

Bioreaktory, buněčná stimulace

Biotechnologie, regenerativní medicína, tkáňové inženýrství,
biomateriály a nanotechnologie, biosenzory

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EVROPSKÁ UNIE
Evropské strukturální a investiční fondy
Operační program Výzkum, vývoj a vzdělávání



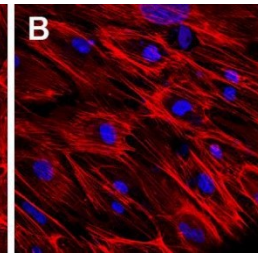
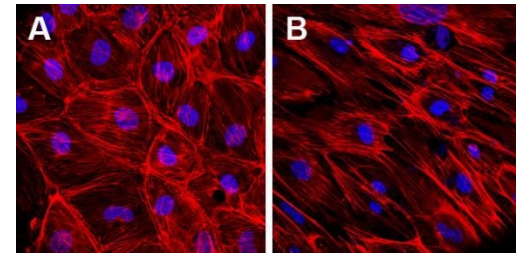
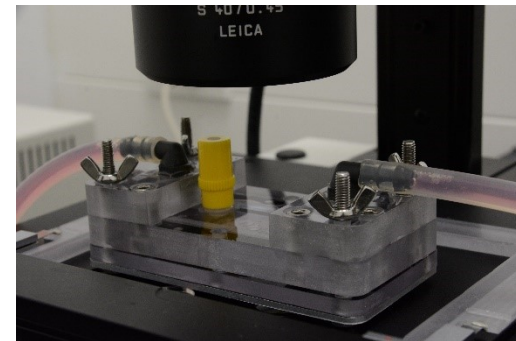
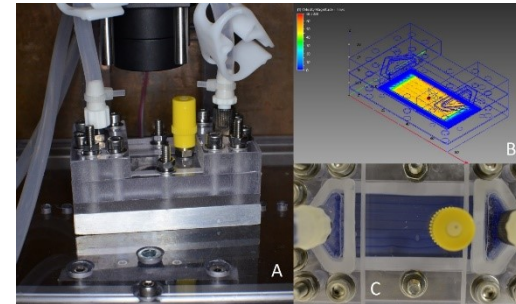
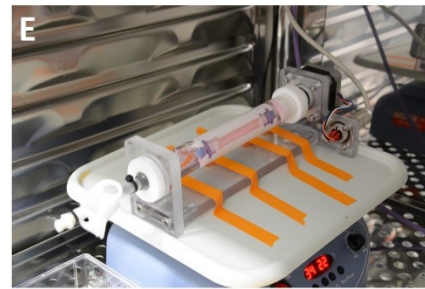
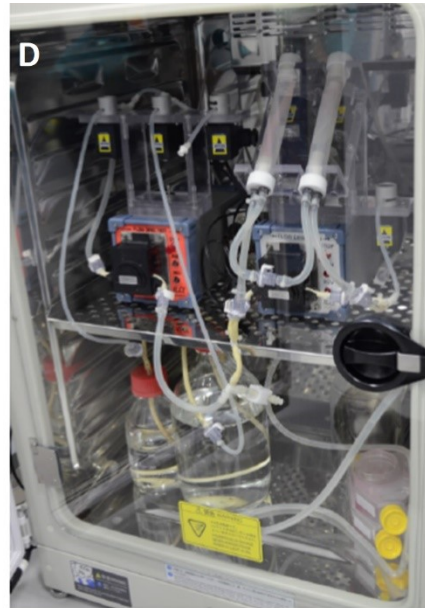
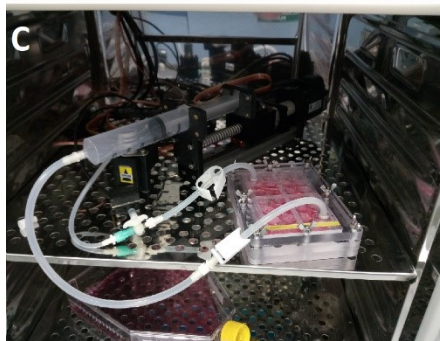
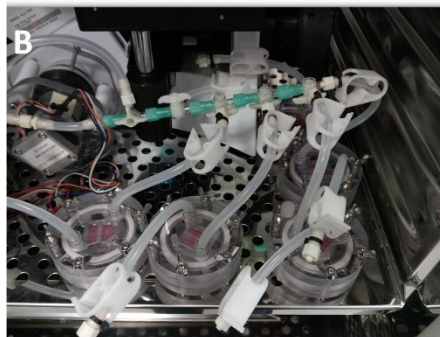
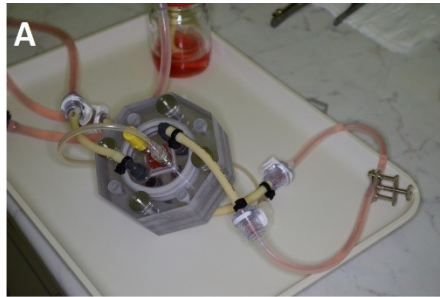
MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY

Vzniklo za podpory projektu Biomedicínské inženýrství pro znalostní ekonomiku
reg. č.: CZ.02.2.69/0.0/0.0/16_018/0002242

Dynamické podmínky

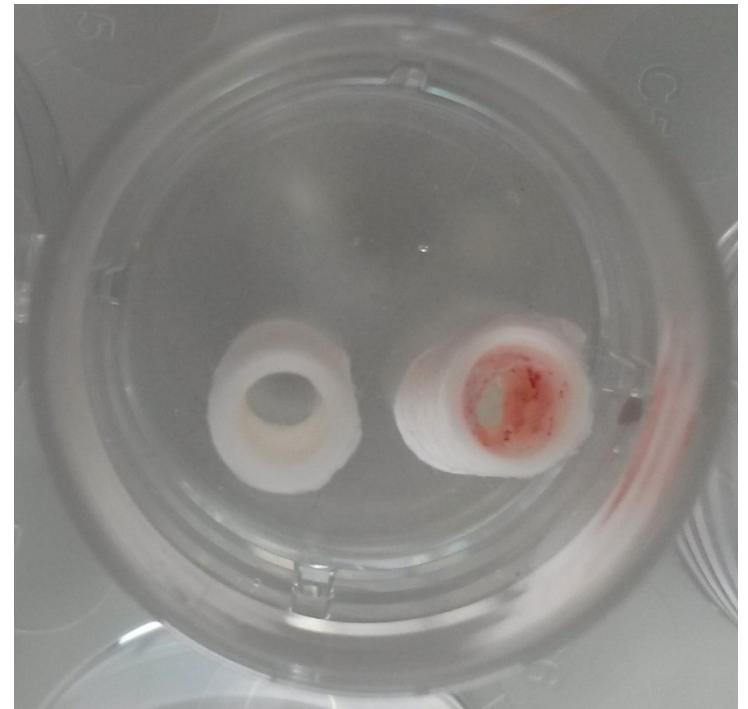
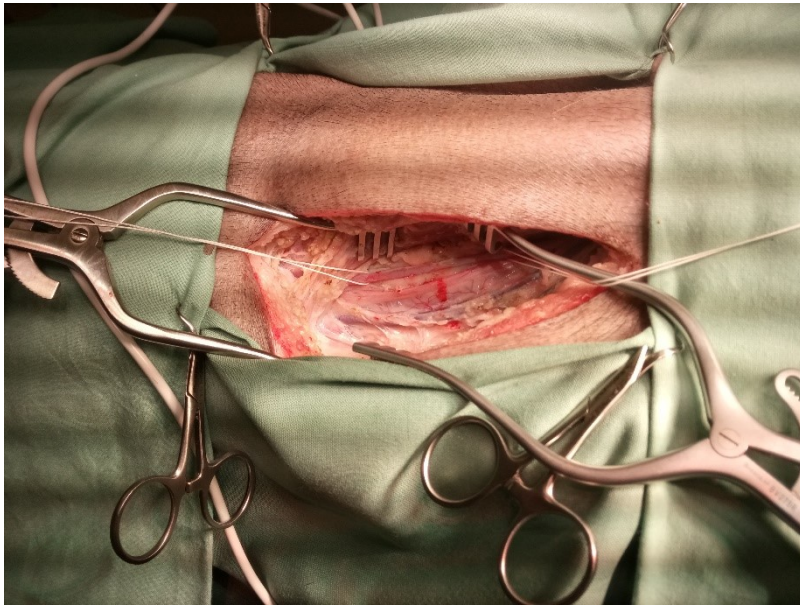
- Vytvoření věrných podmínek simulujících tělo
 - Chemické stimuly – růstové faktory a další látky ovlivňující chování buněk, kompartment.....
 - Mechanické stimuly – proudění a smykové napětí, tlak, tah, ohyb...
 - Elektrické stimuly – elektrické pole, aktivaci a inhibice kanálů...
 - Vzájemná synergie
- Nutnost
 - Metabolismus buněk
 - Fenotypická maturace
 - Diferenciace kmenových buněk
 - Produkce proteinu a tvorba extracelulární hmoty

Dynamické podmínky



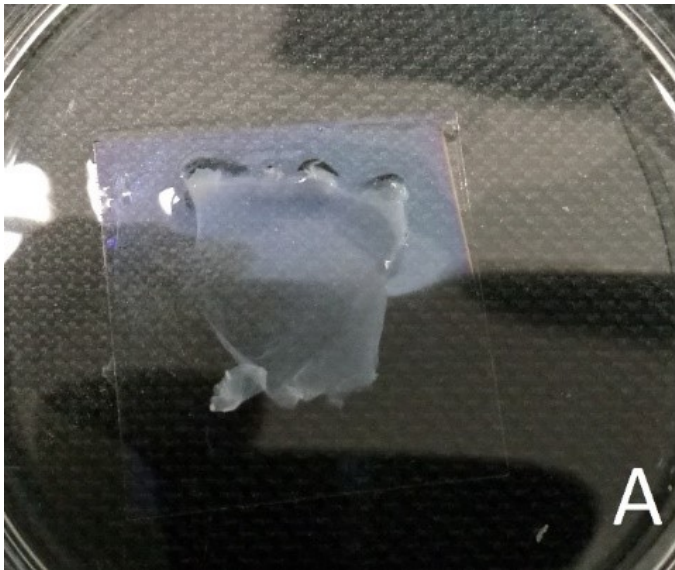
Tkáňové náhrady

- Osídlení umělého / decelularizovaného nosiče buňkami
- Optimalizace povrchů scaffoldů, testování kompatibility,
- Cirkulující buňky
- Maturace před implanatací



