Interpenetrating Networks for Delivery Systems

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Abstract

Interpenetrating networks that are composed of gelatin cross-linked with PEG diacrylate provide a promising solution to decrease healing time for large surface area wounds. However, the current reconstitution and administration methods of this product are clinically undesirable. The current method requires 60ºC water. The goal of this project was to create a novel method to reconstitute the components of an interpenetrating network in order to achieve long-term storage and successful IPN application at room temperature. After extensive testing, a final solution of 20mL acetate/citrate buffer, 2g of 90-110 bloom gelatin, and 20mg I-2959 photoinitiator was determined to provide the best dissolution. Alterations were also made in the design of the spray bottle to improve administration techniques.

Introduction

Background

Large surface area and chronic non-healing wounds significantly impair the quality of life for millions of people in the United States (Harding et al, 2002). These wounds are characterized by a loss of skin and underlying tissue which do not heal properly with conventional types of treatment (Falanga, V., 2004). Instead, intensive treatment is required that is costly and requires a lengthy recovery period. Hence, solutions have been investigated to aid and advance the wound healing process. Numerous “bioactive dressings” as well as “skin substitutes” have been created, however few are currently operational in a clinical setting (Harding et al, 2002). Our client, Professor John W. Kao, has created a biocompatible interpenetrating network (IPN) that offers a drug delivery mechanism and promotes healing in large surface area wounds.

This particular interpenetrating network is a mixture of crosslinked polyethylene glycol-diacrylate (PEG-dA) and dissolved gelatin. PEG-dA, as shown in **Figure 1**, is a polymer which can be synthesized in a variety of molecular weights; of which the three most common are 600 Dalton, 2kD, 3.4kD. 600D PEG-dA is a liquid, while the others are a powder. When PEG-dA is added to a photoinitiator and exposed to a UV light, the diacrylate groups crosslink via free radical polymerization (Nakayama, 1999). When PEG-dA is mixed with gelatin and crosslinked, the gelatin becomes entrapped in the PEG-dA.

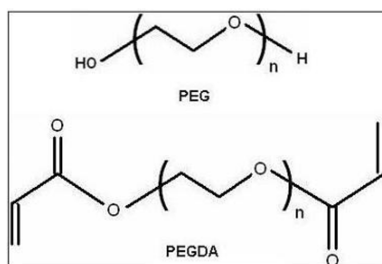

Figure 1: Structure of (top) poly(ethylene glycol) and (bottom) poly(ethylene glycol) diacrylate.

The components from which an IPN is made were carefully chosen by its creator for their desired biological properties. First, PEG-dA is bioinert; meaning that it does not elicit a response from a biological tissue into which it is inserted(Nakayama, 1999). Additionally, gelatin is derived from collagen; a naturally occurring substance in mammals (Rhee, 1999). For this reason, it is biocompatible in solution. When an IPN forms, the photo-polymerized PEG-dA provides a matrix that holds the gelatin. The resulting network provides a perfectly-conforming wound dressing.

Interpenetrating networks are beneficial for healing advancement of large surface area wounds due their physical and chemical properties. First, IPNs are able to cover large surface area wounds that are often irregularly-shaped. The fluid nature of IPNs allows them to properly conform to these irregularly-shaped wounds, promoting rapid and uniform healing. However, IPNs are effective barriers against foreign microbial infections. In addition, IPNs can be created to contain therapeutics in either a solvent form or as a covalent attachment to gelatin (Kao et al, 2003). The drugs are then administered to the patient via diffusion or cleavage, respectively, further aiding in the healing process. Professor Kao's laboratory has obtained positive results in a wound treatment study utilizing IPNs (Kao et al, 2003). However, while IPNs offer an exceptional solution to improved healing time and drug delivery, there are many problems associated with the current administration techniques.

Current Methods

Current IPN preparation and administration methods (**Figure 2**) are only suitable for a laboratory setting. Preparation in a clinical setting has been limited by the necessity for gelatin to be mixed with a heated solvent (at 60 degrees Celsius) for five minutes to ensure complete dissolution. However, in a clinical setting a heating element would not be available, so modifications are necessary. Also, administration methods are inadequate because syringes are currently being used, yet IPNs are intended to treat large surface area wounds. Syringes provide for tedious and uneven administration of the IPN solution. In order to begin using IPNs in a clinical setting, these issues must be resolved.

