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A Cellular Automata Model of Infection Control on Medical Implants

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Abstract

S. epidermidis infections on medically implanted devices are a common problem in modern medicine due to the abundance of the bacteria. Once inside the body, *S. epidermidis* gather in communities called *biofilms* and can become extremely hard to eradicate, causing the patient serious complications. We simulate the complex *S. epidermidis-Neutrophils* interactions in order to determine the optimum conditions for the immune system to be able to contain the infection and avoid implant rejection. Our cellular automata model can also be used as a tool for determining the optimal amount of antibiotics for combating *biofilm* formation on medical implants.

Keywords: Medical implants, *Neutrophils*, *S. epidermidis*, *biofilms*, Cellular automata

MSC (2010) No.: 68Q80, 92C17, 92C50, 92D25

1. Introduction

Medically implanted devices are becoming increasingly important in medical practice, Xue et al. (2007). Due to the abundance of skin-colonizing bacteria, infectious reactions on such implants constitute a problem for modern medicine, Otto (2009). The most common member of the group of coagulase-negative *staphylococci* is *Staphyloccocus epidermidis,* Vuong and Otto (2002), which is a bacterial colonizer of the skin and mucous membranes of humans and other mammals, Otto (2009). It has been characterized as the main pathogen involved in nosocomial bloodstream infections, cardiovascular infections, and infections of the eye, ear, nose and throat, Vuong and Otto (2002). Being a common colonizer of human skin and one of the most often isolated bacterial pathogens in hospitals it is almost impossible to prevent *S. epidermidis* from entering the body while inserting a medical implant, Vuong and Otto (2002) and Otto (2009). Once in the body, *S. epidermidis* can lead to a wide variety of complications including inflammation, thrombosis, infections and fibrosis, Xue et al. (2007). These complications have a direct effect on the stability of the implanted device because they trigger immune responses, including a rapid accumulation of phagocytic cells, Xue et al. (2007).

If the immune system is not able to eradicate *S. epidermidis* during the first hours after it has entered the body then *biofilm* formation is likely to commence. A *biofilm* consists of bacterial cells immobilized in a substratum which is frequently embedded in an organic polymer matrix of microbial origin. *Biofilms* appear in many different forms, including layers, clumps ridges, and even more complex micro-colonies that are arranged into stalks or mushroom-like formations, Costerton (1999) and Eberhard et al. (2005). Once protected by the *biofilm*, bacteria become difficult for the immune system to eradicate, Gunter et al. (2009), and studies suggest that *biofilms* are present on the surface of the implant as early as 16 hours after implantation, Gunter et al. (2009). However young *biofilms* are more vulnerable to *phagocytic* cells than mature ones which have been growing for more than 48 hours, Gunter et al. (2009). In addition, most antibiotics are only effective against the fast growing bacteria which reside in the outer layers of the *biofilm*, while the slow growing bacteria deep inside of the *biofilm* formation tend to be spared and to persist in the body, Eberhard et al. (2005).

Therefore, it is critical that the immune system destroys the majority of the bacteria before a *biofilm* begins to form. Recent studies suggest that *biofilm* formation by *S. epidermidis* is regulated by a chemical communication between the bacteria called the *agr* system, Kong et al. (2006). When bacterial communities reach a certain size they are ready to gather into a biofilm, so they start releasing a specific chemical that will give the signal to start the attachment process. By disrupting the *agr* system these chemicals are never released then the *biofilm* will never form, which allows the immune system to kill the bacteria and contain the infection.

