First clinical experience of biofilm detection on bone in infected open fracture with optical coherence tomography

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INTRODUCTION: In trauma surgery, biofilms that form on surfaces of tissues and orthopaedic devices account for up to 65% of all healthcare bacterial infections, affecting tens of millions of people and leading to >3% annual fatality rate in the US [1]. First-line treatment for this serious surgical complication is typically antibiotic therapy but often results in revision surgery to replace infected hardware following thorough local debridement, all of which are associated with morbidity and significant financial costs. Despite aggressive treatment, a substantial number of cases result in amputation and even death. Notably, biofilms demonstrate 10-1000x higher multi-drug resistance to antibiotics which contributes significantly to treatment failure [2], and therefore new alternative anti-biofilm approaches are needed. Development of these methods are accelerated and enabled by clinically-usable microscopic-resolution imaging techniques for biofilm detection and treatment efficacy assessment [3]. Here we report the first clinical use of optical coherence tomography (OCT) for detection of Methicillin-resistant Staphylococcus aureus (MRSA) biofilms developed on bone surface after open tibia fracture surgery. We reveal optical signatures of these antibiotic-resistant biofilms through macrofluidic *in-vitro* experiments and translate the portable OCT technology to the operating room (OR) to test its potential in MRSA biofilm visualization.

METHODS: OCT system (Ganymede II, Thorlabs, Newton, NJ) with two imaging probes was used for data collection: a table-top OCTG9 model for *in-vitro* imaging (**Figure 1A**) and a hand-held OCTH-900 model for clinical application (**Figure 2B**). For *in-vitro* experimentation, a macrofluidic model of MRSA biofilm growth on orthopaedic hardware was developed, consisting of a 3D-printed macrofluidic device (**Figure 1B**) with 3 titanium and 3 stainless steel Asnis III Stryker washers, connected to feeding and draining syringe pumps (BS-8000, Braintree Scientific, Braintree, MA) through segments of #30 microtubing. Microtubes were attached to 27G needles on 1 mL syringes and plumbed into device inlets. Pumps continuously fed tryptic soy broth and drained each well of the device at 1µL/min flow rate for 72 hours to grow 18-20 µm thick biofilms on washer surfaces. Bioluminescent strain SAP231 [4] of patient-derived MRSA was imaged with IVIS Spectrum (PerkinElmer, Shelton, CT) to confirm stable biofilm development (**Figure 1C**). The texture of volumetric OCT images (**Figure 1E**) was analyzed in Matlab (version 2023a, Mathworks, Natick, MA) using histogram-based multi-parametric fitting approach (**Figure 1F**) to identify planktonic and biofilm MRSA optical scattering characteristics and validate against IVIS-based quantification.
Revealed biofilm optical signature was used to identify MRSA in orthopaedic trauma patients at Dartmouth-Hitchcock Medical Center (IRB approved 40-patient clinical trial #02001786). To date, informed consent was obtained from five participants with confirmed MRSA infection developed after tibia open fracture surgery. The hand-held OCT probe was wrapped into a sterile ultrasound cover and inserted into the wound (**Figure 2A**) for bone visual identification with a built-in white-light camera (**Figure 2C**) and following 3D bone scanning (**Figure 2D**) before irrigation under general anesthesia. Results of texture analysis of acquired OCT images were presented in the parametric space of histogram-fitting parameters (**Figure 2E**) with MRSA biofilms color-coded in a green-yellow depth range.

RESULTS: OCT modification with a hand-held probe (**Figure 2B**) was found to be a convenient tool for use in the operating room, with the 60 seconds average time spent for scanning of each location of interest. Mature biofilms were identified in each patient scanned to date with the thickness of 85 ± 33 µm (p=0.03) before the first irrigation and 57 ± 21 µm (p=0.05) before the second one. Besides biofilms, numerous bacteria colonies were detected on tibia surface (some labeled with yellow arrows in Fig. 2E), whose density reduced 23 ± 8 % (p=0.03) after the first irrigation.