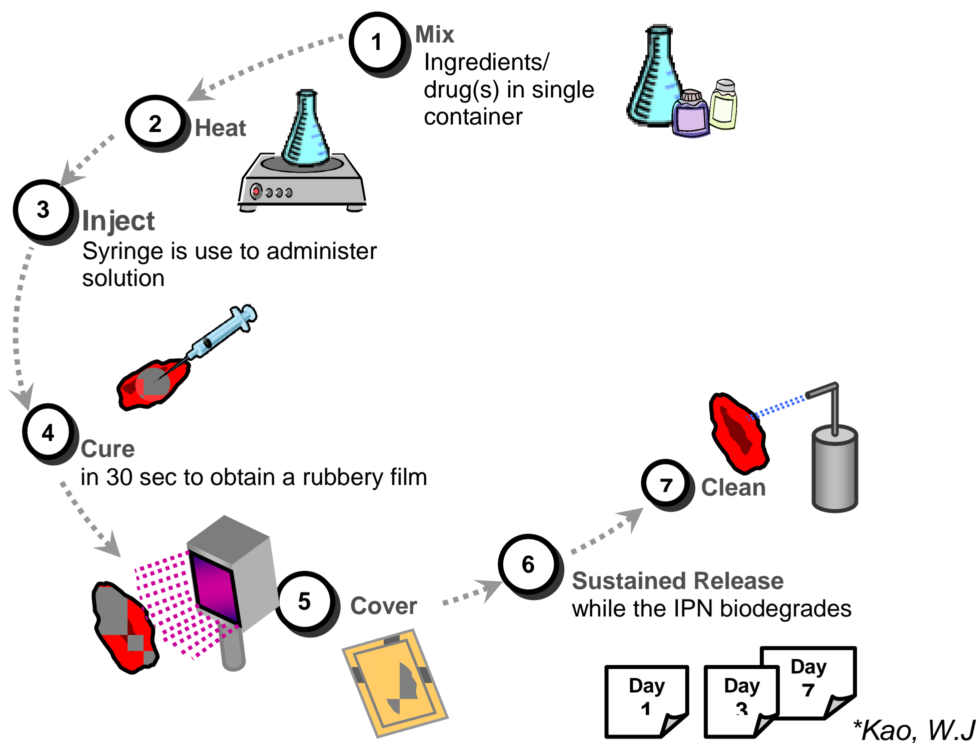

Figure 2 Current method for administering an interpenetrating network to a wound. Main ingredients used include PEGda, gelatin, and a photoinitiator.

Problem Statement

Interpenetrating networks are a type of biomaterials that polymerize in situ and have been used in drug delivery, wound healing, and tissue engineering applications. The goal of our project has been to develop a novel delivery mechanism and create a simple reconstitution method for the components of an interpenetrating network. This design must be suitable for a clinical setting, and the final product must also satisfy the design constraints outlined by Dr. Kao.

Design Constraints

Dr. Kao has instituted several restrictions to our design approaches. The most important restriction to consider is the clinical applicability of the final result. In order for a product to be clinically accepted, it must fit seamlessly into the hospital environment. The utility of our product centers on several factors, including shelf life, the ability to reconstitute each component without the need for additional equipment, and ease of application.

Regarding shelf-life, Dr. Kao has requested that our equipment be one-time use only. Disposable medical equipment is more practical because sterilization is not required after application. Similarly, single use products reduce risk of contamination due to minimized exposure to oxidizing agents and microbial invasion. Overall, the capacity for prolonged storage in a sterile environment could lead to increased product applicability.

In addition, reconstitution is a major barrier of this project because it must be accomplished at room temperature. PEG-dA is a compound that reconstitutes after lyophilization in nearly any water-based environment. Opposingly, gelatin is a thermosetting material. Thermosetting materials strengthen through the addition of energy in the form of heat, so the typical method to reconstitute gelatin is the use of 60ºC water. For this reason it is referred to as a reference solvent. This proposes an interesting predicament for the clinician, since most hospitals do not have readily-accessible 60ºC water. Therefore, our design must

circumvent the problem by modifying the physical properties of gelatin. Reconstitution will be the most important step of application of this product. For that reason, the components must consistently dissolve in entirety.

Another important factor of clinical applicability will be the ease with which our design can be implemented. The reconstitution method must be simple as well as efficient. The entire process must be streamlined and fit seamlessly into the clinical setting. Several complicated steps or a long preparation time could limit the clinical applicability. One way to make this product's use easy will be minimize storage space of the components. Another implementation issue involves the viscosity of the final solution. It must be viscous enough to stay in the area onto which it has been applied, yet not so viscous as to impede spraying. Finally, the time it takes the PEG-dA to crosslink and form an interpenetrating network should be reasonable, with a goal of 60 seconds. This requirement is for the benefit of both the patient and the clinician. In essence, the quicker and easier the IPN can be applied, the better.

Implementation of this product will ultimately hinge on whether it is accepted by the medical community as an efficient and beneficial treatment to its intended wounds. By making the application of the IPN as simple as possible, we can greatly increase the probability of a successfully marketed product.

Competing Products

In the past decade, several advances have been achieved in wound care. The use of silver nitrate dressings has declined as new products have been introduced. Each product addresses the shortcomings of silver nitrate in a different manner.

The first competing product still uses silver as a method to prevent infection. This group of alternatives is known as silver coated wound dressings. They rely on ionic silver to serve as a broad spectrum anti-microbial agent. They protect against both gram positive and gram negative bacteria (Dowsett, 2004). They have also proven to be effective against bacteria that are considered resistant to other methods; currently there are no known strains of bacteria that are resistant to silver ions (Lansdown, 2002). These products have become popular due to recent advances which allow both a fast and sustained release of ionic silver. Dressings in this class are available under several different brand names, such as Actisorb®, Acticoat®, and Contreet®. Aside from the stated benefits, these dressings have shortcomings: they are applied topically, and they fail to penetrate deep into the wound bed.

