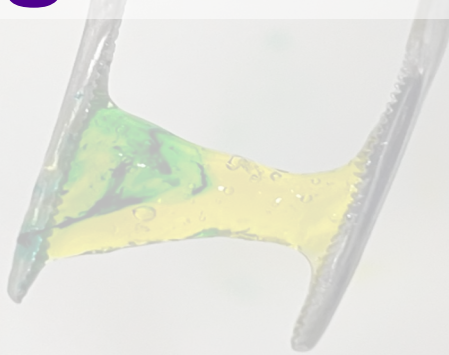
Tampere University

Advanced hydrogel materials for cell and organoid biology



Austin Donnelly Evans, PhD Student, MSc (Tech)

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Tampere University, Faculty of Medicine and Health Technology (MET)

Bioengineering and Nanomedicine Group and Protein Dynamics Group

Finland 3R Symposium 2023

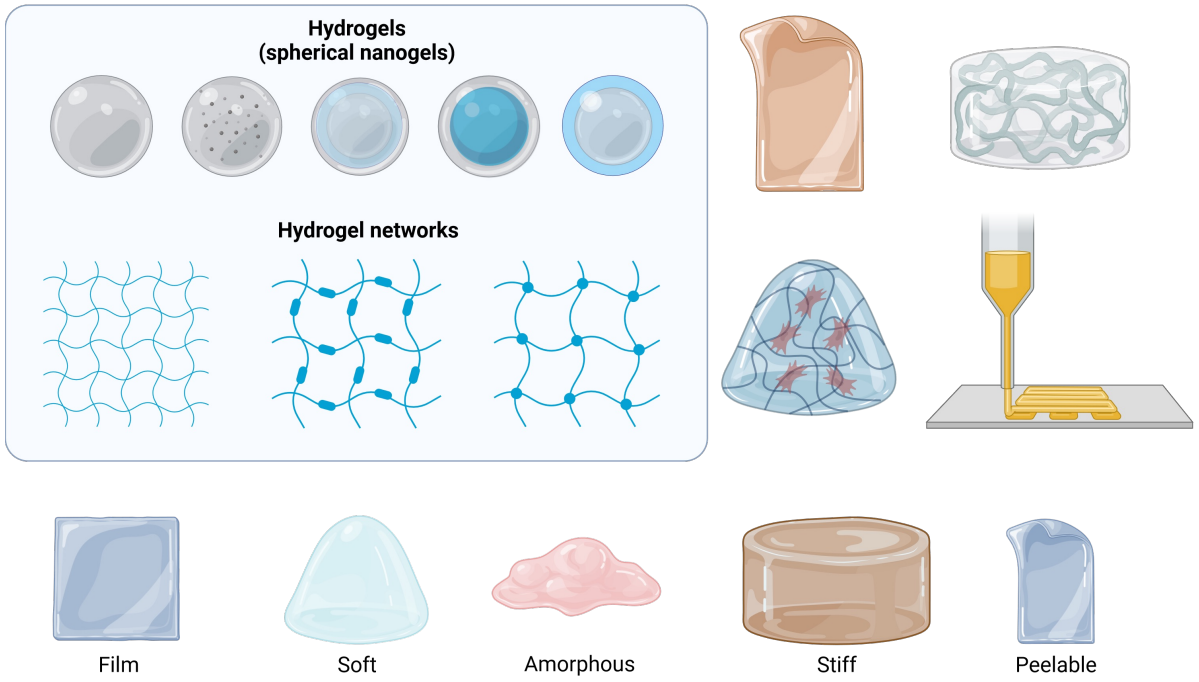
FIN3R

I will talk about the following topics:

- **Hydrogels**: an introduction and background to illustrate why hydrogel materials are especially relevant today
- What **type of questions** can we address with hydrogels?
- **Our approach** towards more sophisticated and feasible hydrogels

What are hydrogels?

- 3D network of polymer chains that absorb and retain water without dissolving.
- They are viscoelastic materials that can exist as solids, liquids, or both with non-Newtonian viscoelastic behavior.