DISCUSSION: Our preliminary results demonstrate that the developed methodology, enriched with other bacteria strains commonly found in orthopaedic trauma patients, may be used for monitoring bacteria colonies' spread, irrigation solutions' effectiveness and potentially - the antimicrobial therapy response. Ability to identify specific bacteria strains (e.g., gram positive or gram-negative) during patient visit may help to make informed decisions in the OR regarding therapeutic strategies to control infection. OCT scanning time (a minute of stable probe holding in the wound at each location) and data post processing for biofilm visualization on the imaging system monitor are currently two major limitations of the method. Their resolution is possible by 5-20x faster commercially-available OCT systems and by translation of data handling to multi-core graphics processing units, allowing near real-time biofilm visualization. Microfluidic and macrofluidic studies for identification of optical signatures of other bacterial strains (*E. coli*, *E. faecalis*, *P. aeruginosa*, etc.) are currently ongoing in our laboratory at Dartmouth and will be used to form an identification library for biofilm detection in infected trauma patients.

SIGNIFICANCE/CLINICAL RELEVANCE: Currently, detection and monitoring of MRSA as well as other bacteria strains in orthopaedic trauma wounds is problematic due to a lack of imaging modalities capable of handling this task *in-situ*. Our initial experience of employing commercially available OCT system for solving this problem and obtaining preliminary data from 5 patients shows its extremely high potential for clinical application.

REFERENCES: **[1]** Saeed et al., J. Orthop. Res. 37, 1007-17 (2019); **[2]** Laws et al., FEMS Microbiol. Rev. 43, 490–516 (2019); **[3]** Yun et al. J. Orthop. Trauma 30, S21–S26 (2016); **[4]** Plaut et al. PLoS ONE. 8(3), e59232 (2013).

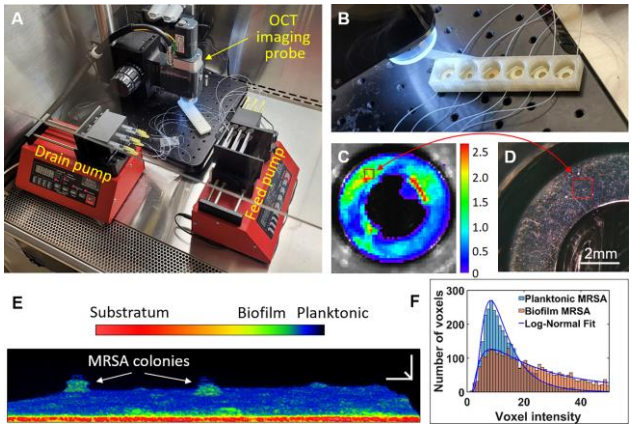

Fig. 1. Identification of planktonic and biofilm MRSA optical signatures: (**A**) Bioluminescent MRSA biofilm growth setup; (**B**) 3D-printed macrofluidic device; (**C**) Orthopaedic implant washer with grown biofilm imaged with IVIS and (**D**) Corresponding white-light image; (**E**) 3D rendering of OCT image taken from location indicated with red rectangles in (C) and (D). Scale is 50µm; (**F**) OCT image voxel intensity distributions - unique planktonic MRSA and biofilm MRSA optical signatures.

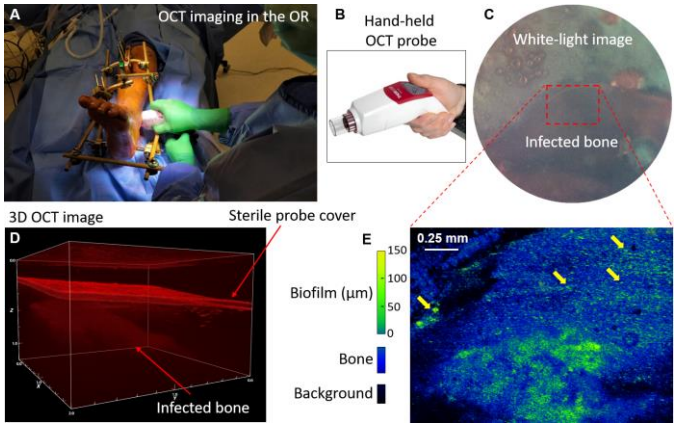

Fig. 2. Biofilm detection on tibia in a patient with confirmed MRSA infection: (**A**) The surgeon holds a hand-held OCT probe (**B**) wrapped in a sterile ultrasound probe cover in the wound for 1 minute to acquire a 3D OCT image shown in (**D**). (**C**) The OCT probe has a built-in camera for imaging area visualization in real-time. (**E**) Parametric image obtained by texture analysis of (D) using MRSA optical signature with bone colored in blue and biofilm in green-yellow. Yellow arrows indicate some of the MRSA colonies on the tibia.