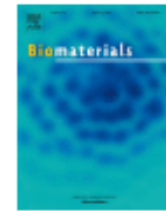




Biomaterials

Volume 75, January 2016, Pages 135-147



A new three dimensional biomimetic hydrogel to deliver factors secreted by human mesenchymal stem cells in spinal cord injury

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FOLAB Chirurgie Journal Club
October 16, 2017

Cell Chip Group
Institute for Applied Synthetic Chemistry
Institute for Chemical Technologies and Analytics
Faculty of Technical Chemistry



Spinal Cord Injury

Level and completeness of injuries^[15]

	Complete	Incomplete
Tetraplegia	18.3%	34.1%
Paraplegia	23.0%	18.5%

ASIA Impairment Scale for classifying spinal cord injury^{[11][14]}

Muscle strength ^[13]		ASIA Impairment Scale for classifying spinal cord injury ^{[11][14]}	
Grade	Muscle function	Grade	Description
0	No muscle contraction	A	Complete injury. No motor or sensory function is preserved in the sacral segments S4 or S5.
1	Muscle flickers	B	Sensory incomplete. Sensory but not motor function is preserved below the level of injury, including the sacral segments.
2	Full range of motion, gravity eliminated	C	Motor incomplete. Motor function is preserved below the level of injury, and more than half of muscles tested below the level of injury have a muscle grade less than 3 (see muscle strength scores, left).
3	Full range of motion, against gravity	D	Motor incomplete. Motor function is preserved below the level of injury and at least half of the key muscles below the neurological level have a muscle grade of 3 or more.
4	Full range of motion against resistance	E	Normal. No motor or sensory deficits, but deficits existed in the past.
5	Normal strength		

- Classified into 3 etiologies: mechanical forces, toxic, and [ischemic](#)
- Divided into [primary and secondary injury](#): the immediate cell death, and [biochemical cascades](#) initiated by the original insult causing further [ischemia](#), [inflammation](#), edema, apoptosis, & [neurotransmitter](#) imbalances which can occur minutes or weeks post injury.

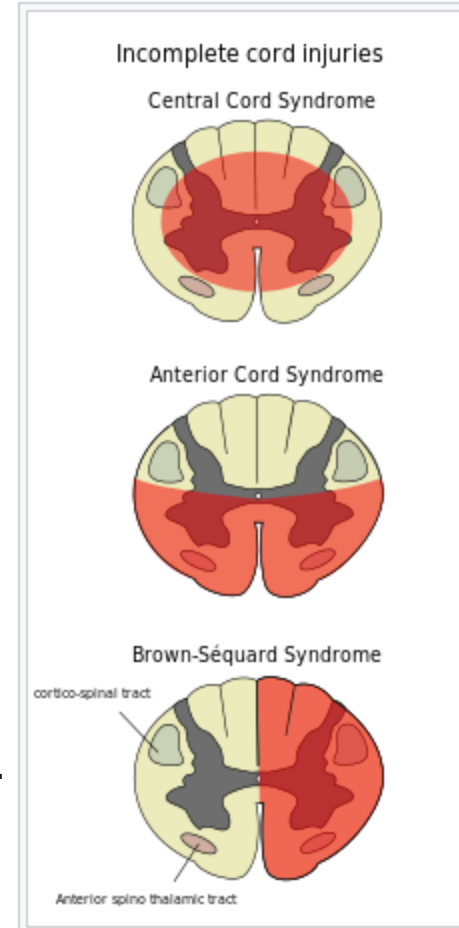
Spinal Cord Injury

Worldwide incidence of 10.4 to 83 people per million per year.

Males under the age of 30 account for four out of five traumatic spinal cord injuries.

The best predictor of prognosis is the level and completeness of injury, as measured by the ASIA impairment scale

The neurological score at the initial evaluation done 72 hours after injury is the best predictor of how much function will return.



Introduction

MOA: paracrine effect of hMSCs

Exogenous trophic factors modulate immune response

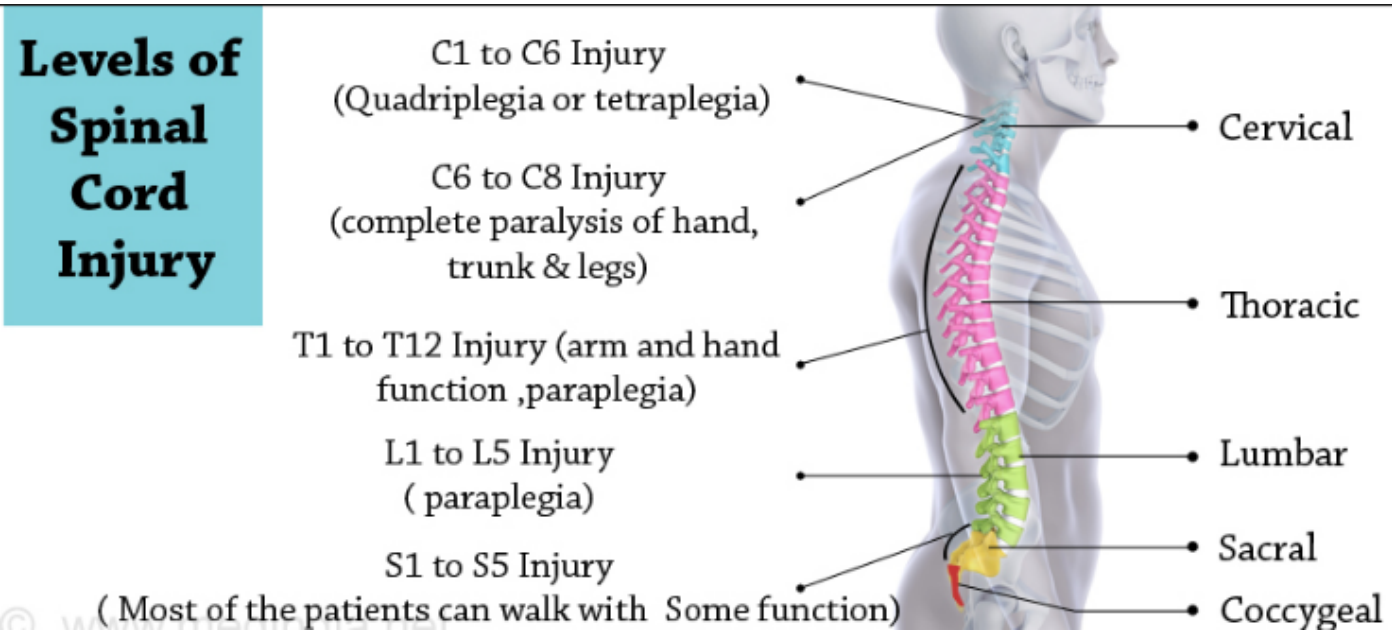
Limitations include unlikely cell migration across BBB after systemic administration as well as risk of adverse reactions (e.g. PE). Intrathecal application could reduce cell survival and increase cell damage. Intralesional injection may reduce MSC viability due to local ischemia / post-injury environment.

Scaffolds maintain short term MSC in vitro although in vivo attempts have been limited due to loss of MSC:scaffold adhesion in the absence of a biological niche



Aim

Propose a new biomaterial coated with 3D extracellular matrix to create a more optimized niche able to better sustain hMSCs viability and health.



