Title: Photoacoustic Tomography as a Therapeutical Brain Imaging Technique for Brain Mapping and Treatment

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A. SPECIFIC AIMS

Big picture

Neuroimaging or brain scanning involves the use of various techniques to image the structure, function, or pharmacology of the brain directly or indirectly. Structural imaging of the brain deals with the structure of the brain and diagnosis of large-scale intracranial disease such as brain tumors. Functional imaging is used to diagnose metabolic diseases and lesions on a finer scale, like Alzheimer's disease. CT, fMRI, PET and SPET are most widely used imaging modalities for diagnostic structural and functional brain imaging.

What is/are Problem(s)?

A fundamental constraint of these modalities is cost and spatial resolution. CT, MRI, PET, and SPECT machines/equipment can range from approximately $65,000 to $2.5 million [1], prior to maintenance costs. This makes having specific procedures done using these imaging modalities expensive for patients with no health insurance with procedure costs ranging from approximately $50 to $2,050 [2]. Additionally, the spatial resolution of these imaging modalities differs with CT having the highest spatial resolution compared to MRI, PET, and SPECT. Spatial resolution is an important factor as it determines the ability to differentiate adjacent structures.

Goal(s) of your proposed work

Photoacoustic tomography (PAT) is an emerging imaging modality that provides non-invasive high-resolution images of the entire brain in a preclinical setting at near real time [3]. It is a hybrid imaging modality that combines rich optical contrasts with a high ultrasonic spatial resolution in deep tissue. PAT is based on the photoacoustic effect which starts with optical absorption by tissue molecules and ends with ultrasonic wave emission through thermoelastic expansion. Thus, PAT is known to offer two advantages over other imaging modalities, the resultant PA signal is sensitive to the rich optical absorption contrast of the biological tissue which allows for functional and metabolic imaging capabilities and the acoustic waves are less sensitive to scattering as light which leads to high spatial resolution in deep tissue and low procedure cost [3].

Aim 1. Utilizing PAT as a functional imaging method while providing drug delivery applications simultaneously.

Aim 2. Reinforce PAT functions with specialized nanoparticles containing polypropylene glycol (PG)

Impact(s) of proposed work:
The expected impacts of the work that we propose are important for the advancement of medical imaging. By using PAT, two advantages are offered over the commonly used imaging modalities including greater depth and higher resolution for lower costs. This creates opportunity for improved diagnosis of brain damage and disease.

B. BACKGROUND AND SIGNIFICANCE

B1. Background

The neuroscience and neuroengineering have been one of the hottest topics in the current world with evolving technology and increasing awareness for mental health diseases, brain tumors, brain disorders, and brain related diseases. There has been a huge gap that was being tried to be closed ever since 1970s when the interest in brain studies skyrocketed. Currently there are no brain imaging techniques that are very accurate, affordable, and accessible to everyone. Up to now, novel works have been done and are still being pursued to bridge that gap and provide a brain imaging device that is accurate and affordable. The main areas of focus for brain imaging modalities include fine resolution, high speed, deep penetration, and new functionality (12).
The main goal of neuroscience is to improve and advance the understanding of brain structure and neuroactivities that results in production of perception, emotion, behavior and cognition. There are no techniques that can provide all the necessary information when used on its own and thus combinations and hybrid modalities needed to be utilized for advancement of the field (11). Success in being able to interpret the brain activity by mapping accurate brain images will help advance other fields like diagnostics and therapeutics related to brain disorders.
For centuries founders of neurology have been creating, developing, and perfecting techniques for brain imaging and some of those techniques included optical intrinsic signal imaging, optical microscopy, magnetic resonance imaging, positron emission tomography, X-ray computed tomography etc. (10).
Advancement within the hybrid photoacoustic tomography technique provides different temporal and spatial scaled images and complement other imaging techniques.
The function of photoacoustic tomography includes emitted a pulsed laser beam in the form of electromagnetic wave for nanoseconds against the tissue of interest. The difference that sets photoacoustic tomography aside is the induction of tissue photoacoustic waves when interacting with electromagnetic waves. The transducer that emits the pulsed laser beam ideally detects the resulting photoacoustic waves reflected. The contrast in this imaging modality results from the optical absorption properties of tissue chromophores which initiate broadband ultrasound (US) waves upon interacting with the electromagnetic waves. Upon tissues getting into interaction with incoming electromagnetic waves, resulting thermoelastic expansion of tissues produces wideband sound emission (11). Near infrared light has been utilized to reach deeper penetrations due to the fact that main dominant chromophores within the body have distinctive optical absorption phenomena within 650-900 nm region of electromagnetic waves (10). Photoacoustic tomography can achieve 100-300 mm resolution with imaging depth extending several millimeters (10).
Photoacoustic tomography provides advantages such as mapping the functional and metabolic activities of brain resulting from optical absorption and signal distribution. The mapping of small brain models achieved to distinguish regions such as central gray, cerebellum, cerebral aqueduct, corpus callosum, hippocampus, hypothalamus neocortex, olfactory bulb, lateral ventricles etc (10).The imaging procedure resulting in lower scattering of the acoustic signal lets the spatial resolution to be fine and the penetration to be deep which surpasses the abilities of multiphoton detection (11).

