

Human iPSC-derived cardiomyocytes: A comparison with primary cells and applications in standard and 3D culture models

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studying cardiomyocyte biology *in vitro*

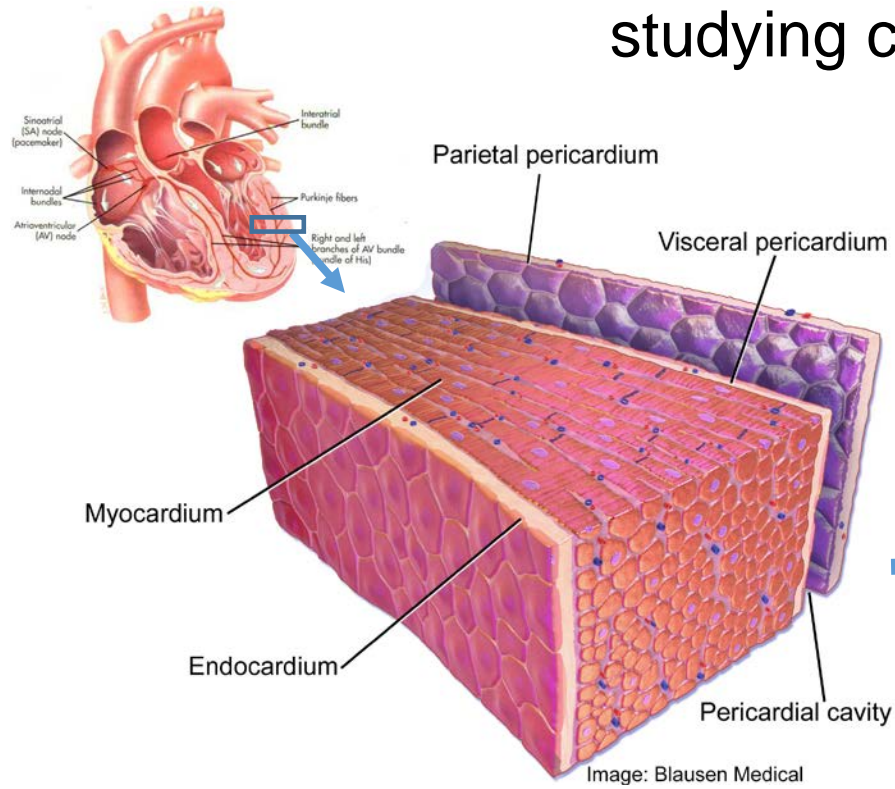
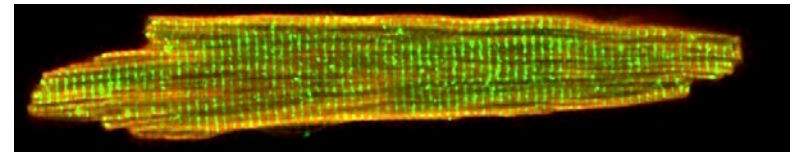
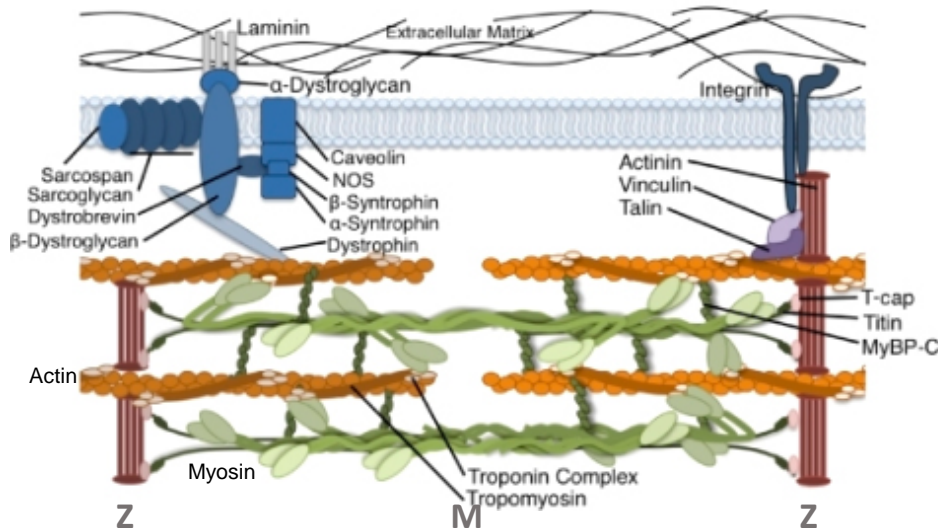
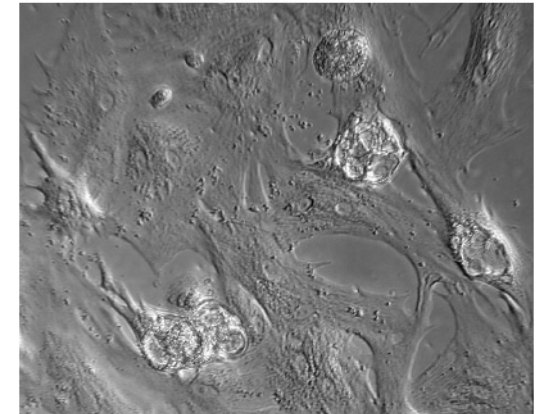
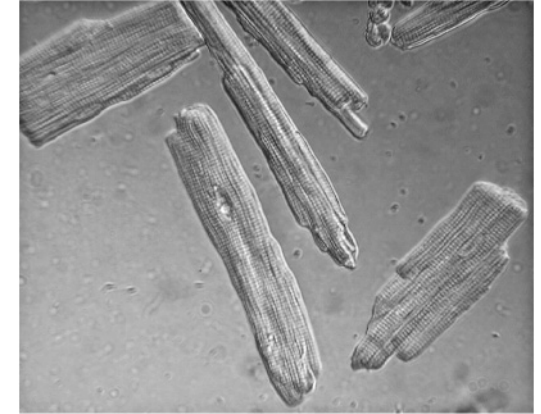
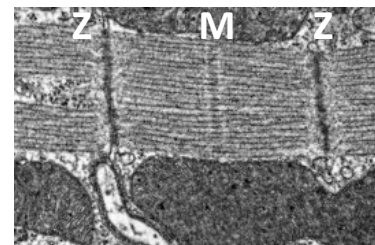


Image: Blausen Medical



Actin T-cap



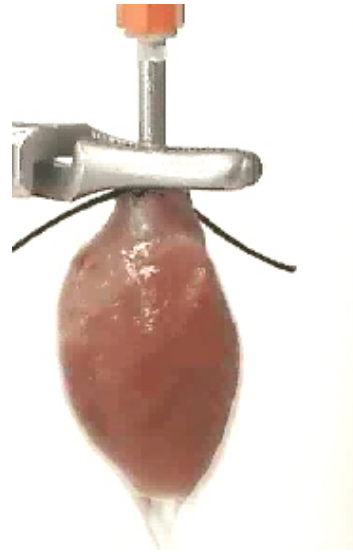
Sources of primary cardiomyocytes and isolation methods



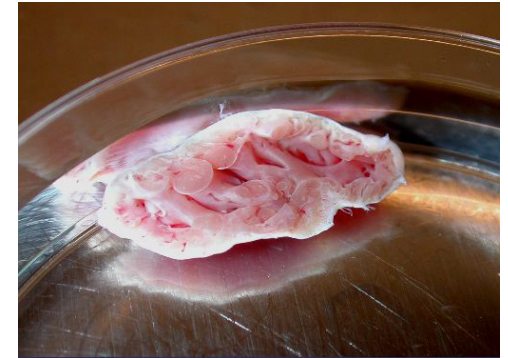
Chicken embryo



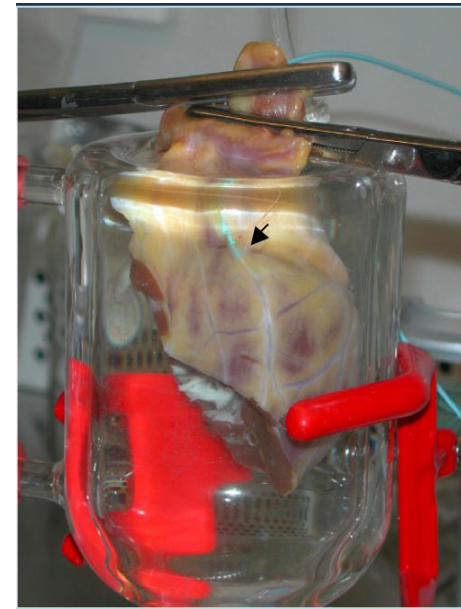
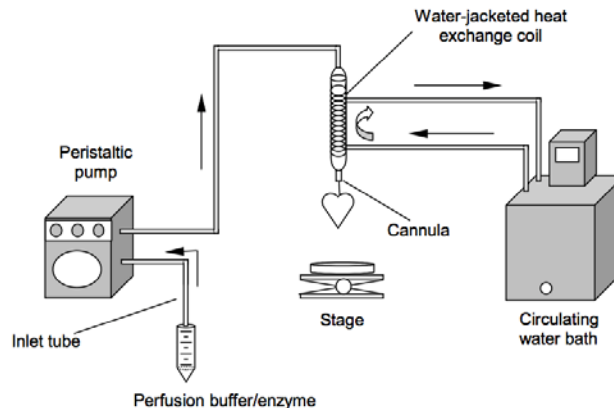
Neonatal mouse or rat



