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ABSTRACT

We develop a pH sensor based on use of X-ray Excited Luminescence Chemical Imaging (XELCI) to non-invasively detect and image changes in pH of a surface with high spatial and pH resolution while minimizing tissue scattering effects. Diagnosis and treatment of infections associated with implanted medical devices is a challenge, as clinical symptoms of implant associated infection are often delayed and can sometimes be completely absent till infection reaches a later stage. Early diagnosis of implant associated infection and non-invasive continuous monitoring of infection to evaluate eradication and success of treatment has not been established yet. Treatment of implant infection without implant removal is possible if infection can be diagnosed at its onset. Our pH sensor can be attached to the implant surface to non-invasively diagnose and monitor implant associated infection in situ. Bacteria and inflammatory responses cause a pH drop in the area and pH shifts to acidic from in situ pH (~ 7.3). Our pH sensor consists of a layered structure of a pH sensitive polymer film over radioluminescent particles. The pH sensor is characterized for reversibility, sensitivity and resolution. XELCI provides high spatial resolution images mainly limited by X-ray beam width with minimum increase from X-ray scattering in the tissue. It allows point by point mapping of the surface with minimum background. We studied pH changes during the formation of biofilm on the pH sensitive sensor film. In summary, our sensor provides a novel approach to non-invasively image surface pH to diagnose implant infection and assess treatment.

SENSOR DESIGN

Sensor Concept

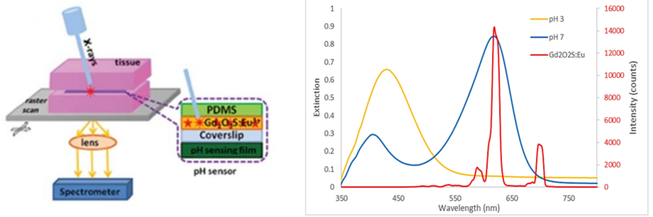
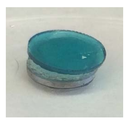


Figure 1. A. Sensor design and schematic drawing of the XELCI setup. **B.** Luminescent spectrum of scintillators (Gd_2O_2S:Eu) (red line, right y -axis) and the extinction spectra of BCG doped PEG films in pH buffer 3.0 (yellow, left y-axis) and buffer 7.0 (blue, left y-axis).

Sensor Fabrication

Sensor films are synthesized from biocompatible Polyethylene glycol (PEG) hydrogel in which a pH dye (Bromocresol green) has been incorporated. There is a layer of scintillating particles (Gd_2O_2S:Eu) enclosed in a layer of PDMS (Polydimethylsiloxane) below the pH sensing film. Both the pH sensing film and scintillating particles can be attached either directly to the implant plate or to a Titanium disc that could fit in the implant plate.



PEG based pH Sensor

Sensor Characterization

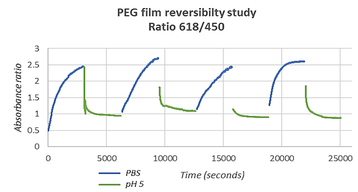


Figure 2. PEG film reversibility study. A spectrum was recorded in water for 50 minutes (not shown) then added PBS and recorded spectra every 1 second for a total of 50 minutes (3000 s). The film was cycled between PBS and pH 5 buffer and spectra recorded for 50 minutes for each solvent. The film cycles between green (in pH 5 buffer) and blue (in PBS, pH 7.4).

Cell Culture Study

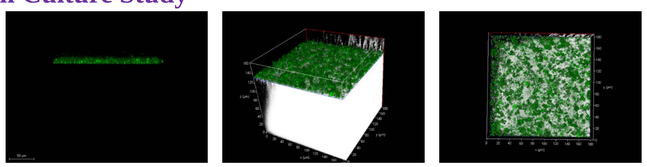


Figure 3. Confocal scanning laser microscopy images (side, volume and top views) of biofilm grown on PEG sensor films (containing 5% PETA) after 24 hour incubation with gfp tagged bacterial culture (staphylococcus aureus). Thickness of the biofilm from the image was 14.7 um (49 slices) with a total coverage of 54%.

CALIBRATION CURVES

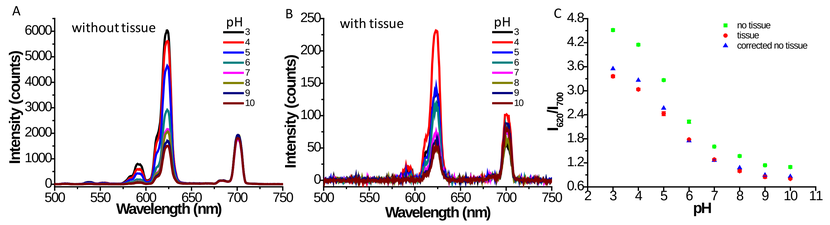


Figure 4. A. Radioluminescence spectra of the pH sensor film in response to different standard buffers range from pH 3.0 to pH 10.0; **B.** Radioluminescence spectra of the pH sensor film in response to different standard buffers after passing through 6 mm porcine tissue; **C.** pH calibration curves of the pH sensor films without tissue (green squares), with passing through 6 mm tissue (red dots) and the no tissue calibration curve corrected by referring to effect of tissue on the radioluminescence spectrum of the X-ray particles (blue triangles).

