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Introduction/Background

According to the World Health Organization, every year 15 million people suffer a stroke, 5 million of whom die, and another 5 million of which are disabled [1]. Due to the rapid loss of brain tissue during stroke, prompt diagnosis and treatment are critical for improving stroke outcomes [2]. At Openwater we are developing the fundamental technology to produce low-cost, portable medical imaging devices capable of both functional and structural imaging. Our first prototype measures tissue hemodynamics using near-infrared (NIR) light. In particular, it is designed to measure differences in blood flow with the goal of reducing the time to diagnosis of ischemic stroke.

Biomedical optics is a rapidly expanding field that is providing biologists and clinicians new ways to detect, diagnose, and study disease. However, most optical techniques can only be used to gain

information near the tissue surface [3]. For example, confocal microscopy is only capable of imaging up to 50 μm below the tissue surface, and even optical coherence tomography only images at depth of up to 2-3 mm in opaque tissues.

The two principal obstacles to looking deep into tissue with visible light are the high degree of light absorption and scattering by tissue. There exists a spectral region in the NIR where the absorption of light by tissue is relatively low, about 650-950 nm, where light can penetrate deeper into tissue. The second obstacle, the high degree of light scattering, limits the use of any of the above techniques to a few millimeters [4]. The mean free path (the average distance a photon will travel before interacting with matter) of visible light in tissue is only about 100 μm, and multiple scattering events will cause the direction of the average photon to be randomized after ~1 mm. Thus, techniques which rely on ballistic or quasi-ballistic light (aka time-of-flight) are inherently limited in depth. The problem is not that NIR light cannot penetrate deeply into tissue; for example, NIR light transmitted through 10 cm of human breast tissue can be detected [5]. The problem is that beyond a few millimeters deep, almost all of the remaining photons have been scattered multiple times, and their directions are random. Although the light exiting the tissue is easily detected, in order to acquire information about tissues deep below the surface, a method is needed which permits this information to be extracted from detected photons that have been scattered many times. Techniques using scattered near-infrared light to interrogate deep tissues are often referred to as Diffuse Optics and/or Near-Infrared Spectroscopy (NIRS).

When laser light is reflected from a rough surface and then detected (e.g. by your retina or a camera) the resulting image contains randomly located light and dark spots commonly referred to as speckle [6]. The light and dark spots are due to the constructive and destructive interference between light waves that travel different distances. This phenomenon also occurs when laser light passes through highly scattering media, such as biological tissue. If the light scattering particles which compose the scattering media are in motion, the locations of constructive and destructive interference of the light waves (i.e. the speckle) change in time. If the change from bright to dark occurs on a time scale equal to or shorter to the exposure time of the light detector, the contrast of the speckle (i.e. the difference between bright and dark spots) decreases. As a result, the contrast of the speckle pattern is related to the motion of the interfaces scattering the light. More motion, due to either the scatterers moving faster, or more of the scatterers moving, results in a decrease in speckle contrast [7]. Openwater's blood flow technology combines diffuse optics with measurements of laser speckles. Short pulses of monochromatic laser light are injected into tissue using a fiber optic. The light diffuses through the tissue. Some of the light remitted from the tissue is collected by fiber optics located at various locations. The contrast of the measured speckles is related to the number of moving blood cells and their speed. A measure of microvascular blood perfusion is then calculated, commonly referred to as blood flow index (BFI), using an algorithm based on a mathematical model of the relationship between scatterer motion and laser speckles.

Using our technology, we performed a study to determine if we could reliably detect left versus right hemisphere blood flow differences after permanently occluding the middle cerebral artery (MCA) in rats following a previously established surgical method [8]. Measurements were taken on the left and right sides of the head both before and after surgery. A total of six rats were measured, out of which 10

baseline pairs of left/right measurements and 5 post-stroke pairs of left/right measurements were made (one rat died during surgery, two rats received multiple baseline measurements). On average, left to right differences were 7x larger after occlusion (see Figure 1). In addition, the smallest post occlusion difference (0.06) in any of the rats was twice as large as the largest baseline difference (0.03) among all the rats, demonstrating that the measurement could be used to determine if a rat received the occlusion.