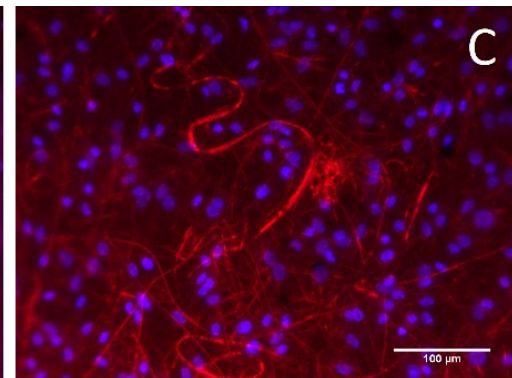
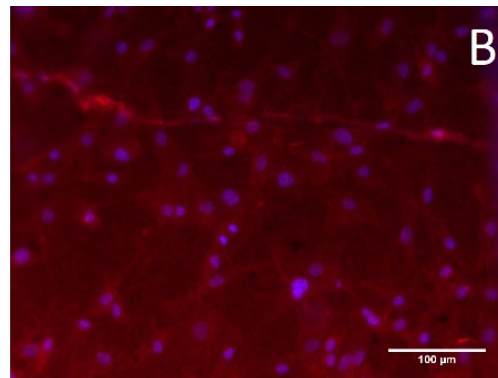
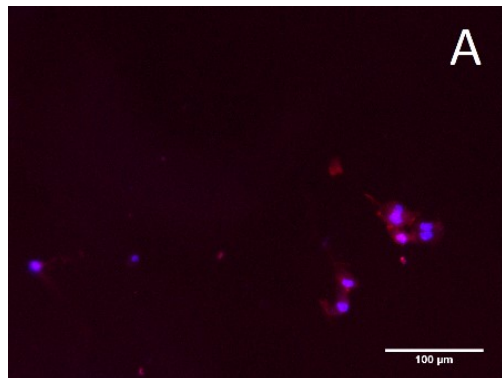
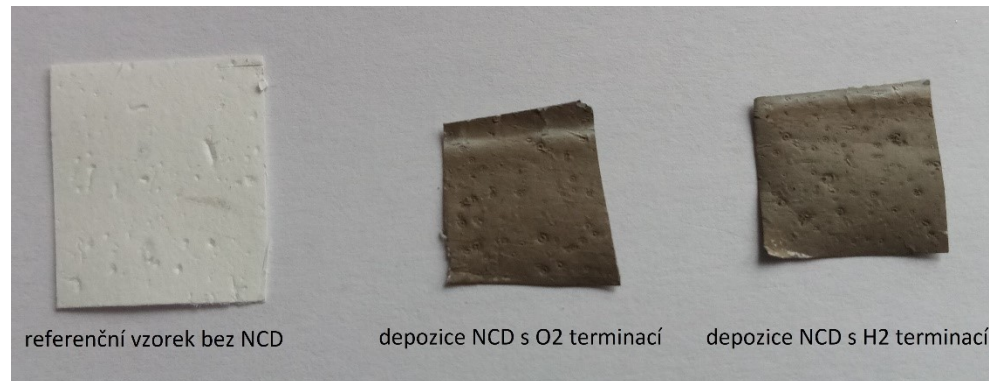
Modelové substráty – gely

- Problém řízení systémů – optimalizace nastavení pro neprůhledné substráty
- Použití skel, průhledných polymerů – nutnost plazmování – gelové nosiče



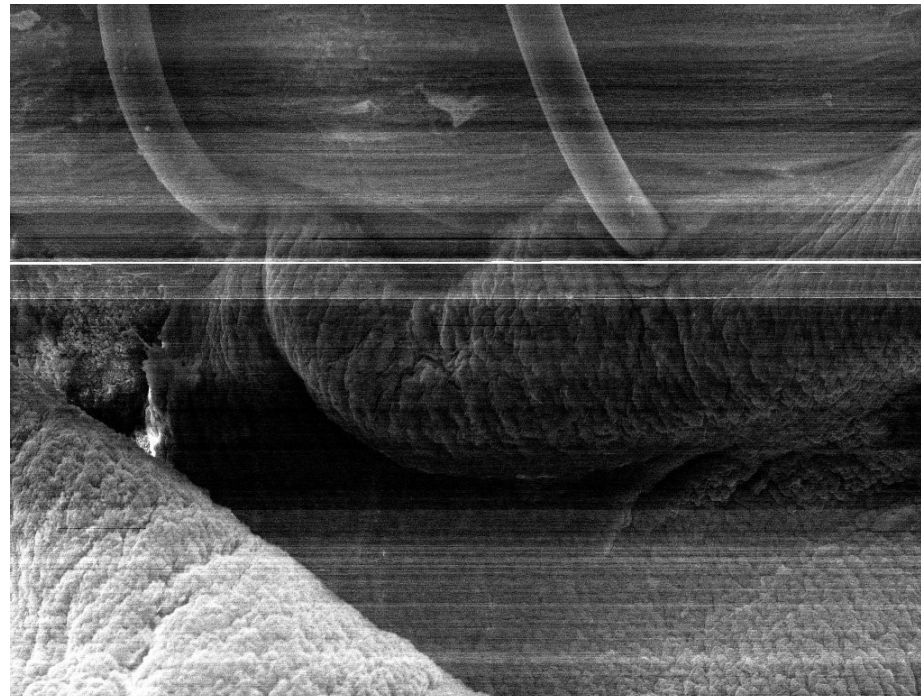
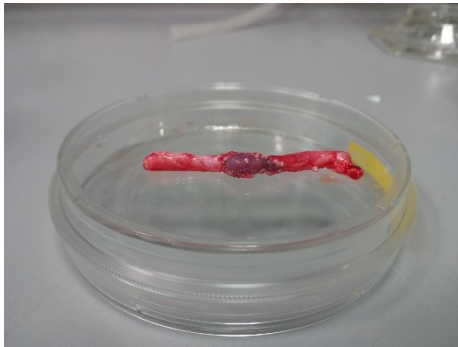
Modelové substráty - nanovláknna

- Polymerní nanovláknna
 - Biodegradabilní
 - Samonosné povrchy



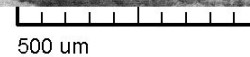
Decelularizované nosiče

- Xenogenní tkáň zbavené původních buněk
 - Prasečí arterie a perikardy → kardiovaskulární aplikace

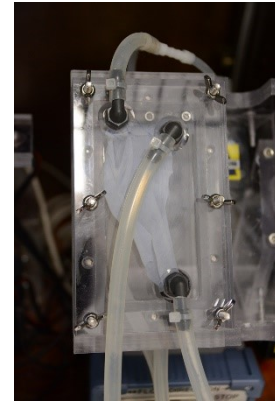


SEM MAG: 100 x
HV: 30.0 kV
VAC: HiVac

DET: SE Detector
DATE: 12/08/17
Device: 5135

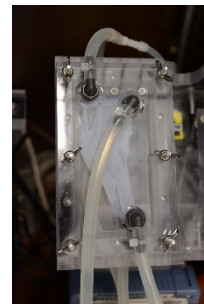
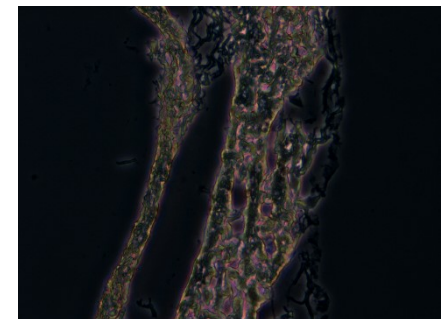
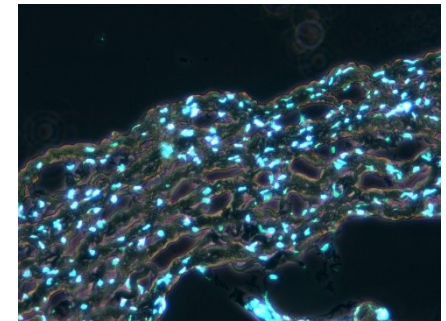
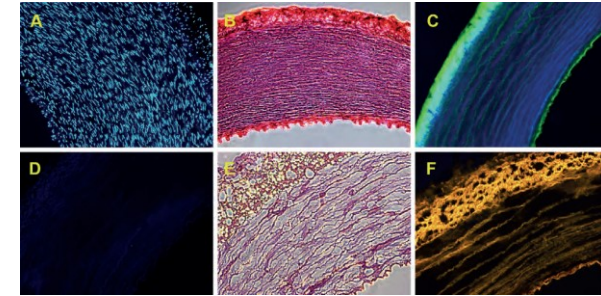
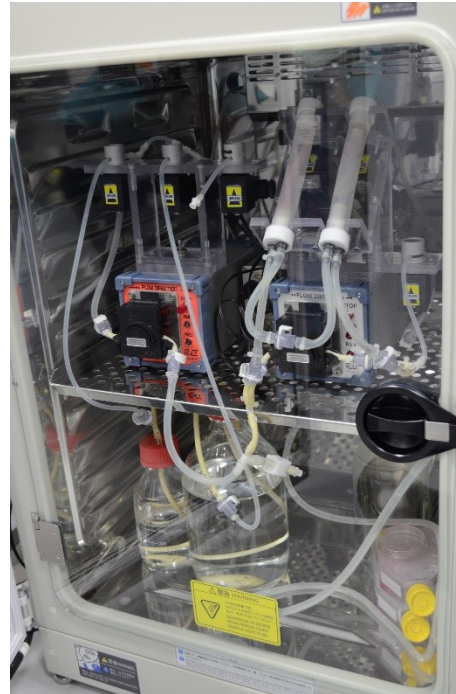


Vega ©Tescan
Digital Microscopy Imaging



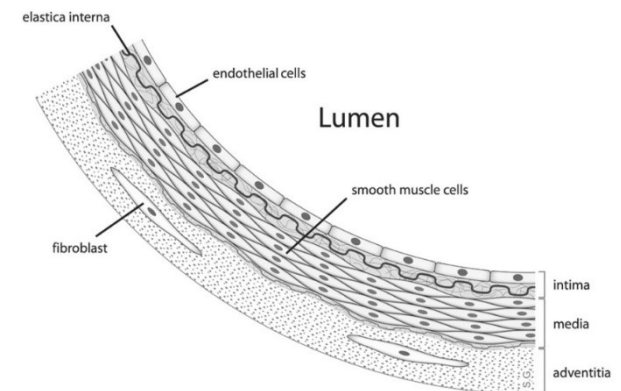
Decelularizované nosiče

- Porcine or bovine vessels and pericardium
- Automation of process
- Used as scaffolds for further cardiovascular applications
 - Surface modification and recellularization
 - Vessel replacements
 - Arterial patches



