Exploration of the Structure and Integrity of Keratin Molecules in Conditions Created by Irradiated Wound Beds

A Technical Report submitted to the Department of Biomedical Engineering

Presented to the Faculty of the School of Engineering and Applied Science
University of Virginia • Charlottesville, Virginia

In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science, School of Engineering

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Spring, 2022

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On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

Lee Drosdak, Sales Manager at Molecular Biologicals

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Abstract

Radiation Dermatitis is a common side effect of radiation therapy, and most commonly results in aggravated and inflamed skin. There is a minority of patients who experience severe symptoms such as skin atrophy, desquamation, and skin ulcers. The current standard of care is simply using corticosteroids. Both radiation and chronic wounds feature stagnated regeneration leading us to investigate repurposing Molecular Biologicals chronic wound healing products. We seek to determine if keratin samples provided by Molecular Biologicals will be able to perform as normal, but in environments simulated to be similar to wound beds of radiation wounds. Our advisors tasked us to test its performance in these minute differences to either support or oppose the continued research and testing of keratin to treat radiation wounds to eventually benefit cancer patients who receive radiation therapy. Our findings were inconclusive due to limitations in lab space and materials, all of which are discussed, but our research into the chemistry and physiology behind these biomaterials allowed us to predict how the materials would perform in these environments. The degradation of keratin in high pH would be slightly higher than keratin in control mixtures and ultimately keratin would improve the healing times for radiation dermatitis. These predictions lead us to support further research and testing of keratin to treat radiation burns and take the next step. We also detailed the types of changes that would be made to the project in the future.

Keywords: Radiation Dermatitis, Keratin, Keratin Dressing, Radiotherapy, Keratin Crosslink

Introduction

What is Radiation Dermatitis

4 million people receive radiation therapy in the United States annually[1]. Of these patients 90% of them suffer from some form of radiation dermatitis[1]. Radiation therapy is the most effective way to prevent the growth of cancerous cells. It damages their DNA, preventing the cells from reproducing. This radiation must be exposed to the skin in order to reach the tumors within the body having a momentous effect on many skin cells. Radiation has the same effect on the skin as it does on tumors. Ionizing radiation causes the creation of reactive nitrogen and oxygen species leading to oxidative damage and increased cytotoxicity in the skin tissue[2]. Apoptosis results in the death of keratinocytes extending the healing process. Another radiation skin injury is radiation fibrosis. Radiation fibrosis is a chronic disease that appears weeks after radiation treatment. This involves an interruption to the natural healing process where the imbalance of proinflammatory and profibrotic cytokines causes a repetitive inflammatory response that leads to major microvascular damage. Transforming growth factor (TGF-β) is one pathway affected by radiation that leads to fibrosis. Mutations in protein signaling pathways lead to overproduction of materials leading to fibrosis (Fig. 1). Overall these radiation-based skin injuries cause a decrease in the quality of life of patients and have virtually no preventive measures. Another effect that the radiation has in lowering the production of key growth factors for regeneration. This is one of the largest deterrents of healing overtime, because the body is proliferating the wrong cells in hope of fostering wound regeneration. Exposure to large amounts of radiation impacts the neovascularization of tissue. An average radiation therapy involves exposing the patient to 45-60 Gy of radiation over the course of a few months. In rat carriers it has been shown that after 10 Gy of exposure there are significantly lower angiogenic factors VEGF[2].

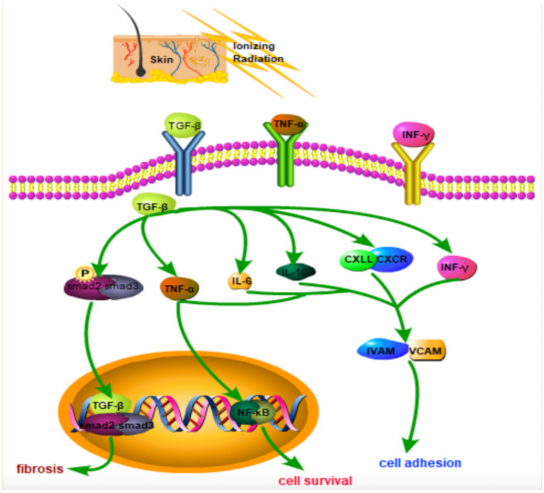

Figure 1 (TGF)-β Signalling Pathway: An example of one pathway overstimulated due to radiation