Materials & Methods: *Hydrogel synthesis & loading*

Carbomer + agarose + PEG in PBS at 80C polymerized with 500 W irradiation

48 multiwell cell culture plate (0.25mL/well)

RGD (arginine-glycine-aspartic acid) functionalized

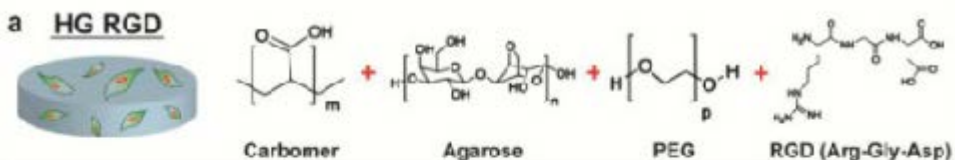
Umbilical blood derived hMSCs after Ficoll, culture flask adherence (P3)

Seeded homogenously below 40C (classic) OR 80 microliters pipetted onto previously polymerized hydrogel, both at ~100,000 cells

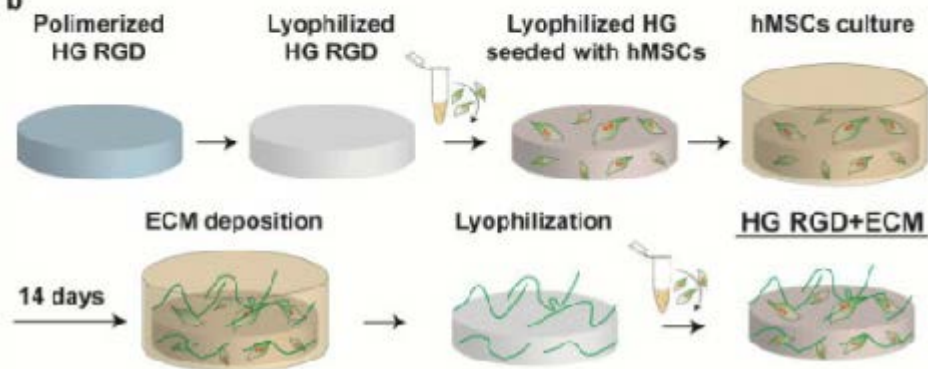
Growth medium

Figure 1

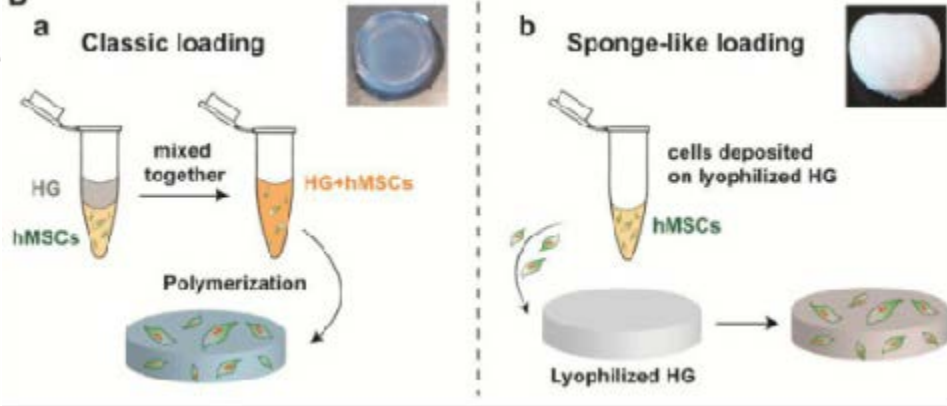
A



b



B



Materials & Methods: *Analyses*

ECM quantification/deposition under Sirius Red at days 1, 7, 14, 21

Cellular density and viability: calcein under confocal counted with Bitplane

SEM at d1 comparing HG RGD and HG RGD+ECM

Cellular differentiation assess by rtPCR (adipo-, osteo-, chondro-) with RNA isolation under RNeasy

ALP, RUNX2, osterix

ACAN, CoIX

FABP4, adipsin

Materials & Methods: cont'd

Previously described rat model: 30g compressive force for 60 s after T12 laminectomy

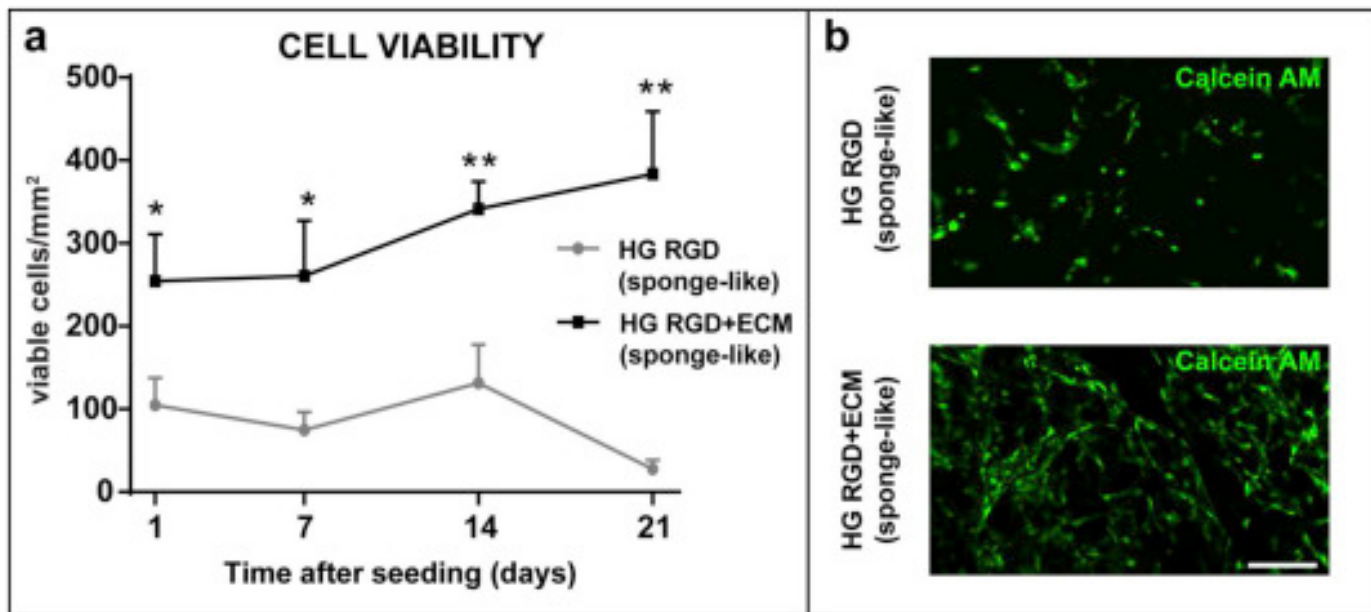
Tx group: 25,000 CFDA+ hMSCs 24hr post-seed (HG RGD + ECM) secured at injury; routine closure in two layers. Day 9 FACS, PCR

Control groups: direct hMSC injection (25K hMSCs/05.mcL 30G needle over 2 min); direct CM injection; CM(80mcL) & HG RGD + ECM

