Continuous Monitoring of Tissue Regrowth Using Optical Biosensors

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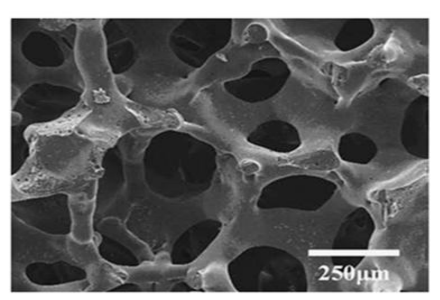
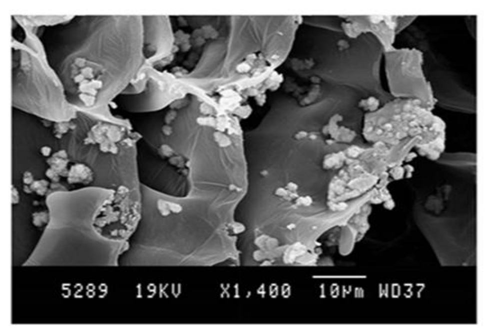
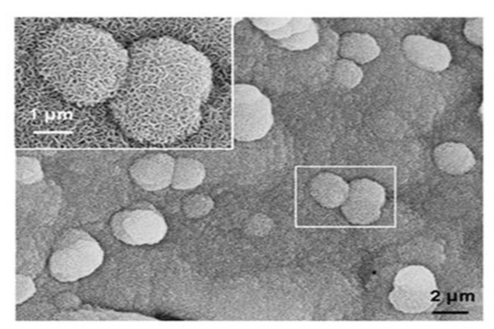
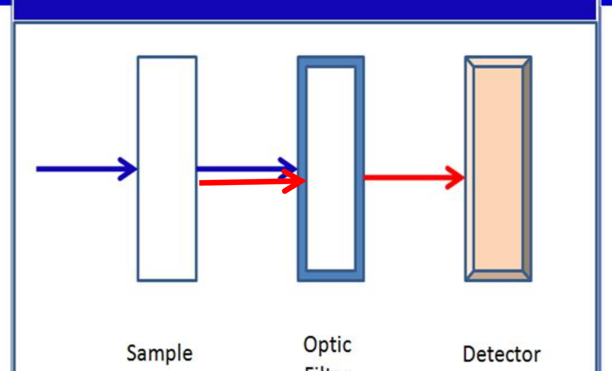
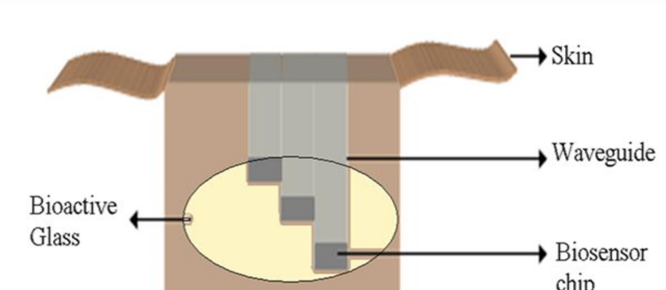
Introduction:

The engineered regeneration of bone is a significant challenge being undertaken to treat conditions such as traumatic casualties, bone cancer, osteoporosis, etc. Advances in regrowth of hard tissue may potentially lead to significantly improved lives for millions of people. Recent work has focused on the use of bioactive glasses as potential materials in the fabrication of scaffolds for hard tissue regeneration. Bone regrowth requires maintenance of optimal levels of oxygen, glucose, phosphate, calcium, and pH. Current work at Missouri University of Science and Technology's Center for Bone and Tissue Repair and Restoration focuses on developing optical biosensors to monitor: conversion of bioactive glass to hydroxyapatite, ease of nutrient transport through the scaffold, diffusion of bioconversion byproducts from the wound site, and general health of the growing cells. Feedback from these sensors aids in material design, allowing researchers to understand how desired levels of analyte molecules are maintained in the complex process of tissue ingrowth.