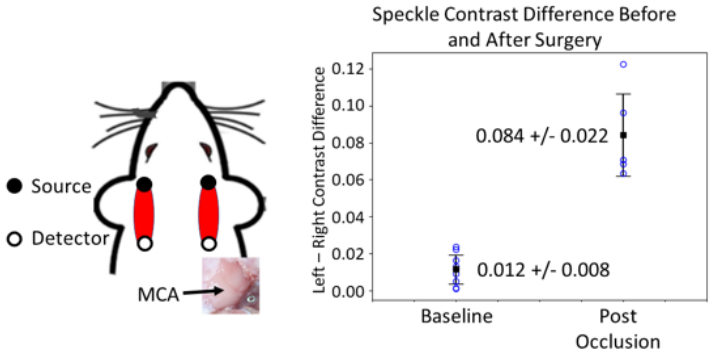

Figure 1: Results of the permanent MCA occlusion study; left and right sides of rats were measured before and after the occlusion. Differences in speckle contrast between the hemispheres were ~7x greater after occlusion than before.

Our initial prototype device for human studies consists of a cart and a wand (schematic is shown in Figure 2). The cart houses the laser, light detectors, computer, and various other optical and electronic components. A cord exiting from the side of the cart carries laser light to the wand. When the wand is pressed up against the patient and its acquisition button is pressed, a safe amount of NIR light is emitted and passes into the patient. Remitted light carrying information about the microvascular perfusion in the brain is collected by the wand's detection fibers and transmitted to the detectors in the cart.

Figure 2: Schematic of the initial first prototype, consisting of a cart (left) and corded wand (right). The next generation will be more compact, with the cart replaced by a small box that can be used in an ambulance.

Purpose/Aim

The purpose of this study is to evaluate differences in cerebral blood flow using near-infrared optical detection in patients hospitalized with supratentorial acute ischemic and/or hemorrhagic stroke with confirmatory imaging (CT, CT angiography, MRI, or MR angiography). In particular we will measure cerebral blood flow in both stroke and non-stroke regions and evaluate for differences in cerebral blood flow.

Research Question(s)/Hypotheses

Patients with acute stroke (ischemic or hemorrhagic) will demonstrate regional reduction in cerebral blood flow within the ipsilateral stroke-afflicted hemisphere compared to the contralateral “normal” non-stroke-afflicted cerebral hemisphere. Furthermore, peri-regional alterations (increase or decrease) in cerebral blood flow will be observed secondary to cerebral autoregulation and collateral blood flow.

Primary & Secondary Endpoints

- Observing relative reduction in regional cerebral blood flow within the stroke affected hemisphere compared to the contralateral, non-stroke hemisphere.
- Observing altered (increased or decreased) cerebral blood flow within the ipsilateral stroke hemisphere outside of the immediate stroke region (peri-regional cerebral blood flow).

Hypotheses:

The metric that we will use to examine the variability of measured blood flow is the difference in measured BFI between the same anatomical position on each side of the head; referred to as the bilateral blood flow difference (BBFD). In addition, ipsilateral and contralateral forehead measurements will be taken and will be used as reference points. The difference between site measurements and reference measurements will be referred to as “Unilateral ipsilateral-reference blood flow differences” (UIrBFD) and “Unilateral contralateral-reference blood flow differences” (UCrBFD) respectively. This will allow us to test the following hypothesis:

1. Compare the variance of BBFD for different locations on the head.
    a. We hypothesize that there will be less variance from the temporal window location and forehead compared to the regions above the superficial temporal vasculature.
2. Assess the variance of BBFD for repeated measurements at all locations,
    a. The purpose of this comparison is to assess how repeatable the BBFD values are, and whether making multiple measurements will help reduce this variability. We hypothesize that making repeated measurements will reduce the variability in BBFD for all locations and subject positions.
3. Evaluate regional differences in cerebral blood flow between stroke regions and non stroke regions

    a. We hypothesize that we may see in addition to BBFD, additional changes will be observed comparing the stroke site to the UIrBFD and UCrBFD, and less variability will exist between reference points measurements vs BBFD measurement sites.

Study Design

This will be a prospective, single-center, non-randomized, unblinded, observational, cross-sectional, no intent-to-treat, non-comparative to current standard methods exploratory study.