Of all the types of phagocytic cells, the most important to the immune system's defence against S. epidermidis are the white blood cells Neutrophils. In order to attack the *S. epidermidis* growing on medical implants, *Neutrophils* cells adhere to the surface of the device and move towards the bacterial formations, Xue et al. (2007). The strength of *Neutrophils* adhesion to the medical implant depends on the type of protein present on the surface of the implant. *Fibrinogen* and *Albumin* are two of the most commonly used protein coatings on medically implanted

devices. *Fibrinogen* facilitates a strong attachment between *Neutrophils* and the implant since it is readily recognized as a malign substance by the immune system. However, *Fibrinogen* also works as a distraction to the *Neutrophils* because the phagocytes place themselves in one spot attacking the *Fibrinogen* covered implant and move very slowly towards the bacteria, Tang and Eaton (1993) and Kuntz and Saltzman (1997). In contrast, *Albumin* is not recognized by the phagocytes as a malign substance and hence the *Neutrophils* cells can move freely around the implant.

Another important distinction between *Albumin* and *Fibrinogen* is the amount of *Neutrophils* each protein coating attracts. Experimental studies suggest that two groups of chemokines *macrophage inflammatory protein* (MIP) and *monocyte chemoattactant protein* (MCP) appear to play a major role in phagocyte-implant interactions, Xue et al. (2007). By releasing chemokines, the *Neutrophils* cells present on the surface of the implant are able to attract more *Neutrophils* to the site. These chemotactic interactions create waves of incoming phagocytic cells, which aid in the fight against the bacterial infection. While *Fibrogen* covered implants are interpreted as a threat to the body and many phagocytes are attracted to them, the *Albumin* coated implant is not perceived as a threat and thus fewer phagocytes are present to fight the infection.

In this paper we examine a variety of mixtures of *Fibrinogen* and *Albumin* implant coatings in order to maximize the effectiveness of the immune system response. Finding the optimum amounts of each of these two proteins will help the immune system destroy most of the bacteria before they start to form *biofilm* communities. This will reduce the number of rejections of medically implanted devices and drastically improve the ability of the body's immune system to combat bacterial infections. Our simulations can also be used to help determine the appropriate amount of antibiotics to use over the implant area so that an *S. epidermidis* infection can be successfully controlled as well as to predict what will happen if *biofilm* formation is avoided.

2. Cellular Automata Models

Cellular automata models are dynamical systems in which space and time are discrete, Eberhard et al. (2005). A cellular automaton consists of a regular grid, each of which can be in one of a finite number of possible states updated synchronously in discrete time steps according to local, identical rules, Mallet and de Pillis (2006). In this paper, we employ a cellular automata modeling approach to simulate interactions between *Neutrophils* and *S. epidermidis* subject to a variety of coatings of *Albumin* and *Fibrinogen* mixtures on a medically implanted device. A set of rules for the movement of the cells and the growth of the bacteria is given for the two different types of protein coatings. The amounts of *Albumin* and *Fibrinogen* in the mixture are allowed to be varied, since they have different effects on the speed of the *Neutrophils* and their ability to control a bacterial infection.

We consider a biased motility model, in which *Neutrophil* cells move with greater probability towards larger bacterial concentrations. The model is divided into three parts. The first part simulates the complex *S. epidermidis*-*Neutrophils* interactions between *4* and *20* hours after the implant is introduced into the body. We consider the reproduction of bacteria at the early stage of a bacterial community formation which triggers the immune response. We also incorporate a series of chemotaxing waves of *Neutrophils* cells in our model. The second part of the model simulates the system dynamics after the *S. epidermidis* have started forming a *biofilm* which

takes place between the 20 and the 52 hours. During this part of the simulation, bacteria experience an increase in the reproduction rate while the immune system response gradually decreases effectiveness as the *biofilms* become stronger. The last part of the model, after the 52 hours, the immune system can no longer fight *S.epidermidis* since they are all gather in fully formed strong *biofilms*.

The novelty of this mathematical approach is the implementation of the cellular automata on different scales. The two-scale discrete CA model includes one scale for the *Neutrophils* and another scale for the bacteria, taking into consideration the much larger size of the white blood cells.

