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Department of Biological Engineering
URCO Grant Proposal

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Abstract

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 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, 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. This drug delivery system is unique in using rapid---sequence, local hyperthermia, hypothermia, and ultrasound at a specific site of interest in the body. The proposed system is designed for patients with compromised liver and kidney function who are unable to safely use normal, oral doses of these medication types.

Significance and Innovation

The proposed drug delivery system is pertinent to the study of drug delivery strategies for patients with compromised kidney and liver function. Studies have shown that non---steroidal anti---inflammatory (NSAID) drugs, such as ibuprofen, have a broad spectrum of adverse side effects on kidney and liver function (Bushra, 2010). According to Bessone, the use of such NSAID drugs could potentially cause transient, severe or sudden hepatic failure (Bessone, 2010). It can then be concluded that the use of such drugs requires constant monitoring of their effects especially on compromised livers and kidneys during the course of treatment. A localized drug delivery method could potentially be a safer alternative as it allows for the administration of drug without exposing the entire body to the same dose (Ibsen, 2012).

Using human serum albumin (HSA) as a drug carrier allows a more localized drug delivery to the tissues of interest. In addition, this drug delivery system can reduce the dosage of drug required as well as the severity of side effects. A programmed device will control the release of drug through localized change of temperature and ultrasound application. The localized change of temperature will induce the denaturation and renaturation mechanisms of the HSA, which will release the drug. In this study, ibuprofen is used as a proof of concept, however, this drug delivery system is designed to work with various types of drugs, such as anti---inflammatory, antibiotic, antiviral and antioxidant drugs. Finally, the success of this drug delivery system will be evaluated using *in vitro* tissue testing. Various methods such as PCR array will be used to determine the effectiveness of the treated HSA on fibroblast cells with induced inflammation.

Specific Aims

1. *Identify a binding method for preparation of ibuprofen---HSA compound:*
    1) Perform viable binding methods
    2) Verify drug binding to the human serum albumin
    3) Select the best candidate based on decision matrices
2. *Select method of drug release:*
    1) Use testing and instrumentation to find optimal heat, cooling, and ultrasound parameters for drug release in phosphate buffer solution
3. *Develop a programmable device to provide localized hyperthermia, hypothermia, and ultrasound for drug release from albumin proteins:*
    1) Following optimization testing in the second aim, an extracorporeal device will be constructed using microcontrollers to orchestrate sequential steps in the conformation change of the HSA and release of the ibuprofen
4. In---vitro *testing of drug release with live tissue cultures:*
    1) Fibroblast cells will be cultured and exposed to an inflammatory inducing agent, followed by the released and unreleased HSA---drug compound
    2) Assays will be used to test the expression of inflammation---related factors by the cells

Approach and Methods

Specific Aim 1 (*Binding Method***)**

Human serum albumin is known for its ability to bind a wide variety of compounds, such as different fatty acids and ibuprofen(Galantini, 2010). HSA is also known to contain a single fluorescent tryptophan near the primary binding site which will be employed for the binding verification using shifts in fluorescence (Thumser, 1998).

HSA will be dissolved in a 10mM phosphate buffer solution. The solution will be stirred for 12 hours and then filtered. Ibuprofen will also be dissolved in a phosphate buffer and will be added to the HSA solution to be bound at a stoichiometric ratio of 10:1 drug to HSA (Galantini, 2010).

Fluorescence and dynamic light scattering (DLS) techniques will be used to verify drug binding. Fluorescence will be measured using an excitation wavelength of 295 nm with emission at 330 nm and 350 nm. It is expected that fluorescence will decrease when the drug is bound to the protein (Thumser, 1998). The instruments for verification and the glassware have already been purchased. The human serum albumin and the ibuprofen are the primary source of lab costs for this section of the project.

Specific Aim 2 (*Release Method***)**

Human serum albumin is a protein composed entirely of *alpha* helices. Upon heating to 75 degrees C followed by immediate cooling, *beta* structures begin to form (Wetzel, 1980). This change in protein conformation is likely to affect the hydrogen bond between the drug and the protein. Ultrasound is proposed to add energy as a final step in localized drug release from HSA. This release method will be tested for success and to verify the drug is still functional after

treatment.

A mixture of the HSA---drug compound will be prepared in a 10 mM phosphate buffer solution. The mixture will be externally heated at a temperature of 80 degrees C (the highest safe temperature for the surface of the body) followed by external cooling at 0 degrees C (Wetzel, 1980). The solution will then be exposed to ultrasound waves. Drug release will be verified by measuring the fluorescence of the HSA, which should change as the drug is released (Thumser, 1998).

Specific Aim 3 (*Device Design***)**

A device attached to the surface of the skin will be devised to cause the optimal temperature fluctuation and ultrasound interaction needed to release the drug from the HSA. Microcontroller technology will be used to operate the three main components of the device. First, a heating element will be used to raise the tissue temperature. A cooling jacket with circulating water will be used to then reduce the temperature and induce *beta* sheet proliferation. Ultrasound will applied last to instil more energy to the molecules, promote drug release, and serve as an additional form of pain relief.

In later stages of development, a depiction of the final product will be drafted using specified drafting software, such as AutoCad or SolidWorks. It is expected that a prototype will be built, tested and used for demonstration. Figure 1 shows the components of the device design process.

