WEBINAR: Introducing NGB-R, a Disruptive 4D Bioprinting System – Application Review

Questions and answers from the March 11, 2021 webinar titled "Introducing NGB-R, a Disruptive 4D Bioprinting System – Application Review"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address. Questions have been grouped into relevant categories.

Publication References

During the webinar a few publications were references, below is the link to access the PDF:

- Laser-Assisted Bioprinted Skin Equivalent for Cosmetic Efficacy Evaluation
  - https://www.axt.com.au/wp-content/uploads/Poietis-BASF-bioprinted-skin-model.pdf
- First Time Use of 3D Micro-Patterning Screening and 3D Skin Printing to Select Firming Activities
  - This was published in IFSCC 2017 – Topic Skin Biology: New and Alternative
    - Please reach out and we will be happy to send you the PDF.

NGB-R Basics & Hardware

1. Can you please explain the concept of 4D printing again?
   a. 4D bioprinting consists of programming a tissue's self-organization by designing tissue constituent organizations – i.e., cells and extra-cellular matrix, that evolve in a controlled way until specific biological functions emerge.
   With laser bioprinting, cells can be spatially distributed in 3D with uttermost precision and precisely organized within the tissue/ECM. Those micro-patterns help guide self-organization of cells and promote biological processes such as cell proliferation, migration, and differentiation. In 4D bioprinting, we investigate the impact of such micro-patterns and fine-tune them to achieve more functional bioprinted tissues that better replicate and mimic organic ones.

2. Can you print micro-tissues for high throughput screening applications into multi-well plates, i.e., 96, 384, etc.?
   a. At the moment, NGB-R is compatible with single, 6 and 12-well plates. Technically speaking, it is possible to print cells into 96 well plates, but the laser-head/receiver bioprinting distance will be greater due to the smaller size of wells, so precision will decrease. To avoid this loss of precision, we recommend relying on removable-grid well

plates – so printing onto a single well plate, then adding the well-separating grid right after printing for screening/high-throughput purposes.

3. Does the presence of the robotic arm, and its movements throughout the study cause an increase in temperature within the BSL-2 cabinet?
    a. No heat is generated by the movement of the robotic arm during the fabrication process; this is something the team at Poietis had carefully tested and a key factor to choose a robotic-arm supplier over another.

4. Is there any heat generated, which may damage the cells, when the laser is used for printing?
    a. No. The energy and heat induced by the laser beam is absorbed by the metalized substrate on which the cell media is placed. That is why we use golden metalized substrates, due to gold's high absorbency properties. Cell integrity is fully preserved. Live/dead viability tests show an average 93-95% cell viability when printed with Poietis' laser technology.

5. Is there any way to perform polymerization during the fabrication steps? For example, using UV light?
    a. Yes, the NGB-R platform can be equipped with an optional dual-wavelength UV lamp for polymerization/crosslinking purposes. As an on-board module, crosslinking can easily be included in the bioprinting sequence, with the robotic arm being able to take the freshly-bioprinted sample straight to crosslinking without having to take the construct out of the cabinet. However, one of the limitations of UV crosslinking is the potential negative impact on cells.
    There are different methods to cross-link collagen depending on the type being used: various chemical compounds/enzymes, UV, temperature…
    We usually rely on inkjet bioprinting for layers of collagen alone, although extrusion can be used if preferred – it depends a lot on the experimental design and/or the type of collagen used. But we have also printed cellularized collagen with the laser head. The way of proceeding depends a lot on the project: with Telocollagen used for skin, for instance, we usually print a single layer of cells over a single layer of collagen, then place the construct in the incubator for a few minutes of gelation before repeating the process. With simple cell-free constructs however, we print several layers of collagen before taking the construct to the incubator.
    For chemical crosslinking, we generally use the inkjet or laser modules. By printing micro droplets directly over the collagen, or just outside. In the latter case, inkjet is generally preferred, because more volume is required and there is no need for the laser's utmost precision.

Cell Media and Bioink Related Questions

6. Who makes the "bio-ink cell ribbon"? Assuming a user makes the ribbon, how difficult is it to make the ribbon? What is the compatibility of bio-ink to different types of cells?
    a. Just like bio-extrusion requires a pressure system (pneumatic, piston or screw) and specific nozzles to function, laser bioprinting requires a substrate, also called ribbon or donor, on which the bioink is spread. This substrate must be metalized with a very thin golden layer. Poietis offers a monthly service package for ready-to-use, disposable metalized substrates, automatically delivered every month. Alternatively, the user can source and purchase substrates themselves, but a metalizer (Plasma sputtering coater) will be required, and they will not benefit from Poietis' cheaper rates achieved through economies of scale, buying larger quantities.
    Laser bioprinting is compatible with pretty much any cell types. What we typically call "bioink" is merely living cells in their own culture media.

7. When working with spheroids or organoids, how large can the cell clusters be?
    a. Spheroids' size depends on the cell type and the experimental protocol used. Size typically increases according to the number of cells printed locally but also to the degree of compaction of these cells. The quantity of cells printed through laser can be adjusted by playing with the cell concentration in the media, with the laser-beam energy, or by repeating locally several prints. The degree of compaction can be modulated by the matrix environment and by the culture medium used. The advantage of the laser is the precise control of the number of cells at a local scale, thus obtaining a homogeneous size of spheroids.
    As an illustration, we have obtained spheroids between 50 and 300um depending on the experimental parameters for our various projects.

8. How do users get the cells to use for printing?
    a. There are no bioprinting-special cells. Cells can be primary human, cell lines, embryonic or pluripotent stem cells. Laser bioprinting is even compatible with plant cells. So, cells can be sourced the same way labs always do.

9. Is there any limitation to the number or types of tissues which can be combined in any printing experiment?
    a. I think this question refers to cell types, not tissue types. With NGB-R, one can perfectly work with several different cell types. Cell types can be printed together, or separately on different substrates and/or through different bioprinting heads. Up to 5/6 cell types can be printed simultaneously, mixing laser bioprinting and nozzle-based bioprinting heads (bio-extrusion and micro-valve)

10. Can you elaborate on the effects of laser assisted bioprinting on cell viability and stress on the cells?

a. Please see answer to question 4, above. In addition, laser bioprinting is a nozzle-free technology, so cells do not experience any shear stress whatsoever, unlike with conventional nozzle-based bioprinting. Cell integrity is preserved, and viability is optimal (93-95%).

Application Specific Questions

11. Are there any publications in which pluripotent stem cells are used with the NGB-R system?
    a. Not yet. We have successfully worked on IPSc at Poietis through European projects which we are involved in but have not yet published results.

12. Has the NGB-R system been used to print tissues or create disease models to screen drugs or target compounds?
    a. Absolutely. Some of this work was presented in the first section of the webinar. For example, we have produced bioprinted full-skin models for anti-aging peptide complex evaluation and in-vitro testing before clinical trial (BASF) as well as liver models for hepatotoxicity screening of possible drug-induced liver lesions (Servier). The platform can also be used for immuno-oncology studies, to produce disease or miniaturized tumor models and many other similar applications.

13. Could you discuss the amenability of the technology to generate implantable bioprinted constructs?
    a. In parallel to NGB-R, Poietis are working on NGB-C, a clinical-grade platform. We have also entered a partnership with the AP-HM (Hospitals of Marseilles, France) and pre-clinical trial phase for implantable skin patches.