3. Numerical Implementation

Our biased motility cellular automata model is implemented on an *SxS* grid. A square in the grid is occupied by bacteria with a variable density while a *Neutrophil* cell occupies a *cxc* square. Each square in the grid is in one of the following four states:

- Empty
- Covered with *S. epidermidis*
- Covered with a *Neutrophil* cell and *S. epidermidis*
- Covered with a *Neutrophil* cell but without any bacteria present

Each numerical simulation consists of a series of iterative steps. We initialize the model with two *SxS* matrices. Every entry in each matrix represents a square in the grid described above. On the first matrix we randomly select *m* blocks of *cxc* numbered squares, each block representing a single *Neutrophil* cell. Each cell has the ability to move in *8* different directions (Figure 1). Direction *i* is chosen with probability P_i, $i = 1 ,..., 8$ where the value of P_i depends on the concentration of bacteria in each direction. In the second matrix, *b* units of *S. epidermidis* are placed randomly, with no limit on the number of bacteria that can reside in a single grid square.

Each block of *cxc* squares in the matrix that represents the *Neutrophil* cells is uniquely numbered. Every time step we check the area under each cell for *S. epidermidis* bacteria. Consequently, one of the following two cases holds:

- There are some bacteria under the area covered by the *Neutrophil*. In this case, the *Neutrophil* doesn't move and consumes one unit of bacteria each time step until there is no more bacteria under the area covered by the *Neutrophil*.

- There are no bacteria under the area covered by the *Neutrophil*. In this case, the cell moves to an available, free from other *Neutrophil* cells, neighboring space *i*, $i=1,...,8$, (Figure 1) with a probability P_i.

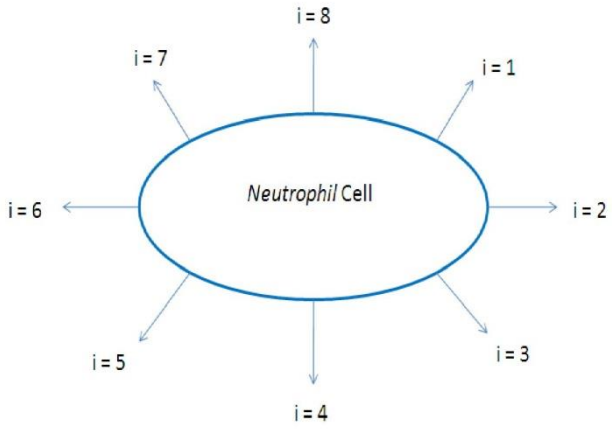

Figure 1. Directions *i=1,…,8* for movement of the *Neutrophil* cells.

The direction *i* of cell movement is determined randomly according to specific probabilities assigned to each direction. The *Neutrophil* cells move toward a higher concentration of bacteria with a greater probability P_i. To compute P_i, we consider a 3*x*3 grid and place the *Neutrophil* cell on the center square in the grid. Then we calculate P_i according to the formula $P_i = A_i/B$, $i = 1,...,8$, where *B* is the total amount of bacteria on each of the 8 squares surrounding the cell and A_i is the amount of *S. epidermidis* on each of the surrounding positions.

To take into consideration the chemotaxis interactions between the *Neurophil* cells, we add *G* additional cells to the system every *dx* units of time, where *dx* is a constant, and *G* is a function of the protein mixture, the amount of bacteria currently present and, the amount of phagocytes in the model at that time. The new cells are placed randomly on available spaces of the implant ensuring that no two cells overlap on the implant. The protein coating is a mixture of *Albumin* and *Fibrinogen*. For convenience, we will use the variable *A* to quantify the percentages of *Albumin* in the protein coating mixture. *A* is a number between *0* and *1* which indicates the fraction of *Albumin* in the protein coating mixture while *1-A* represents the fractional amount of *Fibrinogen* in the protein coating mixture.

