Interpenetrating Networks for Delivery Systems

Team Members

Claire Flanagan
Ashley Huth
Adam Rieves
John Sass

Advisor

Professor Naomi Chesler, PhD
Department of Biomedical Engineering

Client

Professor John Kao, PhD
UW School of Pharmacy &
Department of Biomedical Engineering

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. Three possible designs were proposed to optimize the delivery of the IPN. These designs were a modified syringe, a pressurized spray bottle, and a spray bottle. A design matrix was created and the syringe was determined to be the most promising design to date. Laboratory research will complement the development of this prototype to ensure full dissolution and proper delivery of the IPN.

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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.

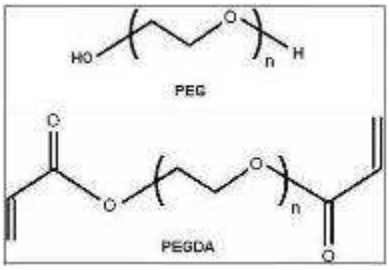


Figure 1: Structure of (top) poly(ethylene glycol) and (bottom) poly(ethylene glycol) diacrylate.

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.

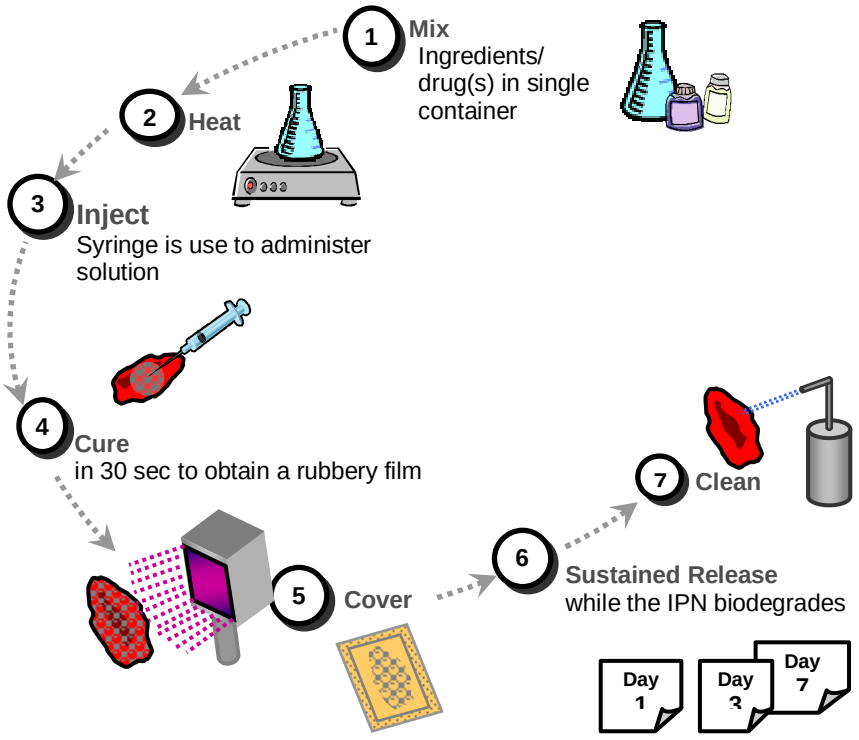


Figure 2 Current method for administering an interpenetrating network to a wound. Main ingredients used include PEGda, gelatin, and a photoinitiator.

Previous Semester

Thus far, our research has focused on eliminating the heating step involved in the aforementioned IPN administration procedure. Since gelatin was deemed the limiting factor in dissolution of the IPN components devoid of heat, we focused on the dissolution of gelatin at room temperature. Experiments involved varying the bloom strength and concentration of the gelatin, as well as the solvent used. In the end, gelatin dissolution was accomplished using 90-

110 Bloom gelatin in acetate/citrate buffer at room temperature. 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 considered to ensure optimal dissolution and spraying capacity. However, at the end of the semester it was evident that further work could be done to streamline and improve upon the administration technique.

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 is to develop a novel delivery mechanism and create a simple reconstitution method for the components of an interpenetrating network. Moreover, the focus of our project will be to develop a novel administration and packaging system to store, reconstitute, and apply IPNs. This design must be suitable for a clinical setting, and the final product must also satisfy the design constraints outlined by the client.

Design Constraints

Our client has delineated several criteria for achieving an optimal product design. In coordination with our objectives last semester, the most important guideline is that the IPN administration mechanism must fit seamlessly into a clinical environment. This goal will be achieved by meeting several, more pointed objectives that will lead to a product that requires minimal preparation and effort to administer the IPN.

