Biofabrication of 3D Adipose Triculture Models via Magnetic Levitation-Driven Assembly

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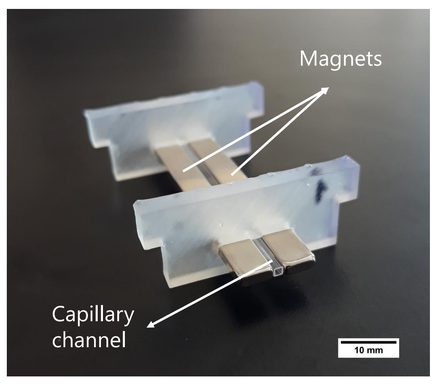
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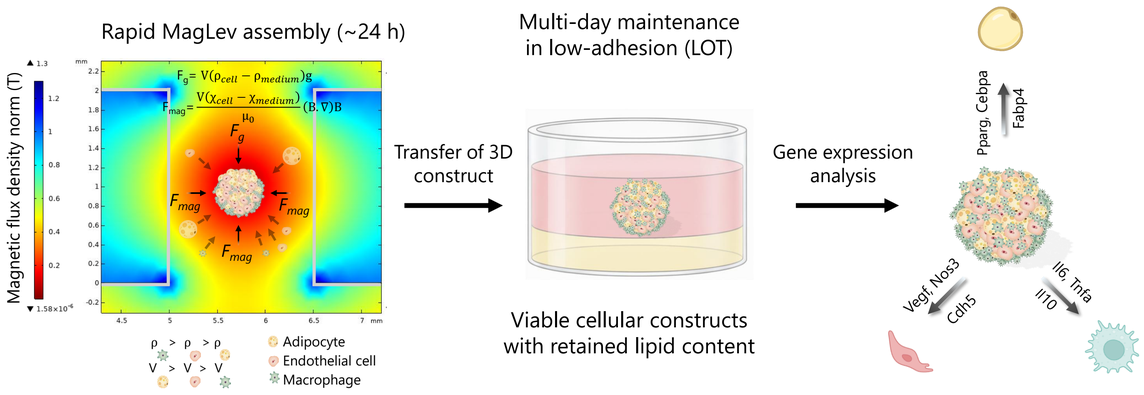
INTRODUCTION

Adipose tissue functions as a complex endocrine organ in which adipocytes, endothelial cells, and immune cells interact to regulate energy metabolism and inflammatory responses[1]. In recent decades, adipose tissue engineering has attracted considerable attention for both elucidating tissue physiology and pathophysiology and developing constructs to repair defects caused by high-grade burns or tumor removal[2]. However, recapitulating the multicellular heterogeneity of adipose tissue *in vitro* remains challenging, primarily due to adipocyte buoyancy and the limited heterotypic interactions in conventional culture systems[3]. To address these limitations, we developed a scaffold-free magnetic levitation (ML) platform capable of generating three-dimensional adipose tissue models incorporating adipocytes, endothelial cells, and macrophages. Furthermore, this approach was enhanced by integrating the liquid overlay technique (LOT), which promotes the formation of more compact and uniform structures and facilitates the investigation of tissue-specific cellular crosstalk.

METHODOLOGY



The magnetic levitation platform consists of two permanent magnets, and a microcapillary channel placed horizontally. Gadavist is used as a paramagnetic agent to levitate cells.