Made with Biorender.com

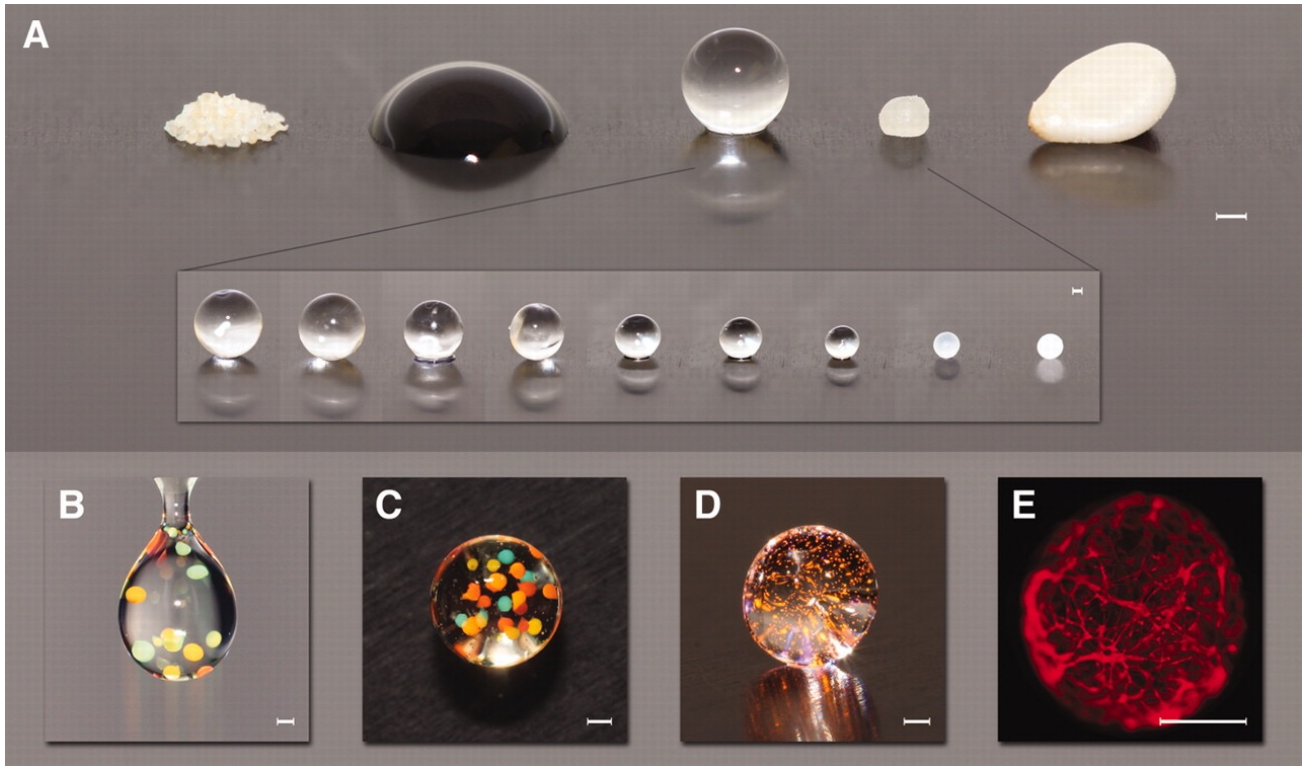
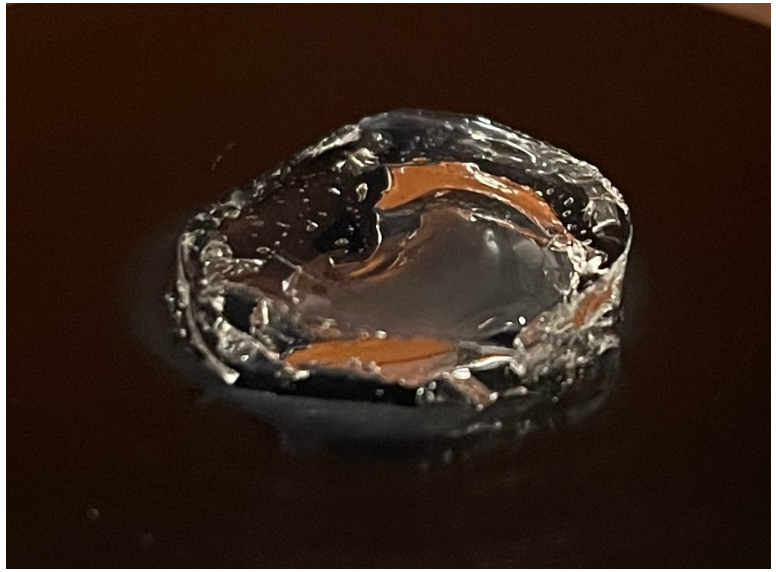


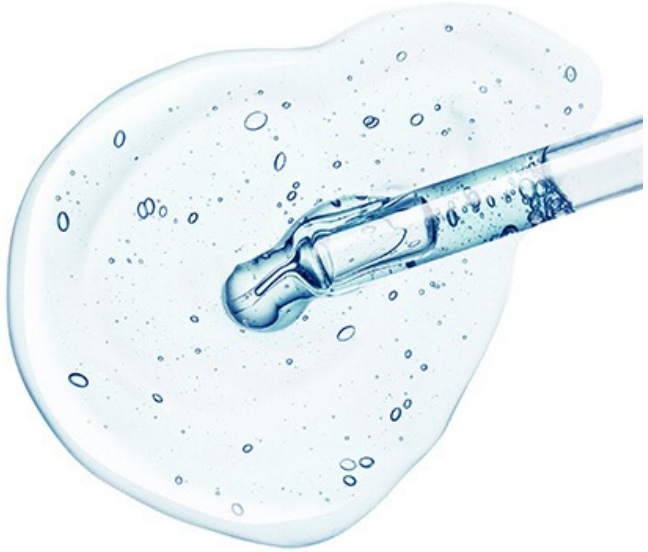
Fig: Hydrogels can be cast into any shape, size or form.

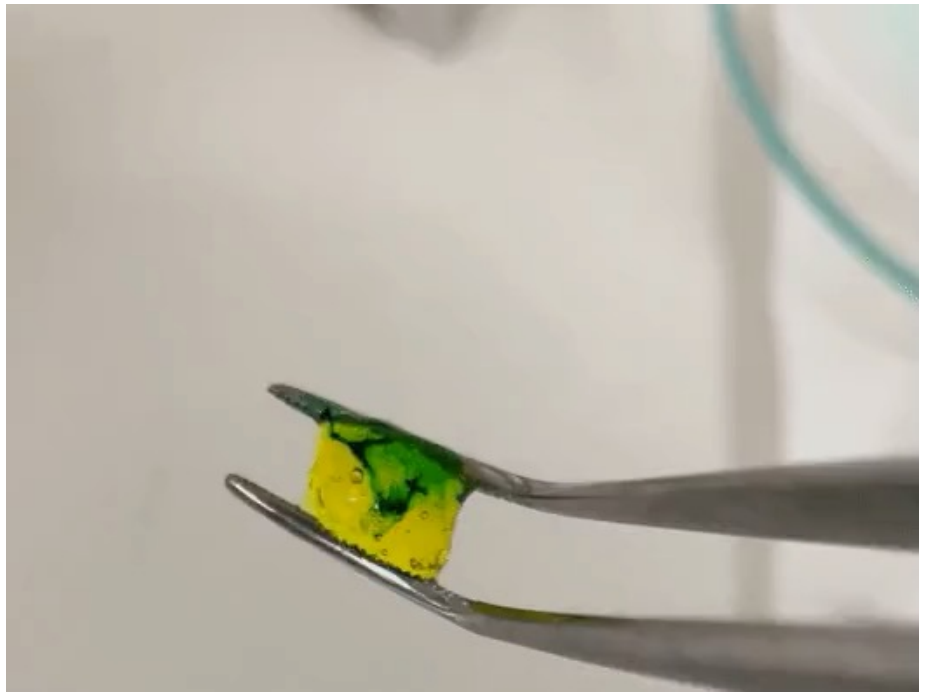
Seliktar, 2012, DOI: 10.1126/science.1214804

