Polymeric Gels As A Means Of Controlling Local Skin Delivery

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Skin cancer is the most common cancer. While most forms of skin cancer have high survival rates if they are caught early, some can metastasize and become very difficult to treat. Inhibition of Matrix Metallopeptidases (MMPs) can be effective in preventing growth and metastasis of existing tumors. For this reason, they may be especially useful in the treatment of skin cancers. Systemic delivery of MMP inhibitors can cause unwanted side effects, so localized delivery is preferable. By incorporating MMP inhibitors into polymer gels, the drug can be administered topically and its distribution within the skin and into the systemic circulation may be controlled. Polymers were formulated to contain a model MMP inhibitor and applied to human skin samples using a high throughput skin permeation screening method. After the permeation study was run, samples were removed and the concentration of the drug that crossed the skin was quantified using High Performance Liquid Chromatography (HPLC). Analysis showed that all of the tested formulations permeated across or were retained in the skin at varying degrees. Further, some differences between the polymers, as well as between the same polymers with altered concentrations of cross-linking agents, were statistically significant. Polymeric gels are shown to be a viable method of delivering MMP inhibitors topically. Due to the permeation enhancing effects of different polymers and the effects of various concentrations of cross-linking agents, formulations could be customized to penetrate the entire tumor without entering the blood stream and causing systemic effects.

Skin cancer is the most common type of cancer afflicting people globally.[1] The rates of skin cancer continue to increase rapidly due to the prevalence of tanning salons and inadequate sunscreen application.[3] A single severe sunburn from a day of forgetting to apply sunscreen can more than double the risk of developing malignant melanoma later in life.[3] Currently, skin cancers kill slightly over half of the people that breast cancer does each year, but this gap continues to close as rates of skin cancer increase at between 7% and 8% each year.[3] However, if a developing skin cancer tumor is caught early, all forms of skin cancer have relatively high survival rates and can generally be surgically removed via an outpatient procedure without further complications. In contrast, if a malignant tumor is not identified and excised early, it can metastasize, at which point it may become very difficult to treat. Five-year survival rates decrease drastically, from 98% to 23%.[1]

While surgery is an effective treatment for non-metastasized tumors, it is often unable to adequately treat cancer that has spread. As such, many have turned to pharmaceutical research to develop tumor-killing medications as an alternative. Matrix Metallopeptidases (MMPs), normally involved in the regulation of cell growth, movement, and death, can become overactive in patients with cancer, leading to the formation and spread of a tumor.[4] Early research suggests that MMP inhibitors (MMPIs) could be utilized to treat many forms of cancer. However, clinical trials in late stage cancer patients demonstrated that their utility was lower than expected.

MMPIs were found to be effective in preventing growth and metastasis of existing tumors, especially those that are still relatively new and have not yet spread.[4] As a result, MMPIs are of particular interest in improving the treatment outcomes for skin cancers by preventing metastasis, thus reducing the urgency of surgical removal. While MMPIs can be delivered systemically, whether orally or intravenously, systemic delivery can cause severe side effects such as reduced ability to heal from injuries and stunted growth in children, making localized delivery preferable.[4] By incorporating MMPIs into polymeric gels, which serve as permeation enhancers, the drug can be administered topically and its distribution within the skin and into the systemic circulation may be controlled. Formulations may therefore be customized to alter the depth to which the drug is delivered, such that it targets the entire tumor without causing systemic effects. Here, it was hypothesized that the polymers that had a larger molecular size, as well as the polymers that engaged in more intramolecular cross-linking would be the most effective, as they would dilute the charge of the MMPI sufficiently to allow it to cross the skin barrier, while holding the drug tightly enough in the gel that it does not cross fully into the bloodstream.

