Microfluidic extrusion of cell-laden hydrogel fibers for 3D-Bioprinting

ISTITUTO ITALIANO DI TECNOLOGIA

Cristina Colosi, Laura Belloni, Giancarlo Ruocco

Center for Life and Nano Science (CLNS) - Istituto Italiano di Tecnologia - iit@Sapienza - Viale Regina Elena 191 - 00161, Roma (RM), Italy

cristina.colosi@iit.it

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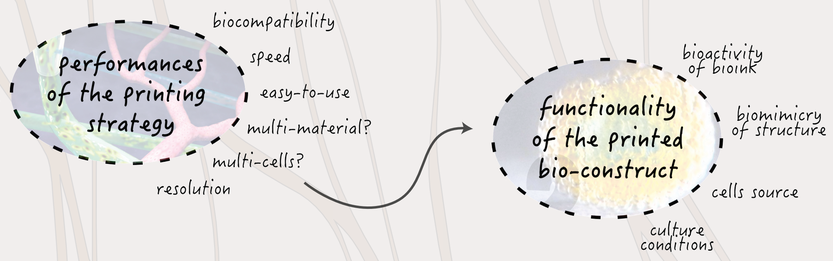
Bioprinting is the technology that aims at creating living&functional tissues *in vitro* by depositing in the three dimensions droplets or fibers of **bioink**, containing living cells and extracellular matrix components. The obtained 3D tissue constructs can be used as biological substitute in regenerative medicine and tissue enginnering applications, as well as more representative, human-derived 3D tissue models for drug discovery and desease modeling[1].

The success of a bioprinting process is determined by many different aspects that play a role over a time span that goes beyond the bioprinting step itself, such as :

- the performances of the bioprinter;
- the choice of cell source(s)
- the choice of supporting biomaterial(s);
- the conditions of culture.



@ the Italian Institute of Technology (iit) we're developing a fiber-based bioprinter that can fastly and easely fabricate three-dimensional heterogeneous tissue constructs embedding living cells inside multicomponent hydrogel systems. In our technique, we exploit the fast physical gelation of alginate in presence of divalent ions (Ca^{2+}) to form fibers with dimensions in the order of 100µm using a coaxial needle assembly (**Figure 1&2**), that separately supply the alginate-containing bioink and the crosslinking solution containing $CaCl_2$. By blending alginate with other cell-active macromolecules (such as gelatin, fibrinogen, hyaluronic acid), that can be crosslinked either contemporary or afterwards the deposition step, we manage to encapsulate cells inside many different extracellular matrixes that can effectively mimic the native environment of each specific cell type.

An advantageous feature of our approach consists in the use of **liquid** and **low viscous bioinks** that undergo gelation simultaneously to the 3D deposition[2] (**Figure 1.b**); in this way, ***it is possible to make the bioink flow inside microfluidic chips prior to its extrusion.***

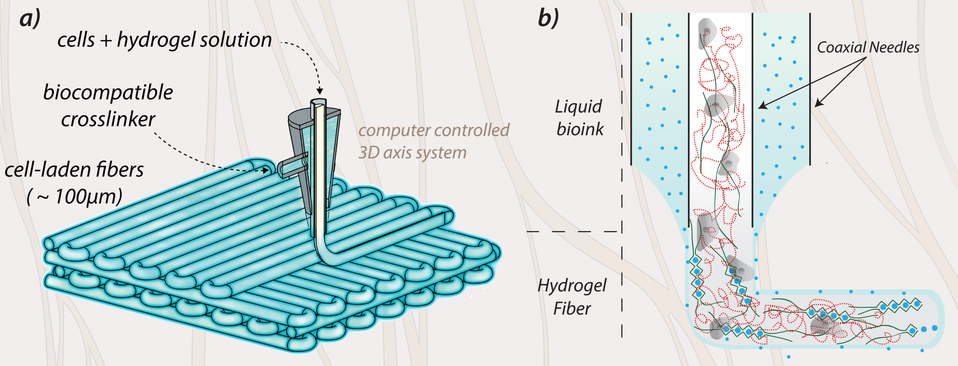


*Figure 1: **a)** Schematic representation of the deposition process with the coaxial needle system. **b)** Schematic representation of the coaxial needle and of the formation of the hydrogel fibers: starting from a liquid bioink, gelation occurs outside the needles during the deposition process.*

