A Fully Controlled Atmosphere Reduces Biofabrication Contamination Risks

Alicia D. Henn, Abigail L. Chapman, Shannon Darou, Randy Yerden. BioSpherix, Ltd. Parish, NY

Abstract

Microbial contamination can bring biofabrication production processes to a halt, costing valuable time and samples. The single largest source of bioburden in a biomanufacturing environment is personnel. The next largest sources are air and surface contamination. Using bioprinters in a biological safety cabinet or laminar flow hood (LFH) can reduce contamination risks during bioprinting, however there is still risky room air present in an LFH, and the rest of the production process is prone to contamination as well. The Xvivo System can separate the entire process from the risks of personnel and room air with a soft, flexible glove-front. Filtered, tanked, medical-grade gases provide a completely fully-controlled and aseptic environment. We decided to study the reduction in contamination risk for bioprinting processes that are associated with separating room air from the bioprinting environment. With the null hypothesis that the setting would make no difference, we printed test samples either inside or outside of the Xvivo System using the INKREDIBLE 3D Bioprinter by CELLINK. We also performed environmental monitoring of the chamber and bioprinter using touchplates and an air sampler. We incubated the constructs in a highly permissive color-changing TSB broth. We found that enclosure with the Xvivo System effectively reduced contamination of the constructs, the environment, and the equipment. Rejecting the hypothesis, we concluded that separation of the process from room air with the Xvivo System does reduce contamination risks for biomanufacturing processes.

Background

- **An aseptic environment is needed to for safe, efficient cell and tissue production [1]**
- **The Xvivo System provides a physical barrier between the tissue production space and room air and maintains ISO 5 conditions inside [1]**
- **Laminar flow hoods or BSC are often not effective at protecting the cell and personnel environments from each other [2]**

Objectives

Comparing bioprinting processes in the Xvivo System and an LFH, assess contamination of:

- **bioprinted constructs**
- **the bioprinting environment**

References

1. Wiley, L.A., et al., cGMP production of patient-specific iPSCs and photoreceptor precursor cells to treat retinal degenerative blindness. Sci Rep, 2016. 6: p. 30742.
2. Hinrichs, T., S. Gragert, and M. Klein, Biological Safety Cabinets Simulation and Quantifying of Airflow Perturbation Caused by Personnel Activities. Applied Biosafety, 2016: p. 1535676016635369.

Experimental Design

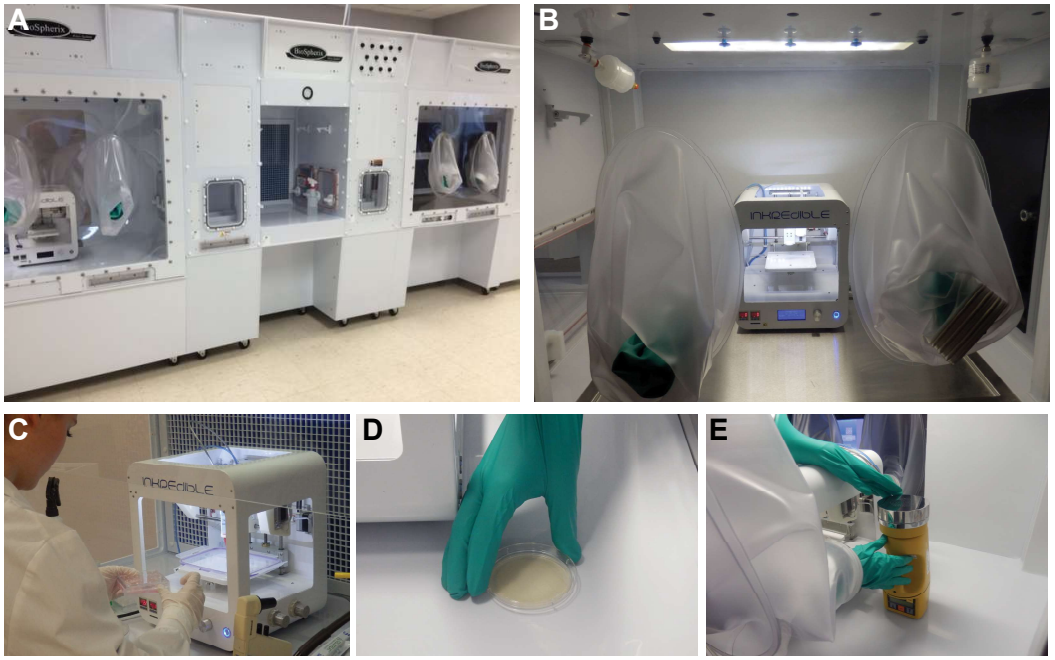

Figure 1. Experimental Set-Up. We compared different bioprinting environments: (A, B) The Xvivo System, which can surround, enclose, and protect any cell or tissue production process with filtered, warmed gases from tanks and (C) a HEPA-filtered room air laminar flow hood (LFH) (C). Before use, all surfaces were disinfected by wiping with gauze soaked with SporKlenz (Steris). The bioprinter was used in three separate trials in each environment to print small grids of CELLINK bioink in standard 6-well plates. The constructs were incubated with highly-sensitive color-changing TSB broth (BioMerieux). Touchplates (D) were applied to three places in each chamber after use (left, right, and center), each finger of each glove, the bioprinter stage, and the chamber doorknobs (Xvivo only). An air sampler (E) was used to draw air across an additional touchplate for monitoring the atmosphere during bioprinting. Constructs and touchplates were incubated for a minimum of 5 days before reading.

Results

Table 1

%Pos Wells	Room Air	Hood	Xvivo
Trial 1	57%	0	0
Trial 2		0	0
Trial 3		3%	0

Table 1. The LFH and Xvivo Both Prevented Construct Contamination. Printing on the open bench in room air, 57% of constructs showed signs of contamination (color change from clear pink to turbid yellow medium) (17/30 tests). When the bioprinter was in the LFH, 3% (1/30) of constructs were contaminated in one trial. Enclosed in the Xvivo, no constructs showed any contamination (n=5 plates/trial x 6 tests/plate x 3 trials/condition = 90 test/condition).

Table 2

CFU	LFH			Xvivo		
Trial	1	2	3	1	2	3
Chamber Floor L	< 1	< 1	< 1	< 1	< 1	< 1
Chamber Floor C	6	< 1	9	< 1	< 1	1
Chamber Floor R	< 1	1	< 1	< 1	< 1	< 1
Gloves	2	7	5	< 1	< 1	< 1
Bioprinter Stage	1	< 1	1	< 1	1	< 1
Air	< 1	< 1	< 1	< 1	< 1	< 1
Doors	N/A	N/A	N/A	< 1	< 1	< 1
Neg Control	< 1	< 1	< 1	< 1	< 1	< 1
Pos Cntrl (%confl)	60	90	90	80	60	60

Table 2. The Xvivo System Dramatically Reducied Contamination of the BioPrinting Environment. We found colonies growing on contact plates touched to the floor of the LFH between the operator and the bioprinter as well as on the operator's gloves. We found a dramatic reduction of environmental contamination in the Xvivo System chamber.

Conclusions

- **The LFH improved construct contamination rates over room air**
- **The full-time protection of the Xvivo System reduced contamination of both constructs and the production environment even further**