Biopackaging of minimally invasive ultrasound assisted clot lysis device for stroke treatment

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Abstract— The biopackaging of a minimally invasive sonothrombolysis device (ultrasound-assisted clot lysis device) for acute stroke treatment is proposed and presented. Instead of using thrombolytic medicine (tissue plasminogen activators) in combination with the ultrasound wave for total blood clot dissolution, the proposed sonothrombolysis device uses only pure ultrasound wave generated from Microelectromechanical Systems (MEMS) -based piezoelectric micromachined ultrasonic transducers (pMUTs) that were designed and fabricated in-house. The proposed biopackaging aims to simplify and shorten the clot lysis procedure which could help speed up the recanalization or surgical removal of the blood clots in acute ischemic stroke treatment. The device packaging is composed of two main integrated parts – the three-dimensional (3D) printed United States Pharmacopeia (USP) Class VI plastic material and the 50-µm thin polyimide flexible printed circuit board (PI FPCB) substrate. The 3D printed USP Class VI plastic material is configured as the drainage catheter of the dissolved clots as well as the custom-fit carrier of the PI FPCB substrate that is coupled and secured onto it. A single layer PI FPCB is used as the substrate of either two or four number of MEMS-based pMUTs, which are attached on it using biocompatible epoxy and wire bonded using 1 mil gold wire. The PI FPCB is also designed such that it is directly compatible with the Flat Flex Cable (FFC) connector of the external circuitry that would trigger the MEMS-based pMUTs to generate acoustic signals as well as measure the viscosity of the blood clot. To drain the dissolved blood clots, the catheter is printed with a number of holes that are placed across and around the pMUT location. The catheter tip is rounded-off to remove sharp corners from the plastic material. Buckling analysis is done to simulate stiffness of the catheter when inserted into the brain tissue leading to the center of blood clot. The buckling load of the 3D printed USP Class VI plastic material at a total deformation of 0 – 1 mm at 1 sec is 2038.4N as compared to the buckling load of the silicone rubber (usual catheter material, without the metal guiding rod) which is only 0.128N. The simulation results showed that the 3D printed USP Class VI plastic material will not buckle easily during penetration in the brain tissues or insertion into the blood clot compared to silicone rubber. In order to validate that the combination of materials used in sonothrombolysis device are non-reactive and are not cytotoxic, in vitro cytotoxicity test based on ISO 10993-5 standards is performed. The materials passed.

Keywords- sonothrombolysis, stroke, piezoelectric micromachined ultrasonic transducer, pMUT, ultrasound, clot lysis device, biopackaging, wire bond, FPCB, biopackaging

I. INTRODUCTION

Stroke happens when the supply of the oxygen to an area of the brain is interrupted or when a weakened blood vessel in the brain ruptures. Major types of stroke could either be hemorrhagic or ischemic and both could render long term neurological damage or could even cause death. In the case of hemorrhagic stroke, brain damage happens when too much pressure on the brain cells is exerted because of the bleeding in the brain and its surrounding that is caused by a ruptured weakened blood vessel or a burst aneurysm. Prevention and surgical therapy may be done to quickly stop and to prevent further bleeding. Ischemic stroke accounts for 87% of all strokes [1]. Blood clots, the main cause of both heart attacks and ischemic stroke, are broken up and dissolved by drugs in thrombolytic therapy. As in the case of ischemic stroke, where blood clots pool to a blood vessel in the brain and prevent blood flow to that area, clot-busting or thrombolytic medication (tissue plasminogen activator or tPA) is used in order to quickly dissolve the clots and therefore help limit stroke damage and disability [2]. Other treatment includes endovascular procedures such as balloon angioplasty, with or without stenting, which is done via catheterization and thrombectomy. The effectiveness of these procedures is dependent on the speed and completeness of the recanalization, or the restoration of the blood flow to the blocked vessel. Similarly, inherent to the action mechanism of these procedures is the increased risk of bleeding and damaged neurological tissues. Therefore, a new therapy called sonothrombolysis is being studied and explored.

