Sol-gel silica controlled release thin film for inhibition of methicillin resistant *staphylococcus aureus*

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Statement of Purpose: The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection has significantly increased [1]. 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. Traditionally, because of the universal resistance of MRSA to β-lactams, the glycopeptides vancomycin became the mainstay of treatment. However, in vitro susceptibility of MRSA to vancomycin is no longer universal. A 1997 report of clinical strains of *S. aureus* with intermediate (MIC, 8–16 μg/mL) susceptibility to vancomycin in Japan was soon followed by descriptions of several frankly vancomycin-resistant *S. aureus* isolates (MIC, 32 μg/mL) in the United States [2]. To combat MRSA with low dose of vancomycin, continuous effort is under way to increase its effectiveness. One of the promising techniques is to use combinational therapeutics. Farnesol is a natural sesquiterpenoid and quoram sensing molecule. In this role, farnesol prevents the transition from yeast to hyphal growth in *Candida albicans* and greatly compromises biofilm formation by this fungus. *In vitro* studies showed that it can greatly enhance the effectiveness of vancomycin when used together. However, the biggest obstacle is that the farnesol is water insoluble which compromises the bio-availability along with vancomycin at the site of infection when the treatment concept would be used *in vivo*.

The objective of this work is to design an efficient therapeutic strategy which can deliver both antibiotic and an adjuvant simultaneously in order to treat the infection with better efficacy. Herein we demonstrates that the vancomycin and farnesol can be adequately incorporated into thin sol-gel silica films coated on to the implant surface with the goal of having controlled delivery of both molecules in sufficient quantity, simultaneously. We have demonstrated the efficacy of the vancomycin and farnesol loaded thin film on the suppression of MSSA and MRSA bacteria on the implant surface. Sol-gel films with only vancomycin, only farnesol and without vancomycin or farnesol were used as controls.

Methods: First a silica sol was created by following method: 2.16 mL of distilled water is mixed with 0.25 mL 1N HCl (Fisher Scientific, Pittsburgh, PA) and 9.63 mL of 200 proof ethanol in 20 mL glass vial and stirred for 5 minutes until pH equilibrates within the solution. Next, 5 mL of the silica precursor, TEOS is added drop-wise while stirred at 300 RPM and then kept stirring for another 2 hours. Calculated amount of vancomycin solution (100mg/mL) was added to make 10 wt% and 20 wt% vancomycin loaded sol. For each of the vancomycin loading we used farnesol loading concentrations of 10 wt%, 20 wt% and 30 wt% respectively by adding the calculated amount of farnesol to the vancomycin containing sols. The notation used to describe the drug loading are the following: 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 dip coating method was used to create 5 layers of uniform thin film of each sol on implant surface. Each of the coated implant was challenged in-vitro with 10^6 CFU/mL of MSSA and 10^4 CFU/mL of MRSA for 24 hrs respectively.

Results: Figure 1(a) shows CFU/mL of the adherent MSSA bacteria on these rods. It shows that the 30 wt% of farnesol alone has no bactericidal effect and 10 wt% of vancomycin alone also has very little bactericidal effects. Whereas, when 10 wt% of vancomycin is combined with 20 wt% of farnesol it can reduce the bacterial growth to 6.00×10^3 CFU. Furthermore when 10 wt% of vancomycin is combined with 30 wt% of farnesol bacteria growth is completely suppressed resulting in no bacterial growth on the implant. Figure 1(b) depicts the colony forming units of adhered MRSA on implant surface. The ability of farnesol to act as an adjuvant is clearly evident. When 30 wt% of farnesol was added to 10 wt% vancomycin containing thin film (SGVF(10, 30)) we observed a 10 times greater reduction in bacterial growth compare to SGV(10). More remarkable effect of farnesol was observed when 30 wt% of farnesol was added to 20 wt% of vancomycin containing film (SGVF(20, 30)) which resulted in no measurable bacterial growth on implant surface.

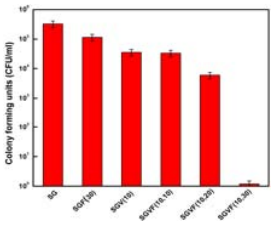

Figure 1(a)

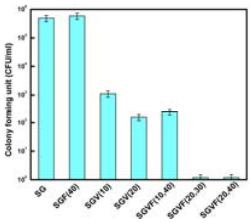

Figure 1(b)

Conclusions: This *in-vitro* study demonstrated that thin sol-gel films can be applied to titanium implants for controlled release of antibiotics with normally bio-unavailable adjuvants. The ability of farnesol to act as an adjuvant and increase the effectiveness of vancomycin against both MSSA and MRSA was demonstrated and forms the basis for proceeding to testify the concept *in vivo*.

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References: 1. Hawkey PM, Molecular epidemiology of clinically significant antibiotic resistance genes. Br. J. Pharmacol, **2008**, 153, S406-S413
2. Tverdek FP, Crank CW, Segreti J. Antibiotic therapy of methicillin resistant *Staphylococcus aureus* in critical care. Crit Care Clin **2008**; 24:249–260.