A Combinatorial Hydrogel Platform to Probe Stem Cell Chondrogenesis in 3D

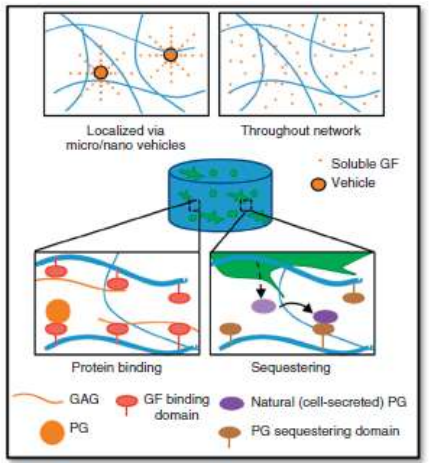
Sebastián L. Vega, Kwang Hoon Song, Chao Wang, Lin Han, Jason A. Burdick

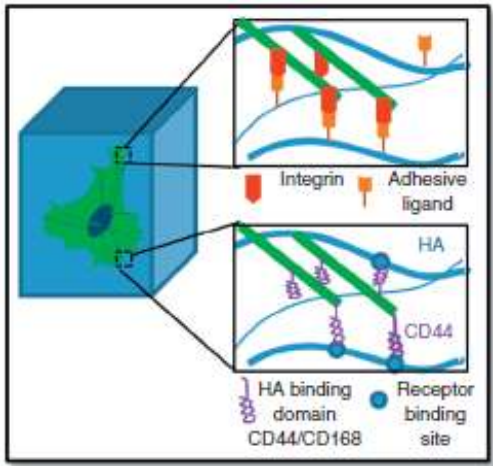
Monday October 24, 2016, 2:05PM

The Stem Cell Microenvironment

growth factors ***cell-ECM interactions***

Biochemical Cues





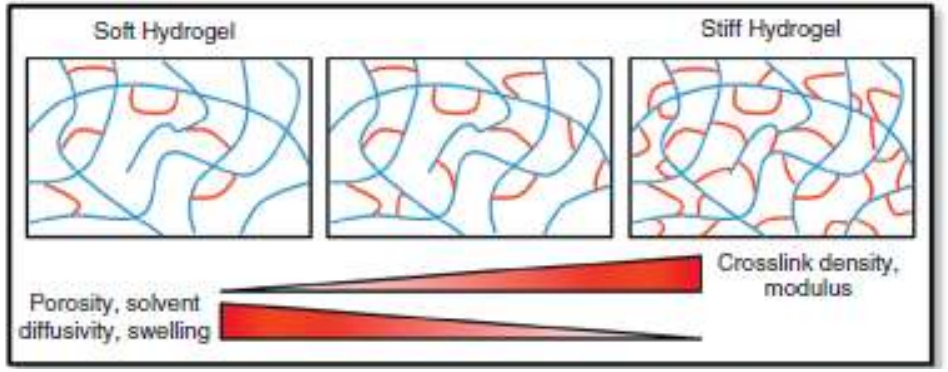
Challenges in deciphering cell-ECM interactions:

- Tedious to evaluate many conditions
- Unable to decouple mechanical and biochemical cues

Need for novel materials and high-throughput techniques to probe cell-ECM interactions