Sonothrombolysis is using ultrasound wave in combination with either microbubbles or fibrinolytic agents (clot dissolving) for dissolution of the blood clot or clot lysis [3]. The downside of using fibrinolytic agents or thrombolytic medicine in combination with ultrasound wave is the high occurrence of bleeding as its side effect [4]. Despite this, several studies have already been conducted using this combination and method and all have shown a higher rate of recanalization, as compared to thrombolytic medicine/therapy only. Further studies were also conducted to optimize the ultrasound frequency and energy safety and efficacy [5].

To address the concerns on high occurrence of bleeding as the side effect of using fibrinolytic agents in combination with ultrasound wave and to simplify the clot lysis process, development of a minimally invasive ultrasound assisted clot
lysis device using pure ultrasound wave is proposed along with the device’s biopackaging and assembly.

II. DEVICE PACKAGING CONCEPT

The concept of the proposed sonothrombolysis device is that it will use only pure ultrasound wave to accelerate the blood clot lysis rate in case of acute ischemic stroke, at the same time provide real-time drainage of the dissolved clots. Thrombolytic medications would not be used in combination with the device with during clot lysis procedure. The device will be in catheter form and will be inserted accurately into the center of the clot using standard neuronavigation techniques. It will be inserted similar to how external ventricular drainage (EVD) catheters are inserted in the skull, but without the use of any metal guiding rod because the catheter will be made from 3D-printed USP Class V plastic material, which will be stiff enough to penetrate the brain tissue without buckling. An option of either two (2) or four (4) MEMS-based piezoelectric micromachined ultrasonic transducers (pMUTs) that are wire bonded on the polyimide flexible printed circuit board (PI FPCB) will be used to generate ultrasound waves. Other biopotential measurement sensor, i.e. temperature sensor, will be attached with the pMUT. The catheter will be printed with holes located across and around the pMUTs and will lead to the center hole which will serve as the main drainage for the dissolved blood clots. The end of the FPCB would be connected to the external circuitry that will trigger the pMUTs as well as measure the viscosity of the blood clot. Figure 1 shows the design concept with of the whole device.

![Design of the proposed minimally invasive ultrasound assisted clot lysis device for stroke treatment.](image)

III. DEVICE STRUCTURE

A. piezoelectric Micromachined Ultrasonic Transducer

The MEMS-based pMUTs were fabricated with an array of piezoelectric layer sandwiched between top electrode and bottom electrode micromachined on thermally grown silicon dioxide layer of an 8 inch silicon-on-insulator (SOI) wafer and the backside of the wafer was etched in order to form an array of membranes. The pMUTs were used to function either as treatment transducer or as measurement transducer, and could operate under low frequencies and high frequencies, respectively. Low frequency, in tens of kilohertz range, activates the pMUT to dissolve blood clots while the high frequency, in megahertz range, performs sensing and measurement of viscosity of the blood clot. The pMUT covering 20 KHz to 100 KHz with power density of the diaphragm surface of more than 0.5 W/cm² has been fabricated for clot lysis.

B. Polyimide FPCB

Polyimide (PI) has been proven a biocompatible material that has an insignificant cytotoxicity level for use on medical devices and implantable biosensors [6]. It has also been demonstrated that flexible printed circuit board (FPCB) made of polyimide with a single layer (1L) of Gold over Copper metal traces and a cover layer is wire-bondable and workable for integration with implantable catheters [7]. For this work, a 50 µm-thick FPCB was designed and configured with the option to cater to either two (2) or four (4) pMUT devices. Utilizing gold ball bonding process, the bond pads of the FPCB has 0.50 µm Gold thickness. The FPCB’s total length from end to end, including the ZIP connector compatible portion, is 153 millimeters. The width of the FPCB was kept at a maximum of 2.0 millimeter so that it will not protrude from the surface of the 3D printer catheter, thus will not create a sharp corner along the body of the catheter. Figure 2 shows how the actual two FPCBs look like. The FPCBs could be cut into two to create two different devices and the dummy “wings”/parts could be disposed of before coupling with the 3D printed catheter. The whole FPCB substrate could then be custom-fit attached with the 3D printed catheter by the use of medical grade cyanoacrylate instant adhesive and sealed with biocompatible UV cure epoxy.