Explanted and perfused animal heart



Human atrial appendage



Human explanted heart

Primary cardiomyocytes in culture: a challenging cell type

- Terminally differentiated: no proliferation, limited life time in culture (2 days or approx. 2 weeks in dedifferentiated longterm culture with serum)
- Almost no immortalized cell lines available (few existing, from animal origin and with little advantages compared to neonatal rodent primary cells)
- Cardiac muscle is depending on uninterrupted oxygen supply, therefore living human organs are rare and mostly used for transplantation, valve surgery etc.

The need for better *in vitro* models

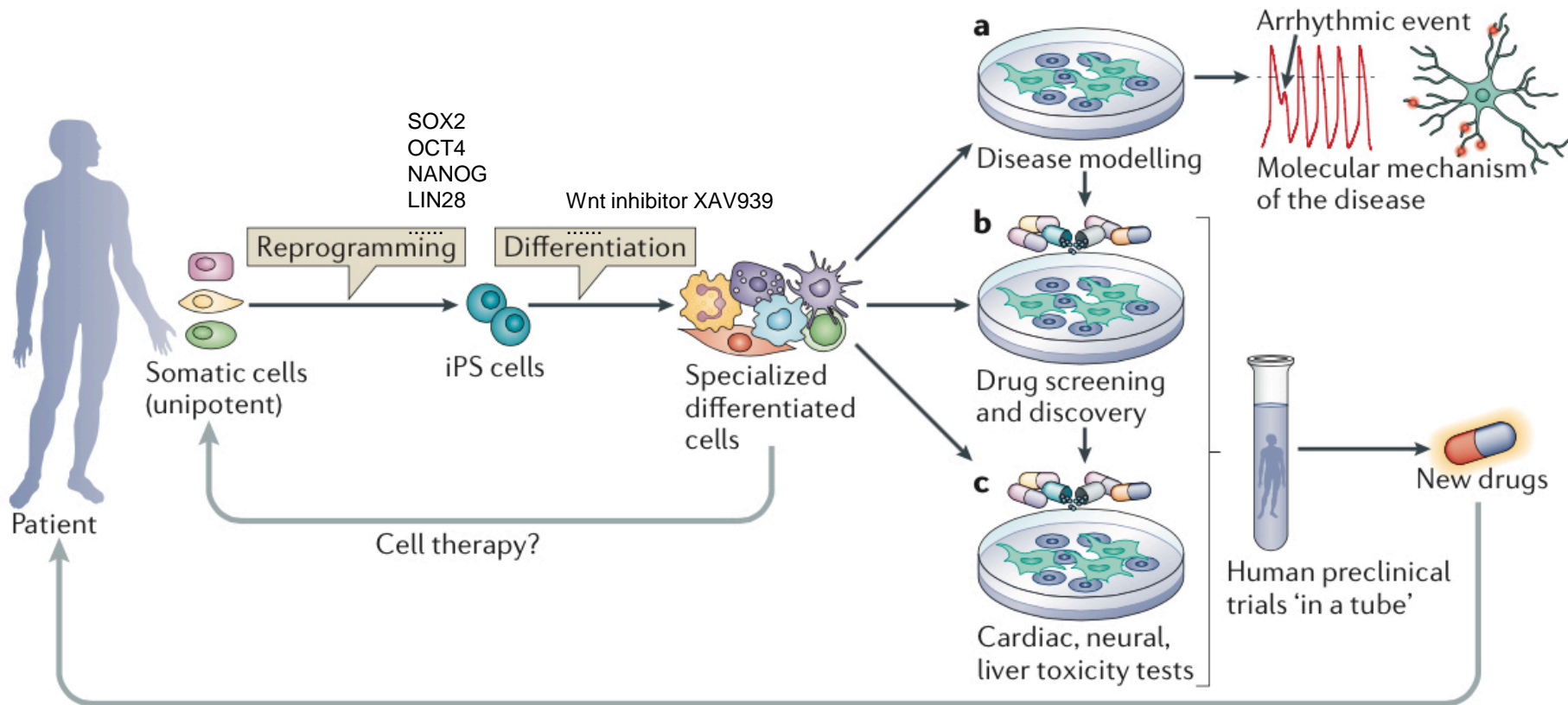
We need:

- **Cardiomyocytes from human origin for taking into account species-specific signaling pathways, genetic mechanisms, (electro-)physiological differences and for testing antibody-therapies**
- **A constant quality of cells in each lot**
- **A cellular environment involving mechanical and paracrine (co-culture) stimuli**

➔ **Human iPSC-derived cardiomyocytes**

➔ **3D culture of scaffold-free microtissues aggregated in the hanging drop with/without other cardiovascular cell types**

Production of induced pluripotent stem cells (iPSC)-derived cardiomyocytes from human origin and potential applications

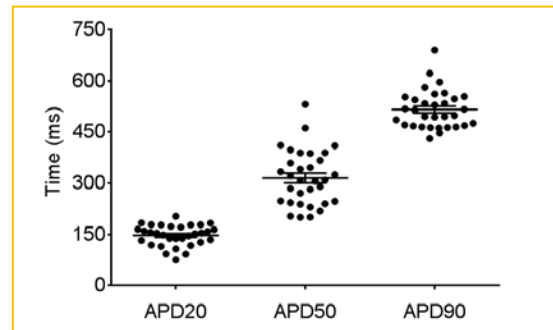
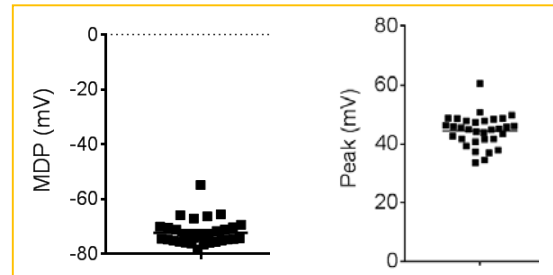
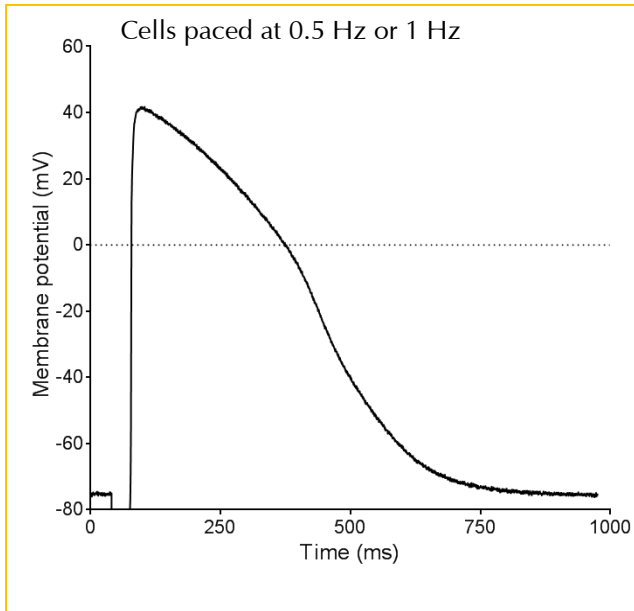