Krevní řečiště

- Krev
 - Není Newtonovská kapalina
 - Tekutina, krevní elementy
 - Viskozita 4 – 6 vyšší než voda
 - Prouděním působí mechanickou zátěží – smykové napětí
- Krevní céva - elastická trubice
 - Tlakové podmínky mění její průměr a délku
- Kultivační láhev a médium
 - Statické podmínky – buňky mají pouze kamaráda
 - Nízká viskozita



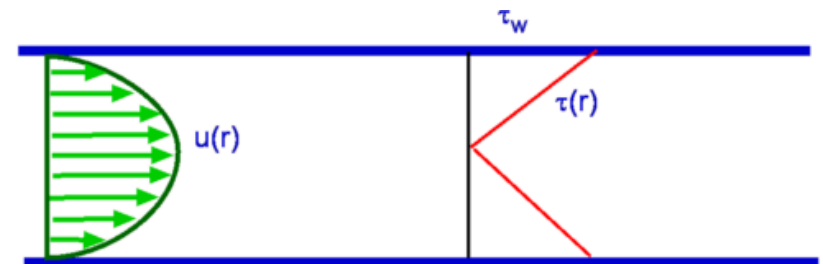
Smykové napětí

- Závislost na geometrickém tvaru, a vlastnostech proudění

Average physiological values of shear stress

| | Shear stress (dyne/cm ²) |
|-----------------------|---|
| Aorta | 12 |
| Large arteries | 13 |
| Arteries | 53 |
| Capillaries | 44 |
| Veins | 18 |
| Large veins | 8 |
| Vena cava | 2 |

$$\tau_w = \mu \frac{dv}{dx}$$

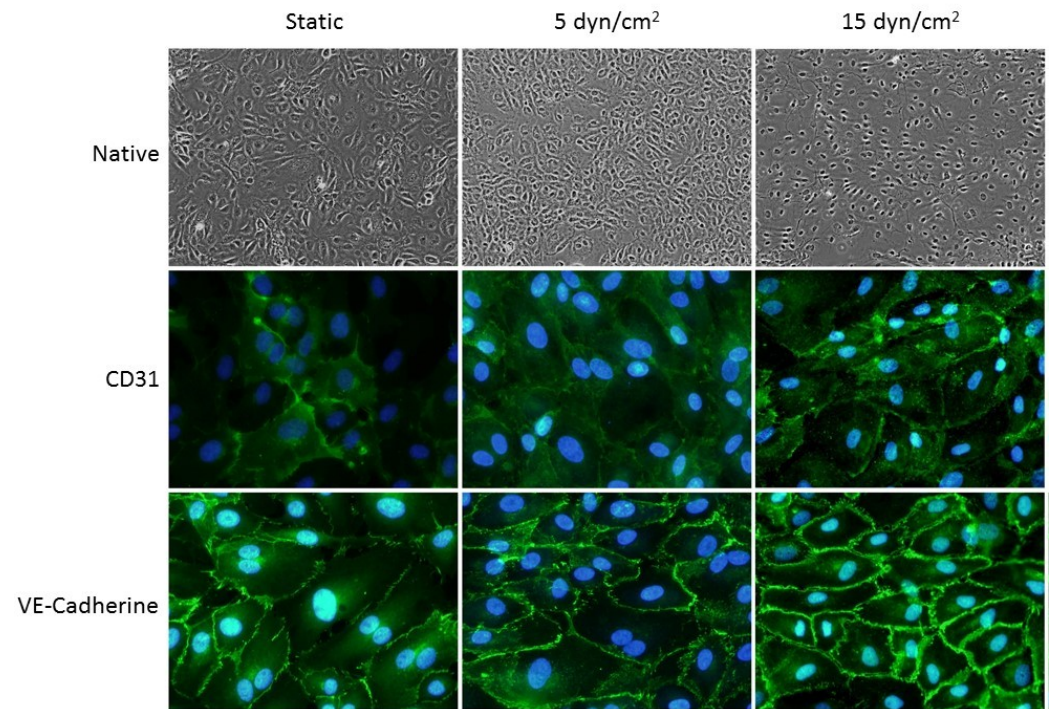
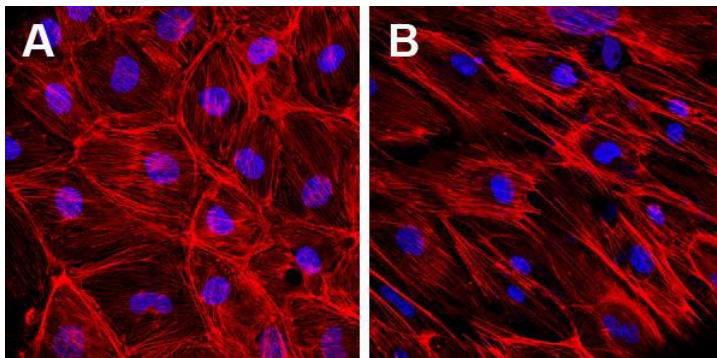
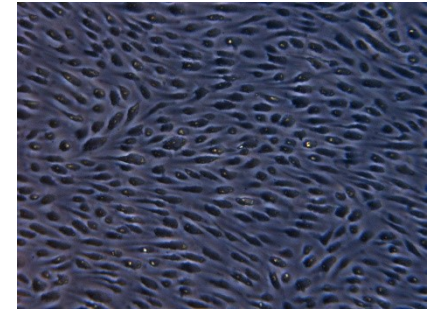


$$Re = \frac{Q \cdot D_H}{v \cdot A} = \frac{2Q}{v \cdot (b + h)}$$

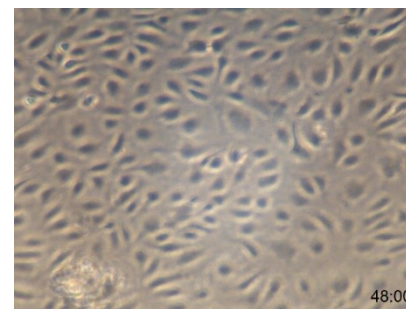
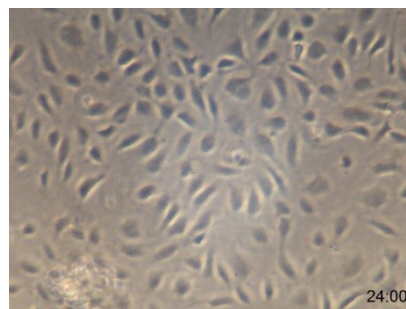
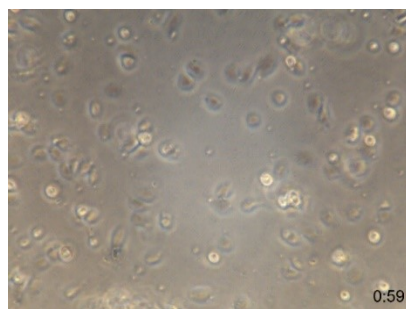
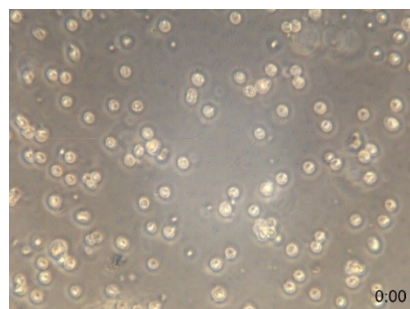
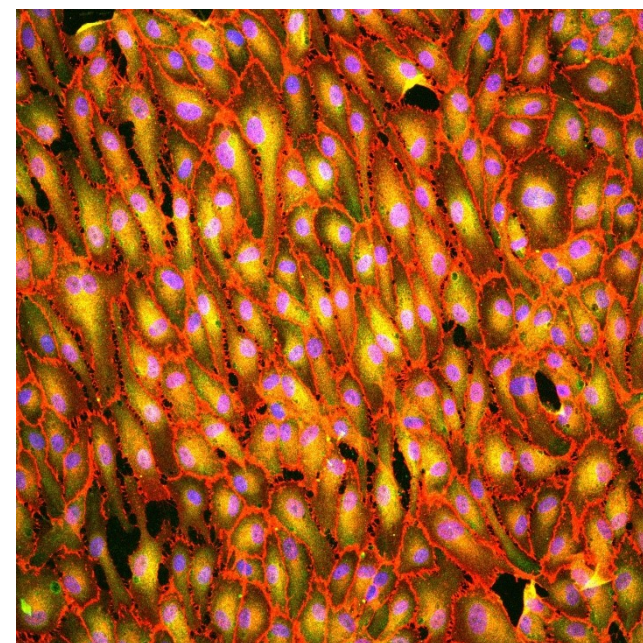
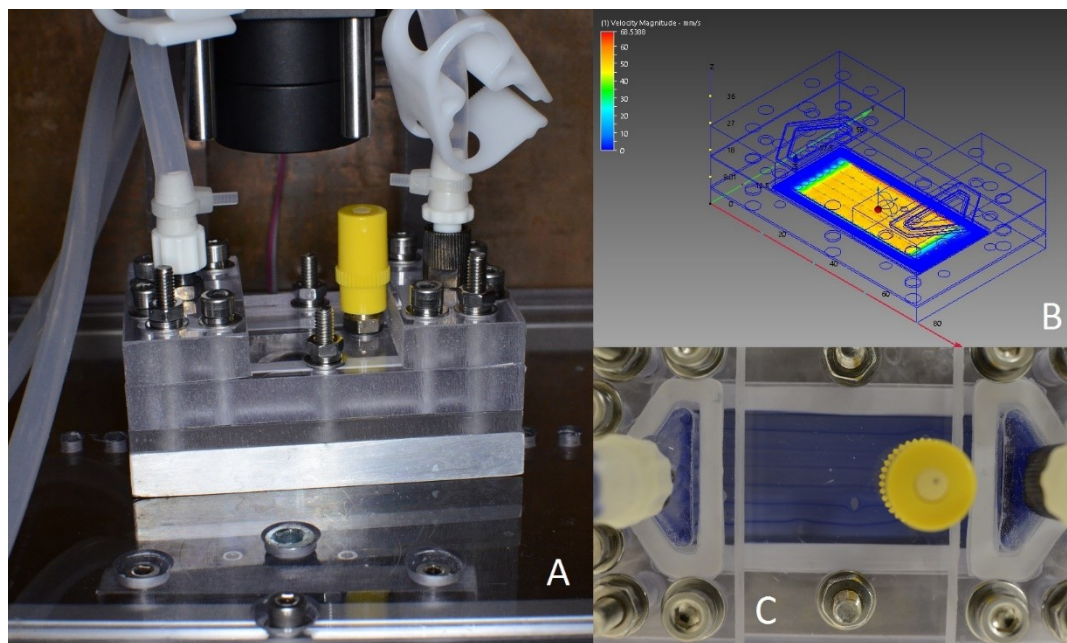
$$\tau_w = \frac{6 \cdot \eta \cdot Q}{h^2 \cdot b}$$

