POLBIONICA

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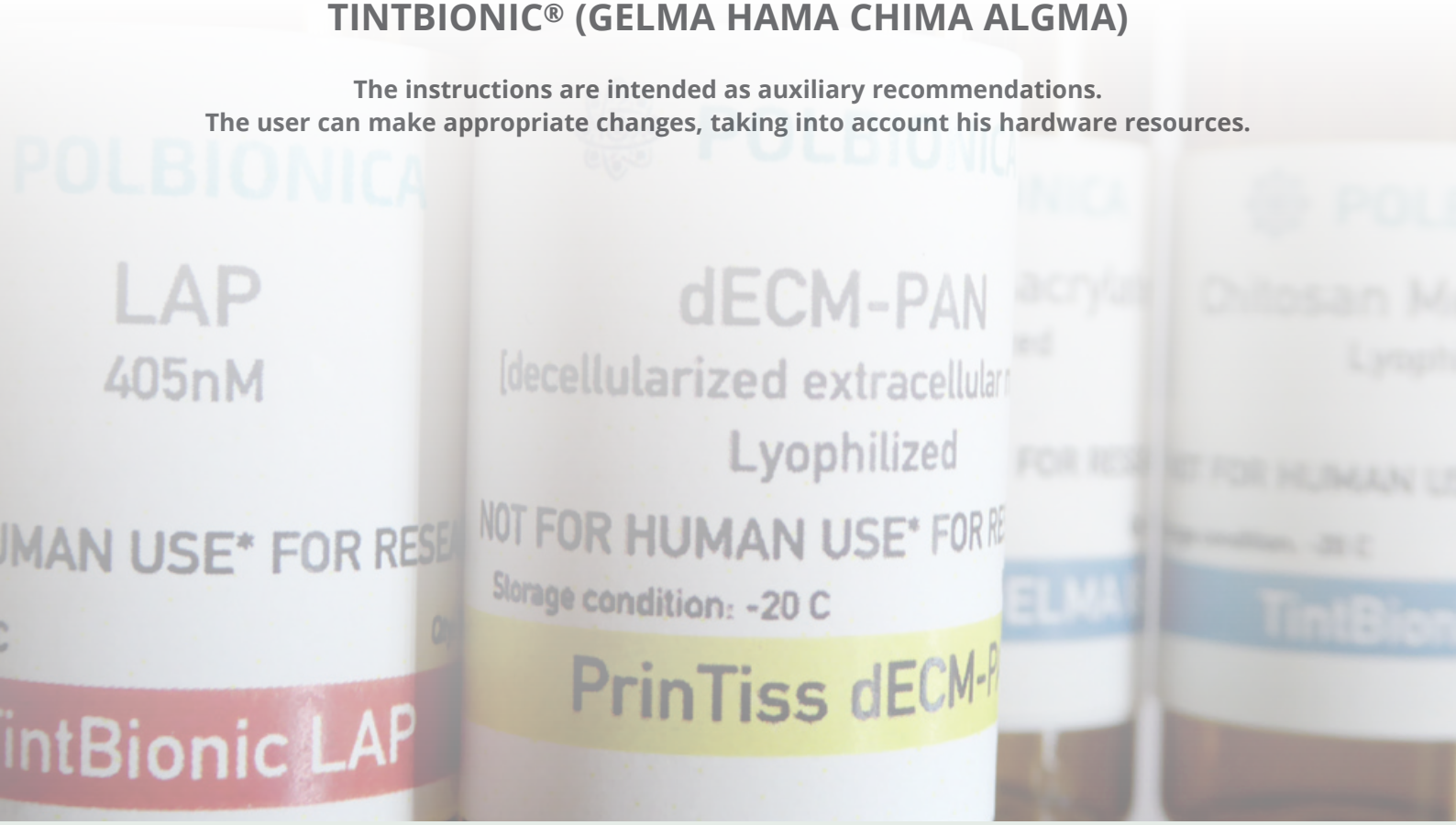
BIOLOGICAL RESEARCH IN 3D CONSTRUCTS WITHOUT THE USE OF A BIOPRINTER

HOW TO USE dECM-PAN (DECELLULARISED EXTRACELLULAR MATRIX FROM PORCINE PANCREA) AS AN ADDITIVE TO TINTBIONIC® RANGE BIOINK PRECURSORS WITH LABORATORY UV-Vis LAMP

TINTBIONIC® (GELMA HAMA CHIMA ALGMA)

The instructions are intended as auxiliary recommendations.
The user can make appropriate changes, taking into account his hardware resources.