iPSC-Derived Ventricular Cardiomyocytes

Safety pharmacology testing



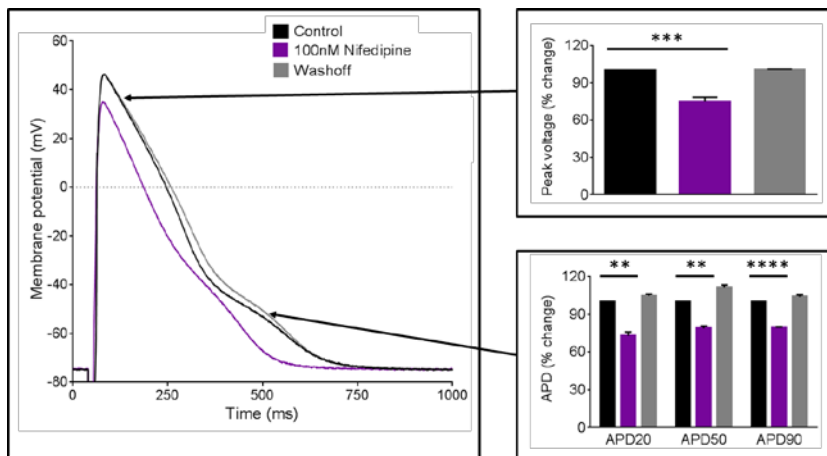
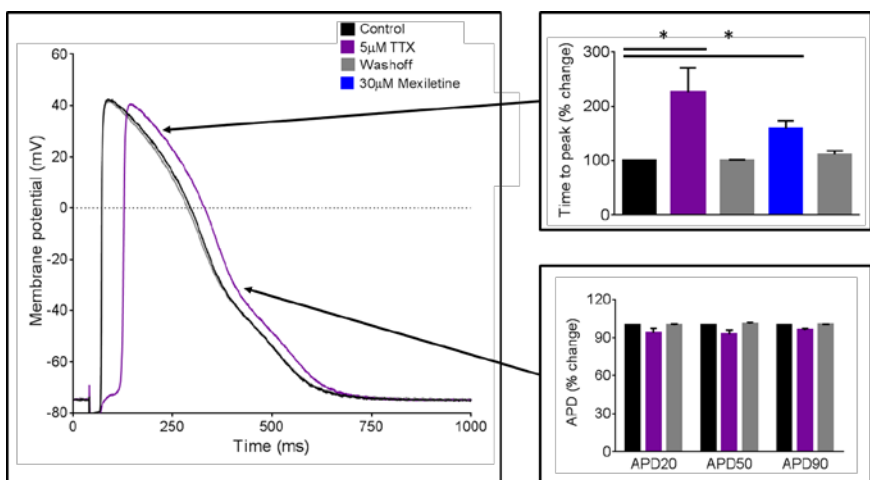
Untreated Action Potential Parameters



Compounds Tested

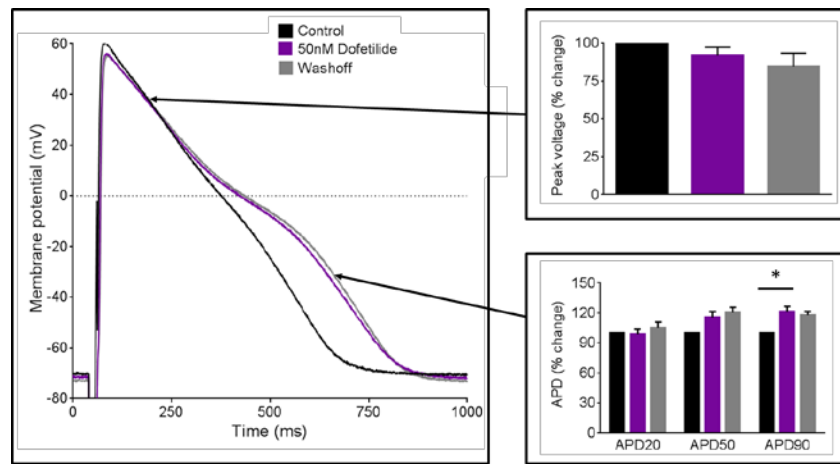
Compound	Ion channel
Nifedipine	I_{Cav}
TTX, Mexiletine and Lidocaine	I_{Nav}
Verapamil	I_{Cav} & I_{Kr}
Dofetilide	I_{Kr}
Carbachol	I_{KACh}
4-Aminopyridine (4-AP)	Non-selective K_V channel blocker
Tertiapin- Q	Selective Kir3.1/3.4 blocker

TTX & Mexiletine (I_{Nav})



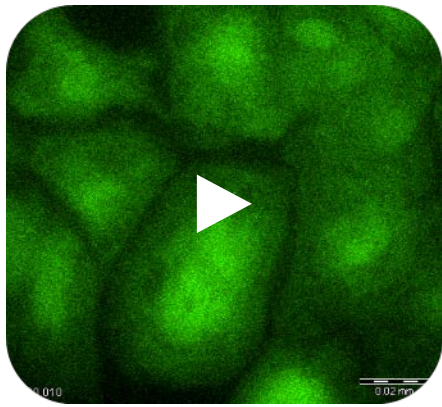
Nifedipine (I_{Cav})

Dofetilide (I_{Kr})



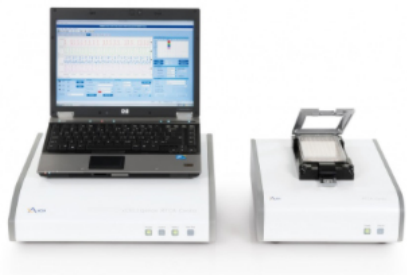
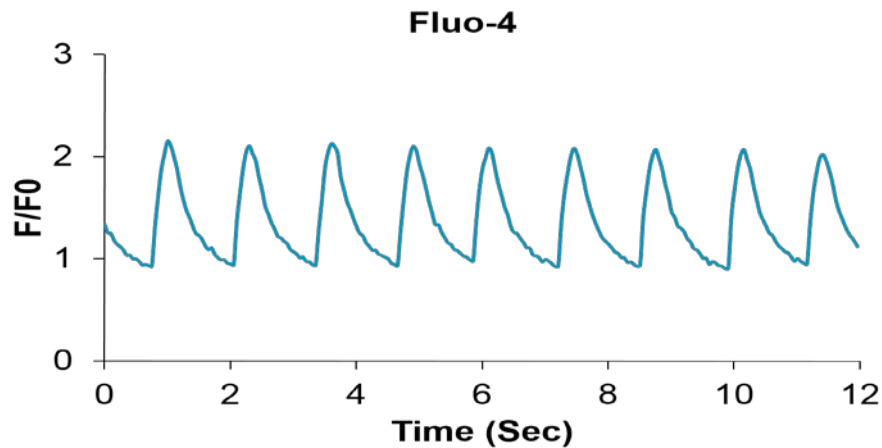
- Consistent and reliable results (n=32),
- Resting membrane potential -75 mV to -80 mV
- Broad action potential duration (APD90>300 ms)
- Relevant ion channel expression of I_{Nav} , I_{Cav} and I_{Kr}