Hydrogels come in many forms but are separated into two main material categories that can overlap with hybrids: Synthetic and Natural



Synthetic Hydrogel

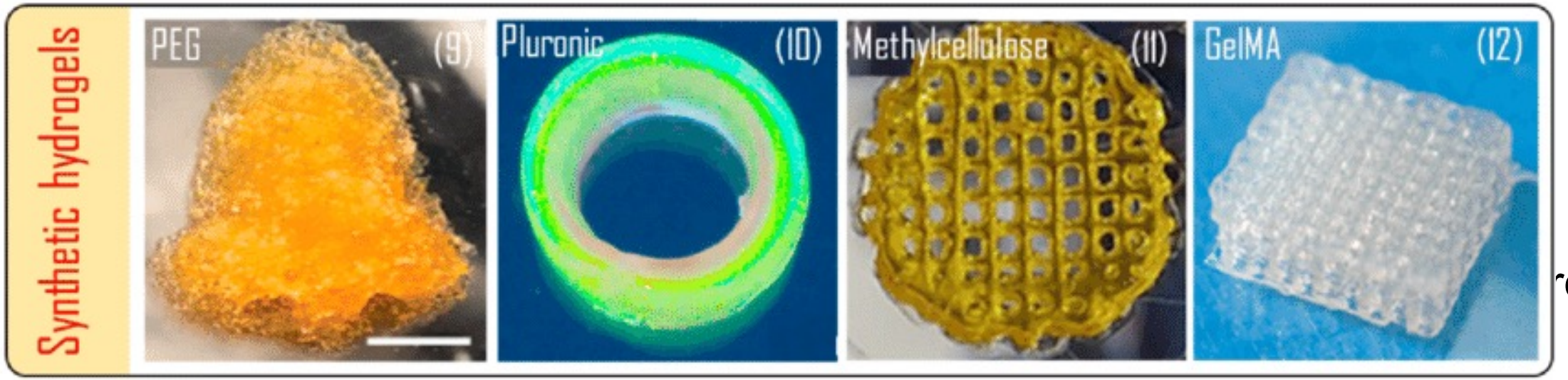




Natural hydrogel (Hyaluronic Acid)

Synthetic hydrogels

Polyethylene glycol (PEG), Polyacrylamides (PAAm), PolyVinyl Alcohol (PVA), Polyacrylic acid (PAA), Pluronics



- F
- V
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  c
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  l
  pathogens
- Longevity (non-biodegradable)
- Low immunogenicity

reign

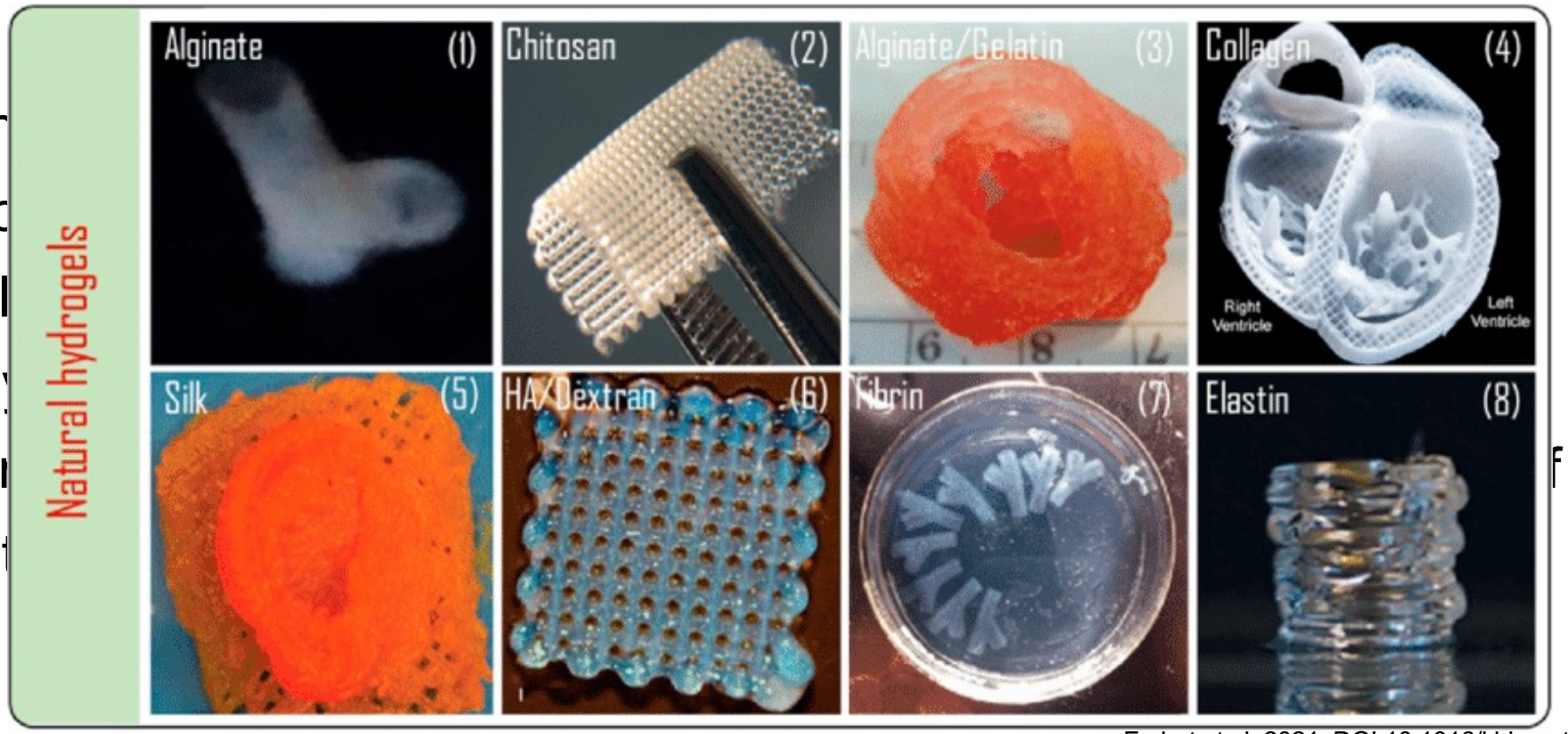
- Sometimes complex and costly manufacturing with toxic substances
- Limited elasticity without additional chemical modifications or mixtures