PREPARATION OF MATERIALS

1. Before starting work, prepare:

a. a suitable bioink precursor or a mixture of them (GELMA, HAMA, CHIMA, ALGMA), in the required concentration, according to the INSTRUCTIONS FOR PREPARING TINTBIONIC® METACRYLATE SOLUTIONS

note: ***materials should be in a liquid form, ready for syringe/pipette collection***

note: ***working with biological material requires sterile conditions. It is therefore recommended that the prepared bioink precursor solution be filtered through a 0.22 µm pore size filter and that it be appropriately handled according to good laboratory practice.***

b. appropriate pancreatic ECM hydrogel solution for testing, according to the enclosed INSTRUCTIONS FOR THE PREPARATION OF DECM Printiss® HYDROGEL

note: ***the hydrogel should be in a liquid state (ready for pipette or syringe collection); to ensure adequate rheological properties, it is recommended to place the solution at 30°C for 10-20 minutes; the hydrogel should be prepared and stored under sterile conditions.***

c. the biological material (cell lines/organoids), which should be suspended in a dedicated cell medium

note: ***the total volume of the mixture should be adjusted to the planned experiment in order to allow cross-linking of the final biomaterial mixed with the cells.***

note: ***select the amount of suspension according to the expected density of cells in the biomaterial***

d. the cell medium dedicated to the continuation of the experiment (on cross-linked constructs).

e. 2 identical syringes, luer-lock syringe adapter, stand, injection needle, culture vessel, medium pipette, lamp

PREPARATION OF 3D CONSTRUCTS WITH THE ADDITION OF dECM

VARIANT I - BIOLOGICAL MATERIAL SUSPENDED IN BIOINK

1. Unpack the two syringes. Remove the plunger from one and set it aside (it will be needed later on).

2. Place an injection needle on syringe No. 1 and draw the prepared liquid sterile solution of the bioink precursor

note: ***do not exceed 37°C when working with biological material***

note: ***the injection needle should be appropriately selected in diameter so that it does not generate excessive shearing forces when dispensing the material with the cells***

note: ***select appropriate syringes adapted in volume to the anticipated amount of biomaterial***

3. Remove the needle from syringe No. 1 and immediately attach the luer-lock adapter. Release air from the adapter.

4. Connect syringe No. 2 (without the plunger inserted) to the other side of the luer-lock adapter. Then place both syringes, connected with the luer-lock adapter, vertically on the stand in such a way that syringe No. 2 (without the plunger) is facing upwards.

5. Add the previously prepared and liquefied ECM hydrogel to syringe No. 2 in an amount consistent with the ratio of components in the bioink assumed earlier in the research plan

6. Add the previously prepared cell suspension to the same syringe No. 2 (choose the amount of suspension according to the expected density of cells in the biomaterial).

7. Place the plunger in syringe No. 2, without forcing it all the way in - just enough to prevent the liquid from spilling out (up to the first resistance), then turn the syringes 180 degrees, twist off syringe No. 1 and release the air from the syringe with the biological material, plug in the syringe with the bioink precursor again and mix gently (make a total of about 10 pushes with the plungers in either direction).

8. Pour the prepared bioink into the bottom of a selected culture vessel (vessel selection according to the research plan) - so that the planned structure (3D construct) can be formed.

note: ***it is recommended that the dimensions of the structure should not exceed approximately 2 cm in diameter and 2 mm in height***

9. The poured construct should be immediately cross-linked with the UV-Vis lamp, Polbionica, according to the parameters presented in Table 1.

   note: *when using precursor mixtures, the cross-linking parameters should be adjusted experimentally (it is suggested to perform these tests before starting the actual experiment)*

10. Pour the previously prepared cell medium over the 3D cross-linked construct using a pipette, so that it is fully immersed in the medium.

11. Carry out cell culture according to your own research plan.

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VARIANT II - BIOLOGICAL MATERIAL SEEDED ON THE SURFACE OF THE BIOMATERIAL

1. Draw the liquid, sterile solution of the bioink precursor with syringe No. 1 using the injection needle

   note: *do not exceed 37°C when working with biological material*

2. Remove the needle and attach the luer-lock adapter to the syringe. Release air from the adapter.

3. Connect syringe No. 2 (without the plunger) to syringe No. 1 using the luer-lock adapter. Then place both syringes, connected with the luer-lock adapter, vertically on the stand in such a way that syringe No. 2 (without the plunger) is facing upwards.

4. Administer the previously prepared liquefied ECM hydrogel using a pipette into syringe No. 2 in an amount according to the assumed ratio of components in the bioink.

5. Place the plunger in syringe No. 2 (without forcing it all the way in, just enough to prevent the liquid from spilling out), then turn both syringes 180 degrees, twist off syringe No. 1 and press the air out of the syringe with the ECM hydrogel, reconnect the syringe with the bioink precursor and mix gently (make a total of about 10 pushes with the plungers in either direction).

6. Pour the prepared bioink into the bottom of a selected culture vessel (vessel selection according to the research plan) - so that the planned structure (3D construct) can be formed.

   note: *it is recommended that the dimensions of the structure should not exceed approximately 2 cm in diameter and 2 mm in height*

7. Cross-link the poured flake with the UV-Vis lamp, Polbionica. according to the parameters presented in Table 1.

   note: *Table 1. shows the cross-linking parameters for the bioink precursor solutions alone, the addition of ECM hydrogel can affect the cross-linking parameters depending on the ratio of components used in the formulation; in general, the higher the ECM hydrogel content in the formulation, the longer the flake cross-linking time*

8. Coat the cross-linked 3D construct with dECM with a suspension of the previously prepared biological material using a pipette (choose the amount of suspension according to the expected density of cells on the material).

9. Carry out cell culture according to your own research plan.

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Table 1. *Range of recommended parameters for cross-linking bioink with Polbionica UV-Vis lamp*

Material	Wavelength [nm]	Power [mW/cm^2]	Cross-linking time [s]
GELMA	405	20,0	20-50
HAMA	405	20,0	20-40
CHIMA	405	20,0	20-40
ALGMA	405	20,0	30-60