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Sol–gel silica controlled release thin films for the inhibition of methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection has significantly increased. Generally, the success of this bacterium as a pathogen is attributed to its ability to adhere to surfaces and remain there, under the protection of an extracellular matrix known as biofilm. To combat MRSA with regular doses of vancomycin, efforts are continuously underway to increase its effectiveness. A promising technique is to use combinational therapeutics. *In vitro* experiments showed that farnesol can be used as an adjuvant with conventional antibiotics. Farnesol is a natural sesquiterpenoid and quorum-sensing molecule. The biggest obstacle to using this concept is that farnesol is highly water insoluble. This compromises its bioavailability if it were to be used along with vancomycin at the site of infection when the treatment needs to be administered *in vivo*. Herein we designed an efficient therapeutic strategy for the simultaneous delivery of both antibiotic and adjuvant in order to treat MRSA infections. We demonstrate that sufficient quantities of both vancomycin and farnesol can be incorporated into sol–gel silica applied as thin films on an implant surface. The incorporation of the hydrophobic farnesol does not affect the stability of the thin films and neither does it affect the controlled release of vancomycin. The data demonstrate the potent adjuvant effect of farnesol on vancomycin in inhibiting MRSA infection. *In vitro* experiments show the complete inhibition (10^6 fold reduction in growth compared to control) of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) when the ratio of vancomycin to farnesol in the sol–gel silica films is optimized. The local delivery of antibiotics minimizes the need for systemic antibiotics. The incorporation of vancomycin and farnesol into thin sol–gel films represents a new treatment paradigm for the topical delivery of antibiotics with adjuvant. The potential clinical benefits are significant and include avoiding the need for revision surgery, preventing surgical site infection and controlling healthcare costs.

1. Introduction

Staphylococcus aureus is among the most prominent of all bacterial pathogens. It is a commensal inhabitant of a significant proportion of the healthy population that colonizes the nares (its primary reservoir), axillae, vagina, pharynx, and/or damaged skin surfaces [1]. *S. aureus* is unique in its ability to invade and cause disease in previously normal tissue at virtually all sites [2,3]. These infections are then typically treated with antibiotics. Yet, microbial resistance against antimicrobials is increasing. This is regarded as the consequence of the proliferation of their use since their introduction nearly 70 years ago. This issue is not abating, but is worsening [4]. In fact, the incidence of methicillin-resistant *S. aureus* (MRSA) infection has significantly increased and the statistics for MRSA infections speak for themselves. From 1999 to 2005, infections outside the lungs or blood tripled [5].

The most frequent primary diagnosis associated with other *S. aureus*–related infections is cellulitis and abscess, followed by postoperative infection, infections with an implanted device or a graft, and osteomyelitis. Surgical site infections are the second most common type of adverse events occurring in hospitalized patients. Surgical site infections (SSI's) account for approximately 16% of nosocomial infections and are associated with prolonged hospital stays and increased costs [6]. Infection develops when endogenous flora are translocated to a normally sterile site.

Generally, the success of resistant pathogens, such as MRSA and *Candida* species is attributed to their ability to adhere to surfaces and remain there under the protection of an extracellular matrix

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known as biofilm. Thus, the presence of a foreign body, such as an implant device, increases the risk for infection organisms and oftentimes leads to persistent infections.

Traditionally, by virtue of the universal resistance of MRSA to β-lactams and the lack of other effective alternatives, the glycopeptide vancomycin became the mainstay of treatment. It provides *in vitro* activity against all staphylococci and demonstrates clinical responsiveness against MRSA infection [7]. Yet, *in vitro* susceptibility of MRSA to vancomycin is no longer universal. In 1997, a clinical strain of *S. aureus* with susceptibility to vancomycin occurring at higher than the normal concentration (minimum inhibitory concentration [MIC], 8–16 μg/mL) was reported by a Japanese group [8]. This was soon followed by descriptions of several frankly vancomycin-resistant *S. aureus* isolates (MIC, 32 μg/mL) in the United States [9].

