Portable Perfusion System With Integrated Incubation and Interstitial Flow Parameters

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Introduction

Perfusion systems and bioreactors have the potential to support **clinical availability of engineered tissue constructs (ETCs)** by facilitating tissue development and maintenance in a mobile biomimetic microenvironment.

Current perfusion system limitations:

- Microfluidic systems offer precise control of the microenvironment but are limited by their size.
- Scaled-up versions are typically stationary requiring pumps and incubation.
- Portable organ perfusion systems aren't designed for ETCs.

We developed a portable perfusion system that offers:

- Integrated incubation.
- Flow rates and velocities at the microfluidic scale.
- Flow regime flexibility with exchangeable bioreactors.

Methods

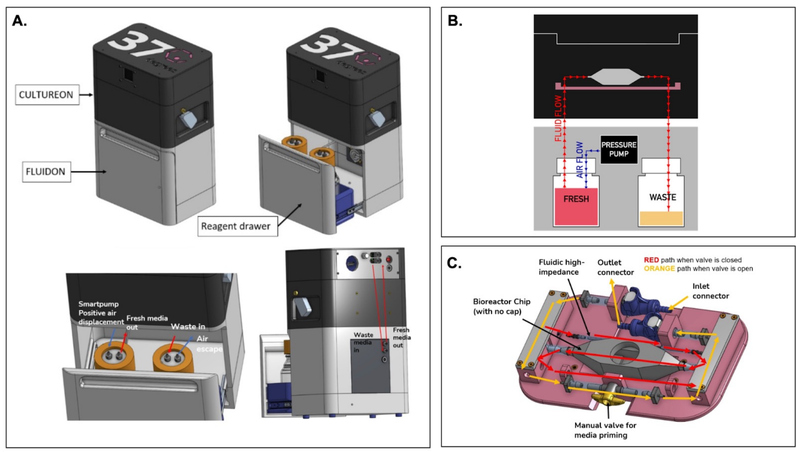
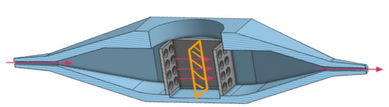


Fig 1. A) Portable system with integrated incubator (CultureOn) and perfusion (FluidOn) modules developed with 37degrees Inc. **B)** Pressure driven flow perfuses media through the bioreactor in the CultureON**. C)** The bioreactor tray contains a high impedance element to reduce the flow rate. A bypass valve can be opened to prime the system with media.

Simplified Navier-Stokes Equation

$$\mu\left(\frac{\partial^2 u}{\partial y^2}+\frac{\partial^2 u}{\partial z^2}\right)=\frac{dp}{dx}$$



The mass flow rate was measured, and computational fluid dynamics (CFD) was used to visualize the flow velocity profile. The **Navier-Stokes equation was solved to obtain the 2D flow velocity and shear stress profiles of the uniform flow regime** by fitting the solution to the CFD data and plotting them in python.

To evaluate **perfusion-mediated molecular transport between bulk flow and ETCs**, mMSC/mEC spheroid-embedded fibrin gels (fibrin patches) were cultured under perfused and static conditions for 3 days. Fibrin patch degradation and inter-spheroid spacing were compared with and without Aprotinin-a serine protease inhibitor (n=3).

To assess the **CultureOn's ability to preserve tissue viability during transport**, fibrin patches were placed either in the CultureOn or a standard plastic container for a 20-minute walk simulating sample transfer between facilities. Samples were Live/Dead stained and imaged via confocal microscopy.

Results

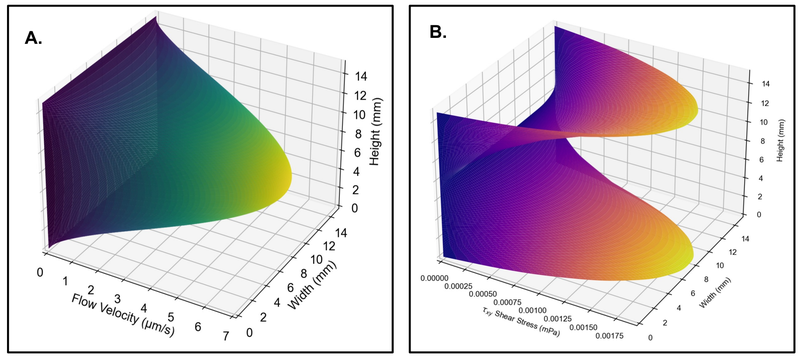


Fig 2. A) Bioreactor flow velocity profile at a had flow velocities within the range of interstitial flow (1.69-4.6µm/s vs 0.1-2.0µm/s in vivo[1]). **B)** However, the maximum shear stress was 10^{-6}-10^{-7} Pa lower than interstitial shear stresses in vivo[2,3]. The profiles shown were simulated for a drive pressure of 2psi.

