3D-Printed Degradable Microneedles for Controlled Drug Release in Central Nervous System

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Background

Acute spinal cord injury (SCI) remains one of the most debilitating pathologies. The permanent paralysis and sensory deficits that follow leads to severe disability, shorter life expectancy, and significant economic cost. The pathophysiology of SCI involves two temporally distinct mechanisms, termed primary and secondary injury. Considerable research has focused on understanding the pathophysiology of secondary SCI in hopes of developing therapeutics and therapeutic delivery systems that minimize further cell loss and tissue damage due to local inflammatory mediators. Corticosteroid administration soon after injury may reduce the extent of permanent sensorimotor deficits, improving the patient's quality of life.

An immune response follows immediately after the primary SCI and consists of a complex reaction involving both cellular and molecular components. The effect of immune cells and regulatory proteins is largely inflammation, a key event in the secondary SCI cascade that persists for several weeks to months. Immune cells at the site of SCI secrete pro-inflammatory cytokines, including interleukin (IL)-1α, IL-1β, IL-6, and tumor necrosis factor-α (TNF-α). Activating an immune response removes cellular debris, a process that promotes the regeneration of surviving neurons. However, an upregulated and persistent immune response damages healthy tissue surrounding the primary SCI. Mitigation of this immune response may decrease inflammation and free radical-based injury exacerbation.

A variety of therapeutics have been tested in SCI patients for the goal of improving functional recovery. A systematic review conducted in 2012 concluded that systemic administration of steroids improves the neurologic outcomes if administered within eight hours of injury. Furthermore, maintenance does of steroid over a 48 hours provides additional improvement in motor neurologic function. Dexamethasone is a corticosteroid that offers neuroprotective effects by dampening the inflammatory response through reduction of local cytokine production and release. However, prolonged doses of systemic steroids elicit a myriad of undesirable side-effects. A therapeutic delivery system for localized dexamethasone administration was developed in hopes of improving outcomes of spinal cord injury while circumventing unwanted systemic effects. This research developed a localized microneedle delivery system. The degradable polymer microneedle patch can facilitate controlled, sustained therapeutic delivery directly to the site of injury.

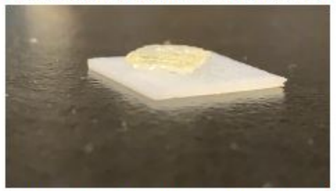

Figure 1: Dexamethasone loaded polymer microneedle with a 15x15 array inserted into collagen dura mater substitute.

Methods

Negative molds were made from polydimethylsiloxane (PDMS). The microneedle monomer solution contained a mixture of vinylpyrrolidone (VP) and polyethylene glycol diacrylate (PEGDA). A mixture if 95:5 VP to PEGDA was made and combined with 1.3% of a radical photo-initiator. For microneedles containing dexamethasone, 3 mg of dexamethasone was added to 10 uL of VP. A 4:1 ratio of VP-PEGDA to dexamethasone-VP was the final monomer solution. To generate a microneedle array, 3 uL of final monomer solution was placed into a PDMS mold and placed into a vacuum for 5 minutes. A microneedle backing of 4 uL of VP-PEGDA without dexamethasone was added to the mold. The PDMS mold filled with is placed under ultraviolet light for 30 minutes and then the microneedle is removed from the PDMS mold.

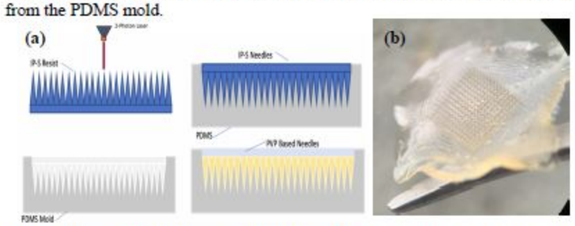

Figure 2: (a) Molding process of PDMS negative and microneedle array, (b) final form of a dexamethasone-loaded 15x15 microneedle array.

For in-vitro experiments, a novel release method was developed to evaluate dexamethasone delivery across a dura mater substitute. A custom well insert was created using a 3D printer. A collagen dura matter substitute produced by Medtronic for neurosurgical repair was placed to the bottom of the well and secured using cyanoacrylate. The well was then filled with 1.8 mL of sterile phosphate buffered saline (PBS). The dexamethasone-loaded microneedle array was pressed into the dura and placed in the incubator at 37 °C for 0.5, 1, 4 and 24 hours. For each time point, the PBS solution was removed and replaced with fresh PBS. A mass spectroscopy assay was developed to quantify dexamethasone delivery across the substitute dura mater and into the PBS solution within the well.

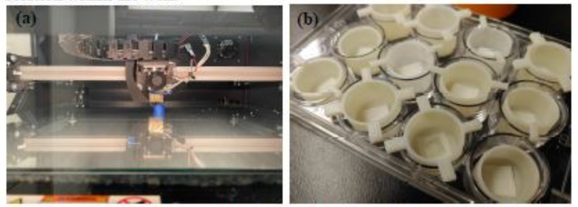

Figure 3: (a) 3D printer constructed well for placement of collagen dura matrix substitute, (b) in-vitro transdural experiment setup with placement of dexamethasone microneedle array.

Results

A scanning electron micrograph of a 15x15 dexamethasone-loaded microneedle array can be seen in Figure 4a. Figure 4b shows the standard curve for mass spectroscopy response as a function of dexamethasone concentration. This standard curve was used to estimate the dexamethasone concentration delivered across the collagen dura mater substitute. The delivery of dexamethasone across the collagen dura mater substitute at 0.5, 1, 4, 24, and 48 hours is shown in Figure 4c. At 48 hours, the concentration of dexamethasone accumulated on the delivered side of the dura mater substitute was approximately 30 ug/mL, a considerably higher concentration that what had been shown to be therapeutically effective in suppressing inflammatory cytokine expression.

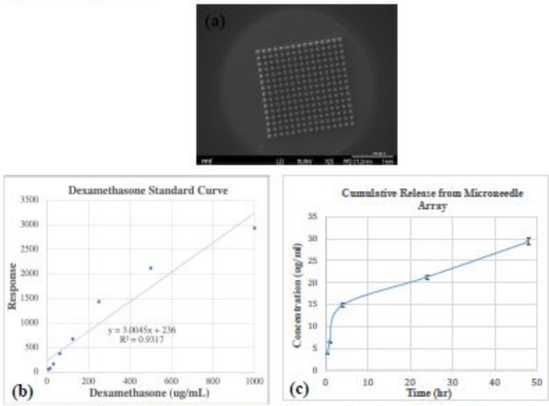

Figure 4: (a) Scanning electron micrograph of microneedle array, (b) dexamethasone standard curve, (c) cumulative release from microneedle array.

Future Work

Future work will involve conducting biological immunoassays to quantify the reduction in inflammatory mediators from microglia cells. Even further work will use rats in in-vivo studies as a SCI model. In. addition, other therapeutics such as the anti-inflammatory cytokine IL-10 may be substituted for dexamethasone in transdural delivery experiments to see the effect on modulating the immune response from microglia cells.

Resources

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