mechanics, topography

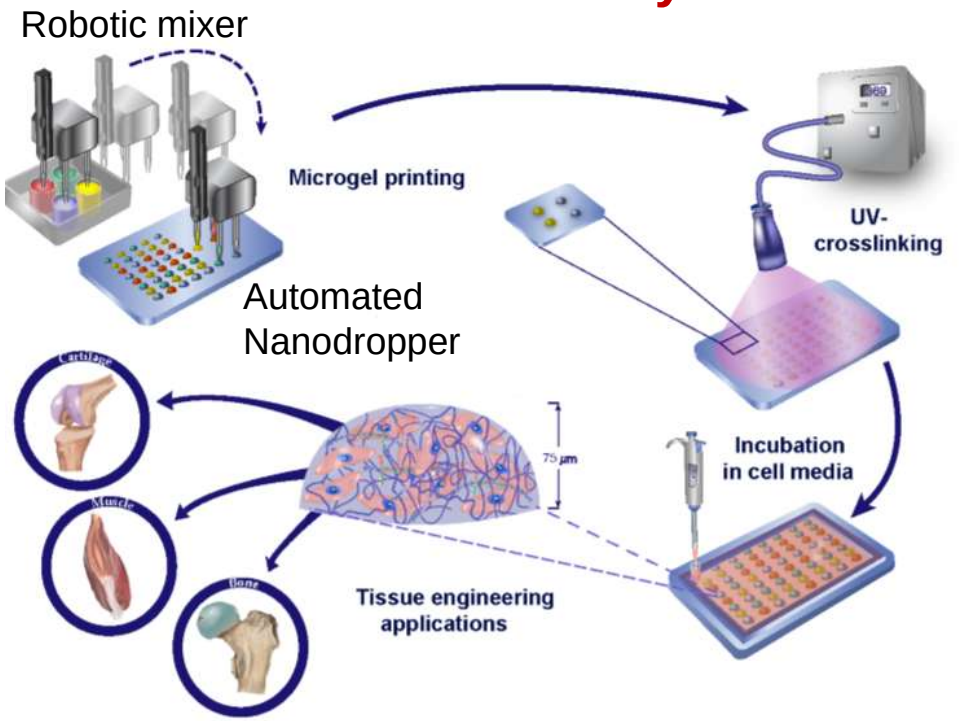
Physical Cues



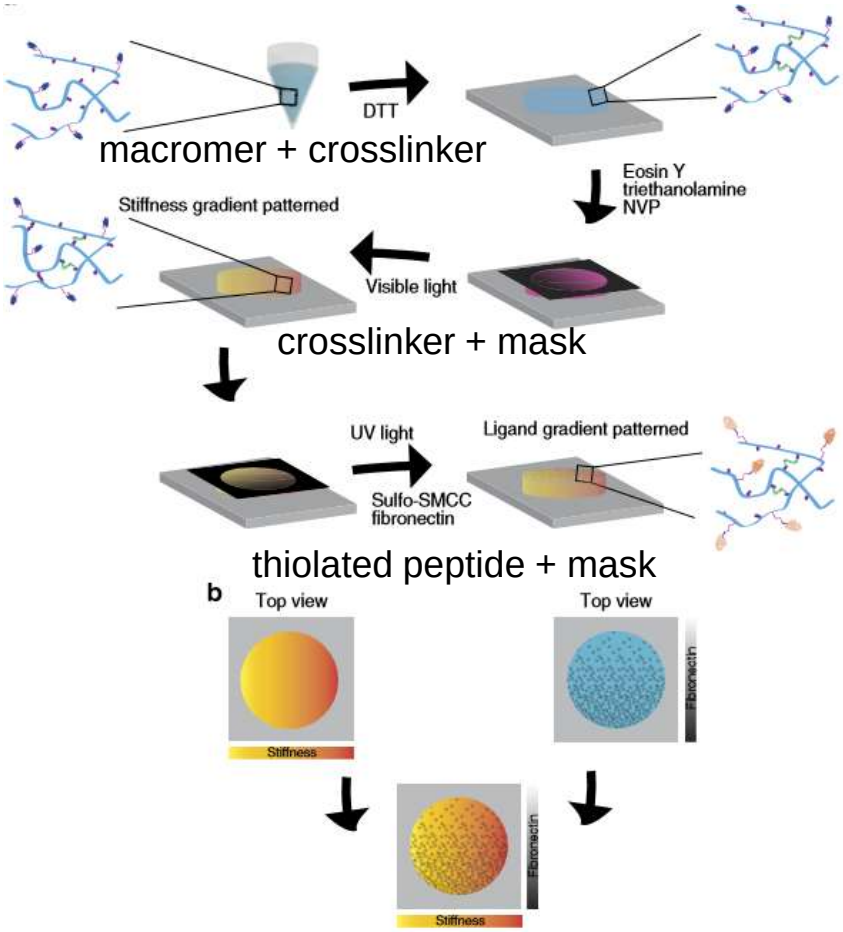
Guvenriden et al., *Curr Opin Biotechnol*, 2014.

Techniques to probe cell-biomaterial interactions

ECM Mimetic Arrays



Combinatorial Hydrogels



ECM Mimetic Arrays

- Complex DOE
- Expensive equipment not readily available

1st Generation Combinatorial Hydrogels

- Mostly explored in 2D contexts
- 2D does not represent 3D environments

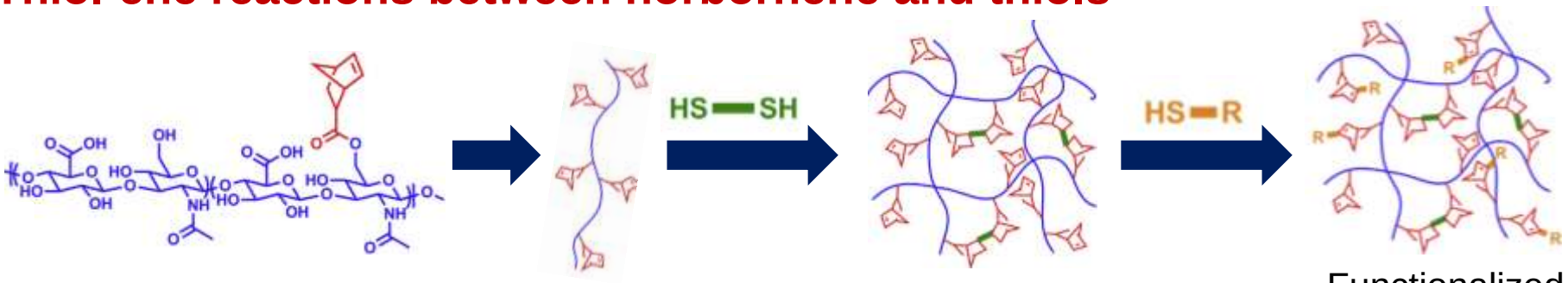
Dolatshahi-Pirouz et al., *Sci Rep*, 2014;
Rape et al., *Nat Commun*, 2015.

