

Specific Aims

Skin cancer is the most common cancer in the United States, currently affecting over 5.4 million.¹ While melanoma only accounts for approximately 2% of skin cancer patients, it is the leading cause of death related to skin cancer.² Over 73,000 new cases of invasive melanoma are estimated to be diagnosed in the United States in 2015.² Typical treatment options for skin cancer primarily involve wide-local excision surgery in which the cancerous mole and a small area surrounding it is removed. Patients who undergo wide-local excision surgery are at a high risk of surgical site infection, potentially leading to death if left untreated. Common chemotherapeutic agents are often prescribed after surgery to help prevent relapse, but they compromise the immune system. This can further increase the risk of post-operative infection. These antibiotic treatments and chemotherapeutic agents are typically applied intravenously in a non-targeted manner, which can cause off-site toxicity and negative effects on the microbiome of the patient.³ A good way to avoid this would be on-site delivery of combined antibiotics and chemotherapeutics via the skin. Chemotherapeutic topical creams have been developed, however, the application is heterogenous and can be easily removed through agitation. **Clearly there is an unmet need for a simple, robust, and versatile delivery mechanism for the controlled delivery of both chemotherapeutics and antibiotics in order to combat both infection and remission post melanoma removal surgery.** The *Overarching Goal* of this proposal is to develop an alternating layer by layer hydrogel, wherein each of the layers are uniquely sensitive to changes in pH and temperature, in order to release therapeutics directionally in a controlled manner. We *hypothesize* that using alternating layers of pNIPAAm and PMA modified to dissolve and release drugs at 34°C and pH 5.5, respectively, will result in a directional controlled release of therapeutics when applied to the epidermis. To realize these goals, we plan to synthesize and characterize the prototype Hydro-Bandaid patches with Professor Kilian at the University of Illinois, Urbana-Champaign.

The specific aims of this proposal are:

Aim 1: Synthesize and physically characterize Hydro-Bandaid in its ability to directionally deliver drug molecules in a controlled manner

- (a) Tune each hydrogel layer of pNIPAAm and PMA to release contents at 34°C and pH 5.5 respectively.
- (b) Assemble layer by layer, and combine with micro-needle array.
- (c) Test physical characteristics of the layered material.
- (d) Test release characteristics directionally in solution.

Aim 2: Characterize the material effectiveness in vitro and demonstrate the superiority of our delivery mechanisms to a topical cream approach.

- (a) Test in vitro.
- (b) Apply Hydro-Bandaid in a guinea pig model.

Background and Significance

The incidence of melanoma has been increasing for the past 30 years and currently has a death rate of 56.6%.⁴ Melanoma tumor cells originate in the pigment-producing

melanocytes in the basal layer of the epidermis, and the tumors typically resemble moles appearing on the surface of the skin.⁵ Melanoma develops from unrepaired DNA damage to skin cells, typically caused by ultraviolet radiation from sunlight or tanning beds.^{6,7} This DNA damage triggers mutations, or genetic defects, that lead the skin cells to multiply rapidly and form cancerous growths. Initial diagnosis of melanoma is generally based on visual cues. Cancerous moles are identified from benign tumors in that they are asymmetrical, with uneven borders, a variety of colors, larger diameters, and/or more rapid changes over time. The only reliable and decisive way to determine if a mole is actually cancerous involves skin biopsy analysis.

Current status for melanoma treatments

The treatments available for patients with melanoma depend heavily on the current stage of growth when diagnosed. Earlier stages (0-2) are pre-metastatic stages with progressive tumor thickness and are prime candidates for successful treatment. Wide-local excision surgery is the established and most common treatment for melanoma diagnosed in these early stages.⁸ In this procedure, the skin cancer and a small margin of healthy surrounding tissue are surgically removed.⁹ The size of this margin depends on the thickness of the tumor. The recommended margins vary from 0.5 cm for in situ tumors, which have not spread below the epidermis, to 2 cm for tumors around 4mm thick.² The edges of the wound are then sutured and the cancerous tissue is sent to the pathologist for margin evaluation via histology. The recovery rate after the surgery depends on the size and site of the cancer.

