Engineering Trojan-horse Bacteria to Trap Virus: A Mechanistic Model for Ebola Virus

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***Abstract*—The outbreak of Ebola virus disease in recent years has resulted in numerous research initiatives to seek new solutions to control and contain the spreading of the virus. A number of approaches that have been investigated includes new vaccines to boost the immune system as well as therapeutic approaches such as transfer of plasma from patients who survived the virus to newly infected patients. An alternative approach is proposed to treat infected patients based on genetically engineered bacteria that are able to trap Ebola virus. The bacteria will act as an engineered trojan-horse that will search for the Ebola and through the surface binding will remove the virus from its host. The study of this approach is based on the analysis of the chemical binding force that attaches an Ebola virus onto a genetically engineered bacterium, the surface area of this attachment, and the calculation of the stable attachment point by considering the opposing force resulting from the hydrodynamic tension force and drag that are acting on the hanging body of the virus. To evaluate the feasibility of the proposed approach, simulations of the bacteria moving to trap the virus within a confined area have been conducted. The results show that the proposed approach is capable of collecting a large quantity of virus within 100 seconds. Through simulations, the paper evaluates the impact of binding energy and contact area on virus pick-up efficiency in three different scenarios. In the worst case, simulations shows (for maximum binding energy) a pick-up probability up to 90% of Ebola virus within a confined area.**

***Index Terms*—Genetically engineered bacteria, Ebola virus, Virus ecological trap.**

I. Introduction

The recent outbreak of Ebola virus disease has resulted in concerns by the research community in developing new solutions that can curb and control the spreading process [1]. While the majority of Ebola virus disease outbreaks are currently found in Africa, their rate of spreading requires immediate attention. The spreading process of Ebola virus is through the exchange of fluids between individuals, animals, as well as with the environment where the virus lies. The poor sanitary conditions in the developing countries also fuel the spreading process, which has detrimental effects on lives and on the socio-economical stability of the affected regions.

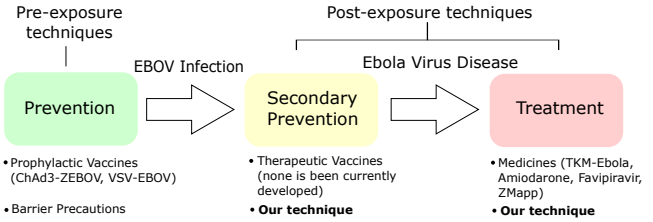

Fig. 1: Summary of current Ebola virus disease therapeutics. For each step, different approaches have been proposed [2]–[10], [15], [16]. In this paper we are focused on secondary prevention.

Figure 1 presents a graphical representation of Ebola virus infection therapeutics. Currently, there are preventive and post-exposure treatments available [2] [3]. Two vaccines were developed and are still being tested in Guinea: one developed by Merck Sharp and Dohme and another by Toyama Chemical [4], [5]. The effectiveness of these vaccines for treatment, and in particular for large scale population, is still under investigation. Current advanced research for solutions to the Ebola virus disease problem is mostly in the domain of molecular biology and biotechnology [6]. A number of therapeutic medicine to treat Ebola virus disease has been developed and tested, and this includes TKM-Ebola, amiodarone, dronedarone, verapamil and ZMapp [3], [7]–[9]. ZMapp is a cocktail of three monoclonal antibodies produced from Tobacco plants (*Nicotiana benthamiana* species) and provides immunity to Ebola virus. Successful tests were made on mice and non-human primates [9], [10]. Also, monoclonal antibodies derived from a person who survived Ebola virus disease protected non-human primates when given as late as 5 days after Ebola virus infection [11], [12]. Other treatments that have compounds capable of blocking Ebola virus-like particles from entry into the cells and a novel peptide vaccine have also been proposed to increase the range of available treatments [13], [14].

The field of *synthetic biology* has received tremendous attention in recent years, due largely to the potential impact of delivering new solutions for biotechnology [6], [15]. The process of synthetic biology allows genetic circuits to be designed and inserted into cells in order to create new properties or characteristics. In order to prevent pathogenic

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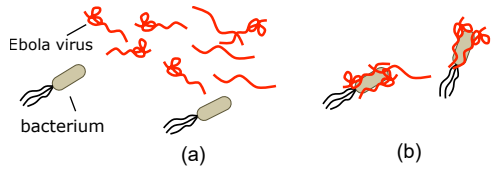


Fig. 2: Illustration of genetically engineered bacterium proposed in this paper to trap the Ebola virus, (a) bacterium move towards Ebola virus scattered in a space, (b) bacterium move away after trapping the virus as it binds to their surface, effectively removing it from the space.

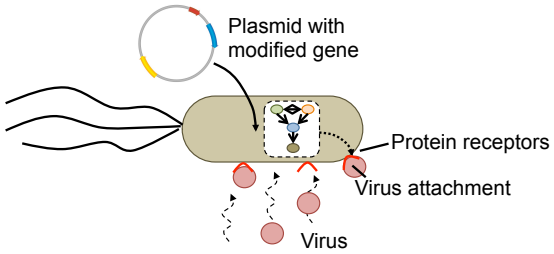


Fig. 3: Genetically engineered bacteria will produce proteins on the surface to attract the virus.

strains, a possible approach is to use a specific gene expression system that suppresses the replication of the target virus. There are several approaches that already exist that could be used, such as engineered *RNase P ribozyme* that has been used to successfully inhibit herpes simplex virus 1 or the over expression of *Tat-dependent MazF* expression system that is used to avoid the emergence of pathogenic strains [17], [18]. Currently, it is possible to produce synthetic vaccines on demand to face pathogens epidemics [16].

