Ref No: PR-TE-0101010101
Date: 8-NOV-2019
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PROTOCOL

VasKit

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

Protocol aim

The aim of this protocol is to provide instructions for how to use the VasKit device. The VasKit perfusable device is a bioreactor developed for channel perfusion and visual inspection of fully or partially bioprinted tissues. It has four female Luer lock connectors serving as either inlet or outlet points, each leading to a gel connector.

Materials needed

Kit components:

- VasKit device
- 2 x 3 mL cartridges of GelMA C with one bottle of Crosslinking Agent
- 2 x 3 mL cartridges of CELLINK PLURONICS
- 2 x Luer lock adaptors
- Bioprinting nozzles

Other components needed:

- 1.5 mm Allen Key
- Stopcocks (4 pcs)
- Luer plugs (4 pcs)
- Luer female to barb connectors
- BIO X*, BIO X6* or INKREDIBLE-series* 3D bioprinter

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

Protocol

The VasKit perfusable device is a bioreactor developed for channel perfusion and visual inspection of fully or partially bioprinted tissues. It has four female Luer lock connectors serving as either inlet or outlet points, each leading to a gel connector.

The VasKit device have bounding box dimensions of 36x85x19 mm, a maximal build volume of 6 CC, the distance between channel and glass is 1 mm and the made of USP Class VI Biocompatible SLA resin, glass, EPDM 70, stainless steel, medical device grade silicone and nylon.

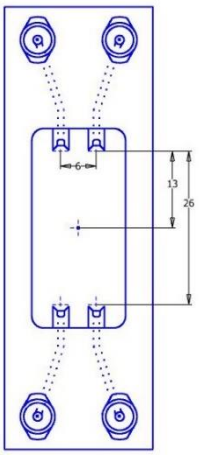

***Figure 1**. Illustration of the VasKit including the distance between channels.*

1. Preparing the VasKit

DESCRIPTION

- The VasKit device is reusable. Sterilization methods: UV, 70% EtOH, autoclavable at 121°C for 15 minutes.
- Before start, autoclave nozzles, Luer plugs and Luer female to barb connectors.

2. Printing tips

DESCRIPTION

Printing of the channels

- To print channels, CELLINK PLURONICS can be easily printed at room temperature, then liquefied below 4°C and removed by suction. See *Printing Protocol Pluronics 40%* for instructions on how to print Pluronics.

Printing of infill:

- For the infill material around the channels, we recommend GelMA C, a GelMA based bioink which can be photocrosslinked. To retain the gel shape during incubation and perfusion, the GelMA C also contains nanocellulose and alginate which makes the bioink crosslinkable using $CaCl_2$ containing Crosslinking Agent.

Printing CELLINK PLURONICS on GelMA C:

- When using a 3D bioprinter, it can be a challenge to precisely layer a CELLINK PLURONICS filament on top of a hydrogel. The CELLINK PLURONICS display a slippery behaviour that would sometimes result in failure in achieving the desired geometry. It is therefore extra important that the casted bottom layer is as flat as possible and to position the nozzle at correct height.
- A way of printing channels embedded in GelMA C is to cast a thin gel layer (at least 500 µL) of incubator warmed GelMA C on the bottom of the flow device and knock it gently against the desk to generate a flat surface. Let the gel cool for a short while to gel. Then print a CELLINK PLURONICS channel between two inlets/outlets. Cast another layer of warm GelMA C in order to cover the CELLINK PLURONICS. Photocrosslink and evacuate the PLURONICS by gently flushing with chilled water. Gravity is often enough to pull cold liquefied out when tilting the device. Make sure not to use excessive forces.

Coaxial printing:

- Another channel printing method is to use co-axial needles where CELLINK PLURONICS is situated in the filament core, surrounded by GelMA C at the periphery. On the bottom of the VasKit device, a thin layer of GelMA C can be casted. When printing between two gel connectors, only CELLINK PLURONICS would be extruded at the start and end points, whereas both CELLINK PLURONICS and GelMA C would be extruded along the channel path. Full channel embedding can be done by casting GelMA C over the channel filament and start/end points, followed by photocrosslinking and CELLINK PLURONICS evacuation by gently flushing with chilled water.