F7ADIBTRM - Preparation of a 3D printed construct for cell culture colonization

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Among the available technologies in tissue engineering, bioprinting has been growing rapidly in the last decade. This method involves printing biomaterials using a three-dimensional printer, but instead of materials such as thermoplastic or resin, a special bio-ink is used. This bioink is mainly based on natural hydrogels creating suitable conditions for cell growth and can directly incorporate living cells. This makes it possible to create homogeneously populated scaffolds with cell integration throughout the volume. Compared to other methods of scaffold preparation (casting the hydrogel into a mold), it also allows a more controlled approach to modifying the shape, surface properties, surface combinations, etc.

An important element is the substrate on which the print is made. From an application point of view, thin film or membrane constructs are suitable, which provide sufficient strength, allow initial adhesion of the printed material and are biocompatible. In this preparation, this substrate will be a nanofibrous membrane consisting of PCL and collagen nanofibers. The PCL will provide suitable strength and the collagen will in turn aid the integration of the substrate with the printed material.

Instruments

- Laminar box
- 3D bioprinter
- System for the preparation of nanofibres by electrostatic wetting
- Inverted fluorescence microscope

Other aids

- Sterile instruments for handling cell culture and media
- Culture media, PBS, Trypsin and EDTA
- Porcine collagen solution for fibre
- PCL polymer solution for fibre
- Bioinkoust - cellink bioink
- Cell culture of PrASC - porcine adipose tissue stem cells
- Solutions for fluorescent staining - blocking, permeabilizing, diluting for Phalloidin and DAPI

Before starting the preparation, place the 3D bioprinter in the laminar box and sterilize the box using a germicidal lamp.

Preparation of a planar composite nanofibre carrier using a needle-free emitter

1. Prepare polymers in 5 ml syringes - each polymer separately syringe
2. Prepare the system for fibre - basic setup
    a. Prepare a two-channel emitter for needle-free softening
    b. Prepare a flat-plate collector and place a layer of sterile foil or non-woven fabric or sterile gauze on its surface
    c. Insert an adapter into the dispenser to use two syringes
    d. Connect both syringes to the emitter

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e. Insert the collector and emitter into the deposition chambers, deflect the syringes in the dispenser
f. Vent the emitter and dispenser, it is necessary to dispense both solutions in

in the same proportion

3. Set the deposition parameters
    a. Deposition distance 15 cm
    b. Set the base voltage to 40 kV
    c. Set the dosing volume to 120 ul/min
    d. Set the air flow in the chamber to 35 l/min, temperature 35 °C
4. Start the threading process
    a. Gradually increase the deposition voltage until fibrous jets are formed from both polymers, approx. 45 - 60 kV depending on the degree of polymer loosening
    b. Check for fibrillation from both solutions
    c. In case of rapid softening of the droplet on the needle, increase the dosage volume to approx. 120-140 ul, in case of polymer accumulation reduce the dosage volume to approx. 80-90 ul
5. Deposit for 15 minutes
6. After deposition is complete, remove the collector with the prepared fibers, cut the fibers into 30 x 30 mm squares using a scalpel, and sterilize under UV light in a laminar box for 30 min. **FROM THIS POINT ON, WORK STERILE!!!** Attach the prepared squares to sterile holders for subsequent 3D printing.

Preparation of 3D printed gel on nanofibre carrier

1. Prepare the 3D bioprinter
    - Start the HeartWare SW
    - Insert empty needle cartridge
    - Reset the printer - make sure the cartridge is inserted
    - Perform Z-axis calibration on the test model
2. Prepare cell culture
    - PBS rinse, T+E, culture
    - Centrifugation 300g 5 min
    - Dispersion at 10 mil.b. / ml
3. Prepare Cellink-Bioink according to the enclosed instructions and mix with the cell culture
4. Load the Circle and Annuloid model in the HearWare cellink, use the Simple shape Bioink template and set the pressure to 25 kPa
5. Insert the prepared nanofibre carrier into the printer and print the model
    - 1 print at 25 kPa
    - 2 printing at 18 kPa
    - 3 printing at 35 kPa

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6. Compare dimensional stability
7. Place in 2 culture 6j plates - half of the samples will be cultured statically and the other half dynamically, add culture medium

Figure 1 - optimal shapes of printed substrates

Carrier analysis

1. Cultivate for 3 days
2. Then fix the samples with 4% PFA and rinse with PBS and azide
3. Permeabilize with the prepared solution for 15 min.
4. Block non-specific proteins with blocking solution 60 min
5. Prepare a 2 ml solution of Phalloidin (1:50) and DAPI (1:500)
6. Drain the blocking solution and add the prepared Ph. and DAPI solution to the samples, incubate for 60 min.
7. Rinse thoroughly
8. Prepare microscopic images of samples - fluorescence, combi filter, 10x or 20x vol.

