Engineering "Building Blocks" for Efficient Cell Growth, Migration, and Differentiation in Tissue Engineering Applications

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Repair Reconstruction Regeneration

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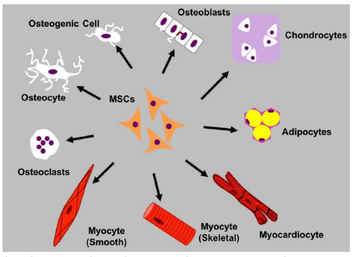
Background

Mesenchymal Stem Cells are Dynamic Cells in Dynamic Environments

Mesenchymal stem cells (MSCs) have the ability to differentiate down a variety of cell lineages, but exhibit a limited shelf-life when isolated from primary human or animal tissues**.** MSCs are currently cultured on 2D plastic surfaces (e.g., well-plates, T-flasks) and in 3D aggregates or in modified materials. Yet, these formats do not fully mimic *in vivo* environments. To prevent cell stratification, researchers must physically detach cells from the growth substrate and dilute cells to be plated on a new growth substrate (Passaging).



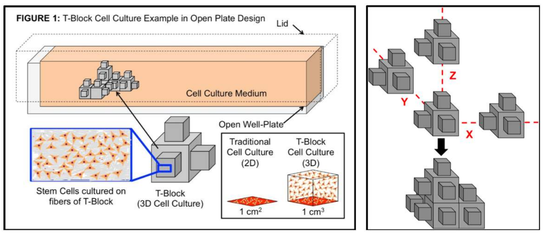
Detachment from the original growth substrate is a traumatic event for stem cells as the cytoskeletal components must rapidly adapt to a non-adherent environment, which can diminish stem cell phenotype and differentiation potential. Repeated passaging can lead to cell senescence and loss of differentiation potential, which ultimately limits the utility of autologous stem cells in tissue engineering applications.

The key problem in expanding viable MSCs in large quantities is that current technologies provide limited growth surfaces.

Purpose

To Demonstrate A Novel Platform Technology for Growing MSCs in 3D

Construction of a 3D expandable substrate could provide a strategy for growing healthy MSCs for use in creation of organoids and sophisticated tissues. **Tissue Blocks (T-Blocks)** are interlocking modular 3D scaffolds that can create a substrate that can be expanded in the X, Y, and Z axial directions **(FIG. 1)**.



Methods

Proof-of-Concept

Punch & Coat T-Blocks

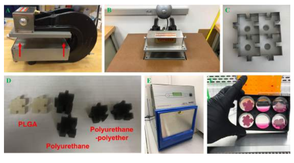


Figure 2. A) Side-view of die press, which compresses die into material using 7 Tons of pneumatic force. **B)** Front view of die press. **C)** Jig-saw shape die. **D)** Jig-saw geometry punches of different materials. **E)** Ethylene oxide sterilization chamber. **F)** Fibronectin coating of T-Blocks.

3D Bioprinted T-Blocks

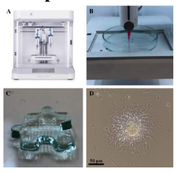
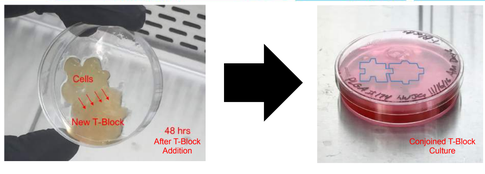


Figure 3. A) CellInk Bio X Bioprinter. **B)** Bioprinting of T-Block. **C)** Bioprinted T-Block. **D)** Micrograph of human Wharton's jelly cells (HWJCs) encapsulated within Matrigel T-Block. HWJCs formed multiple colonies within the Matrigel 24 hrs after bioprinting.

Study

Experiment 1: Validate Concept

Fabricate and EtO Sterilize Scaffolds → Coat Scaffolds with 5 ng/cm² Fibronectin → Seed Scaffolds with 100,000 MSCs → Culture MSCs for 60 Days. Expand Scaffold as needed → Analyze Constructs



Experiment 2: Apply T-Blocks to Spinal Cord Injury Model

Encapsulate MSCs (100,000 cells/mL) in Matrigel → Bioprint T-Block Construct with MSCs → Harvest Embryonic Mouse (Day 13.5) Spinal Cord → Culture injured spinal cord on top of T-Block for 3 Days → Analyze Constructs

Results

MSCs Thrive in T-Blocks Over a Period of 60 Days

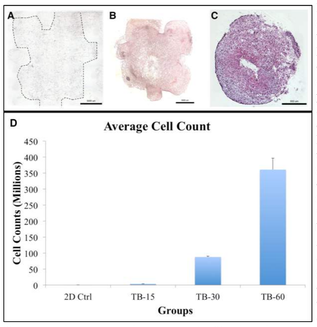


Figure 4. Human Wharton's jelly cells (HWJCs) are a type of MSC found in the human umbilical cord. HWJCs were isolated from human umbilical cords donated under informed consent using the approved KUMC IRB Protocol (STUDY00140532). HWJCs were expanded to passage 2 and used for experiments. HWJCs were seeded on 2D fibronectin (5 ng/cm²) coated glass coverslips or into non-woven mesh PLGA T-Blocks at a density of 100,000 cells/mL. HWJCs were grown until confluent on glass coverslips (~7 days) or for up to 60 days in T-Block constructs. HWJCs were examined at 15, 30, and 60 day time points, post-seeding. T-Blocks were fixed in 4% paraformaldehyde in phosphate buffered saline overnight, dehydrated, cleared, and embedded in paraffin. Ten-micron thick sections were stained with **Hematoxylin & Eosin (H&E) (A-C).** There is a clear increase in cell density from day 15 to day 60. PLGA fibers were observed to be bent, and forming a cylindrical structure after 60 days of culture.