Innovation

There are two main risks associated with wide-local excision surgery; the risk of recurrence and the risk of infection. Recurrence of melanoma occurs 10 or more years after initial treatment in more than one in 20 patients.⁹ The risk of surgical site infection after wide-local excision surgery is also high.¹⁰ It is currently estimated that around three in every 100 patients who undergo surgery develop a surgical site infection, and there are around 157,500 occurrences per year.¹¹ This infection can cause diarrhea, vomiting, inflammation of veins, and blood clotting, which can all be very dangerous for patients whose bodies are already under enough physical stress. The **Overall Objective** of this application is to maximally reduce the number of deaths resulting from post-operative infections, remission, and secondary surgeries/infections following remission. **If Successful**, we expect our Hydro-Bandaïd to suppress infection and remission of cancer through the controlled directional release of chemotherapeutics and antibiotics. Additionally, our Hydro-Bandaïd model would pose as an excellent vessel for the topical delivery of any hydrophilic/hydrophobic therapeutic. The **Innovation** of our work is through layered assembly of alternating hydrogels, each being sensitive to separate environmental release mechanisms. This layer by layer approach should result in an 'insulation' provided by one layer to the other, resulting in a uniform layer by layer dissolution. The Hydro-Bandaïd will address several of the concerns associated with post wide-local excision surgery. The patch will be placed on the skin at the location where the surgery was performed, releasing therapeutics in order to prevent remission, infection and to promote healing. It will include four main drugs; imiquimod to prevent recurrence, a combination of antibiotics to prevent infection, interferon to boost the

immune system, and anticoagulants to prevent blood clotting and vein inflammation. This topical drug delivery method will focus on patient recovery post wide-local excision surgery and can offer a great alternative to intravenous drugs, which can cause off-site toxicity issues such as gastronomical issues. These therapeutics will be layered throughout the Hydro-Bandaïd assembly and require no regulation or work from the patient, making it very easy to use.

Control of Polymer Sensitivity

Hydrogel polymers, which are sensitive to environmental changes have seen particular progress through use as drug delivery agents in a controlled manner.^{12–16} This environmental sensitivity has been especially useful for passive targeting to cancerous microenvironments as these operate in anaerobic conditions (which are both hotter and more acidic than healthy tissue).^{14,17–19} Poly(n-isopropylacrylamide) (PNIPAM) is a favorite among temperature sensitive polymers with a larger number of research papers focusing on that aspect for controlled release of therapeutics. The polymer PNIPAM is also capable of being modified for specific temperatures of release.¹⁶ Poly(Methacrylic acid) (PAA) is a common hydrogel polymer with release mechanisms triggered by decrease in pH.¹⁵ Through similar modifications, the pH sensitivity of PAA can also be modified.

For targeting specific micro environments, the ability to tune the drug release mechanisms becomes especially important in order to prevent offsite toxicity issues due to the drug therapy.^{13–19} We plan on utilizing an alternating layers of modified pAA and pNIPAAAM (Figure 1) to release both chemotherapeutics and antibiotics through environmental sensitivity to the skin microenvironment.

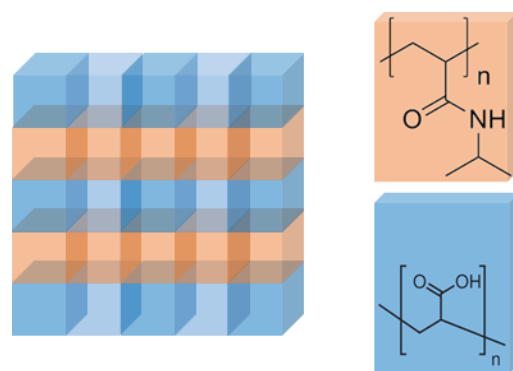


Figure 3. Example diagram of alternating layers of hydrogel polymers

Layer by Layer Assembly

Layer by Layer (LBL) assembly is a popular fabrication method in the biomedical field for several convenient features. This method allows high control over the structure of material, physiological or chemical condition, and loading of other molecules within each layer.²⁰ It is also relatively cheap and easy to prepare and process, making it the most suitable option for mass production of materials. Electrostatic interactions and hydrogen bonds are the most widely used methods to create these layer by layer assemblies. However, there are some unconventional methods use self-assembled building blocks.²¹