Cell viability tracking: day 0, 3, 9

Hydrogel positioning: secured v unsecured under Hoechst for histology at day9

Results: Figure 2





Results: Figure 3

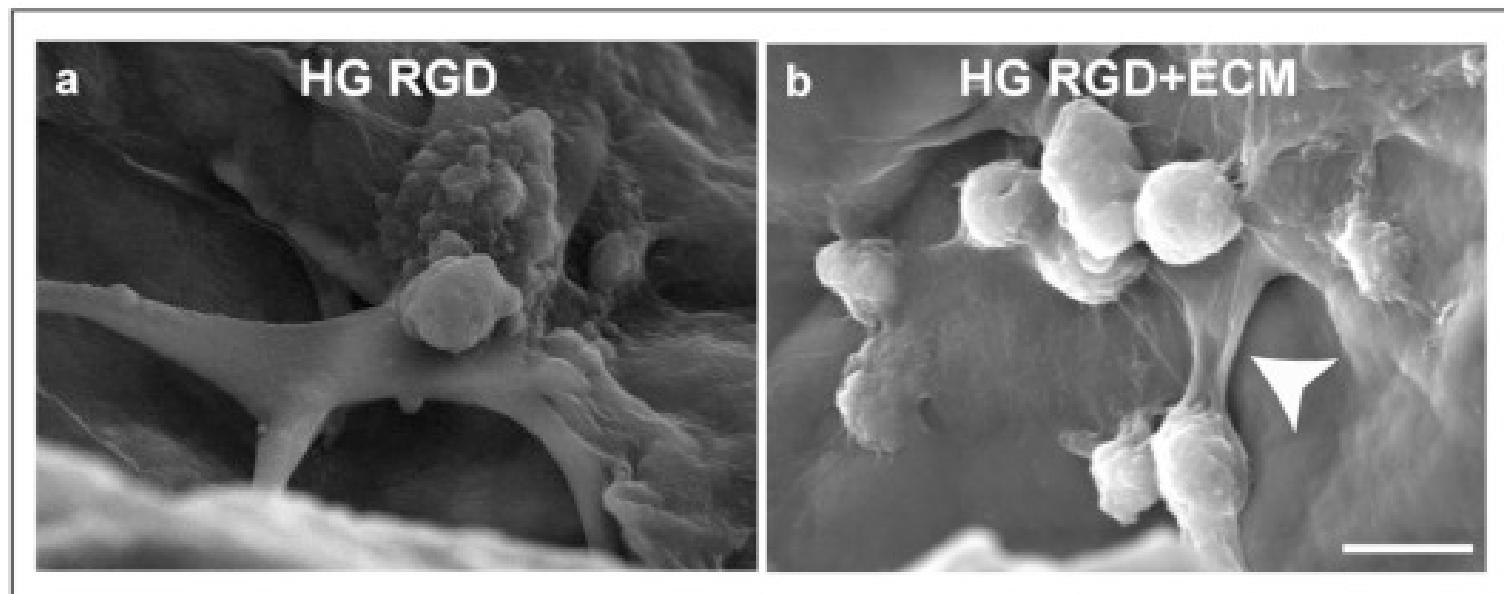
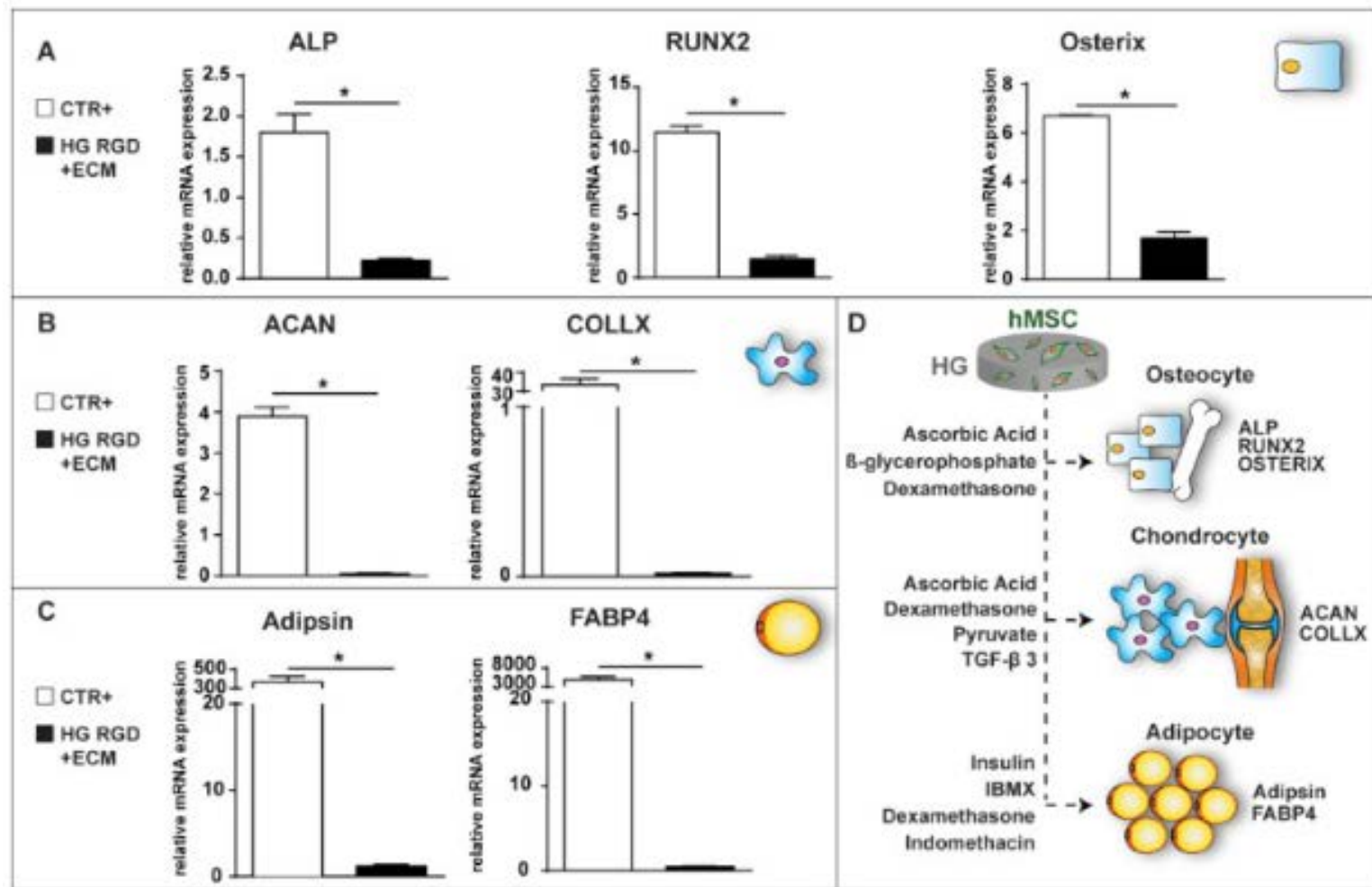
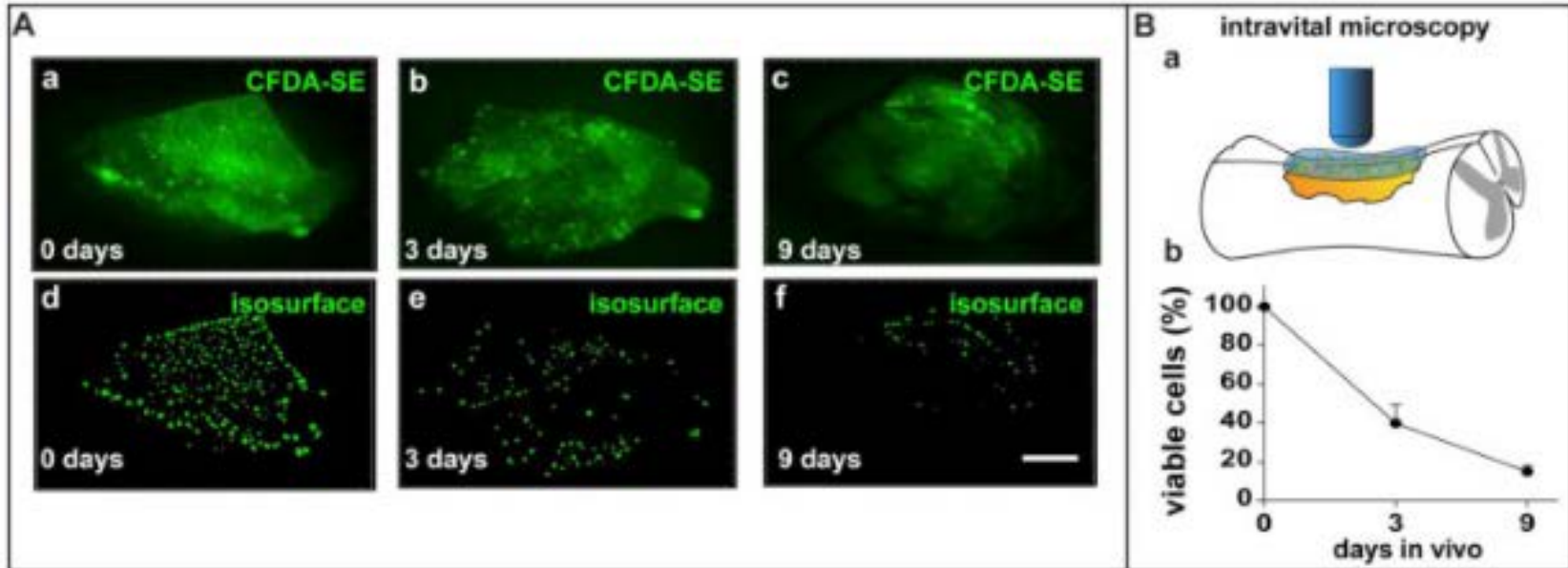