Methods

First, a literature review was performed to assess potential polymers to serve as the basis for the drug-containing gels. Polymers were selected based on their ability to form a gel, biocompatibility, and previous use in drug delivery vectors. Due to the proprietary nature of the work in the lab, the author is bound by an NDA not to discuss the names or specific properties of the polymers. After compiling a list of the twelve suitable polymers, literature was reviewed further to establish general guidelines for preparing the drug-containing gels. The required concentrations of polymer, whether the addition of a cross-linking agent was necessary, as well as ideal temperatures for preparation and storage, were determined through experiments that were guided by published literature. Since the layers of skin are not particularly permeable for polar molecules, it was necessary for the polymer to dilute the polarity with its positive charge, without encapsulating the drug such that it would not disperse into the skin. After determining the ideal method of polymeric gel preparation, the methods were verified as candidates for drug delivery by remaking them and incorporating the model MMPI. Polymer gels A, B, C, D, J, and K were created by dissolving the solid polymers in water using a magnetic stir bar at room temperature. Polymers J and K had differing concentrations of a cross-linking agent added to aid in the gelling process. Polymers E, F, G, and H were formulated by adding the polymer to water chilled to 5° C, mixed in an ice bath until dissolved, then stored at 4° C overnight. After a minimum of twelve hours at 4° C, the polymers were returned to room temperature while mixing, at which point they formed a thick gel. Polymer I was heated as it mixed, and then chilled in a refrigerator. The polymers were individually applied to human skin samples obtained from the National Disease Research Interchange (NDRI) using the high throughput skin permeation screening method developed by Martins et. al,[2] as shown in Figure 1. Since the upper layers of the skin are composed of cells known as keratinocytes that are already dead, no additional measures were necessary to preserve the function of the skin other than storing the samples in a -80° C freezer. The permeation study was run for 24 hours at 37° C, at which point the samples were removed and the concentration of the drug that crossed the skin was quantified using High Performance Liquid Chromatography (HPLC) with a Waters 1525 Binary HPLC Pump, Waters 717plus Autosampler, a Waters 2487 Dual λ Absorbance Detector, and a Waters, Symmetry C18 3.5um (Part No. WAT200632) Column. Additionally, the skin present in each well was separated from the skin sample with a disposable biopsy punch, massed, and then incubated in methanol for three hours at room temperature. After incubation, the tubes were sonicated and centrifuged at 3000 rpm for 30 minutes. The supernatant was subsequently removed and analyzed with the same HPLC machine to determine how much of the drug had been retained in the skin.

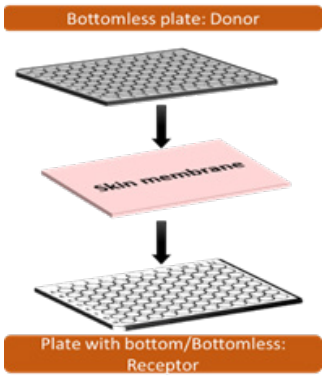

Figure 1. The high-throughput screening assembly.

Results and Discussion

HPLC analysis showed that all the tested formulations permeated the skin, as compared to a control that showed no permeation. The degree of permeation differed between formulations (Figure 2). The results were analyzed using ANOVA and a t-test. The permeation of polymers A, D, and J1 was higher by a statistically significant margin at a p-value of 0.05. Further, the differences between the two concentrations of polymers J and L, which were both binary combinations of polymers that were gelled with different concentrations of a cross-linking agent, were statistically significant. The increased concentration of cross-linking agent increased the intermolecular binding of the gel and subsequently how tightly it held the drug.

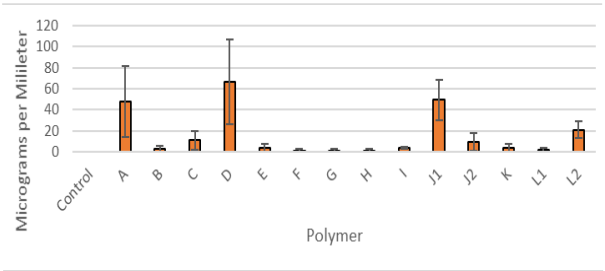

Figure 2. Concentration of MMPI that permeated skin; * denotes a statistically significant difference at a p value of 0.05.

Analysis of the amount of drug retained in the skin revealed statistically significant differences between the polymers that were assessed (Figure 3). When compared to the amount that diffused across the skin, the polymers that had a significant amount of the MMPI retained in the skin had relatively small amounts of drug that made it entirely across the skin barrier. Because tumors originate within the skin, this shows that the formulations were a success, and drug could be delivered to a tumor within the layers of the skin without crossing into the blood stream and causing systemic effects.

