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TARGETED DRUG DELIVERY SYSTEM FOR KIDNEY AND/OR LIVER FAILURE PATIENTS USING HUMAN SERUM ALBUMIN

by

Sean Bedingfield

Thesis submitted in partial fulfillment of the requirements for the degree

of

HONORS IN UNIVERSITY STUDIES WITH DEPARTMENTAL HONORS

in

Biological Engineering in the Department of Biological Engineering

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Fall 2014

Targeted Drug Delivery System for Kidney and/or Liver Failure Patients using Human Serum Albumin

Utah State University

Department of Biological Engineering
Final Report
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10 December 2014

PROJECT SUMMARY / ABSTRACT

Compromised liver and/or kidney function reduces the acceptable dosage of a variety of medications that can be administered to patients. These patients still have a need for drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), antivirals, and antibiotics. The project goal is to provide a drug delivery system to accommodate these reduced dosage limits with added therapeutic benefits to address symptoms of liver or kidney failure. Localized drug delivery allows for a smaller, concentrated dose rather than inundating the patient's system with the drug of interest. Human serum albumin (HSA) is a researched candidate for drug delivery with therapeutic properties. HSA was tested as a drug delivery vehicle for localized, percutaneous drug release aided by a displacing compound and changes in temperature and ultrasound generated by an external device. Dynamic light scattering (DLS), UV/Vis spectroscopy, and optical microscopy were employed in verifying drug binding, release, and effectiveness. The optimization of the proposed drug release method, the design of the device to promote drug delivery, and the testing of the drug delivery system with *in vitro* tissue testing were performed to validate the designed system.

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OBJECTIVES / AIMS

Due to kidney or liver failure, normally harmless doses of prescription drugs can have toxic effects leading to further medical complications (Ing, 1979). Those suffering from kidney and/or liver failure frequently suffer related and unrelated ailments, but are restricted on the medications and the amounts thereof. Site-specific ailments such as arthritis, localized viral infections (viral pneumonia), neuropathic pain, and other illnesses could be treated with smaller doses if the treating drug were only made available at the targeted site.

The project goal is to develop a drug delivery system using human serum albumin (HSA) as a carrier for anti-inflammatory, antibiotic, antiviral, and antioxidant drugs.

In this project, ibuprofen was used as a model drug due to its availability and strong literature support for its binding mechanism to HSA. This drug delivery system is unique in using rapid-sequence, local hyperthermia and ultrasound at a specific site of interest in the body. The proposed system is designed for patients with compromised liver and/or kidney function who are unable to safely use normal, oral doses of these medication types.

Objectives

- Develop a two gel system
    - Investigate and incorporate a drug-carrier binding method using HSA and ibuprofen
    - Investigate and incorporate a drug release mechanism for drug delivery using competitive binding of omega-3 fatty acid
- Develop a device
    - Develop a device to activate and amplify the effectiveness of the dose

DESIGN CRITERIA

Drug binding and release

The success of the drug binding is evaluated by the change of absorbance of HSA measured with UV/Vis spectroscopy. The criterion for success is a 20% decrease in absorbance from HSA-only solution to HSA-drug solution, as this is indicative of a 1:1 molar ratio of HSA-ibuprofen binding (Sułkowska, 2002; Thumser, 1998).

Device

For the success of the device, there are many criteria that must be met. The designed device must be able to successfully facilitate the diffusion of the drug through the skin of the inflamed tissue. To do this, the device must be able to alternate between heating and ultrasound. It is expected that the heating element of the device will reach 40°C within two minutes (Galantini, 2010; Lawrence, 1976). The use of ultrasound will be incorporated to further promote diffusion of drug across skin. The ultrasound must have a holder that allows for direct contact with the skin; the clearance between the ultrasound head and the skin must be no more than one millimeter (basically direct contact with aid of ultrasound gel) (Forrest, 1989). The ultrasound must also be versatile in regards to position on the affected part of the body; it should have the ability to be moved wherever the user deems necessary. Lastly, the device should be non-invasive as possible and should be convenient and affordable for home use.

BACKGROUND / HISTORY

Prevalence of Patients with Liver and/or Kidney Disease

The Centers for Disease Control and Prevention claims that 10% of adult Americans have some level of chronic kidney disease (CKD), and the same percentage suffer from liver disease. Over 800,000 Americans were treated for end-stage renal disease (ESRD) in 2009 (CDC, 2014; CDC, 2010).

Chronic kidney disease is the prolonged reduction of kidney function most commonly caused by diabetes, high blood pressure, and advancing age. CKD patients are advised to specifically avoid further kidney damage by using anti-inflammatory medication unless advised by a doctor. (CDC, 2014)

Liver disease can be caused by cirrhosis, excessive use of certain pharmaceuticals, infectious hepatitis, mononucleosis, the accumulation of fat in the liver, and a host of other illnesses. Treatment of liver disease must be specific to the cause, but all patients must be careful not to overload the liver's reduced metabolic function. (CDC, 2010)

Drug Carrier: Albumin

Human serum albumin is a natural antioxidant that removes reactive oxygen species common to those with liver failure, and has drug-binding mechanisms that are well researched (refer to Figure 1). It is known that the total amount of albumin in the body is about 3.5-5.0 grams of albumin per kilogram body weight, which for a healthy 70 kg adult is roughly 250-300 grams. Most of the albumin (120-145 g) is lost in the extravascular space; however, some does get

digested (about 1 g) and very little albumin passes through the kidneys. Albumin is synthesized in the liver and the rate at which it is synthesized depends on nutrition and the overall health of the liver. It is clear that if the liver is diseased, the rate at which albumin is synthesized decreases, resulting in an albumin deficiency (Nicholson, 2000).

This proposed drug delivery system will not only treat inflammation with the use of ibuprofen, it will also minimize damage to the liver or kidney due to the localized treatment of drug. In addition, albumin can serve as a carrier for a variety of different drugs such antioxidants, antivirals and antibiotics. Because albumin is the most abundant protein in the blood, which accounts for approximately 55% of protein in the blood plasma, it is expected that the body will not have any significant negative response to the HSA-drug compound.

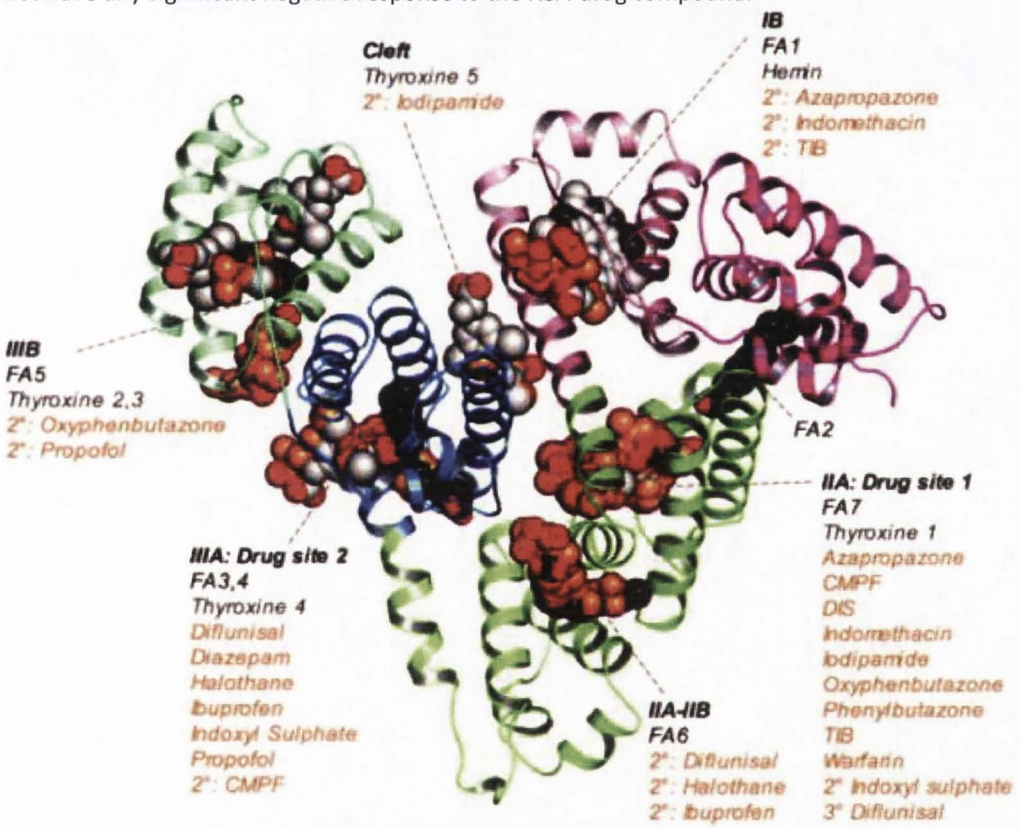

Figure 1. Three-dimensional model of the structure of human serum albumin with drug binding subsites and common pharmaceuticals to which they correspond (Ghuman, 2005); "2°" refers to drugs for which it is a secondary binding site.

Drug of Choice: Ibuprofen

There are over 100 pharmaceutically active compounds shown to bind with HSA, as shown in Figure 2 (Zsila, 2011). These drugs vary in form and function. Ibuprofen was selected for initial testing of this drug delivery system for its affordable price, the usefulness of site-specific delivery, and foundation in literature. Ibuprofen was shown to have a relatively high affinity to HSA. It was shown to bind primarily to HSA binding site II (Ghuman, 2005).

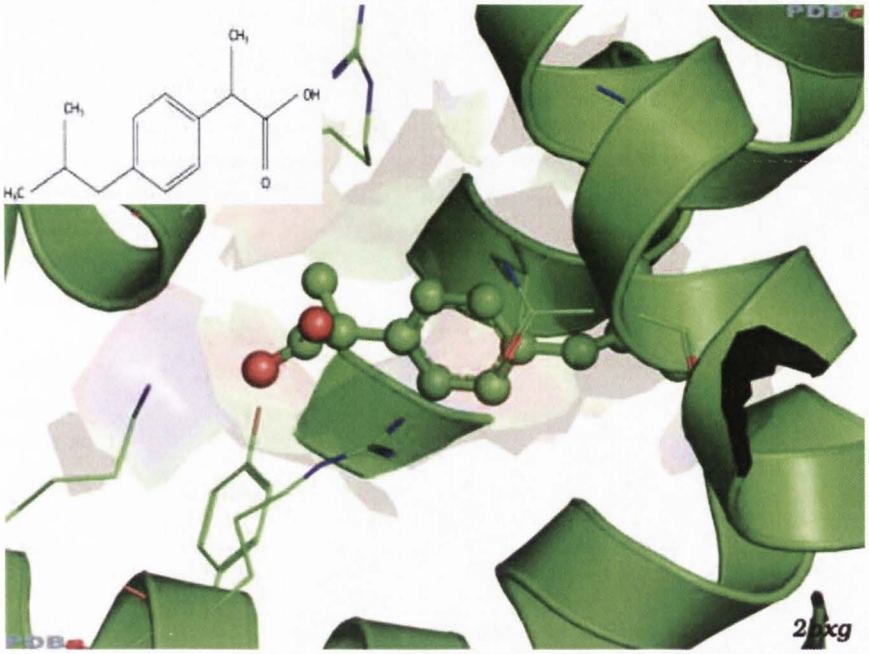

Figure 2. Structure of ibuprofen (top-left), three-dimensional model of ibuprofen bound to human serum albumin at subsite IIIA (Ghuman, 2005)

Displacement Compound: Omega-3 Fatty Acid

Omega-3 fatty acid is a polyunsaturated fatty acid that is an essential nutrient found in plants and animals. This fatty acid is important for normal body metabolism, blood clotting, decrease of blood pressure and inflammation.

