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3-D Culture System For Rapid Expansion of Human Neural Stem Cells

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3-D Culture System

For Rapid Expansion of Human Neural Stem Cells



Introduction

Stem cell research is a fast growing field due to its extraordinary differentiation potential for regenerative medicine. There is an increasing demand for this branch of research because a critical cell count must be met to demonstrate therapeutic effect. Our objective is to create a novel three-dimensional (3-D) culture system for rapid stem cell expansion. The final product design focuses on physical topography and surface treatment of our proposed 3-D system. Current culture systems rely on two-dimensional (2-D) plates; however, 3-D systems offer exponentially increased surface area for cell attachment requisite for cellular expansion. Current 3-D systems include fibers, hydrogels, and microcarriers. Fibrous networks are valued for mechanical stability while aqueous hydrogels mimic natural ECM and allow for customizable degradation. Microcarriers were chosen for their scalability and ease of cell retrieval. Bioactive coating was applied to customize surface features to aid cell adhesion.

Methodology

A polystyrene and dichloromethane mixture was fabricated into beads using a novel piezoelectric system shown in Figure 1. First, a metered syringe pump uniformly released the polymer mixture into a dish of polyvinyl alcohol (PVA). A function generator would then produce a triangle wave against the syringe needle, causing beads to drop at a controlled rate and size through varying concentration, frequency, and flow rate. Beads were dried and separated by size. The final sizes (diameters in μm) included: 90, 150, 175, 211, 250, 500, 600. The beads were sterilized and a peptide coating was applied. Neural stem cells (NSC) were seeded by pipetting into microwell plates that contained beads suspended in media. They were cultured for 12 days and analyzed at days 7 and 12. Cell viability was measured by Trypan Blue. AlamarBlue™ staining was used to calculate percent reduction, a measurement of metabolic activity, which indicates the extent of cellular proliferation.

Results



Figure 1. Piezoelectric Setup

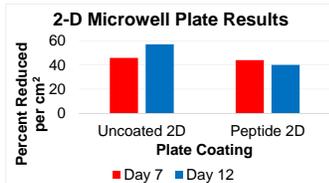


Figure 2. 2-D Results

Results

- Growth rate was approximated as the percentage of reduction of the AlamarBlue™ dye between different conditions of day 7 and day 12
- The growth between day 7 and day 12 is statistically significant for all the groups tested ($p < 0.005$)
- By day 12, the peptide coating improved the cell proliferation significantly for the sizes 175-500 μm ($p < 0.05$)

Table 1. Percent Reduction per Unit Area for 3-D Size Ranges

Size (μm)	Day 7 (growth/ cm^2)		Day 12 (growth/ cm^2)	
	Uncoated	Coated	Uncoated	Coated
90	0.34	0.19	0.73	0.77
150	0.34	0.40	0.64	0.85
175	0.37	0.51	0.72	1.04
211	0.52	0.92	0.78	1.58
250	0.43	0.49	0.99	1.63
500	0.60	0.94	0.60	1.24
600	1.14	0.94	2.49	2.89

3-D Polystyrene Bead Group Results

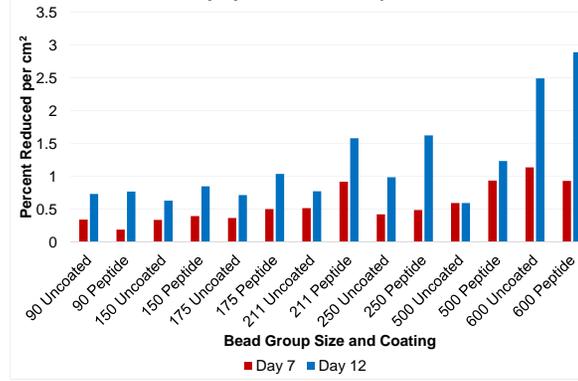


Figure 3. 3-D Results

Results

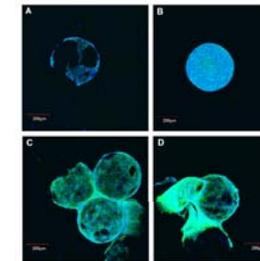


Figure 4. (left) Merged Fluorescent DAPI (blue) and Phalloidin (green) Stained Images of Peptide Coated (A,B) and Uncoated (C,D) Beads at Day 7

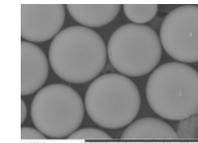


Figure 5. (bottom) SEM image of dry fabricated beads pre-coating

Discussion and Conclusion

- A p-value of 0.001 for growth between day 7 and day 12 in all 3-D size groups demonstrates that there is a significant proliferation of cells between day 7 and day 12.
- From Fig. 2, it can be observed that peptide coating does not show any improvement in the cell proliferation in 2D culture. However, from the tabulated data and Fig. 3, it can be seen that peptide coating significantly improved the cell proliferation for the beads of the size range 175-500 μm
- This shows coating does increase proliferation rate with possible saturation before day 12, causing the cell count to plateau between days 7 to 12. Regardless, the peptide coating has shown to be extremely effective in aiding cell attachment, as the coated beads resulted in much greater cell proliferation than uncoated beads.
- This demonstrates that there exists a size range with greater proliferative capacity with peptide coating, possibly confounded by size extremes.
- When comparing the 3-D system's efficacy on cell proliferation versus 2-D, it may take longer for cells to proliferate due to the time it takes to attach to beads before proliferation. Though 2-D cultures may reach confluence within 7 days, all sizes show significant growth continuing after that period.
- Sources of error included the aspiration of smaller bead sizes and cells while changing media in the pilot study. In the second study, this was corrected with 40 μm well filters. This inconsistency in the techniques employed between the 500 μm pilot study and the second study of all other bead size ranges, was noted.
- Future direction could include repeated trials with more time points sampling.