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

Questions and answers from the February 25, 2021 webinar titled "Introducing NGB-R, a Disruptive 4D Bioprinting System"

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.

Cell Media and Bioink Related Questions

1. What cell concentration do you have to work with when using laser bioprinting?
    a. That is precisely one of the many advantages of laser bioprinting: you can work with either low or very high cell densities – typically from 1 to 100 million cells per ml. As a nozzle-free technology, there is no risk of clogging when increasing the cell concentration in the bioink. What will happen is more cells will be ejected in every single droplet. The optimal cell concentration will depend entirely on the specific application and approach being used, and the end goal of the biofabrication (pattern, tissue morphogenesis, biological functions…). As an example, the bioink concentration range we most often use on our R&D projects is between 10 and 60 million cells per ml.

2. 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 on top of. This substrate must be metalized with a very thin golden layer. Poietis offers a great monthly service package for ready-to-use, disposable metalized substrates, automatically delivered to the customer's every month. Alternatively, the user can source and purchase substrates themselves, but a metalizer (Plasma sputtering coater) will be required, and they won't 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.

3. How do you usually optimize the culture media?
    a. As far as laser bioprinting is concerned, there is no need to optimize the culture media whatsoever. All there is to do, is deposit the cell-loaded media over the laser-head donor and gently spread across.

4. Can you transfer a single cell?
    a. Yes, it is possible to reach single-cell bioprinting or get very close to single cell by adjusting 2 parameters: the cell concentration on the laser-head donor and the laser-beam energy level. There might not be a single cell in every droplet tough: some will indeed carry just one cell, others with carry 2 to 5, some will be empty. Indeed, it all depends on where cells are located on the donor before getting ejected. Generally speaking, we experienced a 70% success rate with single-cell bioprinting (meaning, 70% of the ejected droplets carried a single cell).

5. Can the system identify and select the specific cell to transfer?
    a. Not at the moment, however we are working on an imaging-based system allowing to pre-visualize the laser-head donor before bioprinting and selecting the exact spots to be targeted by the laser beam. We are also thinking about the automation of such a system with automatic selection criteria according to size and sphericity.

6. In case of "potential" single-cell printing, how does the system recognize the location of individual cells in the "ribbon"?
    a. It does not – it all depends on where cells are located on the donor before getting ejected. Generally speaking, we experienced a 70% success rate with single-cell bioprinting (meaning, 70% of the ejected droplets carried a single cell).
    However we are working on an imaging-based system allowing to pre-visualize the laser-head donor before bioprinting and selecting the exact spots to be targeted by the laser beam.

7. How do you print and cross-link multiple layers of collagen; I guess through extrusion via an available extrusion channel?
    a. 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.

    NGB-R can also be equipped with an optional dual-wavelength UV lamp for 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.

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.

8. What is the typical source of the cells you use for bioprinting? Is it primary human cells, or induced pluripotent stem cell (iPSC) derived?
    a. Both cell types can be used and laser-bioprinted. We have worked with primary human cells, cell lines as well as iPSC and ESCs with no issue.

9. Can you comment on the ribbons used in laser induced flow transfer (LIFT) bioprinting? Are these proprietary disposable ribbons? What's the workflow for users to prepare the ribbons with custom bio-inks?
    a. There is an option for a monthly service contract for ready-to-use, disposable metalized substrates, automatically delivered to the customer's every month. Ribbons are not proprietary, and can be sourced elsewhere. However, investing in a metalizer (Plasma sputtering coater) would be required and the cost of substrates would be higher.
    In terms of workflow, it is extremely straight-forward: 1. place a ribbon on top of the laser-bioprinting head, 2. deposit your bioink (cells in their culture media) and gently spread it across the ribbon, 3. you're ready to go!

10. How long can you print with your laser setup until cells start to die because of evaporation; does the source layer have to be renewed after some time?
    a. Evaporation will largely depend on bioink formulation and bioprinting speed. Regarding laser bioprinting speed, the process is extremely fast: up to 10,000 droplets per second. It usually is a matter of a few micro-seconds for an entire patterned-layer of cells to be printed. Regarding bioink formulation, it can easily be optimized by the user based on the requirement. So there is no actual issue with evaporation or cell viability.

Fabrication/Printing Related Questions

11. Is there any limit in the size of the tissue being printed?
    a. The only limit lies in the type of receiver used. NGB-R is compatible with 6 and 12 well plates, so bioprinted tissues are typically 2-3cm^2. It is also possible to print into single

well plates, with tissues as large as 40cm^2, however the process is slightly more complex (2 or 3 substrates will likely be required, depending on the cell micro-pattern).

12. How much time do you need to print a pattern of cells?
    a. Less than a second (the laser technology can print up to 10,000 droplets per second).

13. Turning larger objects upside down during the printing will likely cause damage to the object. Same applies to low viscosity materials as they will flow when being moved around like that. Is it possible to avoid the inversion?
    a. This is, indeed, something to consider when relying on laser bioprinting to incorporate cells onto chips, devices and 3D objects/scaffolds. We have always found ways to ensure the object sticks to the receiver and therefore does not fall when flipped upside-down. For the vast majority of hydrogels and biomaterials, the inversion has never been a problem.