In this paper, we propose synthetically engineered Trojan-horse bacteria (*Escherichia coli* species) that moves and traps Ebola virus in a sponge like manner [19]. Our proposed approach is illustrated in Figure 2. When the bacteria come into contact with Ebola virus, this will lead to protein binding between the two surfaces, which results in the virus attachment.

The process of virus trapping has been investigated previously. For example, in [20] the red blood cells are used to trap virus. The red blood cells are ideal for virus trapping due to the fact that they lose their DNA when grown in the bone marrow. Therefore, when the virus infects the red blood cell, it will have no capability of replicating itself due to the missing DNA, and hence, leading to a trapping process. Another example is in [19], where the authors specifically studied the *Phi-6* virus which typically invades *Pseudomonas phaseolica* cell. This particular bacterium attaches itself to the plants by using its hair like structure that extends from their body. To increase the surface of attachment of their hair, these bacteria retract their body. This retracting process will also lead to the virus being able to infect the bacteria. In order to trap the virus, the authors engineered the bacteria to have excessive amount of hair on the surface leading to minimal amount of space to allow the virus to penetrate through the membrane but enough to trap the virus.

Engineered bacteria has already been used as a therapeutic agent in the past [21]. Patients that suffer from inflammatory bowel diseases have been targeted for therapies that use engineered bacteria to express chemical compounds in the gut to regulate the immune response [22]. A mouthwash was developed using *Lactococcus lactis* strain for the treatment of oral *mucositis* in patients subjected to cancer therapies, reducing the disease symptoms in 30% of cases [23]. Engineered bacteria have also been used to prevent viral infections as well [24]. For example, HIV-1 infection in CD4+ T cells and macrophages were inhibited using *Lactobacillus jensenii* bacteria [25]. Bacteria have also been used to hunt down and eradicate human lymphomas [26].

While current approaches of combating Ebola virus is based on the use of vaccination and drugs, our solution does not require drugs but rather bacteria that can be used as source of traps for Ebola virus. Our focus is **to prevent the Ebola virus from developing and spreading**. The proposed technique can be applied both as secondary prevention and as post-exposure treatment. In this paper we propose to use receptor binding process as the mechanism to trap Ebola virus. Although the viral entry process varies largely depending on the host system, the most common mechanism is through the virus-receptor binding process observed in bacteria and mammalian cells [27]. This process is similar to what we are proposing in this paper. However, there are a number of challenges to be beaten. First, compatible binding process is required between the virus and the bacterium. The compatible receptors on the bacteria can be engineered through synthetic biology as illustrated in Figure 3, where genes that are inserted into the plasmid can lead to expression of proteins on the surface. Second, Ebola virus has a long snake-like structure unlike other types of virus, and usually with a higher molecular weight. Therefore, the binding process may not cover the entire length of Ebola virus, leading to parts of the virus hanging from the bacteria after binding. This means that the binding process must be strong enough to support the momentum of the hanging virus body. To apply this technique in humans, there is a need to switch off the immune system for a brief period to make sure that most of bacteria have enough time to perform the trapping process. This could be achieved using the procedures presented in [28]. Besides applying this techniques to humans, the use of bacteria to hunt Ebola virus could also be adapted for open environments.

The contributions of this paper include:

- **Design of Ebola virus trapping process:** a cleaning process is proposed, where the bacteria are used to collect and trap the Ebola virus in the environment. This trapping process will minimize the replication process and can curb the virus from replicating and spreading.
- **Binding force model:** Developed a protein binding force model to trap Ebola virus in free movement bacteria that considers their swimming and tumbling process, as well as opposing forces resulting from the hydrodynamic tension and drag and the weight of the hanging body of the virus.
- **Simulation evaluation:** Simulations of bacteria motility process are conducted to evaluate the effectiveness of trapping an Ebola virus population in a confined area.

The paper is organized as follows. Section II describes the physical properties of Ebola virus. The engineering of proteins on the bacteria surface to bind to the virus is presented in Section III. Section IV describes the binding force models between the bacteria and the virus. The simulation evaluation and results are presented and discussed in Section V. Lastly, Section VI presents the conclusion.

II. Background on Ebola Virus

Ebola virus belongs to the order *Mononegavirales* and the *Filoviridae* family. Upon infection, it can kill up to 50% of the victims within 6 to 16 days [29]. The virus has a *baciliform* shape, with a uniform width of nearly 80 nm and a length of approximately 970 nm, and its structure is illustrated in Fig. 4. The molecular weight of Ebola virus is 3.82×10^8 [30], [31]. As illustrated in Fig. 4, virus membrane consists of $GP_{1,2}$ (spike glycoprotein) and two other proteins, VP40 and VP24 (primary and secondary matrix proteins) [30], [32]. This glycoprotein on the surface have 7 nm in diameter and has a spacing of 10 nm between each other. The glycoprotein enables Ebola virus to bind and submerge itself into the host cells (process required for viral internalization).

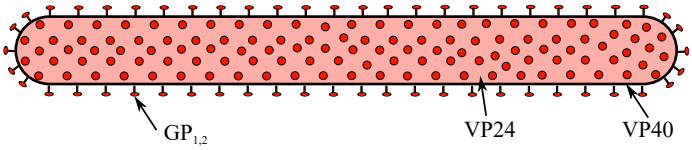


Fig. 4: Structure simplified of an Ebola virus.

