WAVELENGTH ELECTRONICS

Artery Targeted Photothrombosis to Better Model Human Stroke and Forelimb Impairment

ABSTRACT

The traditional method of photothrombosis proves to be a valuable study of upper-extremity motor impairments and recovery mechanisms but fails to have a distinct resemblance to human stroke due to a narrow vascular penumbra after laser exposure to the motor cortex. Researchers have developed a new method to better model human stroke which uses artery-targeted photothrombosis. This model limits laser illumination to specific arterial branches of the cortical surface to induce less damage to surrounding tissue by controlling stability of laser output and minimizing infarct variations. Artery-targeted photothrombosis shows a clear improvement on traditional methods by creating a larger penumbra for a longer amount of time while maintaining the practical results of the traditional model. Thus, the artery-targeted photothrombosis model is suitable for studying impact and recovery from ischemia.

BACKGROUND

The photothrombosis stroke model induces localized focal ischemic infarcts in mice to provide a way to study recovery mechanisms but does not resemble human stroke very well [1]. Photothrombosis simply creates an obstruction of blood supply to a specified region of tissue, by the use of a laser and Bengal Rose, leading to hypoxic death of the cells. Bengal Rose is a photoactive dye which forms blood clots (leading to ischemic stroke) when photochemically activated by a green laser.

One of the main focuses of the photothrombosis stroke model is the impairment and reperfusion (return of blood flow) of the penumbra (the area surrounding the ischemic event where blood flow is reduced). A murine model with wider, graded penumbra that persists over a longer time frame is useful for studying post-ischemic vascular changes in human stroke.

"The electrical function of the brain is intimately related to metabolism and blood flow" [2]. Low levels of Cerebral Blood Flow (CBF) correlates to electrical silence in the brain. The infarctions (local death of tissue as a result of ischemia or low levels of blood supply) are strategically placed to study mechanical recovery and, in this research, human stroke.

Traditional models of photothrombosis illuminate a region of tissue to cause ischemia in the subject while artery-targeted photothrombosis confines the illumination to arterial branches of the cortical surface. This results in less damage to the surrounding tissue and easier imaging of the CBF.

PROBLEMS AND GOALS

Traditional photothrombosis does well to study the recovery mechanisms following stroke or other forms of ischemia. This is possible by accurate placement of the infarction in regions that affect the function of the forelimb to study impairments and recovery. However, traditional photothrombosis does not generate a wide ischemic penumbra to resemble human stroke. Its usefulness to studies is limited.

The researchers' previous study concluded that "wavelength has been shown to significantly affect the penetration depth" of light patterned in photothrombosis. They also found imaging of the system (spatial and temporal resolution) was limited by noise [3].

It was shown in a different photothrombosis study [4] that high variability of infarct size in test subjects was caused by undetected fluctuations in laser power output during testing. This occurred even after several verifications of the output of the laser. For best results during the surgeries, the laser needs to be at a stable power output that will not fluctuate.

In this study, the artery-targeted photothrombosis stroke model overcomes these problems to better resemble human stroke while maintaining the traditional model's benefits/achievements.

The first goal was to determine if there was a wider vascular penumbra with the artery-targeted model compared to the traditional model.

The second goal was to investigate peri-infarct vascularization for post-stroke recovery. This means looking at the formation of blood vessels, vascular densities, and perfusion of blood cells in response to the ischemia created to better understand the effects and outcome improvements post-stroke.

The third goal was to maintain a stable laser output, stable wavelength, and low noise for spatial and temporal resolution of the system described in previous studies.

The last goal was to determine if the artery-targeted model maintained the benefits of the traditional model to study recovery mechanisms.

METHOD

To perform this study, test mice were separated into three groups: Traditional, Targeted, and Sham (control group). A total of 44 mice were used, and equal numbers of male and female mice were selected randomly for each group/testing. All IACUC guidelines (AUP-2015-00182) were followed for the use of animals while being approved by the Animal Care and Use Committee of the University of Texas at Austin.

Small coverglasses were installed to replace a small portion of the skull over the forelimb area of the Motor Cortex (MC). The mice were anesthetized and fastened to a frame with vitals monitored and maintained at a constant level. Rose Bengal was injected followed by illumination of the laser. For traditional photothrombosis, a green laser (532 nm) was coupled with an objective system to illuminate (20-24mW) a region for 12-15 minutes. The sham group was exposed to laser illumination without the injection of Rose Bengal. Targeted thrombosis used a greed diode laser (532 nm) coupled to a digital micro-mirror device (DMD) used to pattern the illumination (20 mW) over specific arteries on the surface. The laser, used for patterned light with the DMD, was driven by Wavelength Electronics' LDD400-1P laser driver to provide stable power output and low noise to the system. The targeted setup can be seen in **Figure 1(b)**.

