3d bioprinting solutions
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The algorithm for reproducible biofabrication of tissue spheroids with optimal size and viability from different cell types

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Introduction

Tissue spheroids are gaining extensively their place in biofabrication as building blocks [1]. In order to print human tissue it is absolutely necessary to elaborate a technology for scalable standardized production of millions tissue spheroids. Tissue spheroids can be prepared from different types of cells, primary and immortalized ones, from benign cells, cancer cell lines, primary tumor cells and cancer tissues depending on further application of produced structures. Tissue spheroids imitate the architectural and functional characteristics of native tissue. Until now the generally accepted standardized protocol for quantitative characterization of tissue spheroids biofabricated from different cell types is absent. In our study we proposed an algorithm for assessment of tissue spheroids and tested it on four cell populations of different origin – HEK293, primary human fibroblasts, primary sheep chondrocytes, and primary sheep osteoblasts. The biofabricated tissue spheroids differ in diameter, circularity, viability, and surface characteristics depending on the cell types as well as initial cell seeding density. Employed algorithm is necessary and sufficient for initial steps of tissue spheroids characterization.

Results

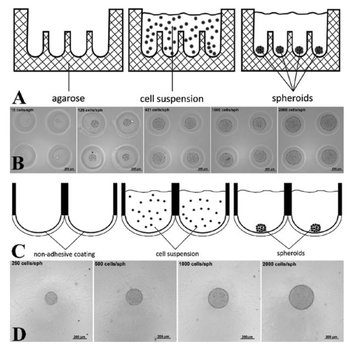

Figure 1. The scheme of tissue spheroids biofabrication using agarose microwells (A); HEK293 spheroids fabricated using agarose microwells (B); The scheme of tissue spheroids biofabrication using spheroid microplates (C); HEK293 spheroids fabricated using spheroid microplates (D).

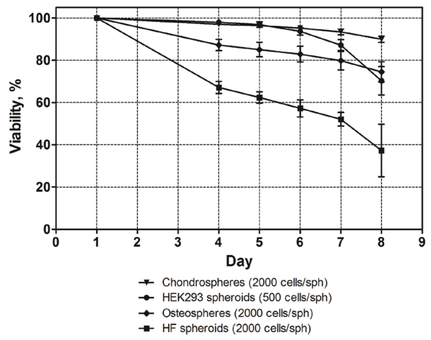

Figure 2. Viability of tissue spheroids fabricated from HEK293 cells, HF cells, chondrocytes and osteoblasts using spheroid microplates. Data represent the mean ± standard deviation (n = 8).

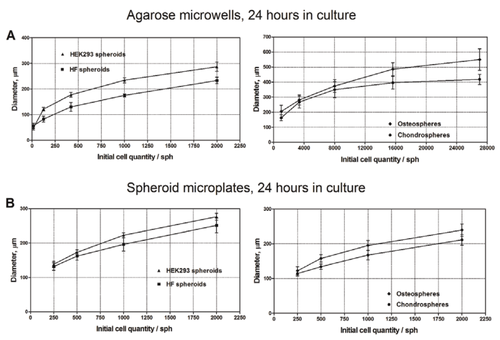

Figure 3. The ratio between initial cell seeding density and resulted diameter of tissue spheroids fabricated from HEK293 cells, HF cells, chondrocytes and osteoblasts using agarose microwells (A) and spheroid microplates (B). Data represent the mean ± standard deviation (n = 80). 24 hours in culture.

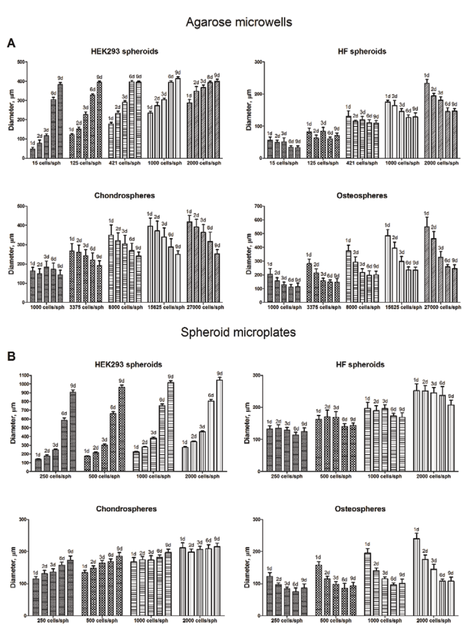

Figure 4. The kinetics of spheroid diameter change. A) Tissue spheroids fabricated using agarose microwells; B) Tissue spheroids fabricated using spheroid microplates. Data represent the mean ± standard deviation (n = 80).