HSA has the ability to be a carrier for fatty acid in the blood as fatty acid has been shown to bind at multiple binding sites of HSA, some of which overlap directly with the two ibuprofen binding sites (Krenzel, 2013). In addition to having multiple HSA binding sites that overlap with many drugs, omega-3 fatty acid is an excellent candidate to be used in a drug displacement study as it has a higher affinity than ibuprofen to HSA (Bhattacharya, 2000; Reichenwallner,

2013). Omega-3 fatty acid is an ideal compound to displace ibuprofen because it is a natural substance in the body, therefore, it does not present any harmful side effects to the body.

Polyunsaturated fatty acids in fish oil are also shown to act as dermal permeation enhancers, promoting the transdermal diffusion of ibuprofen and other drugs (Heard, 2003). Increased diffusion increases the bioavailability of the drug in the tissue, which could mean more rapid results.

DESIGN STEPS

Choosing a drug carrier was the first step of this design process. The drug carrier needed to be biocompatible, stable and versatile enough to be used with a variety of drugs. Next, a model drug was selected to be used as a proof of concept of the designed drug delivery system. The model drug must have a relatively high affinity to the drug carrier to ensure binding.

After a selection of drug carrier and model drug, binding method was developed to bind the model drug to the selected drug carrier and drug binding was verified. Release method was also verified to quantify the release of the bound drug from its carrier. After the success of drug binding and release, a method of drug delivery was carefully considered.

Based on the selected method of drug delivery, an external device was designed and tested against its criteria of enhancing drug delivery. Finally, tissue testing was performed to further confirm the effectiveness of the designed drug delivery system.

Drug Carrier

Drug carriers including polymeric microspheres, synthetic polymer lattices, nanoparticles, protein carriers (particularly human serum albumin), liposomes, and nanofibers were considered for the product. Of protein carriers, human serum albumin was selected above others for complete biocompatibility and an unrivaled versatility among proteins in the transport of multiple drugs. The carrier would need to accommodate the project criteria by minimizing the needed medication, granting control of release, allowing versatility in site treatment, and allotting for ease in manufacturing. Table 1 is a reflection of the evaluation of these drug carriers.

Table 1. Alternative drug carriers. Drug carrier selection is determined by how many of the criteria are met. Some criteria are partially met resulting in a score of 0.5.

	Protein Carrier (Human Serum Albumin)	Liposomes	Nanoparticles	Nanofibers	Synthetic Polymer Lattices	Polymeric Microspheres
Controlled Release	0.5	0.5	1	0.5	1	1
Localized Treatment	1	0.5	1	1	1	1
Biocompatibility	1	1	0.5	0.5	0	0
Biodegradability	1	1	1	1	1	1
Diffusivity	1	1	0	1	0.5	0.5
Availability	1	0.5	0	0	0	0
Ease of Use	1	0.5	0.5	0	0	0
Score	**6.5**	**5**	**4**	**4**	**3.5**	**3.5**

The scores apparent in Table 1 identified human serum albumin as the best option considered for the device. The major advantages of albumin are nearly universal biocompatibility and versatility with a variety of drugs. Major drawbacks are that albumin must be processed to remove lipids before use, and that HSA is only commercially available from animal sources. Other carriers that are synthetically manufactured would likely be less expensive in large manufacturing. Since this product is highly specialized to a niche group, it was decided to favor performance over the high cost of albumin.

Drug of Choice

After HSA was selected as a drug carrier, the selection of drug depends on its binding mechanism to HSA. Drug selection was considered for best fit to exemplify the benefit of the product to patients with compromised liver and kidney function. Groups of drugs were initially evaluated for general concern with such patients, demand for the drug, drug availability, effectiveness in near-surface treatment, drug safety in handling, and the depth of understanding for the functionality of the drug group. Drug group evaluation is shown in Table 2.

Table 2. Classes of drugs thought to possibly fit the criteria required. Drug class selection determined by how many of the criteria are met. Some criteria are partially met resulting in a score of 0.5.

	Analgesics	Antibiotics	Blood Pressure Meds	Antivirals	Anticancer Agents	Hormone Therapy Drugs	Mood Stabilizers
Localized Application	1	0.5	1	0.5	1	0.5	0
Area of Treatment Near Skin	1	0.5	0	0.5	0.5	0.5	0
Damaging to Patients with Liver/Kidney Dysfunction	1	1	0.5	1	1	0.5	0
Drug Widely Used, In High Demand	1	1	1	0.5	0	0.5	0.5
Well-Known Action Mechanism	1	1	1	1	0.5	0.5	0.5
Well-Known Mechanism for Kidney/Liver Damage	1	1	0.5	0.5	0.5	0	0
Drug Availability	1	1	1	0.5	0	0	0.5
Known Drug-Protein Binding Mechanism	1	1	0.5	0.5	0	0	0
Drug is Safe to Handle	1	1	1	1	0	0	1
Score	**9**	**8**	**6.5**	**6**	**3.5**	**2.5**	**2.5**

Upon selecting analgesics as the class from which to select the therapeutic agent, criteria were changed upon reviewing potential drugs (See Table 3). For ease of procurement, the drug must be available without a prescription. As a result, all opioids (codeine, hydrocodone, etc.) were removed from consideration. A rating of the effectiveness of the drug as an analgesic, and a rating of how little damage is done to kidneys and livers were add to select the drug that would be most practical for the application. The change in criteria to find the least damaging drug is an effort to provide the most passive drug within a class of drugs where kidney/liver damage is a concern. Other criteria were eliminated as the criteria were met by all the compounds.

Table 3. Analgesics thought to possibly fit the criteria required. Drug selection determined by score based on weighted criteria.

	Priority	Category Weight	Ibuprofen	Aspirin	Acetaminophen	Diclofenac	Naproxen	Celecoxib (Celebrex)
Drug is Safe to Handle	1	10	10	10	10	10	10	10
Drug Availability	2	10	10	10	10	10	10	3
Drug Widely Used, High Demand	3	8	10	7	8	8	6	3
Known Drug-Protein Binding Mechanism	4	7	10	8	6	8	6	3
Most effective	5	6	8	9	7	0.5	0.5	10
Least Kidney Damage	6	6	10	4	3	7	6	3
Well-Known Action Mechanism	7	5	8	8	8	8	8	8
Well-Known Mechanism for Kidney/Liver Damage	8	5	9	8	9	6	5	4
Score			**543**	**470**	**451**	**435**	**394**	**313**

From the evaluation of analgesics shown in Table 3, ibuprofen was chosen as the ideal candidate based on the criteria. Criteria were rated from the information found in the listed references, and evaluated by relative comparison of the drugs.

Drug Binding Experimentation

The original system design relied on controlled release of ibuprofen within the bloodstream. Binding ibuprofen with a protein stabilizes the drug and reduces activation until the drug is released. HSA is a prevalent blood protein often in inadequate concentrations among liver disease patients and it serves as a carrier for over 14 drugs. Binding procedures were investigated for preparation of an injectable serum.

Of the six protocols found for binding HSA with ibuprofen, the simplest method was tested first. A 10:1 molar ratio of ibuprofen to human serum albumin was prepared with 50 mg of ibuprofen. Lyophilized, lipid-free HSA and powdered *s*-ibuprofen were added to 5 mL distilled deionized water in respective order, giving the HSA two hours to dissolve before adding ibuprofen. After an additional six hours on a shaker table at room temperature, absorbance was measured. HSA is known to contain a single fluorescent tryptophan near the primary

binding site, which was employed for the binding verification using shifts in fluorescence (Thumser, 1998). It was expected that fluorescence would decrease when the drug was bound to the protein (Thumser, 1998). Fluorescence studies and UV-VIS spectrophotometry were employed at excitation wavelengths of 280 and 295 nm with corresponding emissions at 340 nm and 350 nm.

Table 4. Resulting fluorescence of free ibuprofen, pure albumin, and an ibuprofen/albumin solution at 325 nm; no hypothesis is no change in fluorescence

Samples	Mean Fluorescence (RFU)	Standard Deviation (µg/L)	Count (n)	Std Err (µg/L)	α	P-value	Mean % Reduction
HSA	61651	2981	3.00	1721	-	-	-
HSA + IBP	39913	3054	3.00	1763	0.01	0.0065	35.3

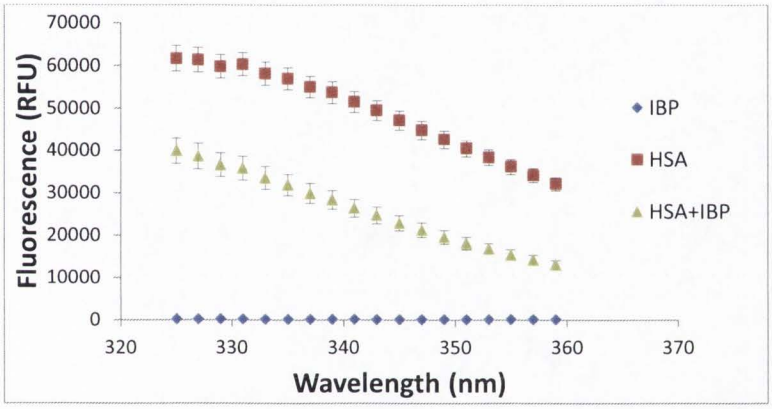

Figure 3. Fluorescence data with HSA showing a drop in fluorescence once mixed with ibuprofen, indicating drug-protein binding; Error bars represent standard deviations of triplicate results

As shown in Table 4 and Figure 3, fluorescence showed a statistically significant reduction with 99% confidence in fluorescence, indicating drug-protein binding (Thumser, 1998). This method was selected for ease of execution. Two methods incorporated removing lipids from HSA, which was skipped by ordering lipid-free HSA. Another three methods involved altering the HSA conformation, which was avoided for simplicity of the product. A major advantage of using standard HSA is the greater extent of the published research on its diffusive and chemical properties over any altered version of the protein. Some disadvantages of selecting lipid-free, unaltered HSA are a weaker HSA-ibuprofen bond, the increased cost of purchasing lipid-free

HSA instead of chemically removing lipids in-house, and the dearth of industrially available non-animal sources.

After the decision was made to use transdermal delivery, there were concerns that the gel used for drug loading and ultrasound would cause premature ibuprofen release or bind with the ibuprofen independently. Testing was necessary to verify the employed gel would act as an inert medium.