Figure 4



Results: Figure 5

100% hMSC survival d0; 40% viability d3: 15% viability d9



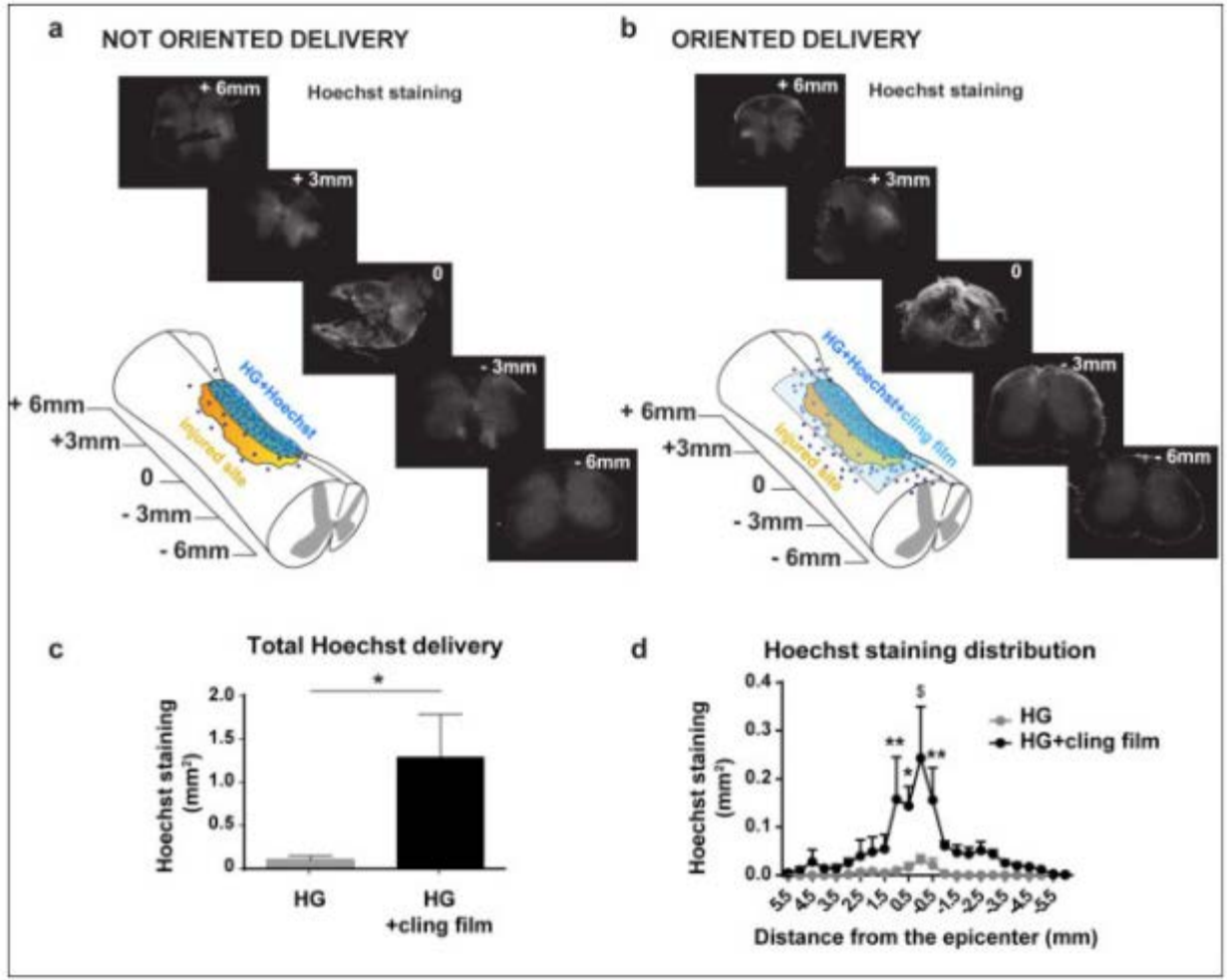
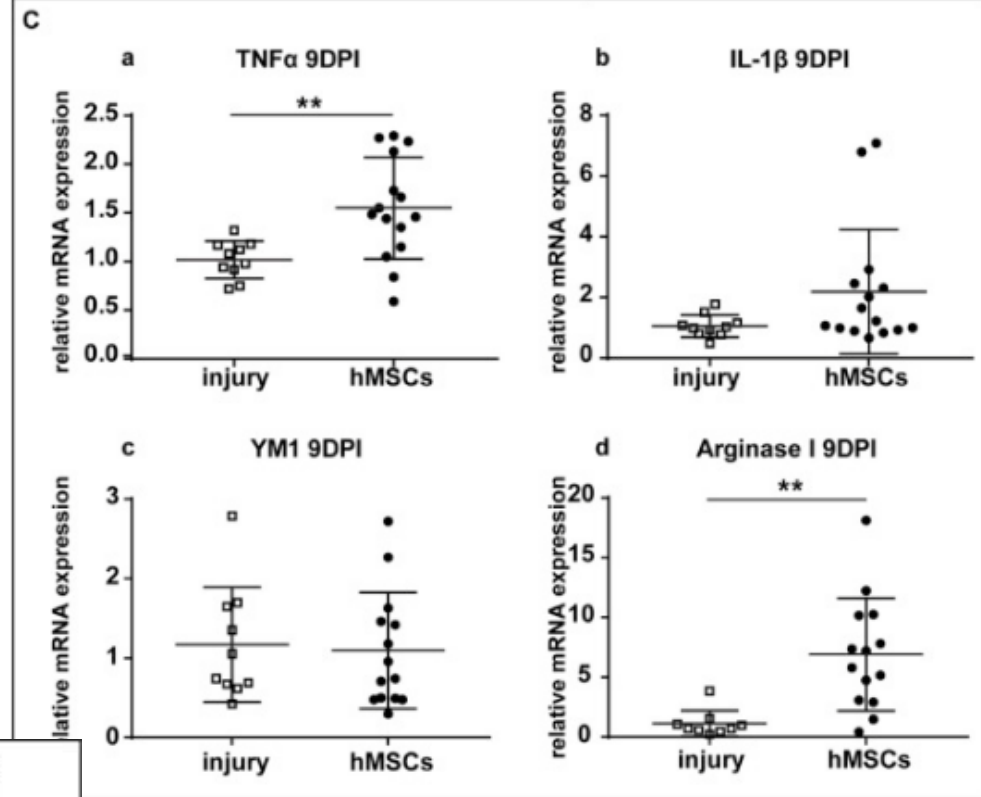
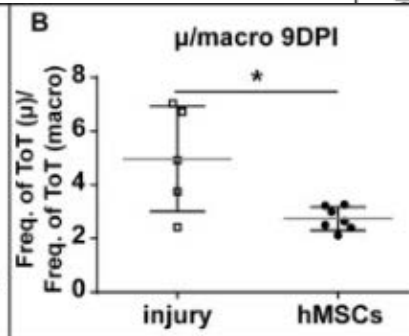
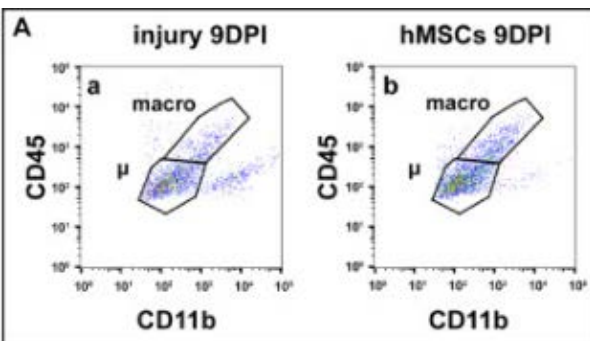


Figure 6

Results: Figure 7

M2 pro-regeneration...



Discussion

Improved hMSC survival with partial spindle-shaped morphology at 14 & 21d after sponge loading v. classic

Intrascaffold preservation of stemness with ECM

No effect of direct injection based on PCR findings

Increase of IL-1beta with (CM)scaffold treatment, reversed with HG+RGD+ECM
hMSC?

