***[5]CORR* Insights®: Is Implant Coating With Tyrosol- and Antibiotic-loaded Hydrogel Effective in Reducing Cutibacterium (Propionibacterium) acnes Biofilm Formation? A Preliminary In Vitro Study**

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Infection remains a major complication associated with orthopaedic surgery and treatment of these infections can be extremely difficult. One of the reasons is that many of the common pathogens form bacterial biofilms which are difficult to diagnose and generally become highly tolerant to a broad range of antibiotics despite being found susceptible by routine clinical methods 1. Many *in vitro* studies have shown that to achieve even modest knockdowns in biofilm bacteria may require dosages which far exceed (high concentrations and long exposure times) those that can be achieved systemically. Local release of antibiotics at the site of the implants by materials such as bone cement, absorbable bone void fillers2 and hydrogel coatings3 offer the potential to achieve such dosages. In addition, concerns with antibiotic resistance has led to the search for novel agents with anti-biofilm efficacy.

The study by Tsikopoulos and colleagues assessed the ability of a hydrogel impregnated with tyrosol, as well as conventional antibiotics, coated on the surface of titanium (Ti) coupons to simulate an orthopaedic implant surface to prevent biofilms formed by *Cutibacterium (Propionibacterium) acnes*. Tyrosol is a quorum sensing (QS) cell signaling compound used by the fungal pathogen, *Candida albicans*, to stimulate filamentous growth from the yeast form when at high cell densities. Two studies (both from the same group) suggest tyrosol also has antimicrobial properties against both Gram positive and negative pathogens 4 5. Farnesol, another QS molecule produced by *C. albicans*, which has better established antibacterial properties, appears to have a dual role of regulating filamentous growth as well as protecting the fungi from colonization by bacteria and tyrosol may have a similar multifunction. Tsikopoulos and colleagues speculated that tyrosol might have potential for protecting shoulder implants from *Cutibacterium (Propionibacterium) acnes*, a low virulence organism which is increasingly being associated with periprosthetic shoulder infections. Although *C. acnes* strains are generally susceptible to antibiotics such as vancomycin when they form biofilms they become highly tolerant6; 7. The team further hypothesized that if tyrosol was incorporated into absorbable hydrogels and applied to the surface of orthopaedic implants it would have anti-biofilm activity localized at the surface. For comparison the team used rifampicin and vancomycin in hydrogels and as solutions. A 96 well plate assay in which Ti coupons coated with a commercially available “Defensive Antibacterial Coating” (DAC) hydrogel alone or loaded with antimicrobial agents was used for the study. The team found that tyrosol did have antibacterial activity against *C. acnes* but the minimum inhibitory and bactericidal concentration’s (MIC and MBC) were very high, 9 and 35 mg/mL respectively. However when tyrosol was in the hydrogel (9 mg/mL) there was no inhibition of planktonic growth. The MICs of rifampicin and vancomycin were 0.008 and 0.06 µg/mL Surprisingly, neither tyrosol (59.7%), vancomycin (2 and 5%) and rifampicin (1%) inhibited biofilm formation when loaded into the hydrogel, even though they were in such high concentrations. Tyrosol (59.7%) in solution caused a greater than 80% reduction in biofilm over controls, a value that the team used as a primary outcome measure with which to power the study. Taken together these results suggest that either the release kinetics did not allow the antimicrobial agents to build up to MIC or MBIC levels or that the hydrogel had an antagonistic effect. It was reported that DAC undergoes complete hydrolytic degradation within 72 hours, which was the incubation time, so that all the antimicrobial agent should have been released (although there was not mention of whether any coating was indeed remaining). These data contradict *in vitro* findings by a different group who showed that indeed the DAC hydrogels loaded with various antibiotics, including vancomycin and rifampicin, did significantly reduce biofilm3. Drago et al. 3 conducted release kinetic assays and used multiwall plates with disks, as well as a number of full size implant models and a range of Gram positive and negative PJI pathogens but not *C. acnes*. It is not clear, therefore, whether differences between these studies might be due to the challenge organism(s) or differences in the experimental procedures. It is also not clear whether tyrosol incorporated in DAC on the surface of implants would have potential to control biofilms formed from other common PJI pathogens.

**Where Do We Need To Go?**

The Tsikopoulos et al. and Drago et al. 3 study titles ask similar questions regarding the efficacy of implant coated antimicrobial loaded DAC hydrogels to control biofilms, but come to opposite conclusions. The most obvious difference is in the choice of challenge organisms, although it is difficult to see why *C. acnes* would be so much harder to kill than the other pathogens, when MICs, MBCs and MBEC values were similar or less than many of the strains tested. Another possibility could be differences in the assays themselves. System specific factors such as contact time scales, residence times, liquid volumes and surface areas within the system, inoculum density and growth phase, maturity of the biofilm and media type likely all come into play. In addition, there are no standardized internal controls (i.e. strain type and antibiotic) which can be used as comparative yard sticks, as there are for standard clinical MIC and MBC methods. Another question that is raised in an *in vitro* test is what constitutes a meaningful biofilm reduction? Here 80% is used as a threshold. This was taken from a previous study 8, although there was no rationale provided, probably because there are little or no data directly correlating *in vitro* efficacy with clinical outcome when it comes to anti-biofilm technologies, leaving researchers guessing at what might be relevant. As the failure of conventional antibiotic therapies to treat orthopaedic infections continue to be recognized there is a concomitant drive to develop new technologies and agents specifically targeted to biofilms. The recognition that treating biofilm associated infections presents very different challenges than treating acute planktonic infections by surgeons, researchers (both in biology and materials science and engineering) and commercial companies is a good thing – necessity is the mother of invention.

**How Do We Get There?**

The pathway to *in vitro* standard methods to directly compare the efficacy of different technologies for treating orthopaedic biofilm associated infections is not straight-forward. Unlike acute infections in which the bacteria tend to be homogeneous and rapidly growing, biofilm bacteria contain multiple phenotypes, including dormant and persister populations. There is large variation in the pathogens, types of surfaces (materials, textures, scale of surface features) and environments (body fluid components, shear stresses, mass transfer conditions) with which biofilms are associated. Thus, the properties of the biofilms are likely to be quite different and difficult to recapitulate in a few simple standard growth models. Similarly, there is much variation in anti-biofilm technologies and in many cases a test system has to be tailored to be compatible with a particular mode of action. Just as the recognition of biofilms has led to growth in the development of anti-biofilm technologies so will the recognition for the needs for developing systems which more closely capture the salient features of the infecting biofilm environment, whether these be physical, biological or both. One approach is to develop a framework for testing with a decision tree incorporating aspects of different types of orthopaedic biofilm associated infection as well as the mechanism of action of a given technology which would lead to one of a number of standard methods for bacterial adhesion, biofilm growth and testing 9.

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