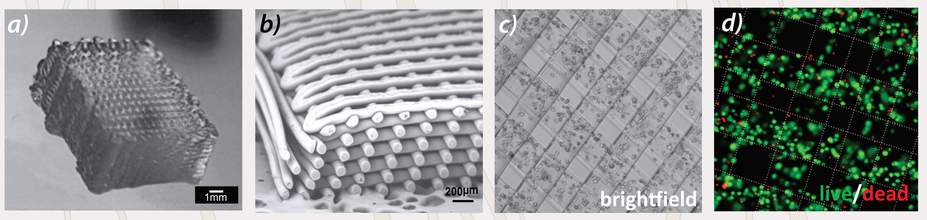


*Figure 2: **a)** Magnified picture and **b)** scanning electron microscopy picture of a 3D printed construct. **c)** Brightfield and **d)** 24h LIVE/DEAD fluorescence miscoscopy pictures of representative cell-containing deposited structures (HepG2 cells) in an alginate-gelatin methacrylate extracellular matrix.*

How can microfluidic platforms enahnce the performances of 3D Bioprinters in the future?

Coupling the extruder with microfluidic chips . . .

Thanks to the reological properties of our bioinks, it is possible to realize a microfluidic feeding of the fiber extruder by connecting the internal needle of the coaxial needle assembly in line with the outlet channel of a microfluidic chip (**Figure 3**). The coupling of the extruder with microfluidic chips offers a major control on the composition and flow of bioinks with the precision, reliability and velocity typical of microfluidic platforms.

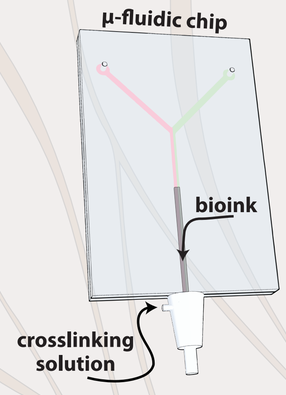


Figure 3: Schematic representation of the coupling between the coaxial needle extruder and elastomeric (PDMS) microfluidic devices

Here, we want to discuss the possibilities that become achievable through the microfluidic manipulation of bioinks in 3D Bioprinting in terms of bioink composition and extracellular matrix-cellular disposition.

Creating heterogeneous fibers . . .

Microfluidic platforms can also be used to create specific flow patterns of different bioinks, allowinf for the deposition of heterogeneous fibers as shown in **Figure 7**.

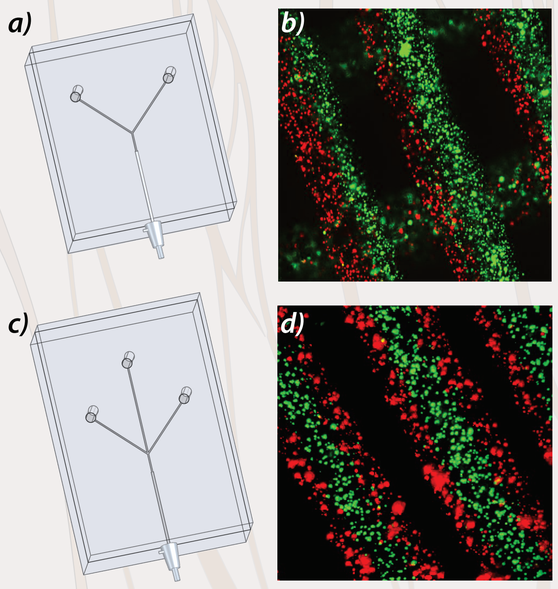


*Figure 7: **a,c)** Schematic representation of the microfluidic chips that generate heterogeneous fibers containing **a)** two or **c)** three parallel flows of distinct bioinks. **b,d)** Fluorescence microscopy pictures of 3D printed constructs with fibers presenting **b)** two or **d)** three different zones (green and red fluorescent beads).*

Changing the composition of the bioink *on-the-fly* . . .

Each tissue is characterized by a specific, heterogeneous cell population and components of the extracellular matrix.

In order to replicate the functionality of the targeted tissue in vitro using 3D bioprinting, it is essential to find a proper composition of the bioink in both its cellular and non-cellular part. The use of simple **micro-mixing fluidic devices** would enable to perform this optimization step in a easier, faster and more reliable way.

An example is given in **Figure 4** where, by mixing two different bioinks at various relative ratios inside a microfluidic chip, it was possible to obtain constructs with different compositions (simulated by the presence of red fluorescent beads) in a single printing procedure.