The second class of alternatives are skin substitutes. These dressings come in several forms - either bioactive or bioinert, collagen derived, or completely synthetic. Skin substitutes contain a scaffold onto which a variety of chemicals can be incorporated. Bioactive skin substitutes contain a variety of growth factors and promote cell growth onto the scaffold. TransCyte® is one such product that contains human dermal fibroblasts on a nylon mesh. This product is a stop-gap measure to be used mostly in burn patients, until a skin-graft becomes available. Another type of dressing is marketed under the name Orcel®. This product is a collagen-derived skin replacement. The most advanced version of this technology is known as Dermagraft®. It is bioactive and uses a polyglactin mesh scaffold to provide the building blocks for a three-dimensional skin substitute. It also contains growth factors as well as human collagen, promoting neogenesis. Dressings of this nature are usually applied topically. TransCyte® is cryogenically frozen and needs to be thawed before its application (Ehrenreich, 2006).

Dr. Kao's IPN does not contain silver; however, it will support the incorporation of different antimicrobial agents, as well as growth factors. It is also capable of infiltrating deep into the wound bed and providing a moist healing environment. This is facilitated by spraying the product directly onto the wound and then curing under a UV light - allowing it to act first as a liquid and then as a solid. We believe that our product incorporates and improves upon each product mentioned above.

Ethical Considerations

Ethics are of utmost importance in our design. First and foremost, the product must be safe and effective to minimize patient risk, regardless of any marketing possibilities. Similarly, it is suggested that consent is given for the application of the IPN, that healthcare professionals are aware of the constituents, and that they have been trained in the methodology for reconstitution. Lastly, ethical considerations will be made during any animal experimentation or clinical trials that maybe necessary.

Design Approach

After thoroughly considering two different design approaches, our design matrix (see appendix) indicated that we should pursue this project from a research perspective. Through a litany of literature research, we discovered that gelatin does not readily dissolve in room temperature water, PEG-dA readily goes into aqueous solution, and not all photoinitators are soluble in water. However, further research revealed that I-2959 is a biologically compatible, water soluble photoinitiator; thus it was established that it would be included in our final recipe. Hence, gelatin was determined to be the limiting factor in dissolution and our testing directed toward achieving gelatin dissolution at room temperature. The optimal gelatin concentration,

gelatin bloom strength, solvent, surfactant, and mixing method needed to be determined. These research factors are shown in the following flow chart.

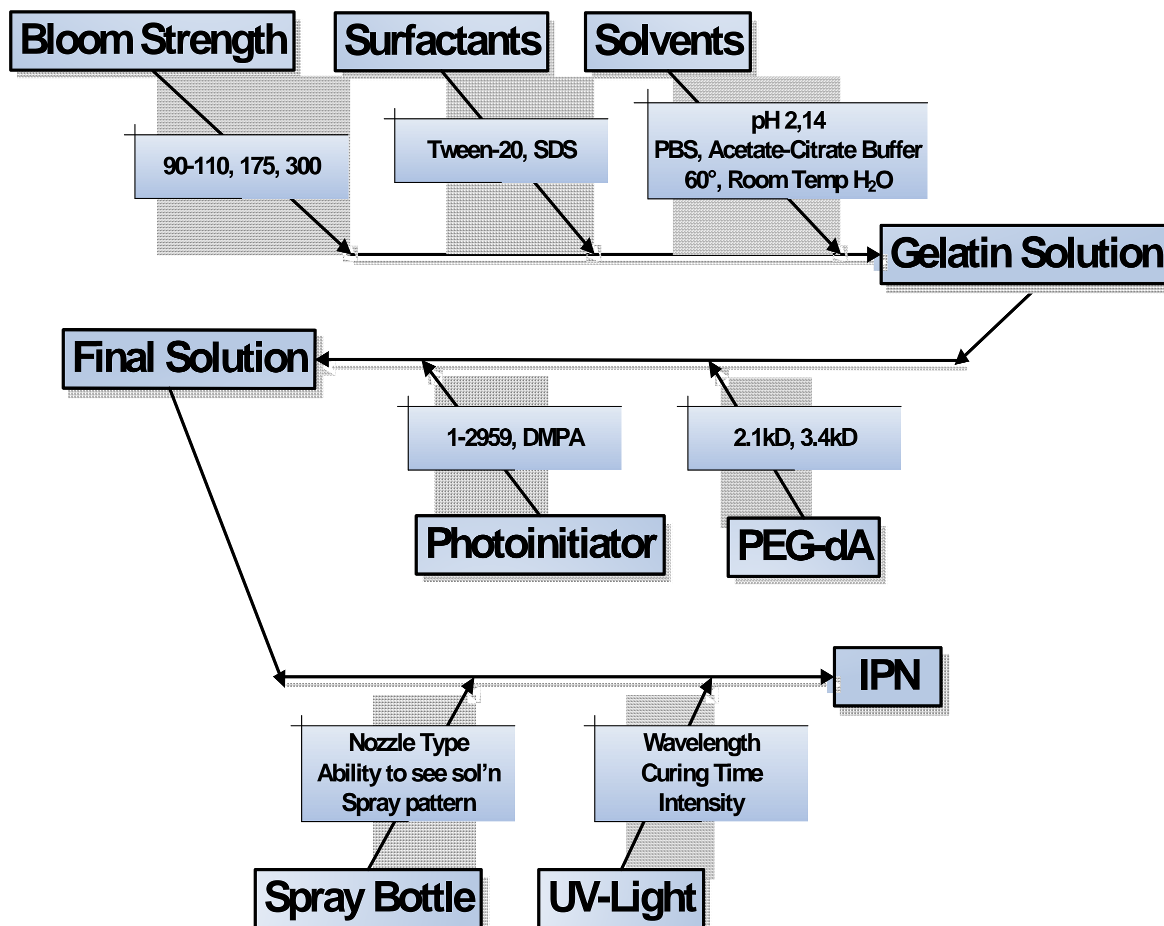

Figure 3: The flow chart suggests the different factors and their respective options that were considered for the final product design.