To ensure even application of the IPN, several objectives that incorporate both research and design components must be met. Within the research realm, the final IPN solution prior to

curing must be homogenous, with each constituent adequately dissolved. Additionally, dissolution must be able to be achieved by establishing a reliable mixing mechanism within the product that can be actuated efficiently. Finally, the device must safely emit the IPN solution in an even spray that requires little effort on the part of the user. In short, the product must provide a way to produce a sustained and uniform spray that can cover a large surface area.

Furthermore, the design of the product must involve some sort of compartmentalization in order to maintain sterility and improve shelf life. Moist gelatin is prone to microbial invasion, so it must be stored separately from the liquid components. Also the solution is not stable if it is exposed to light, so the product must be able to selectively shield the UV-sensitive photoinitiator. Yet, the design must also provide some visibility to the final solution so that the user can monitor dissolution.

In the interest of shelf-life, our client has requested that our equipment be one-time use only. Disposable medical equipment is more practical because after application sterilization is not required. In particular, components that can be individually sterilized prior to packaging are necessary. Overall, the capacity for prolonged storage in a sterile environment could lead to increased product applicability.

Finally, the product must be as cost-effective as possible. This, of course, will open up more market potential and may help justify the incorporation of high-cost pharmaceuticals into the IPN. The use of minimal parts and prefabricated components would largely aid in this objective, as would parts of varying sizes that could be used to treat different wound sizes. The final price will be reflected in making the final product as simple yet versatile as possible, as will its overall clinical applicability.

In general, the success or failure 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 taking the above design constraints into consideration, we can greatly increase the probability of it becoming a successfully marketed product.

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.

Competing Products

This semester the focus of the design is primarily on the delivery mechanism of the IPN. As such, products competing with the final design will be those which require separation of liquid and powder until shortly before use. In medicine several pharmaceuticals are stored in a lyophilized form and must be reconstituted before use. The majority of these drugs are stored in separate vials (one containing liquid, one containing powder). The standard procedure to reconstitute pharmaceuticals is to inject the liquid from its vial to the vial containing the powder, mix the solution by hand, and then use another syringe to draw up the mixture for delivery. These systems require two syringes and leave room for error in mixing. Pharmaceutical companies overfill the liquid vial by as much as 35% (Renoylds, 2007) to ensure full dissolution.

Since these systems are inadequate several products have been created. The West Pharmaceuticals Services' Needleless Transfer Device is an adaptor which connects both vials and has a port which allows a syringe to draw up the mixed solution. This system requires only one syringe, and is one time use only. A drawback of this system, however, is that it requires the liquid portion be stored under vacuum (Needleless Transfer Device product brochure). The Inter-Vial™ system by Duoject® is a syringe/vial adaptor in which the custom syringe is prefilled with diluent and can draw up the pharmaceutical. Products which require separation of liquid from powder are not limited to the medical field alone but even include instant baby formula. Because of its short life after reconstitution the UMix™ baby bottle was created. This product, as described in **Figure 3**, stores the powder in the upper chamber and the liquid in the bottom until the product is ready to use. Although there are other products available, the device we develop will improve upon the shortcomings of the competition and fulfill its own niche.

Fig. 3 The U-Mix Baby Bottle and instructions for use

Syringe Design

Design Summary:

The design alternative which represents the smallest departure from the current method would be to use a modified syringe. In this design the liquid portion of the IPN would be stored in a custom chamber housed in the plunger, and the powder in the barrel. There is a release mechanism similar to a retractable ball-point pen which allows the liquid to fall through a hole into the barrel, where mixing will occur. The stopper on the release mechanism has three positions in which it can reside. The natural

state of the stopper is plugging the opening in the plunger tip. The second state is when the button has been "clicked" once, and the plug is retracted, allowing the liquid to flow. After liquid release, the button returns to the natural position and the solution can be hand shaken. The final state occurs while the IPN is being delivered. In this state the button is fully depressed and forces the plug further into the hole, preventing any of the liquid from backing-up into the plunger.