There are three mechanisms of entry for the Ebola virus: through mucosal surfaces (mouth, eyes, genitalia), skin abrasion or through the use of contaminated needles [33], [34]. After the virus enters the body, its spreads rapidly [33]–[37]. It is capable of overcoming responses from the immune system. The high rate of virus replication inside the immune system cells hinders the human body defenses [35]–[37]. Monocytes, macrophages and dendritic cells are the front door for the Ebola virus infection and preferred sites of viral replication [36]. In addition these cells are used as vehicles to spread the Ebola virus through the lymphatic system [33]. Infected monocytes and macrophages secrete soluble factors to recruit other similar cells inside the lymph nodes and increase the infection. In later stages, hepatocytes and adrenal cortical cells are infected and the production of coagulation factors is decreased which results in internal bleeding [38].

III. Engineering Protein Binding

Our challenge now lies in using synthetic biology to produce surface proteins that can allow Ebola virus to bind to the engineered bacteria. In particular, careful understanding of viral entry and replication mechanisms into the host system is required before suitable genetic circuits can be developed. Past research have used dual color synthetic constructs to observe how a single virus affects the host bacterium and determine the level of infection [39]. Single-virus tracking methods have also been developed to observe the mode of interaction between *E. coli* and bacteriophage lambda [39]. In our proposed model, it is possible to construct a synthetic gene that could increase the expression of Ebola virus proteins binding receptor. Facilitating the binding frequency between the viral proteins and over expressed membrane receptor proteins would be an advantage to harvest the target virus. Specifically, reports have suggested that the cell surface receptor *T cell Immunoglobulin Mucin domain 1* (*TIM-1*) of epithelial cells favourably increases the binding of Ebola virus. A study on over expression of fluorescent tagged *TIM-3* protein in *E. coli* also confirms that TIM-like protein can have a functional property which allows viral protein recognition and binding [40]. Therefore, the TIM-like protein could be one of the possible targets to be expressed in *E. coli* to create engineered bacteria to trap the Ebola virus.

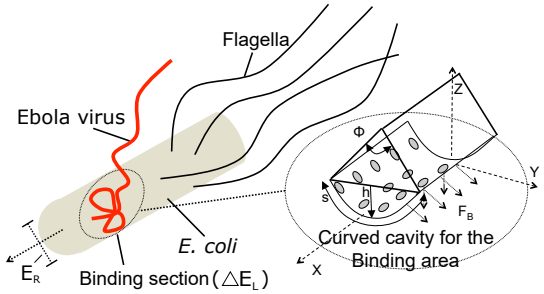


Fig. 5: Illustration of binding area between an Ebola virus and a bacterium.

A. Protein Binding Model

In this section we discuss the binding model between the Ebola virus and the bacteria. We evaluate this model as a function of the chemical parameters characteristics of the protein receptors on the bacterium surface and the glycoproteins on the virus, and for that we compute the geometry of the attachment area and therefore the binding force. We analyse the force required to bind the virus to a bacterium to counter opposing forces (e.g., drag and weight) that can result in the virus breaking its attachment. This process occurs only for a partial engulfment where the virus should sit on the surface membrane. In cell biology, engulfment is the process of particle acquisition through the cell's membrane. In our case, the virus will only deform a bacterium's surface for a certain depth, preventing it from full engulfment into the cell. Since the force exerted on the bacteria surface is sufficiently low, the virus will not be full engulfed [41], [42].

During the binding process, the virus deforms the bacterium's surface creating a curved cavity with submergence angle ϕ (in radians) as shown in Fig. 5 [41].The total binding energy is the energy resulting from the complex formation of the glycoprotein from the Ebola virus and bacterium for the area of attachment B_{Area}. This is represented as [41]:

$$E_{Bind} = -fpB_{Area} \tag{1}$$

where f is the free energy gained from the glycoproteins and cell receptor complex binding, p is the complex density in Ebola virus area. The free energy gained from the complex formation is represented as:

$$f = -k_B T_a ln K_a \tag{2}$$

where K_a is the equilibrium constant of the glycoprotein-cell receptor complex formation, T_a is the absolute temperature and k_B is Boltzmann constant. Based on the number of glycoproteins $N_{GP_{1,2}}$ on the Ebola virus surface, the complex density is represented as:

$$p = \frac{N_{GP_{1,2}}}{SA_{Ebola}}, \tag{3}$$

where the surface area of the Ebola virus is

$$SA_{Ebola} = 2\pi E_R^2 + 2\pi E_R E_L,$$

and this assumes the shape of the Ebola virus as a thin cylinder with radius E_R and length E_L. The total binding area of the Ebola virus to the bacteria, i.e., the area of the virus that attaches, is represented as:

$$\begin{aligned} B_{Area} &= s\Delta E_L \\ &= \phi E_R \Delta E_L \end{aligned} \tag{4}$$

where ΔE_L is the length of Ebola virus that attaches to the bacterium and E_R is the Ebola virus radius. Based on the E_{Bind}, the force of the binding and engulfment process is represented as [41]:

$$F_{Bind} = -\frac{\partial E_{Bind}}{\partial \phi} \tag{5}$$

where ϕ is the submergence angle. Also, we can calculate the probability of successful connection as follows [43]:

$$P_B = \frac{G_c}{k_B T_a} \exp\left[-\frac{E_{Bind}}{k_B T_a}\right] \tag{6}$$

where $G_c = pB_{Area}$ is the density of bound glycoproteins on the bacteria, T_a is the absolute temperature and k_B is Boltzmann constant. The values of these parameters are presented in Table I

IV. Force Model for Virus Attachment

While the previous section discussed the binding force based on the binding energy and engulfment process, in this section we discuss the minimum binding tension force that ensures the Ebola virus remains attached to the bacterium surface during its motility.