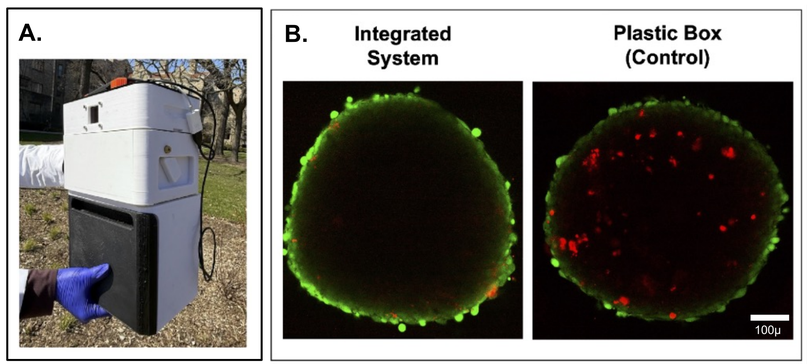


Fig 3. Live/Dead confocal imaging of mMSC/mEC spheroid-embedded fibrin patches after 20-minutes of transportation in a plastic box (control) or integrated system, showing **improved cell viability** in the CultureOn-transported sample.

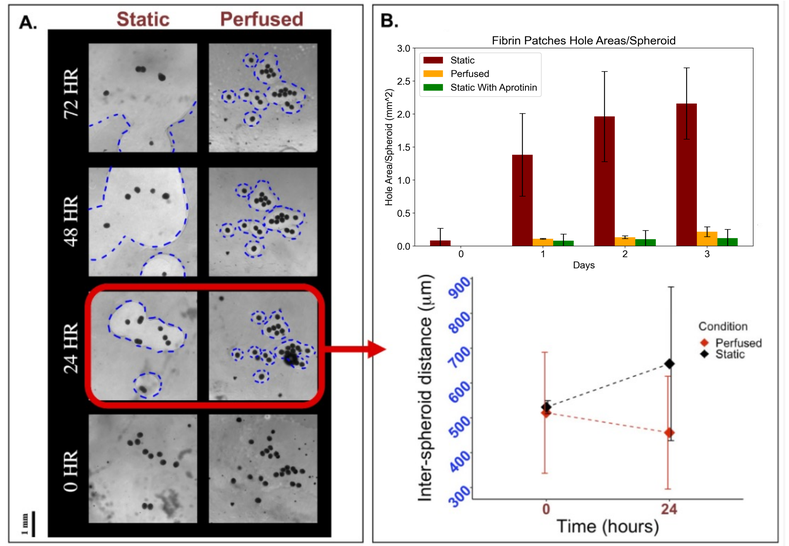


Fig 4. A) The bulk fluid flow over the fibrin patches flushed serine proteases out of the sample, effectively reducing the rate of fibrin gel degradation by 90% **(B)**. As a result, the spheroids stayed clustered together **(C)**.

Conclusions

Our integrated **CultureOn–FluidOn** perfusion system maintains tissue viability in a portable perfusable incubator. The bioreactor achieved **microfluidic-scale flow velocities** that facilitate molecular transport between fluids reducing fibrin degradation and improving spheroid clustering. Although little shear stress was induced, the bioreactor can be exchanged with one with a narrower channel.

Overall, this platform represents a promising step towards **portable perfused culture systems** that can support tissue maintenance during transport between facilities and clinics with customizable fluid-tissue interfaces.

Acknowledgements

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(3) Salinas EY, Aryaei A, Paschos N, Berson E, Kwon H, Hu JC, Athanasiou KA. Shear stress induced by fluid flow produces improvements in tissue-engineered cartilage. Biofabrication. 2020 Aug 10;12(4):045010. doi: 10.1088/1758-5090/aba412. PMID: 32640430; PMCID: PMC8020626.