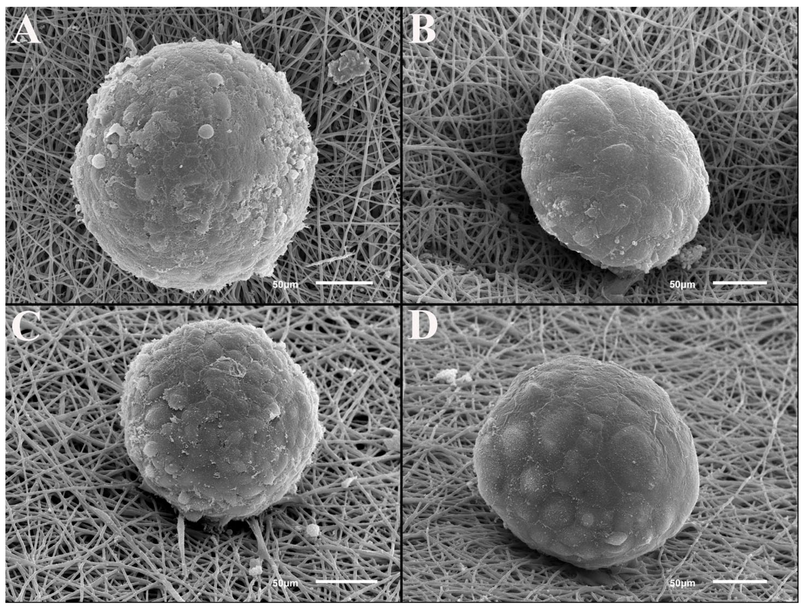

Figure 6 Tissue spheroids on the surface of electrospun polyurethane matrices. Scanning electron microscopy. HEK293 spheroid (A); HF spheroid (B); Chondrosphere (C); Osteosphere (D).

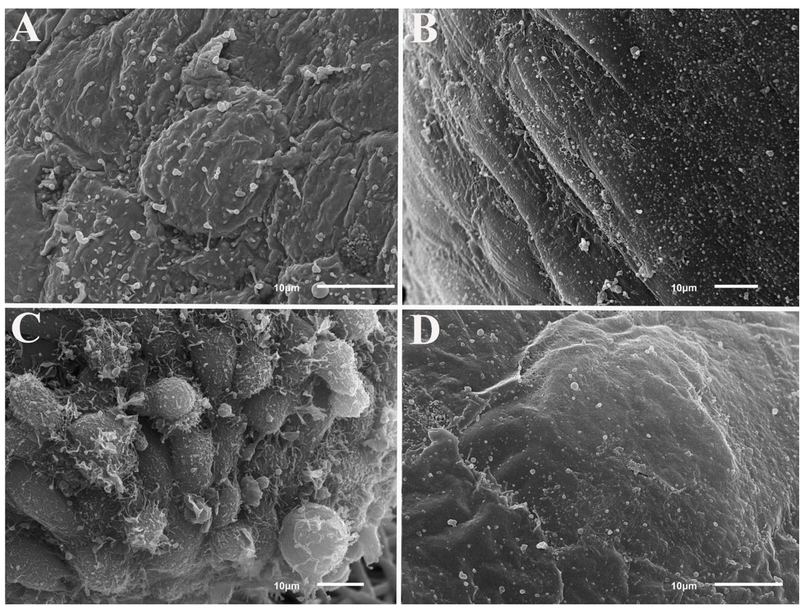

Figure 7. The surface of tissue spheroids. Scanning electron microscopy. HEK293 spheroid (A); HF spheroid (B); Chondrosphere (C); Osteosphere (D).

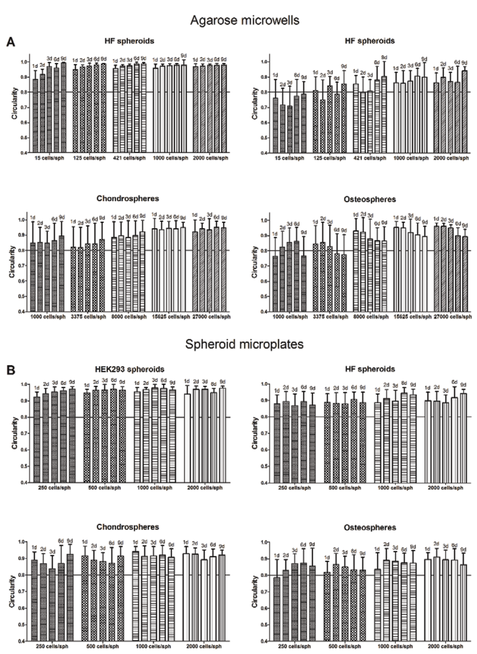

Figure 5. The kinetics of spheroid circularity change. Tissue spheroids fabricated using agarose microwells (A); Tissue spheroids fabricated using spheroid microplates (B). Data represent the mean ± standard deviation (n = 80).

