Influence of *Staphylococcus epidermidis* Biofilm on Collagen Crimp Patterns of Soft Tissue Allograft

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Disclosures

• Dr. Robert A. Duerr - Research support received from Arthrex; Financial and Educational support received from Arthrex, CDC medical
• Dr. Christopher C. Kaeding - Speaker for Arthrex; Paid Consultant for Arthrex; Support received from Active Implants, Vericel, Smith & Nephew, Zimmer
• Dr. Robert A. Magnussen - Support received from Zimmer, Arthrex, Smith & Nephew; Editorial or Governing board of JAAOS, OJSM
• Dr. David C. Flanigan - Paid Consultant for Smith & Nephew, Depuy Mitek, Conmed, Zimmer, Vericel, Hyalex, Moximed; Stocked received from Nanochon; Support received from Smith & Nephew, Depuy Mitek, Vericel, Zimmer, Moximed, Arthrex, Anika Therapeutics, Aesculap
Introduction

• Anterior cruciate ligament (ACL) allograft reconstructions commonly utilize achilles and tibialis tendons\textsuperscript{1}
• Symptomatic infection causes 1\% of graft failures\textsuperscript{2}
• Subclinical infections are likely underestimated and may compromise graft strength\textsuperscript{2}
• \textit{S. epidermidis} has emerged as one of the most common organisms in infected joints\textsuperscript{3}
• \textit{S. epidermidis} bioburden decreases allograft tensile strength and elasticity\textsuperscript{4}
Introduction

• Collagen crimp patterns are essential for proper functioning of tendons and ligaments\textsuperscript{5}

• We hypothesized that increasing incubation time with \textit{S. epidermidis} will lengthen crimp pattern, resulting in increased tendon elasticity and reduced strength
Methods

- Tibialis anterior tendons were sectioned and incubated in $5 \times 10^5$ CFUs of *S. epidermidis* in BHI media
- BHI media alone served as a control
- Bacterial bioburden was assessed after 30 min, 3 hr, 6hr, and 24hr
- Second-harmonic generation imaging allowed for co-visualization of collagen and stained bacterial cells
- Surface area and collagen patterns were measured in ImageJ
Tibialis anterior bioburden increases with greater incubation time

Biofilms were removed from the tendon via sonification and quantified. Hexidium iodide stained both bacterial and host cells, which can be differentiated based on size.
Surface area of tendons increase following *S. epidermidis* incubation

Before and after incubation, tendon sections were imaged, and the percent increase was determined. Length, width, and surface area increased with increasing incubation time.
Collagen structure and bacterial distribution visualized using second-harmonic generation imaging

Representative maximum projection images are shown. Collagen autofluorescence is green, and hexidium iodide stained bacterial cells are red. Host and bacterial cells must be differentiated based on size.
Collagen crimp patterns were differentiated into fine (~15μm) and coarse (~30μm). Crimp length was measured between peak waveforms. Both crimp patterns lengthened with increasing incubation time.
To assess if collagen structural alterations occurred due to *S. epidermidis* biofilm or a secreted metabolite, tendons were incubated in spent media metabolized by *S. epidermidis* but with bacterial cells removed. Compared to incubation in fresh media, collagen crimp lengthened following incubation in spent media; however, the concentration of metabolites in the spent media is likely higher than what was achieved when biofilms were established. An increasing trend in crimp lengthening was not observed.
Conclusions

• Microscopic alterations in tendon structure following incubation with *S. epidermidis* occur before microscopic changes can be appreciated

• Collagen fine and coarse crimp patterns are compromised at a relatively low inoculation time

• *S. epidermidis* infection reduces allograft strength and increases elasticity which may be explained by lengthening collagen crimp patterns

• It is unclear whether a secreted metabolite aids in collagen lengthening

• These results highlight the need for antimicrobial precautions to maintain allograft strength and reduce infection-related graft failures
References


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