Webinar:
Next Generation Bioprinting Platform: A Multimodal Approach for Bioprinting of Cartilaginous Multicellular Spheroids and Skin

Questions and answers from the October 2, 2024, webinar titled: "Next Generation Bioprinting Platform: A Multimodal Approach for Bioprinting of Cartilaginous Multicellular Spheroids and Skin

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address.

1. **Could you please explain more in detail the process with air liquid interface?**

   Certainly. First, we focus on the proliferation and organization of keratinocytes, which form the outermost layer of the skin. In this model, the dermis is immersed in culture media, while the epidermis is exposed to air. This arrangement promotes the progressive differentiation of the epidermis, ultimately resulting in a stratified and functional structure. The use of a liquid culture condition, where only the dermis is submerged and the epidermis is air-exposed, is a common method for cultivating epithelial tissues, especially the epidermis.

2. **Why do you wait a few days between printing human dermal fibroblasts (dermis) and the keratinocytes (epidermis)?**

   We found that waiting a couple of days for the dermis to mature improves the anchoring of the epidermis. If you compare this to a construct where the dermis hasn't been allowed to mature, the results are noticeably different. This is likely because the remodeling of the dermis occurs in the absence of keratinocytes, allowing for greater fibroblast proliferation. From an experimental standpoint, this waiting period has consistently shown better results.

3. **Have you encountered issues with basal keratinocytes not having proper polarity? Have you tried integrating a basement membrane to help mitigate this issue?**

   Yes, we have encountered issues with this. One solution is allowing the dermis to mature properly. There are also specific culture media that promote dermal maturation, which ultimately ensures proper polarity at the dermo-epidermal junction. This helps maintain the correct polarity of the keratinocytes. However, we have not yet tried integrating another component, such as a basement membrane.

4. **Have you considered or tried using organoids rather than spheroids?**

   Yes, indeed, is what we are starting to do in another project funded by the European Commission that deal with brain to our goal is to print mini brains based on organoids. So, it's something that we are just starting now. I would say the principle is the same based on the pixel module.

5. **Are you considering using machine learning/ AI for improving the printing process?**

   Yeah, indeed, we already do that with the pixel model. platform is a robotic platform. That already integrated number of sensors on with a microscope, and so on. All our goal is to use all these sensors to generate data, to be able to use or to leverage AI capabilities afterwards to improve the resolution, or to make the result more predictable, I would say.

6. What approaches would you take to print vascularized, vascularized tissues?

We are also working on printing mini-brains. Currently, we print endothelial cells in combination with mesenchymal stem cells or pericytes. By co-patterning or co-printing both cell types, we aim to create capillary vasculature, which is the main approach we have been developing in the field of vascular bioprinting.

7. What are the most biomaterials used with this technique?

As I mentioned, the technology is quite flexible in terms of viscosity. Typically, cells are suspended in their own culture media, so there's no need to mix them with specific hydrogels or biomaterials. However, for certain applications, we do combine cells with biomaterials. The two main materials we use internally are usually collagen or alginate.

8. Regarding cardiac pathology, have you explored the idea of using cardiac patches?

Yes, we have partnered with a hospital in Paris for this. The project is not focused on printing cell aggregates; in fact, it's quite the opposite. We're more interested in printing exosomes. This approach is expanding our capabilities by allowing us to print cell suspensions, biomaterials, aggregates, and exosomes. It's a relatively new project for us, but it shows great promise for future applications.