4. Numerical Simulations

In order to examine the effect of *Neutrophils* ability to identify bacteria on the progression of the bacterial infection, we run a set of biased motility simulations. The amounts of *Fibrinogen* and *Albumin* are varied in the implant's coating mixture in order to determine the optimal amounts of each protein that facilitate the best immune system response. We use Matlab® to implement our biased motility cellular automata model. The time unit used for the simulations is $\Delta t=20$ seconds, which is the same as the approximate time that it takes for a *Neutrophil* cell to ingest a single *S. epidermidis* bacterium. In our numerical simulations we model the first *76* hours after the implant is introduced to the body. After the initial *20* hours, *S. epidermidis* bacteria start forming a biofilm and the immune system gradually becomes less effective in fighting the bacterial infection. After 52 hours, the immune system can no longer fight the infection.

The specific functions and parameters that are used in the simulations are listed below. The time at which new *Neutrophil*s are incorporated into the simulation, *dx*, is given by

$$dx = 180\Delta t,$$

this represents a one-hour interval and is consistent with the available experimental data that we are basing the model on. When levels of *Albumin* decrease more *Neutrophils* are recruited which means chemotaxis becomes stronger which means that more *Neutrophils* are incorporated into the model each hour. Therefore, we use

$$G(A) = round\left[\left(2(1-A)\frac{b}{\beta} + \frac{1}{2}\right)n\right],$$

to represent the amount of new *Neutrophil* cells that are incorporated into the system every hour. Here, *A* represents the fraction of *Albumin* in the protein mixture; *b* is the current amount of bacteria, *n* is the number of *Neutrophils* currently on the simulation and β is a normalizing factor. For the experiments we are running $\beta=9072$ since this represents the average initial bacteria on the experimental implant, Tang (2010).

The more *Albumin* in the mixture the fewer *Neutrophils* cells are recruited into the implant. As bacteria accumulate on the surface of the implant, more *Neutrophils* are recruited due to chemotaxis which increases the ability of the immune system to fight the infection. According to experimental data, approximately *40*% more *Neutrophils* are found when *Fibrinogen* is the only protein used to cover the implant as opposed to when only *Albumin* coating is used, Tang and Eaton (1993). The amount of initial *Neutrophil* cells on the implant surface, *m*, is modeled by the following function:

$$m(A) = round\left[8(1-A)\frac{b}{\beta}\right] + 2,$$

where the function *m* depends only on the amounts of *Albumin* in the protein mixture and the initial amounts of bacterium since there are no *Neutrophils* on the surface of the implant at this point. There will be more cells recruited when less *Albumins* is present in the mixture.

As said before, it takes *Neutrophils* more time to move on a *Fibrinogen* surface than on an *Albumin* surface. To account for this we use the function T_s which represents the time that it takes each *Neutrophils* cell to move one unit in space (a square in the grid of the model)