Current Forms of Treatment

Radiation based skin injuries are currently viewed as an unavoidable consequence of radiation therapy. Currently there are corticosteroids, and soothing gels to treat the side effects of the injury, but there aren't cures being investigated. There has been difficulty figuring out an approach to jumpstart the proliferation of crucial growth factors needed in wound healing. An oxygen chamber has also been used to promote dermal regeneration and can treat wet and dry desquamation[3], but this treatment is costly and very limited in its availability to all patients receiving radiotherapy. All current treatments do not address the increase in programmed cell death of keratinocytes when exposed to large amounts of radiation.

Keratins Impact on Dermal Regeneration

Keratinocytes have a key role especially needed in chronic wounds. It is responsible for producing collagen and forming granulation tissue. In radiation skin wounds there is an inability to reform granular tissue further delaying healing. This is evident in skin ulcers that can take years to heal.[4] Keratin is a biomaterial derived from keratinocytes that has an intricate filament structure with the ability to crosslink via disulfide bonds. Due to its biocompatibility, biodegradability, and versatility for the use of sponges, casts, and hydrogels companies are investigating its biomedical potential in wound healing. Keratin dressings release keratin peptides into the wound. These activate keratinocytes (skin cells) in the wound bed, stimulating them to proliferate and leading to healing of the wound (Fig. 2). Keratin dressings have been reported to reduce the total cost of care and improve quality of life.

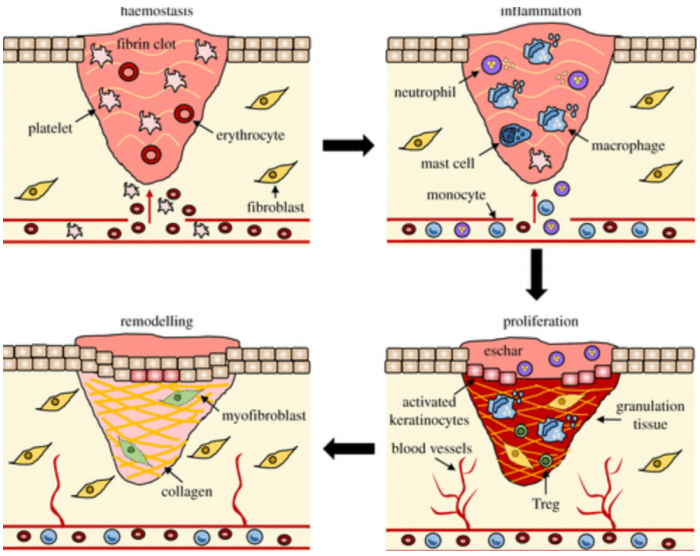

Figure 2: Crucial Steps of Wound Healing Process. Keratinocytes responsible for reestablishing epithelial barrier.

Molecular Biologicals

Keratin is already being used by a few companies, and has been shown that from multiple sources it can be used to heal chronic wounds by jumpstarting the wound healing pathway. Molecular Biologicals uses keratin as a bioactive nanoparticle and has 3 different products. Keragel, KeraGelT, and KeraMatrix. All have their own uses and have shown to rapidly heal chronic wounds (Fig. 3). Keratin dressings release keratin peptides into the wound and activate keratinocytes in the wound bed and lead to proliferation and regeneration of multiple dermal layers, but we were tasked to see if it would be used for radiation-based injuries.