Three samples were used per group (n=3) where bars represent averages and error bars represent standard deviation **(D)**. An average of $3.6x10^5 \pm 5.4x10^4$ cells were identified in 2D controls (2D Ctrl), $3.4x10^6 \pm 4.7x10^5$ cells were identified in T-Blocks after 15 days (TB-15), $8.7x10^7 \pm 2.4x10^6$ cells were identified in T-Blocks after 30 days (TB-30), and $3.6x10^8 \pm 3.2x10^7$ were identified in T-Blocks after 60 days.

MSC Phenotype Identified within T-Blocks

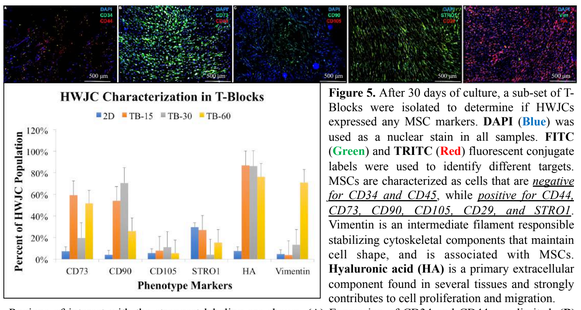


Figure 5. After 30 days of culture, a sub-set of T-Blocks were isolated to determine if HWJCs expressed any MSC markers. **DAPI (Blue)** was used as a nuclear stain in all samples. **FITC (Green)** and **TRITC (Red)** fluorescent conjugate labels were used to identify different targets. MSCs are characterized as cells that are *negative for CD34 and CD45*, while *positive for CD44, CD73, CD90, CD105, CD29, and STRO1*. Vimentin is an intermediate filament responsible stabilizing cytoskeletal components that maintain cell shape, and is associated with MSCs. **Hyaluronic acid (HA)** is a primary extracellular component found in several tissues and strongly contributes to cell proliferation and migration.

Regions of interest with the strongest labeling are shown. **(A)** Expression of CD34 and CD44 was limited. **(B)** CD73 expression was variable, while CD45 was virtually undetectable. **(C)** CD90 was readily identified while less than 10% of cells expressed CD105. **(D)** STRO1 expression was strong in isolated pockets within T-Block culture while CD29 exhibited little to no expression. **(E)** Surprising, HA expression was remarkably strong, while vimentin expression was weak. **(F)** HWJCs grown on 2D Fibronectin coated glass coverslips exhibited poor expression of MSC markers. HWJCs expressed varying levels of MSCs markers over a period of 60 days while cultured in T-Blocks.

T-Blocks Enable MSCs to Interact with Injured Tissues

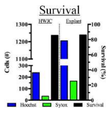
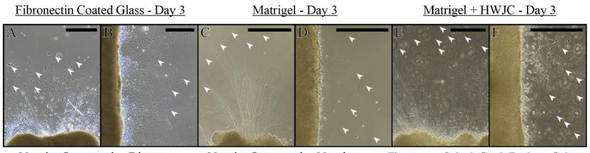


Figure 6. HWJCs were encapsulated at a density of 100,000 cells/mL of Matrigel and were extruded on glass coverslips to form T-Blocks that were 10 mm (L) x 5 (W) x 2 mm (D). Spinal cords were harvested from euthanized mouse embryos (Day 13.5) according to our approved IACUC Protocol (ACUP# 2018-2447). Spinal cords were cultured on top of one of three substrates: (1) Fibronectin Coated Glass, (2) Matrigel T-Block, (3) Matrigel T-Block infused with HWJCs. Samples were maintained for 3 days. HWJCs and neurons within the spinal cord explant were assessed for viability via Sytox staining.



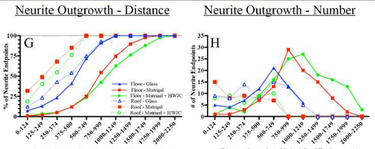


Figure 7. Spinal Cord Explant Culture - Culture Substrates. Neurite outgrowth of spinal cord explants cultured on Fibronectin-coated glass (A-B), Matrigel (C-D), and Matrigel infused with HWJC (E-F) was assessed at both the floor plates (A,C,E) and roof plates (B, D, F). Neurite outgrowth was greatest in both number and distance from the floor plates of explants on HWJC infused Matrigel. All explants analyzed on day 3. (A-F, white arrowheads = neurite endpoint; A-F, scale bars = 500 um)

Discussion

T-Blocks Enable Healthy Cell Growth

HWJCs successfully grow and thrive within PLGA and Matrigel T-Block formats. HWJCs cultured in PLGA T-Blocks exhibited exponential proliferation, and expressed varying levels of MSC markers. Interestingly, HA was expressed in 10X as many HWJCs cultured in T-Blocks than HWCs cultured in 2D. Matrigel T-Blocks enabled HWJCs to interact with an injured spinal cord explant.

Limitations

Only HWJCs have been evaluated in T-Blocks. Other stem cells and primary cells may behave differently in T-Blocks. Composite T-Block constructs, co-cultures, or differentiation protocols that rely on growth factors or genetic manipulation were not evaluated.

Future Directions

Future experiments will focus on evaluating how a variety of different cell types behave in different T-Blocks, and how cells respond when different T-Block constructs are combined.

Conclusion

T-Blocks enable HWJCs to grow efficiently in 3D, and provide a scaffold design that could be beneficial for tissue engineering applications.

Acknowledgments

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