Human Subjects

Inclusion criteria

Patients meeting all of the following criteria will be included:

- All genders can participate
- Adults (aged between and including 18 and 89 years)
- Admitted with acute ischemic and/or hemorrhagic stroke extending to the cortex involving one-third of the MCA territory (or greater), ACA, or PCA territory on CT or MRI imaging:
    - Acute ischemic stroke as defined by the clinical syndrome consistent with a stroke diagnosis and accompanied by radiographic evidence on CT angiography, MR angiography, or catheter-based angiogram of acute ischemic infarction.
    - Acute hemorrhagic stroke as defined by aneurysmal subarachnoid hemorrhage (SAH) with confirmed aneurysm site and clinical or radiographic evidence of cerebral vasospasm, intracerebral hemorrhage (ICH, lobar or non-lobar) extending to the cortex within the same vascular territory, or deep hemorrhage extending to the cortex on CT or MRI brain imaging
- Contralateral acute stroke-free hemisphere relative to the ipsilateral stroke-afflicted side as defined by negative diffusion weighted imaging lesions, parenchymal hemorrhage, or acute subarachnoid blood.
- Patients can be measured with optical blood flow device within 7 days from stroke onset.
- Intact skin at all interrogated regions, no open wounds, with no contraindication to the use of wand/device.
- Reliable optical measurements can be taken without impedance secondary to body habitus, orientation or other environmental factors.

Exclusion criteria

Patients meeting any of the following criteria will be excluded:

- Non-adults (aged < 18 years)
- Pregnant women
- Deep subcortical ischemic or hemorrhagic stroke not extending to the cortex
- Patients with stroke onset > 7 days
- Patients with cerebral venous sinus thrombosis
- Other non-vascular pathology (e.g. brain tumor, encephalitis, demyelinating disease, known history of stroke or intracranial injury)
- Absence of confirmatory imaging (as per inclusion criteria definition)
- Unfavourable measurement due to body habitu or interfering equipment that may affect measurements

Post-enrollment exclusion criteria (if applicable)

- Discovery of exclusion criteria above

Procedures/methods

Recruitment Methods: PAtients who are admitted with a primary diagnosis of stroke will be screened for meeting inclusion criteria. Patients who meet study inclusion criteria (or appropriate surrogate) will be approached and consented to voluntarily participate. Consenting will happen in person, on the day of the measurements and prior to any measurements being made. Consent will be obtained by one of the investigators, clinical research coordinators, or another member of the research team from the subjects in writing. All measurements will be taken in the ER, ICU or post ICU settings at Hartford Hospital.

Once the subject has arrived and completed the consenting and study enrollment process (including demographic factors), the measurement acquisition process will be as follows:

1. Subjects will lie down in the supine position with an approximately 30 degree angle for the upper torso.
2. While lying down, subjects will have their heart rate, respiratory rate, blood pressure, and O2 saturation taken by the technician.
3. The subject will then be fitted with a headband (see Figure. 3) and goggles that will indicate where the blood flow measurements will be made using Openwater's device. Alternatively, if hair types and/or styles do not allow for proper fitting of the headband, markings on the subject's laser safety goggles will indicate where the measurements are to be made.
4. A total of 12 measurements will be made on the head (6 on each hemisphere).
5. This set of 12 measurements will be repeated 4 times back-to-back in order to measure the repeatability of the dataset.
6. After completing the measurements, subjects will have their heart rate, respiratory rate, and blood pressure retaken by the technician.

We expect each measurement to take approximately 7 seconds with an approximately equal amount of time required to move and reposition the wand between measurements. We are expecting the entire process to take around 30 minutes.

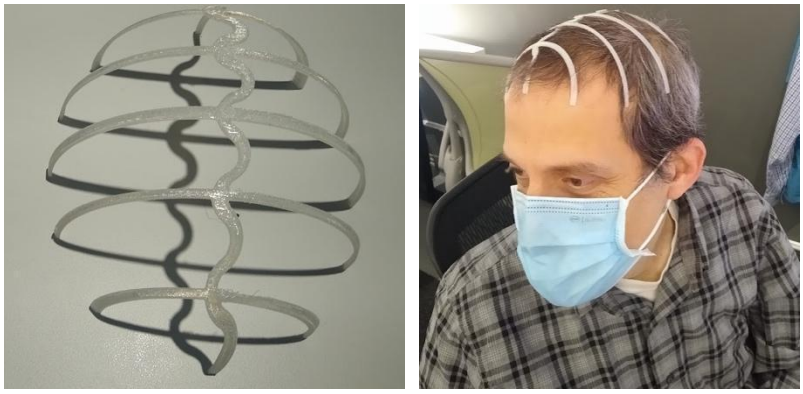

Figure 3: Schematic of custom headband device used to indicate where blood flow measurements will be made on each side of the subject's head.