Combinatorial hydrogels to screen 3D cell-ECM interactions

Design Criteria:

- 3D cell encapsulation
- Cytocompatible crosslinking
- Ability to vary biochemical and mechanical signals
- Rapid single cell imaging possible

Thiol-ene reactions between norbornene and thiols

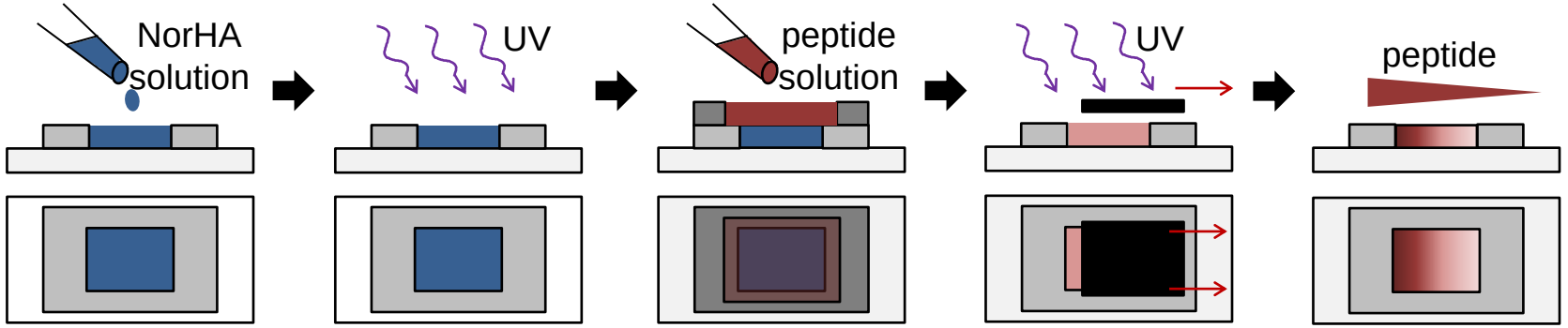


NorHA

Hydrogel

Functionalized with R peptide

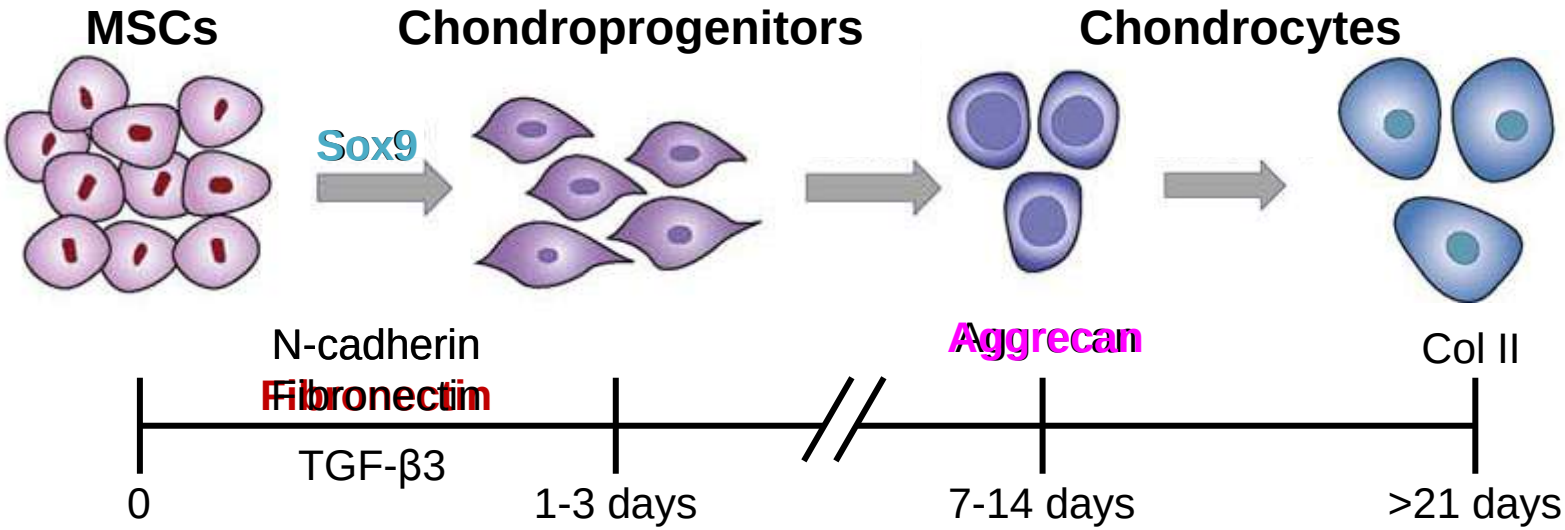
Scheme for fabricating combinatorial hydrogels



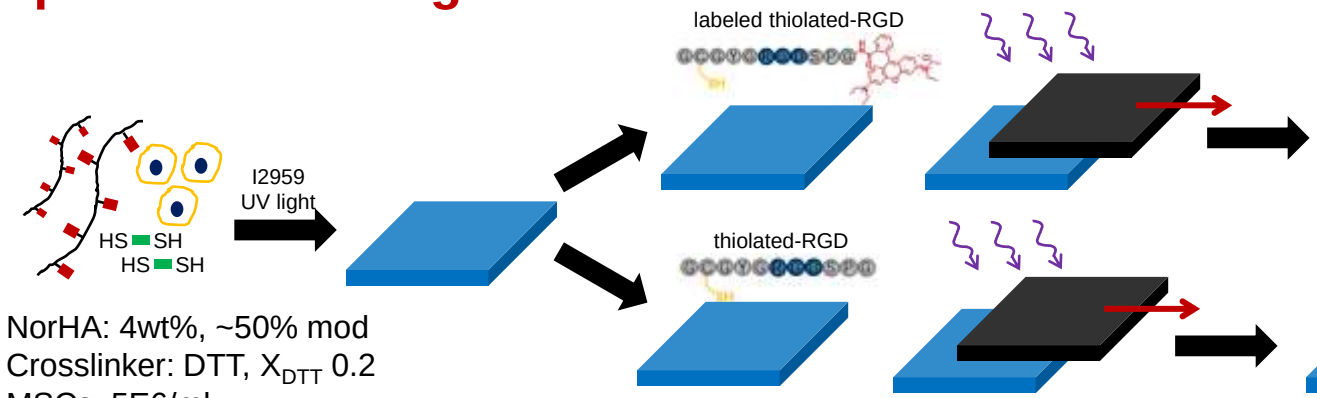
Gramlich et al., *Biomaterials*, 2013.