14. How fast do the droplets, which are being printed with the laser head, travel through the air?
    a. Less than a µ-second (close to sound speed).

15. I noticed cells being printed at a distance of 100-500µm. Can the cells be printed even closer?
    a. Yes, it all depends on the pattern designed with the CAD software, beforehand. Patterns are totally custom-made and adjusted as pleased. Cell droplets can be printed very close or very far from each other, depending on preferences/requirements. Should one want to distribute cell droplets very close from one another, it will be necessary to reduce the laser-beam energy level to obtain smaller droplets and thus avoiding a fusion of drops.

Hardware Component Questions

16. Is there an integrated incubator inside the system?
    a. No, there is not. It would add up to the overall instrument cost, dimensions and footprint and there is no actual need for a built-in incubator.

17. Why is the microscope built-in rather than using an external microscope?
    a. As an on-board device, the microscope is integrated right into the NGB-R workflow and bioprinting sequences, making it extremely convenient, easy and time-efficient to capture a mosaic image right after a layer is printer. With external devices, the user has to take the receiver out of the bioprinter, move to the microscope room, capture an image then head back to the bioprinter and start calibration once again. Very time-consuming and a potentially sample-damaging move! A built-in microscope also enables

target-bioprinting: pre-visualizing the receiver and selecting the spots on which to print cells (very useful when incorporating cells into chips, custom devices and 3D objects).

18. Does the microscope have fluorescent and/or confocal capabilities?
    a. Not at the moment, but it could be in the future and/or if an actual customer makes it a requirement for a purchase.

19. Are the additional extrusion and ink-jet heads thermoregulated?
    a. Yes, thermo-regulated extrusion and inkjet printing heads are available, as an option. Temperature ranges from 4 to 60°C. Up to 3 nozzle-based printing heads (i.e. inkjet & extrusion) can be installed. These can be standard or thermo-regulated. Users are free to choose the number and type of head(s) they want. The standard tri-modal NGB-R pack includes 1x standard inkjet head and 1x standard extrusion head.

20. Do you always print at ambient temperature? Is there any temperature control of the air inside the BSL-2 cabinet?
    a. Yes, we print at ambient temperature, although nozzle-based printing heads can be thermo-regulated if required.

21. What is the resolution of the inkjet print heat? Approximately how many cells per droplet can be achieved?
    a. Up to 100nL. Number of cell per droplet depends on the cell concentration in the bioink.

22. Can the arm be used to move the sample to a new hardware component placed within the workspace of the cabinet?
    a. Yes – NGB-R was designed to offer fully operator-independent processes for high reproducibility and automation. So the robotic arm is calibrated to carry out every single action in the cabinet, from taking the receiver from a printing head to another, capturing an image with the wide-field camera or a mosaic with the microscope, taking the sample to the crosslinking lamp.
    However, a "new" module (different and other than those sold by Poietis) cannot be added to the robotic arm calibration/sequence.

Application Specific Questions

23. Have you ever made a full thickness skin model using neurons, adipocytes, and immune cells?
    a. Indeed, we have worked on models of complexified skin tissue models as part of partnerships and collaborative programs. For confidentiality reasons though, we are not allowed to communicate on these projects at this stage.

24. What is your experience with keeping the fabricated tissues alive for many weeks?
    a. It all depends on the tissue type, its size and the medium used. As far as our skin models are concerned, we are able to maintain them "alive" for a little more than two weeks. What will often be limiting is the thickness of the sample, due to the supply of oxygen and essential nutrients.

25. Can cell migration be seen as a 4D bioprinting feature; change of morphogenesis?
    a. Cell migration per se is not a feature of bio-imprinting. It is indeed a natural phenomenon. However, by using the precision of laser printing, we can accurately organize cells in space both at the local level and at the scale of a global pattern. And from there it is indeed possible to modulate cell migration and thus to orientate/modulate morphogenesis.

26. How do you start to figure out the pattern in which your cells will thrive the best? Is it a trial-and-error process?
    a. Indeed, we initially proceeded through trial and error. However, the experience we have acquired over the years, working on and printing a wide range of cell types, allows us to be very efficient today. This is also part of an instrument sale – our team of biologists can provide customers with support and feedback on how to make the most out of NGB-R – optimizing cell patterns included.

27. Can this technology be used to print a network of micro vessels which will depend entirely on a different type of patterning?
    a. Various strategies to vascularize tissue models using bioprinting are quite well-detailed in the literature. Most of these approaches are based on the use of a unique printing technology. In our case, we can combine these approaches by exploiting the multi-modality of NGB-R (3 bioprinting technologies in 1). Both the option for an integrated microscope and the ViewPrint feature (target-bioprinting allowing to visualize the sample at various stages and print on selected areas) is of great interest. As far as experimental designs are concerned, Poietis' user-friendly CAD software also offers many advantages. Please feel free to request a software demo to find out more!

28. Could this technology be scaled up to print organs?
    a. That indeed is a long-term objective of ours and many other players in the bioprinting industry. However, there are several challenges to overcome before this becomes a reality, such as vascularization, maturation, bioprinting timing…