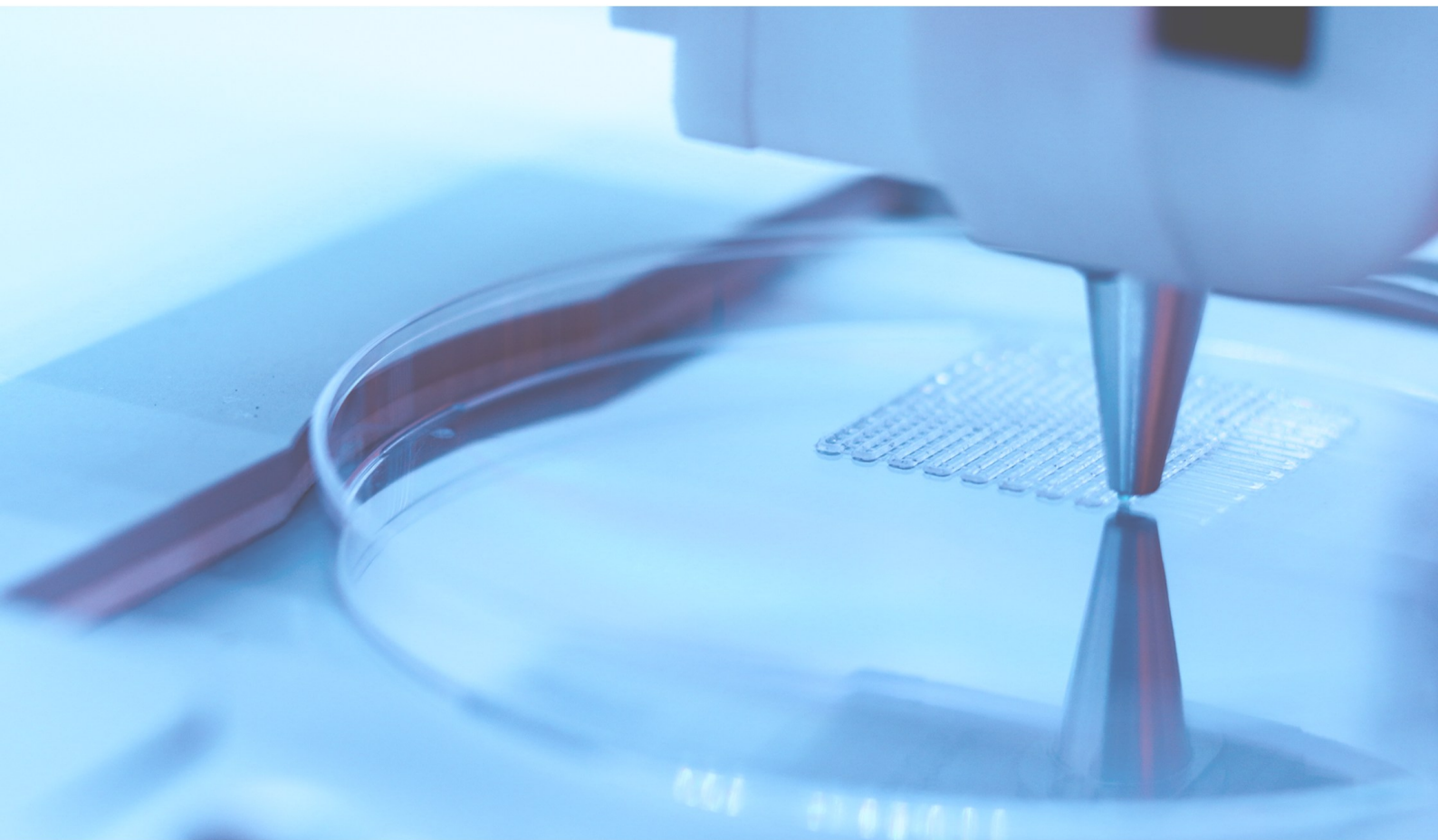




User Guidelines for

EASYGEL  X100



General Information

Storage

EASYGEL INX should be stored in a fridge at 4 °C. Protect it from light. Expiry date of the product is indicated on the sealed pouch. The product can be stored for a maximum of 3 months after opening and should be consumed before the expiry date.