Farhat et al, 2021, DOI:10.1016/j.biomaterials.2020.120465

Natural Hydrogels

Dextran, Hyaluronic Acid, Collagen, Gelatin, Alginates, Agarose, Gellan gum, Xanthan Gum, Chitosan, Pectins, Cellulose, Chitin, Silk Fibroin, Fibronectin

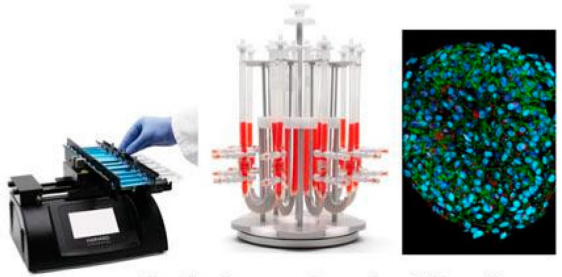
- **Pros:**
- Generally in
- Natural bioc
- Cell controll
- Non-toxic b
- Potential fo
- Can be cost



f animal

Farhat et al, 2021, DOI:10.1016/j.biomaterials.2020.120465

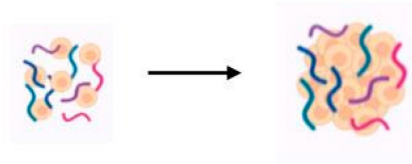
3D technologies for *in vitro* models



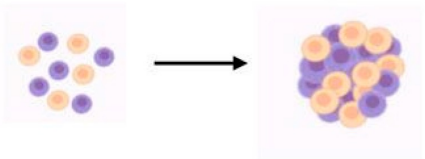
Perfusion system for 3D cell culture and a perfused cell-laden scaffold

Macroporous scaffold

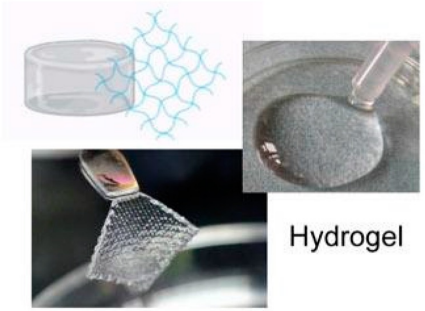
Spheroid



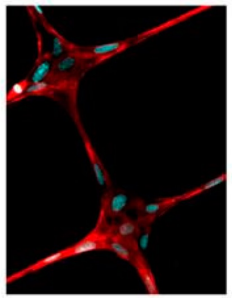
Nanofibers incorporation during spheroid formation



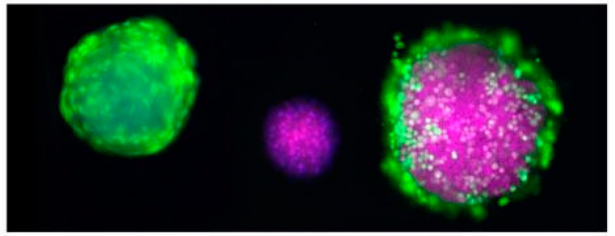
Co-cultured spheroid



Hydrogel

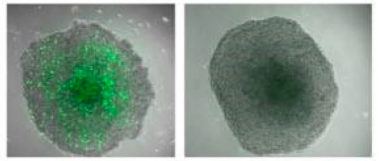


Bioprinted microfibers imaging



Spheroid imaging

Bioprinted scaffold



Imaging of nanobeads incorporation in spheroid

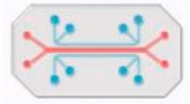
Nanostructured biomaterials

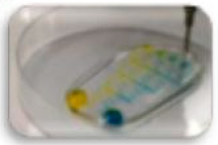
Multicellular spheroids

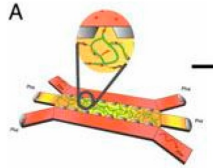
Organoids and organs-on-a-chip

Organs-on-a-chip

Organoids







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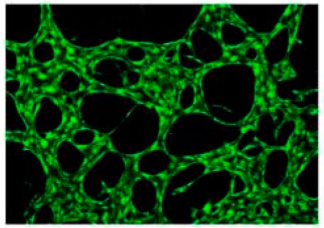
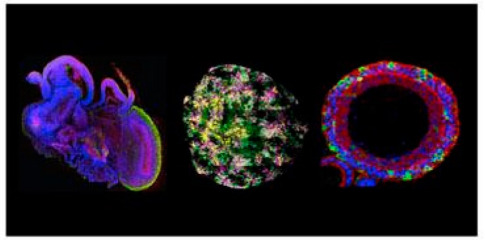
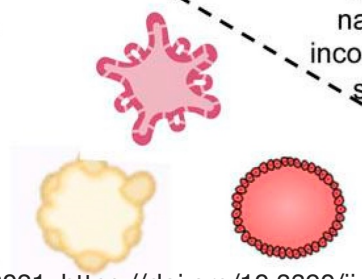


