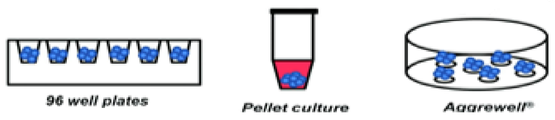
Mesenchymal Stem Cells Aggregation on Silicon-Titanium Diboride Micropatterned Platforms

Arnold Emeh, Jefferson Friguglietti, Dr. Fatima Merchant

UNIVERSITY of HOUSTON

Background

- Aggregation of stem cells *in vitro* mimics the 3D microenvironment cells experience *in* vivo, thus increasing biological function and therapeutic application.
- Current methods for obtaining cell aggregates, such as Hanging Drop Well plates and Centrifuge, are limited by cell viability or limited control over aggregate size. We present a microfabricated platform that introduces stiffness gradients and customizable geometric constraints for tissue culture.
- Human bone marrow mesenchymal stem cells' (hBM-MSC) are easily accessible with applications in a variety of tissue replacement therapies, hence, it makes it an excellent model for evaluating the microfabricated platform.



Objective

- We investigated a novel platform utilizing Titanium diboride (TiB_2) circular micro-patterns on Silicon (Si) wafers for tissue culture.
- Si and TiB_2 are biocompatible, non-toxic, amenable to microfabrication with unique beneficial substrate properties such as TiB_2 stiffness.

Methodology

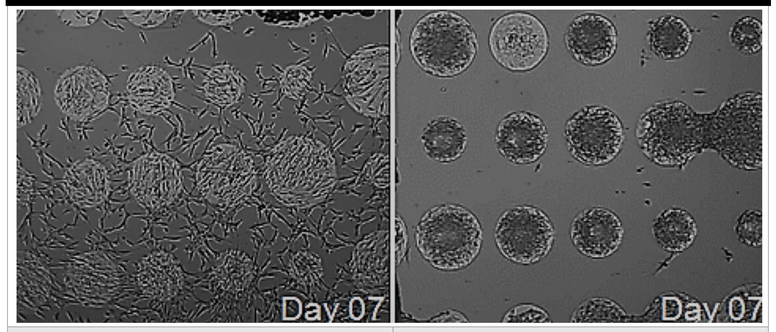
- We evaluated culturing hBM-MSCs on the TiB_2 micropatterned chips using traditional culture media (M199, 20% Fetal Bovine Serum (FBS), 1% antibiotics) only, versus media supplemented with growth factors (50 μg/ml Endothelial cell growth supplement (ECGS) and 1% Heparin).
- Dynamics of cell growth over a period of two weeks were evaluated using viability assays, stereomicroscopy and immunofluorescence-based biomarker analysis.
- X-ray photo spectroscopy (XPS) analyzed the efficacy with which components of the culture media deposited on the micropatterned chips.

Results

- Supplementation of media with ECGS and Heparin enables substrate specificity, limiting cell growth to the TiB_2 micropatterns, and induces aggregation through space constraints provided by geometric patterns.
- Biomarker expression of CD105 is conserved indicating phenotypic stability on chips.
- Analysis of aggregated hBM-MSCs dimensions suggest significant control aggregate formation and larger diameter circular patterns exhibit aggregates with higher viability than smaller patterns.

Results

Cell Growth on Si-TiB_2 platform



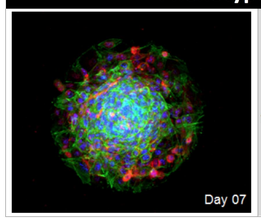
Non-specific growth in monolayers observed in culture media in the absence of growth of factors.

Pattern specific growth with cell aggregation into spheroids observed in media with ECGS and Heparin.

Amount of Exposed Substrate (%)	TiB_2	Si
ECGS	21.5	25.8
Heparin	100	88.8
ECGS & Heparin	21.9	74.4

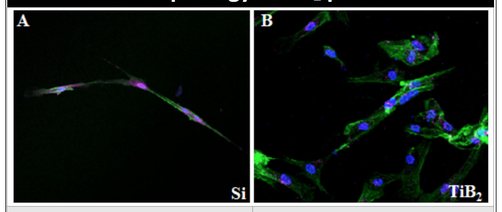
Table 1: X-ray photoelectron spectroscopy revealed that Heparin inhibits the deposition of ECGS on Si at 3 times the rate when compared to only ECGS.

Phenotype Analysis



Immunofluorescence of MSC biomarker CD105 shows that the cells maintain their phenotype when cultured on the Si-TiB_2 platform. Nuclei are stained blue), CD 105 is red and the actin cytoskeleton is stained green.

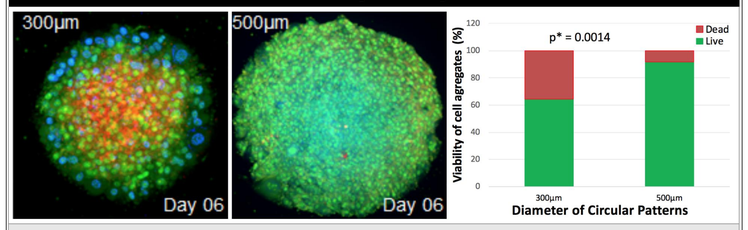
Cell Morphology Si-TiB_2 platform



Nuclear clumping (violet) of intermediate filament vimentin observed on Si surface indicating low adherence of cells. Cell nuclei are labelled blue.

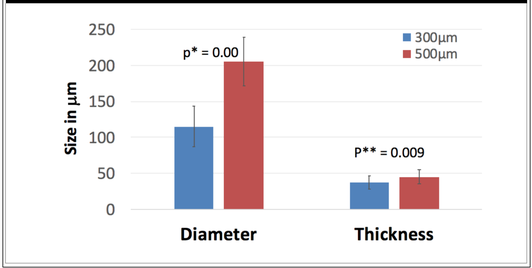
Adherence and motility of cells on TiB2 surface is improved as noted by spreading of both intermediate filament vimentin (violet) and actin cytoskeleton (green).

Viability Analysis



Viability analysis with viable cells stained with acridine orange (green) and propidium iodide (red) indicated that viability was improved in aggregates formed on larger diameter circular patterns.

Morphological Dimensions of Aggregates on Circle Patterns of varying diameter



Conclusions

- This study validates the use of Si-TiB_2 micropatterned platform for use in controlled aggregation of hBM-MSCs, when culture with ECGS and Heparin supplemented media.
- The proposed platform has potential for use in engineering applications relying on scaffold properties such as stiffness and 3D microenvironments for improved therapeutic applications.

Acknowledgements

- Team Members: Julien Washington, Tamsyn Wombwell, & Phi Le
- Advisors: Jefferson Friguglietti and Dr. Fatima Merchant
- UH Office of Undergraduate Research and Houston Methodist Collaborators

References

- T. Bartosh, J. Ylostalo, (2010) "Aggregation of human mesenchymal stromal cells (MSCs) into 3D spheroids enhances their antiinflammatory properties", Proceedings of the National Academy of Sciences
- Sart, S., Tsai, A.-C., Li, Y., & Ma, T. (2014). Three-Dimensional Aggregates of Mesenchymal Stem Cells: Cellular Mechanisms, Biological Properties, and Applications. *Tissue Engineering. Part B, Reviews*, *20*(5), 365–380.