible Spectrophotometer, Oklahoma city, USA), as shown in Fig. 3(b). 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 measurement allows for the calculation of the amount of 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:

\[
A = abc. \tag{3}
\]

In (3), \(A\) is the absorbance measured by spectrophotometry, \(\epsilon\) 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. 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 is measured at wavelength (\(\lambda = 416\) nm), as shown in Fig. 3(c). Second, absorbance of each sample is measured at the selected wavelength. Finally, Beer-Lambert Law is used to calculate the concentration where \(\epsilon = 521880\) as reported in [14] and \(b = 1\) cm. Absorbance is linearly proportional to the concentration of the substance in the same sample. Thus, the increase in the absorbance implies an increase in the concentration and correspondingly an increase in the number of blood cells released from the fibrin fiber network of the blood clot [Fig. 1(a)]. Concentration of samples calculated past the robot and blood clot, under the influence of various frequencies of rotating magnetic fields in the range of 20 Hz to 45 Hz, is shown in Fig. 3(c). Maximum total concentration is measured as \(4.35 \times 10^{-6}\) mol at \(f = 35\) Hz, compared to \(1.05 \times 10^{-6}\) mol in the absence of mechanical rubbing.

The pre- and post-conditions of the blood clots are analyzed following 40 minutes of mechanical rubbing. Three methods are used to validate the influence of mechanical rubbing using helical robots. While the weight of the blood clot samples provides an essential measure (Table 1) to the influence of mechanical rubbing, the samples are affected during handling (extraction from the catheter segments). Therefore, cell count and concentration measurements are used to provide a comprehensive information pertaining to the pre- and post-conditions of the clots. We also find qualitative agreements between the mentioned three techniques. The reduction in weight (Table 1), number of released red blood cells and platelets [Fig. 2(c)], and concentration [Fig. 3(c)] are in qualitative agreement and indicate the influence of mechanical rubbing at relatively high actuation frequency of the helical robot, as opposed to zero-input response. Our measurements do not provide an optimal rubbing frequency owing to the clot-to-clot variability. Nevertheless, maximum removal rate is observed within a frequency range of 30 Hz to 40 Hz.
behavior of helical robot inside animal blood vessels to mimic the physiological environment and our system will also incorporate a medical imaging modality to track the helical robot in vivo [15].

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REFERENCES