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Activated Caspase 3 Analysis in Traumatic Brain Injury Cerebral Organoids

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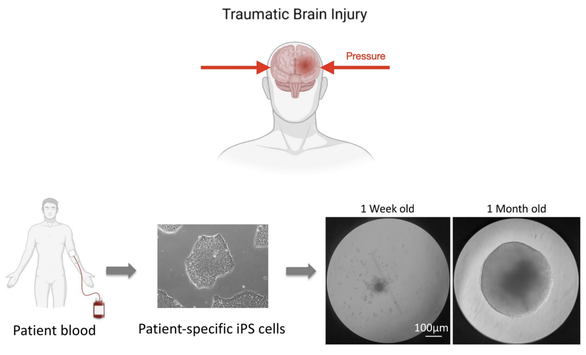
Abstract

TBI stands for traumatic brain injury which causes permanent loss of neural tissue. This experiment focused on pressure caused TBI and its resulting pathology. Cerebral organoids are in-vitro 3-dimensional stem cell cultures that display cerebral cortical regions similar to a developing human brain. Cerebral organoids were used because they potentially offer a more humanistic model of organization. The cerebral organoids were grown using the Pasca protocol and then to model a pressure induced TBI, the organoids are loaded into a tabletop blast chamber. The sections were stained using immunohistochemistry for activated caspase 3, which is an apoptosis marker to determine if the organoids reacted the same as a human brain would. In the cerebral organoids the frequency of the blast and the amount of cell death have a positive correlation. Thus, the dose response to different frequencies gives a threshold of cell death like that of a human blast TBI.

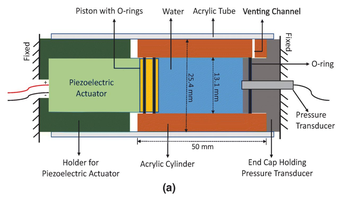
Background

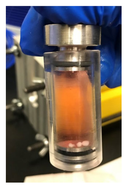
Traumatic brain injury (TBI) are caused by multimodal forces (different combined methods of force), these forces include pressure, sheer, cavitation, and energy. The parameters of a TBI can be characterized by analyzing pathology, using immunohistochemistry. It is difficult to accurately model human TBI and thus stem cell cultures have been developed to form a potentially more humanistic model. Cerebral organoids are made using patient specific IPS cells from a patient's blood and growth medium,



Methods

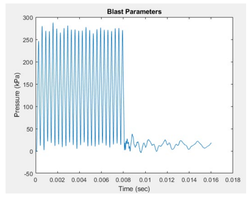
To model a pressure induced TBI, the organoids are loaded into a highly controlled tabletop blast chamber. The chamber has a piezoelectric actuator which allows us to specify exact blast criteria. The chamber was able to record the amplitude and frequency of the blast.



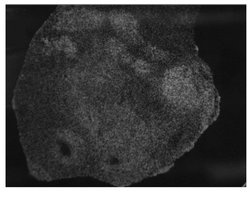


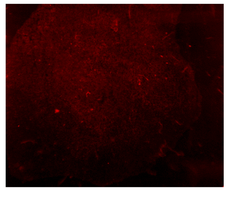
Different frequency and amplitudes of pressure are used to establish a threshold of blast damage.

- Amplitude groups of injury:
  - "mild" 250 kPa
  - "severe" 350 kPa.
- Range of frequencies:
  - 500 Hz
  - 3,000 HZ
  - 5,000 Hz.



The blast organoids were then sectioned using a cryostat and mounted onto slides. The sections were stained using immunohistochemistry for activated caspase 3, which is an apoptosis marker. The image J program was used to measure their surface area to determine a threshold of positive staining, to quantify the percentage of cell death. An ANOVA was run to test for differences between group means.

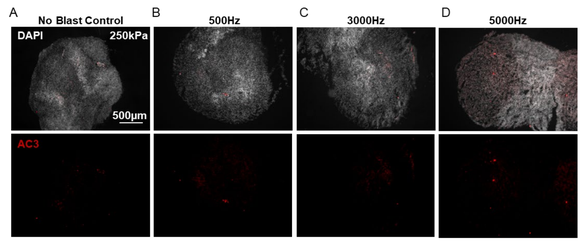


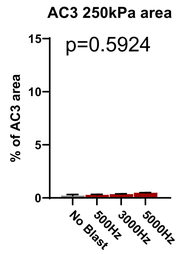


Results

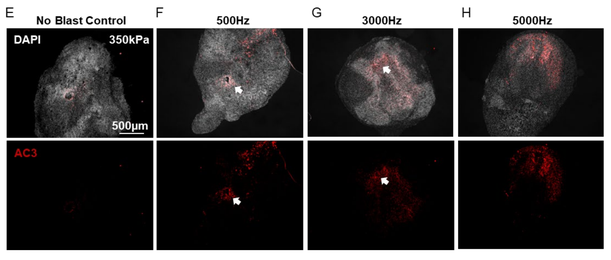
The control organoids have some expected normal apoptosis because the cerebral organoids used don't have vascularization.

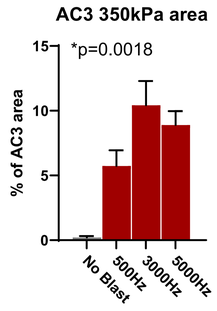
- Mild 250 kPa
  - The low pressure amplitude does not initiate apoptosis
  - Diffuse pattern of apoptosis





- Severe 350 kPa
  - Higher pressure amplitude does initiate apoptosis
  - Apoptosis localized to areas of high cell density





Conclusion

- The frequency of the blast and the amount of cell death have a positive correlation.
- Lower amplitude blasts at all frequencies don't display significant amounts of cell death.
- Higher amplitude blasts display significant amounts of cell death that increases with higher frequencies.
- Thus, the dose responses to different frequencies give a threshold of cell death like that of a human pressure blast TBI.

Steps Forward

With the newfound threshold we can use cerebral organoids to examine what biological changes are occurring that lead up to the characteristics of TBI.

- Co-staining AC3 with Tuj-1 and GFAP to understand which cell types are dying.
- Test how repeated high amplitude blasts can be modeled by cerebral organoids.

References

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