CELLBRICKS THERAPEUTICS

Standardized mass-generation of pre-vascularized organoids to promote angiogenesis in 3D bioprints

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INTRODUCTION



The development of **clinically relevant engineered tissues** remains a promise still beyond reach in regenerative medicine. Despite substantial progress in **3D bioprinting**, generating large, functional tissue constructs continues to be limited by **insufficient vascularization**, essential for the long-term tissue survival and integration[1].

Among emerging strategies, **vascularized organoids** have shown promise as building blocks to **support tissue vascularization**; however, the **lack of scalable and reproducible generation methods** limits their applications in tissue engineering[2]. Advancing such approaches is therefore critical for the development of functional, transplantable tissues.

APPROACH

In the present work, we explored **dynamic suspension culture** methods for the **scalable generation** of pre-vascularized organoids.
We further developed a **quantitative approach** for the systematic evaluation of culture performance.

Finally, we demonstrated the potential of pre-vascularized organoids to **enhance angiogenesis** in 3D bioprinted adipose tissue constructs.

To achieve this, the following objectives were defined:

- Establish a reproducible, donor-independent protocol for mass-generation of pre-vascularized organoids
- Develop a standardized analytical workflow for quantitative assessment across donors
- Integrate pre-vascularized organoids within 3D bioprinted adipose tissue constructs to enhance angiogenesis

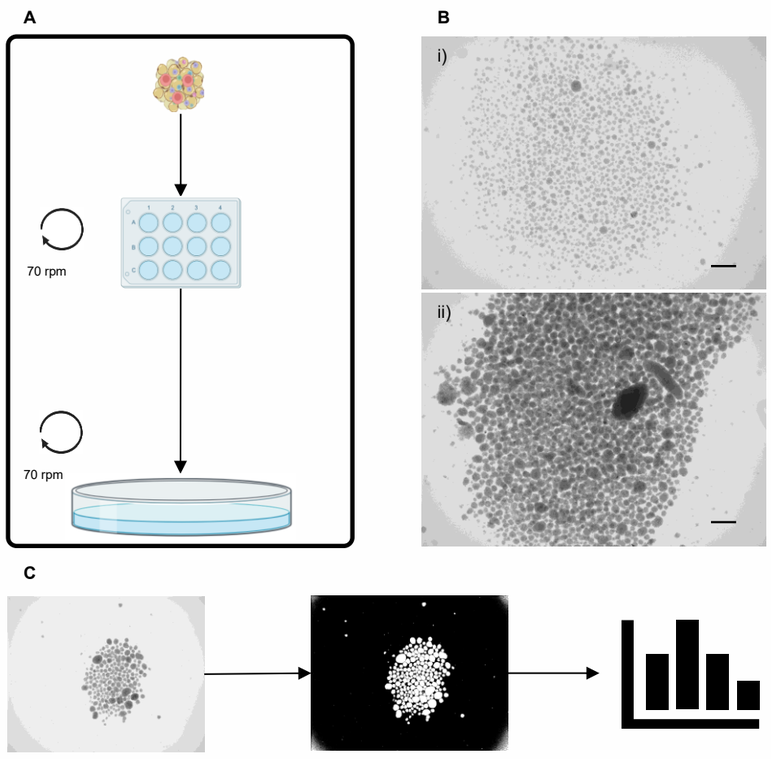
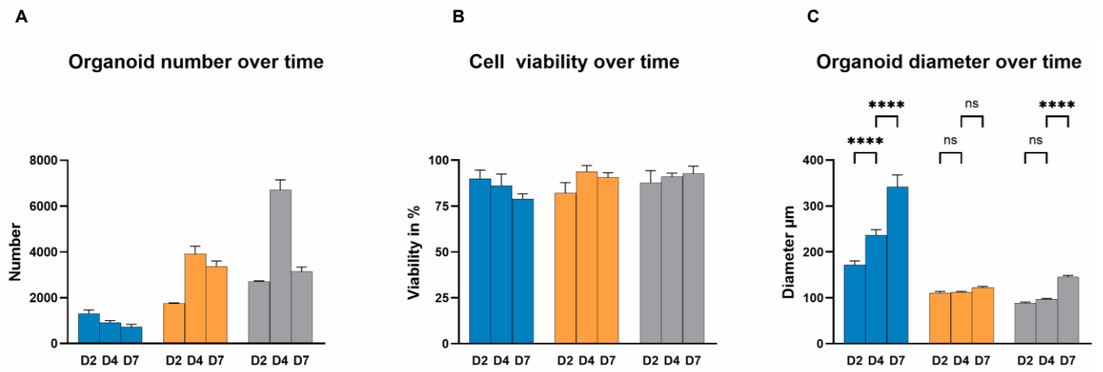


