

# A Multi-Cue Bioreactor to Evaluate the Inflammatory and Regenerative Capacity of Biomaterials under Flow and Stretch

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## Materials

Name	Company	Catalog Number	Comments
advanced Dulbecco's modified EagleMedium (aDMEM)	Gibco	12491-015	cell culture medium for fibroblasts
Aqua Stabil	Julabo	8940012	prevent microorganism growth in bioreactor-hydraulic reservoir
Bovine fibrinogen	Sigma	F8630	to prepare fibrinogen gel to seed the cells on the electrospun scaffold
Bovine thrombin	Sigma	T4648	to prepare fibrinogen gel to seed the cells on the electrospun scaffold
Centrifuge	Eppendorf	5804	to spin down cells and conditioned medium
Clamp scissor - "kelly forceps"	Almedic	P-422	clamp the silicone tubing and apply pre-stretch to the scaffold so the scaffold can be sutured into the engraved groove (autoclave at step 1, step 7)
CO2 cell culture incubators	Sanyo	MCO-170AIC-PE	for cell culturing
Compressed air reservoir	Festo	CRVZS-5	smoothing air pressure fluctuations and create time delays for pressure build-up
Custom Matlab script to calculate the maximum stretches	Matlab	R2017. The Mathworks, Natick, MA	calculate the minimum and maximum outer diameter of the electrospun scaffold
Data acquisition board	National Instruments	BNC-2090	data processing in between amplifier system and computer
Ethanol	VWR	VWRK4096-9005	to keep sterile working conditions
Fetal bovine calf serum (FBS)	Greiner	758087	cell culture medium supplement; serum-supplement

Flow culture chamber compartments, consisting of a pressure conduit with engraved grooves and small holes to apply pressure on silicone tubing, a screw thread, nose cone, top compartment with flow inlet and bottom compartment flow outlet, adapter bushing	Custom made, Department of Biomedical Engineering, Eindhoven University of Technology	n.a.	flow culture chamber compartments (autoclave at step 1, step 7)
Glass Pasteur pipet	Assistant	HE40567002	apply vacuum on electrospun scaffold (autoclave at step 1)
Glass tubes of the flow culture chamber	Custom made, Equipment & Prototype Center, Eindhoven University of Technology	n.a.	part of the flow culture chamber (clean and store in 70% ethanol, at step 1 and 7)
GlutaMax	Gibco	35050061	cell culture medium amino acid supplement, minimizes ammonia build-up
High speed camera	MotionScope	M-5	to monitor the stretch during culture; time-lapse photographs of the scaffolds are captured at a frequency of 30 Hz for 6 sec (i.e. 3 stretch cycles)
High speed camera lens - Micro-NIKKOR 55mm f/2.8 - lens	Nikon	JAA616AB	to monitor the stretch during culture; time-lapse photographs of the scaffolds are captured at a frequency of 30 Hz for 6 sec (i.e. 3 stretch cycles)
Hose clip	ibidi GmbH	10821	block medium flow (autoclave at step 1, step 7)
Hydraulic reservoir with 8 screw threads for 8 flow culture chambers	Custom made, Department of Biomedical Engineering, Eindhoven University of Technology	n.a.	to apply pressure to the silicone mounted constructs (clean outside with a paper tissue with 70% ethanol, rinse reservoir with 70% ethanol followed by demi water, at step 1 and 7)
ibidi pump system (8x) including ibidi pump, PumpControl software, fluidic unit, perfusion set (medium tubing), air pressure tubing, drying bottles with orange silica beads	ibidi GmbH	10902	set up used to control the flow in the flow culture chambers. Note 1: the ibidi pumps were modified by the manufacturer to enable 200 mbar capacity. Note 2: can be replaced by pump system of other manufacturer, as long as same flow regimes can be applied.
Knives (no.10 sterile blades, individual foil pack) and scalpel handle (stainless steel, individually wrapped)	Swann Morton	0301; 0933	to cut the silicone tubing in the correct size for the scaffold and to cut the suture material
LabVIEW Software	National Instruments	version 2018	to control the stretch applied to the scaffolds
Laminar flow biosafety cabinet with UV light	Labconco	302310001	to ensure sterile working conditions. The UV is used to decontaminate everything that cannot be autoclaved, or touched after autoclaving
Large and small petri dishes	Greiner	664-160	for sterile working conditions
L-ascorbic acid 2-phosphate (vitamin C)	Sigma	A8960	cell culture medium supplement, important for collagen production
LED light cold source KL2500	Zeiss	Schott AG	to aid in visualization for the time lapse of the scaffolds during monitoring of the stretch
Luer (female and male) locks and connectors, white luer caps	ibidi GmbH	various, see ( <a href="https://ibidi.com/26-flow-accessories">https://ibidi.com/26-flow-accessories</a> )	to close or connect parts of the bioreactor and the ibidi pump (autoclave at step 1, step 7)