A. Hydrodynamic Drag Force

The Ebola virus will travel through a fluid medium, i.e., blood. Both the Ebola virus and bacteria are subjected to the same hydrodynamic force (drag force) once they are attached to each other. Since we model the shape of the bacteria as a cylinder, the $\mathbf{F_{Drag}}$ can be expressed as follows [44]:

$$\mathbf{F_{Drag}} = -\frac{1}{2}\rho_f v^2 A C_d, \tag{7}$$

where ρ_f is the fluid medium density, $v = v_b - v_f$ is the relative velocity between the bacteria (v_b) and fluid (v_f), $A = \pi B_R^2$ is the bacterium cross-section and B_R is the radius. The drag coefficient C_d is expressed as [44]:

$$C_d = \frac{24}{Re} + \frac{6}{1+\sqrt{Re}} + 0.4, \tag{8}$$

where the Reynolds number (Re) is expressed as [44]:

$$Re = \frac{D_v \rho_f v}{\eta}, \tag{9}$$

where η is the fluid viscosity. Human blood is a non-Newtonian fluid and its viscosity is a function of the vessel diameter D_v and haematocrit rate h_d. Based on this, η_{blood} is represented as [44]:

$$\eta_{blood} = \frac{\eta_{plasma} D_v^2}{(D_v - 1.1)^2}\left[1 + \frac{(\eta_{0.45} - 1)D_v^2}{(D_v - 1.1)^2}\frac{(1-h_d)^c - 1}{(1-0.45)^c - 1}\right], \tag{10}$$

where η_{plasma} is the plasma viscosity, $\eta_{0.45}$ is the relative apparent blood viscosity for a fixed discharge haematocrit of $h_d = 0.45$ and c is the shape of viscosity dependence of haematocrit. The values of $\eta_{0.45}$ and c are represented as [44]:

$$\eta_{0.45} = 6e^{-0.085 D_v} + 3.2 - 2.44 e^{-0.06 D_v^{0.645}}$$

and:

$$c = \frac{D_v^{11}}{D_v^{12}} - (0.8 + e^{-0.075 D_v})\left(\frac{D_v^{12}}{D_v^{12} - 10^{11}}\right).$$

B. Bacteria Motility

Bacteria are known to mobilize and move between different locations when the environment becomes harsh (e.g., depletion of nutrients). Although there are numerous motility process by the bacteria, such as gliding or swarming, in our particular case we focus on the swimming process. Since our proposed bacteria for collecting and trapping virus is envisioned to be realized by engineering *E. coli*, the swimming process will be based on using their *flagella*. The *flagella* are tails that stem from the body of the *E. coli*. The swimming behavior of the bacteria is based on a cycle of run and tumble motion, and is governed by a random walk. During the swimming process, the *flagella* will wrap into a single body, and this will rotate as a propeller allowing them to swim forward. The swimming process will involve the bacteria swimming in straight line for a certain period of time. This average period (λ_{Run}) is based on an exponential distribution [45]. The binding between the bacteria and virus will occur just before the tumbling process, where it is engineered to pause for a short period (τ_s) [46]. This insures sufficient time for a successful binding process [47]. After swimming for a short period, the flagella will unwrap into individual strands and this will lead to tumbling in a fixed location. Once again the average tumbling period is based on an exponential distribution (λ_{Tumble}) [45].

C. Tension Force for Running Motion

In general to achieve the stability of attachment, the binding force $\mathbf{F_{Bind}}$ will highly depend on the opposing forces that include $\mathbf{F_{Drag}}$, which is the force resulting from the resistance due to the fluids (blood, in our case) in the environment, the force due to the hanging weight of Ebola virus $\mathbf{W_h}$, and the tension $\mathbf{T}$ exerted on the Ebola virus to peel it from the bacteria's surface. The equilibrium will depend on the area of attachment B_{Area} between Ebola virus and the bacteria and the motion realized by both bodies. After binding, both bodies can move in a straight line or tumble in a fixed location. For the running motion, the force diagram is presented in Figure 6.

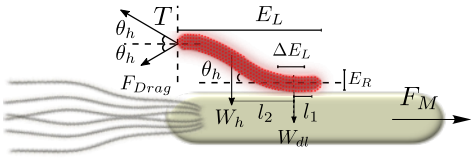


Fig. 6: The binding force representation model between the Ebola virus and the bacteria. The opposing tension force $\mathbf{T}$ is responsible for peeling the virus from the surface of the bacteria while the other forces act to maintain their attachment stability.

In Figure 6 the bacterium's running motion to the right creates forces on the binding area. In order to analyse the forces acting on the binding between two bodies, we use the approach presented in [48]. The description of each variable is as follows: ΔE_L is length of Ebola virus that binds to the bacterium's surface (we are assuming here that only a portion of the virus has bound), $\mathbf{F_M}$ is the force exerted by the bacterium's flagella that enables the movement, $\mathbf{W_h}$ is the weight for the hanging section of Ebola virus, $\mathbf{W_{dl}}$ is the weight of the hanging section of Ebola virus, $\mathbf{T}$ is the tension exerted on the hanging portion of Ebola virus that attempts to peel it from the bacterium due to resistance, l_1 is the distance between the bound mid-section of Ebola virus and the bacterium's centre of mass, l_2 is the distance between the centre of the hanging section of Ebola virus and the bacterium's centre of mass, θ_h is the angle between the hanging portion of the virus and the bacterium, and E_R and E_L are the radius and length of Ebola virus, respectively. We consider both Ebola virus and bacterium as homogeneous bodies. Therefore,

$$\mathbf{W_{dl}} = \frac{m_e}{E_L} g \Delta E_L$$

and

$$\mathbf{W_h} = m_e g - \mathbf{W_{dl}} \, .$$

In order for the body to move linearly in a particular direction, the sum of momentum has to be equal to zero. Therefore,

