Development of Single-Walled Carbon Nanotube-based Biosensors for the Detection of Ions in Ocular Surface

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Statement of Purpose: Potassium ion in the tear fluids plays an important role in osmotic regulation and corneal epithelial maintenance. Presence of appropriate amount of potassium ion is required for various cellular functions. High level of potassium is required to prevent apoptosis in corneal epithelium mediated through different K^+ channels present in corneal epithelial cells (CEpiC) [1]. High potassium levels in in the blood have negative impacts on the nervous, muscular, and cardiovascular system. As such, detecting high concentration of potassium in tear fluid can also be useful biomarker in understanding ocular health and overall psychological conditions in a non-invasive procedure. Nanosensors with optoelectronic characteristics could enable non-invasive detection of potassium ions. Optical sensors based on Single-walled carbon nanotubes (SWCNT) have substantial advantages over conventional processes due to nanotube's non-photobleaching fluorescence in the near-infrared region (900-1600 nm), at which scattering and autofluorescence from biological cells and tissues are minimal, and possess high optical sensitivity. These materials have great potential for a real-time, wireless, implantable, detection method [2]. In this study, we developed SWCNT-based biosensors, via encapsulation of photoluminescent SWCNTs within an FDA approved polymer known to chelate potassium ions, to detect potassium ion and observed its interaction with CEpiC.

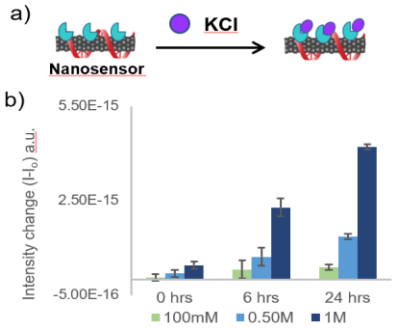

Figure 1: Sensor response to potassium ion: a) utilizing carbon nanotube sensor for the detection of potassium ion. b) sensor response to potassium over time.

Methods: Nanosensors were prepared in an aqueous suspension of non-covalently functionalized semi-conducting SWCNTs using an anionic polymer. Increasing concentrations of potassium chloride solution were added to nanosensor (Figure 1a) and the sensor response was measured via nIR fluorescence measurements from 950 nm – 1250 nm over time (Figure 1b). To observe CEpiC interaction with polymer-SWCNT were added to culture media at varying concentrations (C1-0 mg/L,V2-0.1 mg/L,V3-0.2 mg/L, V4-2 mg/L, V5-5 mg/L, V6-10 mg/L). Live/Dead and Alamar blue assay were conducted in two groups, for first group polymer-SWCNT added media was replaced with culture media after 6 hours and observed 24 hours. For second group, polymer-SWCNT added media was replaced after 24 hours and observed after 48 hours. Immunostaining for Cytokeratin 12 (CK 12) was conducted in similarly except cells were cultured in regular media for 24 hours before adding polymer-SWCNT added media.

Results: A dose-dependent response was observed with intensity-fold increases up to 4.94 for 100 mM, 19.62 for 0.50 M, and 62.25 for 1 M concentrations of KCl (Figure 1b). In Figure 2A, Live/Dead staining showed proliferation of CEpiC with varying concentration of polymer-SWCNT for both with presence of minimal number of dead cells. Similar trend was observed in Alamar Blue Assay (Figure 2C). At higher concentration of polymer-SWCNT (V5, V6), cell proliferation and metabolic activity was lower compared to lower concentration of polymer-SWCNT. CEpiC expressed CK 12 at all concentration of polymer-SWCNT. However, in V5 and V6, the CEpiC had smaller and rounder morphology compared to other concentrations of polymer-SWCNT.

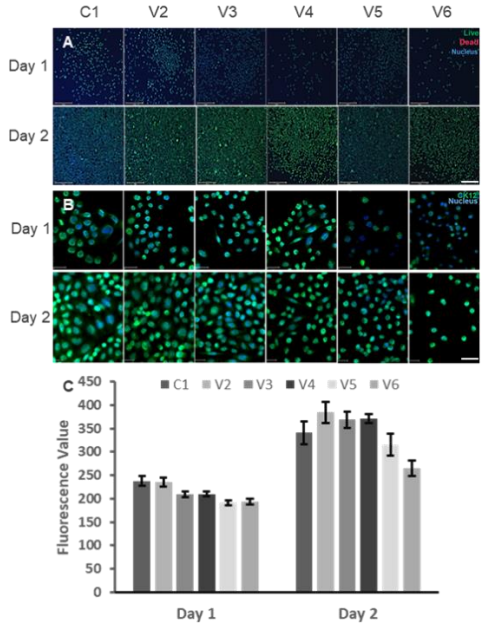

Figure 2: Biocompatibility of CEpiC with varying concentration (C1-0 mg/L,V2-0.1 mg/L,V3-0.2 mg/L, V4-2 mg/L, V5-5 mg/L, V6-10 mg/L) of polymer-SWCNT: A) Live/Dead Assay (scale bar=275 µm). B) Immunostaining of CEpiC specific CK12 protein (scale bar=75 µm). C) Metabolic activity of CEpiC using Alamar Blue Assay.

Conclusion: Our findings show that, developed polymer-SWCNT biosensors can successfully detect potassium ion in solution. Molecular recognition is likely facilitated by the polymer producing an optical response from the nanotubes. Biocompatibility of CEpiC with varying concentration of polymer-SWCNT had been demonstrated. Our next step is developing a library of SWCNT based biosensors and observe their performance in various invitro/invivo/biological conditions.

References:

1) Lu L. Prog Retin Eye Res. 2006;25(6):515-538.
2) Jain A. et al. Biotechnol. J. 2015;10, 447-459.