Pro-regenerative M2 recruitment persists at d9

Ability to locally maintain paracrine effect based on Hoechst assay

Revealed at 52



Ask us anything



Resources in your area



Fundraise with Team Reeve



Living with Paralysis

Get Support

Get Involved

Research

Events

Blog & Forum

About Us

Donate

Research >

Research Consortium on SCI

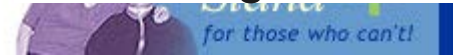
Research Consortium on SCI

Funded by the Reeve Foundation, **The International Research Consortium on Spinal Cord Injury** is a collection of laboratories working towards the identification of potential spinal repair treatments.

tor cure made him real-life hero

CHRISTOPHER REEVE

As of early 2013, the Foundation has awarded more than \$110 million (USD) in research grants and more than \$16 million in quality-of-life grants



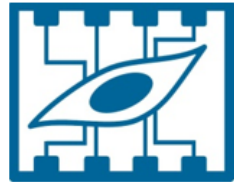
months of therapy at Kessler Rehabilitation Institute in New Jersey in 1995. Reeve would use worldwide interest in his injury to inspire at age 11, but went on to graduate from Harvard University. "It is a remarkable story of somebody who," Reeve recounted to his own mother, Barbara, grew up hope. "She became distraught and began arguing strenuously that Reeve is survived by his wife, Dana, and their son, Will, 12. He also is survived by his parents, Franklin Reeve and Barbara Johnson, and by two children from a previous relationship, Matthew, 25, and Alexandra, 21. With George Rush and Joanna Mealey
Greg Wilson
wh@ntr@cc@reel.org@nytimes.com



Organs on a Chip: a novel research model for disease or tissue regeneration

Julie Rosser, DVM, DACVS, DECVS

Cell Chip Group
Institute for Applied Synthetic Chemistry
Institute for Chemical Technologies and Analytics
Faculty of Technical Chemistry

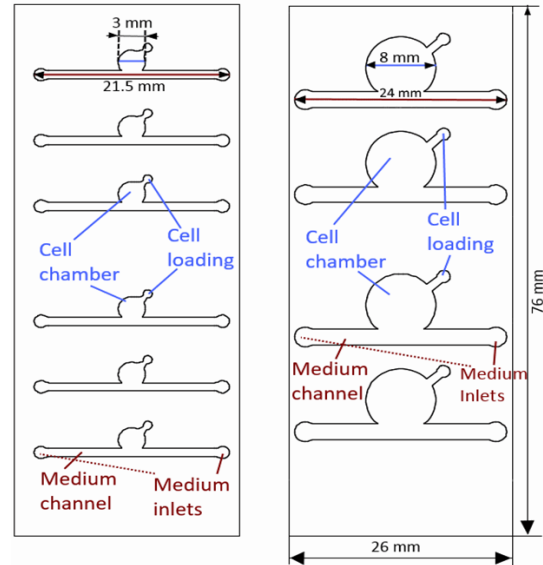


University of Veterinary Medicine, Vienna

vetmeduni
vienna 

Microfluidics Development

- PDMS bottom layer molded using soft lithography on 3D printed molds
- Glass slide as top layer



5-step process to develop microfluidic devices



1.

Design

feasibility
CAD design
simulation
finalization

2.

Mastering

3D printing
lithography
electroplating
milling

3.

Replication

PDMS cutting
 μ -mashing
casting
hot embossing
 μ -injection
molding

4.

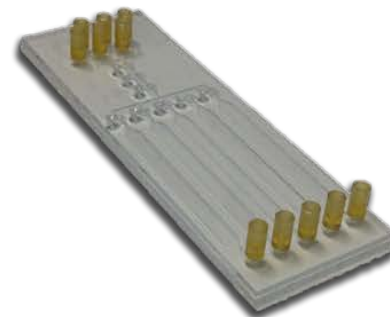
Back end processing

bonding processes
drilling and sealing
assembly and packaging
metallization
surface rendering
biofunctionalization
sensor integration

5.

Quality control

optical inspection
functional test



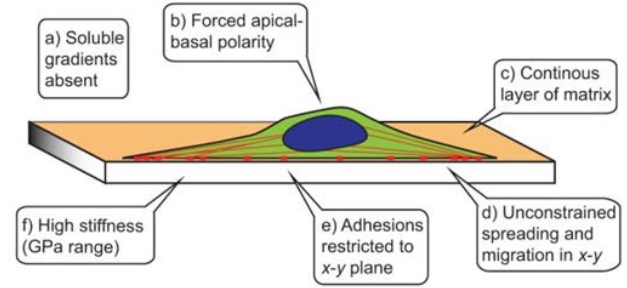
Introduction: Organs-on-a-chip

-3D cell:hydrogel culture to enhance recreation of *in vivo*-like cellular environment

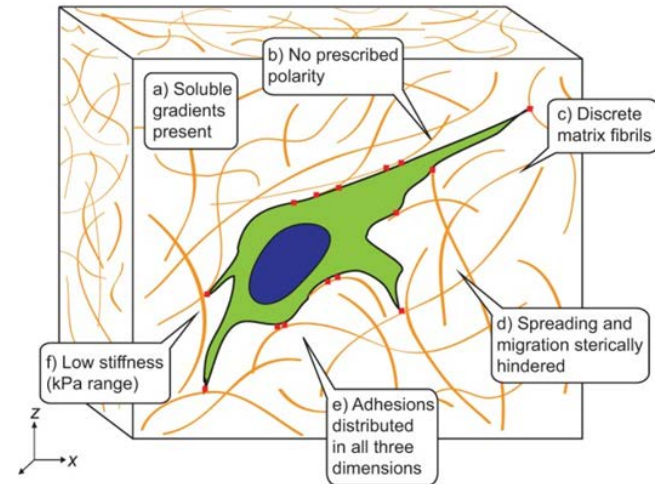
-Specialized cell culture chambers to accurately mimic physiological functions (i.e. nutrient diffusion gradients, etc.)

-Possibility to expose cells to mechanical stimuli

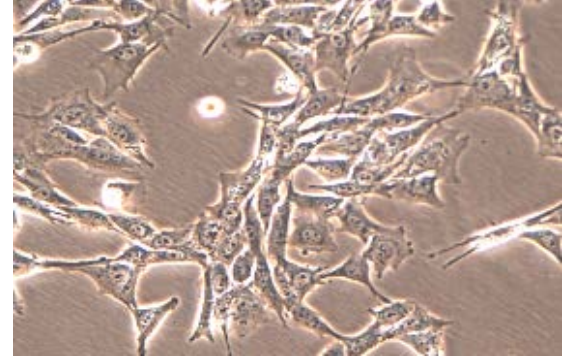
Collagen-coated glass (2D)



Collagen gel (3D)

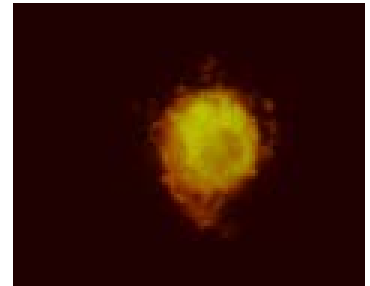


Ongoing Projects



Functional cartilage on a microchip designed for osteoarthritis research utilizing a biochemical injury;

Electrochemically characterize midbrain organoids and neuronal networks cultured in microfluidic hydrogel for future high- throughput personalized therapy for Parkinson's patients.