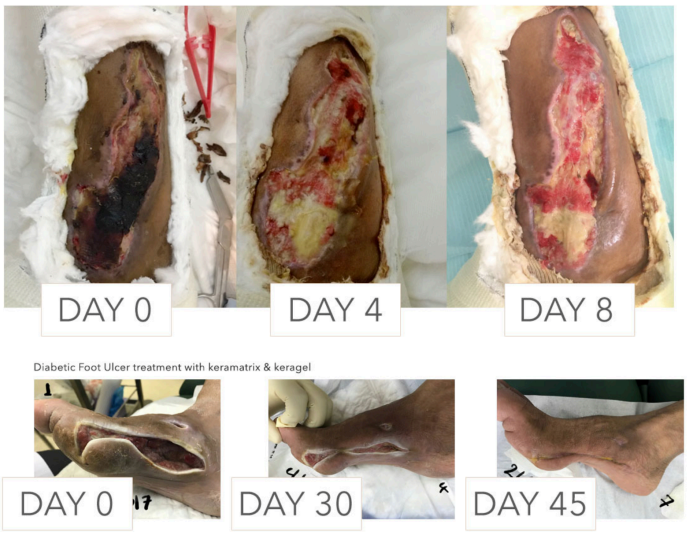

Figure 3: Failed donor skin graft of chronic ulcers on leg and foot. Both of these conditions were classified as chronic wounds that would not heal. This shows the healing process after starting the treatment of keratin on the wound site.

Keratins effect on irradiated wounds

Due to similar issues in the healing process of chronic and radiation wounds, it's absolutely feasible to utilize these products for radiation wounds. However, it becomes necessary to look into the small differences and how that could impact the keratin efficacy as a treatment for radiation wounds. In order to test keratin's efficacy in radiation wounds, testing would be required to support its structural integrity in similar environments. Temperature and pH are the two main differences in the wound bed environment that needed to be tested as a proof of concept. The dysregulation of normal tissue function is caused by waves of radiation and results in higher pH and changes in temperature. Differences between chronic wounds and irradiated wounds are small but have an important influence on keratin structure. Both chronic and Irradiated wounds typically have increases in pH and slowly begin to return to a pH of 6.6-7 as the healing process progresses .[5] Differences in temperature can be attributed to damage to the skin barrier and variations in blood flow and levels of inflammation. Our hypothesis is that the number of crosslinks will decrease in ordinary keratin when placed in conditions similar to radiated wound beds, and that the special OKHP supplied by molecular biologicals will not degrade as rapidly in the same conditions.

Aim 1 - Use electron microscopy to examine the efficiency in keratin crosslink formation and degradation in a variety of conditions. Those conditions being a solution with a pH 8 and 9, a solution with a temperature of 35 and 39 Celsius, and then a solution with no conditions as a control. the number of crosslinks will be recorded 5 times over 24 hours. Data will be extrapolated and an optimal time for maximum crosslink presence will be determined.

Aim 2 - Use an Instron to determine the elastic modulus of keratin. KeraMatrix products from molecular biologicals will be tested to evaluate its structural integrity when subjected to forces that are normally present at radiation therapy sites.

Results

No results were gathered but a discussion of lab procedures, expected results, and alterations to the experiment will be discussed below.

Discussion

Expected Results and Analysis

According to previous literature the line created by the number of crosslinks present will be decreasing at a decreasing rate and indicate an opportune time for reapplication. It is recommended to reapply every 12 hours, but previous literature has indicated that higher pH and a greater temperature degrade keratin fibers more rapidly.[6] With this knowledge in mind, it will be recommended that when using KeraGel on wounds caused by radiation, reapplication should occur every 8 hours for a total of 3 times a day to ensure there are always bioactive keratin molecules present in the wound bed. Data collected by the Instron would indicate that the keratin patch has an elastic modulus lesser than that of the skin located on other parts of the body. This would be expected and accepted due to the fact that irradiated and chronic wounds both display elastic moduli significantly lower than unaffected skin. An elastic modulus recorded to be 20% less than healthy tissue would be acceptable for application in an irradiated wound bed.

Limitations of Lab and sample procurement

Securing lab space while scheduling equipment use and adhering to safety protocols was logistically difficult. The KeraMatrix patch was also not delivered, likely due to a miscommunication between our team and the supplier. So, Aim 2 is no longer prioritized in this project. The KeraGel had similar problems and resulted in only having a powdered sample of High pH Oxidized Keratin (OKHP). The Safety Data Sheet for OKHP indicated high toxicity and limited the ways in which we could test the material due to working in a shared lab space with other undergraduates. The potential benefit of using OKHP is that it is oxidized. This oxidized keratin retains the ability to be placed in a basic solution and make it more neutral, and can be demonstrated by Equations 1 and 2. The free floating OH ions separate, and the oxygen binds to keratin molecules. Meanwhile hydrogen ions are left in solution and begin to make the basic solution more neutral.

