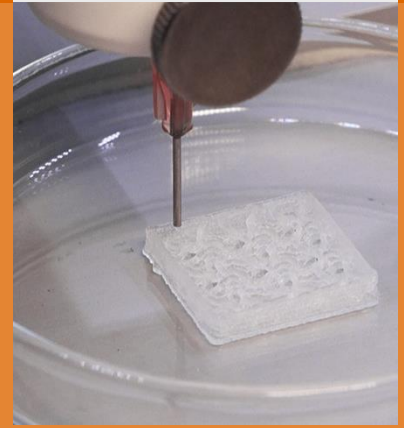


Fibercoll-Flex-A® preparation, adjustment of collagen concentration, printing and neutralization

Fibercoll-Flex-A® is an acidic bioink consisting of complex collagen type I fibers. After 3D printing and neutralization, it gives rise to mechanically highly stable collagen scaffolds without chemical crosslinking that provide a customized matrix for seeding cells on top. Scaffold stiffness can be adjusted by dilution of the bioink. Before cell seeding the printed scaffold needs to be neutralized.

For optimal results please follow this User Guide.

For technical support contact our team at sales@bio.viscofan.com.



Printed Fibercoll-Flex-A® scaffold

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GENERAL INTRODUCTION

This User Guide provides instructions to prepare Fibercoll-Flex-A® bioink for 3D printing by avoiding air bubbles and generating a homogenous mixture. The stock suspension of 5 wt% collagen can be diluted to concentrations between 2 and 5 wt% collagen. If necessary, the protocol can be adapted to an individual final collagen concentration. Examples for printing conditions are included and a neutralization protocol for the printed scaffold before cell seeding is described.

Precautions

- Store the Fibercoll-Flex-A® bioink at 2 to 8°C. Do not freeze.
- Bioprinting may be carried out at temperatures between 4 and 37°C.
- We recommend to use a standard 20G needle (provided) for printing - not conical tips.
- Please use appropriate cell culture plastics, media, and reagents as well as aseptic techniques, and ensure adequate conditions for cell growth.
- The provided protocol is a proposal and may be varied by the user according to his needs.

Required material

- Syringe containing 3 ml of Fibercoll-Flex-A®*
- Sterile 20G needle*
- Sterile syringes (luer-lock or other)
- Sterile connector
- Sterile syringe compatible with your bioprinter
- Sterile NaOH, 0.05M
- Sterile PBS
- Sterile cell culture medium
- Optionally: centrifuge
- Optionally: Sterile dH₂O

*provided in the kit

INTENDED USE

Fibercoll-Flex-A® is intended for research use only. It is neither intended for human nor animal diagnostic, therapeutic use nor any other clinical use.

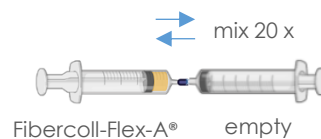
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Table 1: Dilution matrix for aimed collagen concentrations from 2 to 5 wt%

Aimed concentration of collagen [wt%]	Ratio of Fibercoll-Flex-A® stock suspension (stock 5 wt% collagen, 3 ml)	Ratio of sterile dH ₂ O
5	5	0
4	4	1
3	3	2
2	2	3

STEP 1

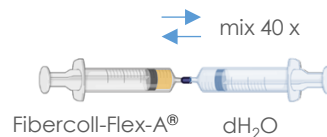
A first homogenization step is recommended: Unpack the syringe with Fibercoll-Flex-A® and connect it with a sterile, empty syringe using a junction. Then pass the bioink from one syringe to the other 20 times, thus generating a homogeneous mixture. Then fill the desired volume of Fibercoll-Flex-A® for printing into one syringe.



STEP 2

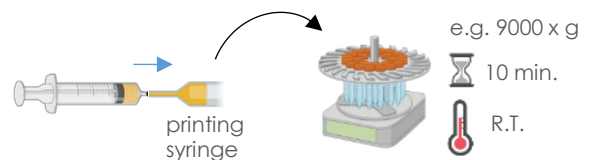
Optionally - if dilution is desired - connect the syringe containing Fibercoll-Flex-A® with a syringe containing sterile dH₂O according to the ratio described in table 1 and pass the content from one to the other 40 times, ensuring a homogeneous mixture.

Optionally:



STEP 3

If necessary, transfer the bioink to a syringe compatible with your bioprinter. Immediately before printing, we recommend to centrifuge the syringe to remove air bubbles. The Fibercoll-Flex-A® is now ready for printing.