$$\sum M = M_{dl} + M_h + M_g + M_d - M_T = 0, \tag{11}$$

where $M_{dl} = \mathbf{W_{dL}} l_1$ is the momentum due to the force exerted on ΔE_L, $M_h = \mathbf{W_h}(l_2 + l_1)\cos\theta_h$ is the momentum due to the force exerted on the hanging section of Ebola virus, $M_g = \int_0^{\Delta E_L} P_n \; x \; dx$ is the momentum due to the force exerted on the glycoprotein-receptor complexes on the bacterium (where P_n is the adhesive pressure of all the glycoproteins and x is the length of Ebola virus binding area), $M_d = \mathbf{F_{Drag}} \sin\theta_h E_L$ is the momentum due to the drag force exerted on the edge of Ebola virus ($\mathbf{F_{Drag}}$ is the hydrodynamic drag force calculated from (7)), and $M_T = \mathbf{T} E_L \sin\theta_h$ is the momentum due to the tension exerted on the hanging section of Ebola virus. Replacing these terms into (11) as well as the relationship of $l_2 = \left(\frac{E_L}{2} - \frac{\Delta E_L}{2}\right)$, which is the distance between the weights $\mathbf{W_{dL}}$ and $\mathbf{W_h}$, the tension $\mathbf{T}$ can be represented as:

$$T = \left(W_{dl} l_1 + W_h \left(\frac{E_L}{2} - \frac{\Delta E_L}{2} + l_1 \right) \cos\theta_h \right.$$
$$\left. + \frac{P_n \Delta E_L^2}{2} + F_{Drag} \sin\theta_h E_L \right) (E_L \sin\theta_h)^{-1}. \tag{12}$$

The adhesive pressure of glycoproteins (P_n) can be expressed as the force exerted within the bound area, as follows:

$$P_n = \frac{\left(F_n + \frac{m_e g}{n}\right) n \Delta E_L^2}{2\theta_h E_R \Delta E_L}$$

Inserting this into Equation (12) will result in:

$$T = \left(W_{dl} l_1 + W_h \left(\frac{E_L}{2} - \frac{\Delta E_L}{2} + l_1 \right) \cos\theta_h \right.$$
$$\left. + \frac{\left(F_n + \frac{m_e g}{n}\right) n \Delta E_L}{2\theta_h E_R} + F_{Drag} \sin\theta_h E_L \right) (E_L \sin\theta_h)^{-1}. \tag{13}$$

D. Tension Force for Tumbling Motion

When bacteria tumbles, depending on the position of Ebola virus binding point, there will be two different types of force models which are *angular* and *flat* binding. Figure 7 illustrates these binding points on the bacterium. As the bacterium rotates at the centre point of the body, the angular binding will occur at the locations when Ebola virus encounters a pulling force (e.g., at the front of the bacterium when it tumbles clockwise), while the flat binding occurs at the tail end of the body when Ebola virus is pushed through the circular force). Figure 8 illustrates the force model for the angular binding, while Figure 9 illustrates the force model for the flat binding.

Since the motion is a continuous rotational spin at a fixed point, the sum of the momentum is represented as follows:

1) Angular Binding:

$$\sum M = I\alpha, \tag{14}$$

where I is the inertial momentum of the bacterium as well as Ebola virus, and the angular acceleration during the tumbling process is $\alpha = \frac{d\omega}{dt}$ rad/s^2. Therefore, the inertial momentum is represented as:

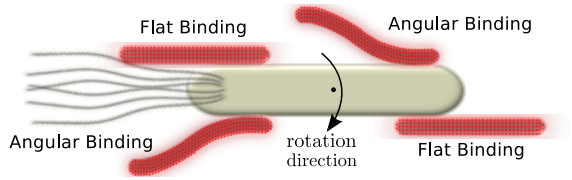


Fig. 7: Illustration of attachment points for angular and flat binding of Ebola virus on the bacterium as its going through a tumbling process. The positions of the flat and angular binding are dependent on the clockwise rotation of the bacterium

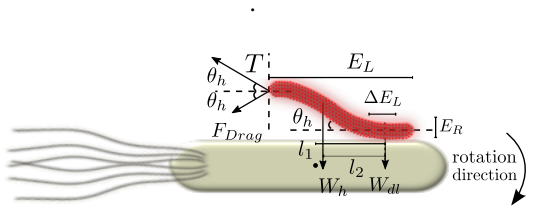


Fig. 8: The force model of Ebola virus on the bacterium that is going through a tumbling process. This illustration shows the forces acting on the angular binding for Ebola virus. The angular binding only happens on locations of turns when Ebola virus is being pulled outwards during the tumbling process.

$$I = \frac{m_B L^2}{12} + m_e \left(E_{hl} \cos\theta_h + \frac{\Delta E_L}{2} + l_1 \right), \tag{15}$$

where E_{hl} is the length of Ebola virus that is hanging, m_e is the mass of Ebola virus and m_B is the mass and L is the half length of the bacterium. For angular binding, considering (14) and (15), (11) can be represented as:

$$I\alpha = W_{dl} l_1 + W_h \left(\frac{E_L}{2} - \frac{\Delta E_L}{2} + l_1 \right) \cos\theta_h + \frac{\left(F_n + \frac{m_e g}{n} \right) n \Delta E_L}{2\theta_h E_R} + F_{Drag} \sin\theta_h E_L - T E_L \sin\theta_h,$$

and from the perspective of the tension $\mathbf{T}$, this is represented as:

$$T = \left(W_{dl} l_1 + W_h \left(\frac{E_L}{2} - \frac{\Delta E_L}{2} + l_1 \right) \cos\theta_h + \frac{\left(F_n + \frac{m_e g}{n} \right) n \Delta E_L}{2\theta_h E_R} + F_{Drag} \sin\theta_h E_L - \frac{m_B L^2}{12} + m_e \left(E_{hl} \cos\theta_h + \frac{\Delta E_L}{2} + l_1 \right) \alpha \right) * (E_L \sin\theta_h)^{-1}. \tag{16}$$

