Frozen in Time: Triumphs in the Cryopreservation and Cultivation of Giant Cell Tumors of Bone

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1. Introduction

Giant cell tumors of bone (GCTB) are rare and account for approximately 5% of all primary bone tumors[1]. The recurrence rate following the most common treatment, curettage, is approximately between 12-27%[2]. The use of cell culture is useful for performing controllable and replicable experiments. The use of cryopreservation is useful for saving tissues and cells for later use. Cryopreservation and subsequent cell culture have been successfully performed using other tumors and tissues, but not yet using GCTB tissues[3,4]. This study aims to test a new cell viability check method following cryopreservation and to be the first to successfully cryopreserve and culture GCTB cells under different freezing conditions.

2. Methods

Step 1: Freezing of Tissue

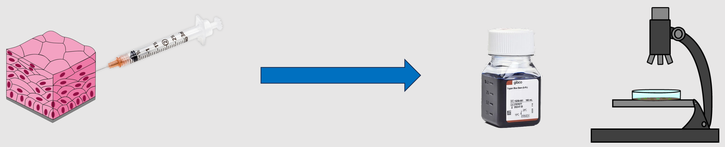
- Tissues were minced and frozen under different conditions:
  - a. Freeze Media
  - b. Dry
  - c. 50% Phosphate Buffered Saline (PBS) and 50% Freeze Media
  - d. Optimal Cutting Temperature Preservative (OCT)

Step 2a: Thawing and Plating

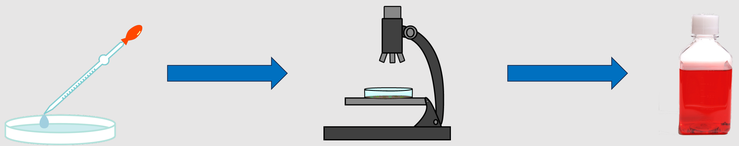
- After thawing and before beginning the cell culture process we performed the cell viability check (see below)
- Following the cell viability check the cell culture plating protocol was followed

Step 2b: Cell Viability Check

- If the cell viability check yielded successful results (lit-up cells) then we continued with the cell culture plating protocol

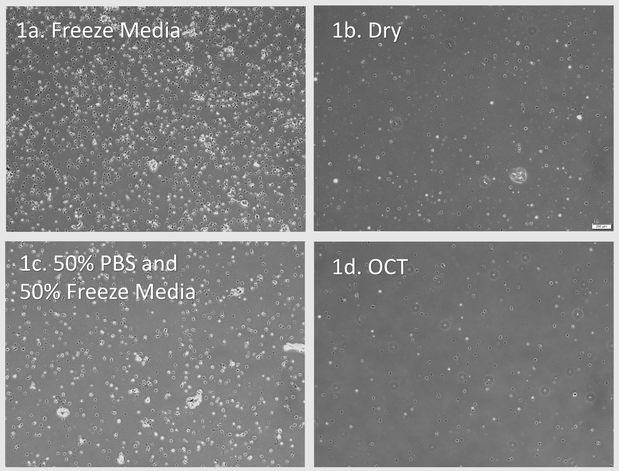


Step 3: Cell Culture Protocol



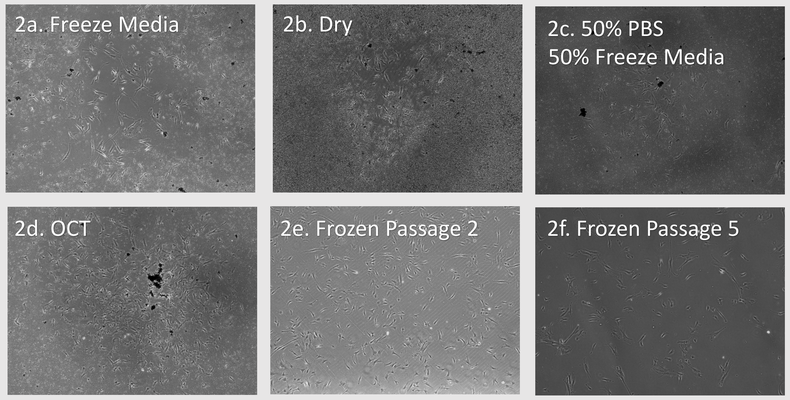
3. Results

Figure 1. Results of the Cell Viability Check Technique



- All cell viability check results contained live and dead cells in trypan blue
- All freezing conditions had similar live-dead cell ratios
- All tissues that had live cells in the cell viability check resulted in successful cell culture

Figure 2. Results of Cell Culturing of Differently Cryopreserved GCTB Tissues

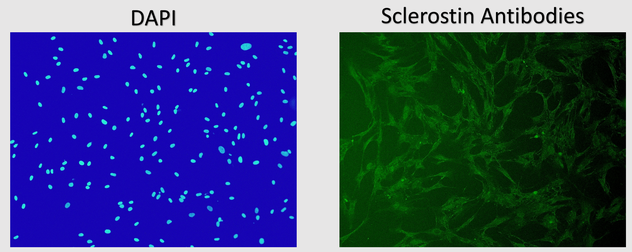


- GCTB cells were successfully cultured from frozen tissues
- All different freezing conditions were successfully cultured
- Different freezing conditions grew at different rates
- Frozen-down cells from different passages were also successfully cultured from frozen as well

4. Conclusion and Discussion

The results of the cell viability check and the subsequent cell culture verify the first successful use of the viability check and cell culture of GCTB cells from frozen tissue. The successful cell culture shows freezing conditions do not matter for the culture of GCTB cells. Successful freezing and thawing of GCTB cells show successful cell storage and the ability to re-culture the cells for later use. More assays need to be performed to diagnose cells as GCTB cells, but our preliminary findings based on immunocytochemistry staining are promising as seen in Figure 3. Possible drawbacks of this study include no controls and no quantitative data for more detailed analysis. The successful cryopreservation also plays a key role in future research into this rare disease as researchers now have an effective way to store GCTB tissues as new cases are not common. Future directions include testing for specific proteins such as sclerostin for possible anti-tumor therapies.

Figure 3. Results of Immunocytochemistry on GCTB Cells



The picture on the left is of cells stained with 4',6-diamidino-2-phenylindole (DAPI). Bright spots are cell nuclei. The picture on the right is cells stained with sclerostin antibodies. Light areas are parts of the cell that contain sclerostin.

5. Acknowledgements

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6. References

1. Sobti A, Agrawal P, Agarwala S, Agarwal M. Giant Cell Tumor of Bone – An Overview. *Arch Bone Jt Surg.* 2016;4(1):2-9
2. Miles DT, Voskuil RT, Dale W, Mayerson JL, Scharschmidt TJ. Integration of denosumab therapy in the management of giant cell tumors of bone. *J Orthop*. 2020;22:38-47. doi:10.1016/j.jor.2020.03.020
3. He A, Powell S, Kyle M, et al. Cryopreservation of Viable Human Tissues: Renewable Resource for Viable Tissue, Cell Lines, and Organoid Development. Biopreservation Biobanking. 2020;18(3):222-227. doi:10.1089/bio.2019.0062
4. Wu SZ, Roden DL, Al-Eryani G, et al. Cryopreservation of human cancers conserves tumor heterogeneity for single cell multi-omics analysis. *Genome Med*. 2021;13(1):81. doi:10.1186/s13073-021-00885-z

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