Vliv smykového napětí

- Organizace buněčné struktury – wall brick
- Orientace ve směru proudění
 - V případě patologického stavu kolmo
- Laminární vs. Turbulentní proudění

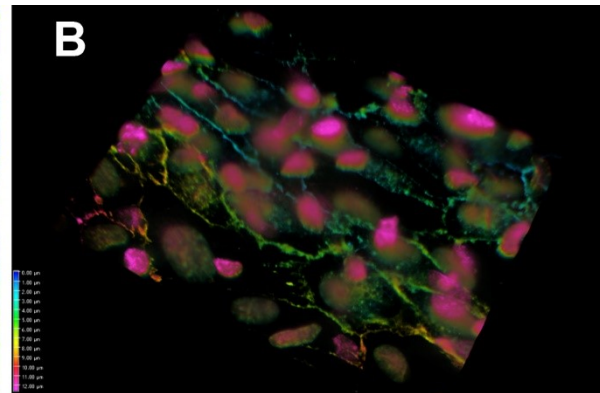
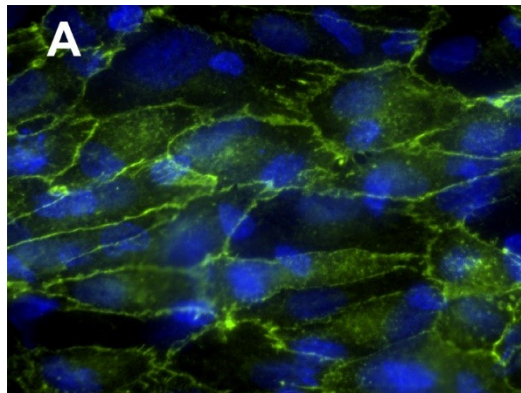
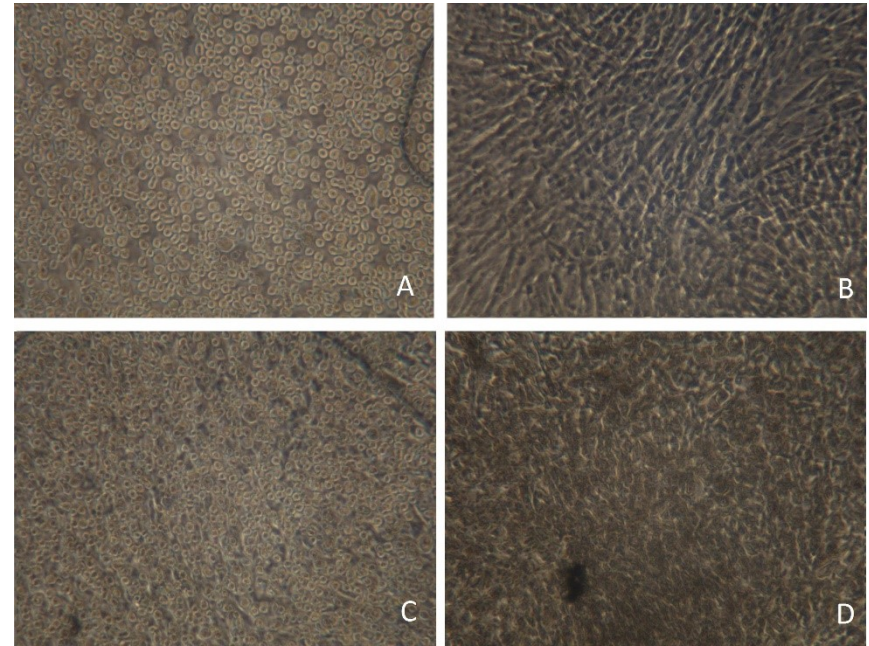


Vliv smykového napětí



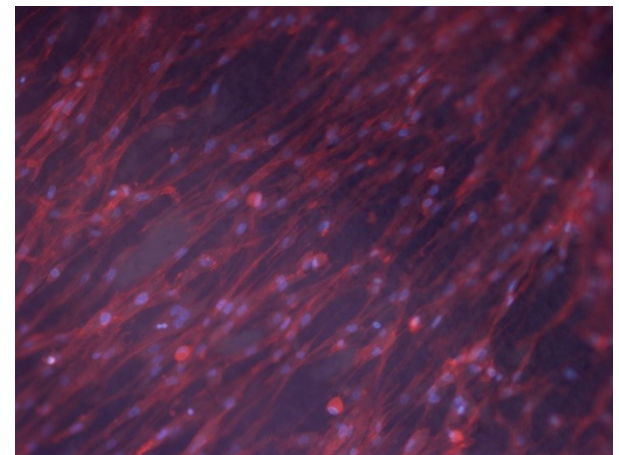
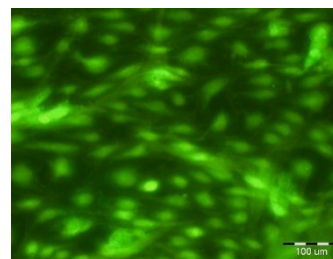
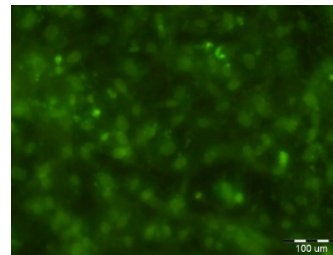
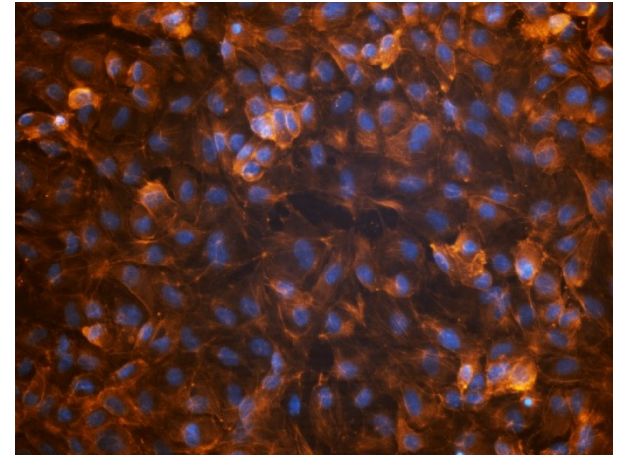
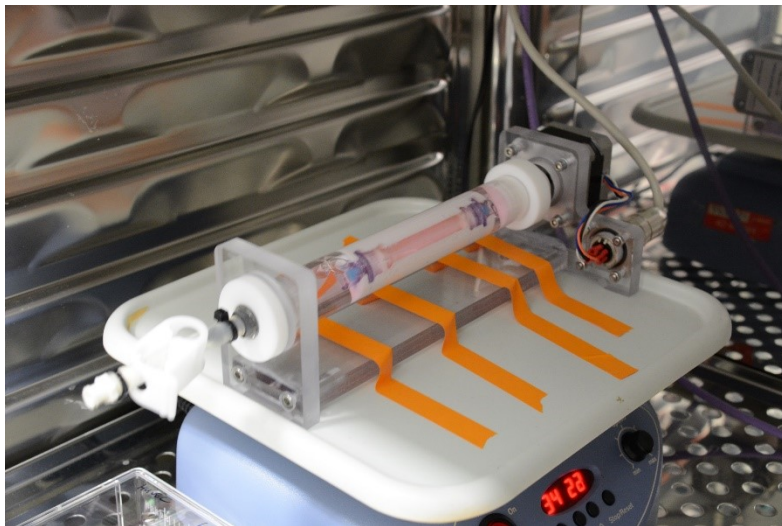
Kokultura v dynamickém systému

- ASC + EC
- EC vytváří ochranu
- ASC diferenciaci do SMC
 - Produkce kolagenu
 - Stavební struktura



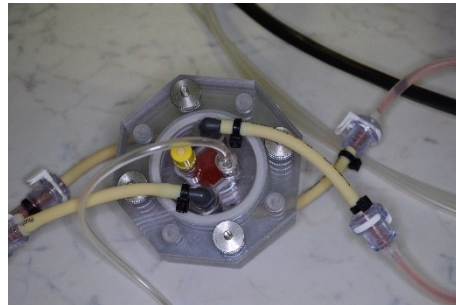
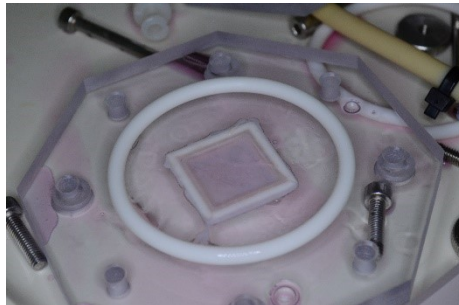
3D systémy – cévní protězy

- Kultivace uvnitř decelularizované cévy
- Nutnost zvýšení viskozity – krev
- Rotační osazení
- Proudění trubicí



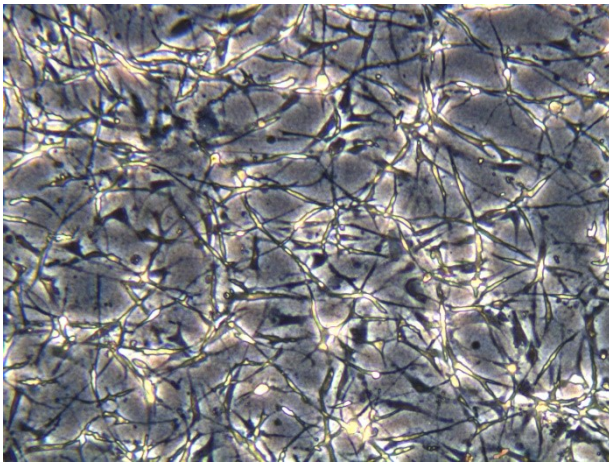
Tlakové poměry

- Dynamické podmínky způsobené srdeční prací
- Střední, systolický a diastolický tlak
- Vlivem tlaku dochází
 - Natažení cévy
 - Změny průměru
- Nastartování metabolismu
- Podpora diferenciacce buněk

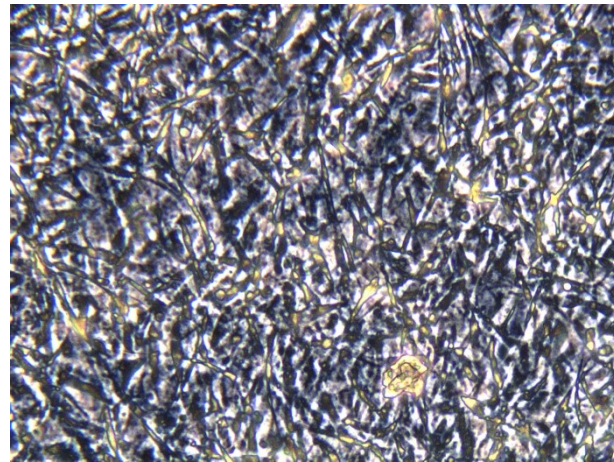


Tlakové poměry

- Thick Fb gel (3D), ca. 300 μm
- Hypertenze 150 / 100 mmHg, 1 Hz
- ASC ca. 350 000 / cm^2
- DMEM + FS and TGF-B and BMP4
- Týdenní kultivace