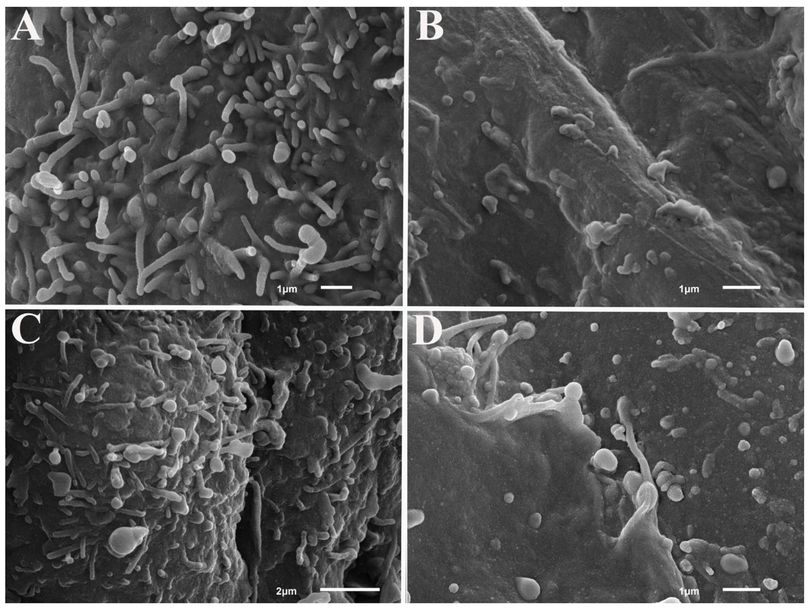

Figure 8. The surface of tissue spheroids at higher magnification. Scanning electron microscopy. HEK293 spheroid (A); HF spheroid (B); Chondrosphere (C); Osteosphere (D).

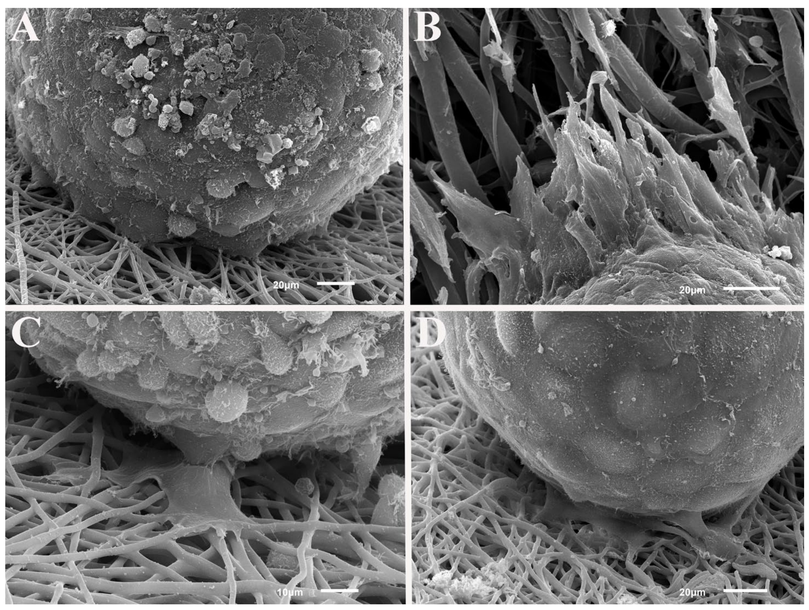

Figure 9. The interaction between cells migrating from spheroids and electrospun polyurethane matrices. Scanning electron microscopy. HEK293 spheroid (A); HF spheroid (B); Chondrosphere (C); Osteosphere (D).

Conclusion

In our work we proposed an algorithm for assessment of tissue spheroids and tested it on four cell lines of different origin – HEK293, primary human fibroblasts, primary sheep chondrocytes, and primary sheep osteoblasts. The biofabricated tissue spheroids differ in diameter, circularity, viability, and surface characteristics depending on the cell types as well as initial cell seeding density. In our study we report an algorithm to apply for any cell line one starts to work with to prepare a new type of tissue spheroids with predictable controllable optimal features. Proposed algorithm includes following necessary criteria: (a) estimation of correlation of tissue spheroids size with initial cell seeding density; (b) estimation of diameter and circularity as a functions of time; (c) estimation of viability as a function of time, (d) estimation of diameter and viability as a function of cell type. For scalable and standardized robotic spheroids production we suggest non-adhesive technology applying coated microplates.

References

[1] Mironov V, Visconti RP, Kasyanov V, Forgacs G, Drake CJ, Markwald RR. Organ printing: tissue spheroids as building blocks. // Biomaterials. 2009. Vol. 30. P. 2164-2174.
[2] Mehesz A.N., Brown J., Hajdu Z., Beaver W., da Silva J.V., Visconti R.P., Markwald R.R., Mironov V. Scalable robotic biofabrication of tissue spheroids. // Biofabrication. 2011. Vol. 3. 025002.

Acknowledgments

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