$$T_S(A) = floor\left[e^{4(1-A)}\right],$$

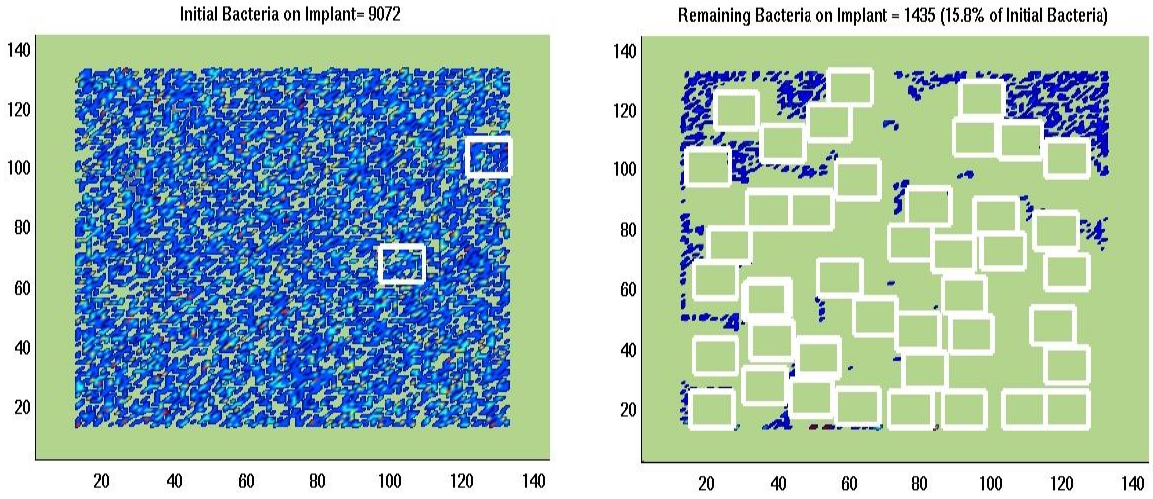

Figure 2. Snapshots of the initial (left) and final (right) state of the system in a *20*-hour simulation

Other parameters used in the simulations include *c*, the size of the *cxc* square on the grid that a single *Neutrophil* cell occupies, and *S*, the size of the *SxS* grid used in the cellular automata models. We use *c=12* since the ratio between the radius of a *Neutrophil* cell and an *S. epidermidis* bacteria is approximately 1:12, and *S=120* which represents a grid of size approximately *.01%* of the area of a biomedical implant used in practice. We also consider the generation time of the bacteria inside a *biofilm* to be *200* minutes, Konig et al. (2001), while the generation time of free bacteria under stress to be *600* minutes, Tang (2010).

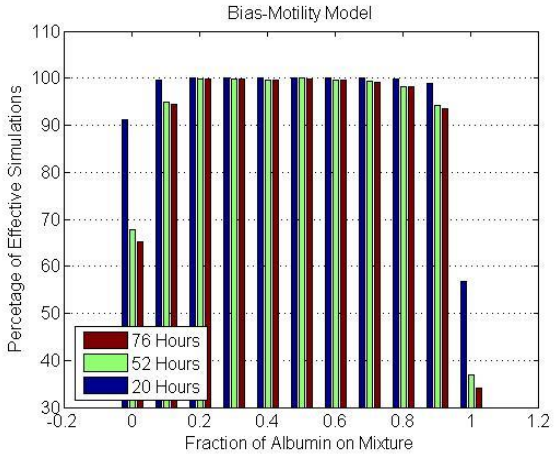

Figure 3. Percentage of effective simulations using a biased motility model

We run the simulation *10,000* times for *76* hours, retrieving the amount of bacteria left in each simulation after *20* (Figure 2), *52* and *76* hours. An effective simulation is defined as a simulation in which at most *1%* of the implant area is covered with bacteria after *76* hours. The graph below shows the percentage of effective simulations for all values of *Albumin* between *0*% and *100%* in *10*% increments (% of *Fibrinogen=100* - % of *Albumin*) after *20*, *52* and *76* hours.

Figure 3 shows the results of the simulations using our biased motility model, which yielded results similar to published experimental data by Tang and Eaton (1995).

In order to improve the results for all *Albumin* and *Fibrinogen* percentages two strategies could be used: (1) medical devices can be pre-coated with antibiotics before implantation; or (2) *biofilm* formation can be blocked, Kong et al. (2006). The model was modified as follows to include both approaches:

- To include the effect of antibiotics in our original model, every certain amount of time some percentage of the bacteria is eliminated at random from the implant. The amount of time and percentages can be modified to describe the effect of different types of antibiotics. The effects of a sample antibiotic on the different mixtures after a series of *76*-hour simulations are shown on the graphs below (Figure 4, left).

- *Bioflim* formation can be avoided by disrupting the *agr* system to prevent the attachment of bacterial cells. Our original cellular automata model was modified to neglect *biofilm* formation by treating the 20-to-76-hour parts similarly to the 4-to-20-hour part of the model. Bacteria are treated as free bacteria, and *Neutrophils* are able to kill bacteria at the same speed during the entire *76*-hour simulation. The results are shown on the graphs below (Figure 4, right) where the effect of disrupting the *agr* system can be easily observed.