Organ-on-a-chip imaging

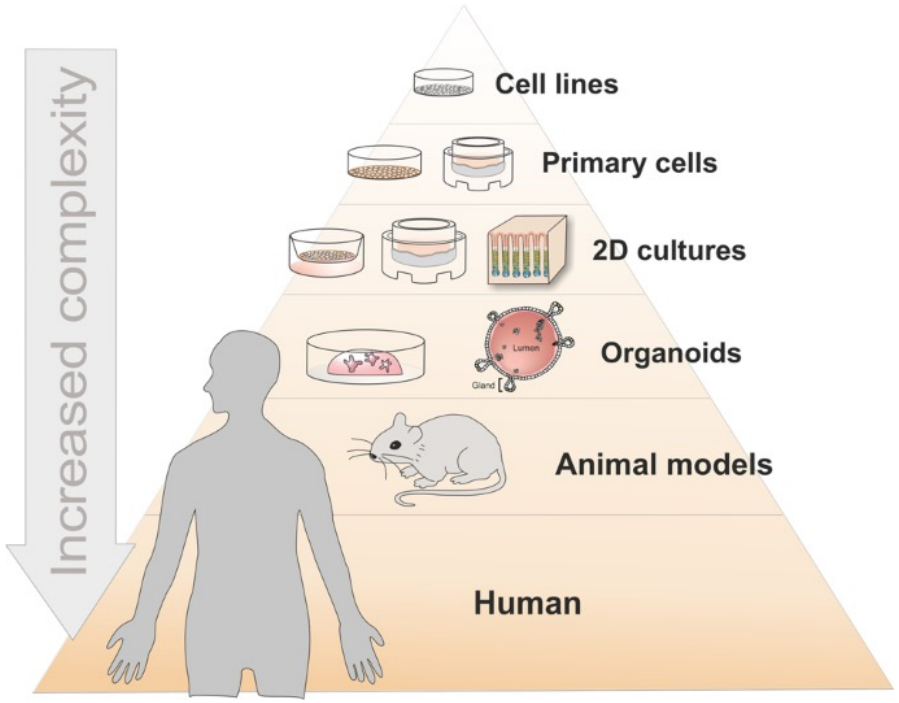


Organoid imaging

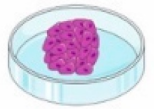
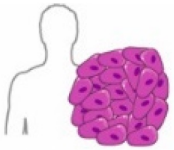
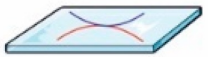


Bassi G et al, 2021, https://doi.org/10.3390/ijms22031195

A comparison between animal models and the move towards advanced hydrogel based in vitro models to bridge the gap



Aguilar et al, 2021, https://doi.org/10.1038/s12276-021-00629-4

	 Animal model	 2D cell culture	 3D cell culture	 Human organoid	 Organ-on-chip
Vascularization	✓	X	X/✓	X/✓	✓
Microenvironment control	X/✓	X	X/✓	X	✓
Ethical consideration	✓	X	X	X/✓	X/✓
Acces to compound	✓	✓	X/✓	X/✓	✓
Cell-cell interaction	✓	X	✓	✓	✓
Imitation of human physiology	X/✓	X	X/✓	X/✓	X/✓
High-throughput screening	X	✓	✓	✓	✓
Model complexity	✓	X	X/✓	✓	✓

Sokolowska P et al, 2022, https://doi.org/10.3390/organoids1010007

Hydrogels are especially relevant now that the FDA and other regulatory bodies have removed the requirement for animal testing for novel therapeutics to reach human clinical trials. (Dec 2022)

- Currently 93 ongoing clinical trials worldwide involving hydrogels of some form as of 26.10.2023
- 473 clinical trials completed using hydrogels
- 20 clinical trials currently ongoing using hydrogels for cancer, 6 for arthritis, 17 for chronic wounds and ulcers, 3 for antibiotic resistance coatings, many for corneal applications

(source clinicaltrials.gov with search term ‘hydrogel’)