RGD gradients to probe effects on chondrogenesis

Chondrogenic Differentiation



Experimental Design



NorHA: 4wt%, ~50% mod
Crosslinker: DTT, X_{DTT} 0.2
MSCs: 5E6/ml
Chemically-defined media +TGFβ3 (10ng/ml)

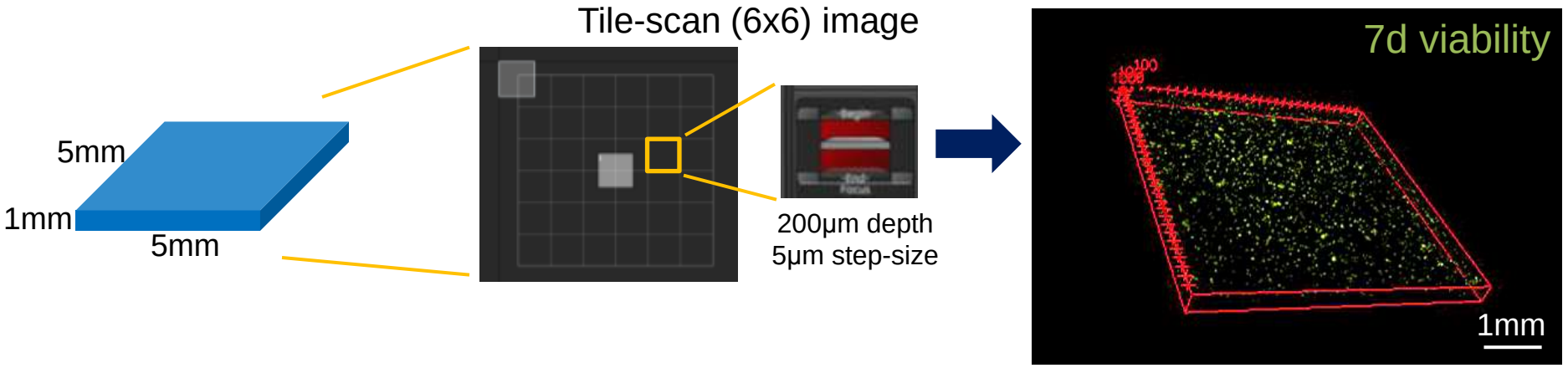
Hydrogel characterization
RGD gradient, mechanics

Cellular studies
Viability (+7d), Sox9 (+1d), aggrecan (+7d)

Vinatier et al., *Trends in Biotech*, 2009.

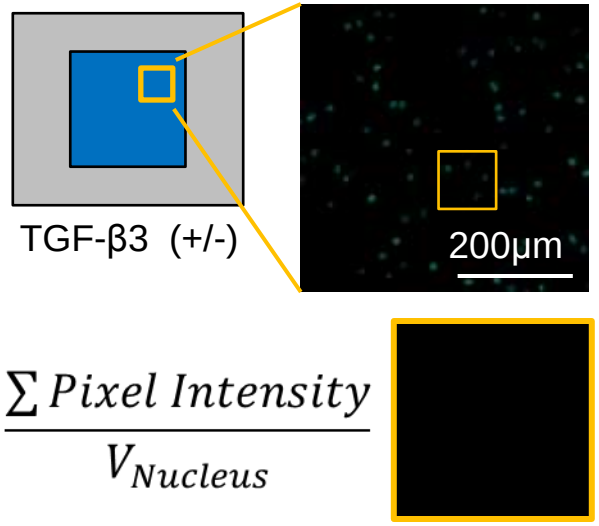
Confocal microscopy of 5x5 mm hydrogels

Develop whole-hydrogel imaging protocol

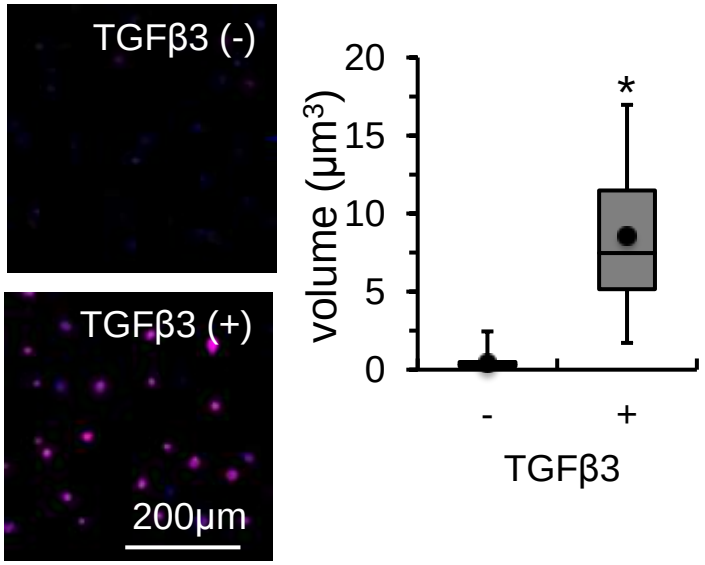


Develop Sox9 and aggrecan image analysis protocols

Sox9 (+1 day)