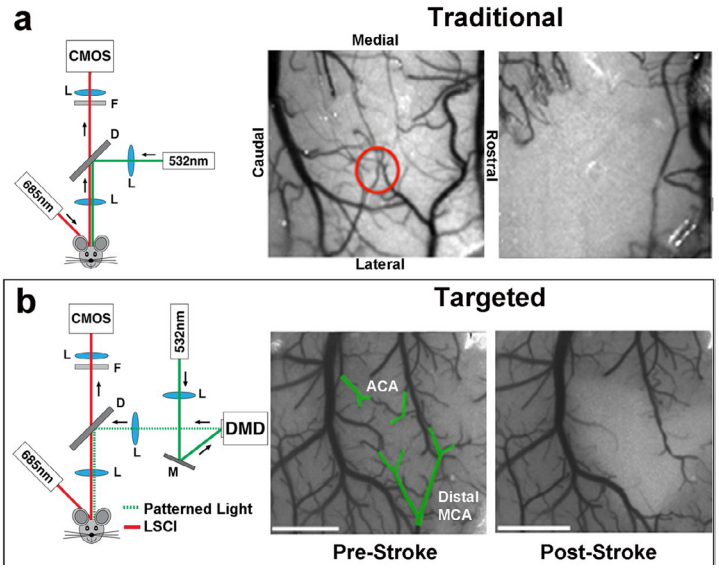

Figure 1. Traditional (a) and Targeted (b) Photothrombosis methods and CBF before and after induced ischemia.

Although the traditional method succeeded in lowering the blood supply and flow in the area of illumination, seen in **Figure 1(a)**, it disturbed the many arteries surrounding the small target area. **Figure 1(b)** shows the patterned illumination on the arteries labeled: Anterior Cerebral Artery (ACA) and Middle Cerebral Artery (Distal MCA). The lack of blood supply, in just those targeted locations post-stroke, is seen in the bottom right image.

Multi-exposure speckle imaging (MESI) was used to capture and monitor CBF after illumination in both the targeted and traditional groups. To perform MESI, a laser was used to illuminate the portion exposed for photothrombosis. This also triggered 15 different camera exposure settings. Light scattered back into the CMOS camera through an objective onto the sensor. Raw frames were converted to speckle contrast images and eventually into inverse correlation time (ICT) images shown in **Figure 2(a)**.

To test the effects of artery-targeted photothrombosis on the forelimb recovery and function, mice were trained to reach for seeds outside of their enclosures. After photothrombosis the mice where given the same evaluations. Each trial the mice received four attempts to grab the seed. A success was when a mouse was able to bring the seed from the training chamber to its mouth. A failure was when the seed was missed, displaced, or dropped before brought to the mouth. Results were plotted as the Mean ± Standard Deviation (SD) success per attempt.

To assess vascular density change, images were taken using a standard light microscope with a reflected fluorescence system. Images of the peri-infarct cortex were collected, and the area fraction of IB4-labeled vessels were calculated. The magnitude of vascularization in peri-infarct cortex compared to the sham group can be determined by the vascular density results.

The size of the penumbra was studied by looking at the CBF surrounding the tissue or arteries illuminated by the laser. Measurements were grouped together in 100, 100-300, 300-500, and >500 µm from the ischemic core. The distance depended on the animal size or location of the core in relation to the cranial window. This is seen in **Figure 2(b)**. Ischemic core was defined as the area of parenchyma (at 48 h) with CBF values ≤20% of baseline. At the 48 h time point, the transition of ischemic tissue to infarct core fate would be complete [1].