2) Flat Binding: For the flat binding during the tumbling process, (16) can be simplified because there is no angle of attachment between Ebola virus and the bacterium. This means that a large part of Ebola virus will lie flat on the the bacterium during rotation. This scenario is presented in Figure 9. In this case, the tension $\mathbf{T}$ is represented as:

$$T = \left(-W_{dl} l_1 - W_h \left(\frac{E_L}{2} - \frac{\Delta E_L}{2} + l_1 \right) - \frac{\left(F_n + \frac{m_e g}{n} \right) n \Delta E_L}{2\theta_h E_R} - F_{Drag} E_L + \left(\frac{m_B L^2}{12} + m_e \left(E_{hl} + \frac{\Delta E_L}{2} + l_1 \right) \right) \alpha \right) (E_L)^{-1}. \tag{17}$$

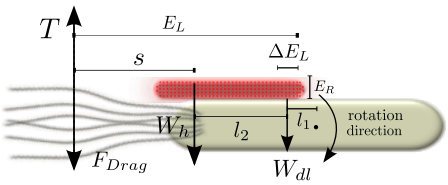


Fig. 9: The force model of Ebola virus on the bacterium that is going through a tumbling process. This illustration shows the forces acting on the flat binding for Ebola virus. The flat binding only happens on locations of turns when Ebola virus is being pushed up against the bacterium during the tumbling process.

E. Binding Force Analysis

The binding force engulfment process (5), as well as the drag force (7), the tension force $\mathbf{T}$ for running motion (13) and tumbling motion of angular binding (16) and flat binding (17) were evaluated using the parameters presented in Table I. The drag force evaluated was equal to 0.41×10^{-19} N. The analyses are plotted and presented in Figures 10, 11a, 11b, and 11c. First, in Figure 10, we considered two different temperatures to observe different behaviours that can arise. Normal human body temperature is 309.65 K and during fever this is elevated to 313 K. The latter is also the average temperature of countries where Ebola virus disease outbreak occurred. As we can observe, the temperature does not produce significant changes in the receptor binding force behaviour.

In the case of top and angular binding analysis (Figures 11a and 11b), we considered four different binding angles between Ebola virus and the bacterium. As we can observe from the plots when the binding angle increases, the tension applied to detach the virus from the bacterium decreases. This is largely due to the tension force $\mathbf{T}$ that is acting on the Ebola virus body, which decreases as its being pulled during the running motion. As the angle decreases towards zero, the top and angular binding will start to have a similar behaviour to the flat binding process.

The binding area also has an important role in the attachment process as can be seen in Figures 11a - 11c. As the binding area increases, the detachment forces tends to decrease. This behaviour is due to the tension that is applied on the hanging part of the virus as illustrated in Figures 6 and 8. Intuitively, as the binding area increases, larger portion of the Ebola virus's glycoproteins will bind to the bacterium leading to larger force of attachment. This high attachment force will therefore, overcome the opposing tension of the hanging portion of the Ebola virus. Figure 11d presents a comparison

TABLE I: Parameters used to evaluate receptor binding force, tensions and drag force applied on the system – bacterium as well as Ebola virus.

Variable	Value
τ_s	250 s
E_R	47×10^{-9} m
E_L	970×10^{-9} m
B_R	0.5×10^{-6} m
v_b	20×10^{-6} m/s
v_f	5×10^{-6} m/s
m_e	5.45×10^{-19} kg
m_b	1×10^{-18} kg
ϕ	from $\pi/100$ rad to $\pi/10$ rad
T_a	310 K
k_B	0.00831446211 kJ/mol/K
SA_{Ebola}	$527,787.57 \times 10^{-9}$ m
$N_{GP_{1,2}}$	1819.96
K_a	4.13×10^9 Å^3
ΔE_L	from 1×10^{-9} m to 970×10^{-9} m
t	from 1×10^{-9} s to 5.5×10^{-9} s
h_d	0.45
μ_{blood}	4.14×10^{-9} Pa.s
ρ_{blood}	1060 kg.m^{-33}
w	3×10^{-6} rad/s
g	9.8 m/s^2
C_{Drag}	0.4181875
μ_s	0.5 Pa.s
R	$8,32$J/mol $*$ K
W_e	1.05×10^{-18} N
η_{plasma}	5×10^{-3} Pa.s
ω	3×10^{-6} rad/s
D_v	100×10^{-6} m

between the tension generated for the three binding types (considering an angle of binding of $\theta = 10°$) and the receptor binding force. The receptor binding force is calculated based on the Equations (1) - (5), and the values from Table I.

As illustrated in the figure, the region that has high tension force will lead to a detachment since this is higher than the receptor binding force. We can observe this for the angular binding below an area of $0.4 \times 10^{-14} m^2$, and for the flat binding this is for the area that is below $0.25 \times 10^{-14} m^2$. We can also observe from the graph that the linear binding for small areas has a tension force that is smaller than the receptor binding force, and so this will maintain the attachment. However, as the area of binding increases, we can see that the receptor binding force slowly increases above the three different tension force, leading to a stable attachment of the Ebola virus on the bacterium (this is shown in the grey shaded area in the graph).