B2. Current approaches

The main techniques that are utilized for brain imaging are two-photon microscopy and functional magnetic resonance imaging (fMRI). Two-photon microscopy provided detailed

analysis of the neuroactivities in small animals and fMRI has been mainly used for human brain activities mapping but the benefits of the two-photon microscopy cannot be advanced for human brain use due to the skull thickness and fMRI has low spatial resolution for a single cell examination (12).

Other commonly used modalities include PET and MRI. The main reasons those modalities are utilized for are excellent sensitivity and penetration depth that let map the functional and metabolic neuroactivities within the intact skull (10). Despite having many applications within the neuroengineering field, these modalities have limited temporal resolution, necessary invasiveness when using exogenous tracers, safety risks, and are very expensive and inaccessible.

Optical modalities are on the other hand safer and are more accessible. Microscopic methods are utilized in optical modalities with concurrence of using contrast agents and genetically encoded reporter proteins to map details of tissues and their neuroactivities at a cell level (10). However, even optical imaging has major setbacks that couldn't be resolved without developing a new technique.

Brain imaging has advanced at imaging the neuroactivities of small animals and a lot of work needs to be done for human brain mapping. It was observed again and again that one modality had too many challenges to map the human brain and that the wise choice would be to fuse some of the leading imaging modalities and obtain a more effective hybrid modality. Photoacoustic tomography is a hybrid imaging modality that has the promising results and future once researchers are able to incorporate new ideas into excelling the technique and addressing the shortcomings of the hybrid modality.

Photoacoustic tomography is a hybrid model that can bridge the shortcomings of other optical imaging modalities in mapping the brain. Photoacoustic tomography (PAT) is currently the only high resolution providing tomographic imaging tool that can provide scans ranging in depths of several centimeters of target tissue (9). In the process, the scattering of photons is scarce and scattering of sound is weak, thus resulting in more accurate image of the brain. Photoacoustic tomography is a diversified imaging technique that can also incorporate endogenous and exogeneous contrasts to provide different image contrasts which gives the opportunity to visualize various anatomical structures such as blood vessels and cell nuclei (9).

The other modalities mentioned have challenging time providing effective imaging depths and high resolution because there is usually a compromise when it comes to imaging depth and spatial resolution. Photoacoustic tomography, on the other hand, provides advantageous phenomena such as rich optical absorption contrast, high spatial and temporal resolutions, and deep penetration of the tissue which are critical in brain studies (12).

B3. Significances of your proposed studies

Photoacoustic tomography reached great accomplishments and opened many doors for various research lanes within the neuroscience. Despite being such a promising technique, photoacoustic tomography has its own shortcomings. Photoacoustic tomography has been very successful and promising within the areas of research such as breast cancer diagnosis, endoscopy, and minimally invasive imaging applications but brain and cancer research has been more challenging. Currently there are very few studies on human brains due to the challenge of penetrating the skull of human subjects. Human skull is much thicker than rat, monkey or mouse skulls and is around 7-11 mm for adolescents and adults (12). The thickness of the human skull results in strong absorbance, attenuation, distortion, and scattering of light and sound. Despite

providing high resolution images, imaging depth of photoacoustic tomography is lower than the commonly used brain imaging modalities such as MRI and US imaging technique (9). Emergence of photoacoustic tomography allowed color visibility in imaging techniques which led to better analysis and visualization of target tissues (9). Thus, it is important to continue exploring new avenues within the photoacoustic tomography because this technique has been one of the most promising imaging techniques for brain imaging so far. Utilization of dyes, exogenous contrasts, contrast agents, and combination with US imaging technique can provide better penetration depth thus eliminating the limitation of imaging depth (9)(10). Application of new methods such as utilizing facial cavities such as nose, ear, and mouth can also provide promising results.