General Research Proceedings

The general progression of our research was net-like in fashion, since acquisition of new insight often involved revisiting previous variables. Since the IPN composition currently in use by Dr. Kao consists of 300 bloom type A gelatin and was readily available, 300 bloom gelatin was initially used to determine an optimal concentration for further testing. Upon selecting an appropriate concentration using the reference solvent, further tests focused on other solvents to

dissolve gelatin at room temperature. Since literature research indicated that surfactants are capable of decreasing surface tension and can promote dissolution. A common surfactant, Tween 20, was used to assess the possibility for surfactants to promote further dissolution. At this point it was deemed necessary to quantify our data, thus UV-vis. spectrophotometry was used to compare dissolution to the reference. Then it was brought to our attention by Dr. Kao that gelatins of different bloom strengths may behave differently under identical conditions. Two additional bloom strengths of type A gelatin were purchased and all initial experiments were repeated.

Once a final recipe was determined other design constraints could be addressed. To assess how well these constraints had been met the final recipe was used to synthesize multiple IPNs. Final considerations included spray bottle design, mixing technique, and packaging.

Results and Discussion

As indicated in the methods section, several factors went into the final composition. Our generalized research approach can be found in the appendix; however, results below describe our progression towards the final composition.

Time until dissolution was monitored for varying concentrations (100, 150, 200, & 250 mg/mL) of 300 bloom gelatin in 60° Celsius water, as shown in **Table 1**. A ten percent weight concentration was set as the minimum concentration, based on design constraints. Solutions were given 5 minutes to dissolve, after which qualitative analysis on their dissolution was noted. Since the test showed that dissolution only occurred at concentrations near this minimum value, only 10% and 15% gelatin solutions (shown in blue) were concluded to be viable concentrations for subsequent experiments.

Table 1: The table below shows which concentrations were considered acceptably dissolved at a constant temperature of 60° Celsius. These concentrations are indicated in blue. It also shows the physical characteristics of the final solution, which served as a qualitative baseline for assessing optimal dissolution in subsequent testing.

Weight % Gelatin (g/mL)	Dissolution Time (min)	Physical Characteristics of Final Solution
10	3	clear gel, dissolved completely
15	3	clear gel, dissolved completely
20	No dissolution	not thoroughly dissolved, gelled quickly
25	No dissolution	not thoroughly dissolved, cream-colored

Solvent Effects on Gelatin Dissolution at Room Temperature

Since the design constraints oppose the use of heat to aid in dissolution, other stimuli that could promote dissolution were considered. With both 10 and 15% weight percent and using 300 bloom gelatin solutions, varying pHs ranging from 1-4 and 10-13 and different biological buffers were tested. The pH standards were created in separate vials by adding 1M HCl or 1M NaOH to deionized water. The initial pHs of these standards and the biological buffers were recorded prior to mixing. Similarly, for all trials the final solutions' pHs were measured and the change in pH from the solvents original value was calculated. **Table 2** depicts the effects of pH variation and biological buffers on gelatin interactions. The rating scale used to judge extent of dissolution was as follows: (+++) represents full dissolution, (++) represents partial dissolution, (+) represents dispersion, and (--) represents phase separated solution.

Table 2: Dissolution of 10 and 15% gelatin was tested at pHs from 1-4 and 10-13 and in five different buffers at room temperature. The extent of dissolution was rated as (+++), (++), (+), or (--), and the change in pH was also noted after 5 minutes. The rows highlighted in blue are the most positive results.

Weight Percent	Buffer	initial pH	final pH	Δ pH	Dissolution	
10%	N/A	1.1	6	4.9	+++	pH Testing
15%	N/A	1.1	6	4.9	+++	
10%	N/A	2.1	5	2.9	+	
15%	N/A	2.1	5	2.9	+	
10%	N/A	3.1	5.5	2.4	+	
15%	N/A	3.1	5.5	2.4	+	
10%	N/A	4.1	5	0.9	+	
15%	N/A	4.1	5	0.9	+	
10%	N/A	10	6	4	+	
15%	N/A	10	6	4	+	
10%	N/A	11	6	5	++	
15%	N/A	11	6	5	+	
10%	N/A	12	7	5	++	
15%	N/A	12	7	5	++	
10%	N/A	13	12	1	+++	
15%	N/A	13	12	1	+++	
Weight Percent	Buffer	initial pH	final pH	Δ pH	Dissolution	Biological Buffer Testing
10%	MES	5	6	1	-	
15%	MES	5	6	1	-	
10%	Acetate/Citrate	6	6.5	0.5	++	
15%	Acetate/Citrate	6	6.5	0.5	++	
10%	Phosphate	7	7	0	+	
15%	Phosphate	7	7	0	--	
10%	Tris	8	8	0	-	
15%	Tris	8	8	0	-	
10%	HEPES	8.5	7.5	1	+	
15%	HEPES	8.5	7.5	1	+	

Table Key:

+++ represents full dissolution ++ represents partial dissolution
+ represents a dispersion -- represents phase separated solution

As shown above, the most promising results included the extreme pHs and the acetate/citrate buffer. However, a color change was also observed with the extreme pHs which may be indicative of gelatin's further denaturation. This renders gelatin bioinactive, so further

experimentation with extreme pHs was ruled out for the time being. Similarly, acetate/citrate buffer showed very promising results by promoting dissolution. However, the final solution remained at a biological pH which meets design constraints. Thus, subsequent experiments sought to quantify and maximize dissolution using acetate/citrate buffer.