Testing of HSA-bound ibuprofen fluorescence behavior suspended in the selected guar gum gel was compared to HSA, ibuprofen in gel and water, and HSA-bound ibuprofen in water. Since the guar gum gel is aqueous, the desired dosage of 50 mg/mL of ibuprofen was achieved by preparing a doubly thick of gel, and using half the needed water to perform the binding protocol before mixture with gel. Fluorescence of the samples was again tested under the same conditions as water-based binding confirmation testing. Results are shown in Table 5 and Figure 4.

Table 5. Fluorescence comparison at 320 nm of compounds in water and compounds (IBP = ibuprofen; HSA = Human Serum Albumin; HSA-IBP = HSA-bound ibuprofen) in gel; no hypothesis was no change in binding

HSA+IBP Sample Matrix	Mean Percent Fluorescence from HSA in Gel	Standard Deviation (%)	Std Err (%)	Hypothetical Mean	α	P-value
Water	24.70	2.63	1.52	24.70	-	-
Gel	24.82	0.41	0.24	24.70	0.10	0.6775

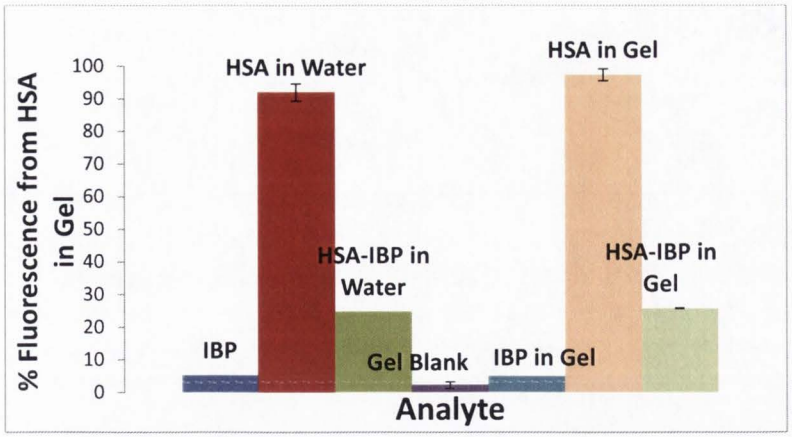

Figure 4. Fluorescence comparison at 320 nm of compounds in water and compounds (IBP = ibuprofen; HSA = Human Serum Albumin; HSA-IBP = HSA-bound ibuprofen) in gel; Error bars represent standard deviations of triplicate results of triplicate sample; unseen error bars have a negligible standard deviation

As shown in Table 5, no statistically significant difference was found between fluorescence behavior in water and gel solutions, which indicated retained binding behavior in both matrices. Drug binding was found to be compatible with the selected gel.

The final concern with drug binding was the reversibility of the drug binding. Successful drug release was the final requirement for an effective drug binding strategy.

Drug Release Experiments

Initially, the HSA-drug compound was planned to be injected and released through the use of the device. The drug was planned to be released through an increase and decrease in temperature followed by ultrasound. These heating and cooling cycles polymerize HSA, causing a release of the drug. Ultrasound was proposed to promote drug diffusion and bioactivity in localized drug release from HSA.

Experiments were performed to test if HSA polymerization seen in literature when cycling between 75° C and 25° C could be observed after only five minutes of external heating at 75° C (Wetzel, 1980). Results are shown in Table 6 and Figure 5. Experiments at all lower temperatures and heating times showed no statistically significant change in HSA size distribution.

Table 6. Measurements of albumin aggregate diameter by dynamic light scattering (DLS); the null hypothesis was no change in aggregate size.

Mean Values of Each Group	Mean Albumin Aggregate Diameter (nm)	Standard Deviation (nm)	Std Err (nm)	α	P-value
Untreated (25 C)	8.15	5.05	1.13	-	-
External Heat (75C)	67.94	62.17	13.90	0.01	0.00039

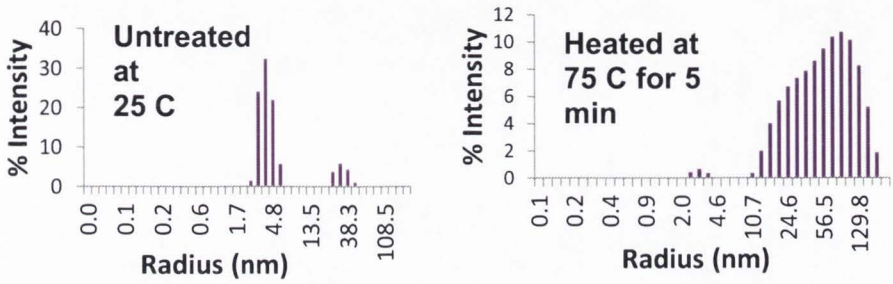

Figure 5. Dynamic light scattering (DLS) measurement of size distribution before heat cycling (left), and after heating with 75° C for 5 minutes before cooling to 25° C (right)

It was found that a minimum of 75° C was needed to polymerize the HSA to release ibuprofen. However, the temperature was deemed too hot for the skin surface. With 50° C considered the maximum tolerable temperature for skin, heating cycling of 50° C for 35 minutes was still not adequate to cause polymerization or release ibuprofen from HSA. It was then concluded that HSA could not be denatured to release ibuprofen within the temperature range that is tolerable for the skin.

In addition, the first approach of the drug delivery system was not reliable as there was not enough data on the circulation time of HSA-drug compound in the body. Moreover, it was not an efficient design as the patient would need to release the drug at his or her own time after being injected with the HSA-drug compound.

Because drug release through the polymerization of HSA through changes in temperature was not adequate in releasing ibuprofen, a second approach of drug release mechanism was needed. Several different alternatives were explored such as pH changes, pressure changes and electrical conduction. However, site-specific changes of pH in the bloodstream would be

invasive, costly, and would likely have adverse effects on the body. In addition, adjusting the pressure applied to the protein would require more complicated equipment. Electrical conduction was also another possible alternative to induce drug release. Applying voltage to the HSA was expected to change the conformation of the protein, releasing ibuprofen. However, this was not a feasible method for use in the body.

Transdermal drug release using a displacement compound was then selected as the new release strategy. There was also consideration of removing albumin entirely from the project. It was decided that albumin could still play the following roles as the drug carrier in a transdermal system:

- Albumin can stabilize a suite of pharmaceuticals in aqueous solution.
- Albumin could mitigate bioactivity until application of pharmaceuticals, such as hormonal derivatives, could be hazardous to handle.
- Region of drug delivery could be tuned by adjusting the time interval between introduction of the HSA-bound ibuprofen and the introduction of a displacing agent.

Various displacement compounds were considered for drug displacement as shown in Table 7. Because HSA is a natural carrier of fatty acid in the blood, fatty acid was deemed sufficient to fulfill the role of a drug displacement compound. Various antioxidants, warfarin, diazepam and bilirubin were also considered for their known binding site that overlap with binding site of ibuprofen in HSA. The use of salicylic acid as a drug displacement compound was frequently studied in literature, however, its binding site in HSA does not overlap with that of ibuprofen. Finally an enantiomer of ibuprofen was also considered to be a potential candidate of drug displacement compound, however, the risk of overdose compromised the safety of the designed drug delivery system.

Table 7. Alternative displacement compounds for drug release experiment. Displacement compound is selected by the highest total score.

	Fatty Acids	Warfarin	Antioxidants	Salicylic Acid	Diazepam	Bilirubin
Safety	1	0	1	0.5	0	0.5
Affinity to HSA (> IBP)	1	0.5	0.5	0.5	0	0
Known Binding Mechanism	1	1	0.5	1	1	0.5
Known Binding Site II	1	1	1	0	1	1
Availability	1	1	0.5	1	0.5	0.5
Score	**5**	**3.5**	**3.5**	**3**	**2.5**	**2.5**

Fatty acids were found to most closely meet the criteria for a displacing comment with a high binding affinity with HSA, binding site overlap, depth of understanding for HSA binding, and compound availability. As these fatty acids were derived from fish oil, there is a variety of unsaturated fatty acids. The unknown variety in fatty acids may be a source of undesired variability in effect. This risk was accepted as the product design calls for excess of fatty acids, and variability should be minimally influential on product performance.

A major role of albumin in the body is safe lipid transport through the bloodstream. Albumin has seven high-affinity fatty acid binding sites as shown in Figure 7. It was decided to try distilled omega-3 fatty acid from food grade fish oil to displace the bound ibuprofen through competitive binding.

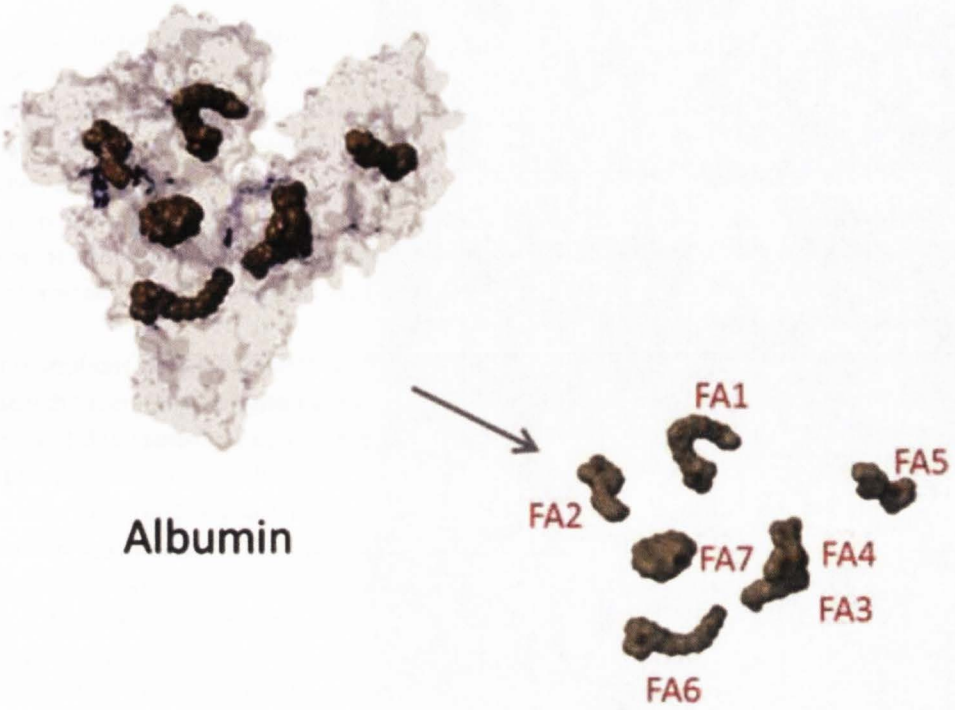

Figure 7. Map of fatty acid binding sites on human serum albumin (Reichenwallner, 2013)

Fatty acid binding sites FA3, FA4, and FA6 depicted in Figure 7 directly overlap with ibuprofen binding sites. Distilled fish oil was chosen for availability and the blend of eicosapentaenoic acid (EPA) and decosahexanoic acid (DHA) fatty acids.

Fatty acid displacement would require a separate introduction of the oil at the time of treatment. Like an epoxy, a two-part gel system would be necessary to cause competitive binding and displacement at the time of use. This creates an undesirable added step in complexity of treatment, but also provides added control over drug delivery that could be considered advantageous. Further literature review indicated that fatty acids from fish oil in excess would also facilitate transdermal drug diffusion (Heard, 2003).