C. 3D-Printed USP Class VI Catheter

Three-dimensional (3D) printing, also called additive manufacturing (AM), is creating an object from a computer aided design and a machine that bonds or adds layer-upon-layer of material, such as plastic, metal, or other layering material, to form a 3D object. It has come a long way from being primarily utilized only in rapid prototyping to create engineering prototypes and molds to being used in medical applications such as custom-made medical implants, devices, and jigs and fixtures for use in operating theaters. 3D printing was initially used to create dental implants and prosthetics, until recent applications and development include bioprinting of a wide range of tissues and organs, customized prostheses, targeted drug delivery and dosage, and 3D printing for use in
neuroanatomy [8]. For this work, 3D printing of the catheter using USP Class V plastic material is proposed.

For this work, material chosen for 3D printing is of excellent humidity and moisture resistance has a post-cured average Tensile Strength of 43.5 MPa/PSI, and post-cured average Tensile Modulus of 2125 MPa/KSI. The material also has a hardness of Shore D post printing. Table 1 below shows the material properties in liquid form, prior 3D printing. Figure 3 shows the actual 3D printed catheter made of USP Class VI plastic material showing the drain holes location and the slots for pMUTs attached on FPCB. The length of the catheter is configurable to 10 centimeters. The outer diameter is 4.0 millimeter and the inner diameter is 2.0 millimeter. Each drainage hole is 1.0 millimeter in diameter, located 10.50 millimeters from each other, with first holes are located and printed at 6.0 millimeter from the tip of the catheter.

### Table 1. USP CLASS VI PLASTIC MATERIAL PROPERTIES (LIQUID PRE-CURE)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value/ Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (Liquid)</td>
<td>1.1 g/cm³ @ 25 degC</td>
</tr>
<tr>
<td>Density (Solid)</td>
<td>1.17 g/cm³ @ 25 degC</td>
</tr>
<tr>
<td>Viscosity</td>
<td>235 – 260 cps @ 30 degC</td>
</tr>
<tr>
<td>Critical Exposure</td>
<td>9.5 mJ/cm²</td>
</tr>
<tr>
<td>Penetration Depth</td>
<td>6.1 mils</td>
</tr>
<tr>
<td>Color</td>
<td>Clear</td>
</tr>
</tbody>
</table>

IV. DEVICE PACKAGING

The sonothrombolysis device packaging assembly process flow is shown in Figure 4. The polyimide FPCB was first prepared after determining if two (2) or four (4) pMUTs will be used for the device assembly. For this work, two pMUTs were used. The FPCB did not require any baking to release any moisture, so the pMUTs were die attached directly on the FPCB along with the temperature sensor. Curing of epoxy at 150 °C for 5 minutes follows. Alternate epoxy cure condition is done at 80 °C for 90 minutes. Plasma cleaning using Argon (Ar) gas was then performed to enhance adhesion of the ball bonds during wire bonding and to clean the surface of the sample.

The pMUT arrays were designed and fabricated with a maximum of five (5) 50 µm x 50 µm wire-bondable bond pads, with any three (3) of the bond pads could be routed or interconnected to the FPCB. Wire bonding using 1.0 mil Au wire was employed to create the interconnection between the bond pads and the FPCB. Reverse stand-off bonding, i.e. ball bond on the FPCB bond pad and ball bond on bump on pMUT bond pads, was done to achieve a very low looping profile. The low profile avoid protrusions on the catheter after assembly. The wire bond clamper was custom-designed, fabricated, and used to hold the 50-µm thin FPCB in place during wire bonding. The vacuum holes of the wire bond clamp and insert were distributed along the length of the FPCB and its dummy portion or wings and none directly below the pMUTs. The wire bond temperature used in processing the sample was kept to a maximum of 150 °C.