Measuring amplifier (PICAS)	PEEKEL instruments B.V.	n.a.	to amplify the signal from the pressure sensor and feedback to LabView
Medium reservoir (large syringes 60 mL) and reservoir holders	ibidi GmbH	10974	medium reservoir (autoclave at step 1, step 7)
Medium tubing with 4.25 mm outer diameter and 1 mm inner diameter	Rubber BV	1805	to allow for a larger flow rate, the ibidi medium tubing with larger diameter is used. Note: the part of medium tubing guided through the fluidic unit valves are the same as the default ibidi medium tubing
Motion Studio Software	Idtvision	2.15.00	to make the high speed time lapse images for stretch monitoring
Needle (19G)	BD Microlance	301700	together with thin flexible tubing used to fill the hydraulic reservoir with ultrapure water without adding air bubbles
Needle driver	Adson	2429218	to handle the needle of the nylon suture through the silicone tube (autoclave at step 1, step 7)
Paper tissues	Kleenex	38044001	for cleaning of the equipment with 70% ethanol
Parafilm	Sigma	P7793-1EA	quick fix if leakage occurs
Penicillin/streptomycin (P/S)	Lonza	DE17-602E	cell culture medium supplement; prevent bacterial contamination
Phosphate Buffered Saline (PBS)	Sigma	P4417-100TAB	for storage and washing steps (autoclave at step 1)
Plastic containers (60 mL) with red screw caps	Greiner	206202	to prepare the fibrinogen solution
Pneumatic cylinder	Festo	AEVC-20-10-I-P	to actuate the Teflon bellow (clean with a paper tissue with 70% ethanol at step 1 and 7)
Polycaprolactone bisurea (PCL-BU) tubular scaffolds (3 mm inner diameter, 200 $\mu$ m wall thickness, 20 mm length)	SyMO-Chem, Eindhoven, The Netherlands	n.a.	produced using electrospinning from 15% (w/w) chloroform (Sigma; 372978) polymer solutions. See Van Haaften et al Tissue Engineering Part C (2018) for more details
Pressure conduit without holes (for static control)	Custom made, Department of Biomedical Engineering, Eindhoven University of Technology	n.a.	to mount electrospun tubes on silicon tubing (autoclave at step 1, step 7)
Pressure sensor and transducer	BD	TC-XX and P 10 EZ	the air pressure going to the pneumatic actuated pump is raised until it reaches the set pressure
Proportional air pressure control valve and pressure sensor	Festo	MPPES-3-1/8-2-010, 159596	provides compressed air to the pneumatic actuated pump
Roswell Park Memorial Institute 1640 (RPMI-1640)	Gibco	A1049101	cell culture medium for monocyte/macrophage
Safe lock Eppendorf tubes (1.5 mL)	Eppendorf	30120086	multiple applications (autoclave at step 1)
Sodium dodecyl sulfate solution 20%	Sigma	5030	Used to clean materials, at a concentration of 0.1%.
Silicone O-rings	Technirub	1250S	to prevent leakage (autoclave at step 1, step 7)
Silicone tubing (2.8 mm outer diameter, 400 $\mu$ m wall thickness)	Rubber BV	1805	to mount the electrospun tubes on the pressure conduits (autoclave at step 1)
Sterile tube (15 mL)	Falcon	352095	multiple applications
Suture, 5-0 prolene with pre-attached taper point needle	Ethicon, Johnson&Johnson	EH7404H	Prolene suture wire 5-0 (75cm length, TF taper point needle, 1/2 circle, 13 mm needle length)

Syringe (24 mL)	B. Braun Melsungen AG	2057932	to add the ultrapure water or medium to the hydraulic reservoir or flow culture chamber
Syringe filter (0.2 µm)	Satorius	17597-K	to filter the fibrinogen solution
T150 cell culture flask with filter cap	Nunc	178983	to degas culture medium
T75 Cell culture flask with filter cap	Nunc	156499	to culture static control samples
Teflon bellow	Custom made, Department of Biomedical Engineering, Eindhoven University of Technology	n.a.	to load the hydraulic reservoir (clean outside with a paper tissue with 70% ethanol at step 1 and 7)
Tray (stainless steel)	PolarWare	15-248	for easy transport of the fluidic culture chambers and the bioreactor from incubator to laminar flow cabinet and back (clean with a paper tissue with 70% ethanol before and after use)
Tweezers	Wironit	4910	sterile handling of individual parts (autoclave at step 1 and 7)
Ultrapure water	Stakpure	Omniapure UV 18200002	to correct for medium evaporation, mixed with aqua stabil mixed and used as hydraulic fluid. (autoclave ultrapure water at step 1)
UV light	Philips	TUV 30W/G30 T8	for decontamination of grafts and bioreactor parts before seeding