FDA U.S. FOOD & DRUG ADMINISTRATION

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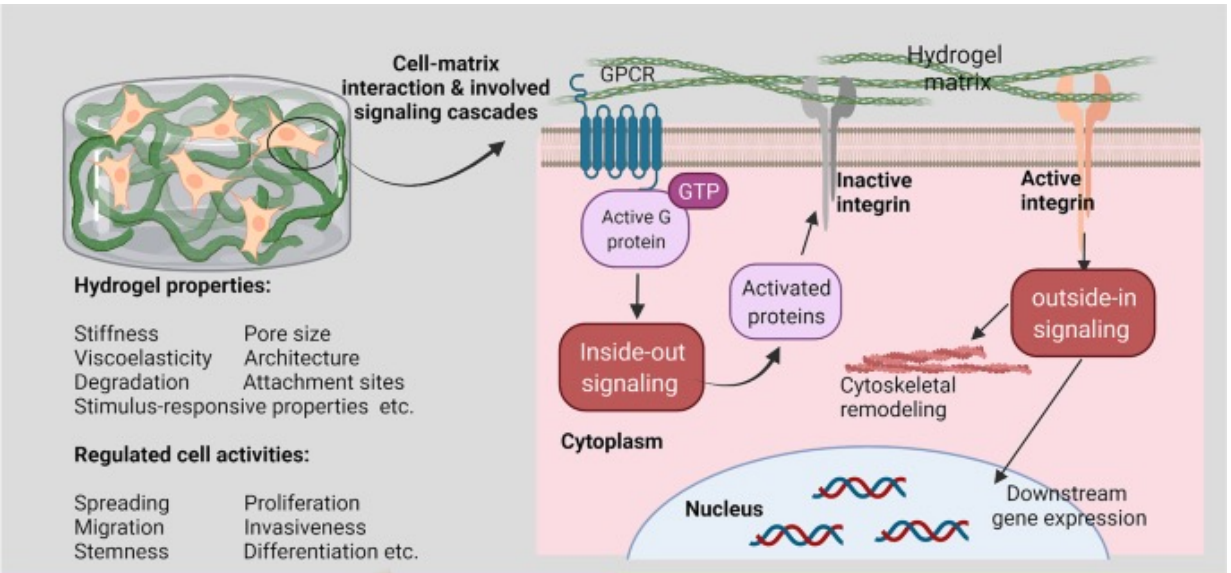
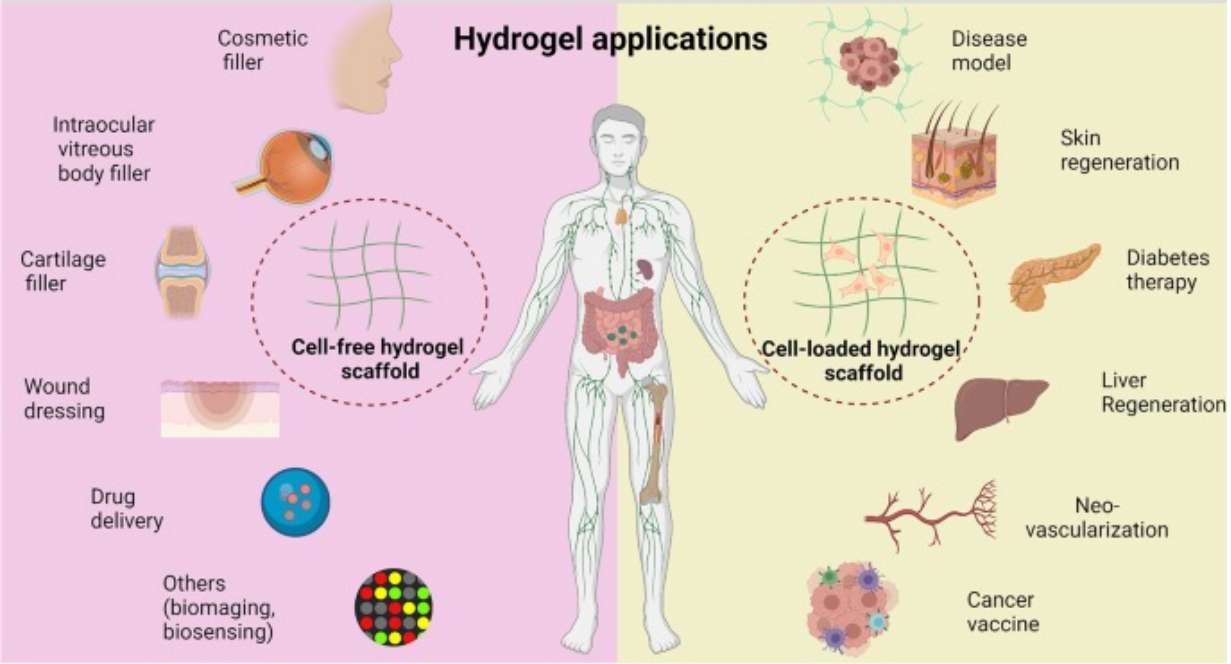
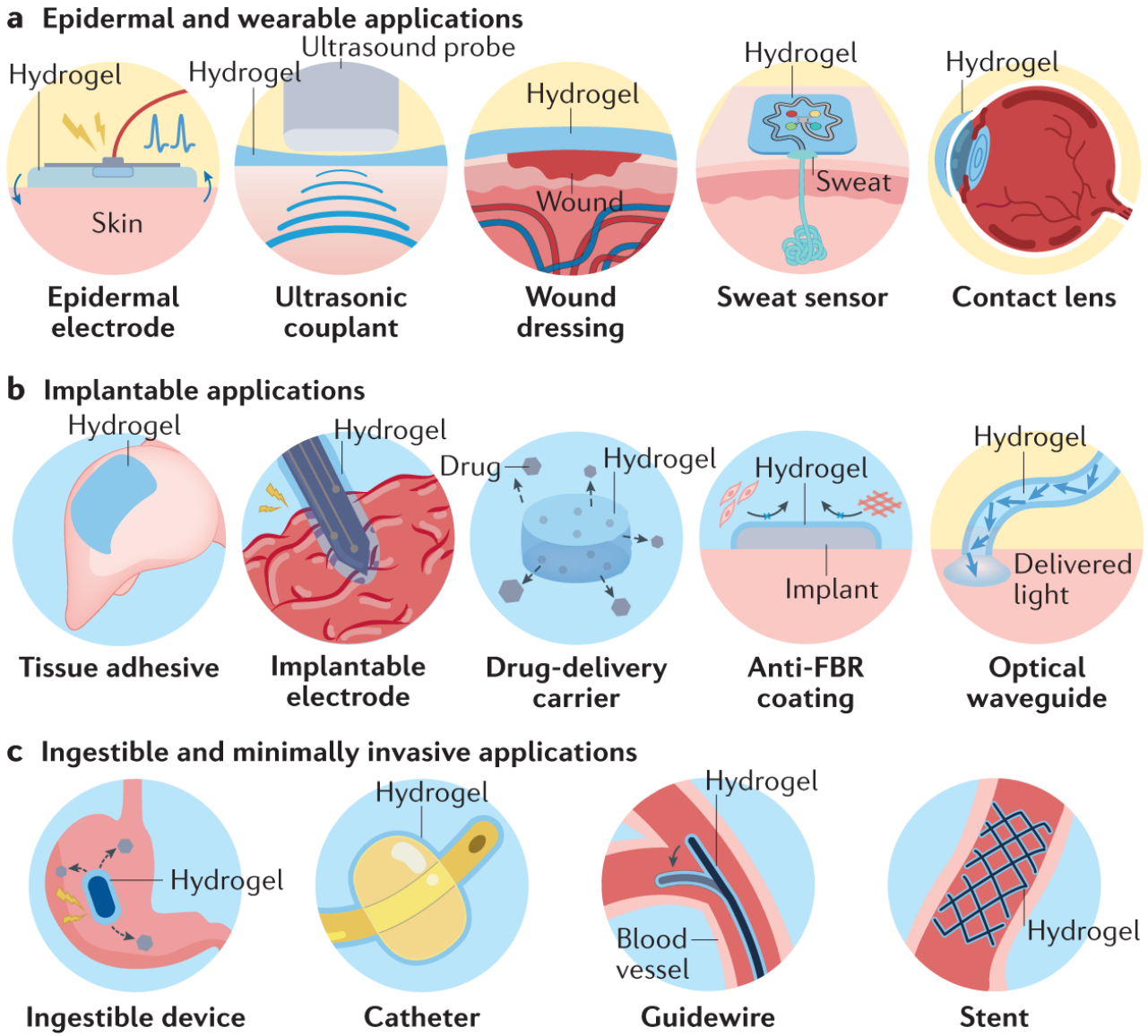
What types of questions can be addressed with hydrogel based materials?





