

HAPPY PRINTING!

Get ready to print
living tissue



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APPLICATION NOTE



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CELLINK[™] can be used not only as a bioink for 3D bioprinting but also as a 3D cell culturing suspension. When you mix the cells with CELLINK[™], you can either directly bioprint your wanted structure, or simply dispense the mixture in a well-plate and cross-link the mixture with our proprietary Crosslinking solution. A 5 min bath is sufficient for structures bioprinted in a well-plate. After that time, remove the Crosslinking Solution and replace it with media, you will see how easy it is to change it every two days (times depends on the cells you are using).

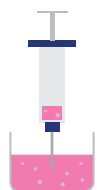
For 3D bioprinting, you can use it as it is, like a biopaper: printing first a layer of bioink then a layer of cells and so on. But what we suggest you to do, is to mix the cells together with CELLINK[™] and bioprint everything in one run with one printerhead. We describe here how to mix the cells manually, but you can use our revolutionary STARTINK-Kit with our CELLMIXER, which is specifically designed to simplify the mixing process and offers a homogeneous suspension with an increased cell viability.

N.B: The CELLMIXER contains a dead volume that does not mix the first 0.5 mL of bioink. As a result, when bioprinting the cartridge, the last 0.5 mL will contain very few or no cells.

Mixing of CELLINK[™] with cells

All work should be carried under sterile conditions.

With CELLMIXER:



- 1 Fill 1 with 0.3 mL of $10 \cdot 10^6$ Cells/mL in media. A needle can be used to fill in the syringe. The large syringe is prefilled with 3mL of bioink.



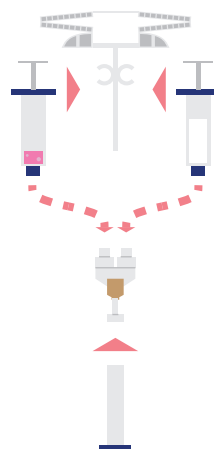
- 2 Clip both syringes to the dispensing unit.



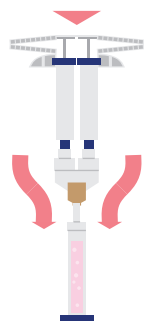
- 3 Screw the mixing unit onto both syringes.



- 4 Screw the empty cartridge onto the mixing unit.



Ensure to clip the collar of the syringes in the bottom part of the dispensing unit and the top part of the plungers in the top part of the dispensing unit.



- 5 Apply gentle pressure onto the dispensing unit to mix the content of both syringes into the empty cartridge.

Without CELLMIXER:

Tools needed (all should be sterile):

- Micro-spatula
- Cells suspension
- Small container for mixing (eg: petri-dish)
- A cartridge and a piston

Harvest the cells and count them. We advise a cell concentration of approximately $10 \cdot 10^6$ cells/mL.

1. The mixing ratio is 1:10. For 1mL of CELLINK[™] mix 100μL of cell suspension in media (eg: containing $10 \cdot 10^6$ cells).
2. Dispense the two amounts together and gently mix them with the micro spatula until you obtain a homogenous pink suspension.
3. Fill the cartridge with the mixed suspension, push everything to the bottom of the cartridge with the use of the piston. Be careful not to have air bubbles in your suspension.

You are now ready to print!

Printing procedure

1. Insert the cartridge (5) into the printer
2. Set your parameters:
For CELLINK bioprinters we advise:
 - 25G, or larger, nozzles.
 - For 25G nozzles, a pressure of around 20 kPa.
 - Printing speed of 10 mm/s.
3. Print

Right after printing

4. Pour the crosslinking solution onto the final printed structure. Ensure to cover the entire structure and wait 5 min (10 min for 5 cm structures).
5. Remove the crosslinking solution and replace it with the appropriate media. Change the media one more time after approximately 30 min.

Your tissue is now growing!