Synthesis of Zwitterionic Sol-Gel Films to Prevent Bacterial Adhesion

Haibo Qu[1], Raymundo Moya[2], Paul Ducheyne[1]
[1]Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104
[2]Department of Chemical Engineering, Rice University, Houston, TX, 77085

Statement of Purpose: Surface modification of orthopedic implants is one of the main focuses to inhibit biofilm formation. In addition to pursuing the release of antibiotics from the implant surface to impede bacterial attachment [1], recent data suggests that zwitterionic films can prevent biofouling, this is, the adhesion of bacteria to substances [2]. Zwitterions are molecules with a neutral total charge, but with opposite charges placed alongside within the molecule. Herein we propose to create zwitterionic sol-gel films to prevent bacterial attachment.

Two approaches to endow zwitterionic nature to thin sol-gel film were studied: incorporating two organically modified alkoxides with opposite charges [3] or incorporating one organically modified alkoxide with both positive and negative charges.

Methods: For the two-alkoxide study, (3-Aminopropyl)triethoxysilane (APTES) and carboxyethylsilanetriol (CES) were used to partially substitute tetraethyl orthosilicate (TEOS). APTES and CES were co-hydrolyzed with TEOS to form sols. Molar concentrations of APTES and CES were varied with respect to TEOS. Three different sols were prepared: 5% A-C, 10% A-C, and 20% A-C with molar ratios of APTES:CES:TEOS being 2.5:2.5:95, 5:5:90, and 10:10:80 respectively. In the single-alkoxide study, N-(trimethoxysilylpropyl) ethylenediaminetriacetate (TEM) was co-hydrolyzed with TEOS. The molar concentration of TEM was varied with respect to TEOS. Three conditions were chosen: 5% TEM, 10% TEM, 20% TEM with molar ratio of TEM:TEOS being 5:95, 10:90, and 20:80 respectively. A sol (control) was also prepared by hydrolyzing TEOS only.

Fresh prepared (<2 days) sols were used for depositing sol-gel films on titanium rods (3mm diameter, 30 mm length) utilizing a dip-coating method [4]. Sol-gel films consisting of four consecutively deposited layers were prepared.

The effect of zwitterionic sol-gel films was evaluated *in vitro*. Titanium alloy rods coated with different sol-gel films were challenged with methicillin resistant S*taphylococcus aureus* (MRSA). The sol-gel coated rods were incubated with 2 mL of bacteria solution (10^3 CFU/mL) for 24 hours. Attached bacteria were then removed from the surface by sonication in 2% Tween solution. The colony numbers of detached bacteria were counted using Petrifilm.

Results: Co-condensation of APTES, CES with TEOS has been reported to functionalize sol-gel materials with NH_3^+ and COO^- groups [3]. In this study, the charged groups in APTES and CES provided both positive and negative charges in the sol-gel films. The equal amount of opposite charges ensured a zwitterionic nature. When challenged with MRSA, the sol-gel films behaved differently in term of bacterial attachment (Fig.1). The CFU of the adherent bacteria was 10^6 for 10% A-C, 20% A-C and control sol-gel films, but was only $4.3x10^4$ CFU MRSA on 5% A-C film, which is a more than one magnitude reduction.

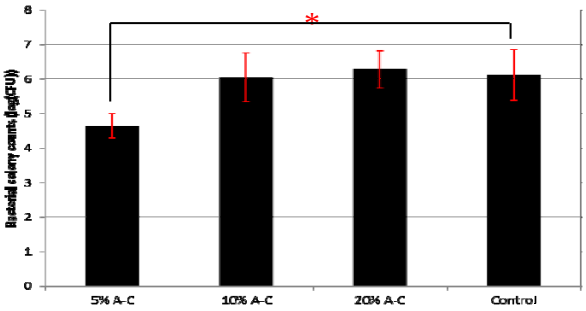

Figure 1 MRSA attachment on various sol-gel films at 24hrs; reported as the mean±SD (n=5).(*: $p<0.05$. Statistical analysis was performed using Student's t-test).

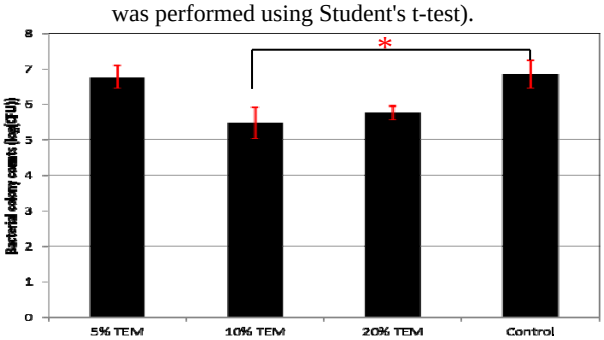

Figure 2 MRSA attachment on various sol-gel coatings after 24hrs incubation (n=5). (*: $p<0.05$, Student's t-test).

TEM has both positively and negatively charged motifs in close proximity. For zwitterionic sol-gel film containing TEM, the results of bacterial colony count showed that higher concentration of TEM reduced bacterial adhesion (Fig. 2). The control and 5% TEM sol-gel films had similar bacterial attachment (~$6.3x10^6$ CFU). The bacterial adhesion on 10% TEM sol-gel films is only $2.5x10^5$ CFU, more than a magnitude smaller than for the control sol-gel film.

Conclusions: Two different groups of zwitterionic sol-gels films have been prepared. *In vitro* bacterial culture results confirmed that zwitterionic sol-gel films can reduce bacterial adherence in comparison to unmodified sol-gel film.

Acknowledgments: We gratefully acknowledge support from the U.S. Army #W81XWH-10-2-0156.

References:

[1] Adams CS, *et al*, J. Orthop. Res., 2009,27,701-709. [2] Cheng G, *et al*, Biomater. 2007, 28, 4192-99. [3] Colilla M., *et al*, Chem. Mater. **2010,** *22*, 6459-66. [4] Shula R, *et al*, Biomater. 2007;28:1721-1729.