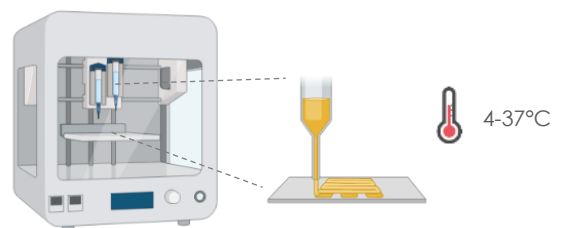
STEP 4

Print the scaffold at the desired temperature between 4 and 37°C.

Recommended conditions for a pneumatic extrusion based bioprinter, using a 20G needle, at 20°C are:

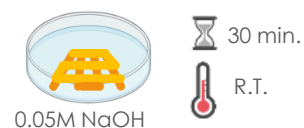
- for 5 wt% collagen: 300 kPa 5 mm/s
- for 3 wt% collagen: 150 kPa 5 mm/s

If needed, adjust the conditions by changing the pressure and speed of the printer.



STEP 5

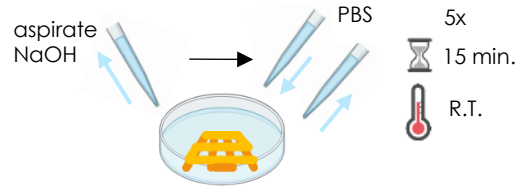
Following printing, neutralize the scaffold by covering it completely with sterile 0.05M NaOH for 30 min. at room temperature.



Fibercoll-Flex-A® preparation, adjustment of collagen concentration, printing and neutralization

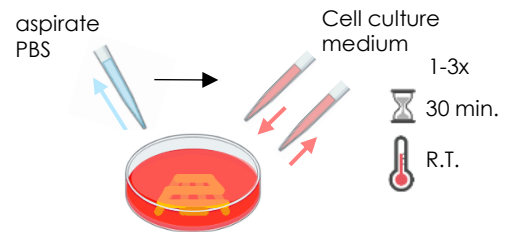
STEP 6

Discard the NaOH and add PBS until the scaffold is completely covered. Incubate for 15 min. at room temperature.
Repeat the washing step four more times.



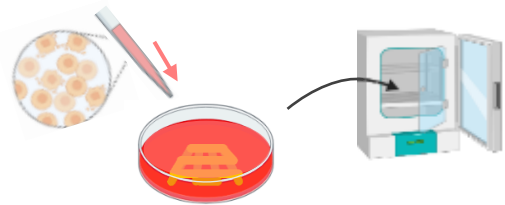
STEP 7

Discard the PBS and cover the scaffold completely with cell culture medium for 30 minutes at room temperature.
The scaffold should have turned rose by now (in case the medium contains phenol red). If the scaffold is fuchsia pink or white, carry out two more 30-minute washes before cell seeding.



STEP 8

The scaffold is now ready for cell seeding. Afterwards, please ensure that the scaffold is completely submerged in cell culture medium and incubate at suitable conditions.



Most tool-symbols are derived from BioRender.

PROBLEM	ANSWER
1. In case my bioink has air bubbles , are they going to interfere with printability?	<ul style="list-style-type: none"> • Small bubbles will not interfere with printing. A centrifugation step can be performed to remove most air bubbles (step 3). • The homogenization step (step 1) will reduce the size of air bubbles to facilitate the printing process.
2. I do not want to use the whole syringe at once. Can I use only a part of it?	After homogenization in step 1, transfer the desired amount of Fibercoll-Flex-A® to a sterile syringe and continue the protocol. To dilute it, follow the ratio collagen : dH ₂ O described in Table 1.
3. Fibercoll-Flex-A® is very viscous , and my printer can't print it.	<ul style="list-style-type: none"> • Option 1: Dilute the bioink to obtain a less viscous solution. • Option 2: Use a slower printing speed to be able to print a scaffold at lower pressure. • Option 3: Use an external pump to print at higher pressure.
4. The extrusion of bioink is not continuous or not homogenous .	<ul style="list-style-type: none"> • Make sure that you are using a needle (provided in the kit) or similar for printing instead of conical tips. • Make sure that the printing pressure is high enough to extrude the chosen collagen concentration of the Fibercoll-Flex-A® (note recommended settings in step 4). • To avoid interference of air bubbles please consider problem & answer 1.

All data and recommendations correspond to the present state of our knowledge; they are published without engagement. We reserve the right to make alterations and additions in line with technical developments without prior notice. The customer is obliged to check whether our products meet the technical requirements. Please contact us for questions or support.



Fibercoll-Flex-A®

VWR catalog number: 76628-720

Contact us for support

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