Additional techniques for drug evaluation with cultured hiPSC cardiomyocytes



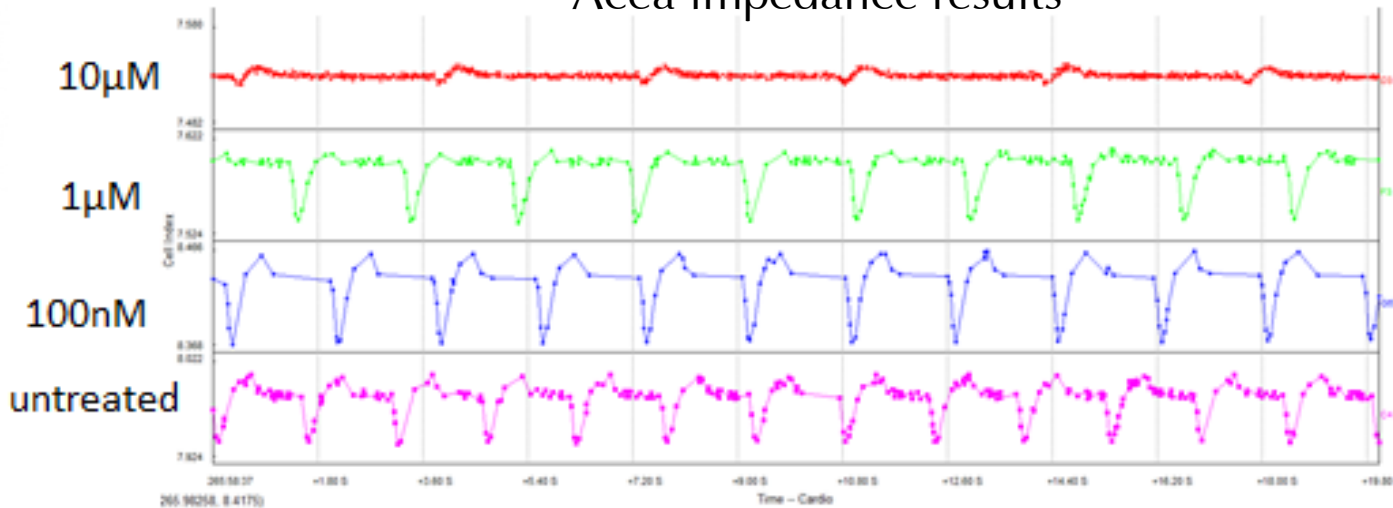
Fluo-4-AM

Calcium Imaging



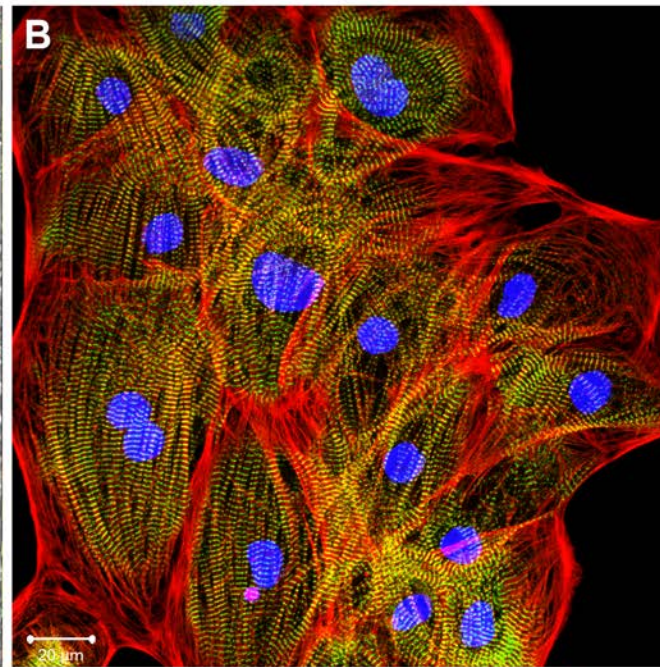
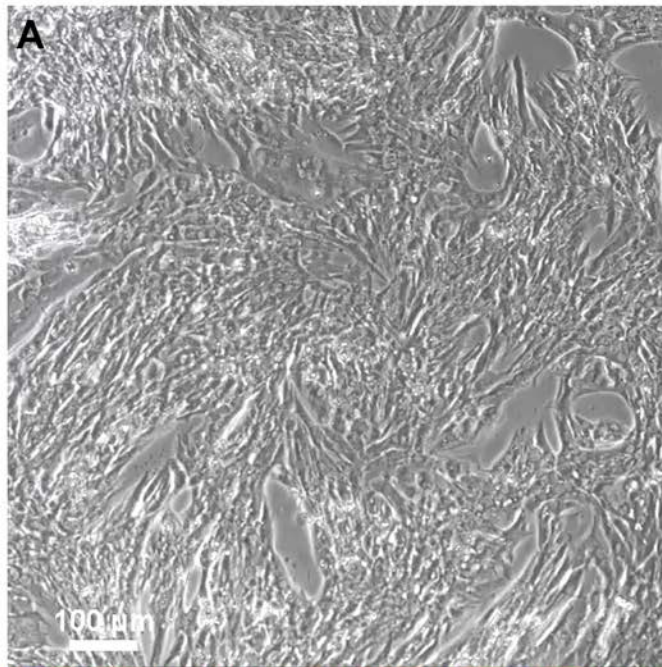
Carvedilol

Acea impedance results

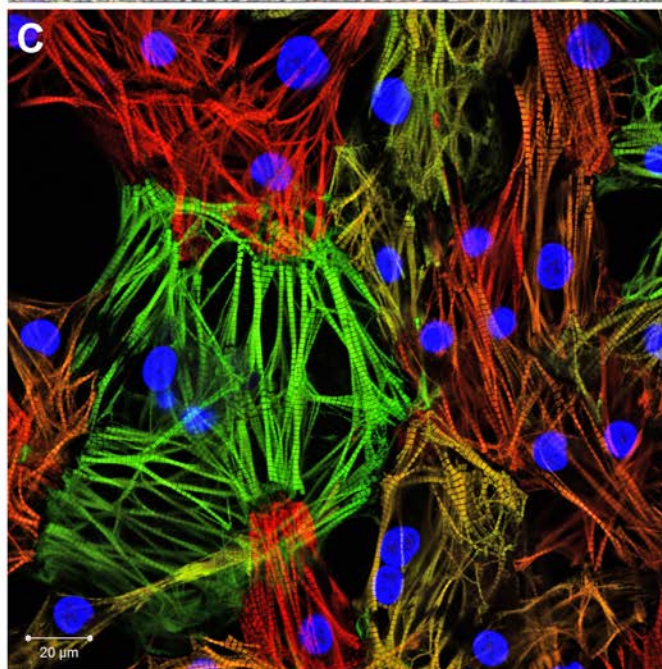


What is the developmental stage of hiPSC- Cardiomyocytes?

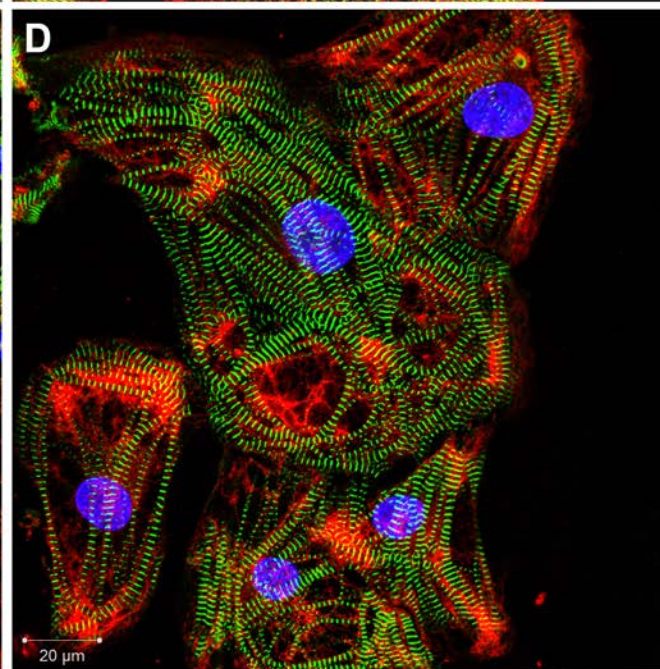
**Organization of cytoskeleton and sarcomeric proteins
in comparison to adult ventricular cardiomyocytes**