Cao et al, 2021, https://doi.org/10.1038/s41392-021-00830-x

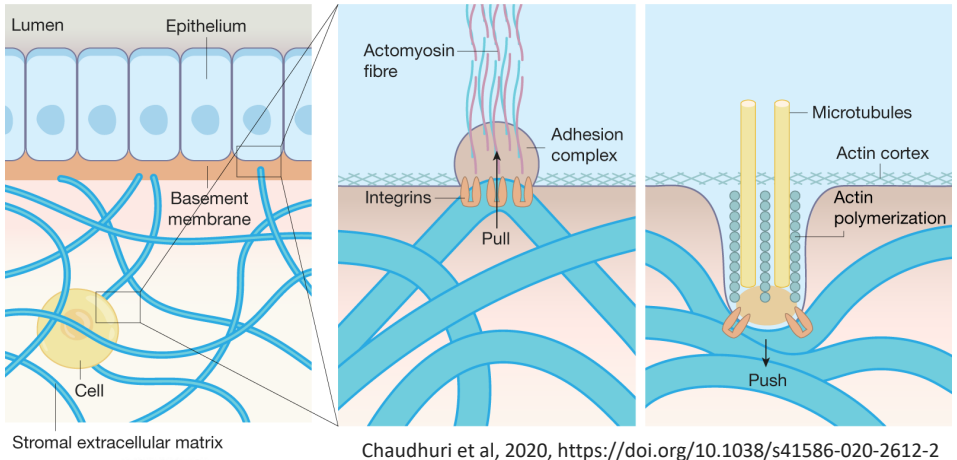


Yuk et al, 2022, https://doi.org/10.1038/s41578-022-00483-4

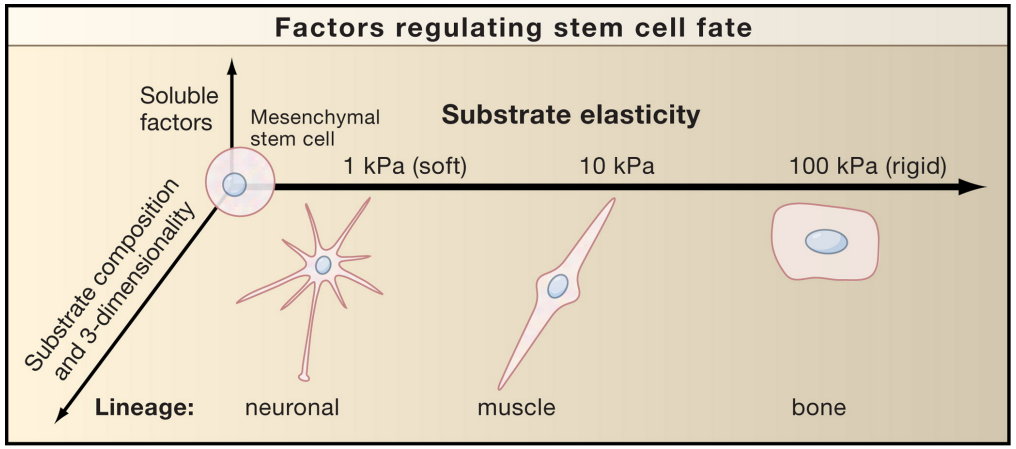
Limitations with many current hydrogels in use:

- Static, brittle, poor mechanical strength, and bioinert materials
- Non-specific biochemical cues or complete lack thereof
- Do not mimic the physiological environment well enough
- Lack structural and biochemical complexity compared to native tissue
- Constructs tend to shrink and lose shape over time as cells pull on the matrices, poor biodegradability

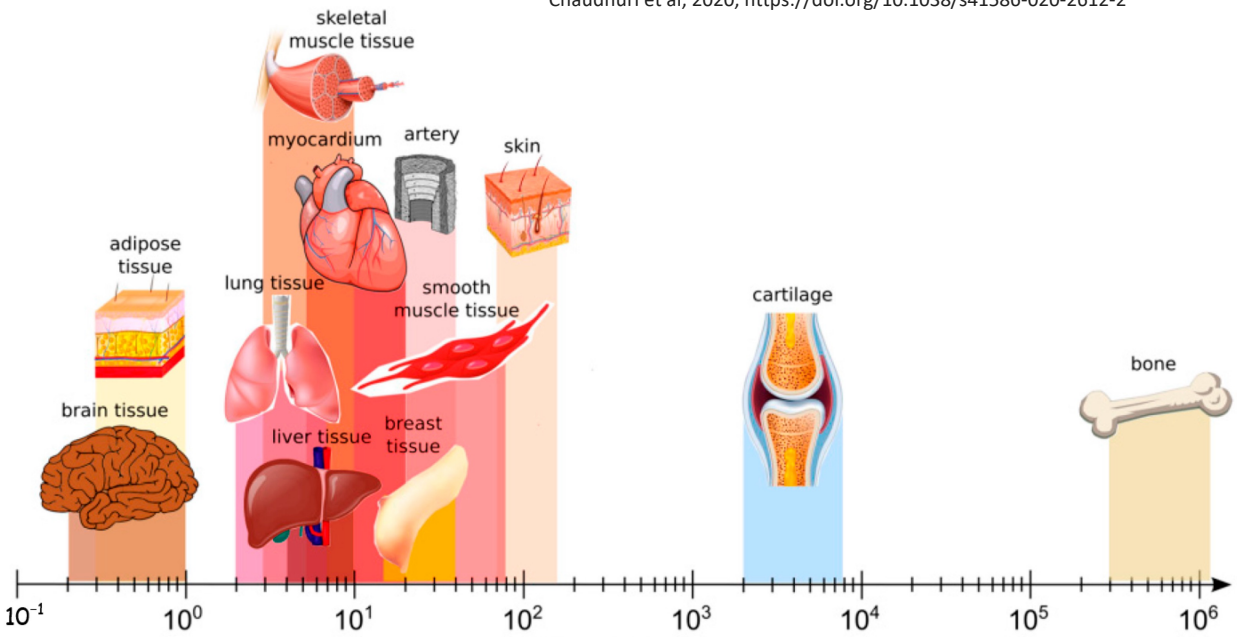
Dynamic interactions between cells and their local environment, the ECM