To combat MRSA with regular doses of vancomycin, efforts are continuously underway to increase its effectiveness. A promising technique is to use combinational therapeutics. Recently, farnesol was described as a molecule with antimicrobial properties [10] and its potential against planktonic cultures of *Staphylococcus epidermidis* was demonstrated [11]. Farnesol is a natural sesquiterpenoid present in several fruits aroma. It is also secreted by *Candida albicans* as a quorum-sensing molecule. In this role, farnesol prevents the transition from yeast to hyphal growth in *C. albicans* and greatly compromises biofilm formation by this fungus [10]. It was recently shown in *in vitro* experiments that farnesol can be used as an adjuvant with conventional antibiotics [12,13]. Although promising, the biggest obstacle to using this finding is that farnesol is highly water insoluble. This compromises its bioavailability if it were to be administered parenterally along with vancomycin, as it may not reach the locus of infection *in vivo* in sufficient concentrations.

Room temperature processed silica sol–gels are highly porous, nanostructured controlled release materials that are extensively studied [14–22]. Large quantities of biologically active molecules can be added uniformly to the liquid sol. After gelation, condensation and drying, the molecules are encapsulated in the resulting glassy solid and are uniformly distributed in the solid matrix. Some of the important benefits associated with silica sol–gel glasses are the excellent biocompatibility, as demonstrated *in vivo* [14], and the extensive control of release kinetics [15]. Various biomolecules such as antibiotics, proteins and growth factors can be released in a time- and load-dependent manner [16,19–22]. As far as the antibiotic vancomycin is concerned, it retains its bactericidal properties upon release [16,19].

In this study we report on an efficient therapeutic strategy for the simultaneous local delivery of both antibiotic and adjuvant in order to treat MRSA infections. We demonstrate that sufficient quantities of both vancomycin and farnesol can be incorporated into sol–gel silica applied as thin films on an implant surface. We measured the effect of the twofold molecule incorporation on the degradation of the thin films. We also analyzed the effect of farnesol on the release kinetics of vancomycin, and we determined, *in vitro*, the efficacy of the vancomycin and farnesol loaded thin films on the suppression of methicillin-susceptible and methicillin-resistant *S. aureus* colonizing implant surfaces. Sol–gel films with only vancomycin, only farnesol and with no vancomycin and farnesol were used as controls.

2. Materials and methods

2.1. Surface treatment of titanium rods and wires

Two sources of titanium alloy rods were used, Ti–6Al–4V titanium rods with diameter 3 mm and length 13 mm, and Ti–6Al–4V anodized Kirshner wires (K-wire) 2 mm in diameter and length of 30 mm. These rods and K-wires were carefully sandblasted utilizing the SandStorm Expert (Vaniman, Fallbrook, CA). Sandblasting was performed using 50 micron Korox Aluminum Oxide (Lincoln, RI) at 40 psi.

The rods and wires were cleaned by sonication in acetone (30 min) and a 2% Liqui-Nox detergent (1 h). After rinsing in distilled water, rods and wires were further sonicated in a 35% solution of nitric acid for 1 h. Finally rods were rinsed in distilled water 3 times and dried in a laminar flow hood for at least 1 day before use. Each dried rod or wire was weighed before sol–gel coating.

2.2. Synthesis of sols

2.16 mL of distilled water was mixed with 0.25 mL 1 N HCl (Fisher Scientific, Pittsburgh, PA) and 9.63 mL of 200 proof ethanol in 20 mL glass vial and stirred for 5 min until pH equilibrated within the solution. Next, 5 mL of the silica precursor, tetra ethyl orthosilicate (TEOS) was added drop-wise while stirred at 300 RPM. Stirring was continued for another 2 h.