myomesin
actin

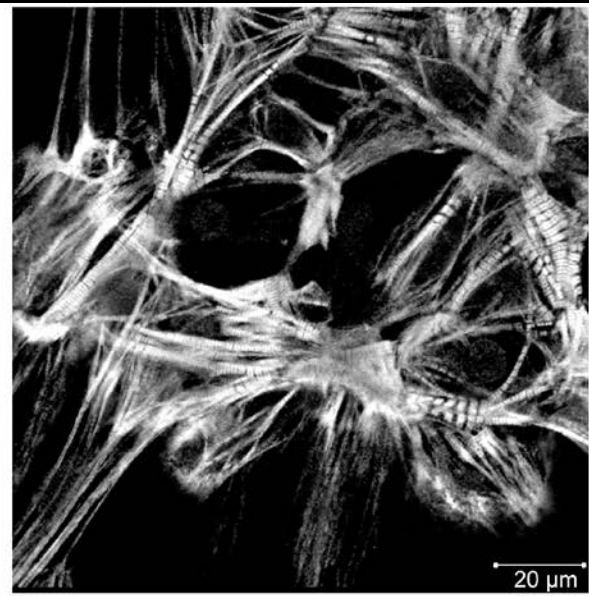
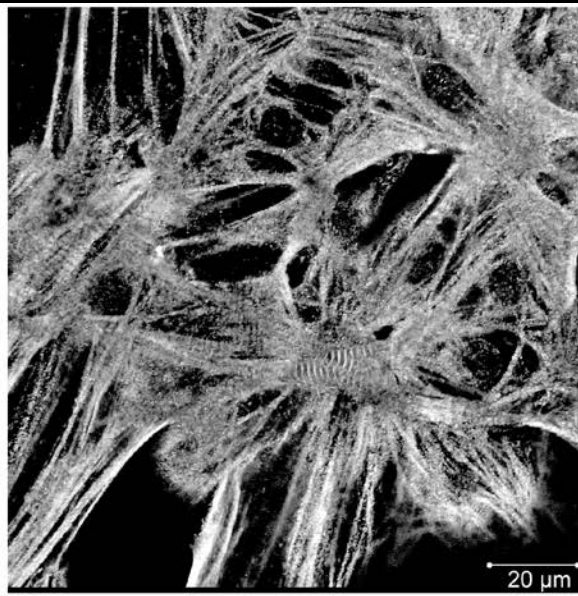
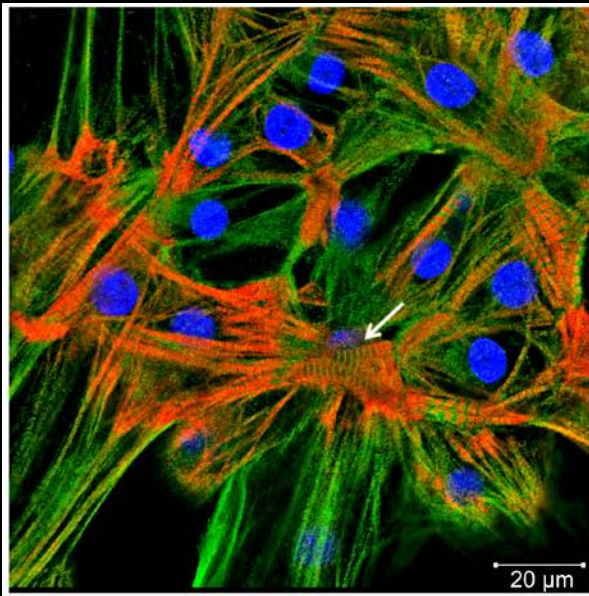


MLC2a
MLC2v

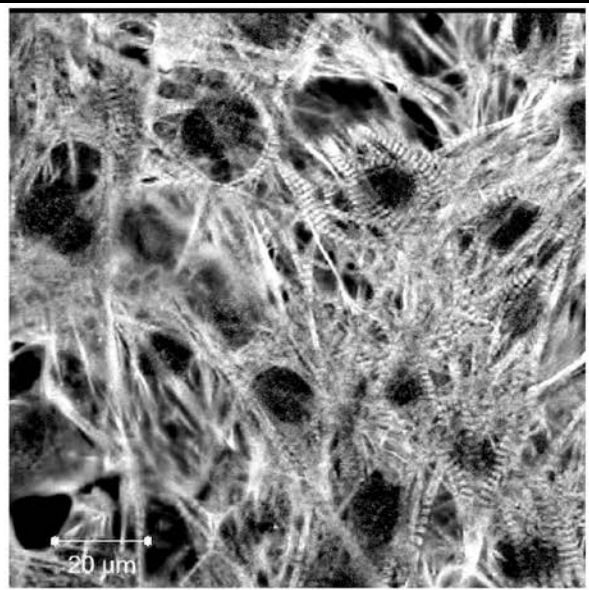
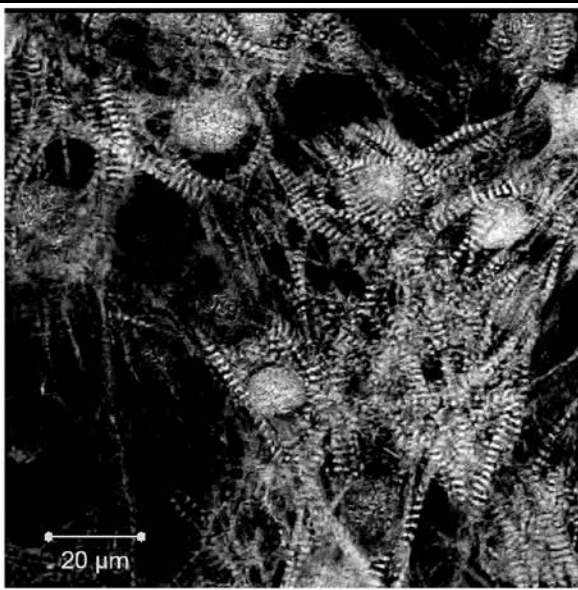
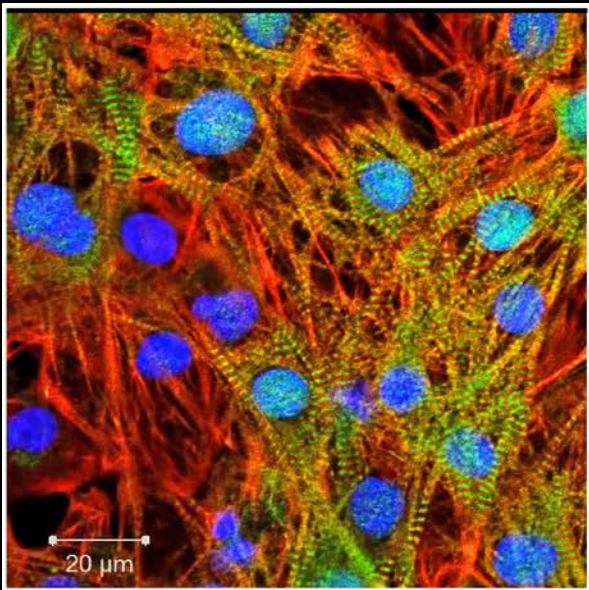