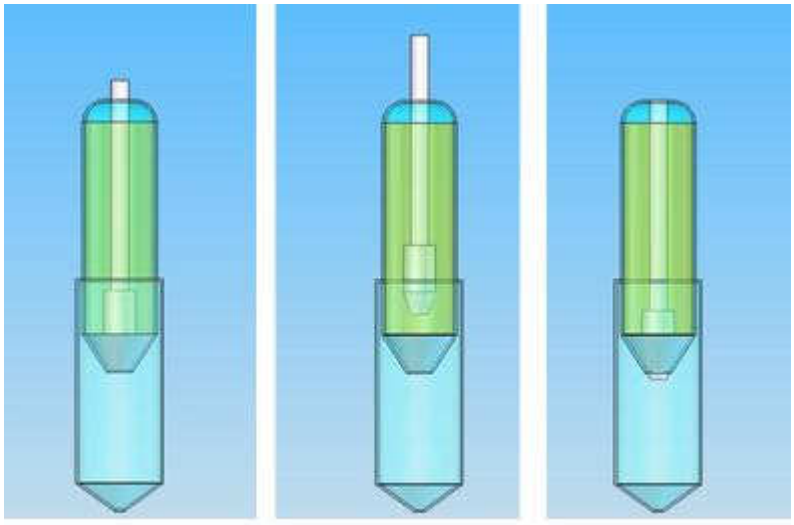


Fig. 4 The syringe design in three positions.
The second position releases the liquid portion of the IPN.

This feature will allow the IPN to be delivered from any orientation. To achieve a fine mist during delivery, an atomizer will be attached to the luer lock connection on the tip of the syringe. Manual pressure applied to the plunger of the syringe will effect spraying.

Pros:

There are several advantages to a design such as this. The principal advantage is the mixing method. The method to release the liquid into the powder is a familiar one for the clinician, and would require little training to properly operate. Another advantage of this system is the ease with which the IPN can be sprayed. The average human hand can create 35N of force between the thumb and forefinger (Human Performances Capabilities, p. 113; NASA-STD-3000 203). That force applied to a small opening can generate a sufficient amount of pressure to dispense the IPN. Another advantage of this system is the ability to easily adapt production to different wound sizes. Syringes come in varying sizes, and the formula can be increased or

decreased to accommodate the size of the wound. This would reduce wasted product or reduce the need to use several packages on one wound, thereby increasing its cost-effectiveness.

Cons:

The system is not without its disadvantages, however. The main issue is the necessity for custom manufacture of the plunger. This could raise the cost of the overall product, and adds another road-block to marketing the product. Also, the release mechanism has moving parts which could malfunction. The photoinitiator needs to be protected from light before the product is to be used. The packaging will shield the product from UV-light, but after opening the syringe and its contents will be exposed.

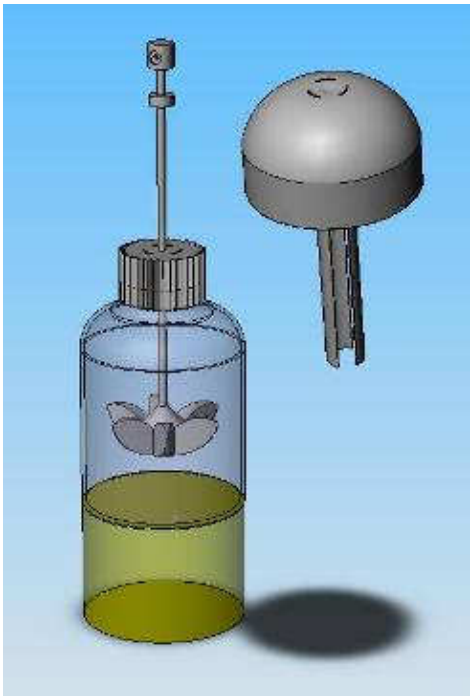


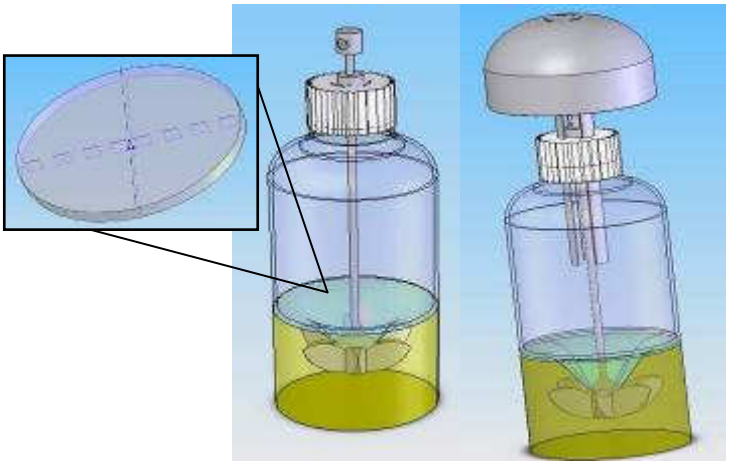
Fig. 5 The pressurized bottle design prior to puncturing the perforated seal.