Fig. 1 Dynamic suspension culture for scalable organoid production. A. Schematic overview of the experimental workflow **B.** Human adipose-derived stem cells (hADSCs) seeded in **i)** 12-well plates and cultured on an orbital shaker overnight. Resulting organoids were transferred to **ii)** 15 cm culture dishes and maintained under orbital shaking conditions (scale bar: 500 µm) **C.** Schematic overview of the image-based analysis workflow. Diluted organoid samples were imaged and subsequently quantified for organoid number and diameter using ImageJ.

RESULTS

Reproducible organoid production across different donors



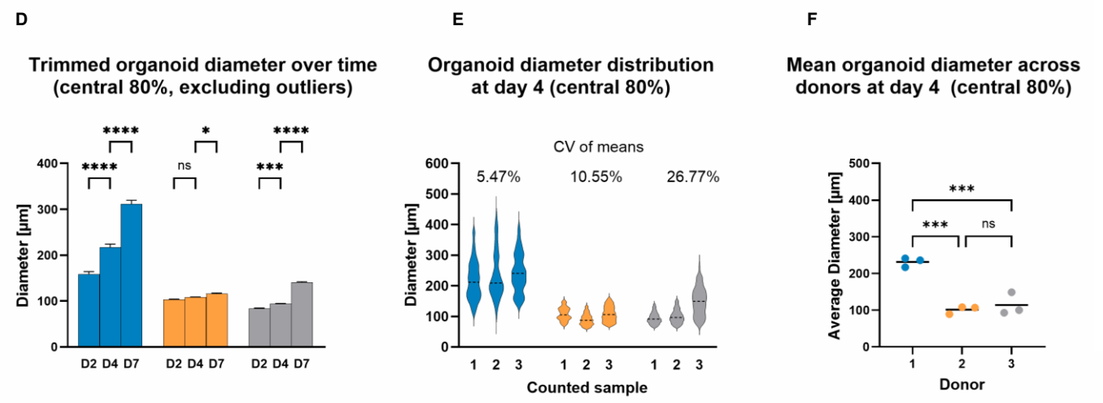
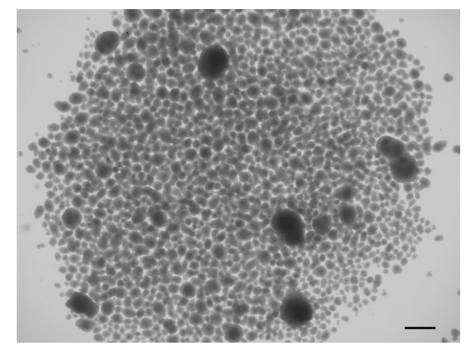


Fig. 2 A. Organoid number over 7 days. **B.** Cell viability of organoids over 7 days. **C.** Organoid diameter over 7 days. **D.** Trimmed organoid diameter (central 80%, excluding outliers) in the sample over 7 days. Data represent N = 3 samples per donor at each time point; error bars indicate ± SD. Statistical Analysis was performed using One-way ANOVA, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. **E.** Violin plots showing the diameter distribution of the central 80% of organoids in the sample on D4. Mean diameter indicated with striped line. Coefficient of variation (CV) of mean diameters indicated above plots. **F.** Average diameter of the central 80% of organoids on D4. Each dot represents one sample. Statistical analysis was performed using one-way ANOVA, *p < 0.05 , **p < 0.01, ***p < 0.001, ****p < 0.0001. Data in (A-F) are shown for three independent donors (Donor 1 in blue, Donor 2 in orange, Donor 3 in grey).