myomesin
vimentin

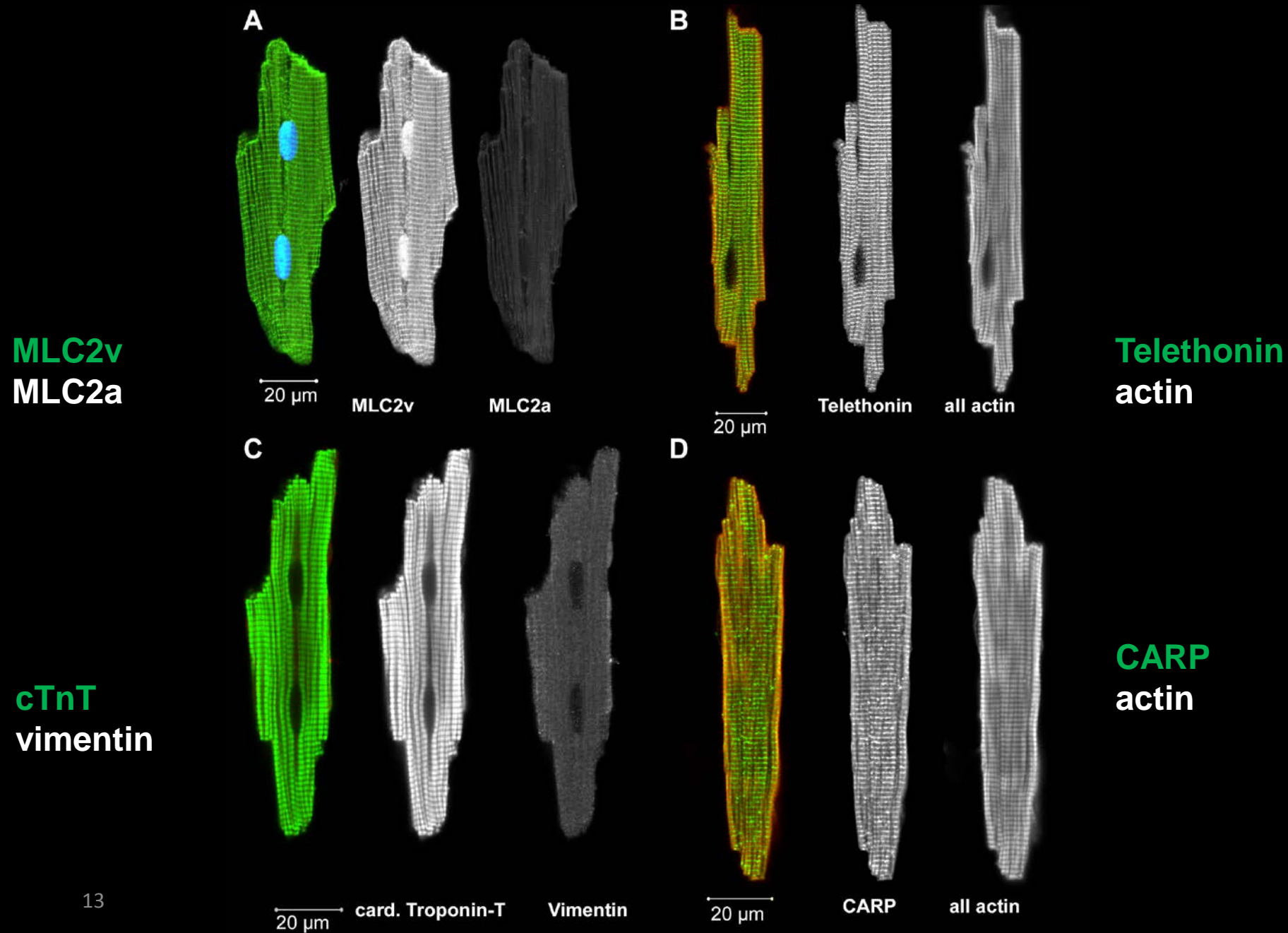
Telethonin (t-cap) all actin



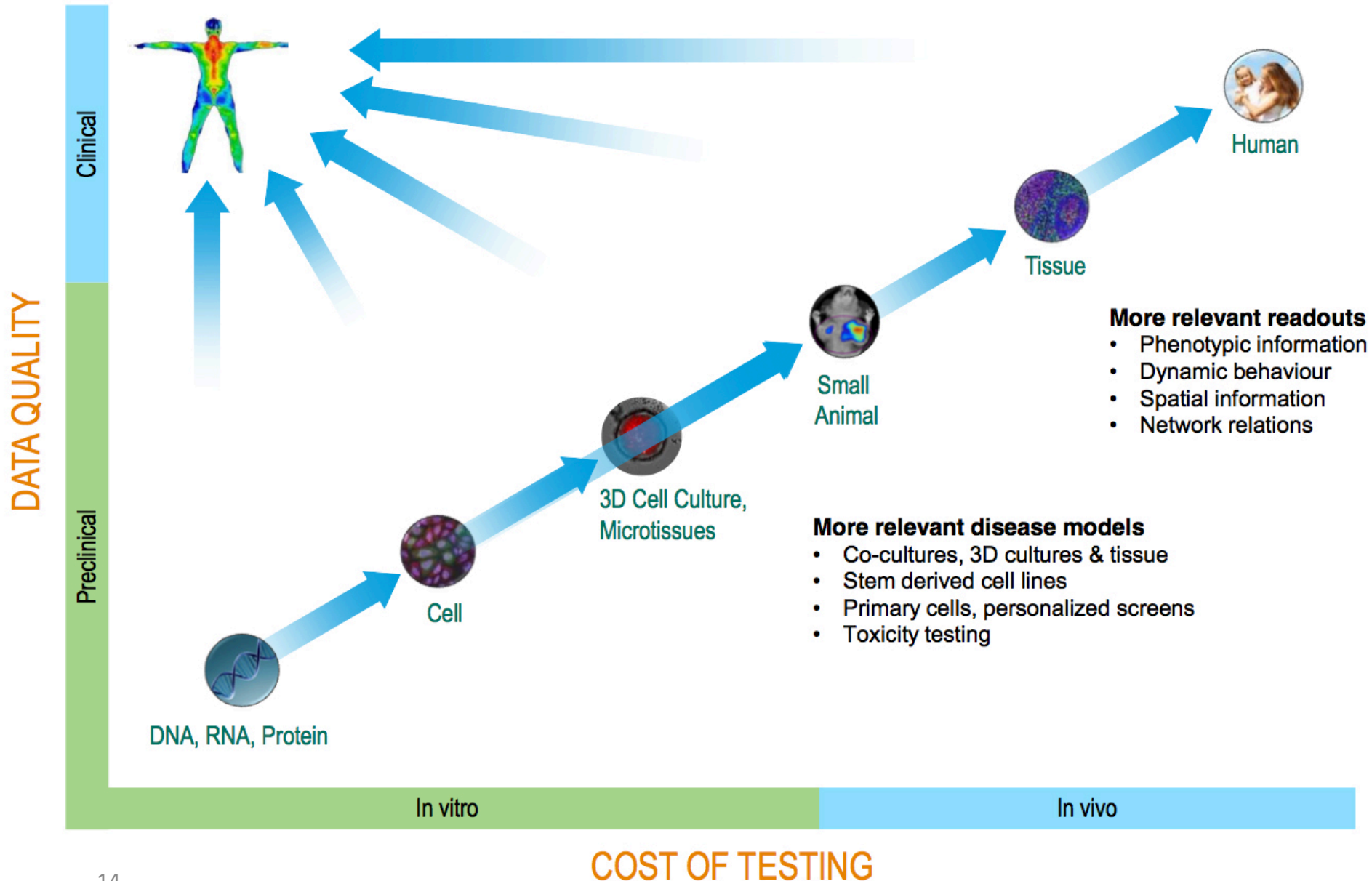
Cardiac ankyrin repeat protein (CARP) all actin



Comparison with fully differentiated cardiomyocytes from adult rats

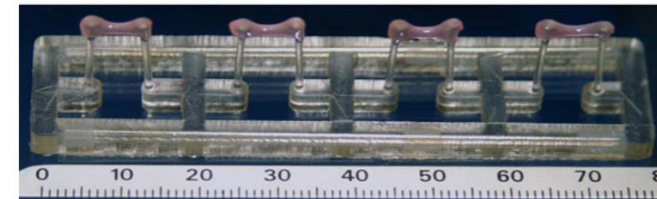
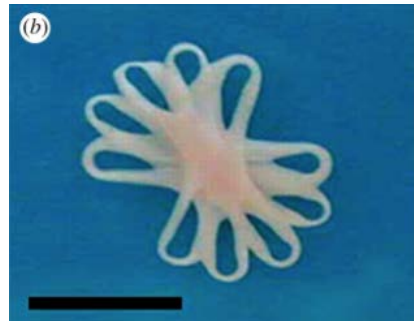
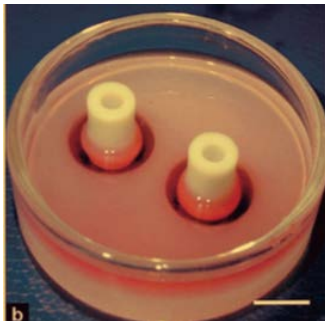
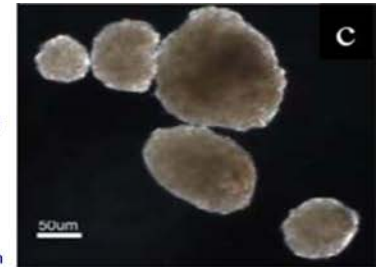
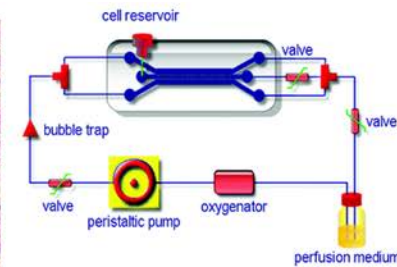
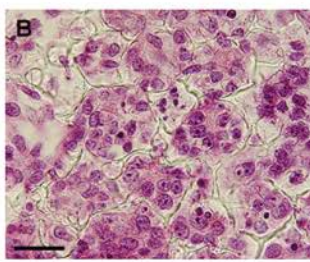
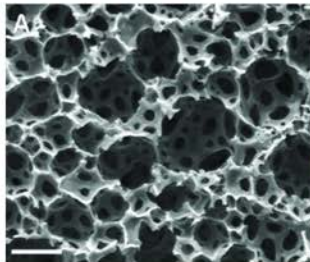