RESULTS

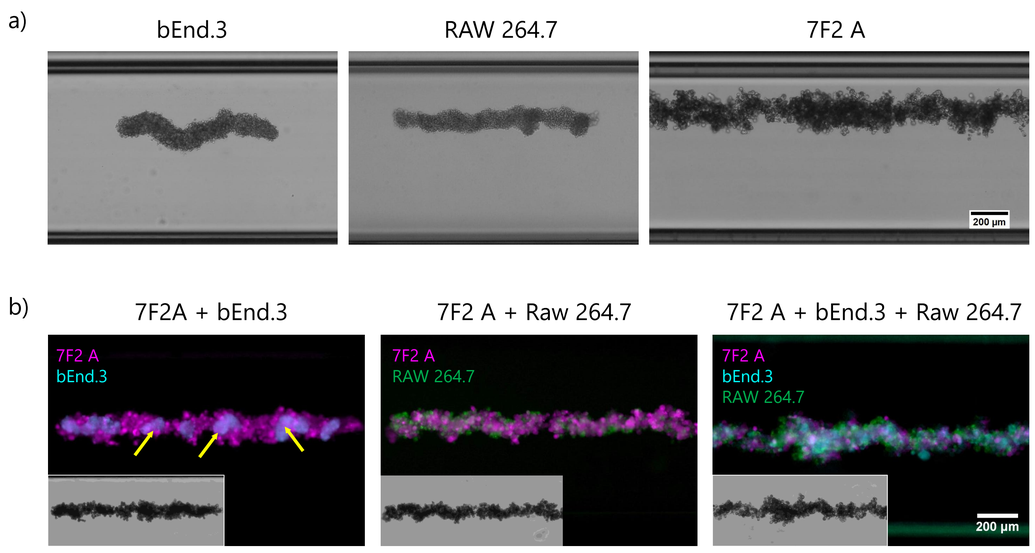


Figure 1. a) *Monoculture of bEnd.3 endothelial cells, and RAW 264.7 macrophages, and adipogenically differentiated 7F2 cells (7F2 A) maintained for 24 h in a magnetic levitation system.* ***b)*** *Co-cultures of adipogenic cells with bEnd.3 and/or RAW 264.7 cells, as well as triculture involving all three cell types.*

REFERENCES

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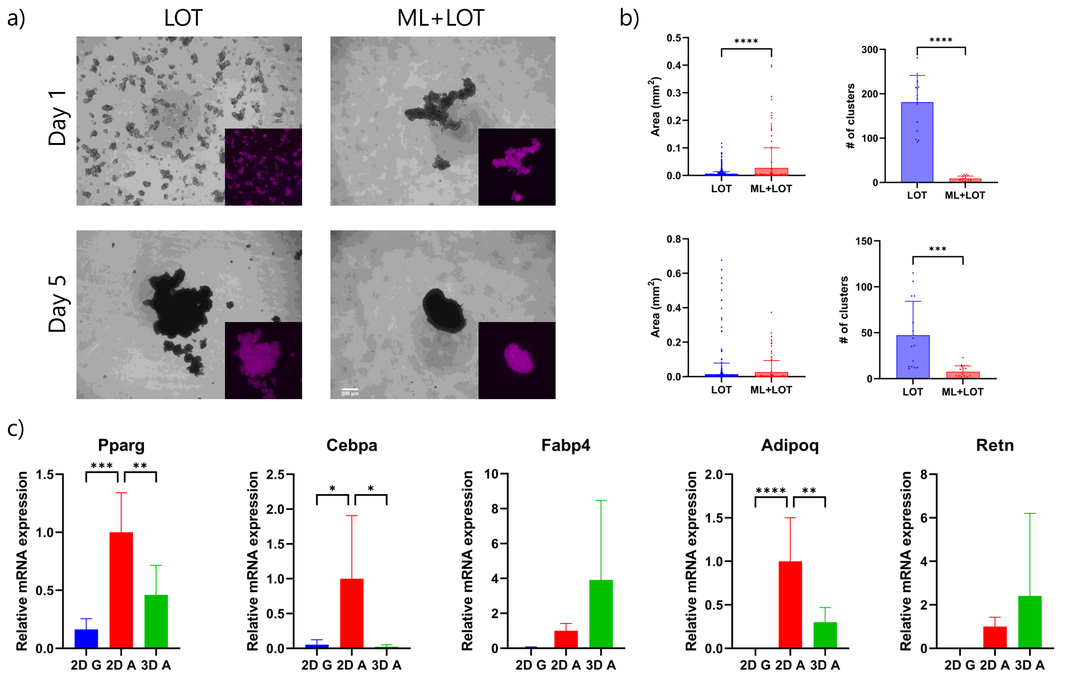


Figure 2. a) *Long-term culture of the adipogenic cells using the liquid overlay technique (LOT) or of pre-formed 3D structure of adipogenic cells created by the magnetic levitation system (ML+LOT) on agar gel.* ***b)*** *Area and number of 3D structures formed in culture wells at day 1 (upper) and 5 (lower).* ***c)*** *mRNA expression of Pparg, Cebpa, Fabp4, Adipoq (adiponectin), and Retn (resistin) in three conditions: 2D G (2D growth controls; undifferentiated), 2D A (2D monocultured adipogenic cells), and 3D A (3D monocultured adipogenic cells using ML+LOT).[4]*