Similar to the external ventricular drain (EVD) catheter, which is used to drain the excess cerebrospinal fluid from the brain and allow for intracranial pressure monitoring, the proposed 3D printed catheter is configured to be inserted into the brain center to where the blood clot is located, but without the use of metal guiding rod like in EVD catheter, and will be used as the drainage for the dissolved clot. For this work, material chosen for 3D printing is of excellent humidity and moisture resistance has a post-cured average Tensile Strength of 43.5 MPa/PSI, and post-cured average Tensile Modulus of 2125 MPa/KSI. The material also has a hardness of Shore D post printing.
After wire bonding of the pMUTs, the wires were encapsulated with polydimethylsiloxane (PDMS). The edges of the pMUTs were also sealed with PDMS to avoid moisture to seep through the membranes. PDMS comprised of a base elastomer and a curing catalyst and when cured for 15 minutes at 100 °C formed into a flexible silicone elastomer. With the wires coated and the edges of the pMUTs sealed, the dummy portion of wings of the FPCB were then cut-off (under the microscope) and disposed.

The FPCB assembled with the pMUTs were then coupled onto the 3D printed catheter. The die paddle, 1.0 millimeter in width, where the pMUTs were wire bonded and sealed were first custom-fitted into the slots intended for FPCB of the 3D printed catheter (see Figure 3,b) and were bonded using medical grade cyanoacrylate instant adhesive. After all the pMUTs are secured in place, the whole length of the FPCB is then sealed with biocompatible UV cure epoxy. The medical grade cyanoacrylate instant adhesive was cured at room temperature, while the coating of the FPCB to seal on to the 3D printed catheter was cured by UV light. The whole assembly process is shortened and simplified, although extra caution in the handling of the pMUT and in bending and cutting the FPCB was observed.

With this proposed device structure, there will not be a need to create a bigger burr/ opening hole on the skull since there is no need for a separate catheter for thrombolytic drug delivery on the blood clot like in a discrete system. Only one burr hole is needed to fit one single catheter since the whole device and system is compact. The assembled sonothrombolysis device is shown in Figure 5.

Figure 4. The biopackaging process flow of the proposed sonothrombolysis device.

Figure 5. Photo of proposed device showing the 3D printed USP Class VI plastic material coupled polyimide FPCB substrate attached and wire bonded with pMUTs.

V. RESULTS AND DISCUSSION

A. Buckling Analysis of 3D Printed Catheter

Buckling analysis is conducted to compare determine how stable the chosen material and to compare the buckling load between the 3D printed USP Class VI material and the silicone rubber, the usual material used for external ventricular drain (EVD) catheters, when used to penetrate the brain tissue leading to the center of the blood clot. Using Ansys simulation software, the materials are simulated to penetrate the brain tissues. The brain’s Young’s Modulus is set at 6000 Pa with a Poisson’s ratio of 0.40 [9].

<table>
<thead>
<tr>
<th>Default Failure Criterion</th>
<th>VonMises</th>
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<tbody>
<tr>
<td>Density</td>
<td>1170 kg/m³</td>
</tr>
<tr>
<td>Elastic Modulus</td>
<td>2270 MPa</td>
</tr>
<tr>
<td>Poisson Ratio</td>
<td>0.35</td>
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</tbody>
</table>

Table 2. USP CLASS VI PLASTIC MATERIAL PROPERTIES USED FOR SIMULATION

Results showed that the buckling load of the 3D printed USP Class VI plastic material, with material properties used in the simulation at Table 2, at a total deformation of 0 – 1 millimeter at 1 second is 2038.4 Newton as compared to the
buckling load of the silicone rubber which is only 0.128 Newton. This means that the 3D printed USP Class VI material will not buckle easily when maneuvered to penetrate or inserted in the brain tissues; thus, would not cause further neurological damage.