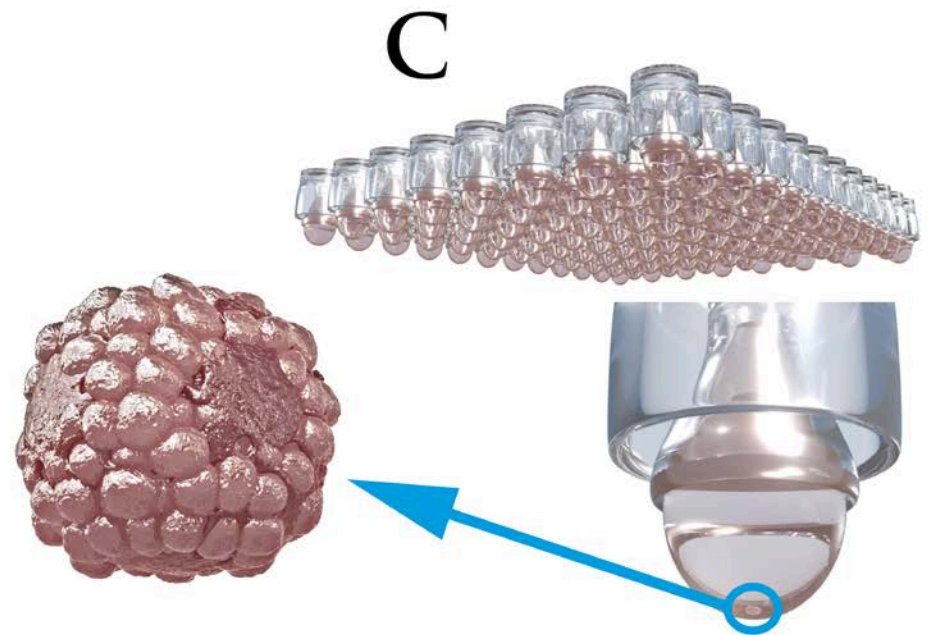
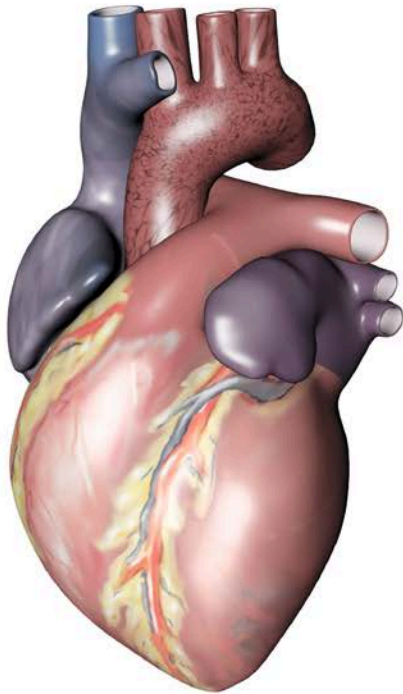
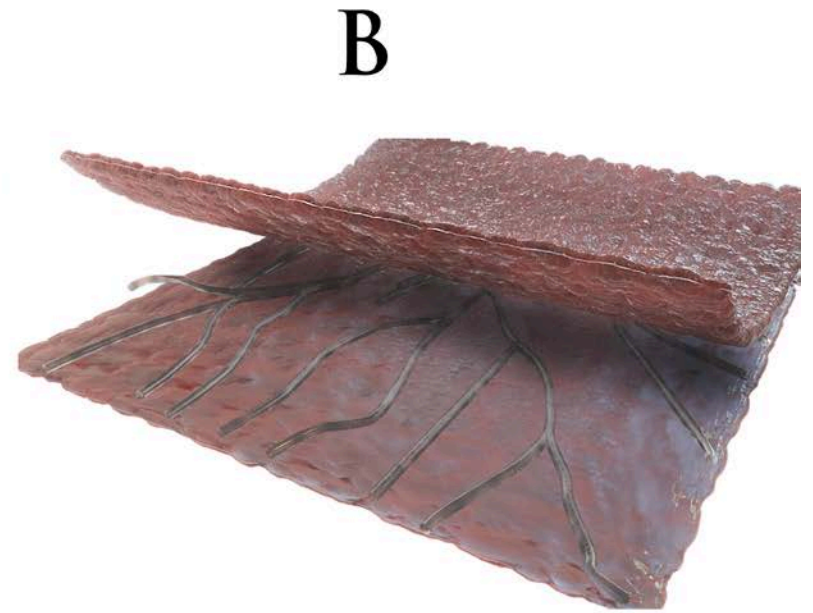
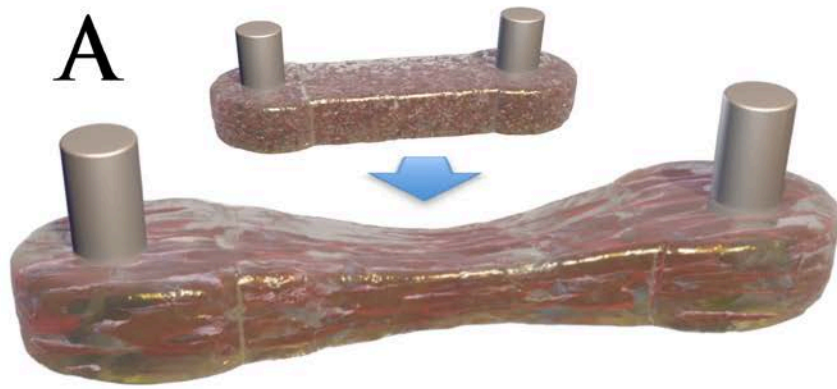
Bridging the gap between cell culture and the patient

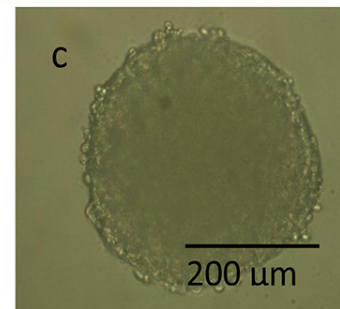
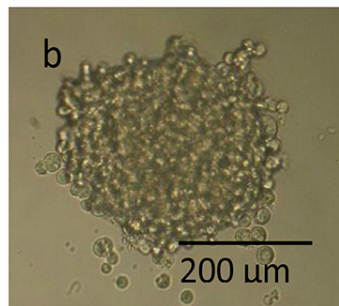
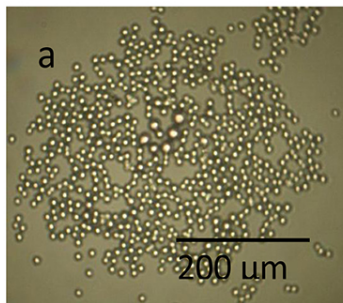
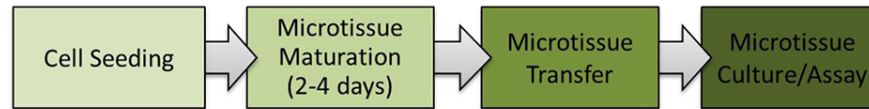
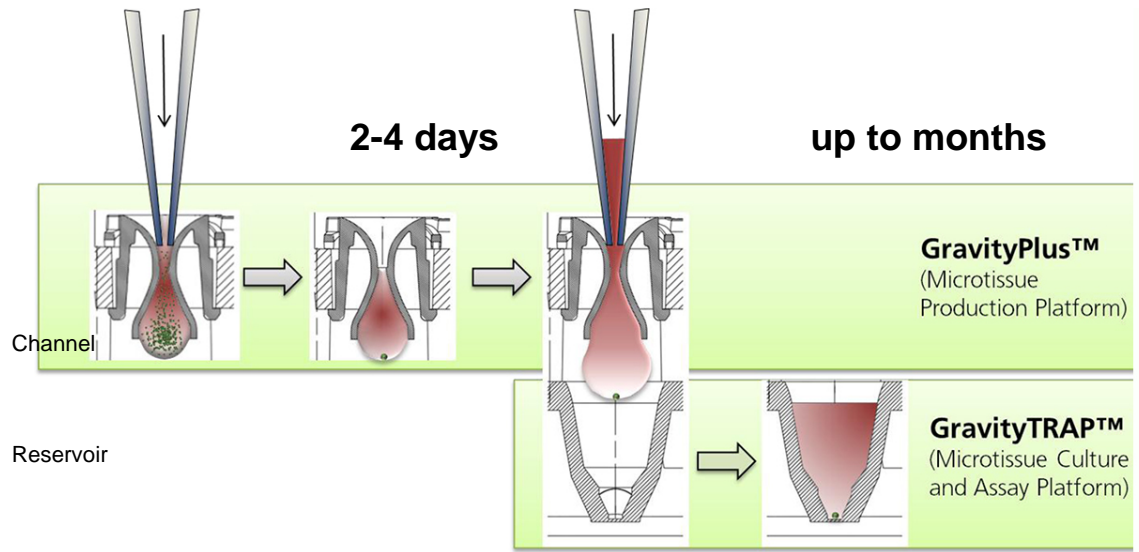
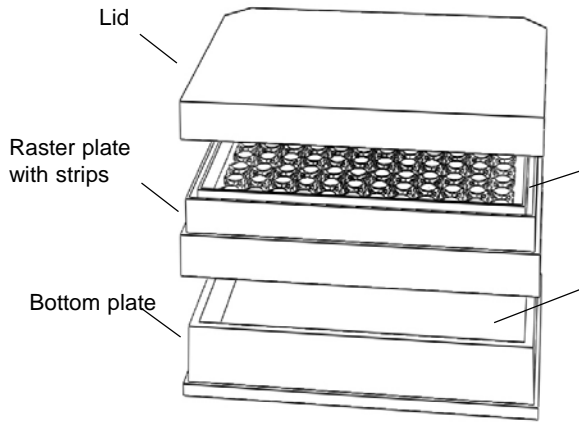


