

Effects of Endothelial Cell Seeding Density and Passage Number On Human EC-Mesenchymal Stem Cell Spheroid Characteristics

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Introduction

- Regenerative medicine strategies often use hydrogels for cell transplants.
- Endothelial cells (ECs) in combination with mesenchymal stem cells (MSCs) may be useful in forming new pods of vascular regeneration.
- Human platelet lysate (PL) hydrogel for 3D culture provides sustained growth factors.

Methods

- Tibial MSCs are isolated from a diabetic patient undergoing below knee amputation at Atlanta VA Medical Center.
- Human diabetic MSCs were mixed with CFSE-stained commercial human aortic ECs at 4 serial passages at 1:1 ratio.
- MSC-EC mixture was embedded in 50% PL crosslinked with human thrombin (5U/mL) in α MEM in 96-well plate, pre-coated with 2% human serum albumin (HSA).
- Cells embedded in PL gel were set for 30 min at 37°C at varying concentrations.
- Growth medium supplemented with 2 μ g/mL aprotinin and 10% FBS was added, and cells were grown overnight at 37°C in 5% CO₂.

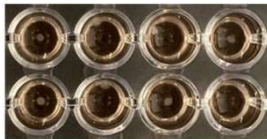


Figure 1: Cells aggregate to form spheroids overnight. Wells are coated with 200 μ L 2% HSA for 2 hours at 37°C, and the cell mixture is cast in 5% PL gel overnight. Cells aggregate to form tight spheroids.

- Spheroids were embedded in 3D fibrin hydrogel, supported by nylon mesh ring scaffolds, and grown in α MEM supplemented with FBS and aprotinin.
- Spheroids were imaged every 24 hours for 3 days.

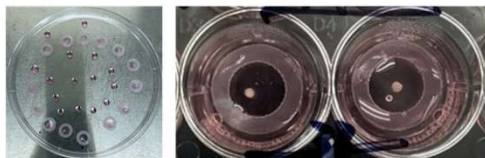


Figure 2: Spheroid embedding. Hydrogel droplets were cast, and the spheroids were placed in the scaffolding nylon mesh rings.

Results

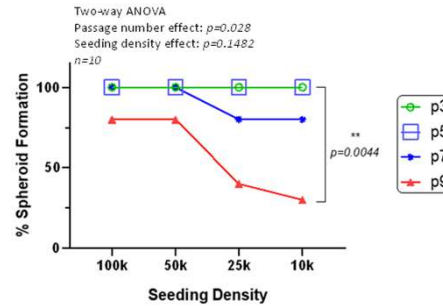


Figure 3: Spheroid formation rate. Higher passage number of the ECs was associated with decreased spheroid formation frequency in PL hydrogel.

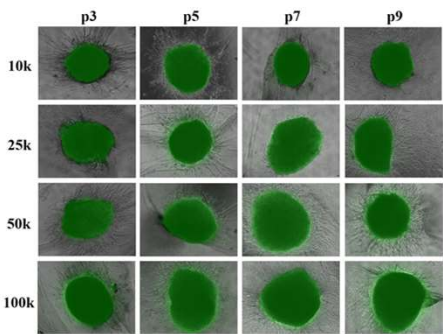
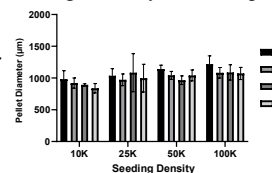


Figure 4: Passage number and seeding density did not significantly affect sprouting potential of MSCs. Spheroid formation was decreased for high passage cells at lower seeding density.

- Spheroid formation frequency depended both on the passage number and seeding densities (Fig. 3).
- Cells at p3 and p5 formed spheroids at any seeding densities.
- Cells at p7 had decreased formation rate, but it was not significant.
- Cells at p9 had significantly decreased spheroid formation frequency at 25k and 10k seeding densities at 30% and 40% respectively ($p=0.0044$).
- Seeding density did not significantly alter the pellet size.

Figure 5: Pellet diameter did not significantly depend on the seeding density. Representative data shown at p9.



Results

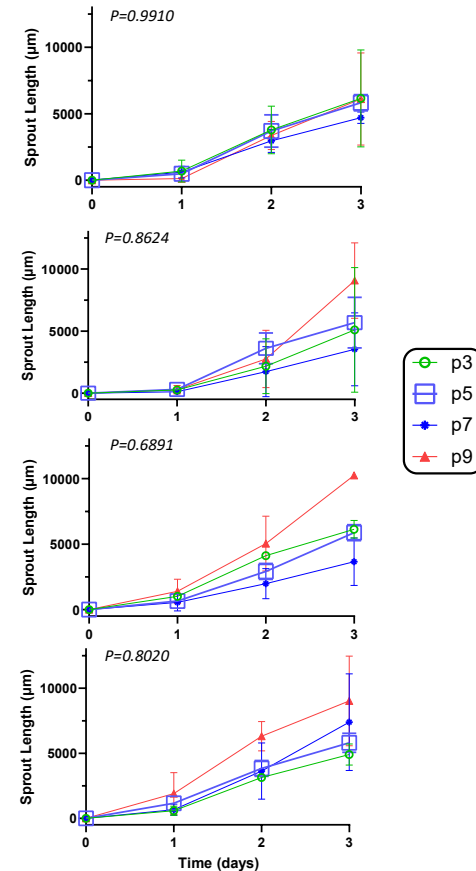


Figure 6: Sprout growth progression. Sprout length at early passage were quite similar in all conditions. Sprout lengths across all passages appeared most consistent when seeded at lower density.

Conclusions

- Altering the seeding density and the age of the ECs while keeping the MSCs' age constant affected the frequency of spheroid formation.
- However, if the cells were able to aggregate adequately to form spheroids, they exhibited similar survivability and sprouting potential as evidenced by the length of the sprouts and the comparable sprout growth rates.