Design 2: Pressurized Bottle

Design Summary:

The pressurized bottle design aims to maximize efficiency of IPN application. The overall design builds off of a previously-developed aerosol spray bottle (Nalgene™, Fischer Scientific), with a few modifications tailored to accommodate the IPN solution **(Figure 5).**

In this design, a plastic bottle houses both solid and liquid components in two chambers that are separated by a perforated seal (**Figure 6**). The seal is punctured by the process described in **Figure 6** by means of the modified spray straw. After puncture, mixing takes place through a unique feature of the straw/cap design, which mimics a childproof medication bottle. Once the cap is screwed on, it can only be unscrewed by pushing down on the cap while



simultaneously unscrewing it, so the cap can then be rotated counterclockwise indefinitely without affecting the extent to which the cap is screwed on. This rotation propels a circle of curved blades attached to the end of the straw.

Fig. 6: The spray nozzle is pressed down until it reaches the ridge in the straw. This motion punctures the seal (shown in inset), allowing the two chambers to mix. Next, the second component is fitted into the cap and pushed to build up the air pressure in the bottle.

Finally, the mixed IPN solution is administered by utilizing a second component, the plastic pump. This pump is fabricated so that three plastic shafts fit into three corresponding slits in the bottle cap. Once inserted, the user pumps the second component up and down to build up the air pressure in the bottle. After 10-15 pumps, this component is then removed from the slits and the spray nozzle is pressed to create an atomized stream of IPN solution that is sustained for 20-30 seconds. The pressure is maintained in the vessel since the slits in the cap are narrow, at approximately 1 mm apart, so minimal air escapes while the user applies the IPN.

Pros:

There are several advantages to the pressurized bottle design, principally its capacity for a sustained spray. The user would not need to devote significant energy to the application while pressure remains in the bottle. Additionally, since the design is based off of a pre-existing bottle design by Nalgene™, many of the parts would be readily available and their technology already perfected. Finally, the product effectively incorporates a mixing mechanism, which can greatly enhance dissolution of the IPN solution.

Cons:

Although the design offers promise in several areas, there are also significant obstacles to its implementation. First, the pressurizing feature comes at a cost. The Fischer catalogue lists the pre-existing Nalgene™ bottle at US $14 (Fischer Scientific, 2007) compared to other non-pressurized spray bottles that are as inexpensive as US $3 (Bel-Art Scienceware Spray Bottle, Fischer Scientific) and syringes that may be less than US $1 a piece (Disposable Plastic Syringes, Fischer Scientific). Additionally, the bottle and pump components must be packaged separately, which may incur an additional cost, and only one size is available using the Nalgene™ design, so there would be a greater likelihood of wasted solution. Finally, the pressure-building feature is somewhat laborious, so its presence may limit its clinical acceptance.

Design 3: Spray Bottle

Design Summary:

The spray bottle design (**Figure 7**) uses a single pump, single spray method as the delivery mechanism for IPN delivery. The photo-initiator is stored in an opaque container at the top of the bottle, separated from rest of the solution. The custom designed straw is threaded on the outside up to the photo-initiator storage container, which allows a specially designed propeller to rotate while traversing the length of the straw. This

