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Demonstration of the bactericidal effects of the boron neutron capture reaction

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HIGHLIGHTS

- Bactericidal effects of boron neutron capture reaction demonstrated on *S. aureus*.
- Bacteria irradiated on titanium diboride disks in thermal neutron beam.
- 2.6×10^{12} n/cm^2 delivered resulted in a surviving fraction of 3.1×10^{-5}.
- Dose modeling estimated a dose of 14 kGy delivered in a 60 min irradiation.

ABSTRACT

This pilot study represents a paradigm shift, using BNCT for the treatment of bacterial overgrowth on surgically implanted medical devices. In this study, titanium diboride disks were inoculated with *S. aureus* and irradiated in a thermal neutron beam. After a delivery of 2.6×10^{12} n/cm^2 the surviving fraction of *S. aureus* on an irradiated disk was 3.1×10^{-5} when compared with non-irradiated controls. This pilot study demonstrates proof of principle of boron neutron capture therapy for infection control (BNCIC).

1. Introduction

Biological implants can serve as a nidus for infection. Direct inoculation can occur during the medical procedure or by hematogenous inoculation from various infections elsewhere in the body, even years after the implantation (Darouiche, 2004). Infections at the implant site, especially at the implant surface, can result in formation of a biofilm, a highly hydrated extracellular structure affixed to the surface of the implant. Infections that have formed a biofilm are difficult to treat with antibiotics. Accumulation of waste products and other substances in this structure cause microbes to either stop growing or force them into a low-growth mode, resulting in a microorganism that is up to 1000 times more resistant to growth-dependent antimicrobial agents than non-biofilm organisms (Jacqueline and Caillon, 2014). In many cases, treating such an infection requires costly remediation (such as additional surgery, removal of implants, and hospitalization) (Peel et al., 2013). In 2013 it was estimated that $9.8B was spent treating hospital acquired infections, with surgical site infections being the leading cause (Zimlichman et al., 2013).

Boron neutron capture therapy (BNCT) has been studied previously, mostly for cancer therapy (Barth et al., 2012). BNCT is a binary therapy that requires placement of ^{10}B in or near the tumor cell and a source of thermal neutrons at the treatment area. BNCT uses ^{10}B, a stable (non-radioactive) isotope of boron which has a 3837 b cross-section for capturing thermal neutrons. Since BNCT is a binary therapy, the deliverable dose to the target volume is dependent on the ^{10}B atom density and the thermal neutron flux in the tumor volume. The neutrons primarily interact with ^{10}B resulting in high LET radiation dose at the location of the boron atom, having minimal effect elsewhere. Thermal neutrons absorbed by ^{10}B produce ^{7}Li (1.02 MeV) + ^{4}He (1.78 MeV) with a branching ratio of 94% and ^{7}Li (0.84 MeV) + ^{4}He (1.47 MeV) + 0.48 MeV gamma ray with a branching ratio of 6%. The Li and He ions deposit kinetic energy within 5 μm and 10 μm of tissue, respectively (Coderre and Morris, 1999). While this distance corresponds to

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approximately five cell diameters of *S. aureus*, the dose rate decreases dramatically beyond 3 μm, imparting specificity to treatment of cells containing or directly in contact with ^{10}B.

In BNCT, targeted dose delivery relies on the delivery of ^{10}B specifically to the target tissues, and non-specific dose unrelated to boron content will, ideally, only slightly affect the delivered radiation dose. Previous studies have demonstrated that BNCT can be administered safely to treat certain pathologies and localizations, and comparison of BNCT with conventional radiotherapy in a randomized study is warranted (Kankaanranta et al., 2012, 2011). While the boron neutron capture reaction has been investigated to treat infectious agents, to our knowledge, BNCT has not been studied or employed for infection control at medical implant sites (Halfon et al., 2009). Such use would overcome arguably the most significant limitation to BNCT, the delivery of B to the treatment area. Incorporation of boron into the surface of implants would place large concentrations of boron precisely at the nidus of infection. In this work, we characterized the ability of BNCT to control bacterial colonies on a titanium diboride (TiB_2) surface, a process we describe as boron neutron capture for infection control (BNCIC).

2. Materials and methods

2.1. Facilities

We used the thermal neutron beamline for neutron capture therapy cell and small-animal radiobiology studies at the University of Missouri Research Reactor (MURR) center (Kueffer et al., 2013). The irradiation position is described in detail elsewhere (J. Brockman et al., 2009). The beamline uses single-crystal silicon for neutron filtering and a bismuth section for reduction of incident gamma radiation. The neutron spectrum at this facility has been measured using an unfolding technique that combines an *a priori* flux spectrum from MCNP5 with activation foil measurements using a weighted least squares approach. The current thermal neutron flux is benchmarked to the previous measurements using Cu/Au flux wires acquired from Idaho National Laboratory. The thermal neutron flux at the irradiation position was measured to be 7.6×10^8 neutrons cm^{-2} s^{-1} (Brockman et al., 2013). The background gamma dose in the facility was previously measured to be 2.7 cGy/min. The background physical neutron dose is approximately 2.5 cGy/min (J.D. Brockman et al., 2009). The beamline has a well-thermalized neutron spectrum with sufficient thermal neutron flux for a variety of BNCT studies (Kueffer et al., 2013).