SPATIAL RESOLUTION

Working principle of XELCI

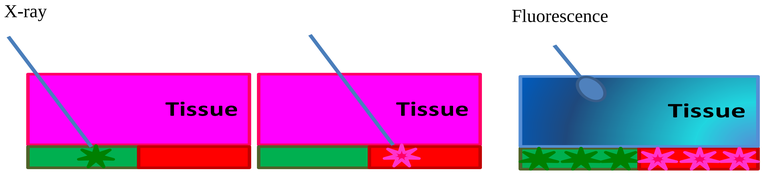


Figure 5. Schematic drawing of the working principle of XELCI as compared to fluorescence tomography. **XELCI:** the sample is irradiated point-by-point with a collimated X-ray beam, which stays collimated after passing through tissue.
Fluorescence Tomography: the illumination beam is broadened after diffusing through tissue.

Target mapping

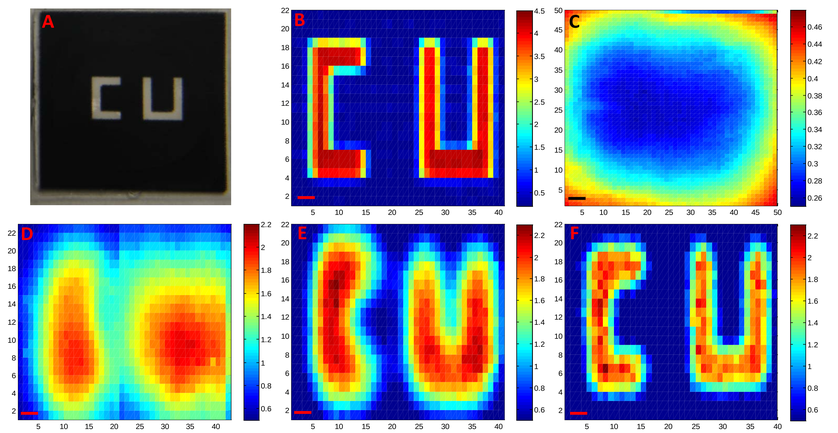


Figure 6. A. Photograph of the "CU" letters (target); **B.** Target mapping, irradiated with a 1.5 mm X-ray beam; **C.** Target mapped through porcine tissue with white light source as the illumination source; **D.** Target mapped through porcine tissue, irradiated with a 3 mm X-ray beam; **E.** Target mapped through porcine tissue, irradiated with a 1.5 mm X-ray beam; **F.** Target mapped through porcine tissue, irradiated with a 1mm X-ray beam. The x and y axis represents each increment (step size=300 μm). The color bars on the right are the ratio of the peak intensity at 620 nm over that at 700 nm. The scale bars represent 1 mm.

SENSOR APPLICATIONS

Antibiotic effect assessment

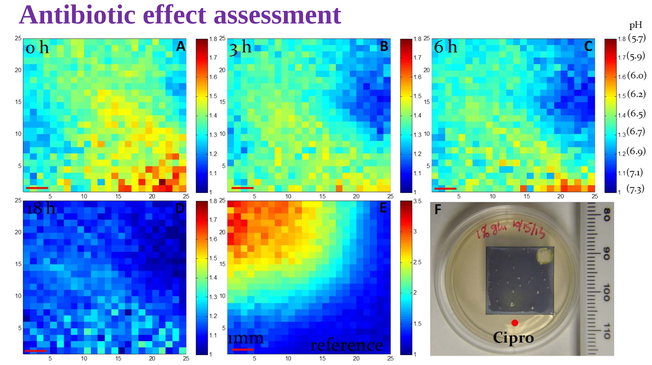


Figure 7. A. Before adding ciprofloxacin (cipro); **B.** 3 h after adding cipro; **C.** 6 h after adding cipro; **D.** 18 h after; E. Reference region; **F.** Photography of the plate

Animal Study

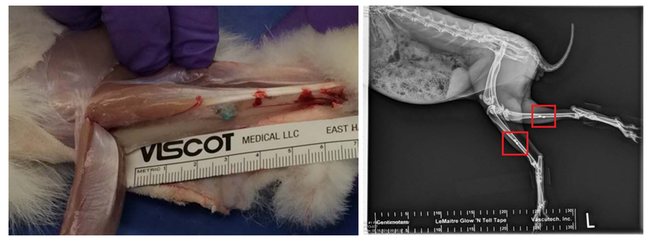


Figure 8. A. Image of implanted PEG based pH sensor (blue) in post euthanasia rabbit still pH responsive. Although there was a dye leaching of about 45% from PEG films (after 80 days in vivo) as determined by absorbance measurements. **B**. X-ray image of control rabbit post euthanasia. The pH sensor and spectral reference implanted discs are visible.

- *Wang et al. Adv Healthc Mater. 2015 Apr 22;4(6):903-10*

CONCLUSION

In conclusion, we constructed pH sensor films based on the inner filter effect between the BCG doped PEG film and radioluminescence of the X-ray particles. The introduction of the X-ray as the excitation source provides high tissue penetration depth with minimum beam broadening and no autofluorescence background. By collimating the X-ray beam and scanning the sample point by point, an image mapping of the "CU" letter was obtained through thick tissue with high spatial resolution. Furthermore, the pH changes during inhibition of antibiotic on the bacterial growth were studied by mapping the pH through thick tissue. An vivo study was conducted to evaluate sensor stability and potential toxicity. These proof-of-principle experiments demonstrate the great potential of our technique in detecting bacterial infection and evaluating treatment on IMDs in biomedical field *in vivo*.

FUTURE WORK

We will optimize the sensor material and characterize for reversibility, sensitivity, pH resolution and specificity. We will also attach our pH sensing films to a mimic implant surface and apply them for *in vivo* pH monitoring in animal model and *ex vivo* cadaver study.

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