The tests also revealed that gelatin may have a buffering capacity, as addition of gelatin to any given solvent usually brought the pH closer to 7.0 from either extreme. This tendency is a useful property, since the final solution must be biocompatible at a reasonable pH. Through this property, it became more permissible to use a slightly broader range of pHs with the assumption that adding gelatin would bring the final solution to a more neutral pH.

Surfactant Testing

Since surfactants are known to decrease surface tension and promote dissolution, a small aliquot of Tween-20 was added to a 10% acetate/citrate buffer and gelatin solution to assess whether or not it promoted further dissolution. It seemed to have no effect on the rate or extent of dissolution, so further testing was deemed impractical. SDS was also considered but ultimately disregarded because of its controversial degree of biocompatibility.

Effect of Bloom Strength on Dissolution

Several factors had been tested on the original 300 Bloom gelatin with acetate/citrate buffer achieving the best overall results in promoting dissolution. The next step was to consider different types of gelatin, so two additional Bloom strengths were compared to the original, 300 Bloom gelatin and rate/extent of dissolution was assessed. To judge extent of dissolution the samples were compared to the reference using the scale outlined in previous tests.

Table 3 below summarizes the results of this test, and once again, acetate/citrate buffer showed optimal dissolution, given the design constraints outlined by Professor Kao.

Table 3: 10% solutions differing Bloom strengths were made up in all of the previously-tested solvents to determine the effect on gelatin's dissolution. The highlighted values show the best results.

Solvent	300 Bloom	175 Bloom	90-125 Bloom
60°C Water	+++	+++	+++
Room Temp. Water	--	--	--
PBS Buffer	--	--	--
Acetate/Citrate Buffer	++	++	+++
HEPES Buffer	+	+	+
Tris Buffer	--	--	--
MES Buffer	--	--	--
pH 14	+++	+++	+++
pH 13	+++	+++	+++
ph12	++	++	++
pH 11	++	++	++
pH 10	+	+	+
pH9.4	+	+	+
pH 9.1	+	+	+
pH 8.2	+	+	+
pH 7	--	--	--
pH 5	+	+	+
pH 4	+	+	+
pH 3.1	+	+	+
pH 2.2	+	++	++
pH 1	+++	+++	+++

Table Key:

+++ represents full dissolution ++ represents partial dissolution
+ represents a dispersion -- represents phase separated solution

Based on these results, 90-110 Bloom gelatin in acetate/citrate buffer seemed to be a qualitatively viable solution for the final IPN recipe. The results can be supported by the **Figure 4** which shows some of the final gelatin solutions after they had been mixed for five minutes.

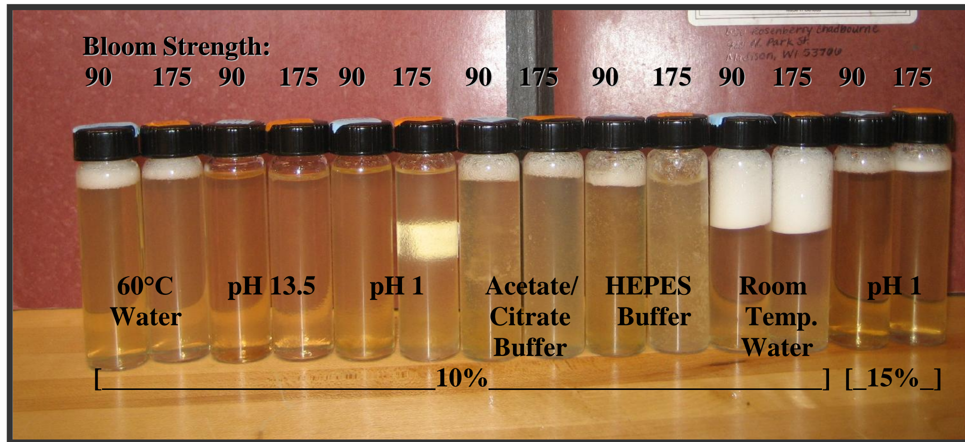

Figure 4: Pictures were routinely taken of each solution vial after 5 minutes. This procedure set a standard for a qualitative rating system. As the figure shows, the extreme pHs at both 10% and 15% showed the most transparent and most dissolved solutions. However, the acetate/citrate buffer at 10% showed positive results, especially after several minutes of being in solution. At this point, the gelatin swelled significantly, and the overall solution remarkably resembled that of gelatin in 60°C water.

These vials were then stored for an indeterminate amount of time, during which it was observed that the vials containing acetate/citrate buffer seemed to increasingly resemble those vials containing reference solvent (gelatin with 60°C water). This was more closely monitored in a subsequent trial, where the claim was supported. It seemed that the lower bloom strengths of gelatin mixed with acetate/citrate buffer swelled over a half hour time period, and the resulting solutions bore a close resemblance to the reference solution.

