00600500480.GOT
Date: 03-JAN-2023
Author: JH,VK. Version: 4

PHOTOCROSSLINKING OPTIMIZATION PROTOCOL

# GelMA 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 photocrosslinking of GelMA and GelXA based bioinks containing photoinitiators (PI) such as LAP or Irgacure. This protocol can be used when recommended photocrosslinking procedure is not sufficient or does not apply, for example at other PI concentrations or dilutions.

## Materials needed

- GelMA or GelXA based bioinks with PI*
- Water/PBS
- BIO X*, BIO X6* or INKREDIBLE+* 3D bioprinter
- UV shielding cartridges, 3cc*
- Conical bioprinting nozzles, 22-27G*
- Well plate or Petri dish*
- Spatula

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

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 or BIO X6 equipped with the Temperature-controlled printhead as well as the cooled print bed. When using the INKREDIBLE+ system, pre-heat a printhead to 24-26°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.

## 1. Preparing the bioink

**MATERIAL**

GelMA or GelXA based bioinks

**DESCRIPTION**

- Heat up the bioink bioink in the cartridge to 35°C until the bioink is liquid. The heating of the bioink can be performed in a pneumatic or Temperature-controlled printhead or incubator.

## 2. Possible dilution

**MATERIAL**

Water/PBS

**DESCRIPTION**

- Simulate a cell suspension dilution or other dilution of the bioink with water or PBS. Mix in according to *Mixing cells and bioink Protocol.*

## 3. Preparation for printing

**MATERIAL**

GelMA/GelXA in 3cc, UV shielding cartridges

Conical bioprinting nozzles, 22-27G

**DESCRIPTION**

- If the cartridge is taken directly from the heat, let sit for 10 min to cool down slightly. Observe any air bubble movement, once it begins to slow down, the bioink is almost ready to print. The viscosity needs to be like a thick syrup or honey.
- Place the semi-gelled GelMA/GelXA in either an INKREDIBLE+ printhead pre-heated to 24-26°C or the Temperature-controlled printhead on the BIO X/BIO X6 pre-heated to 24-26°C. Cap with the desired printing nozzle. If using the BIO X/BIO X6, pre-cool the print bed to 15°C.

Note: The optimal printing temperature will depend on the bioink type and the bioink dilution.

- If proper viscosity and printability is not achieved by extending temperature equilibration time or tuning pressure:
    - Too low viscosity, wide filaments despite using low pressure: decrease the printhead temperature 0.5-1°C to increase the viscosity and equilibrate an additional couple of minutes.

- Too high viscosity, chunky filaments and high pressure required: increase the printhead temperature 0.5-1°C to decrease the viscosity and equilibrate an additional couple of minutes.

# 4. Printing

**MATERIAL**

BIO X, BIO X6 or INKREDIBLE+ bioprinter

Well plate

**DESCRIPTION**

- Bioprint several structures in a well plate according to your experimental needs or according to that specific bioinks *Bioprinting Protocol*.
- Ensure that the bioprinted constructs are thermally gelled after printing by cooling the print bed if using the BIO X/BIO X6 or placing the well plates containing printed construct on ice for 10 s if using the INKREDIBLE+.

# 5. Crosslinking optimization

**MATERIAL**

365/405 nm LED module for photocuring

**DESCRIPTION**

- 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/BIOX6.
- Choose relevant times and distances from light and constructs. 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 LED module. It is recommended to use the 405 nm photocuring module instead of 365 if possible when using cells. Irgacure can only be crosslinked with the 365 nm LED module.

# 6. Incubation

**MATERIAL**

Water/PBS

**DESCRIPTION**

- 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

**MATERIAL**

Spatula

**DESCRIPTION**

- 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.

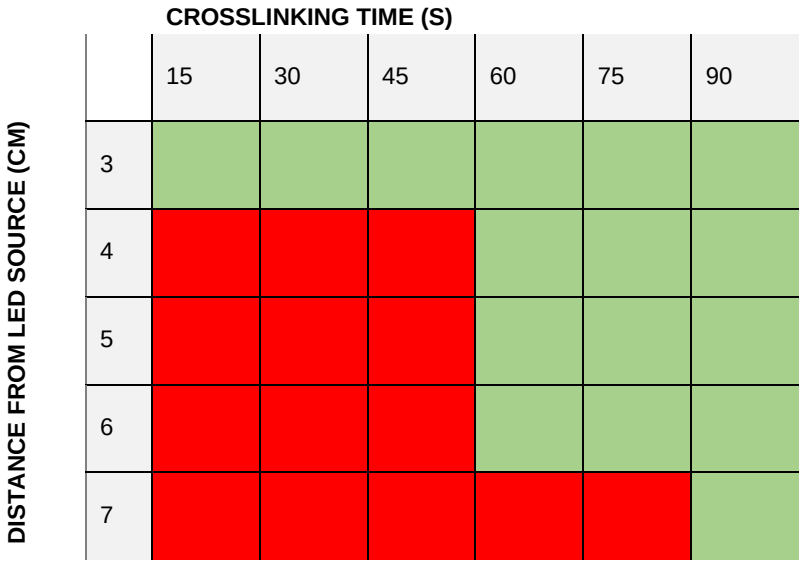


***Figure 1.*** *Example of a crosslinking success rate table that can be generated after testing different times and distance from the construct.*