Continuous Monitoring of Tissue Regrowth Using Optical Biosensors

Prajakta Bhagwat[1], David B. Henthorn[1],
Chang-Soo Kim[2],

[1]Department of Chemical and Biological Engineering, Missouri University of Science and Technology, Rolla, MO [2]Electrical & Computer Engineering, Missouri University of Science and Technology, Rolla, MO

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.

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. Specifically, the pH sensitive membrane employs a ratiometric fluorophore, 9-(Diethylamino)-5-[(2-octyldecyl)imino]benzo[a]phenoxazine (ETH5350), 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.

Current work on oxygen quantification employs the Pt(II) meso-tetra(pentafluorophenyl)porphine complex immobilized in a poly(dimethylsiloxane) membrane. Porphyrin fluorescence quenches with increases in surrounding oxygen levels, and this difference leads to an image of oxygen gradients which develop in the matrix.

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.

Keywords: Hard tissue regeneration, Bone regrowth, Bioactive glass scaffolds, Sensitive ionophore, Colorimetric analysis