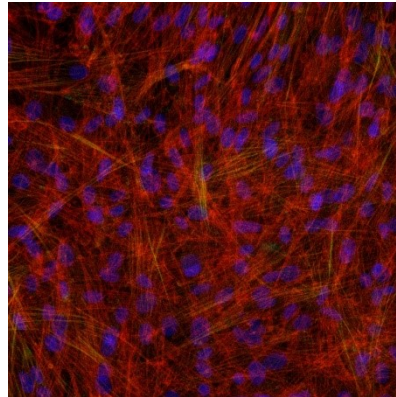
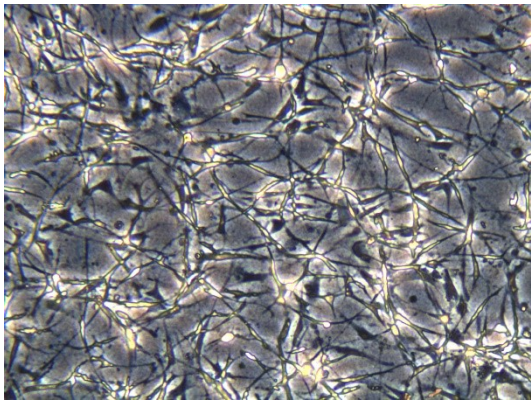
Static



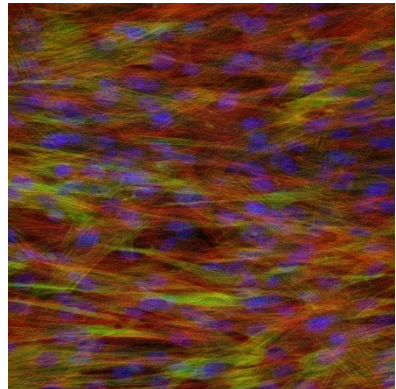
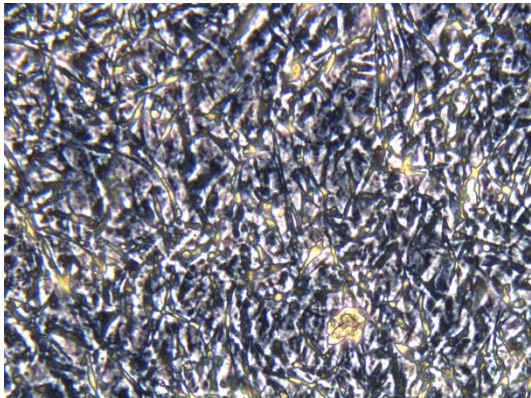
Dynamic

Tlakové poměry

- Diferenciace ASC směrem SMC
- Produkce nové ECM



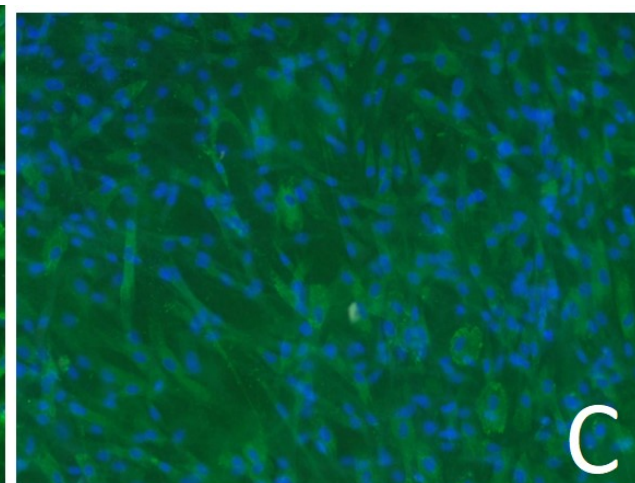
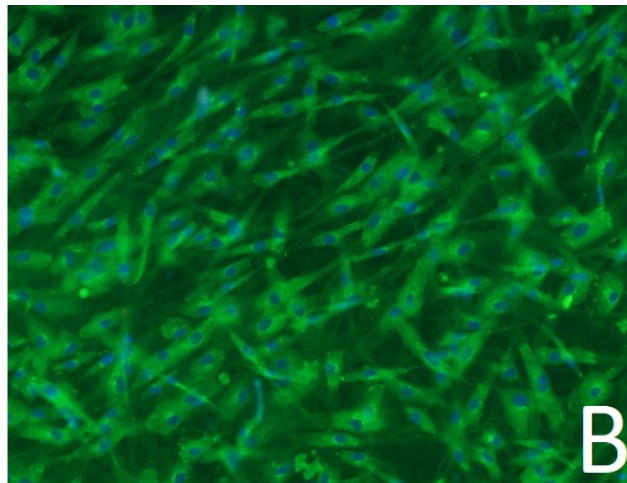
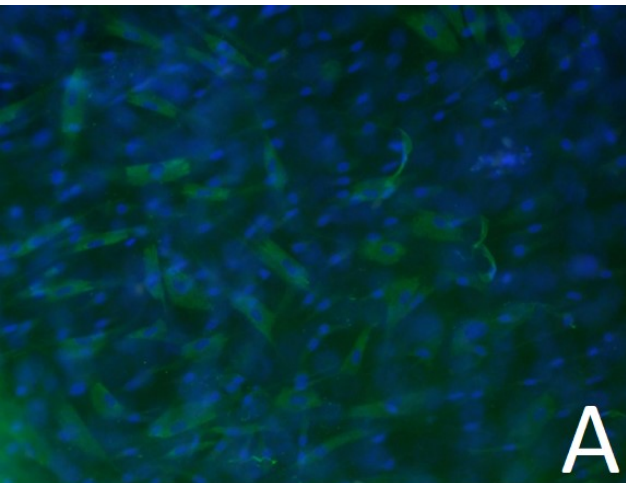
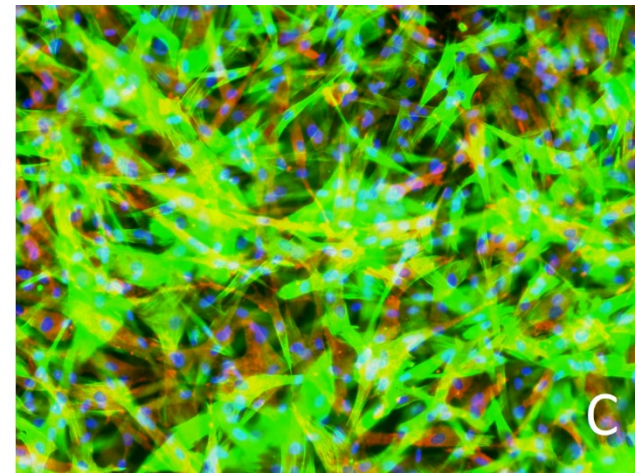
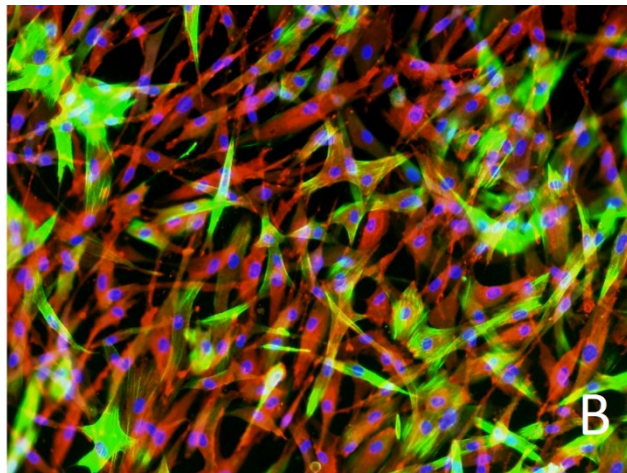
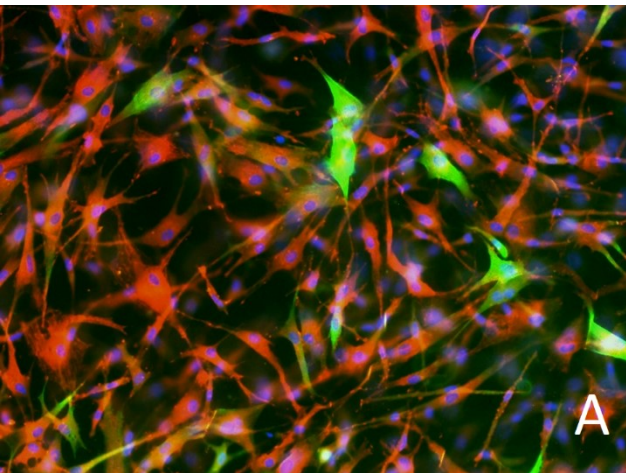
Static