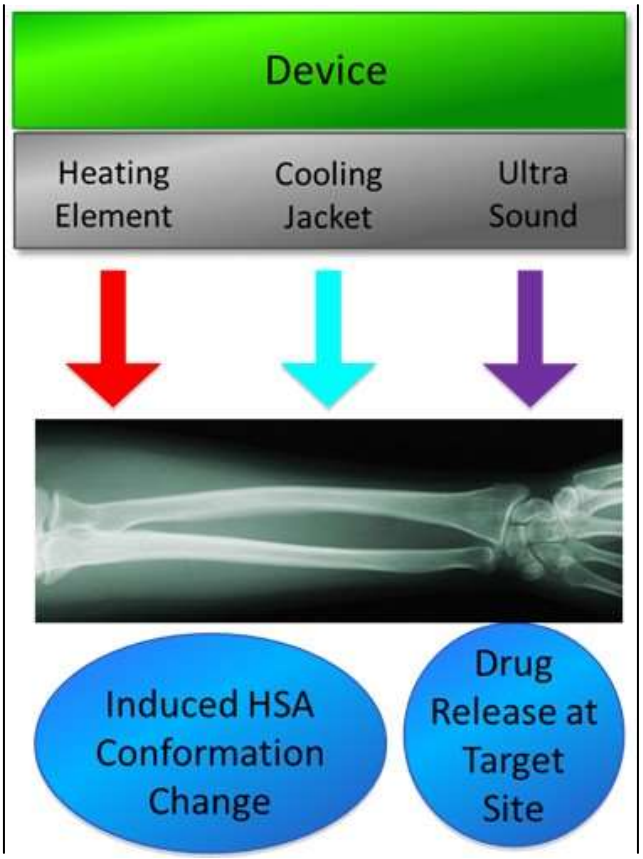

Figure 1. The overarching goal of the proposed research is the development of a drug delivery

system using a device at the skin surface to cause drug release from human serum albumin.

Specific Aim 4 (*Tissue Testing*)

In vitro tissue testing using cultured fibroblast cells will be used as a final means to evaluate the success of the drug delivery system. Fibroblast cell culture will be stimulated using inflammatory cytokine or hypoxic culture conditioning in order to stimulate an inflammatory response (Pooley, 2013). The HSA---ibuprofen compound will then be treated with changes in temperature and ultrasound outlined previously to release the drug. The HSA---ibuprofen compound will then be applied to the inflamed fibroblast cell culture.

Polymerase chain reaction (PCR) array and assay---specific components will be used for the quantitative detection of biomarkers and proteins indicating inflammation present before and after the cells are treated with HSA---drug compound. In addition, cell population growth will be measured on a daily basis to further support the effects of the HSA drug delivery system in treating inflammation as well as to ensure that the HSA does not change the properties of the ibuprofen. A comparison will be made between the cell culture that is treated with the HSA---ibuprofen mixture and the cell culture that is used as a control.

Communication of Results

Findings from research will be presented in the annual review of senior projects to the faculty and students of the Department of Biological Engineering at Utah State University. Any significant findings may be submitted for publishing with a journal with consideration for patenting developed novel technologies.

Project information will be organized into a podium presentation for submission for the American Chemical Society Northwest Annual Meeting in Montana during late June of 2014, and the Biomedical Engineering Society (BMES) Annual Meeting in late October of 2014. Information from this study may also be presented at local student showcases.

Description of Personnel

- Sara: responsible for device design using a microcontroller. She has experience with microcontroller code and medical device design. She also has experience in statistical analysis.
- Sean: responsible for the analysis and optimization of drug binding and targeted release from HSA. He has experience with useful instrumentation (DLS, fluorometer, mass spec, LC---MS/MS, ion chromatography, nuclear magnetic resonance, etc.).
- Stephanie: responsible for tissue testing of drug release. She has experiences in tissue culture techniques such as performing cell passages, enzyme---linked immunosorbent assay (ELISA), immunohistochemistry and data analysis.
- Dr. Elizabeth Vargis: the mentor of this project. She has extensive research experience in cell cultures such as analyzing microfluidic platform with micropatterned surfaces in retinal cells. In addition, she has experienceusing Raman spectroscopy (SERS) to detect biomarkers found in cells.

Timeline

February 15, 2014: Submit URCO Grant Proposals

March 1, 2014: Begin drug binding / release testing

March 15, 2014: Conclude verification of drug binding / release method

March 16, 2014: Start device design and production

April 22, 2014: Interim Report to Senior Design Project Coordinator

May 1, 2014: Complete device design and production

May 5, 2014: Tissue testing using fibroblast cells

June 1, 2014: Completion of tissue testing

June 22---25, 2014: American Chemical Society (ACS) Northwest Annual Meeting

October 22---25, 2014: Biomedical Engineering Society (BMES) Annual Meeting

December 8---12, 2014: Presentation of final project at the senior design review

Spring 2015: Abstracts will be submitted for UCUR, Research on Capitol Hill, and the USU Student Showcase

Budget

Total Amount Requested: $1300

URCO Grant: $650

BE Department Funding: $650

URCO BUDGET SUMMARY		
EXPENSES:	**AMOUNT:**	**PURCHASED BY:**
MATERIAL/SUPPLIES:		
Albumin Protein (500 mg)	$79.00	BE Department
S---Ibuprofen (1000 mg)	$41.30	BE Department
Ibuprofen Sodium Salt (100 g)	$44.80	BE Department
Trypan Blue Dye (100 mL)	$14.90	BE Department
Fibroblast Cell Line	$270.00	BE Department
Cell Media	$22.00	BE Department
Well Plates (50 plates)	$118.00	BE Department
Biomarker Detection Assay	$540.00	URCO Grant
MATERIALS SUBTOTAL	$1130.00	
EQUIPMENT:		
Portable Ultrasound	$110.00	URCO Grant
Arduino & Electrical Components	$60.00	BE Department
EQUIPMENT SUBTOTAL	$170.00	
BE DEPARTMENT TOTAL:	$650.00	
URCO GRANT TOTAL:	$650.00	
TOTAL:	**$1300.00**	

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