Biocompatibility evaluation of skin-wearable silver nanowire based hydration sensors

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Statement of Purpose: Recent advances *in vitro* diagnostics on human skin or organ surfaces rely on the use of sensors. The innovative wearable multifunctional sensors made of silver nanowires (AgNW) has gained considerable interest recently owing to the advantages of large stretchability, high sensitivity, and fast response time (~40ms). However, this engineered silver materials may display unique properties that have impact on human health. Biocompatibility is the ability of a biomaterial to perform its desired function, without eliciting any undesirable local or systemic effects in the recipients. Lack of biocompatibility in a skin-attachable sensor, can result in complications including: cell cytotoxicity, skin irritation, skin sensitization, skin chronic inflammation, and even the recalls on commercial products due to the reported dermatitis. A single test is not sufficient to define biocompatibility, so that a variety of tests are required to determine biocompatibility, based on the device and application. We performed a biocompatibility evaluation of AgNW biosensors using two cell lines and 3-dimensional tissues. Three characterized in vitro assays were used to measure the cytotoxicity, irritation and sensitization of the skin-attachable sensor materials. These *in vitro* assays could meet the FDA requirement, as well as minimize the usage of live animals.

Methods: AgNWs are conductive wires with the diameter of tens of nanometers and length of tens or hundreds of micrometers. It is purchased from BlueNano Inc. with known solvents of ethanol or IPA. The material to fabricated AgNWs includes AgNO3, ethylene glycol (EG) and poly (vinyl pyrrolidone) (PVP). A random networks of AgNWs were embedded inside polydimethylsiloxane (PDMS) to form a conductive and stretchable composite. This composite layer can be patterned and used as electrodes to measure the electrical properties of skin and thus skin hydration.
Human dermal fibroblasts and epidermal keratinocyte were grown 37º C in a humidified incubator with 5% CO^2 and 98% humidity. Cells in T-75 or T-25 culture flasks were checked periodically for their c*onfluency (NHDF 80% and NHEK 60-70%).* Extracts were obtained by immersing fragments of each AgNW materials (6 cm^2/mL) in culture medium at 37 °C for 5 days without agitation. Extracts from zinc fragments, which are considered cytotoxic, were used as a positive control. Fresh medium without extracts were used as a negative control. Confluent cells were trypsinized and diluted in culture medium to a final concentration of 6,000 cells/ml, and then plated in 96-well plates. After 24 hours, the culture medium were removed and replaced with the extract medium. Extracts in culture were change at day 2, 5 and 7 at 37 °C. At each sample time (day 2, 5 and 7), the extracted were removed and cell metabolic activity were measured with MTT assays in triplicate, respectively, as described above. Cell morphology were monitored by fluorescent microscopy at each sample time.

Results: Cytotoxicity and bioactive effects of the component materials, which was assessed with the MTT proliferation assays using cells cultured with the extracts, show that all AgNW and PDMS material extracts decreased cell proliferation less than 20% compared to negative controls (TCPS) for keratinocyte cells (Figure 1).

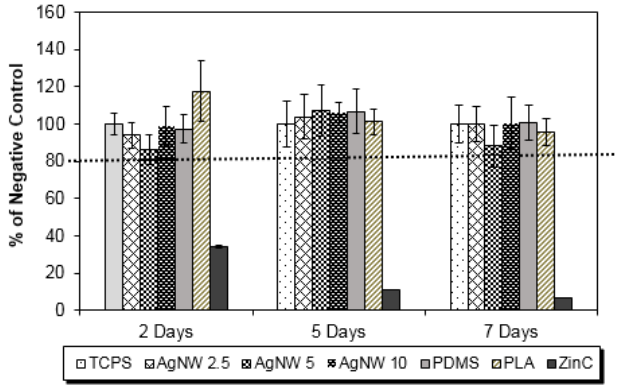

Figure 1. MTT assay with human keratinocyte cell line: time points vs. % of negative control

In addition, fluorescent microscopy show that the morphology of cells cultured in AgNW extracts were not changed compared to these cells cultured in negative control (Figure 2). LC-MS analysis for the extract solution show that the silver ion concentration in these AgNW extracts are about 15 µm, which is less than the minimal inhibitory concentration (23 µm) of human keratinocyte cells[1].

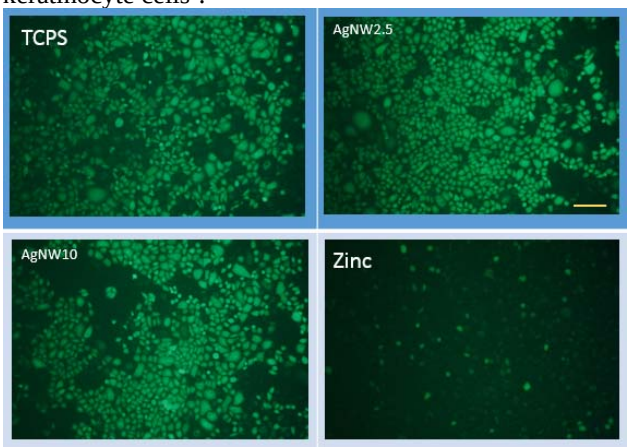

Figure 2. Fluorescent microscopy of keratinocyte cells

Conclusions: Direct contact exposure shown that component material of AgNW doesn't decrease cell proliferation for dermal keratinocyte cells, indicating the non-toxic effects of AgNW for human use.

References: Geraldine Mulley et, al., PLOS one, 9(4): 1–9, (2014).