Considering all the above information, the group decided to proceed with designing and executing the experiments that will involve the utilization of contrast enhancement and combining the photoacoustic tomography with US to be able to successfully penetrate and image the human skull. One of the main struggles with utilizing contrast enhancements in the form of drug delivery is blood brain barrier (BBB). By utilizing microbubbles and US, blood brain barrier can be opened for a short period of time which will be enough for contrast agent and drug deliveries. Thus, photoacoustic tomography will be the main imaging technique to map the brain of the subject where contrast agents will improve the penetration depth thus achieving to overcome the main shortcoming of the photoacoustic tomography and treat the brain tumor that the drug delivery will be targeting.

Summary

Neuroscience field has been developing very fast but due to always emerging limitations and shortcomings of the imaging modalities, no breakthrough has been achieved in the brain imaging field. The closest researchers came to breakthrough was by discovering a photoacoustic tomography technique to image the brain. However, photoacoustic tomography has the major setback of mapping the brain which is inability to penetrate deep enough into the human brain. To overcome this limitation, our new approach will focus on utilizing methods not used before to overcome the barrier of penetration depth.

C. EXPERIMENTAL DESIGN

C1. Overview of proposed work

Utilizing PAT as a functional imaging method while providing drug delivery applications simultaneously.

C2. Aim1.

C2.1. Rationale of aim 1

As was mentioned before there are many difficulties in regards to using PAT for human brain imaging since the thickness of a human skull is about 7 to 11 mm in comparison with smaller animals like mice which have a skull thickness of less than 1 mm however PAT can still be improved to enable functional imaging of the brain so our hope with this process is to be able to use PAT to not only image the brain in a safe way but also be able to use this method to increase the permeability of the blood brain barrier with the help of microbubbles to allow drug delivery in order to treat brain tumors. Using PAT would provide increased vascular permeability while minimizing irreversible damages to the brain tissue. This could be a process similar to FUS-MRI but at lower cost and also with the use of less bulky equipment another advantage in regards with PAT is , it does not use ionizing radiation such as that used in X-Ray which could be harmful to the brain and has a higher temporal resolution in comparison to MRI.

C2.2. Method and evaluation

Here we would use PAT to be able to image tumor associated vasculatures as we know PAT imaging has the capability to visualize blood vessels so we could use this to our advantage in order to detect abnormal vascularization due to the presence of a tumor in the brain as the imaging is going on in real time we could achieve drug delivery to the tumor in the brain.

So to overcome our main issue regarding skull thickness and the light depth penetration we would utilize microbubbles as contrast agent and in order to better focus the light.

Also we know the main issue with drug delivery in the central nervous system is the BBB and even though the vasculature in most brain tumors is unusually leaky its permeability is occasionally similar to that of a healthy brain and for this reason overcoming the blood brain barrier is still necessary and a possible remedy would be the use of microbubbles.

Microbubbles could be introduced to the blood stream prior to US exposure to enable focused light to get a better image and then a second round of microbubbles would be introduced which could assist in opening the BBB where the ultrasound is focused without causing any severe neural damage.

So typically we would have a light source in the form of a photon recycler which would reflect back any scatter photons and this will be directed at the tissue we are imaging, we would use the Nasal cavity in order to emit this light where it would no longer need to pass through the thick skull but only the cribriform plate which separates the nasal cavity and the brain and also we would use a hemispherical ultrasonic transducer as the signal receiver, microbubbles would be introduced in the blood stream further focusing the light for better contrast. The photons energy would be absorbed by the tissue which would bring the molecules to an excited state and when returning to ground state the energy would be converted to heat and due to thermoelastic expansion the increased pressure would propagate the ultrasound wave this ultrasound would in turn be used alongside the microbubbles to achieve drug delivery

C2.3. Expected outcomes

The result we would expect to get is a imaging modality which could simultaneously not only be used to give a functional image of the brain and tumor but also treat the tumor using the combination of ultrasound and microbubbles which could cause an increase in vascular permeability and enhance drug delivery to the cancerous region. Other than what has been mentioned PAT has another benefit. As we know hypoxia is one of the characteristics of tumors and one of the reasons they can grow uncontrollably, PAT has the capability of quantifying oxygen saturation levels which can not only help us find the correct location of the tumor to concentrate the treatment but we can also measure this saturation after treatment to see if it has increased as an indication that treatment is working. So basically PAT can help us continuously study the progress of the treatment and weather oxygen saturation is increasing or not

C2.4. Technical difficulties and alternative approaches

The main difficulty with this approach might be that due to the thickness of the skull we would only be able to achieve any imaging and cancer treatment on the surface of the brain. Another issue could arise due to the reverberation of ultrasound because of the presence of the skull which would hinder a focused treatment on the site of the tumor.