Another approach for measuring compounds in liquid solution is liquid chromatography – tandem mass spectrometry (LC-MS/MS). Tandem mass spectrometry allows for some compounds to be accurately tested at concentrations of 10 ng/L (Hernando, 2006). The binding and release of ibuprofen was verified by measuring the ibuprofen in a solution before HSA introduction, after binding with HSA, and after displacement with fish oil.

Aqueous solutions of ibuprofen were prepared at 1.5 mg/mL concentrations in triplicate, and passed through 0.45 µm Frisenette PVDF filters. A sample of each solution was diluted by a factor of 100, and measured for initial concentration. HSA was added to the solutions for an HSA concentration of 24 mg/mL (for a 10:1 Ibuprofen to HSA molar ratio) and placed on a shaker table at room temperature for 8 hours. Samples were taken, diluted, and measured again in the same manner. Finally, molecularly distilled fish oil was added in a calculated molar excess at a concentration of 32 mg/mL, placed on a shaker table for 6 hours before final measurement. Triplicate samples were measured in triplicate.

Concentrations were determined on an Agilent Triple Quad LC/MS 6490 at the Utah Water Research Laboratory. An Agilent Zorbax Eclipse XDB C-18 Rapid Resolution HT column (50×4.6 mm i.d.; 1.8 µm particle size) was employed. The column temperature was set to 70 °C, and a 30 µL injection volume. A 40:60 volumetric ratio of 0.1% formic acid and acetonitrile (pH=3.0) was used as the mobile phase at a flow rate of 0.15 mL/min. Analysis was done using electrospray ionization (ESI) in positive ion mode. Drying gas flow was 9.0 L/min and drying gas temperature was set at 350 °C. Capillary voltage was set to 4000 V, and nebulization pressure was at 35 psi. Fragmentor and collision energies were 380 V and 20 V, respectively. The precursor ion and quantifier ion mass-to-charge-ratio (m/z) were respectively 204.9 and 161.2. The detection limit for ibuprofen in sample matrix was 202.9 pg on column (24.29 pg/µL), qualified by a signal-to-noise ratio greater than 10. The identified retention time with this method for ibuprofen was 10.08 minutes.

Ibuprofen concentration standards supplied through Agilent were used to generate the calibration curve shown in Figure 8. While standards below 5 µg/L were unreliable, an appropriate quadratic fit was found for the range of 5 - 100 µg/L. Measured concentrations are also included in Table 8.

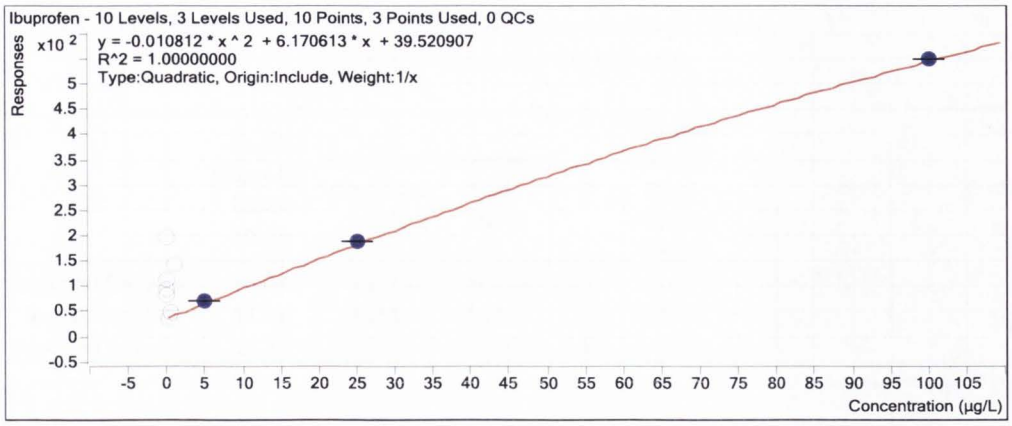

Figure 8. Calibration curve for LC-MS/MS measurement of ibuprofen in water

Table 8. Calibration standard concentrations for LC-MS/MS measurement of ibuprofen in water

Ibuprofen Std (µg/L)	Measured Concentration (µg/L)	Error (%)
100	100.0000	0.00
25	25.0000	0.00
5	5.0000	0.00
1	17.1713	16.17
0.5	1.5278	2.06
0.1	0.0000	-1.00
0.05	11.9411	237.82
0.01	6.6831	667.31
0.001	8.6810	8680.05
0.0001	26.9306	269305.31

Ibuprofen concentrations for each sample are given in Table 9, Figure 9, and Figure 10. There were no observed deviations in preparation between the tested samples. Solution treatments were executed as discussed above.

Table 9. Ibuprofen concentrations for LC-MS/MS measurement in aqueous solutions; Triplicate samples were measure in triplicate

	Mean Ibuprofen Conc. (µg/L)	Standard Deviation (µg/L)	Standard Error (µg/L)	α	P-value (Change from Previous Stage)	Mean Percent Ibuprofen Released	Release Standard Deviation (%)
Unbound Samples	16.05	0.0958	0.0553	0.05	N/A	-	-
Bound Samples	14.22	0.0581	0.0335	0.05	0.0159	-	-
Displaced Samples	15.23	0.0578	0.0334	0.05	0.0471	51.45	10.25

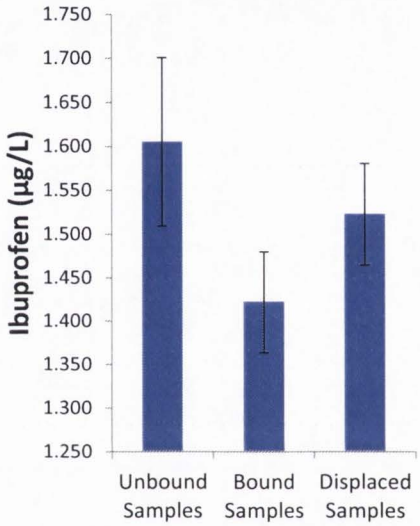

Figure 9. LC-MS/MS measurement of ibuprofen solutions through the drug binding and displacement treatments; Error bars represent standard deviations

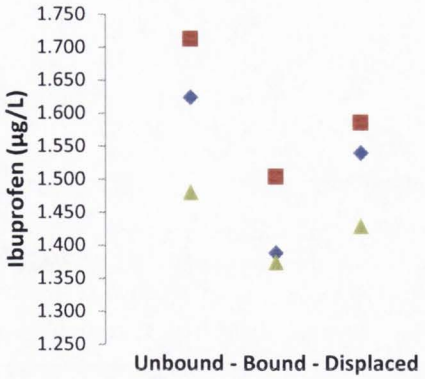

Figure 10. LC-MS/MS measurement of ibuprofen solutions through the drug binding and displacement treatments; Standard deviations of each sample were all less than 0.01 µg/L.

Using a one-sample t-test, it was found that the null hypothesis - that there was no release of ibuprofen from HSA – could be rejected with 95% confidence. This indicates that there is ibuprofen released from fatty acid displacement. It was decided that a one-tailed t-test was appropriate for use with the manageable size of the data used, and the consistent increase between samples after displacement.

While not as apparent in Figure 9, Figure 10 illustrates a consistent trend of ibuprofen reduction after the binding process, and increase after displacement. The significance of the release is further demonstrated in Table 10 by the mean percent of ibuprofen released when compared to the reduction from the binding step, 51.45%.

Delivery Approach

Means of drug delivery were evaluated according to the parameters given in Table 10. Transdermal diffusion was considered for its localized treatment and minimal invasiveness. Bloodstream injection and drug-loaded hydrogel were initially considered for their possibility of local concentration of the drug dosage. Finally, oral ingestion was seen as common, but considered for its effective method of drug delivery.

Table 10. Drug delivery methods evaluated against ranked, weighted criteria

	Priority	Category Weight	Transdermal Diffusion	Bloodstream Injection	Oral Ingestion	Loaded Hydrogel
Requires Minimal Dosage	1	10	10	7	1	7
Possibility to Locally Concentrate the Dose	2	8	10	8	1	10
Safety from Accidental Overdose	3	8	7	3	3	1
Ability to Self-Administer the Drug	4	7	7	4	10	1
Minimally Invasive Implementation	5	6	10	7	10	1
Score			**345**	**244**	**172**	**171**

Bloodstream injection was initially selected to be a means of drug delivery in this project because it provided a reliable way of drug delivery. However, after the drug release method changed, drug delivery by means of transdermal diffusion was selected. In addition, bloodstream injection was deemed inefficient as the patient would need an invasive clinical administration to acquire the HSA-drug compound in the body before releasing it later at home.

As shown in Table 10, transdermal drug diffusion was determined to be the best considered means of drug delivery for this project. Primary strengths of transdermal delivery were the minimally invasive nature of treatment, and the potential to concentrate the dose in the area of treatment.

Device Design

First Design

The overall objective is to design a device that is noninvasive, comfortable, and affordable. Originally, the design idea was to focus on application to the arm, but also have the flexibility to design products for the leg, shoulder, and hand. The plan for the device was to have a tight, but breathable, material and elastic bands sewn in at the wrist; this would provide a more controlled environment as well as controlled blood flow in the desired area. Two holes, at the inside of the elbow and inside of the wrist would provide areas where ultrasound could be

applied. The ultrasound would have been mounted on the forearm, with the ability to pivot and to move laterally with the aid of a notch system. Small heating pads and cool water pumps would be applied both on the upper arm and lower arm, allowing the user to choose which area he or she would like to treat. A depiction of this design can be seen in Figure 11. Another goal of this device was to design a product that could be programmable and mostly automatic.

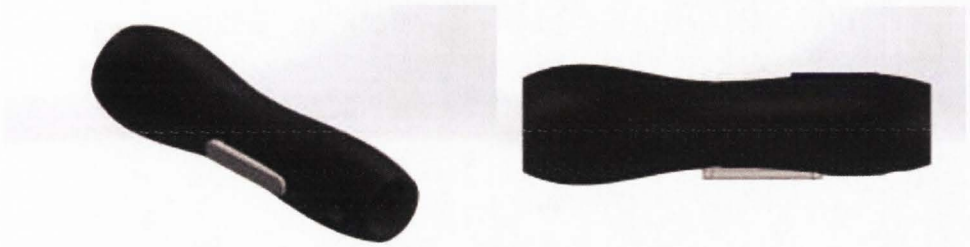

Figure 11. First product design that incorporated an ultrasound (seen in gray) and an LCD screen (seen in blue) as well as heating and cooling elements inside that ran along both the upper and lower arm. A hole near the wrist area for ultrasound application can also be seen.

Since the incorporation of ultrasound was crucial in the overall design of the device, it was important that the ultrasound be mounted in close proximity to the arm as well as have flexibility in position. Therefore, the design of the ultrasound holder came from the idea that every person's arm length is different and that there were two spots within the sleeve design where ultrasound could be applied, so a pivoting and locking system was needed to fulfill the above problems. This original holder design can be seen in Figure 12.