Dynamic



Tlakové poměry



ASC – 17H belly
2nd passage

DAPI
Calponin
 α -actin

Dynamic conditions:
100/80 mmHg
1 Hz

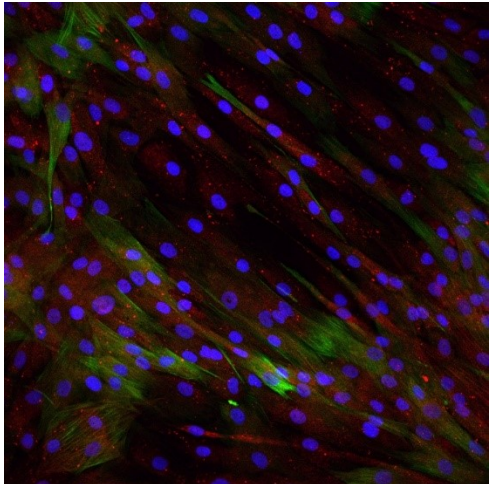
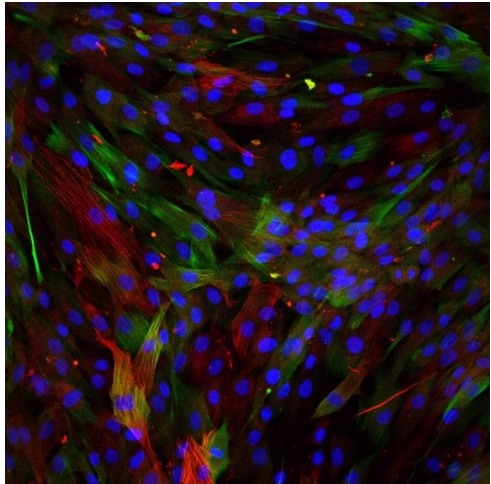
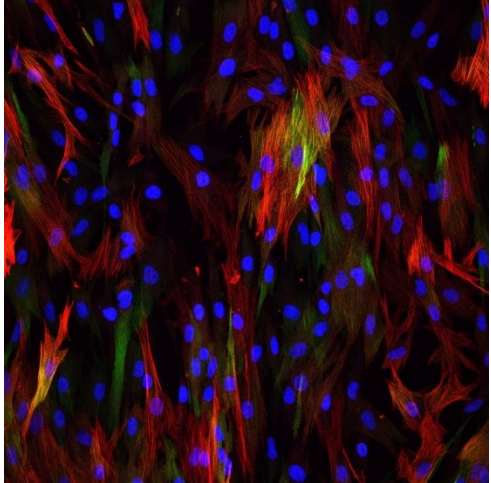
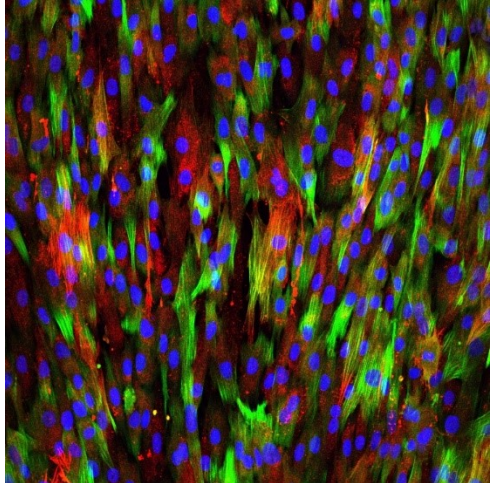
Differentiation medium:
DMEM (HG) +
1 % ABAM
10 % FS +
2,5 ng/ml TGF- β +
2,5 ng/ml BMP4 +
50 μ g/ul Ascorbic Acid

Proliferation medium:
DMEM (HG) +
1 % ABAM
10 % FS +
10 ng/ml FGF2

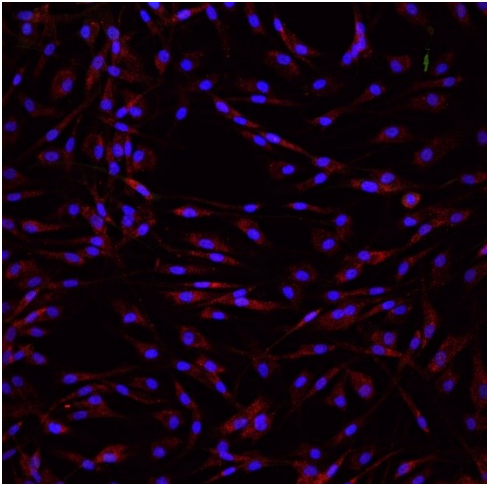
Dynamic – differentiation medium Static – differentiation medium

Fibrin

Glass (plasma treated)



Static – proliferation medium



PrASC – 001

2nd passage

DAPI
Calponin
 α -actin

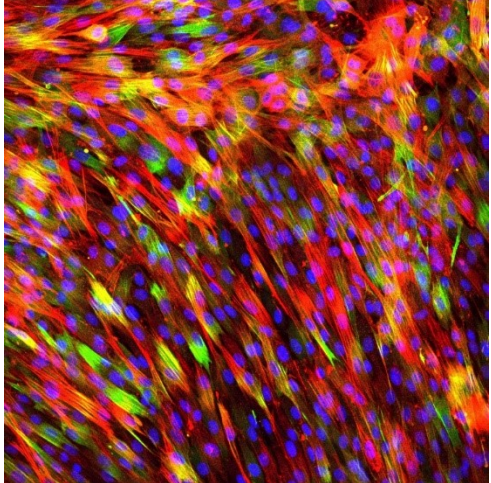
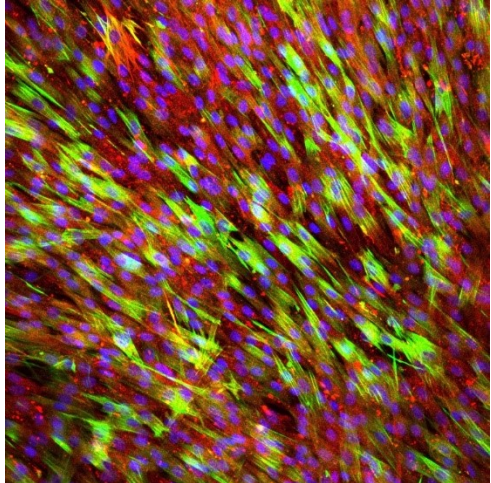
Dynamic conditions:
100/80 mmHg
1 Hz

Differentiation medium:
DMEM (HG) +
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10 % FS +
2,5 ng/ml TGF- β +
2,5 ng/ml BMP δ +
50 ug/ul Ascorbic Acid

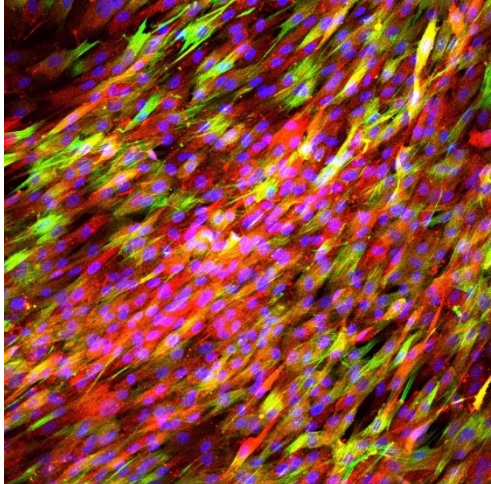
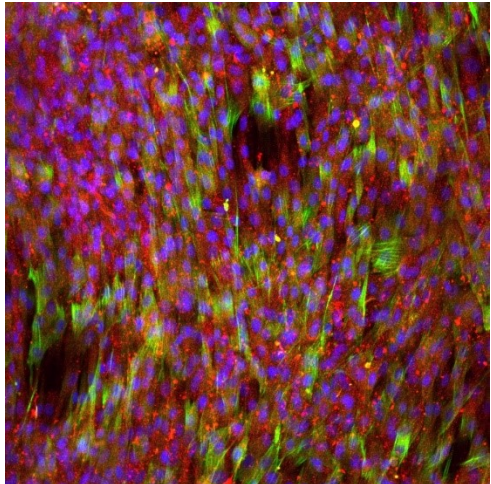
Proliferation medium:
DMEM (HG) +
1 % ABAM
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10 ng/ml FGF2

Dynamic – differentiation medium Static – differentiation medium

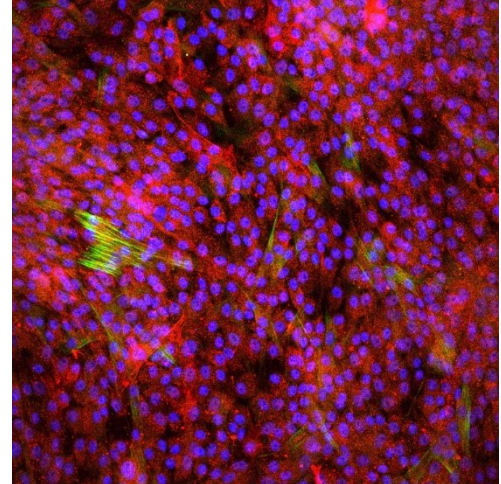
Fibrin



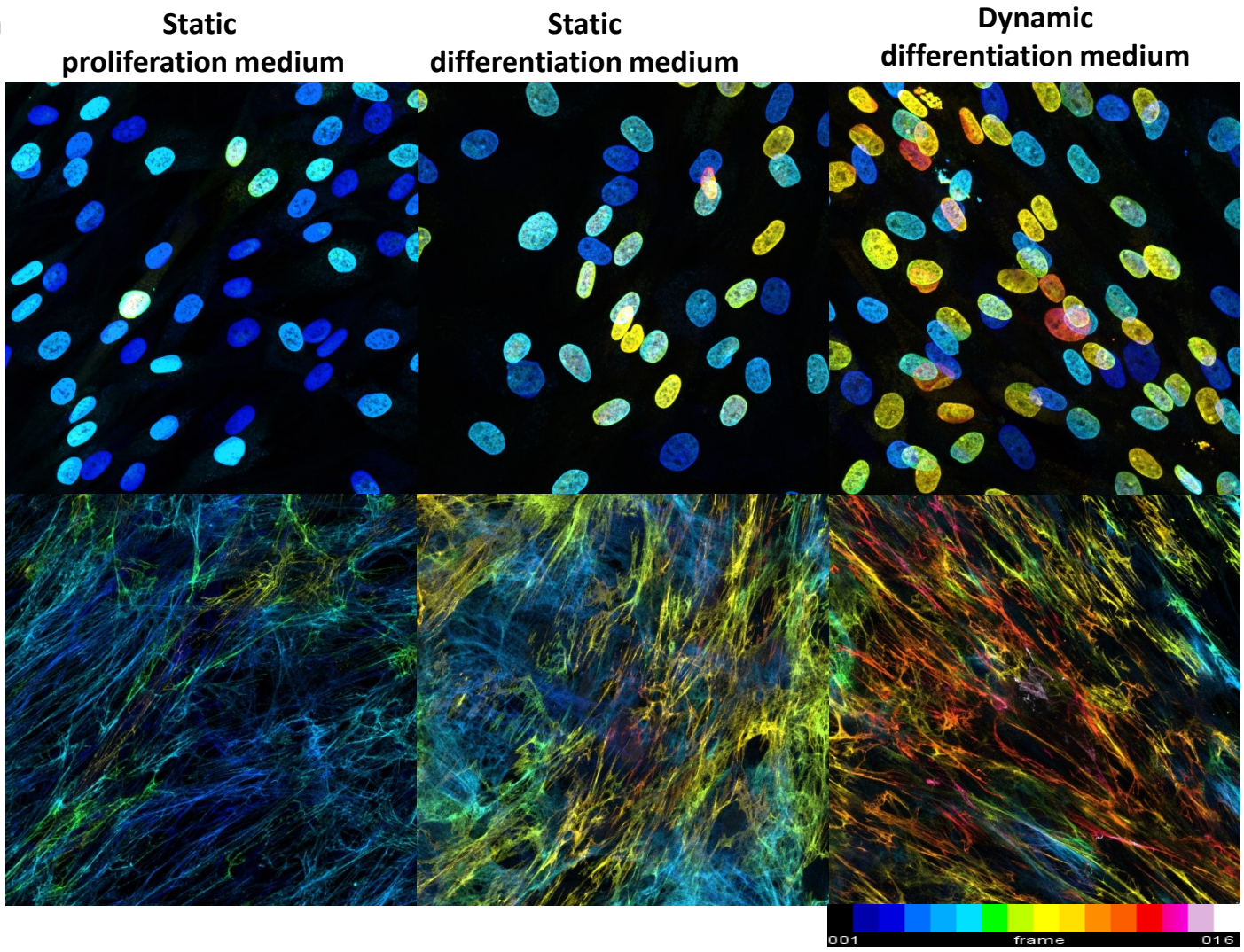
Glass (plasma treated)