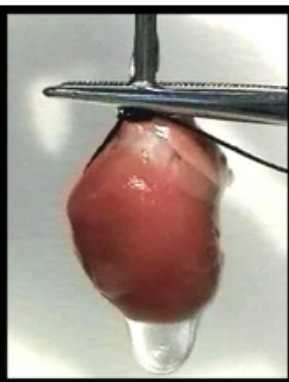


3D cell culture for cardiac cells?

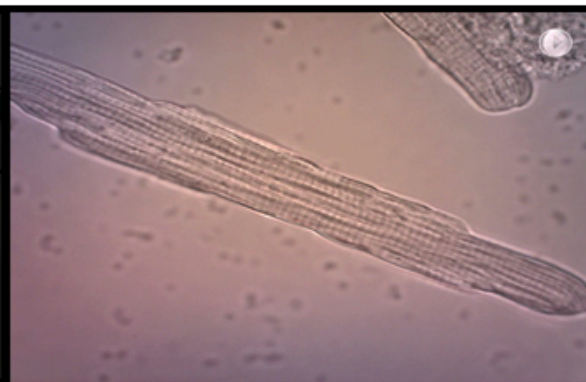




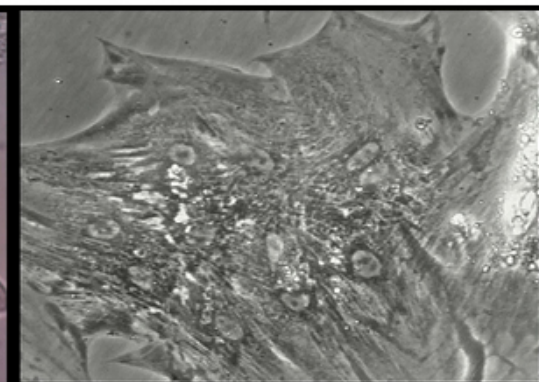




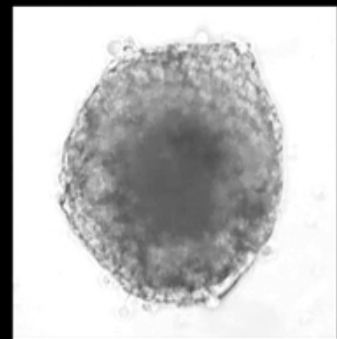
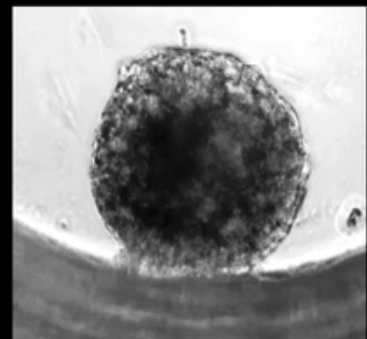
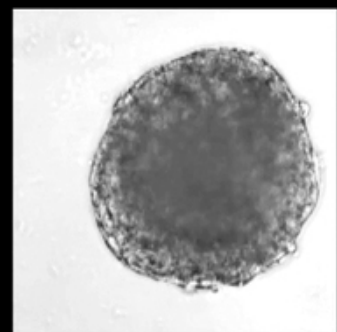
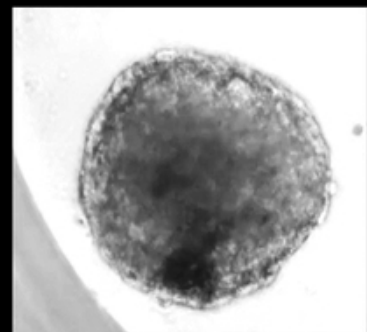
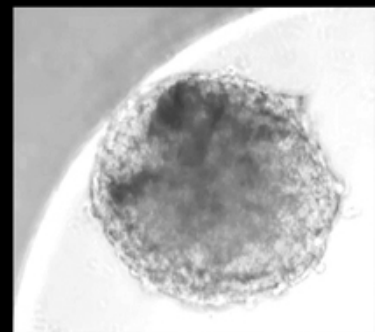
perfused rat heart



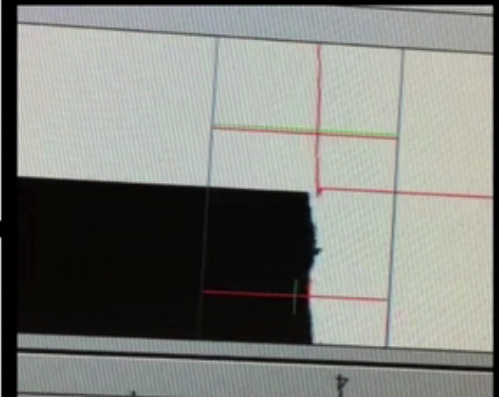
freshly isolated rat CM (paced)



spontaneously contracting long-term cultured rat CM



spontaneously contracting human cardiac microtissues after 14 days in culture



Edge-detection of surface motion in the IonOptix system

Is the proposed difference between standard culture and 3D models - or superiority of 3D culture - a claim supported by actual data ?

Spheroid tumor models: striking differences between 2D and 3D regarding chemotherapy efficacy

IC50 [μM]	Cisplatin	Doxorubicin	Etoposide	Vinblastine
Monolayer	3.0 ± 0.5	0.2 ± 0.1	3.2 ± 0.3	0.008 ± 0.001
Spheroids	81 ± 15	7 ± 1	521 ± 80	53 ± 6

3D model: orbital agitation

A549 lung carcinoma cell line

Resistance due to:

- Reduced uptake
- Resistance to apoptosis
- Differences in proliferation profile
- Hypoxic and necrotic center

B. Desoize Crit Rev Oncol Hematol. 2000 Nov-Dec;36(2-3):193-207.
Nederman T, Carlsson J. Cancer Chemother Pharmacol 1984;13:131-5.

Slide: InSphero

For controlled co-culture of different cardiac cell types: 3D-culture is the way to go

- **Approximation to original tissue structure**
- **To avoid inclusion of animal proteins (naturally forming extracellular matrix)**
- **Functionality of artificial tissues is improved**
- **Drug actions on paracrine pathways and inter-cellular mechanisms (for example fibrosis, myocarditis) can be tested in co-culture**

Outlook

- **iPSC-derived cell models have come of age, can be used for drug development and disease modeling**
- **Improved maturation of iPSC-derived cardiomyocytes is preferred and currently in development**
- **More sophisticated 3D models will be used including multiple cell types, in situ sensors/imaging, miniaturization of incubators (organ on chip)**
- **Bioprinting and self-assembly strategies for artificial organs**



InSphero

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& Group

Financial support:

Federal Commission for
Technology and Innovation
(CTI/KTI)

Swiss Heart Foundation

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Physiology Inst.

Nina Ullrich

