

## Collagen Methacrylate Printing Protocol

In this section, COLMA printing protocol is explained in detail.

Necessary materials can be purchased from [www.zetamatrix.com](http://www.zetamatrix.com)

Before starting, please prepare COLMA solution as described below

Take the desired amount of lyophilized powder form COLMA, it is suggested to keep the concentration between 1.5 to 8 mg/mL. For the best results please ensure the neutral pH 7.0 to 7.4 for the COLMA solution. Please keep COLMA solution 2-10 °C during all process.

1. For gelation heat up COLMA to 37 °C. Be careful to not exceed the temperature
2. If crosslinking is desired photoinitiator should be added before the operation, it is suggested to dissolve Irgacure in methanol and mix with the COLMA solution (for ex: 100mg Irgacure for 1 mL methanol)
3. If needed COLMA solution can be mixed with cells before transferring to the cartridge (All transfers can be easily done with a female/female luer lock adaptor)
4. Insert the suitable bioprinting nozzle
5. Insert the cartridge into the bioprinter
6. Turn off the lights before starting to print (if a photoinitiator is used)
7. Set the desired design and suitable printing parameters
8. Try to pressure flow to check clogging of the nozzle and start to print
9. After printing wait a few minutes before applying UV
10. UV curing time and distance should be selected according to the application and loaded cell type and amount
11. Wait few more minutes before removing the design.

**\*Be aware that bioink with photoinitiator is light sensitive please keep it away from the light even through the transfers to prevent crosslinking before printing.**

**\*Collagen is temperature sensitive so avoid from excessive amount of temperature more than 37 °C and keep the duration maximum 90 minutes.**