Pre-vascularized organoids



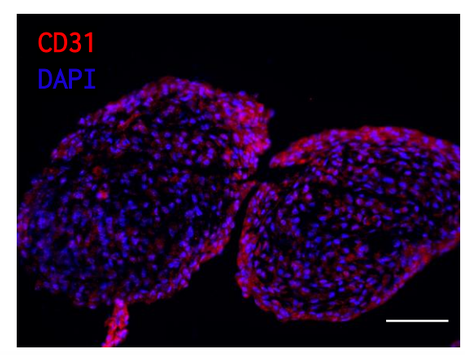


Fig. 3 A. Brightfield image of organoids in suspension culture (scale bar: 500 µm) **B.** Immunofluorescence staining of organoids at day 4 of dynamic suspension culture. CD31 in red and DAPI in blue. From left to right: Donor 1, Donor 2, Donor 3 (scale bar: 100 µm).

Pre-vascularized organoids enhance angiogenesis-related genes in 3D bioprints

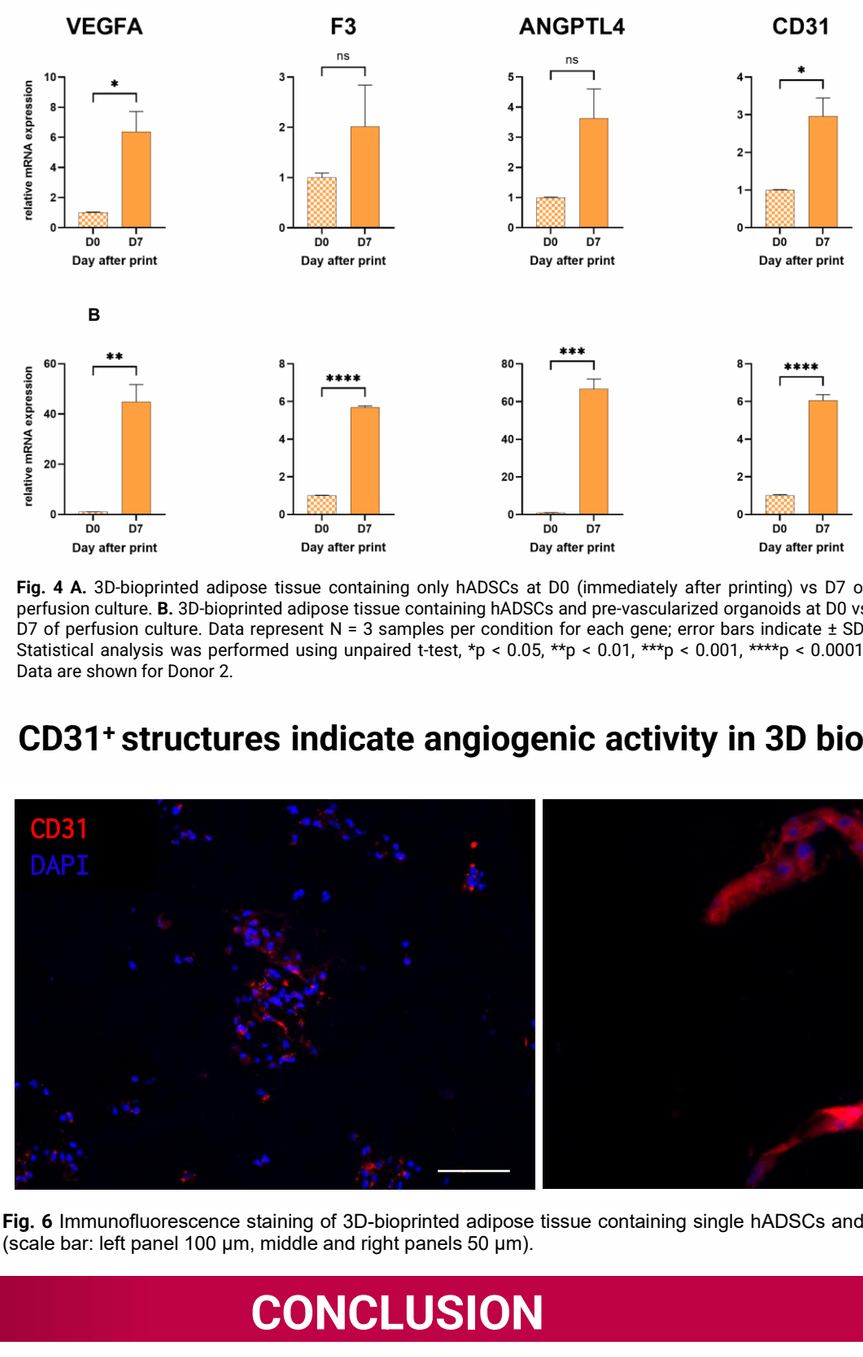


Fig. 4 A. 3D-bioprinted adipose tissue containing only hADSCs at D0 (immediately after printing) vs D7 of perfusion culture. **B.** 3D-bioprinted adipose tissue containing hADSCs and pre-vascularized organoids at D0 vs D7 of perfusion culture. Data represent N = 3 samples per condition for each gene; error bars indicate ± SD. Statistical analysis was performed using unpaired t-test, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Data are shown for Donor 2.

Formation of vessel-like structures in 3D bioprinted constructs

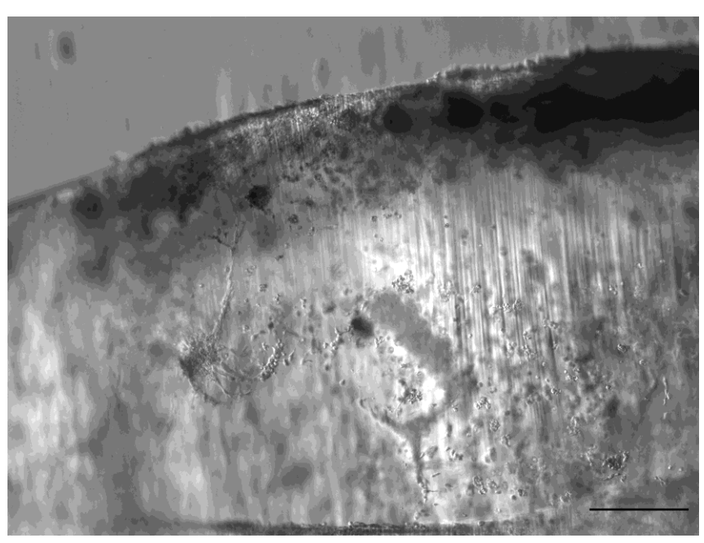


Fig. 5 Brightfield picture showing vessel-like structure in 3D-bioprinted adipose tissue containing single human adipose-derived stem cells (hADSCs) and pre-vascularized organoids at D7 of perfusion culture (scale bar: 500 µm).

CD31⁺ structures indicate angiogenic activity in 3D bioprints

Fig. 6 Immunofluorescence staining of 3D-bioprinted adipose tissue containing single hADSCs and pre-vascularized organoids at D7 of perfusion culture. CD31 in red and DAPI in blue (scale bar: left panel 100 µm, middle and right panels 50 µm).

CONCLUSION

We successfully established a **dynamic suspension culture protocol** for the scalable generation of **pre-vascularized organoids** and their integration into 3D bioprinted adipose tissue constructs. Our biofabrication strategy, combining organoid production, quantitative analysis of culture performance, and light-based bioprinting, enabled the creation of **large-scale constructs exhibiting angiogenesis-related structural and molecular responses**. This proof-of-concept demonstrates the potential of pre-vascularized organoids as functional building blocks for vascularized tissue engineering and provides a foundation for further optimization and translation toward clinically relevant biofabricated tissues.

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