Risks/Benefits to Patients

Potential risks

- This optical imaging device uses non-ionizing radiation for tissue interrogation.
- The amount of light delivered to tissues is intended to generate diagnostic information and not to treat or generate any changes in tissues.
- Devices (including commercially available ones such as functional NIR spectroscopy, optical coherence tomography, laser Doppler flowmetry, laser speckle imaging, and pulse oximetry) employing similar wavelengths of light at comparable energy, or higher, were previously used in patients for tissue diagnosis and are not known to generate any phototoxic effect.
- Both the subject and device operator will be wearing laser safety glasses to ensure ocular safety.
- The device is not intended to be implanted in subjects nor will it be used in supporting or sustaining human life.
- The device will not be used in diagnosis, mitigation or treatment of disease, or otherwise prevents impairment of human health.
- The device does not present a serious risk to the health, safety, and welfare of subjects.

The output light incident onto the skin from the NIR laser used in the system will comply with maximum permissible exposure (MPE) regulations set in IEC 60825-1. We have evaluated the laser to be Class 3R, safe for body tissue other than the eye area for medical applications. Several mechanisms are in place in order to prevent the patient from receiving too much light. The device has a laser safety board (LSB) which monitors every laser pulse (both optically and electrically) and checks to ensure that the system will not exceed the MPE limits. If either of the two redundant LSB sensors detects that an unsafe amount of optical or electrical energy has been generated, the LSB will prevent light from being transmitted to the wand by turning off the power to the laser.

For each subject a disposable waterproof transparent dressing (e.g. Tegaderm) will be applied to the face of the wand that will come into contact with the subject's scalp. After the procedure, the entire wand head and its cord will be sterilized with Caviwipes1 disinfecting wipes following its Instructions For Use. The laser safety goggles will also be sterilized following their IFU (70% alcohol wipe down).

The device operators will be one APRN and one Physician Assistant at Hartford HealthCare who have gone through the Openwater laser safety training as a part of their general training on use of the system. Openwater staff will train the [redacted] personnel conducting the measurements. Completion of training will be documented and can be provided to the IRB upon request. All operators will wear adequate eye protection during any alignment or maintenance.

The device will only be transported, calibrated, and operated by authorized personnel who have the appropriate expertise in operating it. Unauthorized use of the device is further prevented by keys to access the inside of the cart, as well as to turn on the laser. Use of the fully automated device for acquisition is also limited by a software password which is only known to the operators. The cart is also equipped with an external safety shut-off switch in case of an emergency. Openwater laser personnel will be available for a laser safety orientation and training to all hospital personnel who will be associated with the study.

Although the primary purpose of the wand is to transmit and collect light, the wand does contain two low voltage and low current electrical lines that are used by the safety proximity sensor adjacent to the emitting fiber and the trigger button used to start data acquisitions. The cart has been fitted with an electrical isolator transformer and the entire cart frame along with each subcomponent have been grounded following electrical safety guidelines IEC 60601-1-20.

Potential benefits

There will be no direct benefit to the subjects by participating. However, it is possible that this technology could benefit the subjects in the future if they, or someone they know, have a stroke.

Adverse Events

If an AE or SAE does occur, the PI will promptly report the AE or SAE to the IRB following the guidelines described in [redacted].

Data Use, Collection

- Demographic:
  - gender, age, race, hair color, hair density, hair texture, hair length, body mass index (BMI)
- Clinical:
  - Patients last known well time and stroke onset
  - Admission vitals including BP, HR, O2 saturation, respiratory rate
  - All active medications including IV sedation
  - Intubated vs non-intubated status
  - Serum hemoglobin, hematocrit, BUN, Cr (lab value most proximal to CBF measurement time)
- Diagnosis
  - Stroke subtype (ischemic, hemorrhage, SAH)
    - Ischemic stroke subtype by etiology per TOAST criteria : Cardioembolic, Large vessels atherosclerotic, small vessel ischemic, cryptogenic, other
    - Hemorrhagic stroke: Lobar ICH (spontaneous vs. traumatic), secondary ICH (vascular malformation) and current ICH score
    - Aneurysmal subarachnoid hemorrhage aneurysm site, side, size and Hunt & Hess score
    - Time from onset to first CBF measurement
  - Admission and CBF measurement time NIHSS
  - Admission modified Rankin score
  - Clinical stroke syndrome by vascular territory (e.g. RMCA, LMCA, LPCA
- Radiographic
  - De-identified radiographic imaging data - full data from DICOM
  - Radiology report data (CT, CTA, CP, MRI, MRA)
  - Stroke site and vascular territory
  - CT ASPECTS score (most proximal to the CBF measurement time)
  - Stroke core size if applicable by CT or MRI perfusion
  - Penumbra size if applicable and mismatch ratio
  - CT perfusion and/or MR Perfusion data
  - Modified fisher scale for aneurysmal SAH
  - CTA/MRA or catheter based angiography findings
- Complete past medical history:
  - Vascular risk factors including but not limited to: hypertension, coronary artery disease, congestive heart failure, arrhythmias, diabetes mellitus, hyperlipidemia, COPD
  - Current medications, surgical history, allergies (e.g. adhesives etc.)
  - Social history
- Optical blood flow measurements at each anatomical location (see above)
- Discharge NIHSS and mRS