2.3. Drug loading

After completion of the hydrolysis of the sols, pharmaceuticals were added and stirred for 30 min. We used 10% and 20% loading (% by weight) of vancomycin and for each of the vancomycin loading we used farnesol loading concentrations of 10%, 20% and 30% (% by weight) respectively (weight % is the % of drug weight to SiO_2 weight). The notation used to describe the drug loading is as follows: sol–gels film with no drugs SG; sol–gels film with "*x*" weight percentage of vancomycin SGV(*x*); Sol–gels film with "*y*" weight percentage of farnesol SGF(*y*), sol–gels film composed of both "*x*" weight percentage vancomycin and "*y*" weight percentage farnesol SGVF(*x*,*y*).

The following sol–gels film were deposited onto sandblasted Ti rods: SG, SGV(10), SGF(30), SGVF(10,10), SGVF(10,20), SGVF(10,30).

The following sol–gels film were deposited on sandblasted Ti K-wires: SG, SGV(10), SGV(20), SGF(20), SGF(30), SGF(40), SGVF(10,20), SGVF(10,30), SGVF(10,40), SGVF(20,20), SGVF(20,30) and SGVF(20,40).

2.4. Sol–gels film deposition on titanium rods and wires

Uniform thin films on rods and wires were deposited using dip-coating utilizing a dipping device with a stepping motor [22]. The Velmax NF90 Series stepping motor (Bloomfield, NY) allows for controlled withdrawal speed for a controlled vertically mounting gliding system. The withdrawal speed was set at 0.569 mm/s (34.17 mm/min). Five layers of drug loaded sol–gel were deposited onto each rod or wire. After five layers were deposited on the rods/wires, all specimens were dried in air under laminar flow hood.

2.5. In-vitro vancomycin release kinetics

The rods used for the quantification of *in-vitro* release included SGV(10), SGVF(10,20), and SGVF(10,30) and the K-wires that were used were: SGV(10), SGV(10,30), SGV(10,40), SGV(20), SGVF(20,30), SGVF(20,40).

Each rod/wire was placed in an individual 2 mL Biopure eppendorf tube (Eppendorf, Hamburg, Germany) with 2 mL of phosphate buffer solution (PBS). These were kept in static conditions in an incubator at 37 °C. The tubes were removed from the incubator every 24 h and the PBS was replaced by 2 mL of fresh PBS. PBS was collected until there was no longer any vancomycin release.

The detection and quantification of vancomycin was carried out by UV spectrophotometry using the appropriate blank and calibration curve at 281 nm. The standard solutions had concentration ranges between 10 and 500 μg/mL, which were the same as those predicted in the samples from the cells. The calibration curve gave the specific extinction coefficient $E = 6230\ M^{-1}\ cm^{-1}$ at 281 nm of vancomycin hydrochloride with a correlation coefficient 0.9997.

2.6. In-vitro farnesol release kinetics

In-vitro release of farnesol was quantified for SGF(30) on K-wires. Each wire was placed in an individual 2 mL Biopure eppendorf tube (Eppendorf, Hamburg, Germany) with 2 mL of phosphate buffer solution (PBS). The samples were kept in static conditions in an incubator at 37 °C. The tubes were removed from the incubator every 24 h and the PBS was replaced by 2 mL of fresh PBS. PBS was collected until there was no longer any farnesol release.

Farnesol was detected using HPLC methods. Detection was achieved at 215 nm at a retention time of 1.7 min with a mobile phase flow rate of 1.2 mL/min.

2.7. In-vitro bactericidal study

Bactericidal studies were performed with *S. aureus* (MSSA) subspecies Aureus Rosenback (ATCC strain 25923) and methicillin-resistant *S. aureus* (MRSA) subspecies Aureus Rosenback (ATTC strain 33591). Sol–gel coated rods were tested against MSSA and sol–gel coated K-wires were tested against MRSA. Methicillin-sensitive and methicillin-resistant *S. aureus* subspecies were purchased from ATTC (Manassas, VA).