Bioactive glass scaffold: Q. Fu et al./ Acta Biomaterialia 4 (2008) 1854 – 1864	Hydroxyapatite formed on bioactive glass: S G. Kazarian et al./ Biomaterials 25 (2004) 3931 – 3938	Cauliflower morphology of hydroxyapatite on bioactive glass scaffold surface, inset – needle like hydroxyapatite crystals: K. Rezwan et al./ Biomaterials 27 (2006) 3413 - 3431	Osteoblasts attached onto the bioactive glass mesh: H. Kim et al./ Advanced Functional Materials Aug (2006)
			

Proposed work:

A bioactive glass (bioactive glass 45S5 – 45% SiO_2, 24.5% Na_2O, 24.5 CaO, 6% P_2O_5) scaffold is placed in the wound site (replacing the damaged bone's missing portion); with the biosensors embedded in the bioactive glass scaffold, so as to record the changes accompanying the tissue healing process. In the first part of this work, we will monitor the pH changes which occur *in vivo*. When bioactive glass is implanted in the body; the surrounding physiological neutral aqueous environment and high alkaline content of the glass, cause the bioactive glass scaffold to degrade via a rapid ion exchange. In the laboratory, the pH during bioconversion spikes to almost pH=12, a value unsustainable for healthy wound repair. However, this large pH increase is not noticed in vivo, indicating that the body is capable of mitigating these events. Integration of pH microsensors at the wound site will allow us to track bioactive glass conversion and transport of alkaline byproducts. Effect of this alkaline byproduct transport and simultaneous $Ca_3(PO4)_2$ layer deposition on the degrading porous gel-like glass - on the wound site oxygen distribution also needs to be monitored. As such, the second phase of this work will center on the tracking of oxygen in the scaffold microenvironment as the former is vital for tissue survival and growth.

Bioglass coated on artificial implants to make them biocompatible	Bioglass patch at the wound site	Fluorescent Monitoring of Analyte	Biosensor Implantation
			

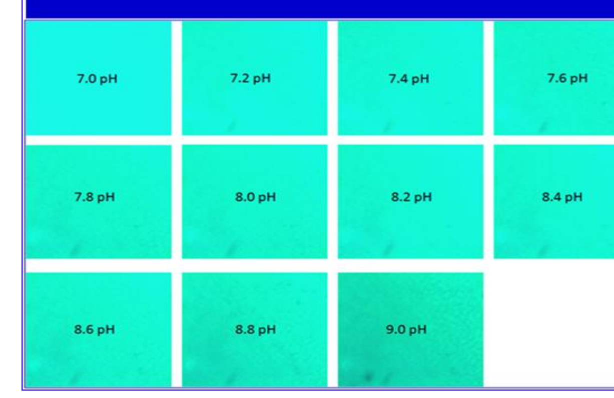
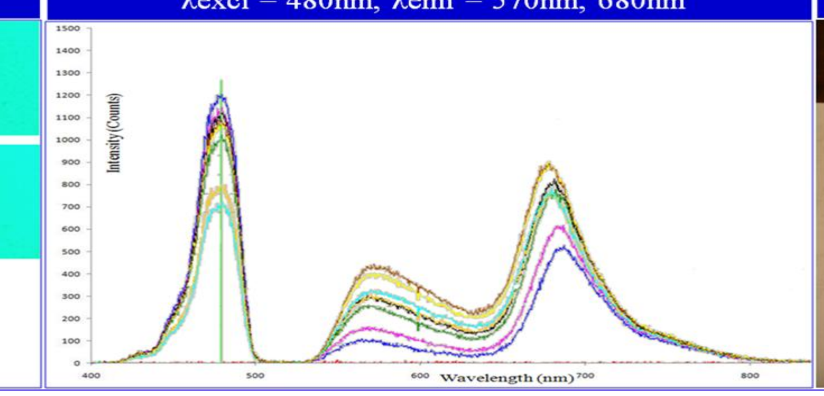
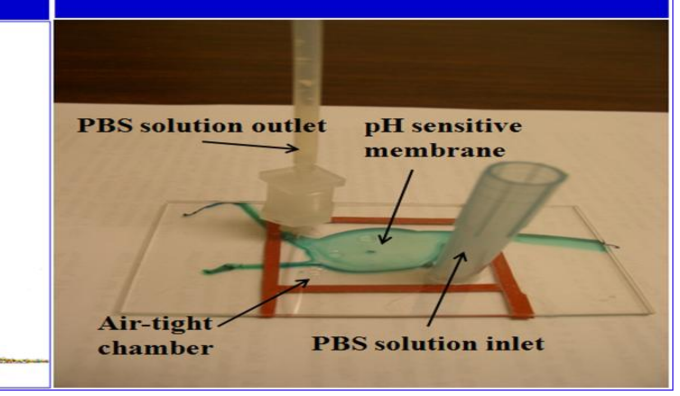
Current in-vitro Experimental:

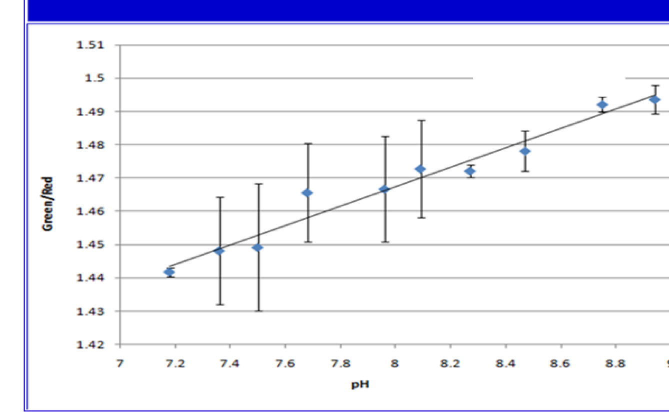
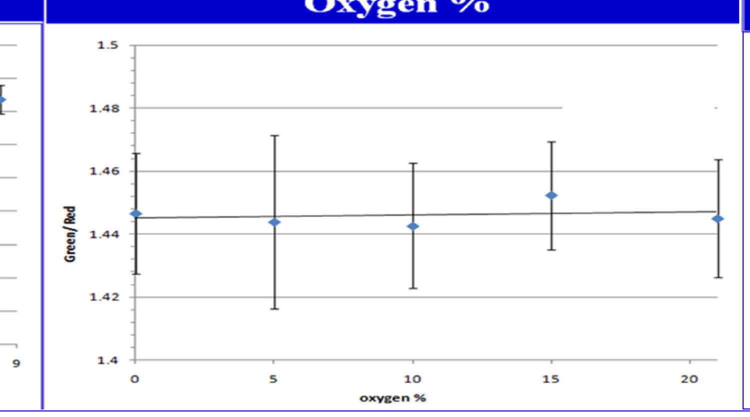
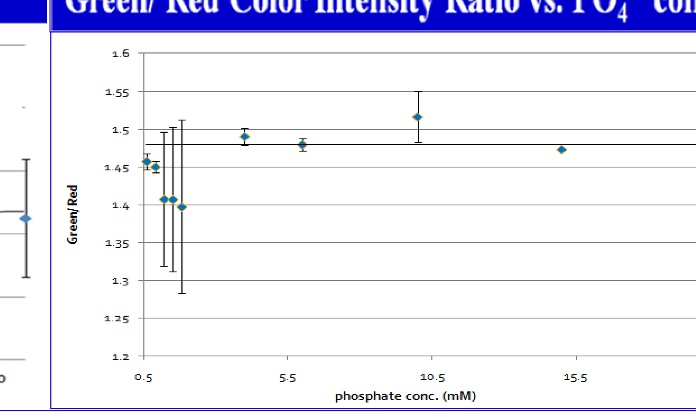
Our work currently focuses on development of pH and oxygen fluorescent biosensor elements. A CCD camera and processing software is used to colorimetrically quantify levels of these two at the microscale. Image processing is done in either the RGB (red, green, blue – native to the camera) or the HSI (hue, saturation, intensity) color space, giving detailed information about analyte concentration throughout the scaffolds and allowing for real time, *in situ* monitoring of cellular ingrowth.

For pH detection, a sensitive ionophore is immobilized in a flexible polymer membrane and cast to form a 2 - dimensional film. Fluorimetric analysis of this film allows us to generate a color-coded picture of the pH gradient that exists in the degrading bioactive glass scaffold. Also, current work on oxygen quantification employs the Pt(II) porphyrin complex immobilized in a polymer membrane, with porphyrin phosphorescence quenching with increase in surrounding oxygen levels, and this difference leads to an image of oxygen gradients which develop in the matrix.