Static – proliferation medium

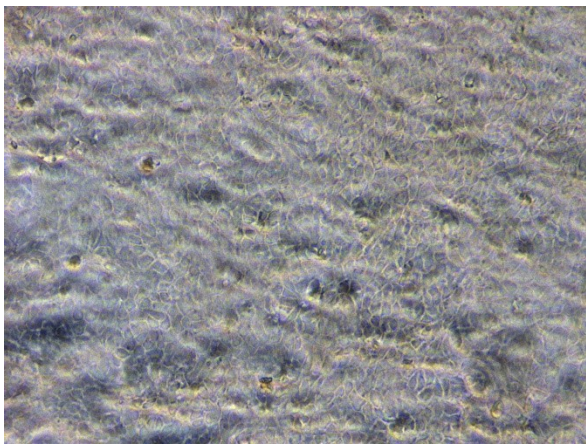


ECM production
7 days
Fibronectin

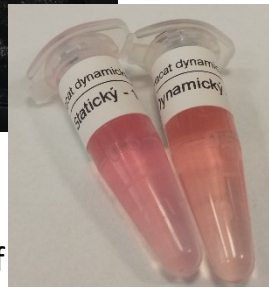
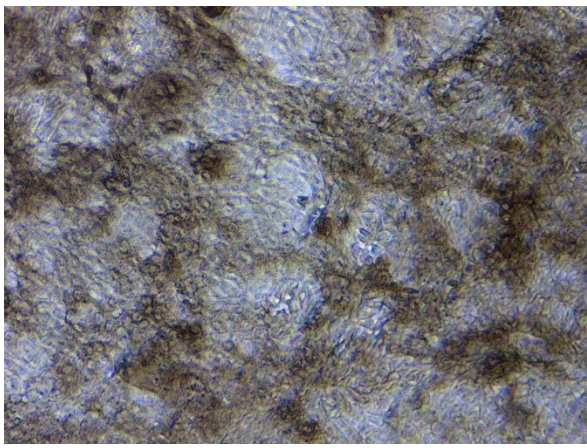


Elastic chamber, plasma treated
Static pre-cultivation till confluence
Dynamic stimulation peak 200 mmHg, 0,25 Hz
approx. 4-5% strain
<https://vimeo.com/292307309/387895ee3a>

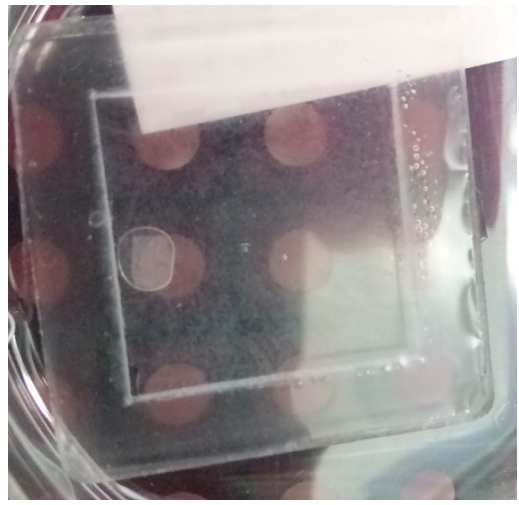
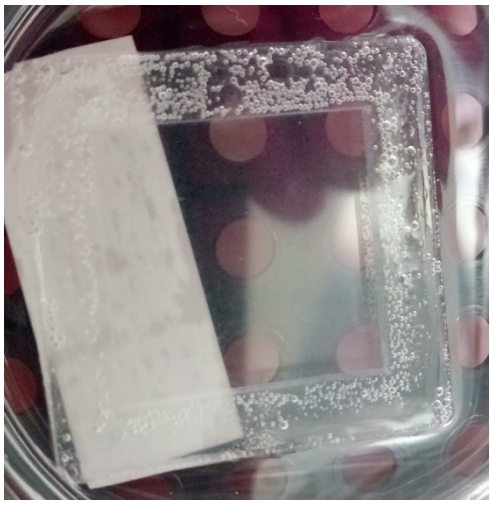
Static cultivation



Dynamic cultivation



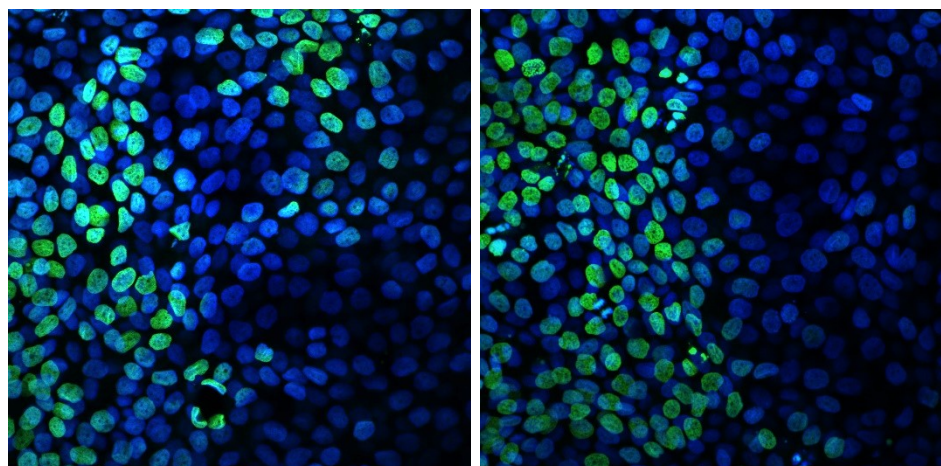
Metabolic processing
equal volume of culture
media, same time, stat. vs diff



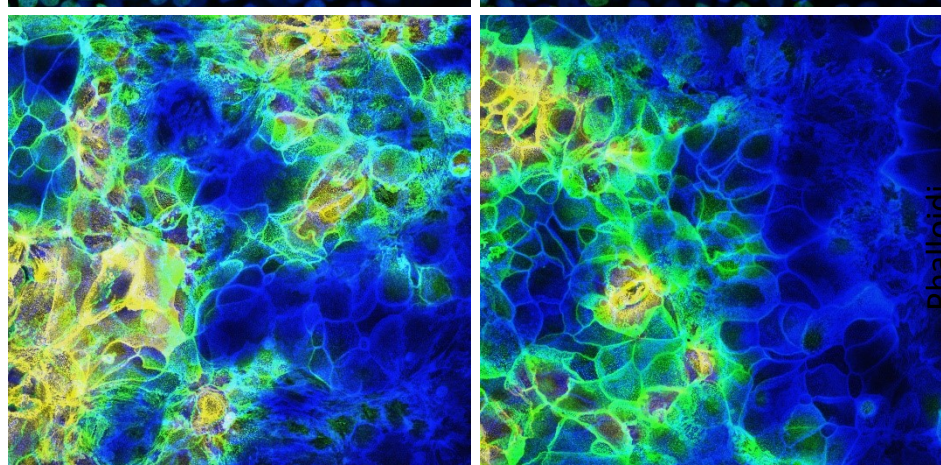
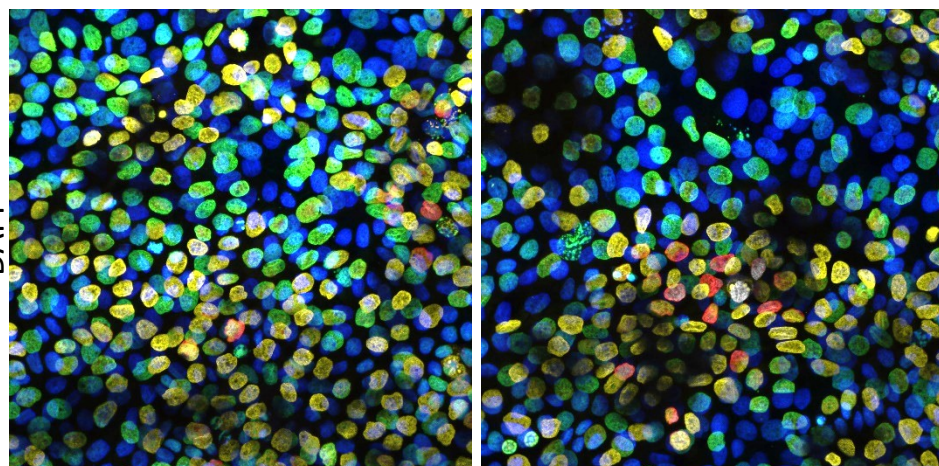
Opaque layer on surface

Static cultivation

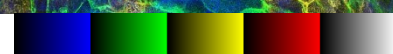
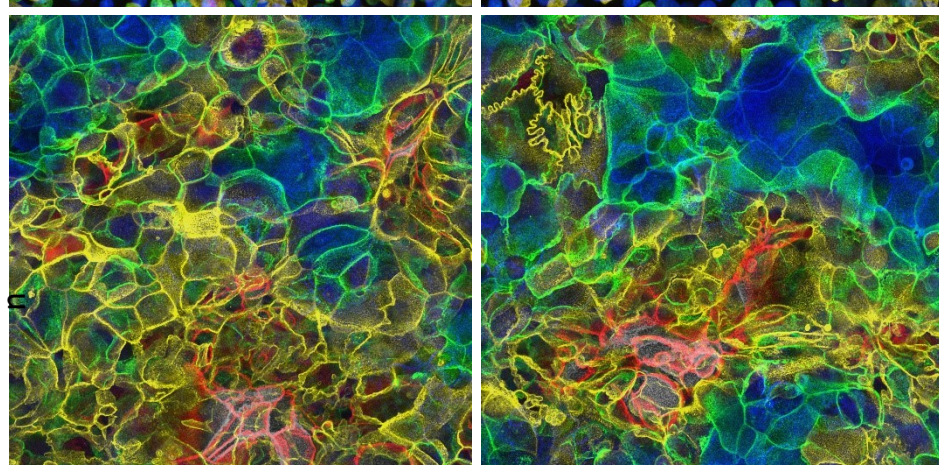
Dynamic cultivation