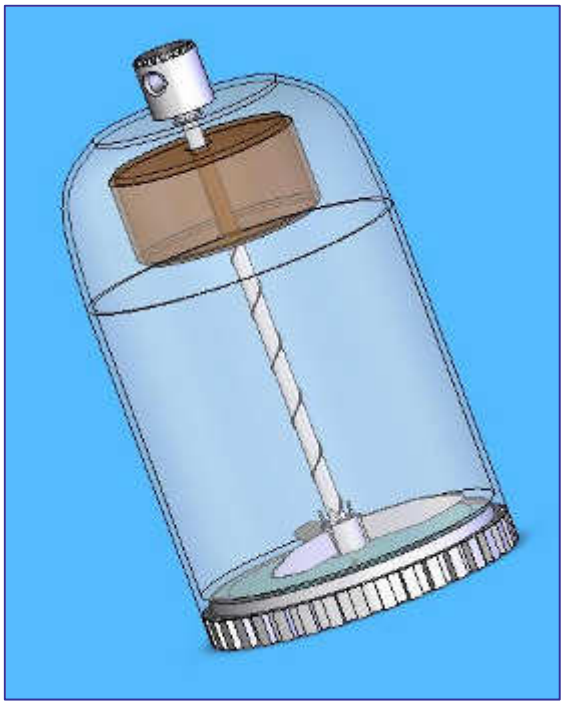


Fig. 7: Spray Bottle Design

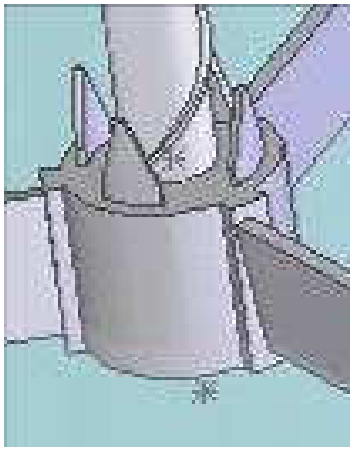


Fig. 8: Close-up of blades on stirring mechanism

propeller acts as a tool that punctures the container housing the photo-initiator by rotating the grooved base of the bottle. As seen in **Figure 8**, a series of four blades around the circumference of the stirring mechanism provide a means of puncturing the photo-initiator container, allowing the IPN components to combine. The propeller also acts as a stirring mechanism as it is rotated back down the length of the straw.

Pros:

The spray bottle design presents an all in one packaging system that separates the components in an effective manner. The opaque photoinitator chamber shields the photoinitior until it is mixed, while the transparent bottle allows the user to assess the extent of dissolution. Another significant advantage to the spray bottle design is the mixing propeller that doubles as a tool used to combine the elements. This mechanism should ensure a uniform solution. The design provides a large volume for mixing, which can improve the homogeneity of the solution after combination.

Cons:

This design contains parts such as the threaded straw and the stirring mechanism that are non-standard and would need to be manufactured. Because the design is to be used only once, expensive manufacturing costs are unwanted, and this design presents such a problem. Another disadvantage to the bottle design is that it must be pumped for each individual spray, which may be strenuous on the user when covering large surface area wounds.

Continued Laboratory Research

The end utility of the IPN product is limited by the effectiveness of the IPN solution, so an additional facet of our work this semester is to continue to investigate gelatin dissolution

under varying conditions. Last semester, we concluded that acetate/citrate buffer provided the best possible dissolution of standard Bloom Type-A porcine gelatin at room temperature, based on qualitative observation and quantitative analysis of dissolution using UV-Vis. spectrophotometry. However, when gelatin in acetate/citrate buffer was observed on a microscope slide relative to a gelatin in 60°C water solution, the characteristics were drastically different (**Figure 9**), suggesting that gelatin did not fully dissolve in acetate/citrate buffer.

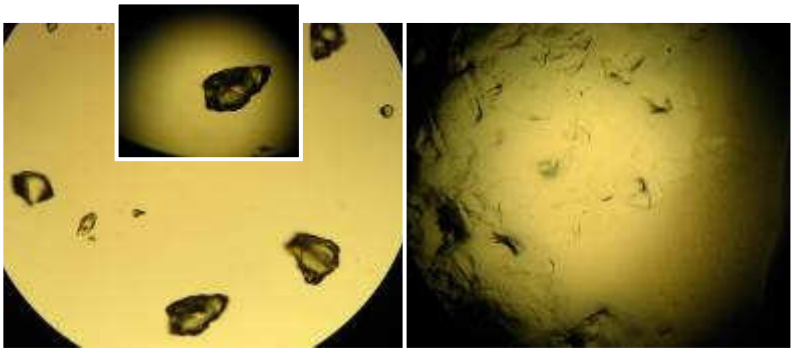


Fig. 9: On left: Solid 90-125 Bloom gelatin particles under 20X magnification (inset shows 40X magnified view). On right: Gelatin dissolved in acetate/citrate buffer under 20X magnification. Gelatin dissolved in 60°C water showed no irregularities, but rather, the solution appeared homogenous and translucent.

Without full dissolution, gelatin loses its bioactivity, and its function in the final IPN would not be expressed. In order to promote optimal efficacy of the final IPN, further research must be performed to determine a way for full dissolution to occur at room temperature. Cold-set gelatin is one option that may have the capacity to meet this design requirement.