1) pH Biosensor Element

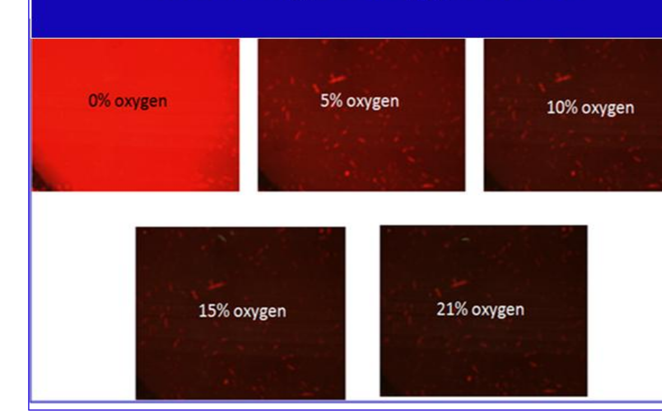
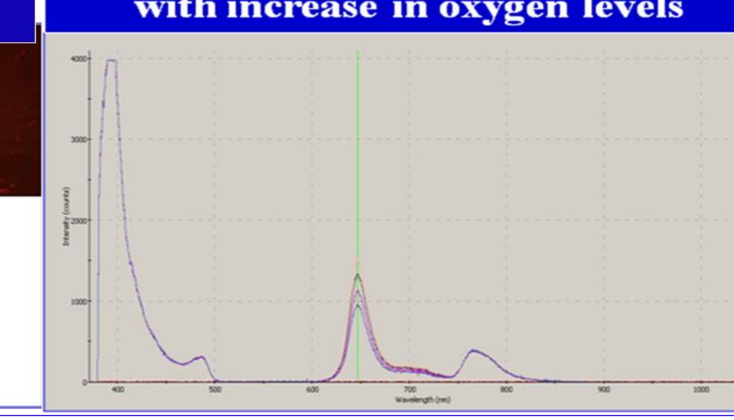
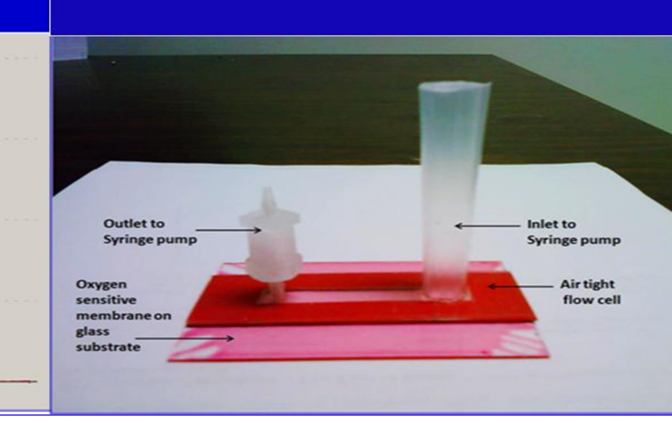
- A ratiometric fluorophore, 9-(Diethylamino)-5-[(2-octyldecyl)imino]benzo[a]phenoxazine (ETH5350), is entrapped in a poly(vinyl chloride) matrix, with bis(2-ethylhexyl) sebacate to promote membrane plasticity and a lipophilic salt, tetrakis (4-chlorophenly) borate, for aiding proton selectivity
- The fluorophore is uniquely suited for colorimetric analysis with off-the-shelf CCD camera equipment as excitation occurs in the blue region and emission, dependent on pH, has peaks in the green and red regions
- The ratio of the red and green intensities therefore may be used to quantify pH, making the technique relatively insensitive to variations in excitation strength
- Green: Red intensity ratio increases with rise in pH
- Membrane 'Response Time', 'Luminance Intensity Ratio vs. Physiological pH', PO_4^{3-} ion and oxygen interference with the fluorophore are documented
- Thus, at any time the pH at the wound site can be referred to from the documented data, for any luminance intensity ratio recorded

