Simulating Bacteria-Materials Interactions via Agent-Based Modeling

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Abstract. This work reports the outcomes of *in silico* simulations of the interactions between *S. aureus* bacteria and an antibacterial polymeric coating developed onto titanium substrates. The aim of the theoretical analysis is to develop a computational approach suitable of predicting the effective amount of antibacterial agents to load onto the polymeric coating in order to prevent titanium implants infections and at the same time to minimize cytotoxicity. The simulations results will be contrasted with experimental data.

Keywords: Antibacterial activity · Titanium implants · Infections · In silico modeling

1 Introduction

In biomaterials science, the development of innovative antibacterial surfaces is one of the most active research fields: infections, indeed, often cause orthopedic and dental implant failure. Implant associated infections and antibiotic resistance are still rising, with *S. aureus* and coagulase-negative *staphylococci* accounting for 45–55 % of infections [1–4]. Therefore, antibacterial biomaterials are essential to improve *in vivo* implant duration [5–8]. Antibacterial agents, loaded and released at the implant site, are an effective approach to address this urgent issue. Silver ions are among the most promising non-conventional antimicrobial agents, although their mechanism of action, as well as their toxicity, are controversial and poorly understood [3].

Wet laboratory experiments on cell-biomaterial interactions are often combined with computational studies because they are cost effective and time saving. These interesting advantages suggest the opportunity for *in silico* methods to become, in the next future, valuable tools to design, test and optimize functional biomaterials [9–11].

In this research field, *agent-based models* are a class of computational techniques that appear to be highly promising since they have been largely applied to describe the macroscopic properties of dynamical systems characterized by heterogeneity and self-organization [12]. They simulate the simultaneous operations and interactions of *agents*, the essential elements describing the studied systems, in an attempt to re-create and predict the appearance of complex phenomena. In fact, *in silico* complex behaviours

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to a higher (macro) level can be observed starting from setting simple rules of agent-agent and agent-environment interactions. This bottom-up method has been exploited to investigate the dynamics of complex populations at different levels, from the sociological to the cellular and atomic scale [13]. On the other hand, biological systems are particularly challenging for classical computational methods, because the information flux between single elements over time, as well as between the elements and the surrounding environment, must be taken into account. When also the spatial distribution plays a crucial role, such as for biomaterials, the agent-based approach is particularly suitable: agents are free to move and act independently from each other [14]. Moreover, in the specific case of antibacterial surfaces, agent-based models enable the study of the effects of surface stimuli on bacterial populations [15].

In this work, an agent-based modeling approach has been adopted to simulate *S. aureus* interactions with a titanium substrate loaded with silver ions, in order to choose the least effective amount of the antibacterial agent. The outcomes of the *in silico* simulations are compared with preliminary experimental observations.

2 Materials and Methods

2.1 Preparation of the Antibacterial Surface

The polymeric coating has been synthesized on 1 cm^2 titanium substrates according to an electrochemical procedure previously described and different amounts of silver ions per unit surface area have been loaded onto the polymeric coating as antibacterial agents [16]. All electrochemical experiments have been carried out using a PAR Versa STAT 4 potentiostat - galvanostat (Princeton Applied Research). The coated titanium samples have been sterilized by UV treatment before *in vitro* antibacterial evaluation.

2.2 *In vitro* Antibacterial Activity

All the experiments have been performed in triplicate according to ISO 22196:2011. *S. aureus* has been grown in Luria Bertani Broth (LB) (Difco, Detroit, MI, USA).

A suspension of 10^4 bacteria has been incubated onto each UV sterilized titanium substrate (and onto sterile substrates as control) for 24 h at 37 °C. Then, bacteria have been serially diluted and plated on agar. After 24 h at 37 °C bacterial viability has been calculated setting to 100 % the control growth (bacteria grown onto sterile substrates) and comparing bacterial number onto the bare coatings and onto silver-loaded coatings.

2.3 *In silico* Simulations

The NetLogo platform has been used to simulate the time behaviour of *S. aureus* bacterial population deposited on a titanium surface coated by a polymeric layer loaded by different amounts of silver ions [17].

Simulation consists in a random walk of bacteria on a squared surface discretized in 33×33 lattice. Each node of the lattice corresponds to a bacterium binding site. Since

the cell diameter of *S. aureus* is approximately 0.6 μm, by assuming a spherical shape, the surface area of a bacterium is around 0.3 μm^2 and therefore 33 × 33 lattice corresponds to a surface of 300 μm^2 with about 10^3 binding sites. The simulation time is expressed as clock ticks, that are time steps in which all bacteria can move, die or reproduce. The length of the clock tick has been estimated equal to 10 min and the simulations last for 144 ticks, corresponding to the *in vitro* test duration (24 h).

At each step of the simulation bacteria are allowed to do:

- a random movement, that is to move in an adjacent binding site by chance. A positive score of 2, called *energy*, has been assigned to bacteria (turtles) moved on a polymer patch. The latter changes colour after bacterial passage;
- bacteria standing onto randomly-positioned silver ion patches get killed and disappear from the lattice;
- when bacteria have reached the division energy, equal to 250, they reproduce (*hatch*) creating two new-born bacteria, each having a starting energy of 25.