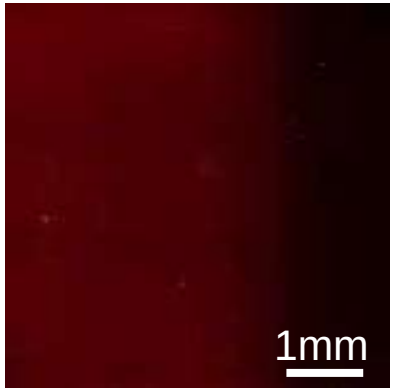


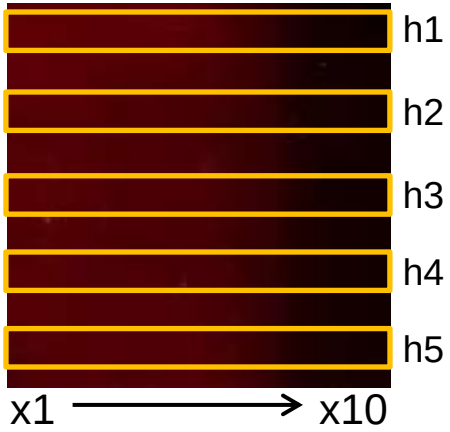
Aggrecan (+7 days)



Combinatorial hydrogel characterization

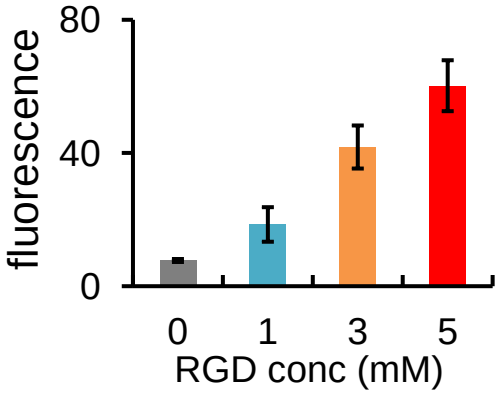
Hydrogel characterization
•RGD gradient

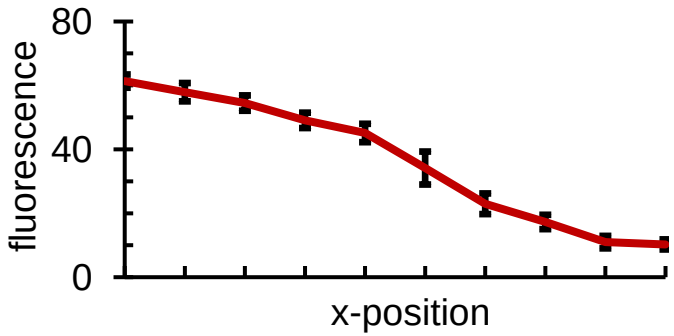


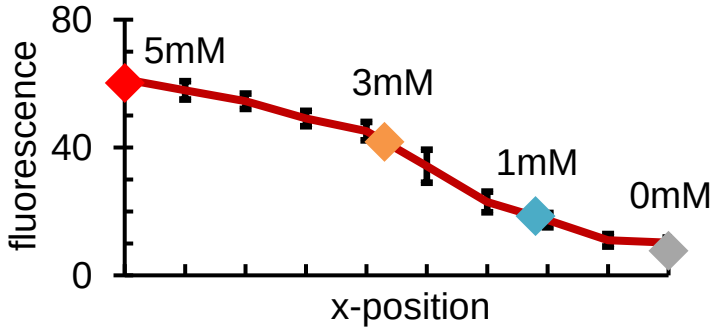


- Tile-scan confocal imaging
- Measure horizontal fluorescence (x1 → x10)
- 5 horizontal regions (n=3)

- Irradiate gels, measure fluorescence of known RGD concentrations





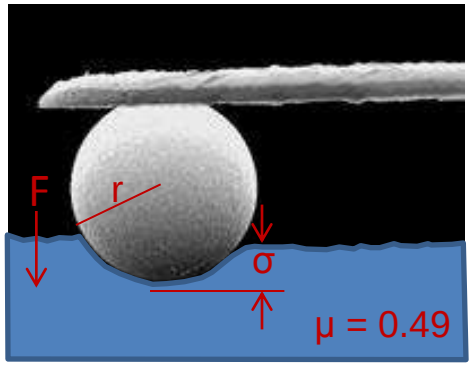


Combinatorial hydrogel characterization

Hydrogel characterization
•Mechanics

7.1 ± 1 kPa

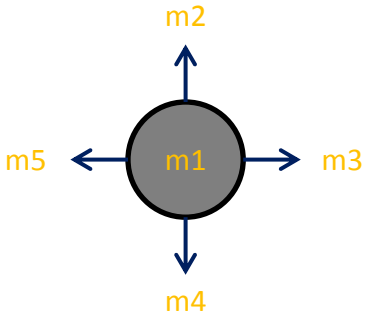
Hertz indentation model



$$F = \frac{4}{3} \cdot \frac{E_S}{1-\mu^2} \cdot \sqrt{r} \cdot \sigma^{\frac{3}{2}}$$