C.3.1. Rationale of aim 2

The main purpose of aim 2 is to reinforce the concepts introduced in aim 1. Shallow imaging depth is one of the principal obstacles for the clinical advancement of PAT. Wang & Valery (2002) verified that polyethylene glycol (PG) is a viable method to increase imaging depth in optical imaging techniques such as OCT. PG is also commonly found in ultrasound gels, therefore an upgrade of both the optical and ultrasound aspects of PAT is expected. Since we are already using microbubbles, nanoparticles coated with PG are a convenient choice for delivery, as the nanoparticles can be stored in the microbubbles and released upon ultrasound activation.

C.3.2. Method and evaluation

The central structure of evaluation would be to compare PAT image quality with and without applying the PG coated nanoparticles. The starting point would be murine trials with in vivo images taken both in near real time and post-acoustic activation. In applications involving cancer therapy, the use of dyes would likely be implemented to track microbubble and nanoparticle localization around tumors [7]. Gold and other metallic particles are two of the most well documented nanoparticles used for brain imaging.

C.3.3. Expected outcomes

After application of PG-nanoparticles, analysis of imaging data is expected to reveal a significant quality increase in both ultrasound and optical portions of the PAT setup. Imaging depth, contrast, resolution, and spatial accuracy should all benefit from the specialized nanoparticles [6]. There should be no toxic interactions or functional interference between the microbubbles, nanoparticles, or any other components.

C.3.4 Technical difficulties and alternative approaches

Nanoparticle safety, especially when involving the blood brain barrier, is a critical issue. Keeping track of where exactly the nanoparticles are located and whether they migrate into systemic circulation will be a challenging process. Furthermore, there exists the possibility of

different aspects of the PAT setup causing interference, for example the thermoelastic expansion caused by light stimulation might displace or even disrupt the PG-coated nanoparticles rendering them ineffective. To avoid harmful interactions, an alternative approach would be foregoing the nanoparticle portion of the design entirely and delivering polyethylene solely via the microbubbles.

C4. Conclusion

In conclusion, brain imaging is one of the most researched topics that will help diagnose and treat the brain related diseases and disorder. Photoacoustic tomography is currently the most promising new imaging technology that has benefits of inexpensive price and fine resolution that other brain imaging modalities do not have. The main principle of the photoacoustic tomography is utilizing the ultrasound and optical properties of tissues to image them. However, the challenges of utilizing the photoacoustic tomography include low penetration depth. Human skull is 7-11 cm deep and for the photoacoustic tomography to get past that depth, new approaches such as utilization of contrast agents or utilization of facial cavities such as nose, ear, and face can provide better results. In our approaches, contrast agents will be utilized to image the brain as well as imaging through the facial cavities will be attempted. These approaches are promising because photoacoustic tomography can provide high resolution images that will be able to image brain abnormalities such as tumors and our approach can also introduce tumor treatment after finding the tumor in the live imaging of the brain using photoacoustic tomography.

D. Timeline

obtaining FDA approval would be the longest portion of the timeline. The average time for 510(k) clearance is around 6 months. Obtaining the required materials including PAT imaging components, murine specimens, microbubbles and nanoparticles should take a few weeks. Conducting the studies with microbubbles and PG-nanoparticles would have an observation period of 2 weeks per specimen, depending on the lab setup that could take at most a few months [8]. In total the entire process would take about a year.

E. Requested Budget

The PAT imaging system is the most costly of the required items coming in at around $500,000 [2]. A steady supply of microbubbles could be obtained for about $300. A group of 20 mice specimens would come in at about $2000. The FDA 510(k) clearance takes up $30,000. Finally, gold nanoparticles go for about $80,000 per gram. Depending on our needs, 1 gram may be adequate. In total our expected expenditures total $700,000.

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