Quantitative Verification Using UV-Vis. Spectrophotometry

UV-vis. Spectrophotometry was used to help verify qualitative interpretation of dissolution in a quantitative manner. A combination of pHs and buffers were tested and absorbance values were compared to the reference's absorbance. All absorbance readings were done at 200 nm (established with a wavelength scan) and measured initially and again after 5 and 10 minutes had passed. **Table 4** below shows the results of this assay.

Table 4: Absorbance readings were taken at 200 nm immediately after mixing and then again after 5 and 10 minutes had passed. These readings were then compared to the absorbance of water after each time period. As shown, acetate/citrate buffer solution was consistently close to those solutions containing 60°C water.

Absorbance	Initial	ΔAbs. With H_2O	5 min	ΔAbs. with H_2O	10 min	ΔAbs. with H_2O
60°C H20	0.533	0	0.416	0	0.392	0
Acetate/Citrate buffer	0.519	0.014	0.397	0.013	0.496	-0.106
pH 6	0.445	0.088	0.478	-0.068	0.564	-0.174
pH 9	0.557	-0.044	0.46	-0.05	0.464	-0.074
HEPES buffer	0.445	0.088	0.64	-0.224	0.472	-0.08
pH8	0.425	0.108	0.509	-0.093	0.517	-0.127

These results coincided with the qualitative results previously determined, so dissolution of varying Bloom strengths were then compared using the same method.

Furthermore, based on the results of previous experiments, 90-110 or 300 bloom strength gelatin was added to either acetate/citrate buffer or 60° water and UV-vis. Spectrophotometry was used to assess the dissolution. Absorbance values were read at 200 nm, each minute, over a 5 minute period. To analyze our results the percent absorbance difference between the control for each bloom strength and the citrate solution were calculated and compared against time, as seen in **Figure 5.** As shown, the lower Bloom gelatin showed very similar readings to an ideal gel in 60°C water.

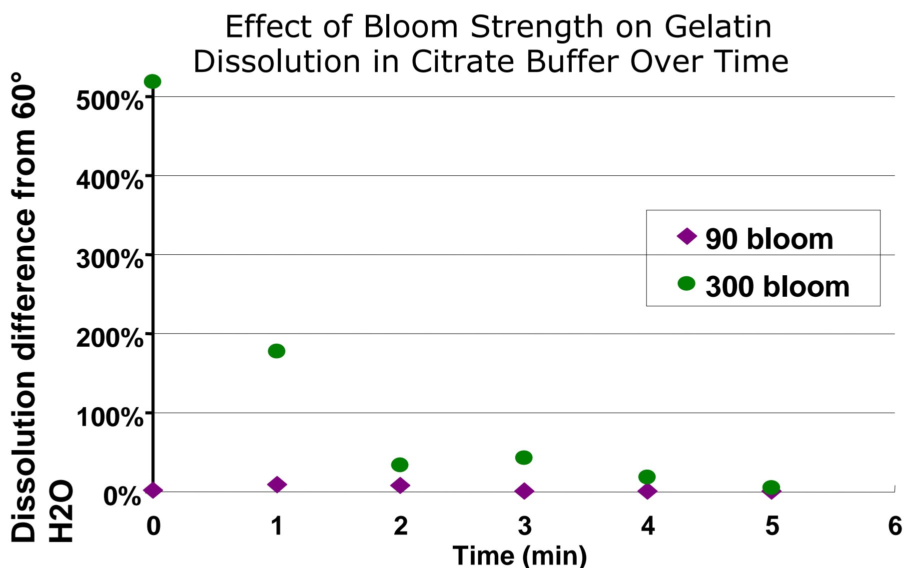

Figure 5: The highest and lowest gelatin Bloom strengths were added to acetate/citrate buffer, and dissolution was evaluated by measuring the absorbance of 200 nm light passing through a cuvette containing the gel solution. Readings of each solution were taken every minute along with absorbance readings of 300 bloom gelatin in 60 degree Celsius water as a control. The percent absorbance difference was then calculated between the control and the citrate solution for each Bloom strength and plotted against time. As shown, the higher Bloom gelatin varies considerably in its absorbance when compared to absorbance in an aqueous solution, despite the fact that the two solutions share similar absorbance readings after five minutes. By contrast, 90-125 Bloom gelatin consistently mirrors the absorbance readings in aqueous solution, and, therefore, its dissolution can be concluded to be very similar as well.

Creation of Full Interpenetrating Networks

To support optimal gelatin dissolution, the final step was to create a full IPN, using our components, which cured under UV light in a reasonable amount of time. The five sample IPNs made cured in an average of 3.5 minutes and afforded a final gel that resembled the sample in **Figure 6**.