Micro-needle Patch Delivery

The main purpose of using micro-needle is to minimize the pain when inserted into the skin and to enhance the success of transdermal drug delivery.^{22–24} The micro-needle patches are designed to be significantly less painful than 26-gage hypodermic needles, and the effect can be enlarged by making the length of each needle shorter and using a

larger number of needles. Controlling the needle length will enable drug delivery to cell layers in different depths, but long needles may induce a lot of pain.^{24–26}

Micro-needle patches can be made from a variety of materials, such as metals or polymers, depending on the environment and the purpose of the patch. For our hydrogel patch, we are choosing to use a 316L stainless steel, as metals have higher conductivity for heat and water than polymeric materials do.

316L Stainless steel was chosen to be the material composing the micro-needle array. It is relatively cheap among biocompatible metals let alone its thermal conductivity.²⁷ Although this material is known to corrode over time within the body, our product aims for usage periods to be no longer than a month (which will not allow for considerable pit erosion). Earliest detection of corrosion after implantation was reported to be two months.

Research Plan

To complete **Aim 1** and synthesize and physically characterize Hydro-bandaïd in its ability to directionally deliver drug molecules in a controlled manner, we will do the following tests and experiments to confirm our working hypothesis.

(a) Tune each hydrogel layer of pNIPAAm and PAA to release contents at 34°C and pH 5.5 respectively.

To finely tune our therapeutic delivery system, we must first modify each polymer layer so their release mechanisms are each sensitive to the correct pH and Temperature. In order to do so, we will experimentally determine the correct ratio of pNIPAAm or PAA to cellulose ratio.

pNIPAAm

To correctly modify or pNIPAAm layer to selectively release drug molecules from skin temperatures we will take an approach similar to Pan *et al.*²⁸ using a cellulose macro-initiator, NIPAM monomer, PMDETA and 1, 4-dioxane. To experimentally determine the ratio of NIPAM:Cellulose, we will prepare several experimental polymers determining the gelation temperature of each through progressive heating in PBS.

PMA

To correctly modify or pMA layer to selectively release drug molecules due to the acidic conditions of the skin we will take an approach similar to Pan *et al.*²⁸ using a cellulose macro-initiator, MA monomer, PMDETA and 1, 4-dioxane replacing the NIPAM monomer with a MA monomer. To experimentally determine the ratio of MA:Cellulose, we will prepare several experimental polymers determining the gelation pH of each through progressive addition of dilute citric acid.

(b) Assemble Layer by layer, and combine with micro-needle array.

Hydrogel will be synthesized by adding water and aqueous drug to dry polymer (either PNIPAM or PMA). The hydrogel will then be shaped into a large, thin film. We will use a

robot laser x-y cutter to cut the hydrogel sheet into smaller pieces. Each hydrogel piece should have surface area ranging from 4cm^2 to 200cm^2 , and thickness of 200nm using a gel cutting machine (Dumbbell Co., Ltd.).^{29–31}

To construct the assembly, alternating sheets of hydrogels will be placed on top of one another with hydrophobic drugs sprayed via aerosol in between. Layering of hydrogels will continue until the hydrogel mixture layer reaches intended thickness ($<2\text{mm}$). The layer-by-layer hydrogel assembly will be placed on top of a bi-directional (needles facing toward and away from gel assembly) micro-needle array made of stainless steel (figure 2). This bi-directional needle assembly will promote adhesion as well as drug elution compared to single directional needles. Each micro-needle will be 500nm in length, 100nm in width, 30nm in thickness, and placed every 4mm^2 . The dimension and number of needles were selected based on the pain scores recorded by H. Gill et al.²⁵ Total thickness of the stainless steel array should be less $50\mu\text{m}$ thick.