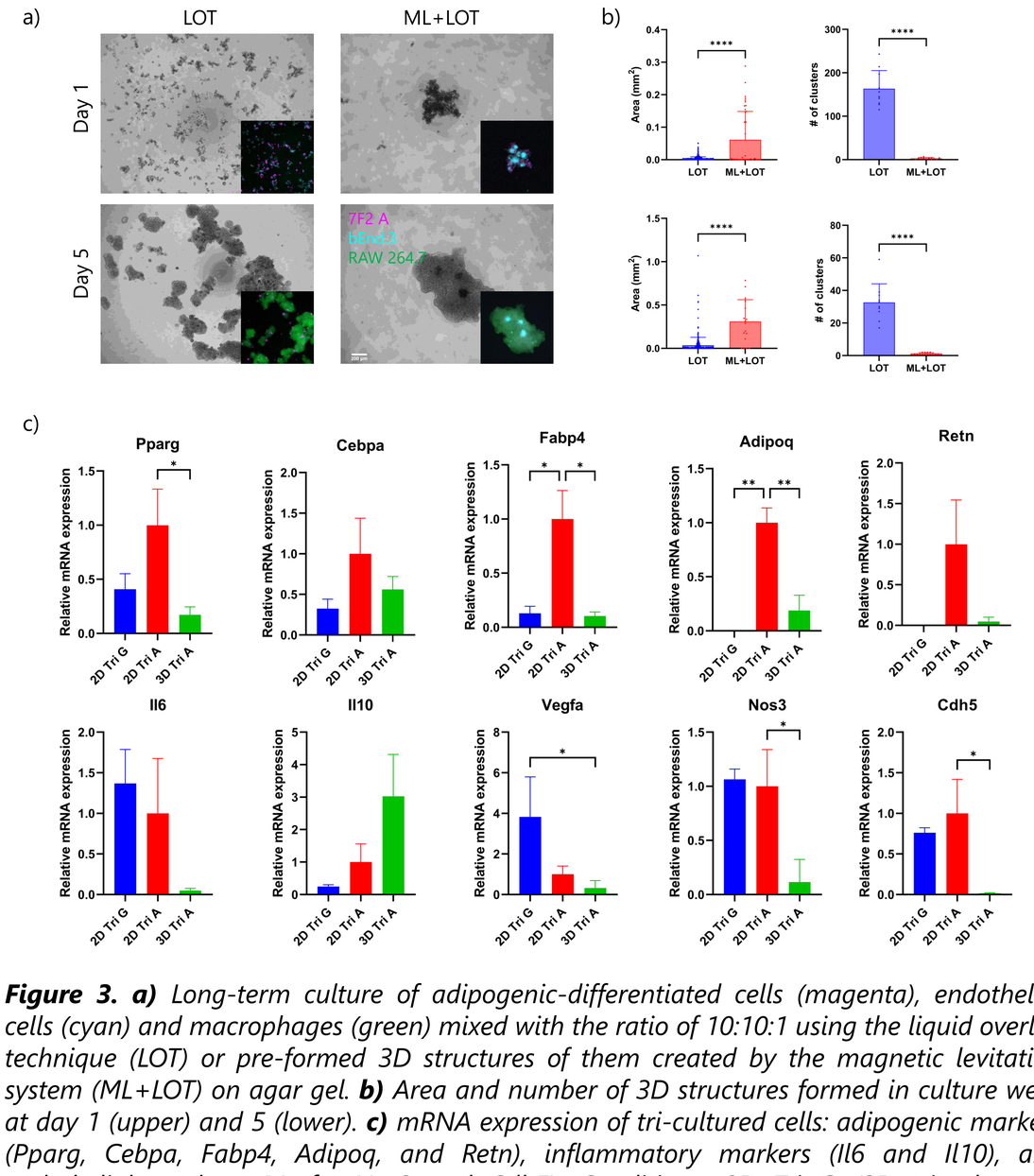


Figure 3. a) *Long-term culture of adipogenic-differentiated cells (magenta), endothelial cells (cyan) and macrophages (green) mixed with the ratio of 10:10:1 using the liquid overlay technique (LOT) or pre-formed 3D structures of them created by the magnetic levitation system (ML+LOT) on agar gel.* ***b)*** *Area and number of 3D structures formed in culture wells at day 1 (upper) and 5 (lower).* ***c)*** *mRNA expression of tri-cultured cells: adipogenic markers (Pparg, Cebpa, Fabp4, Adipoq, and Retn), inflammatory markers (Il6 and Il10), and endothelial markers (Vegfa, Nos3 and Cdh5). Conditions: 2D Tri G (2D tri-culture of undifferentiated cells, endothelial cells and macrophages), 2D-Tri A (2D tri-culture of adipocytes, endothelial cells and macrophages), and 3D-Tri A (3D tri-culture of adipocytes, endothelial cells and macrophages using ML+LOT).[4]*

CONCLUSION

This study aimed to develop uniform and stable 3D adipogenic constructs capable of recapitulating the complexity of adipose tissue using a magnetic levitation–assisted culture system. Neither the ML nor the LOT alone was sufficient to generate compact, well-organized 3D adipogenic structures. However, their combination (ML+LOT) produced highly uniform, spheroid-like aggregates, revealing a clear synergistic effect. ML facilitated rapid initial cell aggregation, while the subsequent overlay culture enhanced structural integrity and promoted maturation. mRNA analyses further confirmed the functional characteristics of the resulting constructs and highlighted the influence of co-existing cell types on their molecular phenotype. Overall, this approach establishes a robust and straightforward biotechnological platform with strong potential for integration into future adipocyte tissue engineering applications.