Senior Design Project
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ISlice: A Method of Reducing Cluster Size While Maintaining Tissue Volume

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Abstract

In regenerative medicine, cell products are based on cell clusters, which rely on nutrient diffusion for their long-term survival. Optimal cluster size for nutrient diffusion is 150μm. When cluster size exceeds 150μm, cell viability and functionality are compromised. ISlice is designed to resize clusters for optimal diffusion unlike traditional filters that remove clusters larger than 150μm. ISlice utilizes titanium mesh to reduce the cluster size without damaging the cells or decreasing the cell product volume.

Introduction

Cells need to maintain their functionality by both intaking nutrients and secreting bioactive molecules through diffusion. While proper nutrient diffusion is critical for cell survival, proper diffusion of bioactive molecules from the cells is critical to maintain cell functionality in regenerative medicine applications.

Diffusion allows molecular transport across the cell clusters. Diffusion through the clusters is inversely proportional to clusters size. Given a fixed concentration of nutrients in the cell culture media surrounding the clusters and fixed cell consumption rate, the larger the clusters, the lower the nutrient concentration in the cluster core. When this concentration reaches values that are below the minimum concentration for cell survival, cells undergo apoptosis. The optimal cluster size for nutrient diffusion to preserve viability of the entire cluster is 150μm diameter. When clusters are larger than 150μm in diameter, cells in the core undergo apoptosis and the cell product loses overall functionality for regenerative medicine applications (**Figure 1**).

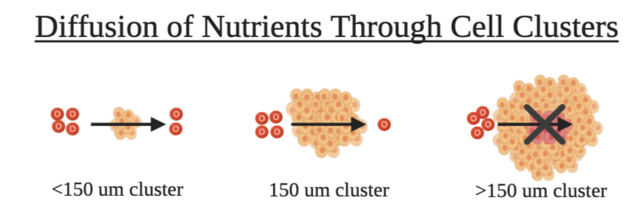


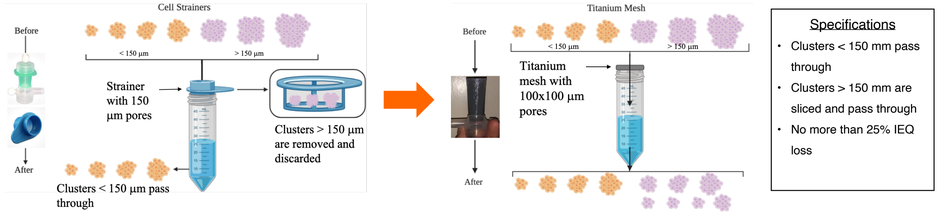
Figure 1: Diffusion of nutrients like oxygen for cell clusters of increasing diameter. 150μm is the maximum cluster size that allows for every cell within the cluster, including the core to receive the amount of nutrients necessary for their survival.

Methods I Design I Analysis

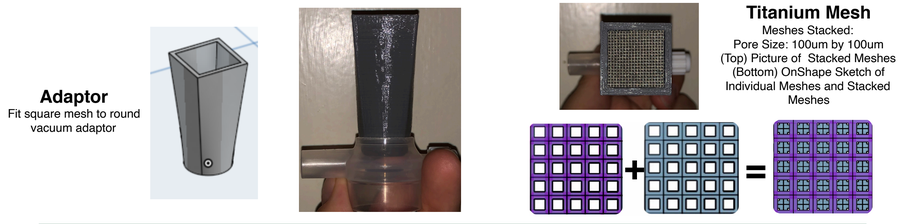
Methods:

- Cell product: MIN6 insulinoma cell clusters produced in Dr. Tomei's lab
- Cell cluster quantification: automated cell counter ICC4 provides quantitative characteristics of cell clusters, including diameter, size distribution.
- Cell product yield compared to commercially available devices can be estimated

Device design:

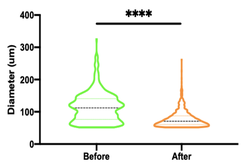


ISlice Device V1



Results

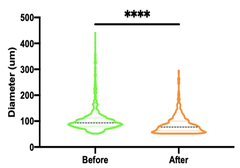
Minimum Viable Product



ISlice	Before	After
Total IN	475	882
Total IEQ	370.06	243.43
Yield	65.78%	
Commercially Available Filters	Before	After
Total IN	475	388
Total IEQ	370.06	160.80
Yield	43.45%	

Figure 2: Violin Graph showing the proportion of clusters at each diameter before and after processing with ISlice mesh. MIN6 insulinoma cell clusters were used

ISlice Device V1



ISlice	Before	After
Total IN	1428	1219
Total IEQ	1109.13	667.27
Yield	60.16%	
Commercially Available Filters	Before	After
Total IN	1428	1178
Total IEQ	1109.13	422.92
Yield	38.13%	

Figure 3: Violin Graph showing the proportion of clusters at each diameter before and after processing with ISlice device V1. MIN6 insulinoma cell clusters were used

Conclusion

ISlice was developed to minimize the size of cell clusters in order to optimize diffusion of nutrients and improve the rate of survival of these cells. ISlice is a titanium mesh system that allows cell clusters below 150μm to successfully pass through the meshes and clusters above 150μm to be sliced into the optimal cluster size of 150μm or less without damaging the cells. ISlice V1 was successful as it significantly decreased the size distribution of cell cluster samples through the two tests that were performed. In order to improve the device considering the issues of clogging and pore size deformation, a more appropriately sized adaptor can be developed along with the use of a higher resolution 3D printer. With these future improvements, ISlice will be a product that will be marketable to any researcher using cell clusters, organoids, or encapsulation in long-term culture that have issues with cell death.

Acknowledgments

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