37°C for 18 hours on an orbital shaker at 200 rpm. After formation of biofilm, the 96-pin lid was placed into a micro-titre tray containing dilutions of tetra-sodium EDTA(0, 5, 10, 15 and 20 mg/ml) and incubated at 37°C for 24 hours. At time intervals of 1, 3, 6 and 24 hours, triplicate pins were removed, for each concentration of tetra-sodium EDTA, washed by inversion in 5ml in phosphate buffered saline (PBS) and then sonicated in a sonicating water bath, for 15 minutes in 3ml PBS. Triplicate log dilutions of the biofilm suspension were then plated onto cysteine lactose electrolyte deficient (CLED) agar plates by automated spiral-plater, and incubated for 24 hours at 37°C, before performing automated colony counts. The MBEC was designated as, the lowest concentration at which growth was reduced by at least 99% when compared to the control. Results: Initial biofilm viable cell count levels averaged log 6 cfu/peg/ml. Of twelve Gram-positive bacteria tested, nine, including Staphylococcus aureus (3), methicillin resistant Staphylococcus aureus (3), Coagulase negative staphylococcus (CNS)(2), and Enterococcus sp. (1), had an MBEC of <5 mg/ml. The remaining three Gram-positive bacteria were CNS with MBEC of 20-40 mg/ml. In comparison, of the twelve Gram-negative bacteria tested, seven, including Klebisella sp. (3), Enterobacter cloacae (1), Proteus sp. (2), E. coli (1), had an MBEC of <5 mg/mL. The remaining five including Enterobacter cloacae (2), Stenotrophomonas maltophilia (1), Pseudomonas aeruginosa (1) and Acinetobacter baumanii (1), had MBEC values of 10-20mg/mL. Conclusion: The MBEC of all the organisms tested, when using the LMCD, were <40 mg/ml tetra-sodium EDTA.

318(C)

DIFFERENCES IN ADHESION AND BIOFILM FORMATION OF SEVERAL CLINICAL STRAINS OF *STAPHYLOCOCCUS EPIDERMIDIS*

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Staphylococcus epidermidis and similar coagulase-negative staphylococci (CoNS) are now well established as major nosocomial pathogens associated with infections of indwelling medical devices. The major virulence factor of these organisms is mainly due to their ability to adhere to devices and form a biofilm.however, it is not known if adherence and biofilm formation are closely linked phenotypes for clinical isolates. since different clinical isolates of *s. epidermidis* would be expected to exhibit different phenotypic behaviours it is further expected that strains of *s. epidermidis* might have different abilities to adhere to synthetic surfaces and subsequently produce biofilms. in this study the initial adherence and subsequent biofilm formation properties of 9 clinical isolates of *s. epidermidis* along with biofilm^{+} and biofilm$^{–}$ control strains were assayed. the adherence results were interpreted in terms of the physico-chemical interaction established between the cells and the adhesion substratum as assessed by contact angle measurements.as expected, the clinical isolates exhibited different abilities to adhere to hydrophilic glass and to form biofilms. moreover, the strains that produced the highest amounts of biofilms were not the ones able to adhere to the largest extent and vice-versa. for example, the biofilm^{-} control strain actually showed the highest level of initial adhesion capability and did not produce biofilm. these results indicate that high levels of initial adherence do not necessarily lead to strong biofilm formation and that some strains do not have a high initial adherence but can subsequently form a strong biofilm. these two aspects of the pathogenesis of medical device related-infection may need to be evaluated independently to ascertain the contribution of each to the virulence of cons causing device related infections.

319(A)

CONTROLLED ULTRASONIC ANTIBIOTIC RELEASE FROM HYDROGEL COATINGS FOR BIOFILM PREVENTION

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Medical devices, such as intravascular and urinary catheters, are routinely employed in healthcare settings since they provide clinicians with a useful means of administering nutrients, drawing blood samples and drug delivery. In spite of these advantages, local and systemic infections are frequently associated with their use. In fact, implanted devices often provide a highly suitable surface for bacterial adhesion and colonization resulting in the formation of complex, differentiated and highly structured communities known as biofilms. Once a biofilm infection is established, conventional treatments frequently fail as bacteria growing in biofilms are much more resistant to antibiotics than their planktonic counterparts. As a result, a variety of implantable drug-delivery systems have been developed. However, drug release tends to decay over time and these systems are prone to uncontrollable leaching. To overcome this problem the University of Washington Engineered Biomaterials (UWEB) group developed a novel drug-delivery polymer matrix consisting of a poly 2-hydroxyethyl methacrylate hydrogel coated with ordered methylene chains forming an ultrasound-responsive coating. This system is able to keep the drug inside the polymer in the absence of ultrasound but will show a significant drug release when low intensity ultrasound was applied. The drug embedded within the polymer is ciprofloxacin, an antibiotic well known for its action against Gram-negative bacteria. In collaboration with UWEB we have designed a flow cell system incorporating the hydrogel coatings which allows simultaneous real time digital or confocal time-lapse microscopy and the application of ultrasound. *Pseudomonas aeruginosa* biofilms were grown on the hydrogel surfaces in the flow cells with a bulk fluid flow of 1 ml/min. The development of cell clusters could clearly be resolved on the hydrogels using transmitted light and single GFP expressing cells could be observed using epi-fluorescence microscopy. Propidium iodide was used to assess the combined and single effect of antibiotic and ultrasound on viability. The uptake of PI correlated well with plate count reductions of cultures exposed to ciprofloxacin. Further testing will include the measurement of biofilm cell viability after exposure to ultrasound at different frequencies. The results of our studies may ultimately facilitate future development of medical devices sensitive to external impulses (ultrasound) capable of treating or preventing biofilm growth via “on demand” drug release.