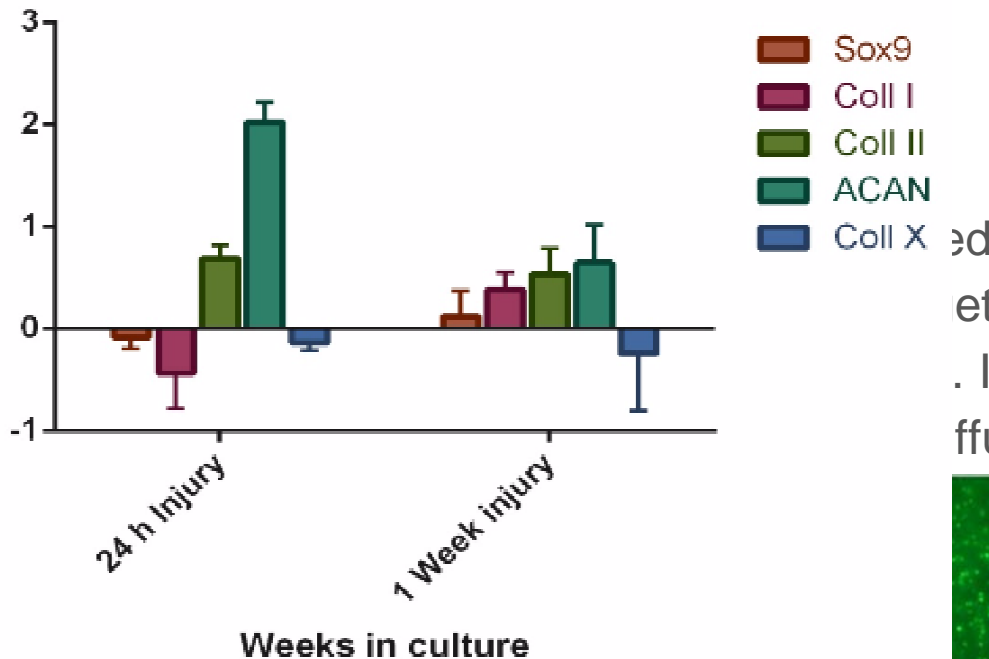


OA Results

Chemical properties in spontaneous cellular self-organization and subsequent engineering and subsequent culture technique, cells properties, cell shape,

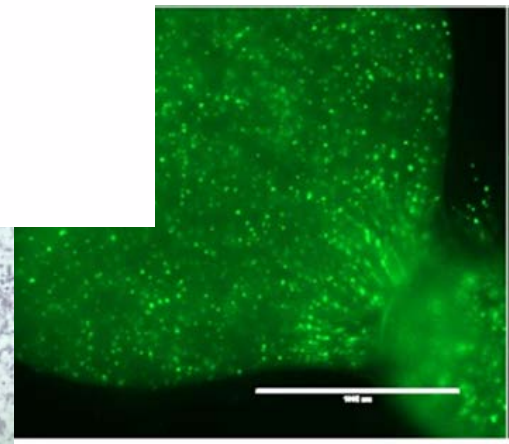
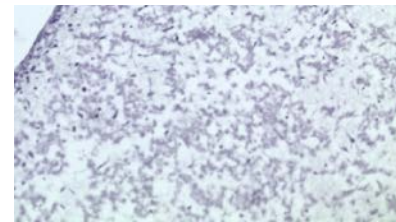
Fold Expression Change rel. to GAPDH [log 2]

Gene expression control vs injury

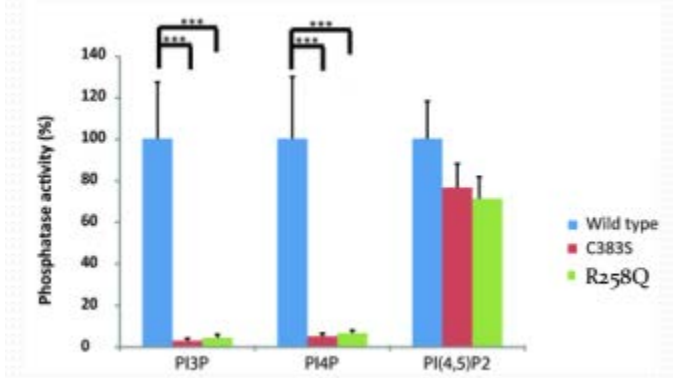


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*Existing animal OA model impact gives us the unique opportunity to validate microfluidic 3D organ on a chip directly with in vivo, ex vivo, and preclinical trials



iPSC Generation



Mutation in the *SYNJ1* gene associated with autosomal recessive, early-onset Parkinsonism. Quadri M¹, et. al., *Hum Mutat.* 2013 Sep;34(9):1208-15

iPS line number	clone #	Gene	mutation	Family member	gender	age	Fibroblast line number	pluripotency	differentiation
EMC47i.nE452	6	FBXO7	R498X	BO53	F	42	nE452	+	+
EMC47i.nE452	13	FBXO7	R498X	BO53	F	42	nE452	+	+
EMC48i.o8Eo407	7	FBXO7	T22M + IVS7+1G/T	NIJ002	F	39	o8Eo407	+	+
EMC48i.o8Eo407	8	FBXO7	T22M + IVS7+1G/T	NIJ002	F	39	o8Eo407	+	+
EMC49i.13Eo908	3	SYNJ1	R258Q	NAPO16	M	50	13Eo908	+	+
EMC49i.13Eo908	10	SYNJ1	R258Q	NAPO16	M	50	13Eo908	+	+
EMC50i.13Eo910	4	SYNJ1	R258Q	NAPO17	F	35	13Eo910	+	+
EMC50i.13Eo910	5	SYNJ1	R258Q	NAPO17	F	35	13Eo910	+	+

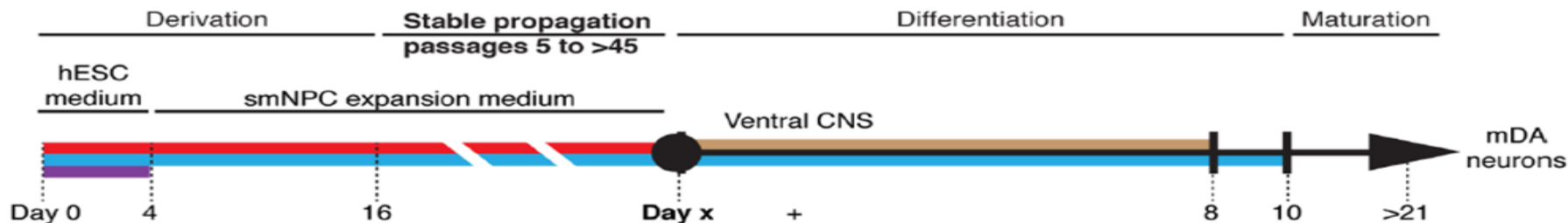
Disease vs Healthy: CRISPR

- Design and construction of sgRNAs targeting wild type and mutated FBXO7 and SYNJ1 loci and introduction of sgRNAs into modified Cas9-harboring plasmids
 - pX330A: plasmid harboring Cas9, GFP and puromycin resistance gene
 - pX335B: plasmid harboring Cas9 D10A nickase, GFP and puromycin resistance gene

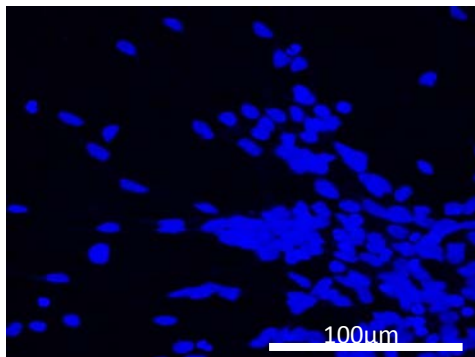
	FBXO7		SYNJ1	
Constructs for gene correction	Cas9	✓	Cas9	✓
	Cas9 D10A	✓	Cas9 D10A	✓
Constructs for introduction of mutation	Cas9	✓	Cas9	✓
	Cas9 D10A	✓	Cas9 D10A	✓