V. Simulation

In order to validate the Ebola virus trapping process by the bacteria, we conducted simulations for two different scenarios based on the setup presented in Figure 12. As shown in Figure 12, Ebola virus were placed in a small area, 100x100 μm^2, while bacteria are placed in an external compartment. As the bacteria are released from the compartment they will swim into the small area and capture the Ebola virus. For both

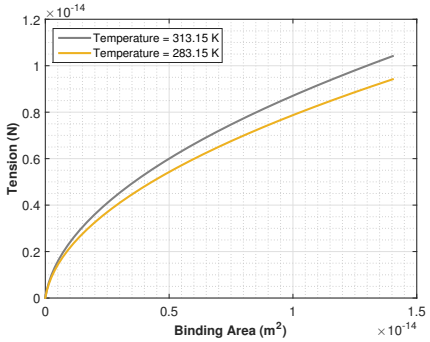
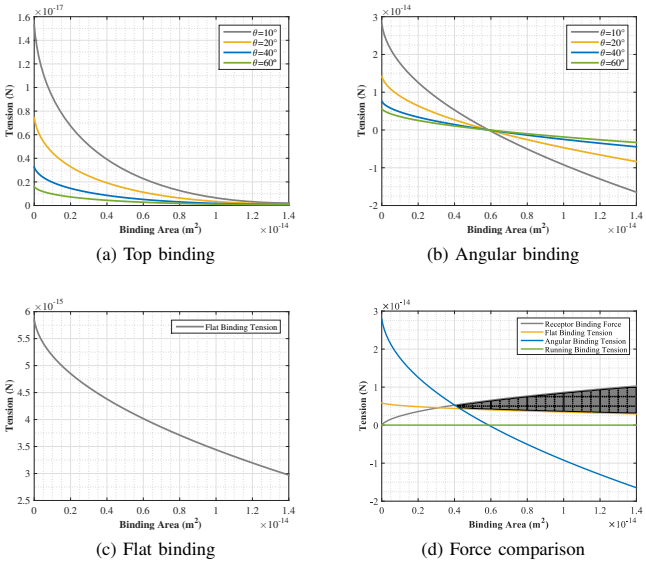


Fig. 10: Analysis of receptor binding force for two different temperatures. The difference of 30 K between them is not sufficient to produce significant change of behaviour. This plot shows that our technique can be used to remove Ebola virus from open environments (temperature around 313 K in countries where Ebola virus disease outbreak occurred) as inside human body (temperature range between 309.65 K–313 K).



(a) Top binding (b) Angular binding

(c) Flat binding (d) Force comparison

Fig. 11: (a) Analysis of the Binding Force of Ebola virus for a running movement of the bacterium. Four different binding angles are considered in this analysis. (b)Analysis of the binding force between Ebola virus and the bacterium as it goes through the tumbling process. In this case, the angular binding is evaluated and four different angles are considered. (c) Analysis of the binding force between Ebola virus and the bacterium for flat binding as it goes through tumbling process. (d) Comparison between the different binding types (running, angular and flat) and the receptor binding force. This analysis used the $\theta = 10°$ binding angle between Ebola virus and the bacterium. The grey painted area is the optimum location to maintain stable attachment of Ebola virus and the bacterium.

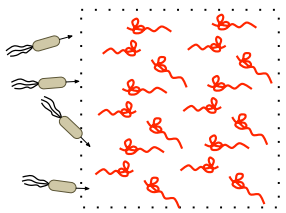


Fig. 12: Simulation scenario of the bacteria released to hunt and trap Ebola virus. The bacteria are placed in a separate compartment on the left and are released simultaneously. The bacteria will swim into the area and capture the Ebola virus which are distributed evenly.

scenarios, the number of Ebola virus are considerably higher than the number of bacteria. Within the area, the Ebola virus are randomly distributed.

In the first scenario, the number of bacteria and Ebola virus

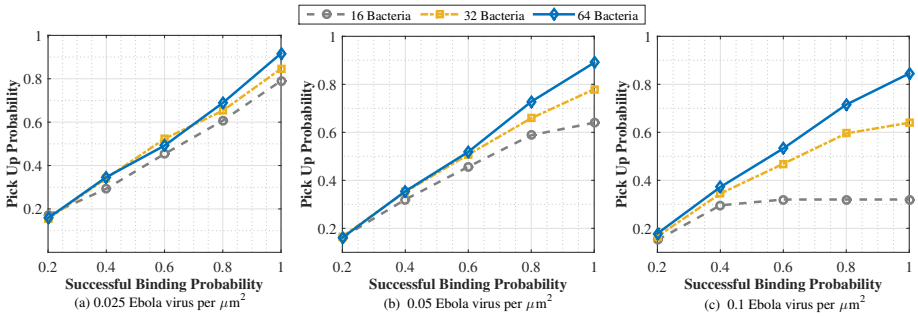


Fig. 13: Pick up probability for three different Ebola virus density on an area of 100x100 μm^2.

