PhD Thesis: Technical Summary

In Vivo Steroid Sensing Using Corona Phase Molecular Recognition: Design, Synthesis, and Applications

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Steroid hormones dictate a number of underlying biochemical processes controlling human physiology and disease. Changes in steroid hormone concentration and activity are often either indicators or direct causes of disease. In the clinic, steroids are measured as biomarkers of health and have been studied in relation to various diseases, including various cancers, endocrine disorders, and mental illnesses. However, measurements of these important signaling molecules are commonly restricted to the analysis of blood samples using chromatography or immunoassays, which lack temporal resolution and are labor-intensive. Given the dynamic behavior of these steroids, we argue in this thesis that their diagnostic value is highly constrained because of limitations of existing measurement technology. Hence, this thesis explores and develops the engineering tools for the design, synthesis, and application of a continuous biosensor capable of measuring *in vivo* steroid hormones in the human body.

A pharmacokinetic model was developed describing the concentrations of cortisol throughout the body as a function of time under normal physiological conditions. Previous mathematical models and parameters describing cortisol production, circulation, and clearance were compiled and combined in a unified model, and used to describe cortisol values in the adrenal gland, blood, adipose, muscle, and brain. The model was validated against physiological literature and used to tune a theoretical affinity sensor implanted in the interstitial space of adipose in terms of its geometry, sensor site concentration, and binding kinetics/equilibrium. An optimal set of parameters was collected, and the same sensor was shown to operate robustly in both a healthy patient and a patient with Cushing's disease. A major conclusion of this portion of the thesis is that the sensor output of most value for this problem is accurate measurement of the first derivative of concentration.

To address sensor development experimentally, we develop a compositionally controlled templated version of Corona Phase Molecular Recognition (CoPhMoRe) to produce unique molecular recognition sites for steroids. In the CoPhMoRe method, a single-walled carbon nanotube (SWNT) is wrapped with an amphiphilic polymer. The pinned polymers form a corona phase that modulate analyte binding. Upon analyte binding, the fluorescence spectrum may be modified in terms of its intensity and/or peak emission wavelengths. In this work, we synthesized a library of 16 polymers containing various amounts of acrylic acid, styrene, and a template

cortisol molecule. The hypothesized mechanism was that the template cortisol monomer would occupy a free volume within the pinned polymer, which would produce a binding shape in the approximate shape of a steroid, allowing free steroid to competitively displace the template and modulate fluorescence. Selective constructs were found for cortisol and progesterone. The progesterone sensor was translated to an implantable hydrogel form factor. Utilizing the reversibility of the sensor, we performed proof-of-concept experiments demonstrating the functionality of the progesterone sensor in an SKH1-E mouse.

To examine potential application spaces, the feasibility of using CoPhMoRe sensors for aquatic organism biologging was explored. In recent years, biologgers have attached sensors to animals to characterize environmental and animal-derived parameters as they behave normally in their environment. By collecting orthogonal datasets describing environmental parameters (e.g. temperature) and animal movement, biologists have elucidated a number of insights regarding migration, predator-prey relationships, reproduction, feeding etc. Currently, however, biochemical information is underutilized and represents a potentially new frontier in biologging. In this study, we examined basic feasibility questions of the use of CoPhMoRe sensors in aquatic biologging. We developed implantation procedures for intramuscular delivery of CoPhMoRe hydrogel sensors and characterized the maximum implantation depth for extraction of the optical signal. Furthermore, we demonstrate that for best fluorescence extraction, hydrogels should be placed into lightly colored tissues. We also demonstrate generally favorable biocompatibility results, with implants causing no observable changes in physiology or behavior.

In both human health and biologging applications, biocompatibility of biomaterials is an important parameter that dictates organisms' tolerance of the material and lifetime of the material. For any long-term use of implantable biosensors, minimizing adverse tissue reactions is critical to prevent chemical modification of the sensor, dislodgement of the sensor from the implantation site, and encapsulation leading to increasing diffusional barriers of analytes from reaching the sensor surface. There have been a number of studies reporting varying degrees of cellular responses depending on SWNT synthesis method, impurity content, SWNT wrapping, and cell type, but the effect of formulation has not been explored systematically. The same parameters that dictate cellular response (e.g. wrapping) also dictate which analytes can be tracked, so discovering orthogonal formulation parameters that can control tissue response while leaving SWNT sensor ability intact is critical. In this study, we tracked tissue responses to five different SWNT-hydrogel formulations to determine design rules to minimize tissue response. Through analysis of the cellular infiltrate, we found that decreasing the hydrogel pore size accelerated the healing process after gel implantation, though all hydrogels were equivalent in inflammatory status by day 28. Furthermore, we demonstrate that the acute inflammatory response has the potential to deactivate hydrogel sensors in a time-dependent manner, pointing to the importance of modulating the tissue response to maximize sensor longevity.

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