Neuron differentiation protocol

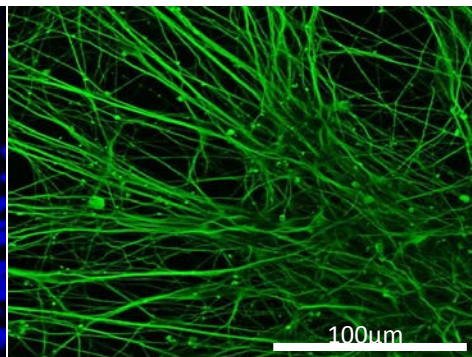
- Differentiate to mDA neurons



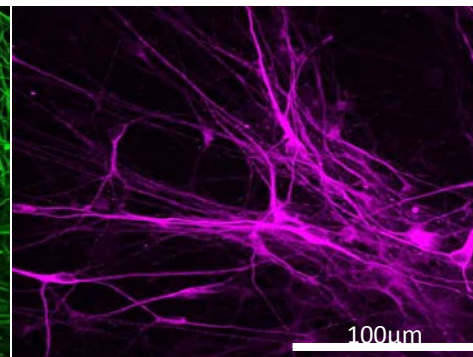
Reinhardt et al, 2013



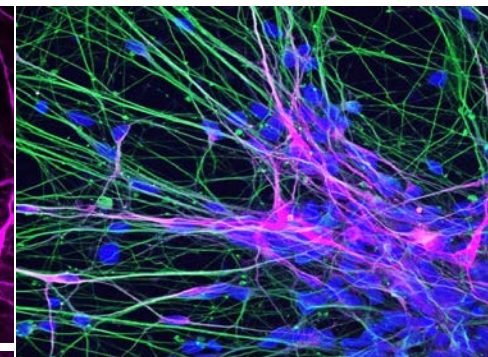
DAPI



TUJ1



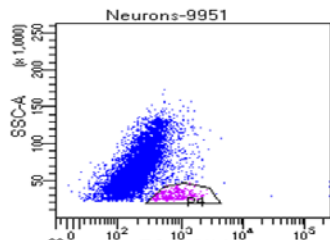
TH



Overlay

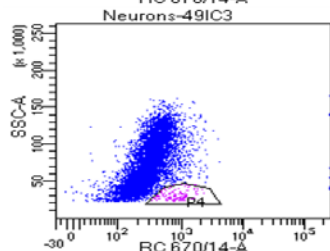
Dopaminergic neuron development

- Fewer TH+ cells in patient-line differentiations



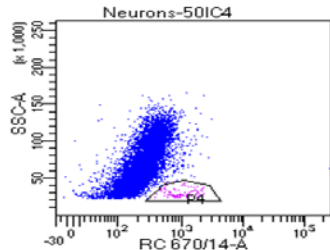
Tube: 9951

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	9,124	91.2	91.2
P2	8,029	88.0	80.3
P3	8,010	99.8	80.1
P4	360	4.5	3.6



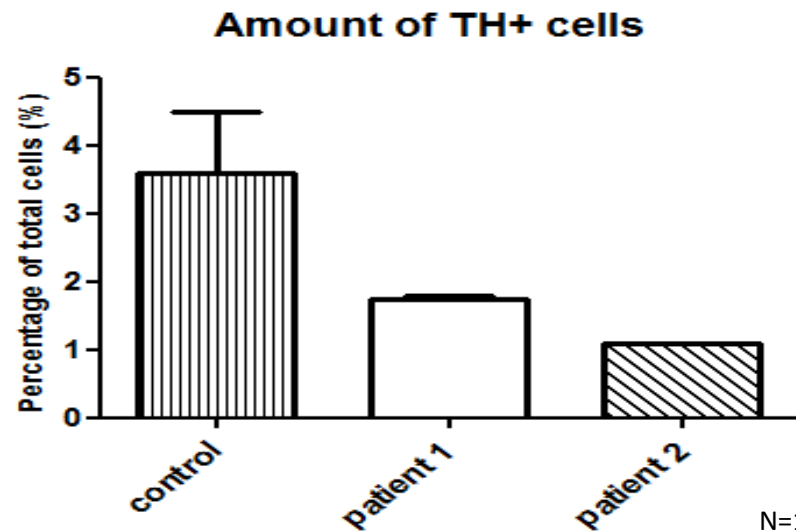
Tube: 49IC3

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	8,502	85.0	85.0
P2	7,491	88.1	74.9
P3	7,473	99.8	74.7
P4	137	1.8	1.4



Tube: 50IC4

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	9,158	91.6	91.6
P2	8,334	91.0	83.3
P3	8,319	99.8	83.2
P4	92	1.1	0.9



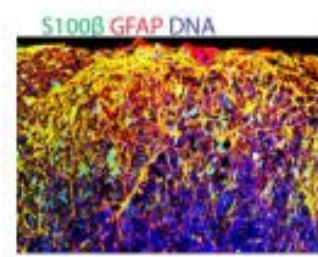
N=1

3D Midbrain organoids

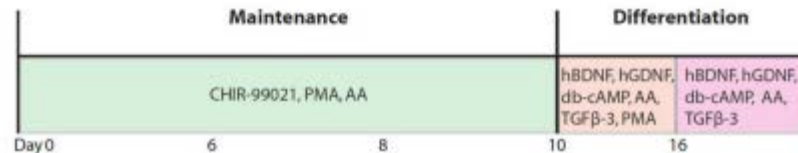
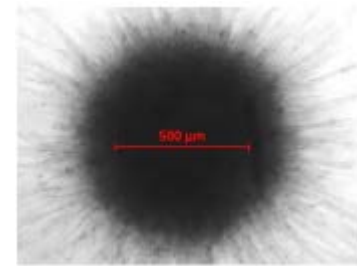
Midbrain organoids exhibit neuronal, astroglial and oligodendrocyte differentiation

Midbrain organoids exhibit spatial asymmetry of mDA neurons

- Midbrain specific, due to mDA marker expression
- Midbrain organoids are functional



Monzel AS. *et al.* 2016

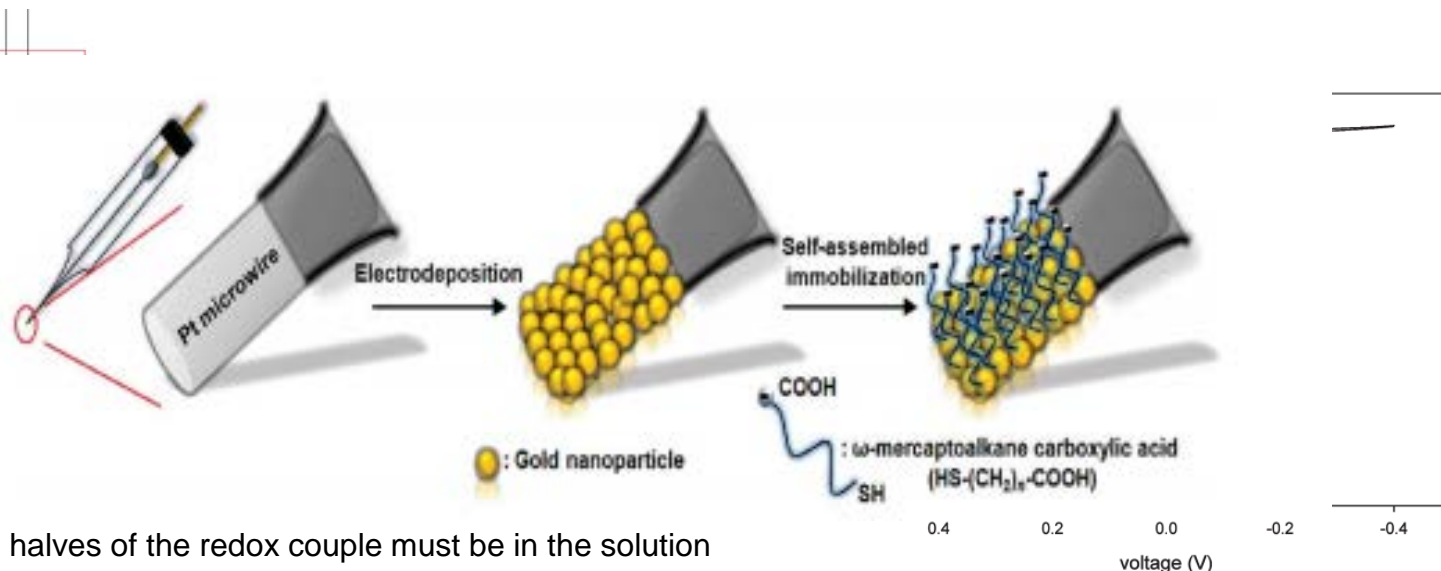


Microelectrodes with gold nanoparticles and self-assembled monolayers for *in vivo* recording of striatal dopamine†

Tien-Chun Tsai,^a Chun-Xian Guo,^{bc} Huan-Zhang Han,^a Yu-Ting Li,^a Ying-Zu Huang,^d Chang-Ming Li^{bc} and Jia-Jin Jason Chen^{*ae}