Last semester, we began to consider cold-set gelatin as a viable alternative to a heated gel. Cold-set, instant gels are considered amorphous; contrary to standard gels, which exhibit crystalline properties (Dick, 1999). These instant gels demonstrate the same rheological properties as gels obtained by dissolving normal gelatin in hot water, so the final viscosity of the solution should remain consistent with standard gels. However, the rates of gel formation are different, since instant gelatin attains 90% of its hardness after 30 minutes, while standard gelatin hardens only after 15 hours (Dick, 1999). Variability in the properties of cold-set gelatin comes

from how it is prepared. Most of this gelatin utilizes a spray or drum-dried method (Cole, 2000) but there is a chemical approach to preparing this gelatin as well (USPTO #2834683). These gelatin solutions would have a more flexible range of application without affecting the composition of the final IPN.

However, if cold-set gelatin does not seem to produce IPNs that resemble those made from 60°C water solutions, there are several additional routes that can be taken. To quantify the amount of gelatin dissolved in solution, a known amount of gelatin can be added to a given volume of solvent. The resulting solution can then be filtered through a Buchner funnel to separate the dissolved gelatin from its undissolved solid. When dried, the undissolved solid can help quantify how much gelatin is able to go into solution. Additionally, communication with Dr. Samuel Gellman from UW-Madison last semester led to the notion of a pseudo-surfactant created from gelatin fragments. If some amount of solid gelatin is added to a solution with an extreme pH, it will most likely denature into gelatin fragments. These fragments can be neutralized using dialysis and can then be added to standard, solid gelatin, with the anticipated effect of eliciting further dissolution, like a surfactant would do. Since the fragments may prove to be more hydrophilic than original gelatin, they may interact with the hydrophobic portions of the original gelatin and bring it into solution. With these options in mind, the possibilities for creating a functional IPN are even more tangible.

Design Matrix

To aid in deciding which design our team should pursue, we constructed a design matrix (Figure 10) that rates each design on multiple categories. Categories were based on design constraints and were weighted with respect to their importance in the final design. Mixing procedure was determined to be the most important category. Without a successful mixing

procedure, the design is likely to fail, regardless of how well the remaining requirements are met. Other criteria that are important include scalability and application ergonomics. Scalability refers to the design's ability to be manufactured in different sizes. Application ergonomics refers to the work required by the user to apply the product as well as the efficiency of application. After each design was rated, the matrix suggested that the syringe design would be the most effective design.

Criteria	Weight	Design 1 Syringe	Design 2 Pressurize	Design 3 Spray
Mixing Procedure	15	10	9	12
Uniform Solution	10	6	7	7
Compartmentalization	10	9	5	8
Parts Availability	10	7	9	6
Application Ergonomics	10	8	6	4
Safety	10	8	6	8
Cost	10	5	3	7
Sterility	5	5	3	4
Scalability	5	5	2	4
Spray Pattern	5	4	5	2
Client Preference	5	5	5	3
Photo-initiator Protection	5	3	5	4
TOTAL	**100**	**75**	**65**	**69**

Fig. 10 The design matrix used to determine the final prototype design.

Future Work

We plan to pursue our syringe design because it fits our design constraints with the fewest limitations. The first step in this process is to finalize the physical design and to construct a precise model using SolidWorks™. We will also need to research materials and parts available on market to construct our design and establish a budget. Materials will be selected based on their biocompatibility, ability to be sterilized, and price. Moreover, at the request of our client we will favor pieces that are currently available on market to minimize the overall cost of

production. With our final design and the chosen materials and/or parts, we will be able to fabricate a device that can reconstitute the components of an IPN and administer the final solution to a large surface area wound. The effectiveness of our design will be evaluated on its ability to deliver a constant spray, the extent of dissolution, as well as the useful life after reconstitution through both computer simulation and laboratory tests. Additionally, if after testing our design proves to satisfy all of the design requirements, we will consider scaling the product for different size applications.

In addition to addressing the product's administration technique and packaging, we would like to optimize the final composition of the IPN solution in terms of its chemical properties. To do so, our research will be extended through additional testing. In particular, we would like to explore methods for creating cold-water soluble gelatin. Our first step would be to modify our current gelatin by following the previously-outlined procedure. Next, the modified gelatin's compatibility in a full IPN will be assessed by measuring both standing cure time and cure time with UV exposure.

Our final design will incorporate both a final recipe for the IPN components as well as an effective design for packaging, reconstituting, and administering IPNs to large surface area wounds. Ideally these efforts will increase the clinical applicability of IPNs.

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