Figure 12. First design of the ultrasound holder. This holder consists of multiple locking notches and required the holder to have a matching piece to ensure proper locking.

When designing the ultrasound holder, it was obvious that this first design was not an effective design for many reasons:

- It relied on the user's ability to push and pull the holder in and out of the locking mechanism. The repetitive pushing and pulling would wear the piece out more quickly in terms of keeping the original shape. This motion would also cause extra movement and pressure, which could cause further pain to the individual.
- It was too complicated for its intended function; it required too much work for the user, when the whole purpose was to make it easier to adjust the position of the ultrasound.
- This system would not be removable; the holder would be protruding out of the armband, which could become uncomfortable over time, especially if the user did not want to use the ultrasound.
- The 3D printer would have had a hard time with this design because of the long slender parts as well as the holes within the piece, and it was crucial that the ultrasound holder be 3D printed.
- This design was based on the first design for a full-length armband.

Second Design

After considering the fact that the first design was dependent on the injection of the protein-drug complex, had a lack of flexibility to be solely used at home and heavily relied on the circulatory system (which made the objective of controlled drug delivery not possible), it was obvious that a new design was needed to accommodate the original objective of creating a noninvasive and portable device with the ability to control drug delivery. A more feasible approach to the problem was to depend on the diffusion of the drug through the skin. In order to facilitate this, the second device design consisted of the following:

- A simple cloth band (similar in shape to a blood pressure band)
- A heating element
- A cooling element
- A sheet of copper (to help heat transfer)
- An ultrasound with a Velcro holder that could be adjusted to anywhere on the arm.

Similar to the first design, the focus was on the application to the arm while allowing flexibility to design for any other part of the body.

Like the original design, the inclusion of the ultrasound was key in further facilitation of transdermal drug delivery. Because the first ultrasound holder design was not ideal, it was necessary to design a holder that could be removable, more user-friendly, and less likely to

wear out. As seen in Figure 13 (left), this design incorporates all of the parameters mentioned previously. In comparison to the first holder design this design was:

- Easily removable, meaning the user can decide whether he or she wants ultrasound or not.
- The ease of moving it is simple; the user just has to undo the Velcro, move the ultrasound to the area where he or she needs it, and then tighten and Velcro to secure it.
- This design was also much less likely to wear out; it did not rely on the user forcing it into matching holes. Instead, it relied on an elastic or rubber band to hold the ultrasound on the holder.
- Because this holder was easily removable and did not rely on extra force to hold it in place, it was deemed more user-friendly than the first design.

However, even though this design met the above criteria, it did not have the capability of securely holding the ultrasound in place. Because of this flaw, it was necessary to add a slight curve to the top of the holder.

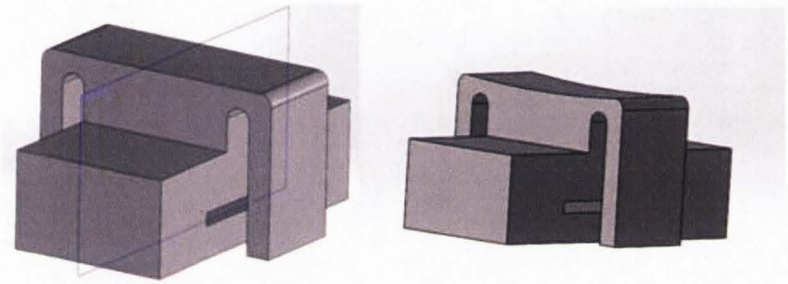

Figure 13. Second design of ultrasound holder. This design includes a slit for Velcro, and two arms to attach an elastic band, which keeps the ultrasound on the holder. The image on the right is the same design just with a slight curve to better hold the ultrasound.

As seen in Figure 13 (right), the addition of the curve to the top of the holder made it much easier for the ultrasound to fit to the holder, therefore making it more stable. Once this design was 3D printed and tested on the arm, the following problems were observed:

- It could not provide the ultrasound sufficient contact with the skin; when the ultrasound was placed on top of the holder, it rested about one centimeter above the skin, which did not meet the criteria of having a clearance of one millimeter or less.
- The holder was not very stable when strapped to the arm; this was not ideal because any additional movement could not only cause more pain to the user, but also position the ultrasound in an undesirable location on the affected area.

This led to another change in the overall design of the device as well as a change in the design of the ultrasound holder.

Third Design

Even though the second overall design fit most of the criteria, there was still one problem regarding the use of the cooling element. Originally, the cooling element was essential in the heating and cooling cycles for HSA polymerization and the release of ibuprofen. The cooling element was kept in the second design for inflammation purposes because cooling helps reduce inflammation. However, the cons of incorporation of this element outweighed the pros. The negatives of the cooling bladder are as follows:

- It would require more energy to power the device, which could become costly.
- The use of water or other cooling fluid would have been involved, which if a leak were to arise, it could damage electrical components as well as introduce the risk of electrocution.
- It would be cumbersome to have to carry around a cooler, which defeats the purpose of the device being easily portable, not to mention that some of the consumers might not have the ability to lift said device; therefore, the third design phases out the integration of the cooling element.

In terms of the ultrasound holder, it was crucial that the problems with the clearance between the ultrasound and the skin as well as stability of the ultrasound on the body part were addressed in the next design. As seen in Figure 14, the third design incorporated a curve and an inclined surface (roughly 21 degree incline) in order to maximize the contact of the ultrasound with the skin. This design also included a wider slit for Velcro and a bigger base for better stability. When tested on a human arm, it was obvious that the ultrasound was touching the skin; it was also obvious that when the arm moved back and forth, the ultrasound stayed in place, therefore maintaining stability and contact with the skin. It was decided, based on the above observations, that this design would be the final design for the ultrasound holder.

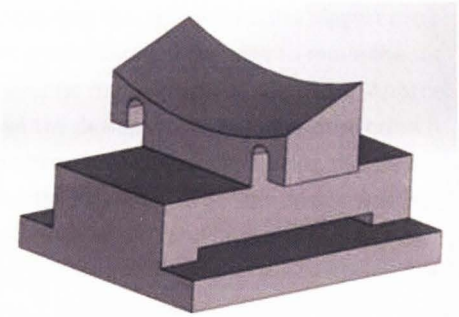

Figure 14. The third design incorporated a more stable base as well as an inclined and curved top to maximize the contact of ultrasound with the skin.

Even though a design for the ultrasound holder had met the specified criteria, the overall device was still lacking the following components:

- It was difficult to draw the design, and it was even more difficult to build a prototype, which building a prototype was one of the crucial steps for the success of the project.
- There was not an easy way to incorporate the microcontroller and the LCD screen into the armband without making the whole product bulky and cumbersome.
- An ultrasound should have the ability to move around, since it should not rest in one place for an extended period of time; the design would not have allowed the ultrasound to move, which could cause additional problems for the user.

Ultimately, a new design was needed to allow ease of prototyping, the ability to house the microcontroller and LCD screen, and the potential to be easily moved around the surface of the skin.

Fourth Design

Using the overall objectives and the flaws of the previous designs as a guide, a fourth design was created. Instead of relying on an armband to ensure proper delivery of the ibuprofen, it was easier to create a handheld device. This design included:

- A laundry-iron type mechanism where the heating and the ultrasound were on the bottom of the device along with a layer of polydimethylsiloxane (PDMS) to increase the efficiency of the ultrasound as well as provide a barrier between the heating element and the ultrasound.
- Places for the microcontroller and the LCD screen as seen in Figure 15, unlike a few previous designs that could not incorporate these elements.
- The last design of the ultrasound holder because it had fit the criteria so well.
- A beveled hole for the ultrasound and holes for tubes of gel and LED lights as device status indicators.

In comparison to the other devices, this one solved the biggest problem of a stationary ultrasound component; this device allowed the user to move the ultrasound (along with the rest of the device) to wherever he or she deemed necessary. Another positive to this design was that the handle to move the device was the actual ultrasound handle, therefore ensuring constant movement of the ultrasound head. Fortunately, this device was 3D printed and tested with the necessary components. However, after this design was 3D printed, it was obviously too bulky for its intended purpose. A fifth and final design was deemed necessary to make the product more user-friendly.

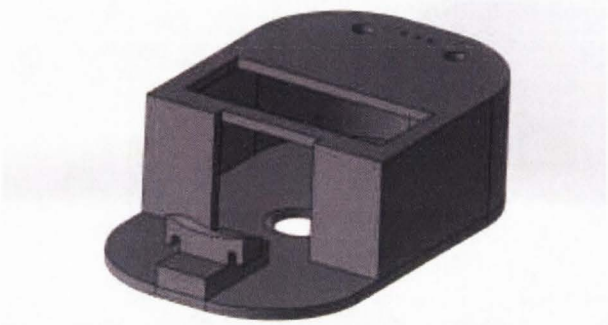

Figure 15. Fourth overall device design. As seen above, there is room for an Arduino and an LCD screen in the front. There is a beveled hole for the ultrasound to ensure proper contact with the skin. The holes in the top of the device are for tubes of gel as well as small holes for three LED lights.

Fifth Design

When comparing the fourth design with the fifth (and final) design, the differences between them are quite small, however the fifth device was designed to be much slimmer; this design feature makes the device easier to hold and maneuver, allowing it to be more user-friendly. Similar to the fourth device design, this new design has a space underneath where the copper, the heating pad, a layer of PDMS, and the temperature probe can go in order to provide sufficient heating and ultrasound. Differences between the two designs include:

- A parallel LCD screen opening to make the device slimmer. The screen opening in the fourth design was perpendicular, which had contributed to the extra width of the device.
- A wider beveled hole (roughly twice the size) to ensure proper contact with the skin.
- A forward shift in the position of the ultrasound holder in order to maximize the amount of handle present; this change was made to ensure the consumer would have a comfortable grip. It would also enable the device to be easily manipulated to wherever the user considers necessary.

- A slight decrease in the length of the device because the Arduino is mounted on the side of the device instead of on the bottom. With that said, the device needed to be slightly taller to accommodate the placement of the Arduino on the walls of the device (refer to Figure 16).

Figure 16. Fifth overall device design. As seen above, this final design features a slimmer look without compromising on functionality.

When the different designs are compared to each other, it was obvious that the fourth and fifth design were the best because they were so similar, however the fifth design was slightly better. Looking at Table 11, the fourth and fifth designs ranked the highest in consistent performance, versatility, durability, and ease of use categories; they also tied with the second and third design in the noninvasive and power consumption categories. As was described earlier, there were many pros and cons to each design. The first design was an acceptable first design because it met a few of the selected criteria, however it still required an invasive treatment; it also was not very versatile or easy to use. The second design was much better in comparison to the first because it was a bit more versatile and it was noninvasive. Unfortunately, it included a cooling element that was not needed and it did pose a slight threat of possible electrocution if the water pump were to leak. The third designed phased out the cooling element hazard and focused on heating and ultrasound. This product was very noninvasive, safe, durable, and affordable, although it could not be 3D printed, which was a critical part for building a prototype. It also did not allow for movement of the ultrasound, which could cause a variety of additional problems. Taking the negatives of prior designs, the fourth and fifth designs were created. These designs were very versatile, more durable, and the easiest to use. The three best parts of these designs were they allowed for ultrasound movement, were able to be 3D printed, and had housing for all the necessary components of the automated system (Arduino, LCD

screen, lights, temperature probe, etc.). With that said, the fourth design was still too bulky, therefore making the fifth design slightly more versatile and easier to use.