Eqn. 1 $\text{Keratin} + \text{Oxidizing agent} \rightarrow \text{Oxidized Keratin}^{+} + e^{-}$

Eqn. 2 $\text{Oxidized Keratin}^{+} + \text{OH}^{-} \rightarrow \text{Keratin} + \text{H}^{+}$

Changes in Experimental Design

After internal corrections in the experimental design it was found the KeraMatrix was never intended to act as a Band-Aid that would move and flex with the skin and body. It was created to lay in the wound bed and dissolve while a secondary bandage and a restraint was used to hold it in the wound. Due to the keratin patch's ability to dissolve, the team suggested that if changes were to be made, the replacement of the toxic OKHP with a keratin patch would be favorable (Fig. 4). Switching these materials would eliminate the need for greater safety precautions, electron microscopy, and decrease the complexity of overall logistics. In this case, a small patch could be placed in solution and be imaged in house. Instructions for the KeraMatrix patch detailed that the patch will need to be replaced every 4-7 days at which point the patch would be fully dissolved. So, the time period of imaging would need to be increased from 24 hours to 7 days.

Figure 4: KeraMatrix developed by Molecular Biologicals. Has no significant toxic effects, and is the new proposed material if changes were made to the experimental design.

Future Studies

Through research and deductive reasoning, it is reasonable for us to recommend that further research and testing is conducted in the application of keratin for irradiated wounds. More rigourous testing would involve directly exposing murine samples to low levels of radiation (30 Gy) in the presence of keratin dressings and comparing rates of healing with keratin and no keratin present. By proactively treating the mice with keratin dressings the experiment can determine if keratin has preventative abilities as well as regenerative abilities.

Materials and Methods

Lab Space and equipment

Provided by UVA BME department with the approval of Timothy Allen. Has many materials and glassware needed to produce the samples for electron microscopy.

Keratin samples and solutions

The proprietary keratin samples supplied directly by Molecular Biologicals. A 20g tube of KeraGel was requested and 5 plates had 1g of the gel smeared onto each one. 10 more grams would be used to repeat 2 more trials and 5g would be left for mistakes or alterations to the procedure. The samples of KeraMatrix were cut into strips with the dimensions of 1cmx5cm.

Electron microscopy

5 solutions were created to be mixed with the keratin samples; 2 basic solutions and 3 solutions of deionized water. The basic solutions were created by adding NaOH to deionized water to achieve pHs of 8 and 9 and then checked with a litmus test. NaOH was chosen as the base because it was abundant and required the least amount of restrictions and regulations while still providing the same reducing aspect required for the solution. The 3 solutions of deionized water were placed in an incubator and kept at temperature of 35, 37, and 39 Celsius. It was important to use deionized water because free floating ions may inadvertently impact the structural stability of the keratin molecules. The dish with deionized water at 37 C was the control. Each one-gram sample was placed in a dish and imaged at t = 0 then one of the five solutions were added to the dish. Images were captured every 3 hours with an electron microscope at the Fontaine Research Center. It was anticipated that the exact timing of the image collection would not be feasible so timestamps would be recorded with each image for precise analysis. The number of crosslinks would be determined by the images captured by Fontaine and be uploaded to Matlab. In Matlab the data would be displayed as a scatter plot with a line of best fit to indicate the total number of crosslinks left intact.

Elastic modulus testing

The elastic modulus would be determined by placing the 5cm strips of KeraMatrix lengthwise in the Instron. The Instron was supplied by the Ideas Lab supervised by Timothy Allen. Screw grips would be used to ensure zero slipping of the keratin patch and the zero-displacement mark was set at the point when the strip had no slack. The amount of slack was determined by pushing against the side of the sample and seeing changes in the strips form. A sample with no slack would return to its original shape and a strip with slack would stay in a slightly curved position. The crosshead limit for the Instron would be 7 cm to allow stretching and eventual failure of the material.

End Matter

Author Contributions

Both authors worked equally in research and design.

The authors declare no conflict of interest.

Acknowledgements

Thanks to Lee Drosdak our Advisor for providing a product of Molecular Biologicals.

Special thanks to Noah Perry and the entire BME department at UVA.

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