E_s = hydrogel modulus
μ = Poisson's ratio
r = probe radius

10x10 array, 15 measurements/position



Elastic moduli (5 – 9 kPa)

7.8	8.4	8.6	8.6	8.0	8.9	7.9	7.9	8.5	6.6
8.2	8.9	8.6	7.1	8.6	8.9	8.3	8.9	8.6	8.6
8.1	8.4	8.4	7.9	8.8	8.5	8.2	6.6	6.2	5.7
7.8	7.6	7.6	7.9	7.6	8.1	6.5	5.8	5.8	6.0
6.9	7.9	7.9	7.4	8.0	6.7	6.3	5.8	6.7	5.6
7.8	7.9	8.5	6.7	6.0	6.0	5.7	6.8	5.3	5.6
7.8	7.2	5.9	7.1	7.6	6.5	6.5	5.8	5.7	5.6
5.9	7.2	7.2	7.5	7.2	7.6	6.3	6.3	5.8	5.6
7.2	6.9	7.5	7.8	6.5	6.3	6.4	6.2	5.6	5.5
7.4	7.9	6.7	7.2	7.5	7.0	6.2	6.0	5.2	5.6

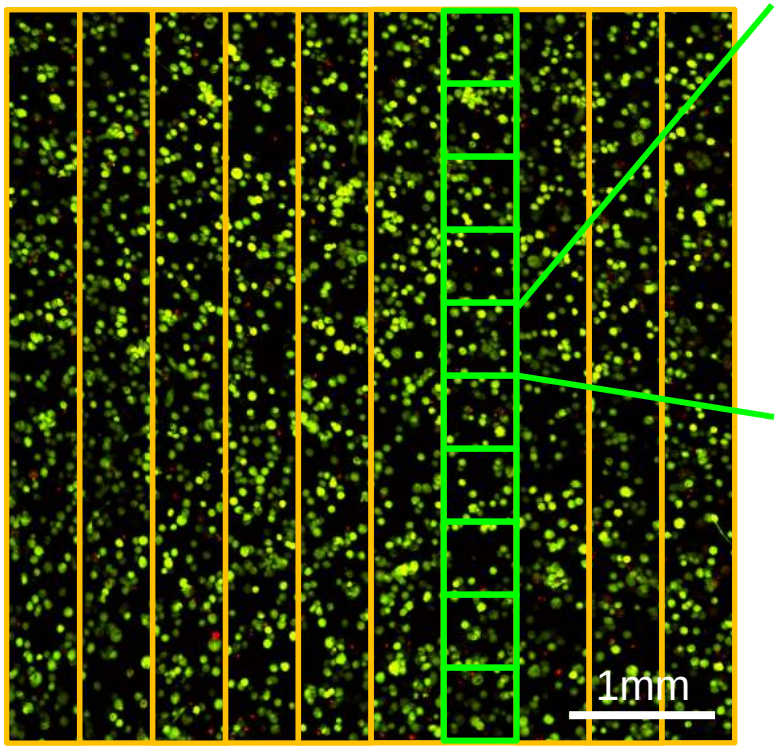
Variation from mean (< 30%)

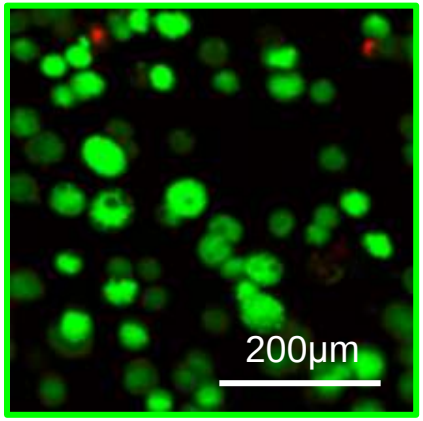
0.09	0.17	0.21	0.20	0.12	0.25	0.11	0.11	0.19	0.07
0.14	0.25	0.21	0.01	0.20	0.25	0.16	0.25	0.21	0.21
0.14	0.17	0.18	0.10	0.23	0.19	0.15	0.08	0.13	0.20
0.10	0.06	0.07	0.11	0.06	0.14	0.10	0.19	0.19	0.15
0.03	0.11	0.11	0.04	0.12	0.06	0.12	0.19	0.07	0.22
0.09	0.10	0.19	0.06	0.15	0.15	0.21	0.05	0.26	0.21
0.10	0.00	0.17	0.00	0.06	0.09	0.08	0.18	0.21	0.21
0.17	0.01	0.01	0.05	0.01	0.06	0.11	0.12	0.19	0.22
0.01	0.03	0.05	0.10	0.09	0.12	0.10	0.14	0.21	0.23
0.04	0.11	0.06	0.01	0.04	0.02	0.13	0.16	0.27	0.22

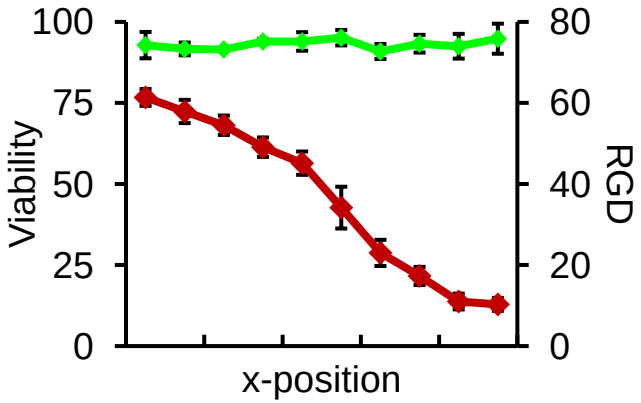
Assessment of MSCs encapsulated in combinatorial hydrogels

