Exploring the Mechanisms Regulating Toroid Formation by Adipose-Derived Stem Cells Cultured on 3D Collagen Hydrogels

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ABSTRACT

Adipose-derived mesenchymal stem cells (ADSCs) are of therapeutic interest due to their great abundance and ease of accessibility relative to other types of stem cells. We observed that ADSCs migrate and self-organize into a toroid when applied to the top of a 3D Type I collagen hydrogel. ADSCs fail to form toroids when embedded within collagen hydrogels. This raises the question of what specific environmental factors and cellular mechanisms regulate toroid formation. The binding of the ligand CXCL12 to the chemokine receptor CXCR4 initiates chemotactic signals in ADSCs. The goal of this study is to determine if the CXCL12:CXCR4 signaling axis is essential to the migration and self-organization required for ADSCs to achieve toroid formation. **We hypothesized that the binding of the ligand CXCL12 to the chemokine receptor CXCR4 is essential for toroid formation by the telomerase immortalized human ADSCs used in our culture model.** To test our hypothesis, we compared toroid formation in ADSCs cultured in two concentrations of the selective CXCR4 inhibitor AMD3100 to control conditions. We used phase-contrast microscopy to qualitatively monitor toroid formation and integrated software to measure the geometry of any structures formed 24 hours after plating. We found that there were significant differences in the distribution of the types of geometries formed. **Our results suggest a possible role for CXCL12:CXCR4 in toroid formation; however, additional testing is necessary.** Future directions include testing different numbers and sources of ADSCs and using selective inhibitors of downstream pathways known to be important for cellular migration and self-organization.

INTRODUCTION

We observed that telomerase immortalized human ADSCs form a toroid when placed on top of collagen hydrogel. Physical forces and biochemical cues cause the cells to migrate together and take on a toroid geometry (Fig. 1A). The binding of the ligand CXCL12 to the chemokine receptor CXCR4 initiates chemotactic signals in ADSCs. The selective inhibitor AMD3100 can be used to block the interaction between the CXCL12 and CXCR4. Thus, if toroids fail to form when AMD3100 is present, then we can conclude that CXCL12 and CXCR4 interaction is required for toroid formation (Fig. 1B). If the toroid still forms when AMD3100 is bound, then we can conclude that the interaction between CXCR4 and CXCL12 is not essential for toroid formation under the conditions tested, and we could instead consider investigating the role of other signaling pathways that may be involved in toroid formation.

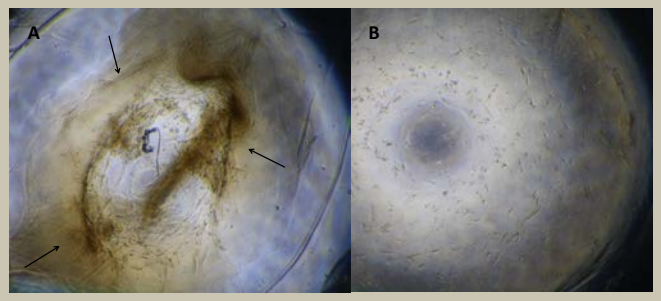

Figure 1: ADSCs placed on top of collagen hydrogels self-organize into toroids, while other ADSCs do not achieve cell organization. A) Human ADSCs plated on top of hydrogels. Arrows indicate the cell migration into the toroid formation. **B)** Human ADSCs plated on top of collagen hydrogels that did not achieve toroid formation.

METHODS

Collagen hydrogel formulation:

- 80% PureCol type I Collagen, 10% 10X MEM, 10% 1M HEPES, Titrate to neutral pH with 0.5 M NaOH

ADSC cell culture:

- Telomerase immortalized human ADSCs were employed in this study (Fig. 2)
- Cells were cultured until they reached 70% confluency prior to being transferred to the gels