Table 11. Device designs evaluated against ranked, weighted criteria

	Priority	Category Weight	Fifth Design: Slim iron device with heating and ultrasound	Fourth Design: Iron device with heating and ultrasound	Third Design: Small arm band with heating and ultrasound	Second Design: Small arm band with heating, cooling, ultrasound	First Design: Full length arm brace
Noninvasive	1	1	10	10	10	10	2
Safety	1	1	10	10	10	8	8
Consistent Performance	2	0.8	9	9	8	7	7
Versatility	3	0.7	9	8	5	4	2
Durability	4	0.6	9	9	8	6	4
Power Consumption	5	0.5	9	9	9	7	7
Ease of Use	6	0.4	10	9	6	4	2
Affordability	7	0.3	8	8	9	6	4
Score			**49.8**	**48.7**	**44.3**	**36.9**	**24.9**

Tissue Testing

In vitro tissue testing was incorporated into the design process as it provided a reliable and valuable test to evaluate the drug functionality in a more relevant biological setting. Primary cells are cells that are extracted directly from living tissues of interest; therefore, they provide a more representative model to cells *in vivo.* Primary fibroblast cells was used because they are more robust and provided a longer culture life span when compared to other primary cells (Takahashi, 2006).

Although *in vitro* drug release test provided an excellent way to evaluate the effects of the drug on cell culture, it did not provide an accurate estimation of how much of the drug was able to diffuse through skin *in vivo*. The second phase of tissue testing was added to determine the diffusivity properties of the gel containing Ibuprofen through skin. The use of a natural or artificial skin layer is a common method to evaluate the efficiency of drug delivery method in drug release studies. The use of a porcine skin layer was then incorporated into the tissue testing to mimic the human skin barrier and to simulate the diffusion of the drug through skin.

The original design relied on changes in temperature to release ibuprofen. The goal of this project was to show that after treatments of HSA-ibuprofen compound by changes in temperature, ibuprofen was able to be released and perform through the inhibition of inflammation in tissue culture. In tissue engineering, exposure of cell culture to hypoxia and inflammatory cytokine are two common methods of inducing cell inflammation *in vitro*. In the first phase of tissue testing experiment, exposure to hypoxia was chosen for its feasibility in inducing inflammation in cell culture. Oxygen is a vital substance to life because of its role in the final acceptor of electrons in the electron transport chain. Stress caused by a lack of oxygen has been shown to affect cell survival and viability (Yun, 1997).

Exposure of cells to hypoxic condition is commonly done by placing the cell culture flask in an incubator with 2% or less oxygen concentration. In the first phase of tissue testing, hypoxic condition was achieved by covering the cell culture flask using plastic paraffin film to prevent the flow of oxygen into the flask in the incubator. To determine the time it takes to effectively induce inflammation, 3T3 fibroblast cell was exposed to hypoxia for 5 hours, 12 hours, 24 hours and 48 hours. The 24-hour exposure was found to be the most effective in inducing inflammation compared to the 5 and 12-hour exposure as it significantly decreases the cell population. On the other hand, 48-hour exposure led to complete cell death as shown in Table 12.

Table 12. Average cell population count after hypoxia induced inflammation.

	Average Cell Population (10^6 cells)				
Experiment	**Control**	**5 hours**	**12 hours**	**24 hours**	**48 hours**
1	2.8	2.76	2.52	1.5	0
2	3.3	2.98	2.84	1.62	0
3	3.04	2.96	2.8	1.44	0
4	2.98	2.74	2.2	1.12	0
Average	3.03	2.86	2.59	1.42	0
St. Dev.	0.21	0.13	0.29	0.21	0.00

Cells were exposed to solutions of ibuprofen, ibuprofen+HSA compound and ibuprofen+HSA+fatty acid complex. T75 polystyrene flask was seeded with $7x10^4$ cells on day 1 of the tissue testing experiment. Cells were allowed to grow for 48 hours. On day 3, the flask was covered with paraffin film to prevent the flow of oxygen and was subjected to hypoxic condition for 24 hours. After 24 hours, the cells were trypsinized and stained with trypan blue dye to calculate the cell population count as shown in Table 13.

Table 13. Average cell population count after cell exposure to ibuprofen, ibuprofen+HSA compound and ibuprofen+HSA+fatty acid.

	Average Cell Population (10^5 cells)			
Experiment	**Control**	**Ibp**	**Ibp+HSA**	**Ibp+HSA+Fatty Acid**
1	14.2	15.9	14.05	15.1
2	15.1	15.55	13.95	16.15
3	14.9	14.1	14.2	15.2
4	14.75	15.5	14.8	16.3
Average	14.73	15.26	14.25	15.68
St. Dev.	0.38	0.79	0.38	0.54

Because the mechanism of drug release changed, a second phase of tissue testing was added to prove the release and the diffusion of ibuprofen. While the first phase of tissue testing provided a good support that the effectiveness and properties of Ibuprofen remained the same, it did not provide enough proof that the drug release was a result of the drug delivery treatments. In the second phase of tissue testing, an endotoxin called lipopolysaccharide was used to induce inflammation in cell culture. Lipopolysaccharide is a cell wall component found in gram-negative bacteria, which plays a role in the process of septic shock (Martich, 1993).

The second phase of tissue testing included the use of a natural skin layer as final step to determine the diffusivity of the drug delivery complex. A porcine skin layer was used to cover a petri dish containing cell culture to mimic human-skin interface. Treatments including the application of gel containing the HSA-Ibuprofen-fatty acid compound and also ultrasound were applied on the skin. The skin was treated with heating pad for 2.5 minutes and followed by ultrasound for 5 minutes. Treatment on the porcine skin using gel that contained only the fatty acid without the HSA-Ibuprofen compound was used as a method control in addition to a negative control as seen in Table 14. Treatment on the porcine skin using gel and ibuprofen-HSA-fatty acid complex showed the highest average cell population count as shown in Figure 17.

Table 14. Average cell population count after tissue testing using porcine skin layer.

	Average Cell Population (10^5 cells)		
Experiment	**Control**	**Gel+Fatty Acid (Method Control)**	**Gel+Ibp complex (Experimental)**
1	14.45	13.85	15.05
2	12.85	13	13.35
3	12.5	12.5	13.6
4	12.8	12.3	14.05
Average	13.15	12.91	14.01
St. Dev.	0.88	0.69	0.75

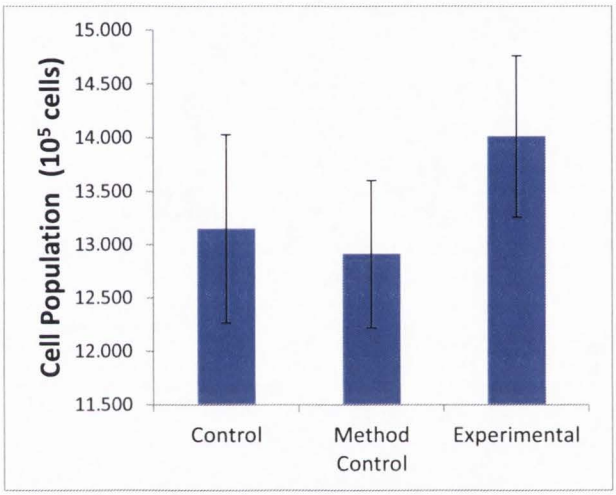

Figure 17. Average cell population after treatment on natural porcine skin layer; Error bars represent standard deviations.

Statistical analysis shown in Table 15 revealed that treatment on the porcine skin using gel which contained the Ibuprofen-HSA-fatty acid complex significantly increased the cell population. Because the P-value is less than the significance level of 0.1, it can be concluded that the null hypothesis that the treatment does not increase the cell population could be rejected with 90% confidence interval. In addition, the method control showed that there was no statistically significant difference in cell population compared to the control.

Table 15. Statistical analysis of tissue testing result.

Mean Values of Each Group	Mean Cell Population (10^5 cells)	Standard Deviation (10^5 cells)	Std. Err. (10^5 cells)	α	P-value
Control	13.15	0.88	14.02	-	-
Method Control	12.91	0.69	15.54	0.1	0.27
Experimental	14.01	0.75	16.18	0.1	0.05

Modeling

The focus of the modeling section is to create a 2D model of diffusion of ibuprofen through skin. This section will be divided into three smaller models:

1. Model of heat transfer from the drug delivery system device
2. Model of competitive binding of ibuprofen and fatty acid in HSA
3. Model of diffusion of ibuprofen across skin

Model of Heat Transfer

This model focused on the heat transfer both on the surface of the skin as well as within the body and how external heating affects the transdermal delivery of the drug of interest. When developing the model, a few assumptions were made. The body part of interest was the lower arm, which was to be modeled as a cube. Convection on the surface of the arm was negligible, and radiative heating from the body was ignored. It was assumed that a layer of dead skin cells could be described using a 2D model with changes in two positions ("x" that runs parallel with the skin and "z" that goes into the skin) as well as changes in time, which can be seen in the following equation:

$$\frac{\delta T}{\delta t} = \alpha \left[\frac{\delta^2 T}{\delta x^2} + \frac{\delta^2 T}{\delta z^2}\right]$$

where α is the thermal diffusivity; x, the change in distance parallel with the skin; and z, the change in distance within the skin layers (Bruch, 1974). On the surface of the skin, without the influence of the device, the temperature was assumed to be 30 °C when the temperature of the surrounding air was 25 °C (Cross, 2008). Because there was not any external heat being applied to the surface of the dead cell layer, this layer was assumed to be in steady state, therefore following a linear temperature distribution, assuming the live cell layer remained at a constant 37 °C. The equation used for the linear temperature distribution is as follows:

$$T_d(z) = \frac{T_l - T_{surf}}{z_d \cdot k} + T_{surf}$$

where $T_d(z)$ is the temperature of the dead cell layer at a given depth, z; $T_.$, the temperature of the top of the live skin layer; T_{surf}, the temperature of the top layer of skin when surrounding air temperature is 25 °C; z_d, the depth of the dead cell layer; and k, the depth iteration.

For the live cell layer, a 1D heat transfer model for blood flow was created using the following modified Pennes' bioheat equation:

$$\frac{\delta T}{\delta t} = \alpha \frac{\delta^2 T}{\delta x^2} + \left[\omega_0 + \omega_1 \frac{T - T_a}{T_a}\right](T_a - T)$$

where T_a is arterial blood temperature; T, local temperature of tissue; α, the thermal diffusivity; ω_0, temperature-independent (basal) perfusion rate (volumetric flow rate of blood per volume of tissue); ω_1, temperature-dependent (vasodilation) perfusion rate; and x, the distance from the heated surface (Lakhssassi, 2010). For modeling purposes, T_a and $\omega_1$were kept constant. The thermal diffusivity was also assumed to be the same for both dead and live skin cells.