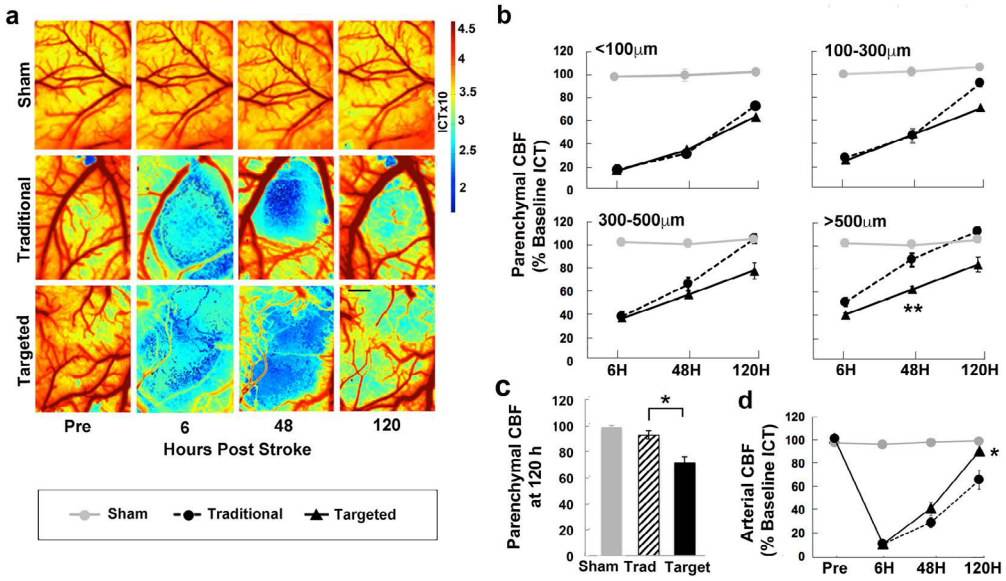

Figure 2. Reduced CBF from photothrombosis. (a) Blue indicates areas of lower CBF and red indicates areas of higher CBF. (b) Post-ischemic CBF deficits represented as a percent of baseline ICT values measured at different distances. (c) Parenchymal (tissue) CBF at 120 h across all measured distances. (d) CBF in occluded or obstructed arteries was higher at 120 h in the targeted group compared to traditional. **p < 0.001, *p < 0.02.

RESULTS

Penumbra Results

Measurements were taken of the CBF at 6, 48, and 120 h following targeted and traditional photothrombosis at the distances from the ischemic core marked in **Figure 2(b)**. Differences were seen between the two groups at 48 and 120 h. CBF was significantly greater at 48 h at distances >500 μm for the traditional group and slightly greater at distances 300-500 μm compared to the targeted group. At 120 h, the differences are clear between the two groups: traditional showed much higher CBF compared to the targeted group without much variation in distance.

This shows that the artery-targeted photothrombosis created a larger penumbra for a longer amount of time. This study resulted in a more graded distribution of CBF following artery-targeted photothrombosis. This creates better resemblance to human stroke for future studies regarding effects and recovery post-stroke.

vascularization Results

Because the traditional photothrombosis model created increased levels of vascular density, the goal was to maintain these increased levels with the artery-targeted model.

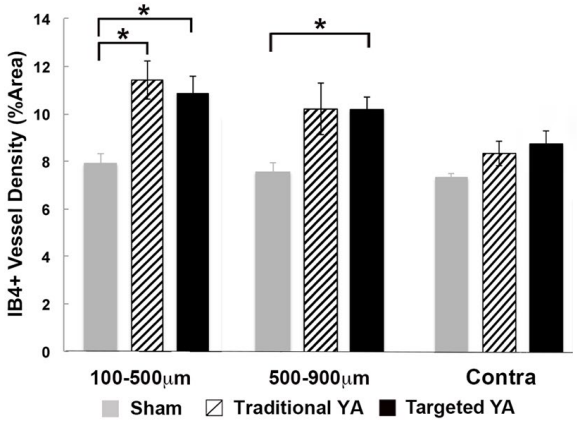

Figure 3. Vascular Density represented as the % area of IB4-labeled blood vessels. *p < 0.02 versus Sham

Figure 3 shows the density levels (as an area fraction of IB4-labeled vessels) of both photothrombosis groups as well as the sham group. It is clear that the infarct groups both had an increase in vascular density following infarct. Both had similar results of increased levels which showed that the targeted model can maintain the same vascularization results as the traditional model. Because vessel densities reflect vascularization and both groups had similar levels of vessel densities, both models created similar magnitude of vascularization even with the differences in CBF deficits shown in **Figure 2**.

Impairment Results

Artery-targeted photothrombosis was able to maintain the results produced by traditional photothrombosis relating to impairing forelimb function. **Figure 4** shows the results of repeated tests. Although the deficits in the forelimb function seem to vanish at the last data point (20 days), it is most-likely due to the small infarct sizes made from the illumination. Another study indicates longer impairments/ deficits result from larger infarct sizes. This study shows that the present model has similar ability to create these deficits in forelimb function as the previous traditional model.

Stability Results

Wavelength of the laser, patterned by the DMD, was controlled by the LDD400-1P. This allowed penetration depth of the photothrombosis to be consistent and accurate. Variation in lesion volumes were small in both traditional or targeted groups. However, artery-targeted photothrombosis produced the smallest lesion volume SD at ±0.21 mm^3 compared to traditional at ±0.47 mm^3. Researchers were able to get accurate and reproducible results with the help of the laser driver.