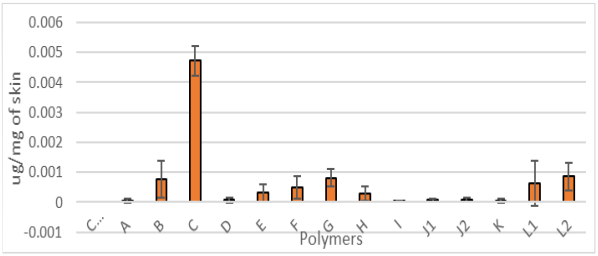

Figure 3. Amount of Drug Retained in the skin; * denotes a statistically significant difference at a p value of 0.05.

The MMPI drug permeated the skin in all screened formulations, although some in very small concentrations, and was retained in most, though to varying degrees. Polymers A, D, and J1 increased permeation by a statistically significant margin when compared to the other polymers. Polymers J1 and J2, as well as polymers L1 and L2 had statistically significant differences in permeation after a change in cross-linker concentration when analyzed with a t-test. This means that the permeation of the drug could be altered by adjusting the concentration of the cross-linking agent, which would allow more control than simply changing the polymer. Based on these data, gels could be customized by altering the polymer or concentration of cross-linker to control drug penetration to the desired depth for a skin tumor without excess systemic absorption.

Further Studies

While the data are promising, further studies are necessary to develop a model that could predict the depth to which the drug would penetrate based on the concentration of various polymers and cross-linking agents. Gels will be formulated to contain a fluorescent dye with a similar structure to the MMPI to visualize how the drug diffuses through the skin over time. Additionally, the polymeric gels formulations were only screened on healthy human skin. The same formulations will be screened on diseased skin, as the presence of tumors can significantly alter the properties of human skin. After a diseased human tissue model, a mouse model of melanoma would need to be used as a translational study before moving on to a clinical trial. Although all the tested polymers have previously been shown to be safe for use on human subjects, several were used in higher concentrations than they had been previously, and thorough safety screenings will need to be conducted.

Implications

The varying degrees of permeation among the polymers suggests that a 3D scan of a tumor could be obtained via MRI or other imaging technology. The formulation could then be customized for the patient such that the MMPI would diffuse through the entire tumor without entering healthy tissue or the bloodstream. This could effectively stop the spread of the tumor and reduce the likelihood of metastasis.

FUNDING INFORMATION

Support was provided by NSF Research Experience for Undergraduates Award (1461192). The author and mentor acknowledge the use of human tissues procured by the National Disease Research Interchange (NDRI) with support from NIH grant U42OD11158.

REFERENCES

1. Diepgen, T. L., & Mahler, V. (2002). The epidemiology of skin cancer. *The British Journal of Dermatology*, 146 Suppl 61, 1–6. https://doi.org/10.1046/j.1365-2133.146.s61.2.x.
2. Martins PP, Estrada AD, Smyth HD. A human skin high-throughput formulation screening method using a model hydrophilic drug. *International Journal of Pharmaceutics*. 2019 Jun 30;565:557-68.
3. Miller, D. L., & Weinstock, M. A. (1994). Nonmelanoma skin cancer in the United States: incidence. *Journal of the American Academy of Dermatology*, 30(5 Pt 1), 774–778. https://doi.org/10.1016/s0190-9622(08)81509-5.
4. Winer A, Adams S, Mignatti P. Matrix metalloproteinase inhibitors in cancer therapy: turning past failures into future successes. *Molecular Cancer Therapeutics*. 2018 Jun 1;17(6):1147-55.

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Simon Blanchard is a member of the Class of 2022. He is a Chemical Engineering major and has been researching in the Polymer Science and Drug Delivery Lab of Dr. Noelle Comolli since the start of his freshman year. During Summer 2019, he had the opportunity to further his research experience by participating in a biomedical engineering REU at the University of Texas at Austin in the lab of Dr. Hugh D.C. Smyth. After graduation, Simon plans to obtain an M.D. and engage in clinical research studying the immune system and autoimmune disorders. Simon is a Villanova Presidential Scholar.

Mentor

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Dr. Smyth is a PI working in the school of Pharmacy at UT Austin. Dr Smyth's group focuses on Drug Delivery, Formulation Science, and Pharmaceutical Engineering. Work in Dr. Smyth's lab focuses on the development of novel methods for drug delivery including inhalation, nasal, transdermal, ophthalmic, and oral delivery systems for a variety of diseases. Translation of these technologies to the clinic is the long-term goal of the lab and is supported by developing a mechanistic understanding of the complex physical and biological systems.