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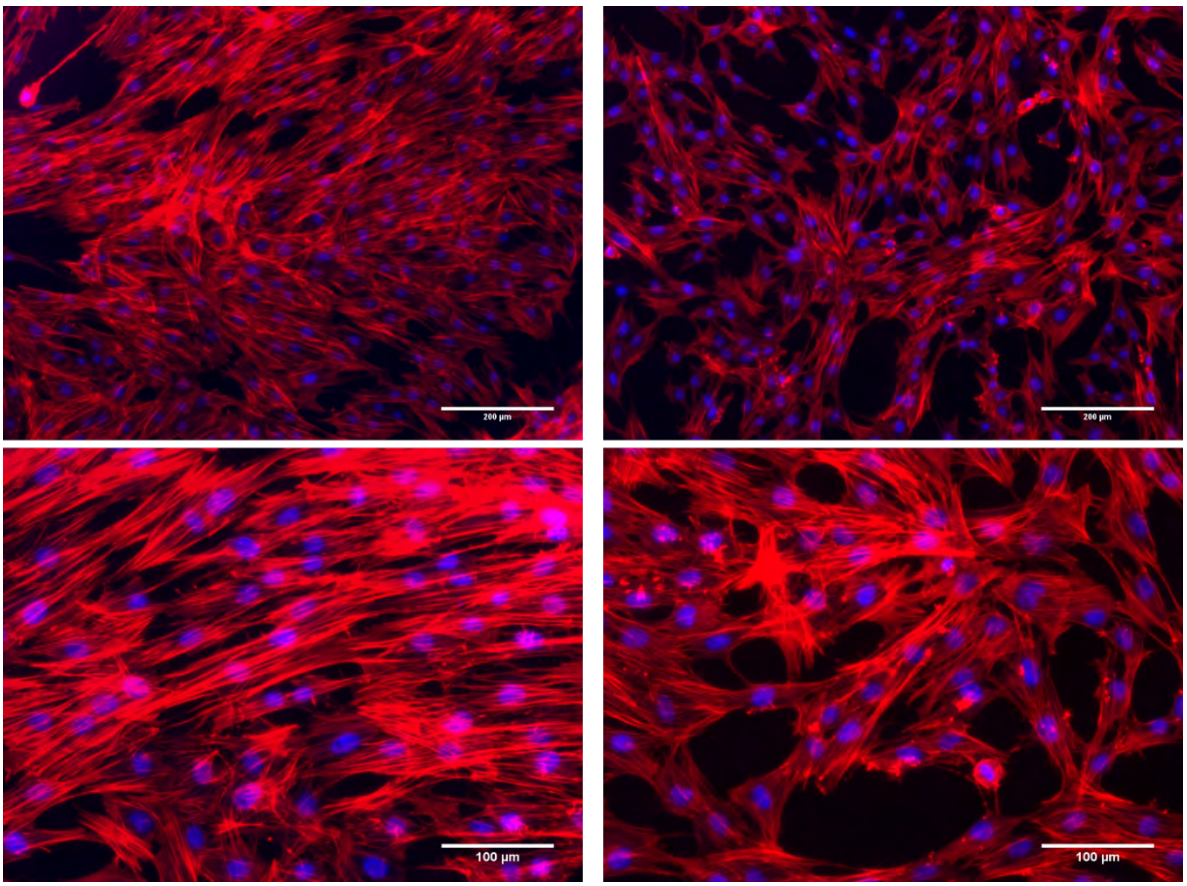

Image 2 - Comparison of dynamic (left) and static (right) cultivation in printed carriers. Staining of F-actin (red) and nuclei (blue).

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