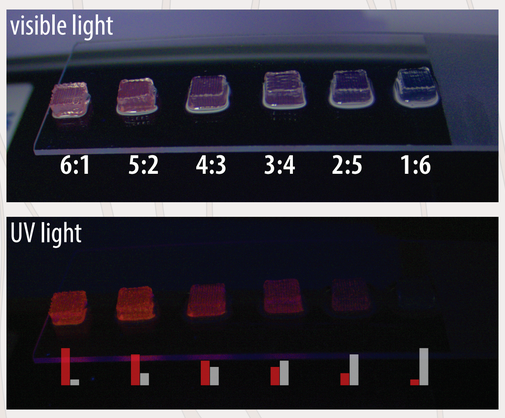


*Figure 4: Magnified photographs of 3D printed constructs obtained using a micro-mixing microfluidic device in line with the extruder. Two hydrogel-precursor solutions, one loaded with red fluorecent beads, were mixed at different proportions inside the microfluidic chip. The ratio between fluorescent and non-fluorescent ink was changed for each construct as reported in figure. The constructs were all produced in a single printing experiment. **a)** Constructs illuminated with ambient light; **b)** constructs illuminated with UV light.*

The compartmentalization of different bioinks in predetermined zones of the deposited fibers can be translated in the production of **bioprinted 3D co-culture cellular models** that enable the replication of specific tissue architectures.

The presented microfluidic platforms (micro-mixing, micro-switch and parallel-flow generator chips) are simple examples of the potentialities of microfluidics in 3D Bioprinting. The combination of these and other microfluidic geometries can make these potentialities explode in virtually infinite possibilities.

Creating heterogeneous structures . . .

Mixing microfluidic chips can also be used to change the composition of the bioink continously within a single construct, creating gradient structures such as that in **Figure 5**.

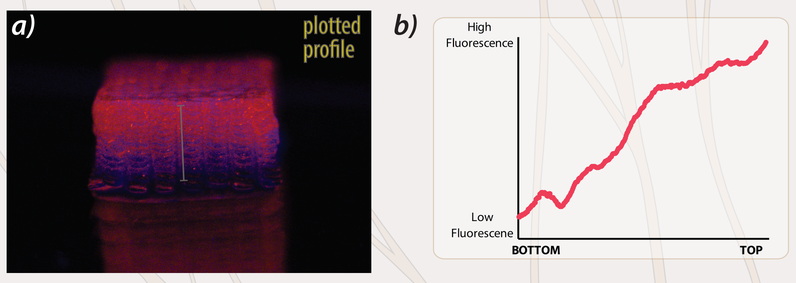


*Figure 5: **a)** Magnified photographs of 3D printed constructs obtained using a micro-mixing microfluidic device in line with the extruder. Two hydrogel-precursor solutions, one loaded with red fluorecent beads, were mixed at different proportions inside the microfluidic chip. The ratio between fluorescent and non-fluorescent ink was changed linearly during the printing of the construct, generating a gradient in the z-direction. **b)** Plot of the color profile in the z-direction (yellow line in a)) highlighting the gradient formation.*

Microfluidic platforms can also be used as **switches**, to alternate the flow sent to the extruder between different bioinks. In this way it is possible to easely create heterogeneous structures (containing different ECM, cellular population or both) deposited in different parts of the construct, as shown in **Figure 6**.

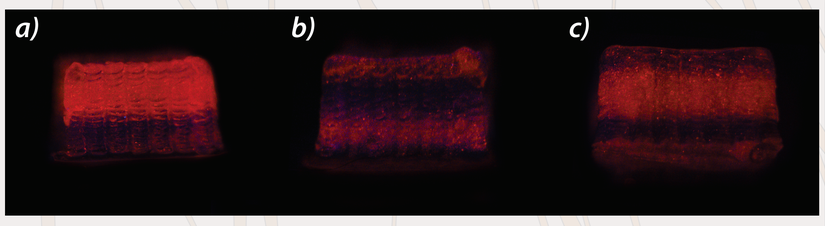


*Figure 6: Magnified photographs of 3D printed constructs obtained using a micro-switching microfluidic device in line with the extruder. Two hydrogel-precursor solutions, one loaded with red fluorecent beads, were alternatively sent to the extruder to form **a)** two, **c)** three or **c)** four distinct layers in the structure.*

In conclusion . . .

The reliability of microfluidic platforms and of Computed Aided Manufacturing can make the creation of multicellular living tissues *in vitro* extremely repeatable. In the future, this could lead to the establishments of new tissue models that will enable a sistematic study of complex tissues and cellular interactions without requiring, or drastically reducing, the use of animal models. The creation of functional tissues outside of the body can also represent a valid strategy in transplantation, solving the problem of extremely long waiting lists or the lack of compatible donors.

[1] MURPHY, Sean V.; ATALA, Anthony. 3D bioprinting of tissues and organs. Nature biotechnology, 2014, 32.8: 773-785.

[2] COLOSI, Cristina, et al. Microfluidic Bioprinting of Heterogeneous 3D Tissue Constructs Using Low-Viscosity Bioink. Advanced Materials, 2016, 28.4: 677-684.

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