In order to combine the two models, it was assumed that the top layer of the live cell layer would be equal to the bottom layer of the dead cell layer. Therefore running them together could give an approximation about the temperature distribution throughout the skin when a 40 °C heating element is applied to the surface of the skin. From preliminary runs of the model, it is apparent that the skin approaches 40 °C within the allotted time of 150 seconds (2.5 minutes).

As seen in Figure 18, when heating a section of an arm with an external temperature of 40 °C, the temperature through the layers of skin quickly approaches this external temperature. Between time equals 0 and 60 seconds the most heat transfer occurs between the surface of the skin and between the tissue and blood vessels. Once the time reaches 90 seconds and beyond, the difference in temperature become much less, indicating equilibrium. From this model, it was discovered that the inside layers of the skin will approach 40 °C, therefore indicating that the design device has the potential to further promote drug diffusion. As a note: the horizontal axis labeled "x (mm)" is the direction parallel to the arm, the depth axis labeled "z (mm)" is the depth into the skin, and the vertical axis labeled "Temperature (C)" is the temperature values used to help create the temperature distribution.

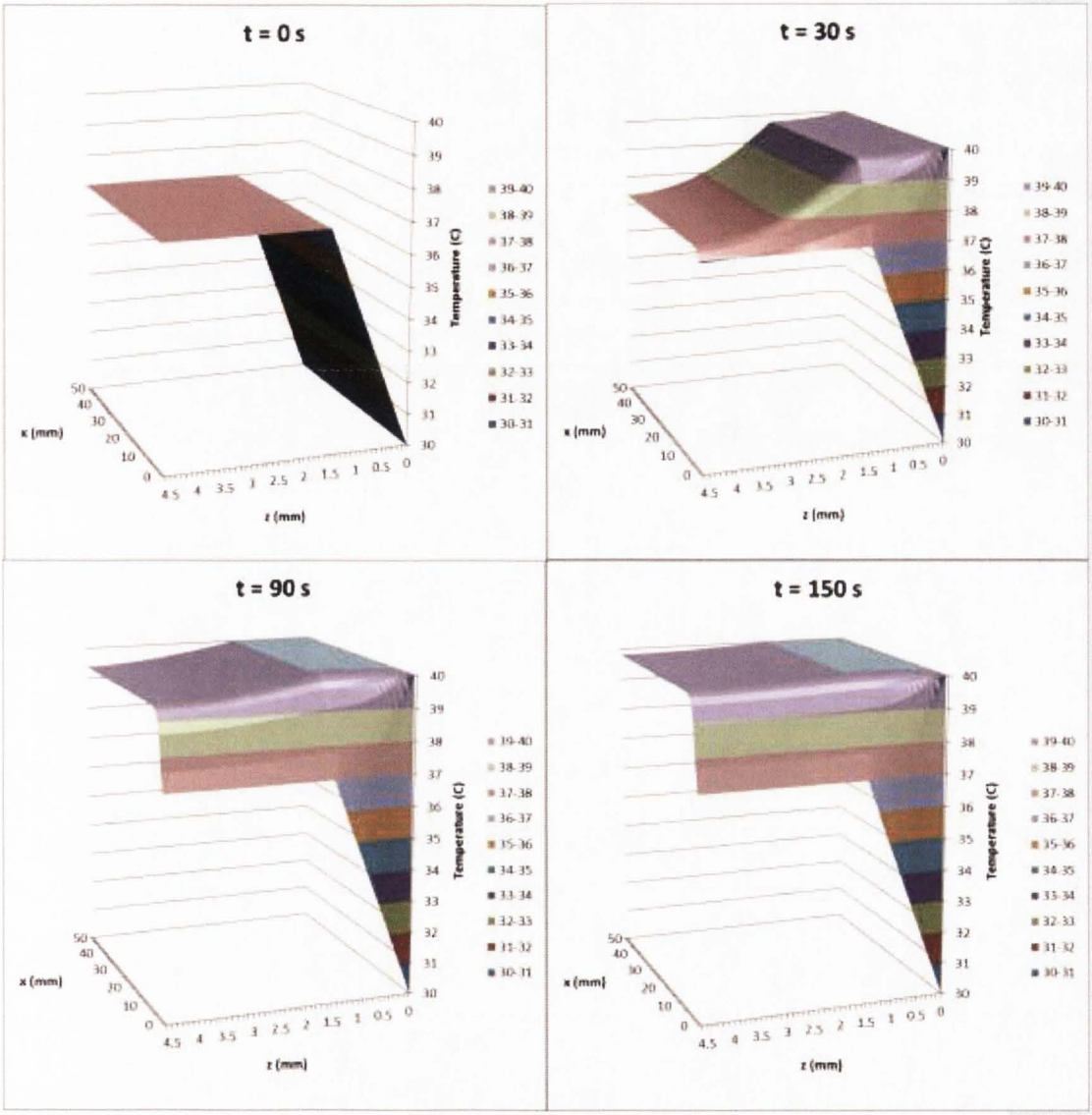

Figure 18. Temperature distribution of 2D surface heat transfer with 1D blood vessel to tissue heat transfer. As seen above, the temperature approaches equilibrium rather quickly, between 90 seconds and 150 seconds (around 120 seconds or 2 minutes).

Model of Competitive Binding of Ibuprofen and Fatty Acid with HSA

The competitive binding component is based on molecular interactions upon application and blending of the two inert gels containing HSA-bound ibuprofen and distilled fish oil fatty acids. The approach takes the dissociation constant (Kd) values for HSA–ibuprofen and HSA–fatty acid binding (Hage, 1995; Simard, 2006), the recorded treatment temperature profile, and published minimum time periods to equilibrium (Baroni, 2001), and influence on Kd by temperature (Brodersen, 1974) over time to calculate concentrations of free and bound molecules. Values from early mass diffusion were calculated concurrently for improved accuracy in both binding and diffusion calculations.

The following equations were applied for the concentration of albumin-bound and free ibuprofen, eicosapentaenoic acid (EPA) fatty acid, and docosahexaenoic acid (DHA) fatty acid as applied in other published applications (Wilkinson, 2004).

The following equations regarding the concentrations of Ibuprofen, Albumin, EPA, DHA and their respective bindings are given. These concentration models were then developed in graphical form (see Figures 19 and 20).

$$L_1 = Ibuprofen$$
$$L_2 = EPA$$
$$L_3 = DHA$$
$$R = Albumin$$
$$L_1R = Ibuprofen - Albumin$$

$$\frac{d\,[L_1]}{dt} = -k_{ass}[L_1][R] + k_{diss}[L_1R] - k_{ass}[L_1][L_1R] + k_{diss}[L_2R] + k_{diss}[L_3R]$$

$$\frac{d\,[R]}{dt} = -k_{ass}[L_1][R] + k_{diss}[L_1R]$$

$$\frac{d\,[L_1R]}{dt} = k_{ass}[L_1][R] - k_{diss}[L_1R] - k_{ass}[L_1][L_1R] + k_{diss}[L_2R] + k_{diss}[L_3][R]$$

$$\frac{d\,[L_2R]}{dt} = k_{ass}[L_1][L_1R] - k_{diss}[L_2R] - k_{diss}[L_3R]$$

The EPA and DHA concentration equations were fashioned following the Ibuprofen model.

$$\frac{d\,[L_2]}{dt} = -k_{ass}[L_2][R] + k_{diss}[L_2R] - k_{ass}[L_2][L_2R] + k_{diss}[L_1R] + k_{diss}[L_3R]$$

$$\frac{d\,[L_3]}{dt} = -k_{ass}[L_3][R] + k_{diss}[L_3R] - k_{ass}[L_3][L_3R] + k_{diss}[L_2R] + k_{diss}[L_1R]$$

$$\frac{d\,[L_2R]}{dt} = k_{ass}[L_1][L_1R] - k_{diss}[L_2R] - k_{diss}[L_3R]$$

The models for were then analyzed using association and dissociation constants (k_{ass} and k_{diss}) for each compound with ibuprofen. Constants were correlated to temperature profile with a respective constant values found in literature (Hage, 1995).

It was found that 95% of equilibrium in competitive binding would be reached by 0.5 seconds after introduction of the fatty acids, according to the considered parameters of the model. As shown in the Figure 19, the summed binding of the three compounds never exceed the total number of binding sites available, indicating appropriate relationships between separate compound calculations. Similar comparisons were made between bound and unbound compounds to find the same conservations within the calculations, further supporting validity of the model within the scope of considerations. Equilibrium seemed to follow temperature equilibrium, as can be observed in Figure 20.

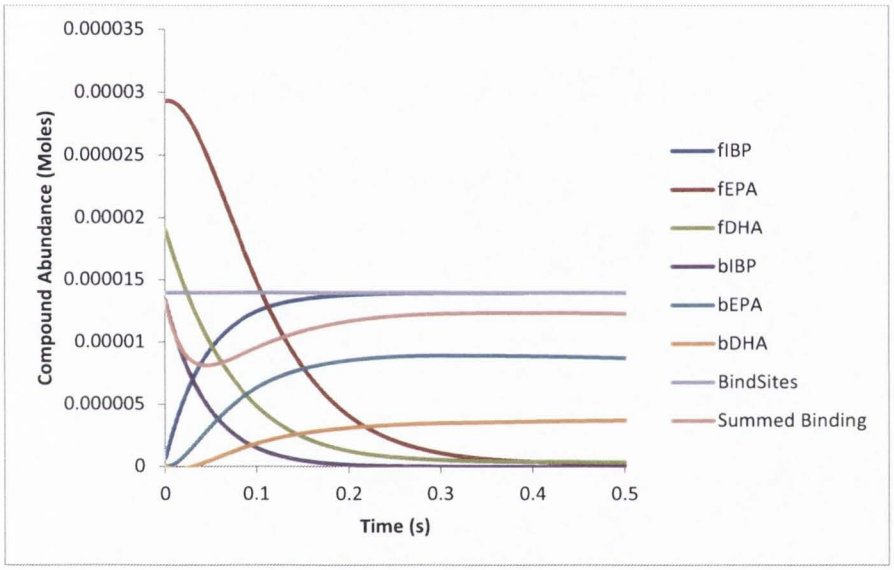

Figure 19. Compound abundance in moles over time; compounds preceded by f are "free", compounds preceded by b are "bound"; Summed binding is given to compare with binding sites and demonstrate obedience of separate compound calculations to constraints .