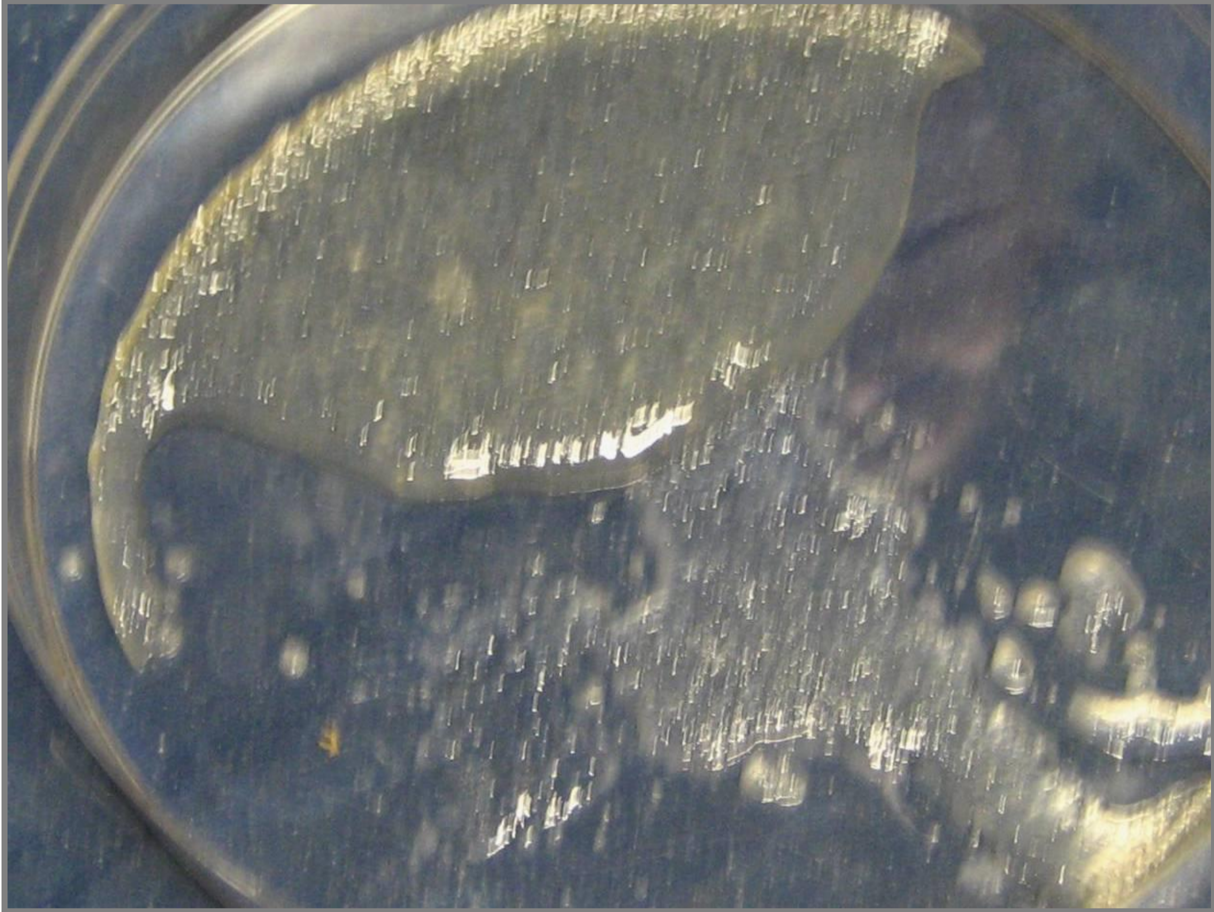

Figure 6: This image represents an IPN solution after it was sprayed/poured into a Petri dish. After 3-4 minutes, the solution became a solid gel.

Some of the gelatin particles were still visible in the final gel; however, dissolution was not reversible over time. The final gel was of a rubber-like consistency with a slight sol fraction noticeable on the bottom surface of the gel. After three days' incubation in a commercial-grade refrigerator, the gel did not seem to degrade at all.

Design Modifications for Product Administration

In addition to research components of the project, modifications to the spray bottle have been developed in order to maximize clinical applicability and gelatin dissolution. The current design consists of one spray bottle containing pre-measured gelatin, PEG-dA, and I-2959 and a

vial containing pre-measured citrate buffer solution. The pre-measured quantities provide a streamlined mixing procedure, as the citrate buffer would simply be added to the spray bottle. In order to enhance shelf life, the spray bottle containing I-2959 must have an opaque coating to prevent photoinitiator polymerization. Oppositely, the medical personnel mixing the IPN components must be able to visually verify complete dissolution. Facilitating both storage and mixing requirements a dissolution strip with a removable sticker has been created (**Figure 7**). Additionally, a clear bottom will provide a second mode of dissolution visibility without compromising the photoinitiator. Furthermore, the spray bottle has a wide diameter which increases surface area exposed to the citrate solvent during initial mixing process and facilitates. Lastly, the spray bottle straw has a wide diameter which allows for a smooth spray distribution.

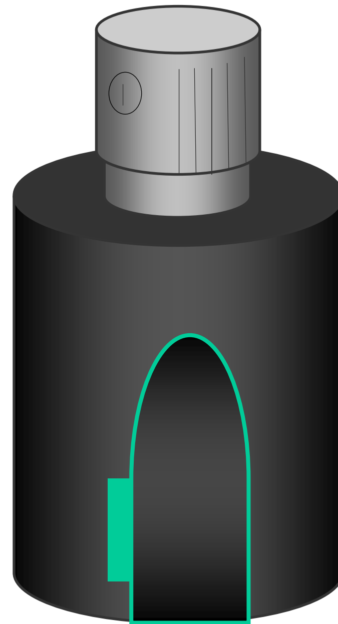

Figure 7: Opaque spray bottle with dissolution strip and removable sticker

Future Work

Further efforts toward optimal reconstitution of the components of Dr. Kao's IPN should be made to help incorporate his product into medical facilities, upon FDA approval. Future tasks include additional testing as well as spray bottle modifications.