Intended Use

Research use only. This product is not intended for use in diagnostic or therapeutic procedures.

Safety Information

For more information, please refer to the material safety data sheet.

User Guidelines

Preparation

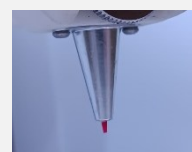


EASYGEL INX X100 was produced under sterile conditions. To ensure optimal performance and prevent contamination, it is recommended to handle this product in a **sterile environment**.

1. Preheat EASYGEL INX cartridge in a water bath at 37 °C for 10 min before printing.
2. Pre-heat the printhead at 37 °C.
3. Remove the cartridge from the water bath. Remove the tip cap and end cap. Insert the selected nozzle (see processing guidelines for recommended nozzle types.)
4. Insert the cartridge in the pre-heated printhead.



For an optimal printing performance, the use of a metal conical **nozzle insulator** is required. Insert the nozzle tip in the insulator as shown in the image.



5. Start printing using the suggested printing parameters (See processing guidelines)

Processing

Recommended processing parameters are listed below.

Nozzle size	22G
Nozzle geometry	Conical
Printhead Temperature	37 °C
Printbed Temperature	22 °C
Pressure	30 ± 10 kPa
Infill Speed	6 ± 1 mm/s
Layer Height	0.22 ± 0.02 mm

⚠ The printing parameters have been validated for printing a square with dimensions 15 x 15 mm (W x L) up to 10 layers.

⚠ Use of rectilinear infill pattern is recommended.

⚠ **Photo-crosslinking:** During printing, structure should be irradiated with light (λ : 365 nm or 405 nm, Dose: 70 mJ/cm²) after every layer. This step is required for partial crosslinking of the structure for a better shape retention. After completion of printing, the final structure should be promptly placed under UV light for complete crosslinking. (Recommended parameters for post-printing photo-crosslinking: λ : 365 or 405 nm, Dose: 10000 mJ/cm²)

For photo-crosslinking kinetics of EASYGEL INX at two different wavelengths, see Figure 1.

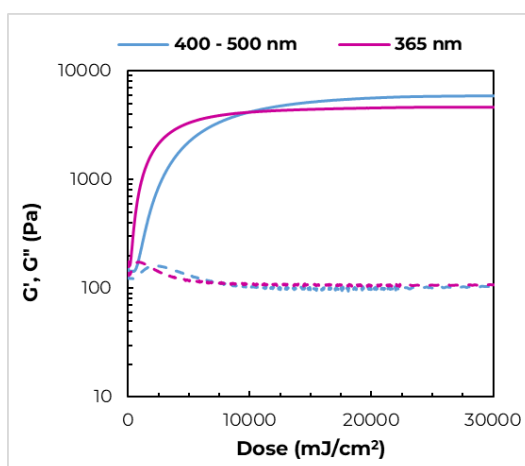


Figure 1. Storage (G', solid lines) and loss (G'', dashed lines) moduli of EASYGEL INX X100 as a function of irradiation dose at 365 nm and 400-500 nm wavelengths

⚠ After photo-crosslinking, place the sample in a buffer or cell culture medium to prevent drying.

Cell Culture

a) Cell seeding

The scaffolds can be readily seeded with cells after overnight incubation in cell culture media without the need of further coating.

b) Cell encapsulation



Preheat EASYGEL INX cartridge at 37 °C.



Connect EASYGEL INX cartridge to a syringe via a female luer-to-luer adapter.



Inject the desired amount of EASYGEL INX into the syringe.



Connect the second syringe containing the cell suspension via a luer-to-luer adapter. Shift the hydrogel back and forth between the syringes until the cells are homogeneously mixed in.



Inject cell-laden EASYGEL INX back into the cartridge via a luer-to-luer adapter.

⚠ For encapsulation, the cell suspension/gel ratio should not exceed 1/10 (100 µL cell suspension per mL of final bioink).

⚠ The cell encapsulation protocol has been validated using human fibroblasts; however, it is important to note that the results obtained from this validation may vary when applied to different cell types.