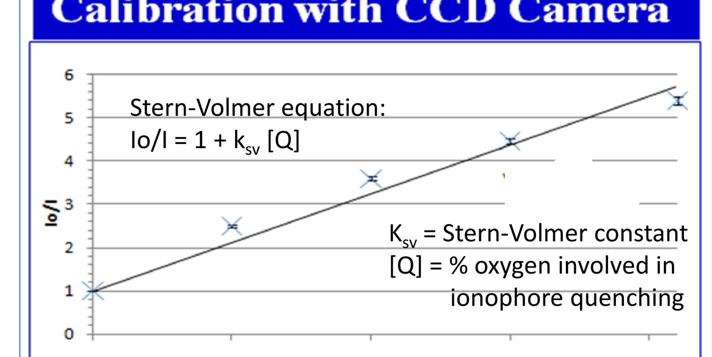
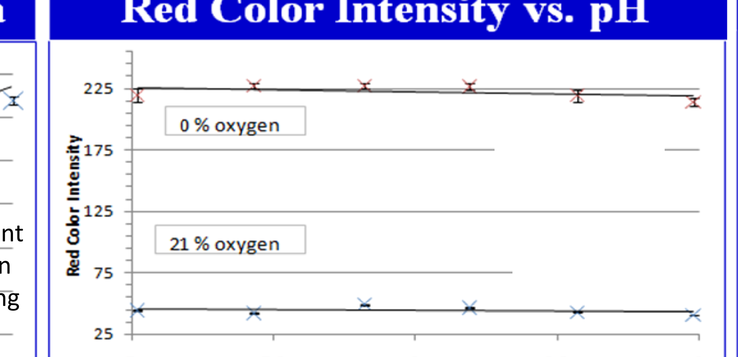
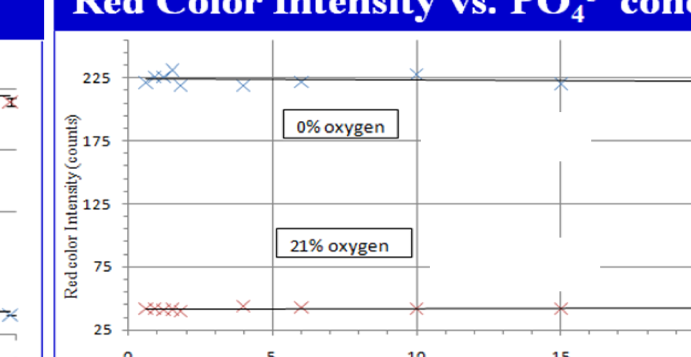
Color Gradation with pH	Intensity vs. Wavelength λexci = 480nm, λemi = 570nm, 680nm	pH Sensor Element in a Continuous Flow-Cell
		

Membrane Calibration with Camera: Green/ Red color intensity vs. pH	Oxygen Interference: Green/ Red Color Intensity ratio vs. Oxygen %	Phosphate Ion Interference: Green/ Red Color Intensity Ratio vs. PO_4^{3-} conc.
		

2) Oxygen Biosensor Element

- Pt(II) meso-tetra(pentafluorophenyl)porphine complex immobilized in a poly(dimethylsiloxane) membrane (Sylgard 184) is tested for its phosphorescence changes when subjected to (0 - 21)% oxygen levels
- A CCD camera and processing software is used to colorimetrically quantify oxygen at microscale. Images of differently oxygen saturated solutions are compared to calibrate our image processing algorithm
- Repeatability of the algorithm is checked and confirmed

Red color intensity gradient with change in oxygen levels	Drop in porphyrin phosphorescence with increase in oxygen levels	Oxygen sensor setup
		

Oxygen Sensitive Membrane Calibration with CCD Camera	pH Interference with porphyrin: Red Color Intensity vs. pH	Phosphate Ion Interference: Red Color Intensity vs. PO_4^{3-} conc.
		

Future Objectives:

The near - term goal is to develop a sensor platform to monitor bioactive conversion process in simulated physiological saline solution environment. Future work will focus on the implantation of sensor membranes at the site of bone injury to study its *in vivo* operation. Levels of pH and oxygen at the wound sites will be monitored and correlated with optical images of tissue regrowth.

Acknowledgements:
Center for Bone and Tissue Repair and Restoration, Department of Defense (US Army grant number: W81XWH-08-1-0765)