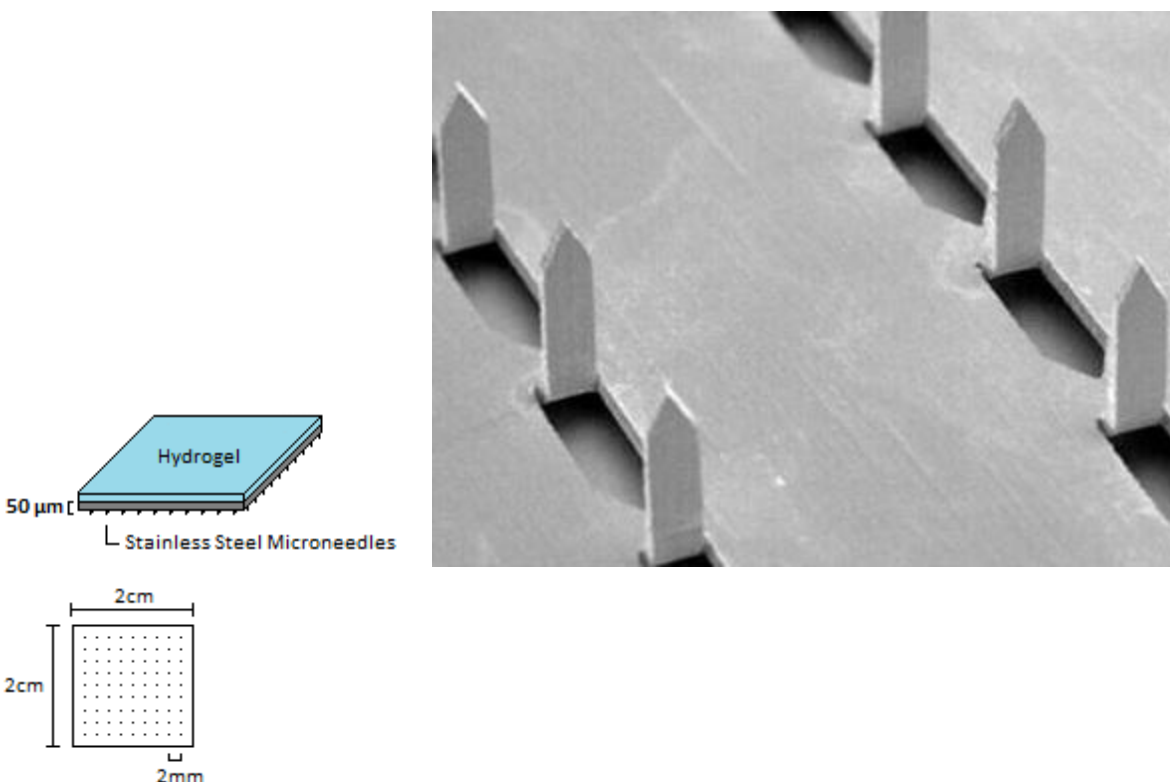


Figure 2. a. schematic design of the hydrogel patch (left) b. microscopic picture of micro-needle (middle) [<http://www.micropoint-tech.com/company/technology/>] c. stainless steel micro-needle array layout on $2\text{cm} \times 2\text{cm}$ size patch (right)

(c) Test Physical characteristics of layered material. (Ayako)

In order to ensure that the Hydro-Bandaïd will be able to accommodate the movement of the patient, various materials tests will be performed. First, to measure tensile strength and elasticity, strip extensimetry of both the pMA and pNIPAAm hydrogels as well as the final pMA and pNIPAAm layered conformation will be performed. To achieve this, we will use a Zwicki-Line Testing Machine (Zwick, Ulm, Germany) at a speed of 1 mm/min at applied forces of 0.25 N, 0.5 N, 1 N, and 5 N.³² The strain of the material will be plotted over time to examine the elongation of the material with stretch. Furthermore, to create a stress/strain curve for use in mechanical analysis and determining the tensile modulus, we will perform compression testing with the Materials Testing System (MTS Corp., Eden Prairie, MN) with a 5.8 mm plunger at a speed of 1 mm/min to compress to a 0.5 mm/mm strain. The load will vary from 0 kPa up to 250 kPa, in increments of 50 kPa.

(d) Test release characteristics directionally in solution.