Cellular Studies

- Divide gel into 10 vertical regions (x1 → x10)
- Divide each region into 10 squares
- For each square:
  - **Viability:** count live/dead cells
  - **Sox9:** nuclear fluorescence
  - **Aggrecan:** secreted volume







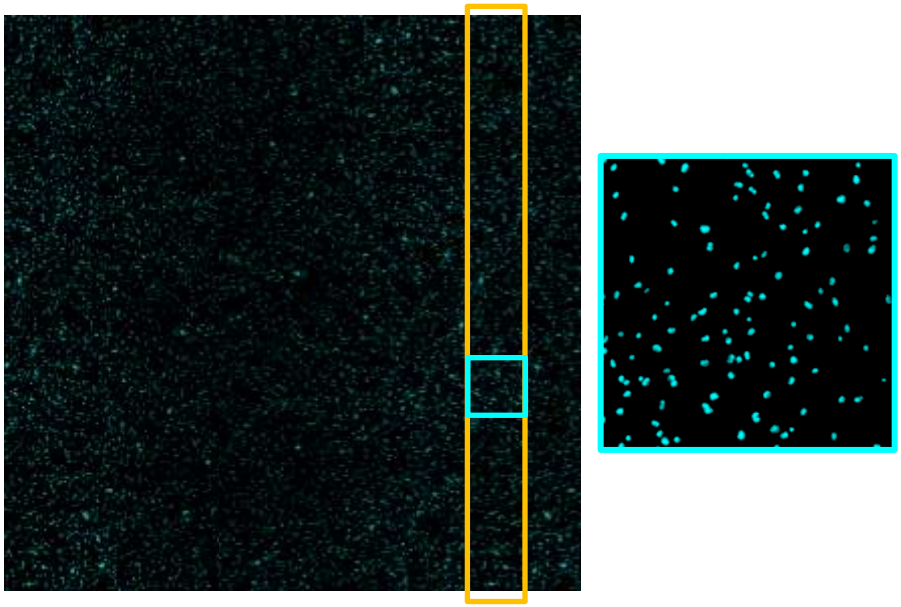
Viability

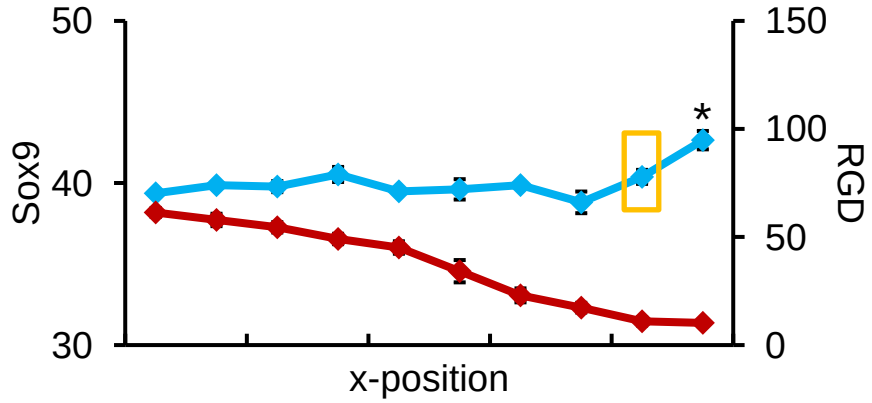
- High MSC viability for at least 7 days in combinatorial hydrogels
- Rapid imaging & image analysis developed (>300 cells analyzed per position)

Assessment of MSCs encapsulated in combinatorial hydrogels

Nuclear Sox9 fluorescence (+1d)