The output of the simulation consists in a plot of the total number of bacteria against time, combined with a 3D animation showing the time evolution of the bacterial population on the coated titanium surface. In Fig. 1, three snapshots of this animation have been reported, that enhance the visualization of the system's behaviour.

In order to easily simulate samples containing different amounts of silver ions, a slider bar has been inserted in the graphical user interface of the NetLogo model, allowing the rapid tuning of the system without any code changes. With the same purpose, other sliders (starting bacterial number and energy gained from polymer) have been added to create a more user-friendly interface.

The antibacterial activity of the simulated substrate is assumed to reach the lower threshold of effectiveness when the initial bacterial population is not allowed to grow and fluctuates within 10 units around the initial value.

3 Results and Discussion

The antibacterial activity threshold for silver-embedded polymer coating is reported in Table 1. It has been estimated by *in vitro* experiments and refers to a surface coverage of silver ions equal to 0.9 %. Loading samples with lower amounts of silver results in limited activity, while a 3.6 % surface coverage of silver ions proves a higher efficiency *in vitro*. Conversely, bacteria grow with no significant difference onto the bare polymeric matrix compared to the control culture (viability 103 ± 1 %). As further control, bacterial growth onto uncoated titanium has been studied and no differences have been seen compared to the control culture (data not shown).

The *in silico* simulation outcomes are in agreement with the *in vitro* observations. When silver ions are absent on the polymer-coated titanium surface after a 144 tick simulation, i.e. 24 h *in vitro*, the entire lattice is covered by bacteria and this corresponds to the viability observed by *in vitro* experiments. On the other hand when silver ions are present with a cover percentage of 0.9 %, i.e. setting 10 lattice sites as silver patches, starting from a bacterial population of 2 % coverage (20 bacteria on the 33 × 33 binding

site lattice), the bacterial population do not grow indefinitely but it fluctuates around its starting value, see Fig. 1. According to the *in vitro* experiments, a higher amount of silver ions exhibits a stronger antimicrobial activity and the bacterial population is highly reduced also *in silico*. Conversely, the system with the lower silver amount than 0.9 % coverage fails to maintain bacterial population into the starting range. Indeed, it has been possible to simulate the performances of the titanium coating loaded with different amounts of silver ions, reproducing *in vitro* experiments.

Table 1. In *vitro* antibacterial activity of the proposed coating

Surface coverage of silver ions	Bacterial viability
0.2 ± 0.1 %	18 ± 1 %
0.9 ± 0.1 %	2 ± 1 %
3.6 ± 0.1 %	0
Polymer only	103 ± 1 %

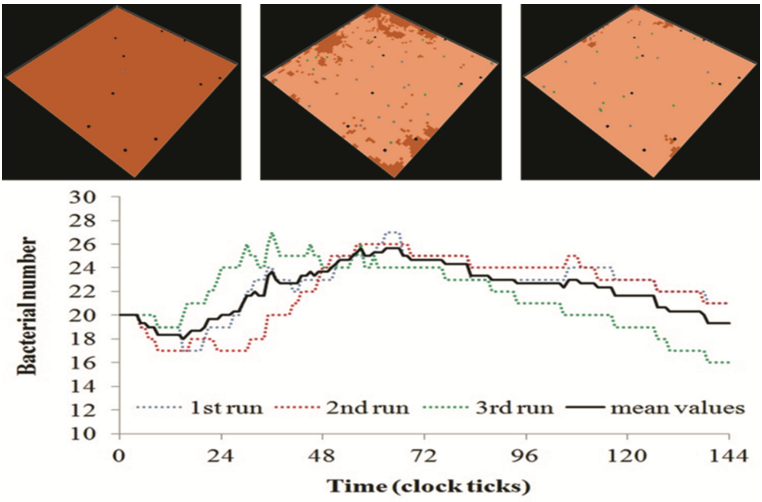

Fig. 1. The lower plot reports the bacterial number against time obtained as outcomes of three different simulations (dotted lines) all of them starting from an initial population of 20 bacteria on a polymer-coated titanium surface loaded with silver ions at 0.9 % coverage; the black line is the average time course of the bacterial population. On top, three different snapshots are shown taken at different simulation times (clock tick 0, 72 and 144), during the run reported in green in the upper plot; initial bacteria are reported as grey dots while new-born bacteria are in green, dark squares represent silver ions.

4 Conclusions

The proposed simulation describes agent-environment interactions in agreement with *in vitro* microbiological tests. As a further improvement, it would be interesting to study the same bacteria-material interactions for a time longer than 24 h, looking for an

agreement between *in vitro* and *in silico* observations. The simulations would be closer to reality taking into account *S. aureus* ability to aggregate forming *grape* clusters, or to produce biofilms to attach to substrates and shield from antimicrobials.

Even though very simple, this computational approach could help reducing time and costs of wet lab experiments, improving the antibacterial performances of current orthopedic and dental implants.

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