2.2. Dose modeling

In silico dose modeling of the experiment was performed prior to *in vitro* studies. The physical radiation dose was calculated using the Monte Carlo N-Particle (MCNPX 2.7) radiation transport code. The MCNPX model used the unfolded neutron spectrum measured at the thermal neutron beam facility at the MURR center (Brockman et al., 2013). The model geometry consisted of a 1 cm diameter TiB_2 disk positioned in the neutron beam. The TiB_2 target was covered with a 30 μm thick layer of glycerin that was sub-divided into 2 μm thicknesses, about the size of two *S. aureus* cells. Glycerin was included in the MCNPX modeling to accurately simulate the physical experiment, where a layer of glycerin was needed to keep the bacteria hydrated on the surface. The model was normalized using a constant derived from the measured neutron flux in the irradiation facility using a Cu/Au flux wire. The neutron flux in the MCNPX model was monitored above the TiB_2 material using an F4 tally. The calculated physical dose (Gy) from the ^7Li nuclei and the alpha particle produced from the ^{10}B neutron capture reaction in the glycerin layer above the TiB_2 surface was determined using the neutron capture ion algorithm (NCIA) option in MCNPX (Pelowitz, 2005; Zheng et al., 2016; Van Der Ende et al., 2016). The 7th entry on the phys:n card was set to 5 to transport the alpha particles and ^7Li nuclei while preserving angular momentum. The physical radiation dose was calculated as the sum of the kinetic energy deposited by the α particle and the ^7Li ion in the glycerin volume above the TiB_2 surface.

2.3. Microbiology study

The *Staphylococcus aureus* (*S. aureus*, ATCC 29,213) bacterial culture was maintained on Trypticase Soy Agar (TSA) with 5% Sheep Blood (Thermo Fisher Scientific) plates at 37 °C. Microbiology studies involved 18 disks inoculated with *S. aureus* in a thin layer of glycerin. The glycerin maintained the viability of the bacteria by retaining moisture at the surface of the TiB_2 disk. The antimicrobial activity of TiB_2 with irradiation was determined by a colony-forming unit (CFU) counting assay. Prior to inoculating onto the TiB_2 disks, a 20 μL aliquot of 2×10^6 CFU/mL bacterial suspension in the minimal growth medium (100-fold diluted Mueller-Hinton broth) was mixed with 5 μL glycerol (final concentration = 20%). The mixture was inoculated on the surface of the TiB_2 or Ti (control) disks. Each treatment or control group consisted of three disks mounted inside of a sterile petri dish using epoxy resin.

The inoculated disks were treated in the thermal neutron beam at MURR by mounting each petri dish into an irradiation position (Fig. 1). Control groups consisted of one group of TiB_2 disks which were not irradiated and one group of non-boronated Ti metal disks irradiated for 60 min to serve as a neutron beam exposure only control. Two separate experiments were performed. In the first experiment, treatment groups were irradiated to 15 min, 30 min, 45 min, and 60 min of neutron exposure. A second experiment was conducted with irradiation times of 15 min, 30 min, 37 min, 45 min, 53 min, and 60 min to better evaluate the sudden decrease in survival at high doses. The thermal flux in each experiment was measured using Cu/Au activation wires from Idaho National Laboratory. The measured flux from each experiment was 7.2×10^8 n/cm^2/s and 7.6×10^8 n/cm^2/s. After irradiation, bacteria on each disk was collected by a sterile swab and suspended in 1 mL of physiological saline, ten-fold serially diluted, and 10 μL of each dilution was plated on LB agar plates and cultured. The numbers of bacterial colonies were enumerated after 16 h of incubation at 37 °C(Quinn et al., 1993). The antimicrobial activity was expressed as surviving fraction using the following formula: (CFU on TiB_2 with irradiation) / (CFU on TiB2 without irradiation). No Institutional Review Board, Animal Care and Use Committee, or other ethics review board approval was required for these studies.

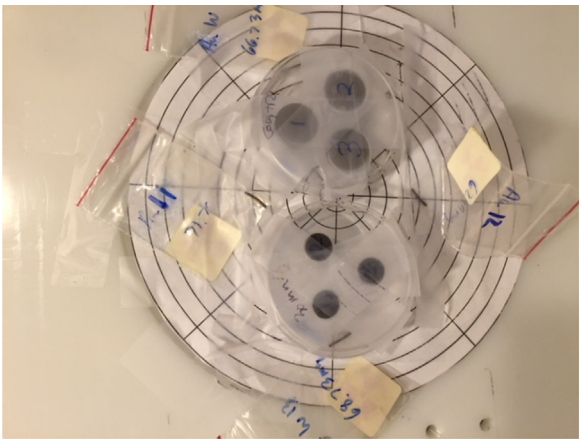

Fig. 1. Sample irradiation procedure. In this example, Ti (dish labeled control) and TiB2 disks are being irradiated. Au/Cu wires are positioned in the bags to measure flux.