To examine the release rates of our layered hydrogel, a dialysis membrane will be stretched over a 500 mL glass beaker filled citrate buffer pH 5.5 containing a magnetic stir bar and our drug eluting layered hydrogel placed on top of the membrane (figure 3). The contents of the beaker will be heated to 37C with magnetic stirring and the



Figure 3. Layered hydrogel resting on dialysis membrane

contents analyzed spectroscopically every 15 minutes for the duration of 2 hours, hourly for the duration of 4 hours then daily for a total duration of a week.

The release kinetics experiment will be tried in triplicate as well as for the conditional combinations with pH 7.4 using PBS and room temperature at 22C

To complete **Aim 2** and characterize the material effectiveness in vitro and demonstrate the superiority of our delivery mechanisms to a topical cream approach, we will do the following tests and experiments to confirm our working hypothesis.

(e) *In vitro* testing.

To evaluate the biocompatibility of our Hydro-Bandaïd prior to animal testing, we will conduct a direct contact test. A 6-well plate will be seeded at 400,000 cells/well with PCS-201-012 human dermal epithelial cells, and incubated for 24 hours at 37C at 5% CO₂ in reconstituted fibroblast basal medium. The pMA, pNIPAAm, and Hydro-Bandaïd samples with and without the drugs, cut in 1 cm by 1 cm squares, will be placed inside the wells and allowed to incubate for 48 hours in the reconstituted fibroblast basal medium at pH 7 and pH 5.5, adjusted using sodium bicarbonate and sodium citrate, respectively. The materials and the old media will be removed, and a live/dead assay will be conducted to examine the effective release of the drug as well as the cytotoxicity of the hydrogels.

For the elution test, each hydrogel material with and without drugs will be placed into the reconstituted fibroblast basal medium, then removed after 24 hours. PCS-201-012 cells will be placed in a 96-well plate seeded at 10,000 cells/well with the media for 48 hours. To examine cell viability, MTT, LDH, and WST cell assays will be performed.

(f) *In vivo testing.*

To further ensure the biocompatibility of the Hydro-Bandaïd *in vivo*, it is advantageous to test the hydrogels in a hairless guinea pig (HGP) model as the permeability of its skin is similar to that of humans.[doi:10.1016/j.tiv.2008.10.008] All animal experiments will be conducted in accordance with accepted standards of humane animal care approved by the University of Illinois Division of Animal Resources. For the experimental group, 10 HGPs will be injected with 25% Matrigel solution containing 2×10^4 C32 melanoma cells near the hind limb under isoflurane anesthetization. The tumor will be allowed to develop for 2-4 weeks. When the tumor has reached a size of 300 mm², we will excise the tumor but leave approximately 1 mm² of the tumor in order to observe the drug release efficacy of the Hydro-Bandaïd. We will adhere the Hydro-Bandaïd with and without the drug to the remains of the tumor on the HGPs. For the first test, we will collect blood samples from the HGPs at 1h, 6h, 12h, and 24h and daily for the next 7 days to develop a drug release profile. For the second test, we will sacrifice the HGPs and surgically remove the skin under where the patch was placed. Then, using classical histopathology methods, we will fix and stain the tissue to observe signs of inflammation, vascularization, and fibrosis. To compare the efficacy of our Hydro-Bandaïd, we will also transdermally apply the FDA-approved Imiquimod, performing the same two experiments.

Anticipated Problems and Proposed Solutions.

A few problems can occur with the use of hydro-bandaïd. Additionally, similar patches intended to be place on top of the skin, carry their own set of anticipated risks. The latter can be closely analyzed since some flaws associated with skin patches have already been previously discovered with skin patches.

Acidic pitting from stainless steel micro-needle array and gel sloshing off or falling apart are a couple potential risks of hydro-bandaïd. Chloride ion reactions are the primary reason behind acidic pitting³³ As a protective measure from pitting corrosion, chromium, molybdenum, and nitrogen, can be incorporated into the metal of the microneedle.^{34–36} These alloying elements provide a resistive characteristic to the metal from pitting; this can be a potential solution to incorporate from the very start.

Hydrogel patches have their own set of issues such as discoloration, infection, inflammation, and more. More often than not, skin changes are harmless and involve easy treatment. Even small infections fall under this category. The benefits of the patch outweigh the treatment in this case so the hydrogel patch treatment would proceed or be based on case-to-case patients.

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