

# The Antibacterial Properties of the Infrapatellar Fat Pad Organ

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## INTRODUCTION:

Lipids from natural biological sources have been shown to inhibit a broad range of pathogens, including viruses, fungi, and both Gram-positive and Gram-negative bacteria. Their antimicrobial effects are often concentration-dependent, lipid-specific, and microorganism-specific, with minimal toxicity to host cells compared to many conventional antibiotics. While sebaceous gland lipids are well studied, lipids from other human tissues have received less attention. The infrapatellar fat pad (IPFP), routinely resected during total knee arthroplasty (TKA), is a rich source of fatty acids, approximately 43% of which is oleic acid. We sought to determine whether crude lipid extract from the IPFP possesses antibacterial activity against *Staphylococcus aureus* in vitro.

## METHODS:

**Sample Collection and Lipid Extraction:** With IRB approval, discarded IPFP tissue was collected from patients undergoing primary TKA. Samples were anonymized prior to processing and there were no exclusion criteria. Using a scalpel and forceps, fat was carefully separated from the connective tissue matrix. The freed fat globules were transferred to a mortar and mechanically morselized with a pestle for approximately 15 min until visible oil was released. The crude oil was pipetted off, taking care to avoid inclusion of any solid tissue.

**Cell Attachment Assay:** 24-well plates contained 0.2 mL IPFP oil (100–10% in 10% decrements), 0.2 mL *S. aureus* in tryptic soy broth (TSB), and 0.2 mL TSB. Controls included *S. aureus* in TSB, TSB only, and PBS only. A comparator group contained 45% oleic acid in identical volumes. Plates were incubated at 37 °C for 16 h, washed three times with PBS, fixed with methanol, and stained with crystal violet. Absorbance at 590 nm was measured using a 9-point grid, with triplicate readings averaged per well. Three independent trials were performed for each assay, and results were averaged for final analysis. Percent inhibition was calculated using the equation: Percent inhibition =  $[1 - (\text{OD treated} / \text{OD untreated})] \times 100$

**MIC and MBC Testing:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) testing was performed for concentrations of 100–10% in 2 mL glass tubes to prevent wall adhesion. Following 16 h incubation, 0.4 mL PBS was added (final volume 1 mL) before centrifugation to separate the oil layer. The top 0.5 mL was removed, and the remaining bacteria were resuspended in 0.5 mL PBS. Absorbance readings were taken as above. Samples then underwent a  $10^{-1}$  serial dilution and were plated on tryptic soy agar for CFU counts after overnight incubation. MIC was defined as  $\geq 90\%$  inhibition relative to the growth control; MBC was determined from the  $\log_{10}$  reduction in CFUs compared to the control.

## RESULTS:

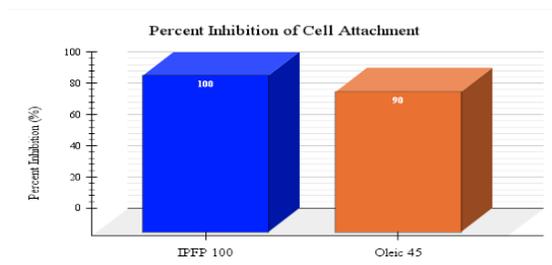
100% IPFP oil inhibited *S. aureus* attachment by ~100%, surpassing the inhibition observed with 45% pure oleic acid (~90%). Inhibition decreased with stepwise dilution ( $R^2 = 0.701$ ), with minimal inhibition below 40% IPFP concentration. MBC testing showed that 100% IPFP oil achieved the largest  $\log_{10}$  CFU reduction (~2.83). The 80% IPFP condition showed a higher apparent log reduction (~4.43), likely due to experimental variability rather than true increased bactericidal effect. Concentrations from 60–100% achieved  $\geq 2$ - $\log_{10}$  reductions, while  $\leq 50\%$  showed limited killing.

## DISCUSSION:

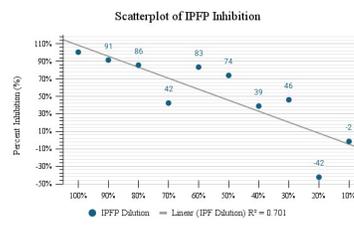
Crude IPFP oil demonstrated both potent anti-adhesive and bactericidal effects against *S. aureus*, outperforming oleic acid alone in preventing bacterial attachment. These results suggest that the combination of fatty acids and lipid mediators in crude IPFP oil may provide enhanced antimicrobial effects. Limitations include a small sample size, hydrophobicity of IPFP oil, potential measurement and handling errors inherent to manual processing, and the general limitations of in vitro assays, which may not fully replicate the in vivo environment. Further work should include compositional lipid analysis and testing against a broader range of pathogens.

## SIGNIFICANCE/CLINICAL RELEVANCE:

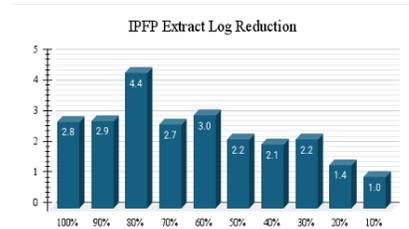
IPFP oil is a routinely discarded surgical byproduct with demonstrable antimicrobial activity against *S. aureus*. These findings suggest that preserving a greater portion of the IPFP during knee arthroplasty could provide an antibacterial benefit and potentially aid in infection prevention.



**Figure 1.** Percent inhibition of *S. aureus* cell attachment for 100% IPFP oil and 45% oleic acid. 100% IPFP oil showed the highest inhibition (~100%), exceeding 45% oleic acid (~89.8%).



**Figure 2.** Scatterplot of *S. aureus* cell attachment inhibition across 100–10% IPFP oil concentrations. A concentration-dependent decrease was observed ( $R^2 = 0.701$ ).



**Figure 3.** Log<sub>10</sub> CFU reduction (*S. aureus*) after exposure to IPFP oil concentrations. 100% IPFP oil achieved the greatest bactericidal effect (~2.83 log reduction); the 80% value was likely due to experimental variability.