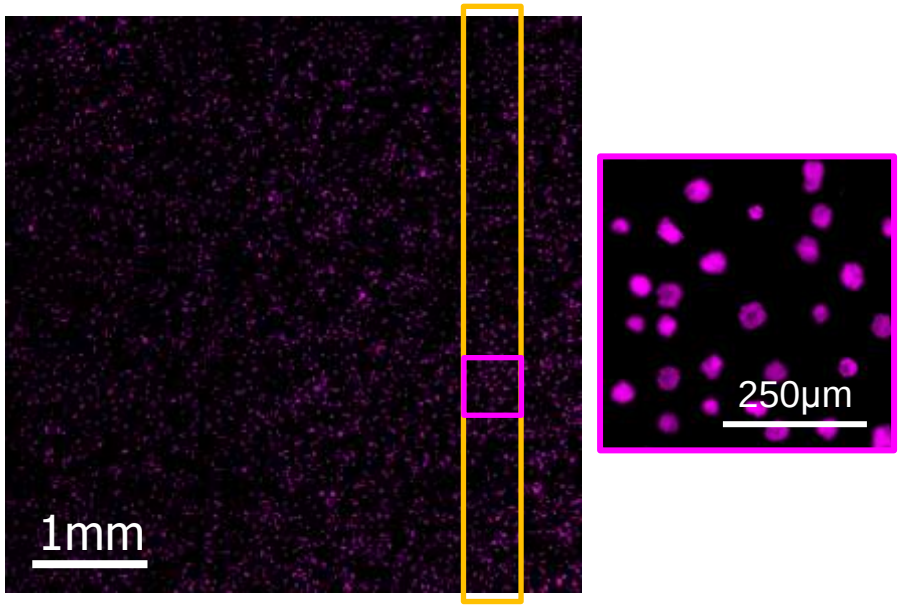
•No RGD (x10) → highest nuclear Sox9

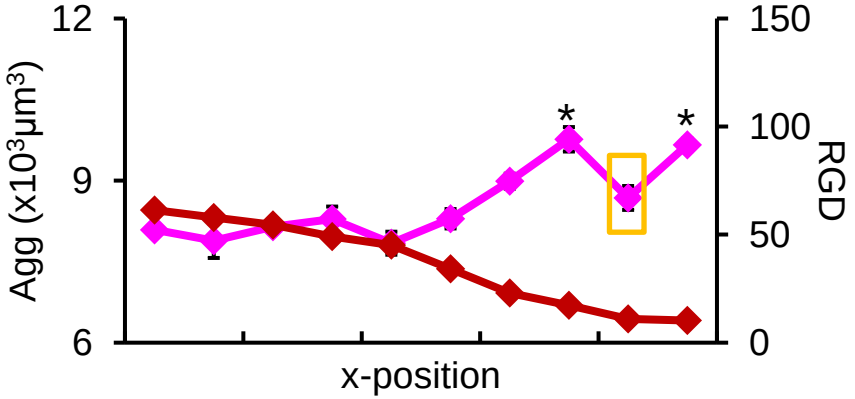




Aggrecan production (+7d)

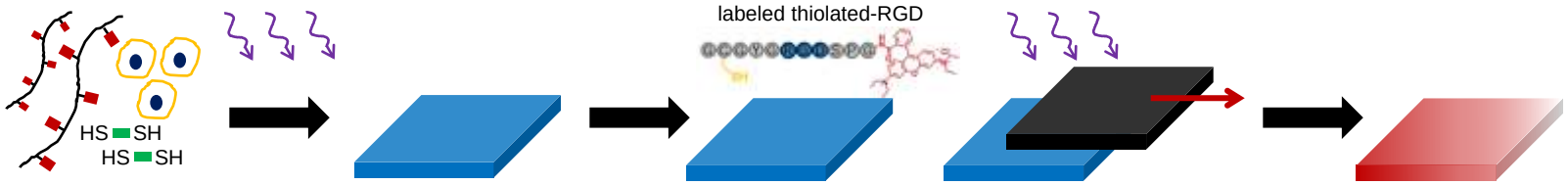
•Best: x8 (~1mM RGD), x10 (no RGD)





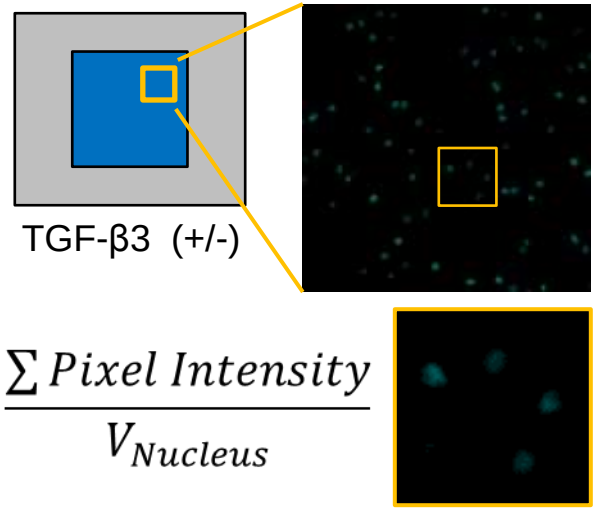
Summary

Developed combinatorial hydrogel system

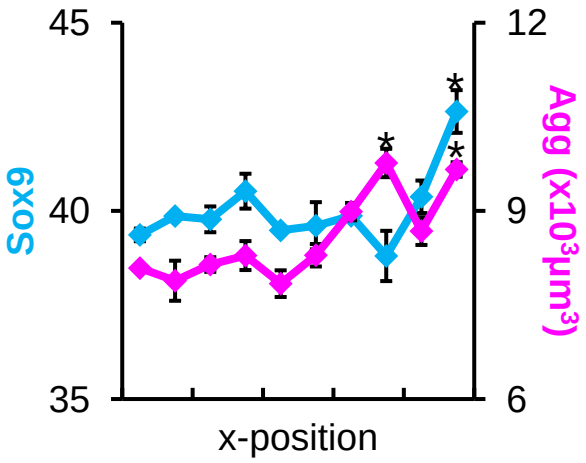


- High viability
- Wide range of biochemical cues (0 → 5mM)
- No significant change in elastic moduli

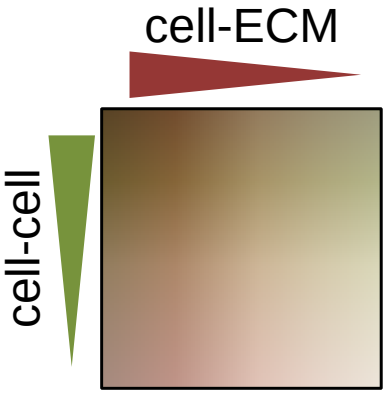
Rapid confocal imaging and image analysis



Significant differences in chondrogenic markers



Can add additional biochemical cues



Acknowledgments and questions

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