Determination of the sol fraction throughout the curing process will quantitatively evaluate the interactions of the IPN components at room temperature in citrate buffer. Based on these results, an alternative direction to research could be utilized. A proposed approach would

consist of dissolving gelatin in an extreme pH followed by dialysis to neutralize the solution. Subsequently, the solution will be lyophilized to return the gelatin to a powder form. This powder would hypothetically consist of gelatin fragments that could be reconstituted with standard (non-fragmented) gelatin in a solvent. This mix of fragmented and standard gelatin will likely enhance extent of gelatin dissolution.

Design modifications will include an all-in-one packaging spray bottle and a stir-stick straw. The all-in-one packaging (**Figure 8**) will confine all IPN components to a single bottle which will reduce: potential human error, cost, and sterility concerns due to multiple packages. By housing a stirring mechanism within the bottle (**Figure 8**) the sterility of the system can be maintained and the solution can be mixed more effectively than by shaking alone.

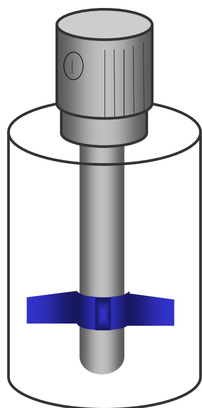

Figure 9
Stir-stick straw

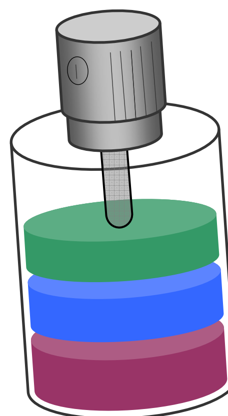

Figure 8:
All-in-one packaging

Conclusions

Interpenetrating networks offer a novel solution to wound care, especially of large surface wounds, and these research results and design solutions offer an effective means for administration of this product. Several components of the existing design were analyzed and modified to streamline the product and maximize its performance. Most importantly, gelatin

dissolution was accomplished using 90-110 Bloom gelatin in acetate/citrate buffer at room temperature. Thus, the final recipe contained one equivalent of gelatin, one equivalent of PEG-dA, ten equivalents of acetate/citrate buffer, and a 1% solution of I-2959 photoinitiator. Additionally, the spray bottle design was modified to accomplish optimal dissolution and spraying capacity. Tests concluded that a wide-diameter bottle with a wide-diameter spray tube met both of these objectives. Although some future work is proposed to further enhance and optimize the design of the final IPN product, the modified recipe and bottle design offers a clinically-applicable solution that meets each of the identified design goals for this product.

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APPENDIX

Design Matrix

In order to decide which approach will be pursued, a design matrix was compiled. Categories were created and weighted between five and fifteen points based on importance to the problem statement. The two approaches were then compared with each other in order to provide a numerical value for each category. Although many categories had comparable values, client preference, feasibility, and cost were the aspects of separation. Although the heating element provides a more feasible approach because the necessary components currently exist, the cost added and client preference outweighed the heating element approach. Overall, the design matrix favored the laboratory research which will be the approach pursued for this project.

Criteria	Weight	Heating Element	Research
Client Preference	15	4	15
Feasibility	15	12	8
Viscosity	15	10	7
Reconstitution Time	10	7	9
Safety	10	6	6
Cure Time	10	9	9
Mixing Errors	10	8	7
Sterility	5	5	5
Shelf Life	5	5	4
Cost	5	1	4
TOTAL	100	67	**74**

Summary Chart of Components Tested

Gelatin	Solvent	Surfactant	Solution Concentration	PEG-dA	Ratio of PEG-dA to Gelatin	Photoinitiator	Spray Bottle Design	Spray Nozzle Design
300 Bloom	PBS Buffer	Tween-20	10%	600	50/50	DMPA	Narrow diameter tube, no stirring	Thin Diameter Tube
175 Bloom	Acetate/Citrate Buffer		15%	2K		I-2959	Wide diameter tube, no stirring	Thick Diameter Tube, notched bottom
90-125 Bloom	HEPES Buffer			3.4K			Narrow diameter tube, stirring	
	Tris Buffer						Wide diameter tube, stirring	
	MES Buffer							
	60°C Water							
	Room Temp. Water							
	pH 14							
	pH 13							
	ph12							
	pH 11							
	pH 10							
	pH9.4							
	pH 9.1							
	pH 8.2							
	pH 7							
	pH 5							
	pH 4							
	pH 3.1							
	pH 2.2							
	pH 1							

90-125 Bloom gelatin and Acetate/citrate buffer in a 10% solution with no additional surfactant was added to 3.4K PEG-dA and I-2959 using a 50/50 PEG-dA to gelatin ratio. This powder solution was contained in a wide-diameter tube and mixed using a stirrer/spatula before it was ultimately sprayed through a wide-diameter sprayer. The solution was then cured under UV light for 3-4 minutes until it became a solid interpenetrating network gel.