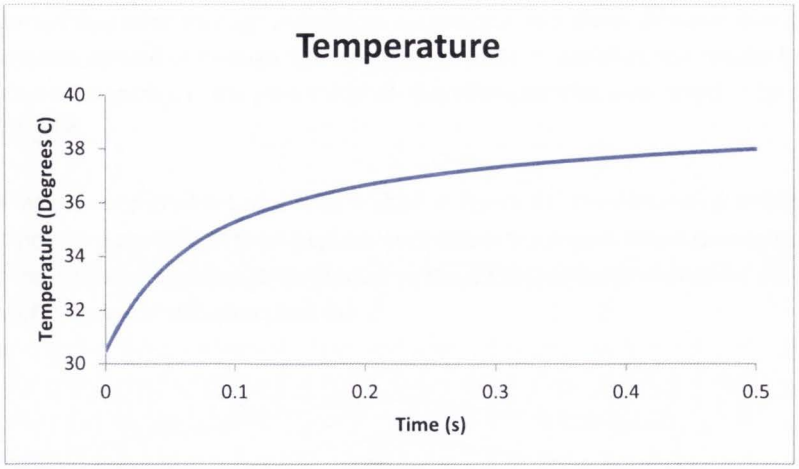

Figure 20. Temperature profile for the skin at 1 mm depth

Model of Diffusion of Ibuprofen

In this model, concentration of ibuprofen was calculated in terms of time, x direction and z direction. The diffusion of ibuprofen across skin was dependent on the previous two models discussed: heat transfer from the drug delivery system device and the concentration of ibuprofen after being displaced by fatty acid. A top section of the arm was assumed to be a cube. The diffusion coefficient D was calculated as a function of temperature from the heat transfer model using the equation:

$$D = \frac{k_B T}{6\pi\mu r_0}$$

Where k_B is the Boltzmann constant, T is the temperature, μ is the viscosity of the gel at surface temperature, and r_0 is the molecular radius of ibuprofen.

Fick's Law of Diffusion, $\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$, was used to derive an equation to calculate the concentration of ibuprofen that diffuses across skin in 2D model. The equation was derived to be:

$$C_{i,j,k} = dt * D\left[\frac{C_{i-1,j+1,k} - 2C_{i-1,j,k} + C_{i-1,j-1,k}}{dx^2} + \frac{C_{i-1,j,k+1} - 2C_{i-1,j,k} + C_{i-1,j,k-1}}{dz^2}\right] + C_{i-1,j,k}$$

Where C is the concentration of ibuprofen across skin, D is the diffusion coefficient, dt is the change of time, dx is the change in position with respect to x and dz is the change in position with respect to z.

The transport of ibuprofen through skin will be assumed to be a direct diffusion through stratum corneum instead of through hair follicles and ducts. In addition, the change in the concentration of ibuprofen in the gel is minimal, therefore, the flux is assumed to be 0 at the gel-skin interface.

Comment [SB1]: Also should include our figure from the presentation with some data

The ibuprofen diffusion gradient over time is given in Figure 21. The diffusion gradient accounts for the temperature profile and drug displacement within the model. There is an initial sharp slope, and the diffusion becomes more gradual as the difference between depths decreases. This behavior is typical of diffusion patterns.

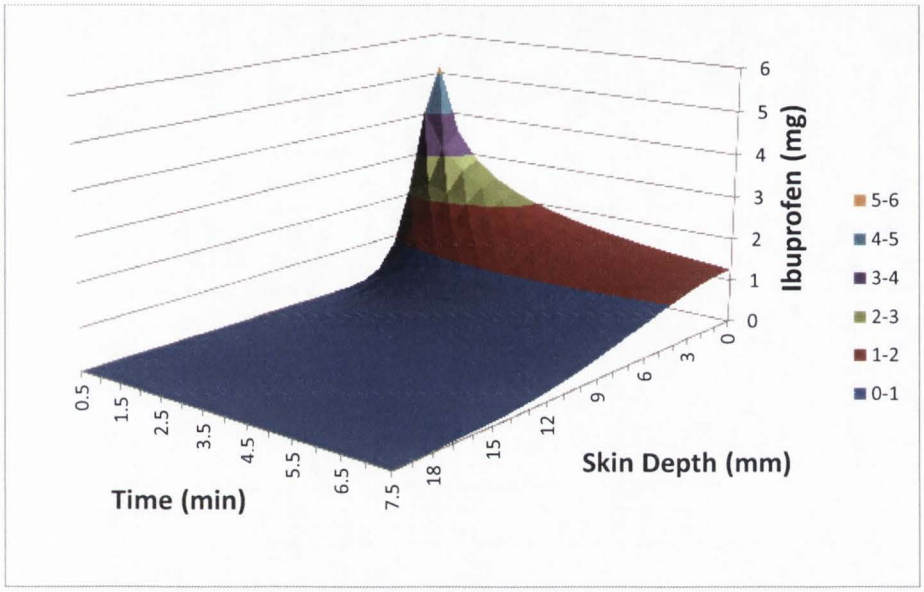

Figure 21. Concentration of ibuprofen with respect to skin depth over time

FINAL DESIGN

As stated in the objectives section of this report, the overall goal of this project is to design a product that will transdermally deliver drugs to an affected area using a two gel system, one with the protein-drug complex and the other with a displacement factor, and a device that provides heating and ultrasound.

The final design for drug delivery is a two gel system with one gel that carries the drug payload, and another gel to displace the drug at time of application. The system is designed for a 1 mL gel application, 0.5 mL of each type. A model drug, ibuprofen, is bound with human serum albumin and suspended in a guar gum gel. The ibuprofen concentration is 40 mg/mL, 5% of the prescribed oral dose (400 mg). Drug complexation was performed with 90 mg/mL human serum albumin. The guar gum was prepared by the dissolution of 3 grams guar gum per 100 mL of water at 80 C. The 10:1 mass ratio of fish oil to HSA was prepared for the displacing gel by blending 900 mg fish oil per mL gel.

In conjunction with the two gel system, the device will serve as a facilitator in the diffusion of the drug through the skin. The device consists of two major parts: a heating element and an ultrasound. The heating element facilitates drug diffusion by opening up the pores in the skin as well as relaxing the surrounding tissues. The ultrasound also helps in opening of the pores, but it also provides a therapeutic component. As seen in Figure 22, the final ultrasound holder design is incorporated, however its base was removed in order to reduce the size of the overall device. The final device was designed to contain an LCD screen (opening at the top), three LED status indicators (small holes to the left of the LCD opening), a large beveled opening to maximize ultrasound contact with the skin, and an opening where the ultrasound can be placed, therefore providing the user with a substantial handle to maneuver the device (square opening in front of ultrasound holder). Underneath the device is a recessed space for the copper sheet, the heating pad within the PDMS, and the temperature probe (to make sure the device does not exceed 40 °C). As can be seen, this device has the ability to hold all the necessary components, to be easily manipulated and moved around, to be very user-friendly, and most of all, to help promote localized transdermal drug delivery of ibuprofen.

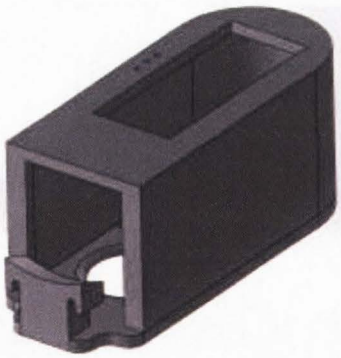

Figure 22. The fifth and final design of the device.

EVALUATION OF FINAL DESIGN

Comment [SG2]: Need to provide qualitative support for all of these

The two-gel system met drug binding and release criteria. The criterion for drug binding in this project was a 20% reduction in HSA fluorescence at 320 nm. Binding was verified with a mean decrease of 35.3% in fluorescence of HSA by the use of fluorometer as shown in Table 16.

Table 16. Resulting fluorescence of free ibuprofen, pure albumin, and an ibuprofen/albumin solution at 325 nm

Samples	Mean Fluorescence (RFU)	Standard Deviation (µg/L)	Count (n)	Std Err (µg/L)	α	P-value	Mean % Reduction
HSA	61651	2981	3.00	1721	-	-	-
HSA + IBP	39913	3054	3.00	1763	0.01	0.0065	35.3

Drug release was ensured through LC-MS/MS analysis before and after displacement treatment. The criterion for drug release was a 50% release of bound ibuprofen concentration. After ibuprofen displacement by fatty acid, it was revealed that there was a 51.5% mean release of bound ibuprofen from HSA as shown in Table 17. This confirmed that our criterion for drug release was met.

Table 17. Ibuprofen concentrations for LC-MS/MS measurement in aqueous solutions; Triplicate samples were measure in triplicate

	Mean Ibuprofen Conc. (µg/L)	Standard Deviation (µg/L)	Standard Error (µg/L)	α	P-value (Change from Previous Stage)	Mean Percent Ibuprofen Released	Release Standard Deviation (%)
Unbound Samples	16.05	0.0958	0.0553	0.05	N/A	-	-
Bound Samples	14.22	0.0581	0.0335	0.05	0.0159	-	-
Displaced Samples	15.23	0.0578	0.0334	0.05	0.0471	51.45	10.25

In terms of the device, the criteria have been completely met. When 11 volts of energy are applied to the heating element, it heats up to 40 degrees C (and above) in only two minutes. As shown in Table 18, each run had a time below the expected two minutes (120 seconds). The mean time for the three runs was around 100 seconds with a standard deviation of 12 seconds; therefore, the heating element meets the original criteria. Figure 23 demonstrates this as well. Because the ultrasound holder was designed to maximize the contact between the ultrasound and the skin, it was concluded that this criteria was successfully met. In addition, when the ultrasound was placed on the holder (as well as on the holder of the 3D printed device), it was obvious that the ultrasound head had direct contact with the skin, thus further meeting the specified criteria. Since the device was designed to be both noninvasive as well as having the ability to be applied anywhere on the body, it can be said that this design meets the noninvasive and versatility requirements.

Comment [SG3]: label

Table 18. Time distribution for three different runs of the heating element.

Comment [SG4]: label

Temperature (degrees C)	Run 1 (sec)	Run 2 (sec)	Run 3 (sec)
30	0	0	0
31	1	0	0
32	11	18	0
33	18	27	24
34	26	24	36
35	33	44	50
36	42	54	63
37	51	65	73
38	61	59	85
39	72	91	97
40	83	107	112

Mean Time (sec)	100.67
Std. Dev (sec)	12.66

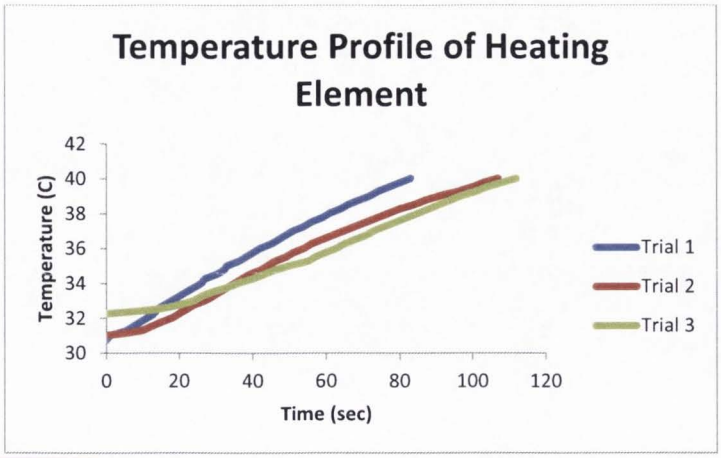

Figure 23. Temperature profile of a heating pad during three timed runs.

Comment [SG5]: label

In addition, tissue testing with the use of porcine skin layer demonstrated that treatment using the gel containing the drug complex increased the average cell population of 3T3 fibroblast cell culture. Tissue testing further supported the displacement of ibuprofen from HSA by fatty acid and that the designed drug delivery system was successful.

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