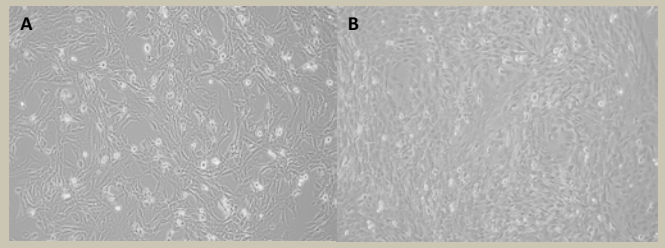

Figure 2: Telomerase immortalized human ADSCs at 70% or more confluency. A) 70% confluent ADSCs **B)** ADSCs over 70% confluent

CXCL4 selective inhibition via AMD3100:

- Control: no AMD3100
- Experimental group 1: 5 nM AMD3100
- Experimental group 2: 50 nM AMD3100

Toroid formation:

Hydrogels were incubated for 1 hour before plating 100,000 cells on top of the gel. After allowing the cells to sit down on the gel for 1 hour, the gel was released from the walls of the well using a sterile dental probe.

Phase-Contrast Microscopy:

- Phase-contrast microscopy allowed us to qualitatively monitor toroid formation (Fig. 3)
- Measurement software allowed us to obtain measurements of the geometry of the structures formed 24 hours after plating. We obtained measurements of the long and short axis of each structure formed.

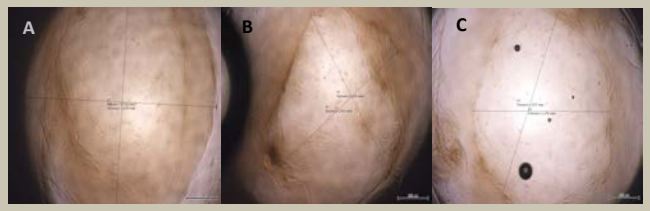

Figure 3: Comparison of the geometry of structures formed under control and experimental conditions. A) Well of control group with long axis of 3.158 mm and short axis of 2.638 mm. **B)** Well of 5nM experimental group with a long axis of vertical 2.563 mm and a short axis of 2.526mm. **C)** Well 50nM experimental group with a long axis of of 2.607 mm and a short axis of 2.290 mm.

RESULTS

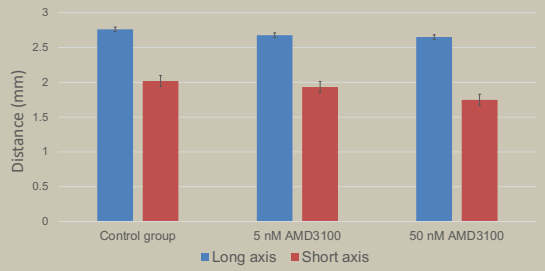

Figure 4: Comparison of the average vertical and horizontal axes of formed structures between control ADSCs and ADSCs exposed to AMD3100. t-test indicates significant differences between the long and short axes within each group ($p < 0.05$). ANOVA indicates no difference in the length of the long axis between experimental groups.

- The toroids formed under all experimental conditions were not symmetric and did not differ in size.

	Toroid	Partial toroid	No toroid or cell organization	Other
Control	17	3	0	0
5 nM AMD3100	12	7	1	0
50 nM AMD3100	8	7	3	2
Total	37	17	4	2

Table 1. Comparison of the types/categories of cellular organization observed for control ADSCs and ADSCs exposed to AMD3100. Chi-square test of independence showed that there is a statistically significant difference between the distribution of toroids, partial toroids, no toroid/organization, and other observations between the three groups ($p < 0.05$).

- ADSCs organized as toroids with and without added concentrations of selective inhibitor AMD3100; however, toroids were less compact than expected.

CONCLUSIONS

- There is a statistically significant difference between the distribution of observed toroids, partial toroids, no toroid/organization, and other observations between the three groups, which suggests a possible role of CXCL12-CXCR4 signaling in the process of toroid formation.
- Inhibition of CXCL12-CXCR4 did not affect the size of toroids that formed.
- Statistically significant differences in the lengths of the long and short axes of toroids indicate that their geometry was not symmetric.

FUTURE DIRECTIONS

- Testing different numbers and sources of ADSCs in our system to better replicate geometry of previously observed toroids
- Using selective inhibitors of downstream pathways known to be important for cellular migration and self-organization.

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