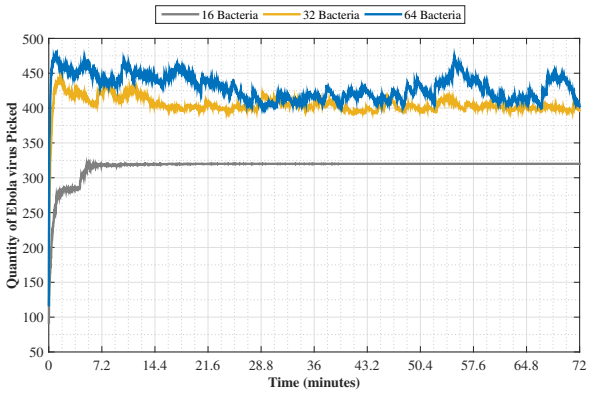
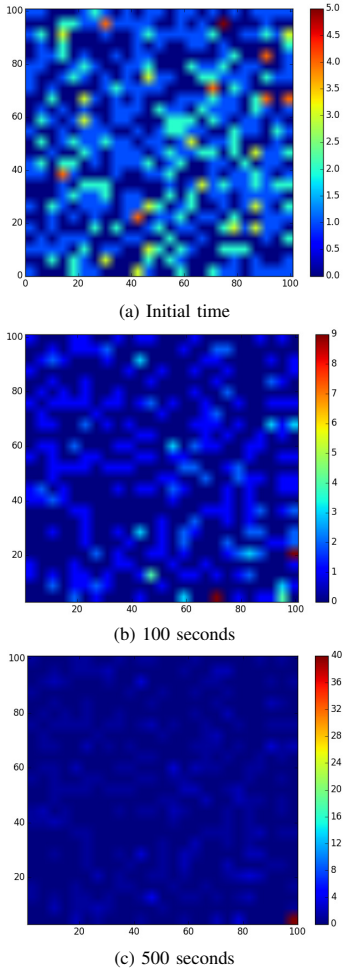


Fig. 14: The quantity of Ebola virus that are captured with respect to time as the quantity of bacteria are varied. The area considered is 100x100 μm^2.

varies: 16, 32, 64 bacteria and 250, 500 and 1000 virus. The results for this scenario are presented in Figure 13. This configuration allows us to observe how the spatial density of Ebola virus affects their probability of being captured. For 0.05 virus$/\mu$m^2 the pick up probability is quite similar for 16, 32 and 64 bacteria. However, as the density increases, more bacteria is needed to increase the picking up efficiency.

For the second scenario, the number of Ebola virus were fixed at 1000 and three quantity of bacteria were considered (16, 32, 64). The simulation time was fixed at 72 minutes and we consider that only half of the total connections are successful. Since each bacterium is set to capture a maximum of 20 Ebola virus, after a certain amount of time the system is expected to became saturated. This scenario was designed to measure the time required before saturation occurs. In Figure 14, the amount of bacteria used achieves their saturation point in less than 15 minutes. This is due to the small area that is considered, and the swimming process of the bacteria (the swimming speed is 20 μm$/s$) which diffuses into the area in a short period of time and runs into a high density of Ebola virus. Depending on the area of binding and the process of capturing the Ebola virus, a number of the virus can peel off and this shows the random fluctuations observed in Figure 14.

To better illustrate the bacteria cleaning process, Figure 15 presents a set of heat maps that shows the reduction in Ebola virus density with respect to time. Figure 15a presents the initial stage where there are Ebola virus distributed throughout the area. As the bacteria swims towards the Ebola virus, they



(a) Initial time

(b) 100 seconds

(c) 500 seconds

Fig. 15: Illustration of pick-up by the bacteria. The heat map shows 500 Ebola virus that are placed randomly in an area of 100x100 μm^2 and been captured by 16 bacteria in 100 seconds.

are slowly captured. At 100 seconds (Figure 15b) we can start to observe the numbers of Ebola virus in the environment dropping. At the end of the simulation time only a few Ebola virus continue to occupy the area, as can be seen in Figure 15c.

VI. Conclusion

The emergence of Ebola virus in recent years has motivated the need for effective treatment solutions to curb the spreading

process of the virus as well as treat patients who have been infected. One treatment approach could be through synthetic biology, which we have seen in recent years has led to solutions that can treat complex diseases based on engineering internal genetic circuits of cells. In this paper, we present an approach where bacteria, genetically engineered, are capable of trapping Ebola virus that binds on its surface. Our approach includes the engineering of receptors on the surface of the bacteria that are compatible to the glycoprotein found on the membrane of Ebola virus. Due to the shape and size of Ebola virus, portions of the body may hang off the bacteria after binding. Therefore, we analyzed the binding force considering linear running motion as well as the tumbling process of a bacterium. Our analysis found that the binding process of the Ebola virus on the bacteria is highly dependent on the binding angle as well as the area of attachment. The paper also presented a simulation model of the bacteria hunting process of the Ebola virus within a confined area. The analysis includes the saturation time of the Ebola virus collection process, as well as the collection and trapping performance when the number of bacteria and Ebola virus varies. Our results show that for a small area the saturation speed can be within a period of minutes and the performance of Ebola virus pick up quantity is highly dependent on the number of bacteria that are deployed.

Our proposed approach can lead to both pros and cons as listed below:

Pros: Ebola are known to release proteins that dampen down the immune system response. This means that using our solution will not require the immune system to fully switch off, since releasing the bacteria can trigger the immune system to eradicate them. The Ebola virus targets connective tissue which is rich in collagen fibers that helps maintain the organs in its place. This property can be exploited by enabling the bacteria the capability of tropism towards collagen.

Cons: Ebola causes small blood clots in the bloodstream which could lead to the flow to slow down. The blood clots can also increase in number and size as the disease progress. While this is a negative aspect of our proposed approach, the limitations can be achieved by releasing the optimum quantity that can capture the required Ebola virus while minimizing the risk of blood clots.

The proposed approach of engineering Trojan-horse bacteria to hunt Ebola can provide a new alternative of eradicating virus that is not only limited to Ebola. Although this paper only concentrated on the *E.coli*, the approach can also extend to attenuated strains of bacteria such as *Salmonella* [49]. This shows the flexibility of utilising synthetic biology as a tool to engineer microbes to eradicate various types of virus, and will open new opportunities for the future that are alternative approaches compared to utilising traditional vaccination.

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