Overall Conclusion

In this study, researchers showed that artery-targeted photothrombosis maintained the strengths of the traditional photothrombosis model in regards to increased vascular densities and impairment of forelimb function. Artery-targeted photothrombosis also increased the size of the ischemic penumbra, strengthening its resemblance to human stroke for future studies on recovery from ischemic stroke

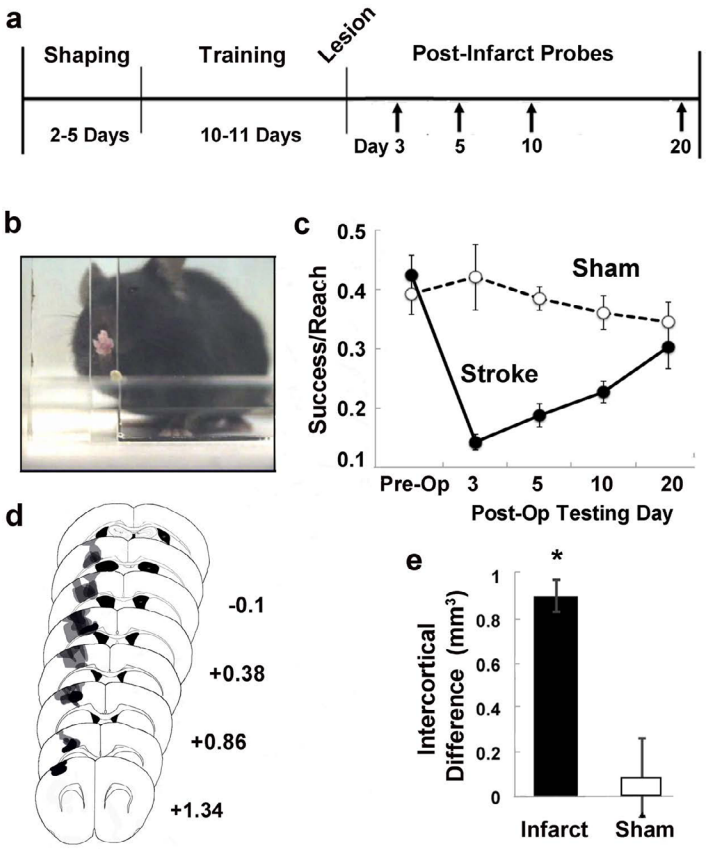

Figure 4. Forelimb skill testing. (a) Timeline of study. (b) A mouse performing the retrieval task. (c) Performance of mice before and after photothrombosis. (d) Lesion reconstruction of infarct group overlayed on coronal sections templates. (e) Intercortical difference between the infarct and sham groups. *p = 0.001 versus Sham

WAVELENGTH'S ROLE

When it was essential to have high performance in laser output and stability, researchers turned to the low noise laser driver that offered excellent current stability in a compact size. This provided stability for the small size and area of illumination required to target individual arteries which increased penumbra size for a photothrombosis model better suited to human stroke.

The LDD400-1P provided up to 400 mA of current to the laser with noise as low as 5 µA. Laser power stability was crucial in controlling the results and minimizing variability between test subjects regarding infarct size. Because wavelength has been determined to greatly impact the penetration depth of the illumination and noise was the ultimate factor in spatial and temporal resolutions of imaging, the LDD400-1P was the optimal choice to reduce all factors hindering the previous methods/results.

With a size of approximately 1.3" x 2.05" x 0.43" (33.0 x 52.1 x 10.9 mm) and a weight of less than 1 oz (<28.3 grams), the driver is able to fit virtually anywhere.

The LDD400-1P has 50 ppm stability to provide precision power output needed for the duration of patterned illumination for this application. The laser driver aided the researches in accomplishing their goal of finding a photothrombosis stroke model (artery-targeted) that could parallel human stroke.

PRODUCT USED
LDD400-1P

KEYWORDS
Photothrombosis, vascular penumbra, stroke, impairment, artery targeted, peri-infarct vascularization, ischemia, cerebral blood flow, laser driver

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USEFUL LINKS

- LDD400 Product Page

OPEN ACCESS

Figure 3 was cropped from original material. No other changes were made to the other images. They are presented here in their original form.

All captions have been modified from their original form.

REVISION HISTORY

Document Number: CS-LD02

REVISION	DATE	NOTES
A	October 2019	Initial Release