DAPI

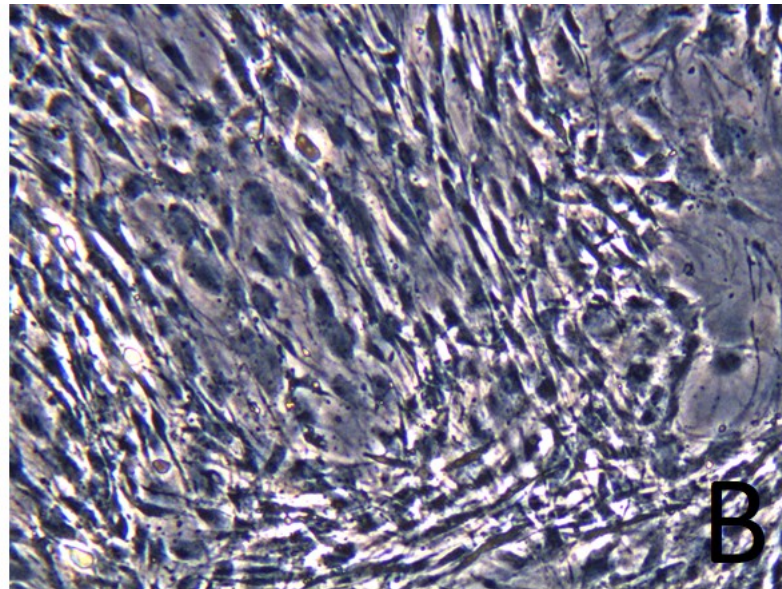
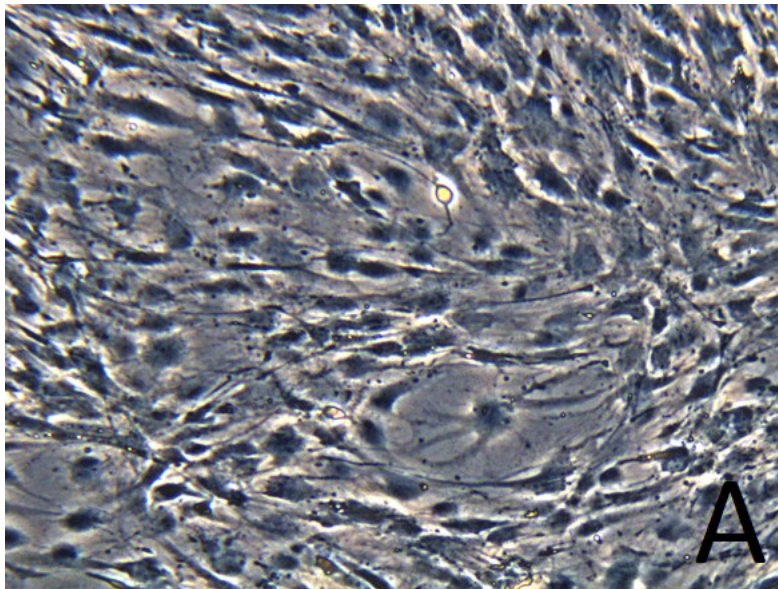
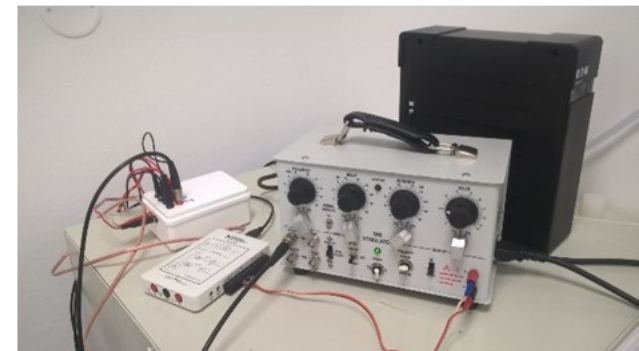


Phalloidin



Elektrická stimulace

- Plošné elektrické pole – sklo s deponovanou elektrodou



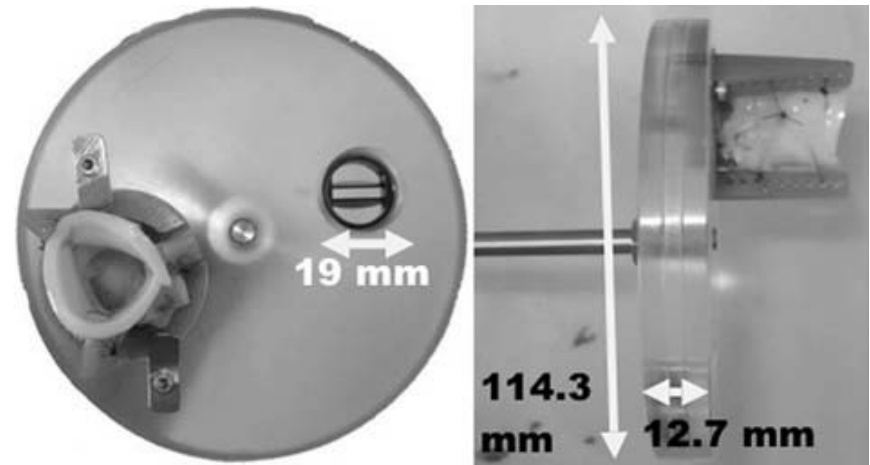
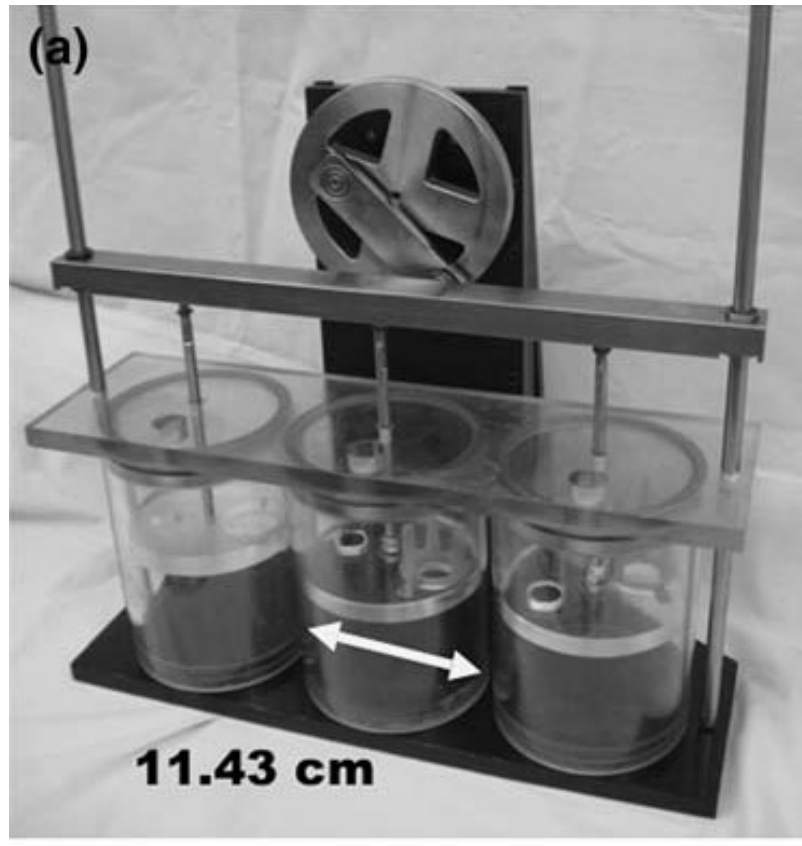
Orientace elektrod

Elektrická stimulace

- Externí generátor
- Proudové pulzy 5 – 100 μA
- Orientace buněk podle elektrického pole
- Diferenciace směrem ke kostním buňkam

- Využití piezomateriálů
 - Tlakové impulzy generující elektrický proud

Bioreaktor na přípravu bioarteficiálních chlopní

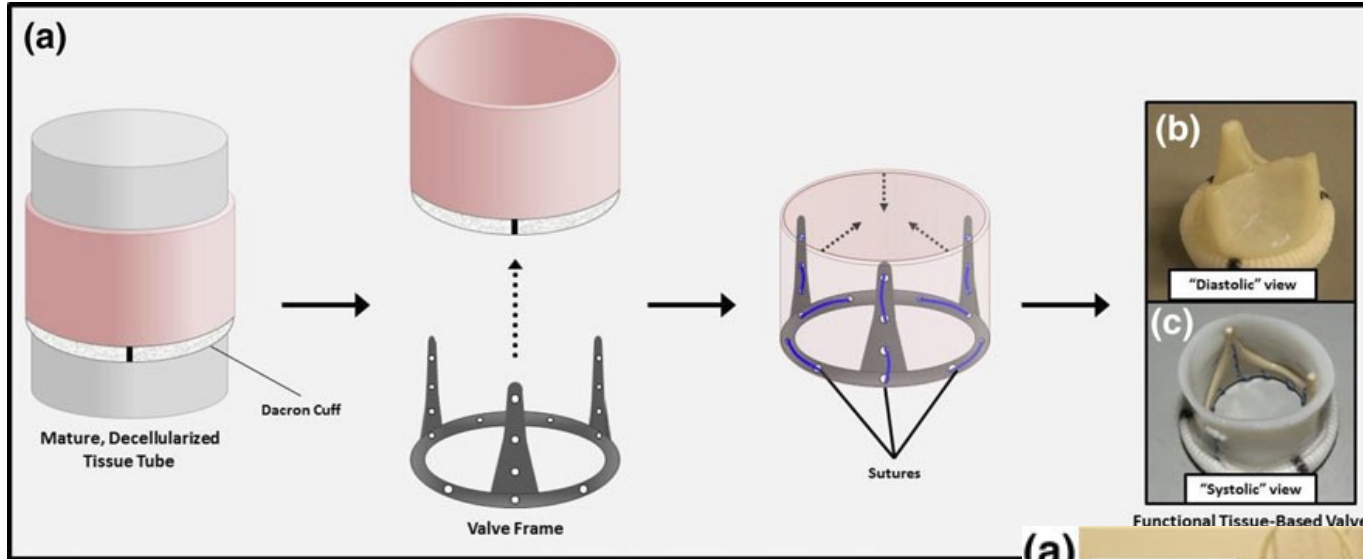


Píst se zabudovanou mechanickou a biologickou (prasečí) chlopní, chlopeň se otvírá pohybem pístu v médiu

internal flow ($Q = 0.193$ L/s), stroke volume (651 mL), static pressure (761 mmHg)

- C.A. Durst and K. JANE GRANDE-ALLEN. Annals of Biomedical Engineering, Vol. 38, No. 2, 319–325, 2010

Chlopeň z fibrinu



Fibrinový gel s ovčími kožními fibroblasty ve formě trubičky o průměru 22 mm byl mechanicky zatěžován v dynamickém systému, dokud se kolagenní vlákna obvodově neseřadila