Data Storage and Security

- No protected health information will be used or collected for the study.
- Only the investigators and the Sponsor named on this study (Open Water Internet Inc.) will have access to the data.
- [redacted] administration will maintain a list of anonymized patient IDs together with patient identifiers on a secured network folder in the event that patient identification is needed.
- Data will be stored in Excel spreadsheets and the raw data (used to calculate BBFD values) will be stored in comma-separated values (CSV) files. All data will be saved locally on the scanning system and backed up in cloud storage.
- All radiologic data will be deidentified
- The data will be maintained for a total of 6 years after the end of the study.

Statistical Methods

Power Analysis and Sample Size Estimate

A total of 30 patients will be enrolled in this study. A preliminary analysis will be completed after 5 patients to evaluate measurement consistency and efficacy to ensure adequate quality measurements are taken. An interim analysis will be completed at 15 patients and final analysis at completion of the study.

There is currently no data available to conduct an appropriate sample size calculation. Results from this observational study will provide insight into the means and variances of blood flow measurements at various locations of the head, using our optical imaging device. This will be the first study to use this device on humans and therefore results from this observational study will provide data to develop a protocol framework for future studies (e.g., with stroke patients) using this device.

Data and Statistical Analyses

Descriptive analysis for all variables will be performed using standard techniques (e.g., measures of frequency, measures of central tendency, and measures of dispersion or variation). Appropriate

transformation of the data or analogous nonparametric methods will be used as necessary. Means and standard deviations (SD) will summarize continuous, normally distributed data; continuous, non-normally distributed data will be summarized with medians and interquartile ranges (IQR). Categorical data will be presented as frequencies, using percentages. All analyses will be performed using SPSS version 26.0. Level of significance will be established as $p < 0.05$.

The following statistical techniques will be used for each of our listed Aims:

1. Compare the variance of blood flow between opposite sides of the head for each of the locations to determine BBFD.

2. Compare the BBFD for each of the locations on the head

3. Compare BBFD, UIrBFD and CIrBFD

4. Assess the variance of BBFD, UIrBFD and CIrBFD for repeated measurements at all locations.

   For the three aims listed above, the variance in blood flow will be compared between locations on the head using either Bartlett's test for homogeneity of variances (if data are normally distributed) or a Levene's test (if data are non-normally distributed). Following tests for homogeneity of variance, for aims one and two, we will perform a paired samples T-test to determine if there are any significant differences in blood flow (Aim 1) between each location on opposite sides of the head as well as differences in BBFD (Aim 2) between lying supine and sitting. For aim three, we will perform a repeated measures ANOVA to determine any difference in BBFD for each of the locations, over the five repeated blood flow measures. If the repeated measures ANOVA is significant, we will conduct post hoc analyses with Bonferroni correction to determine differences between individual time points.

5. Assess whether any demographic factors (age, gender, race, hair color, hair texture, hair density, hair length, BMI) or medical histories (hypertension, coronary artery disease, congestive heart failure, arrhythmias, diabetes mellitus, or hyperlipidemia) correlate to the average variance of BBFD, UIrBFD and CIrBFD for all measurement locations.

6. Evaluate stroke subtype, location and time from onset to measurement for each patient and compare BBFD, UIrBFD and CIrBFD to evaluate the distribution of measurements across our patient population

Clinical Significance
Stroke is the number one cost of long-term disability in the U.S. and the second largest cause of death worldwide; improving the time to diagnosis remains one of the largest challenges to overcoming it. Findings from this study could lead to significantly improved and earlier diagnosis of stroke at a significantly lower cost than any currently approved methods.

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