Crosslinking Optimization Protocol

GelMA and GelX based bioinks

This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions for how to optimize the crosslinking of bioinks with photoinitiator (PI), eg. LAP or Irgacure. This protocol can be used when recommended crosslinking procedure is not sufficient or does not apply, for example at other PI concentrations or dilutions.

Material needed

- Bioink with PI, e.g GelMA and GelX variations*
- Water/PBS
- BIO X* or INKREDIBLE+* 3D Bioprinter
- UV shielding cartridges, 3cc*
- Conical Bioprinting nozzles, 25G*
- Well plate or Petri dish
- 365/405 nm UV module for photocuring
- Spatula

*The product can be purchased in the CELLINK store at *www.cellink.com/store/*.

KEEP THE INK PROTECTED FROM LIGHT IF TRANSFERRED FROM THE ORANGE UV PROTECTED CARTRIDGES TO AVOID CROSSLINKING BEFORE PRINTING. WORK WITH 3D PRINTERS IN DARK MODE. THE PHOTOINITIATOR IS SENSITIVE TO REPEATED OR PROLONGED EXPOSURE TO HEAT.

Protocol

This protocol works best with the BIO X and the Temperature Control Printhead as well as the cooled printbed. When using the INKREDIBLE+ system preheat a printhead to 26°C to achieve the same temperature maintenance as the Temperature Control Printhead. After deposition, a Petri dish or well plate being printed on should be placed on ice or another cooled surface to stabilize the construct prior to photocrosslinking.
Note: Room temperature is within 20-25°C.

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Step	Title	Material	Description
1	Prepare Bioink	- GelMA or GelX based bioinks	- Heat up the bioink in a cartridge to 33-37°C. The heating of the bioink can be performed in a pneumatic printhead, water bath or incubator.
2	Eventual dilution	- Water/PBS	- Simulate any cell suspension dilution of the bioink with water or PBS. Mix in according to *Mixing cells Protocol*. If using GelMA based bioinks continue to step 3a, for GelX based bioinks go to step 3b.
3a	Cool and load the cartridge	- UV shielding cartridges, 3cc loaded with GelMA - Conical Bioprinting nozzles, 25G	- Place cartridge on counter for 20 min to reach room temperature. The cartridge can be placed on ice or in the refrigerator briefly for faster cooling. Every 15-25 sec remove the GelMA cartridge from the ice and flip. Observe the air bubble movement, once it begins to slow down, the GelMA is almost ready to print. The viscosity needs to be like a thick syrup or honey. - Place the semi-gelled GelMA in either an INKREDIBLE+ printhead preheated to 26°C or the Temperature Control Printhead on the BIO X preheated to 26°C. Cap with the printing nozzle. If using the BIO X pre-cool the printbed to 10°C.
3b	Cool and load the cartridge	- UV shielding cartridges, 3cc loaded with GelX - Conical Bioprinting nozzles, 25G	- Place cartridge on counter for 20 min to reach room temperature. The cartridge can be placed on ice or in the refrigerator briefly for faster cooling. - Place the room tempered GelX in the printhead and cap with the printing nozzle. If using the BIO X, pre-cool the printbed to 15°C. Note: When printing with GelX the recommended printhead temperature is between 20-32°C.

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4	Printing	- Bioprinter (BIO X or INKREDIBLE+) - Well plate	- Bioprint several structures in a well plate according to your experimental needs or according to *Bioprinting Protocol*.
5	Crosslinking optimization	- 365/405 nm UV module for photocuring	- Ensure that the bioprinted constructs are thermally gelled after printing by cooling the printbed if using the BIO X or placing the well plates containing printed construct on ice for 10 sec if using the INKREDIBLE+. If photocrosslinking during bioprinting, set the crosslinking parameters appropriately in the G-Code for the INKREDIBLE+ or the printhead setup page for the BIO X. - Choose relevant times and distances from light according to the example in Table 1. Crosslink 1-3 constructs per chosen parameter. - Let the structure sit for 1-5 min to allow crosslinking after the light source is turned off. Note: Bioink with LAP can be crosslinked using the 405 or 365 nm photocuring module. It is recommended to use the 405 nm photocuring module instead of 365 if possible. Irgacure can only be crosslinked with the 365 nm module.
6	Incubation	- Water/PBS	- After photocrosslinking, add warm water or PBS in the wells to cover the constructs and agitate the plate for 2 min. - Incubate the constructs at 37°C for a few hours or over-night.
7	Crosslinking check	- Spatula	- Check if the constructs are holding their shape by lifting the construct with a spatula. - Fill in the success rate according to Figure 1 of the constructs that hold their shape and those that has dissolved. - Choose the successful crosslinking with the lowest time and distance for your experiment since over exposure to the constructs might damage the cells.

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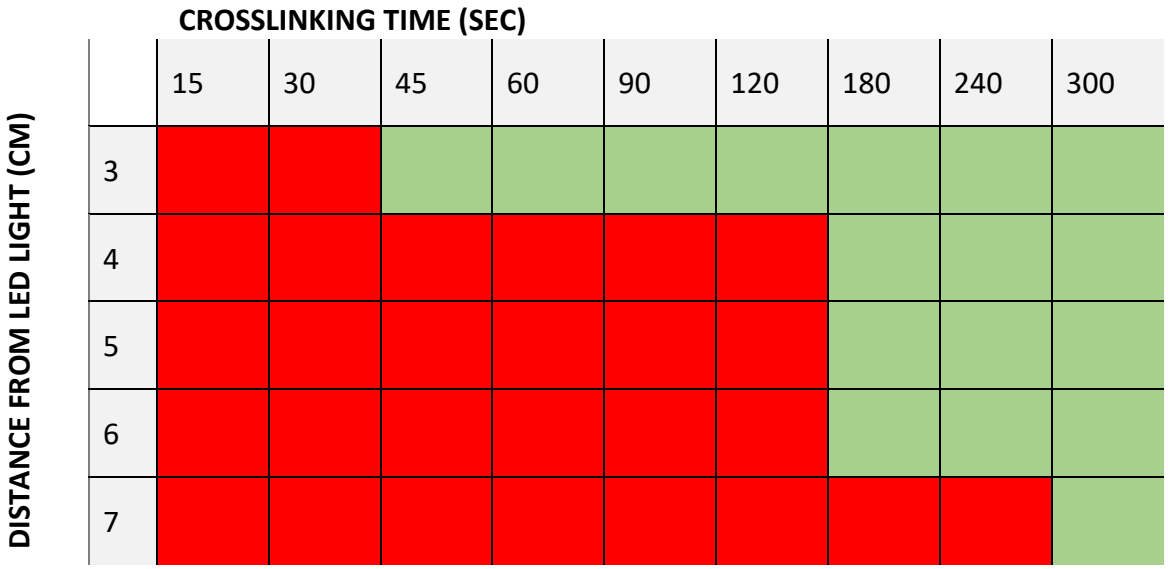

Figure 1. Example of success rate of crosslinking at different times and distance from the construct.

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