Ref No: BPR-IK-D12000
Date: 03-JAN-2023
Author: EZ,VK,JH. Version: 2

NEUTRALIZATION AND CASTING PROTOCOL

Collagen PREMIUM

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

Protocol aim

The aim of this protocol is to provide instructions for neutralization and casting of the Collagen PREMIUM using the INKREDIBLE, INKREDIBLE+, BIO X or BIO X6, with and without cells. It covers both dispensing of the biomaterial with encapsulated cells and post seeding of cells on casted gels. Collagen PREMIUM is crosslinked through thermal induced gelation. This protocol was optimized for undiluted Collagen PREMIUM as well as for a 10+1 cell suspension diluted version. Changing the concentration of solution to cell suspension ratio will change the gelation time.

Materials needed

- Collagen PREMIUM*
- Collagen PREMIUM Neutralization Solution*
- Ice bath
- 3 mL syringes with Luer lock connection
- Female/female Luer lock adaptor*
- Cells* + culture medium*
- Cartridges, 3cc*
- Conical bioprinting nozzles*
- BIO X*, BIO X6* or INKREDIBLE series* 3D bioprinter
- Well plate or mold

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

Protocol

This protocol works best with the BIO X/BIO X6 and the Temperature-controlled printhead. If using the INKREDIBLE+ system, the dispensing procedure should be performed fast to prevent the solution from warming and gelling in the cartridge prior to dispensing.

1. Cooling of material

MATERIAL

Collagen PREMIUM

Neutralization Solution

Ice bath

DESCRIPTION

- Place the bottle of Collagen PREMIUM and the bottle of Neutralization Solution on ice to keep cool.

2. Neutralization

MATERIAL

3 mL syringes, two units

Female/female Luer lock adaptor

DESCRIPTION

- Transfer equal volumes of Collagen PREMIUM and Neutralization Solution into two different 3 mL syringes.

Note: Remove the syringe plunger, cap the syringe with a tip cap and pour the solution in the syringe. Insert the plunger, flip the syringe and release the tip cap to evacuate the air.

- Connect the syringes via a female/female Luer lock adaptor and mix thoroughly back and forth. Be careful not to introduce air bubbles.
- Work fast to prevent materials from warming.
- *If not casting with cells or if seeding cells on-top, move directly to Step 4.*

3. Mixing Collagen PREMIUM with cells

MATERIAL

Cell suspension in syringe

Cooled Collagen PREMIUM solution in syringe

Female/female Luer lock adaptor

DESCRIPTION

- Mix ten parts of cooled Collagen PREMIUM solution with one part of cell suspension without introducing air bubbles to the mixture. For detailed instructions see the *Mixing cells with bioink Protocol*.
    - Attach the Collagen PREMIUM solution syringe to the syringe with cell suspension, using a female/female Luer lock adaptor.
    - Carefully mix the solutions with the cell suspension by gently pushing the solutions back and forth between the syringes.

Note: Suggested cell suspension density is $1x10^6$ cells/mL to $5x10^6$ cells/mL of Collagen PREMIUM.

Note: To avoid an air gap when mixing the solution and the cell suspension, carefully pre-fill the Luer lock adaptor with Collagen PREMIUM solution before attaching the syringe with the cell suspension.

4. Preparing the bioink

MATERIAL

Cartridge, 3cc

Conical bioprinting nozzles

DESCRIPTION

- If using the BIO X, pre-cool the Temperature-controlled printhead to 15°C and pre-heat the print bed to 37°C. If using the INKREDIBLE-series, cool down the cartridge for 5 min if needed.
- Transfer the solution (with or without cells) to the cartridge and cap the cartridge with a bioprinting nozzle of choice. Place it in the printhead and connect the cartridge to the air adaptor.

5. Casting

MATERIAL

BIO X, BIO X6 or INKREDIBLE series bioprinter
Well plate or mold

DESCRIPTION

- Dispense the required volume of solution in a mould or well plate.

Note: If waiting too long between extrusions, the solution can warm in the nozzle causing it to clog. If this occurs, replace with new nozzle.

6. Thermal crosslinking

DESCRIPTION

- Collagen PREMIUM thermally crosslinks.
    - Incubate the constructs at 37°C until gelation occurs, approximately 10-15 min for volumes below 100 µL. For larger constructs, prolong the incubation to 30 min. The BIO X/BIO X6 print bed heated to 37°C can also be used for the thermal gelation.

Note: To verify that the crosslinking is sufficient, add 37°C media to one construct and observe that it doesn't dissolve.

- *If not seeding cells on-top of the casted collagen, move directly to Step 8.*

7. Cell seeding

MATERIAL

Cell suspension

DESCRIPTION

- Dispense the cell suspension in the middle of the casted hydrogel. Suggested cell suspension density: $2x10^4$ cells/cm^2 to $5x10^4$ cells/cm^2. A highly concentrated cell suspension is suggested.

8. Incubation

MATERIAL

Cell culture medium

DESCRIPTION

- Add the desired medium to submerge the constructs. If cells were seeded on-top, wait for 1-2 h or until the cells have started to attach to the collagen surface before submerging the constructs in medium.
- Incubate the constructs in cell culture medium in standard culture conditions (37°C, 5% CO_2 and 95% relative humidity) or according to your application.