Bacterial bioburden analysis of on-label povidone-iodine surgically prepared skin samples in porcine model support patient-originated infection hypothesis

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Introduction: Infection is the most dangerous and costly postoperative complication following implantation of biomaterials, significantly increasing morbidity rates and often exceeding $30,000 per case.[1] The quantitative bacteria threshold for infection on foreign bodies is significantly less than the generally accepted bioburden levels generating infection without any biomaterials.[2]
Skin hosts an extensive microbiome that varies significantly with anatomical location. Cultures from surgical site infections (SSIs), often involving the implantation of devices such as pacemakers, artificial joins, and screws, display growth of organisms specific to the endogenous flora of the region. For example, in SSIs of the shoulders and breasts, where pilosebaceous follicles are abundant, *Cutibacterium acnes* is overrepresented; surgical sites below the anus are more frequently infected with *Escherichia coli*; and finally, transdermal sites are commonly infected by *Staphylococcus epidermidis* and *Staphylococcus aureus*—common skin flora members.[2] This data suggests that most SSIs are derived from patients' own skin flora. More thorough elimination of the skin flora represents a promising target for future biomaterial engineering to reduce SSI rates.

The highest standard of aseptic care is skin preparation using an alcohol-based scrub, either povidone iodine (PI) or chlorhexidine gluconate (CHG). However, we hypothesize there is insufficient antiseptic diffusion and exposure time to eradicate biofilms dwelling in the deeper tissue regions, thus allowing bacterial survivors to contaminate surgical sites and contribute to infection. In this work, we quantified, isolated, and identified biofilm-forming bacterial isolates that survived in porcine skin tissue following an on-label preoperative skin preparation with PI.

Methods: We selected a porcine model based on its similarity to human skin. All surgical samples were collected following local institutional animal care and use committee (IACUC) approvals. Skin samples were harvested from surgery following an on-label use of PI surgical skin preparation kits which contained a 7.5% scrub and a 10% paint. Each sample was 4x4 cm and cut to the surface of the musculature to ensure adequate collection of bacteria residing deep in the follicle. Control samples were collected from four necropsies following a sterile water scrub for a baseline bioburden.
Each sample was transferred to a sterile container (within ~ 30 minutes) with Dey-Engley neutralizing broth. Each sample was homogenized in a blender for approximately 5 minutes. The mixture was then vortexed for 60 s, sonicated for 10 min, and vortexed again for 10 s. A 10-fold serial dilution was performed and aliquots plated on Columbia blood agar in duplicate. Bacteria were grown aerobically and anaerobically and incubated for 48 and 72 h, respectively. A representative count of colony forming units (CFUs) for the PI prepared skin and control skin were compared. We did the same procedure with a control mixture of Dey-Engley broth and plated to ensure there was no contamination.

Results: Aerobic and anaerobic bacteria CFUs following PI skin preparation were $1.20x10^3$ CFU/g of tissue and $7.36x10^2$ CFU/g, respectively. Control tissue had approximately $2.27x10^4$ CFU/g and $2.15x10^4$ CFU/g for aerobic and anaerobic, respectively. Compared to controls, data indicated that PI reduced CFU/g tissue by 1.28 $\log_{10}$ units and 1.47 $\log_{10}$ units, respectively.

Discussion: Data showed that the minimal 2 to 3 $\log_{10}$ reduction in bioburden required by the FDA was not achieved as the product claimed, indicating that bacteria dwell deep in the skin and persist in the presence of an alcohol-PI scrub.[3] Furthermore, the number of surviving organisms were sufficient to achieve a minimum infectious dose for implanted biomaterials, as the quantitative bacteria threshold for infection on foreign bodies is significantly less than the levels without any biomaterials.[4] Currently, preoperative skin preparation techniques vary widely and as these data show, may be insufficient to reduce biofilm burdens to below minimum infectious dose levels for biomaterials. Additional work is needed to determine if bioburden levels are similar in human skin. If a similar pattern is observed, it could indicate that biofilm-dwelling host flora are a major contributing factor to SSIs despite antibiotic strategies. In short, data indicate that if pig skin and human skin respond similarly, surgeons using the very best aseptic technique and procedures may introduce significant levels of bacteria into implant-related surgical sites and contribute to post-operative infection.

References: (1) Kurts et al. *J Arthroplasty* (2012). (2) Williams. *Targeting Biofilms in Translational Research, Device Development, and Industrial Sectors* (2019). (3) Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record, 80 Fed. Reg. 25166 (proposed May 1, 2015) (to be codified at 21 C.F.R. pt. 310). (4) Elek, Conen. *The virulence of Staphylococcus pyogenes for man; a study of the problems of wound infection* (1957).