Photocrosslinking Optimization Protocol

GelMA Series 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 photocrosslinking of bioinks using photoinitiators (PI) such as 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.

Materials needed

- GelMA Series bioinks with PI*
- Water/PBS
- BIO X* or INKREDIBLE+* 3D Bioprinter
- UV shielding cartridges, 3cc*
- Conical bioprinting nozzles, 22-27G*
- Well plate or Petri dish
- 365/405 nm light module for photocuring
- Spatula

*The products 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 using the BIO X equipped with the Temperature-controlled printhead as well as the cooled print bed. When using the INKREDIBLE+ system, preheat a printhead to 26°C to achieve the stable temperature maintenance. After deposition, printing substrates such as Petri dishes or well plates should be placed on ice or another cooled surface to stabilize the construct prior to photocrosslinking.

Note: Room temperature is within 20-25°C.

Step	Title	Material	Description
1	Prepare bioink	- GelMA Series 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 GelMA series*.
3	Cool and load the cartridge	- UV shielding cartridges, 3cc loaded with GelMA - Conical bioprinting nozzles, 22-27G	- 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 s 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-controlled printhead on the BIO X preheated to 26°C. Cap with the desired printing nozzle. If using the BIO X, pre-cool the print bed to 10°C.
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 light module for photocuring	- Ensure that the bioprinted constructs are thermally gelled after printing by cooling the print bed if using the BIO X or placing the well plates containing printed construct on ice for 10 s 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.

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			- 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 overnight.
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 overexposure to the constructs might damage the cells.

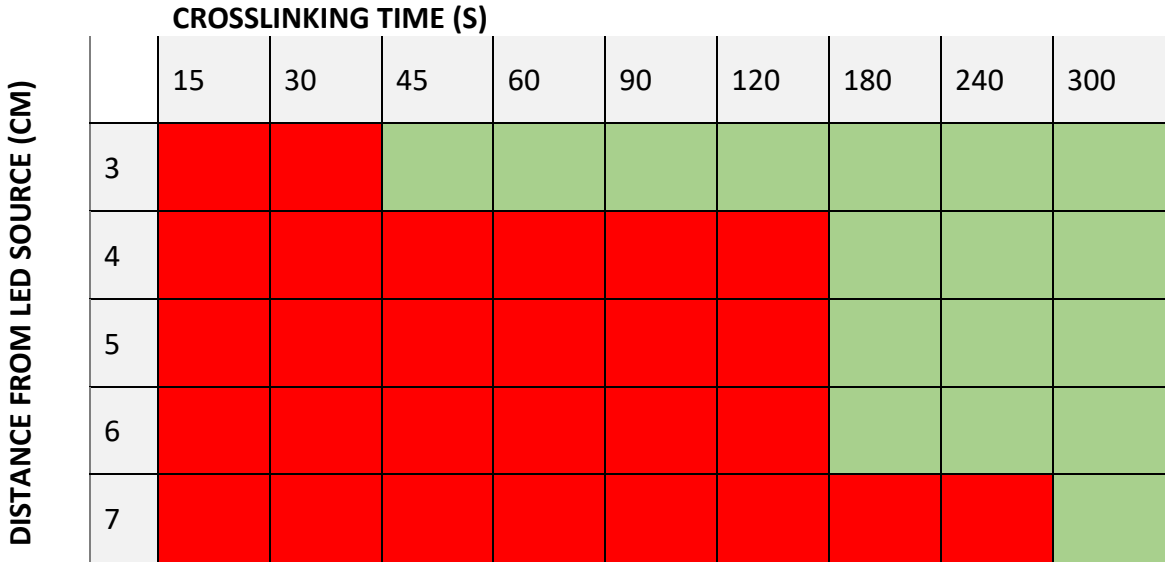

Figure 1. Example of success rate of crosslinking at different times and distance from the construct.