Figure 6. Buckling simulation result between (a) 3D printed USP Class VI plastic material and (b) Silicone Rubber, show that the 3D printed material will not buckle as much as the silicone rubber.

**B. In vitro Cytotoxicity Assessment**

To validate that the biocompatibility of the combination of the chosen materials that they will not cause damage to human cells, i.e. not cytotoxic, an *in vitro* cytotoxicity test performed is according to ISO 10993-5:2009 standards [10]. For this test, mammalian cells, specifically L929 cells, are used and checked/tested *in vitro* to determine if the device or a part thereof would and could cause any toxic or adverse biological effect when in contact with the living and healthy cells in the body. As per the ISO 10993-5:2009 standards, the device/sample tested is compared with three controls. Table 3 shows how the samples are prepared. First control is a blank sample that is neutral and non-toxic to the living and healthy cells. Second control is a positive sample, which produces the cytotoxic response, i.e. near or complete destruction of the cell layers when tested. The last control is a negative sample that does not produce cytotoxic effect or there is no reduction in cell growth. The tests were performed in triplicates. Extracts from the each control samples as well as from the device is then taken and compared.

After the incubation, the extracts were examined microscopically at 100x magnification. The extracts from the sonothrombolysis device/sample showed no reactivity or no cytotoxic effect and are comparable to the extracts from blank sample and negative sample. The extracts from the positive sample remain cytotoxic as expected. Figure 7 shows the extracts of the cells after in vitro cytotoxicity testing.

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Tested Device/ Sample (pMUT catheter)</th>
<th>Reagent Blank Control</th>
<th>Positive Control (Zinc Sulphate solution)</th>
<th>Negative Control (HDPE material)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction Medium</td>
<td>Complete MEM10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraction condition</td>
<td>37˚C for 24 to 48 hrs. in a CO2 incubator containing 5% carbon dioxide with manual agitation for 3 minutes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Qty of sample in extraction medium</td>
<td>1pc (9.7g) of sample in 48.5ml of Complete MEM10 (extraction ratio: 0.2g per ml)</td>
<td>Complete MEM10</td>
<td>0.016g Zinc Sulphate in 20ml of Complete MEM10</td>
<td>1pc of 60cm² of HDPE panel in 20ml of Complete MEM10</td>
</tr>
</tbody>
</table>

Figure 7. Photos of the extracts from the cells cultures of the samples that undergo *in vitro* cytotoxicity test. The tested device/sample shows no destruction in cells, same as in negative control. The positive control shows severe destruction of cells.

<table>
<thead>
<tr>
<th>(a) Extract for the tested device/sample</th>
<th>(c) Positive control</th>
<th>(b) Negative control</th>
</tr>
</thead>
</table>

Table 3. **SUMMARY OF SAMPLE PREPARATION FOR CYTOTOXICITY TEST**
VI. CONCLUSION

The biopackaging of a minimally invasive sonothrombolysis device using pMUTs and 3D printed catheter is presented. The whole devise was assembled using a 3D printed USP Class VI plastic material that served as the drainage catheter for the dissolved blood clot and was coupled with a 50-µm thin single layer polyimide flex PCB substrate where 2 up to 4 pMUT chips and temperature sensor were epoxy attached. Low looping profile was employed using reverse bonding at wire bond and encapsulation and sealing of the wires and the whole device was done by the use of PDMS, medical grade cyanoacrylate instant adhesive, and UV cure epoxy. The 3D printed catheter material chosen was simulated to penetrate the brain tissues safely at a buckling load of 2038.4 Newton. The proposed device packaging is non-cytotoxic. It is compact and will not require thrombolytic medication to function. And because it is compact, it will neither require a metal guiding rod for penetration to brain tissue leading to center of blood clot nor will require large burr hole during insertion to the skull.

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REFERENCES