Received 28th December 2011, Accepted 5th April 2012

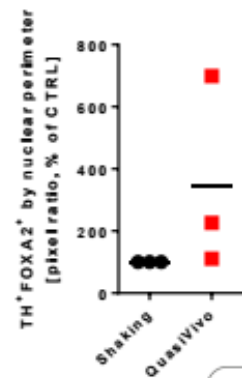
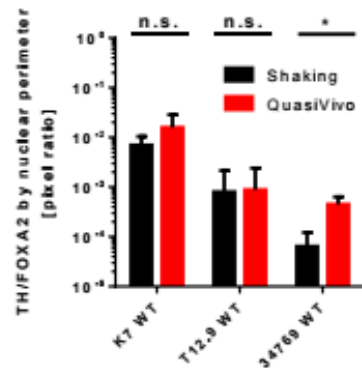
DOI: 10.1039/c2an16306c



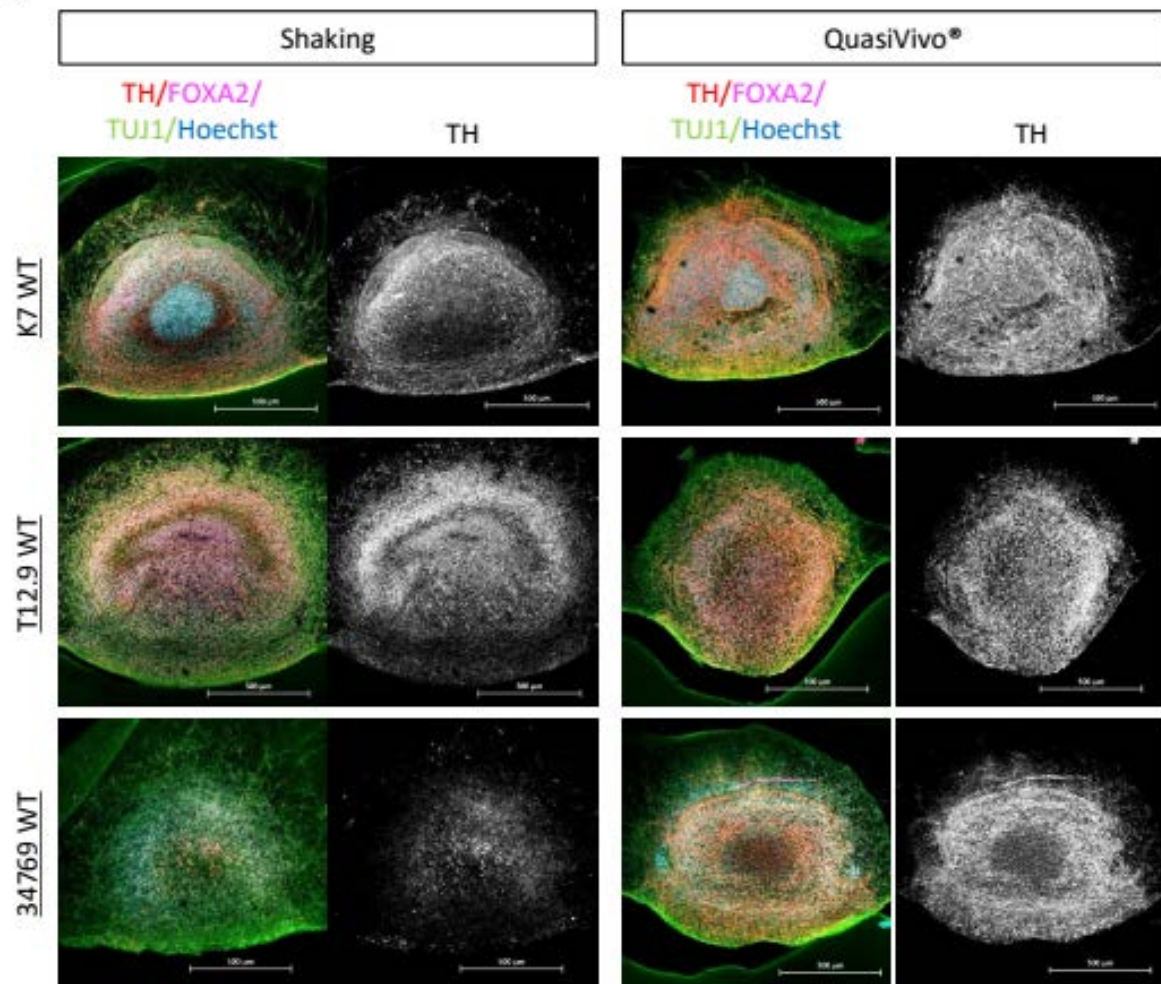
Cyclic Voltammogram (with redox cycling)

- Both halves of the redox couple must be in the solution
- Amplification of the signal through multiple redox cycling of closely spaced electrodes

3DPD Results



A



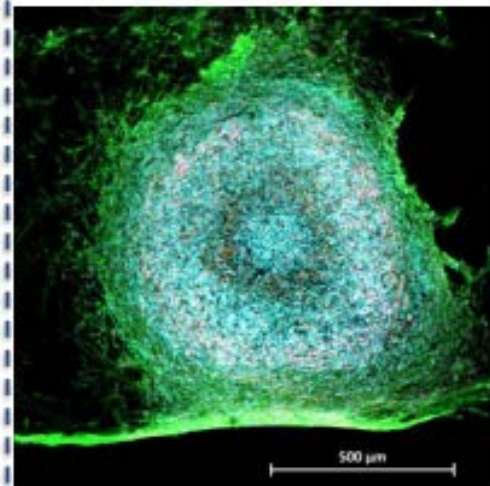
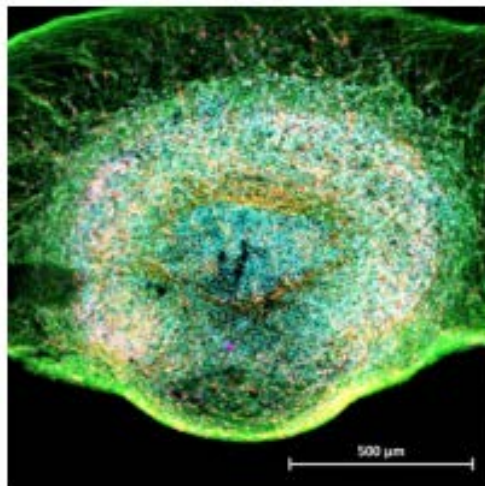
Geltrex

PEG-Fibrinogen

Geltrex

(day 1, 21, 42)

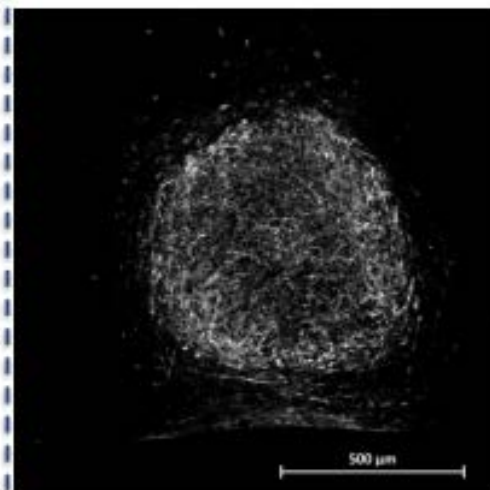
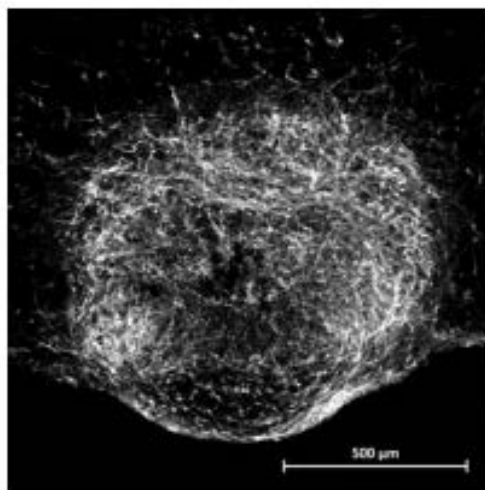
TH/FoxA2/
TUJ1/Hoechst



PEGf

(day 1, 28, 42)

TH



p