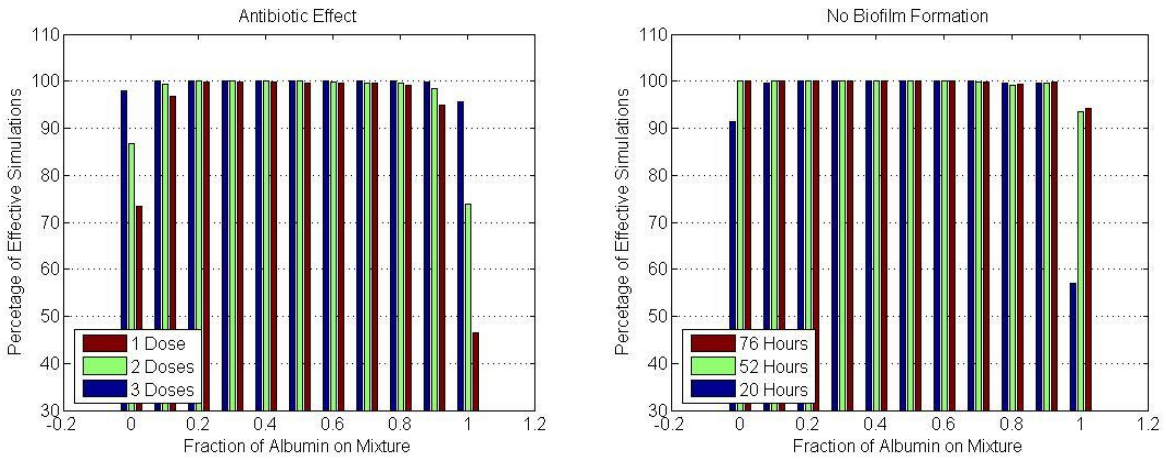

Figure 4. The effects of different doses of antibiotics (left) and the absence of biofilm formation (right) on bacterial infections

5. Discussion and Conclusions

Using a biased motility cellular automata model we have numerically investigated the interactions between *S. epidermidis* and *Neutrophils* on the surface of a medically implanted device with protein-coating mixtures of *Ablumin* and *Fibrinogen*. By using our model, we found an array of different protein-coating mixtures that maximize the immune response while

minimizing the rejection caused by infection or inflammation. We also found the different protein-coating mixtures that prevent *biofilms* from forming on the surface of the implant altogether.

Using this CA model we were able to obtain a range of protein-coating mixtures which maximizes the percentage of effective simulations. Over *99.7*% of the experiments with mixtures between *30*% *Albumin (i.e., 70*% *Fibrinogen)* and *70*% *Albumin (i.e., 30*% *Fibrinogen)* were successful in eradicating the bacteria. Inside that range, mixtures of *40% Albumin* and *60% Albumin* were the most efficient (with *99.9*% effective simulations).

The model was also used to determine the effects of pre-coating implants with antibiotics before insertion. We ran the simulations for different doses of antibiotics to determine how many doses are needed to prevent *biofilm* formation. We were able to conclude that with three doses of antibiotics all protein coating mixtures yield effectiveness above *97.0%*. For different antibiotics the simulation can be rescaled to represent accurate amounts of that specific antibiotic needed to successfully avoid *biofilm* formation under any protein-coating mixture.

Finally the biased motility model was used to determine what will happen if *biofilm* formation can be prevented completely. The simulation showed that in this case all protein coating mixtures will control the infection over *97%* of the time. If we could keep *S. epidermidis* from gathering into a *biofilm,* then very low percentages of *Fibrinogen* can be used on the protein coating mixture while having *99.9*% effectiveness. This could mean greater efficiency in spite of low *Neutrophils* recruitment, which will lead to less inflammation.

In this work, we also implemented an unbiased (random) motility model, in which *Neutrophil* cells move at random on the surface of the implant. The results from the random motility model were found to be biologically inaccurate, and therefore were not presented in the paper.

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