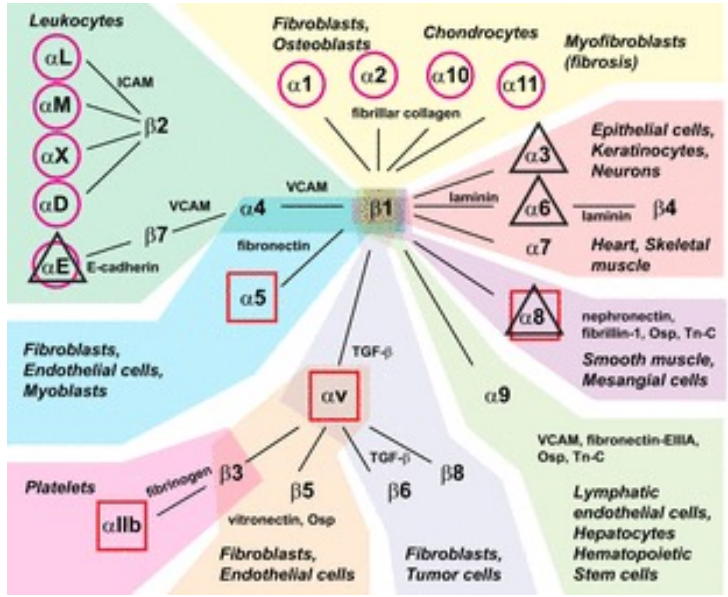
Chaudhuri et al, 2020, https://doi.org/10.1038/s41586-020-2612-2

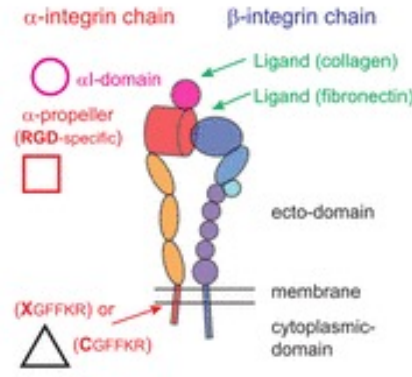


Even-Ram et al, 2006, https://doi.org/10.1016/j.cell.2006.08.008



Biosensors **2023**, *13*(5), 551





Buchmann et al, 2019, https://doi.org/10.1152/physrev.00036.2018

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What we hope to achieve with our hydrogels…

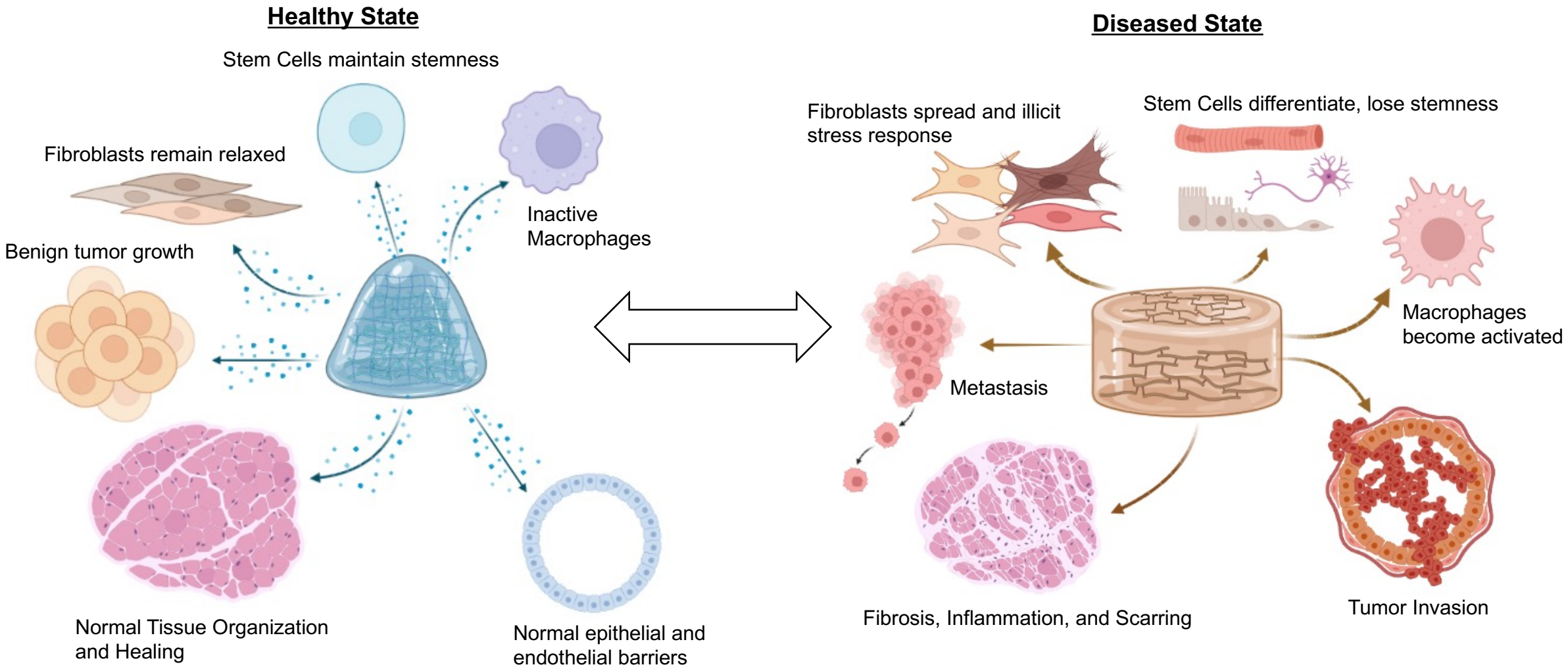
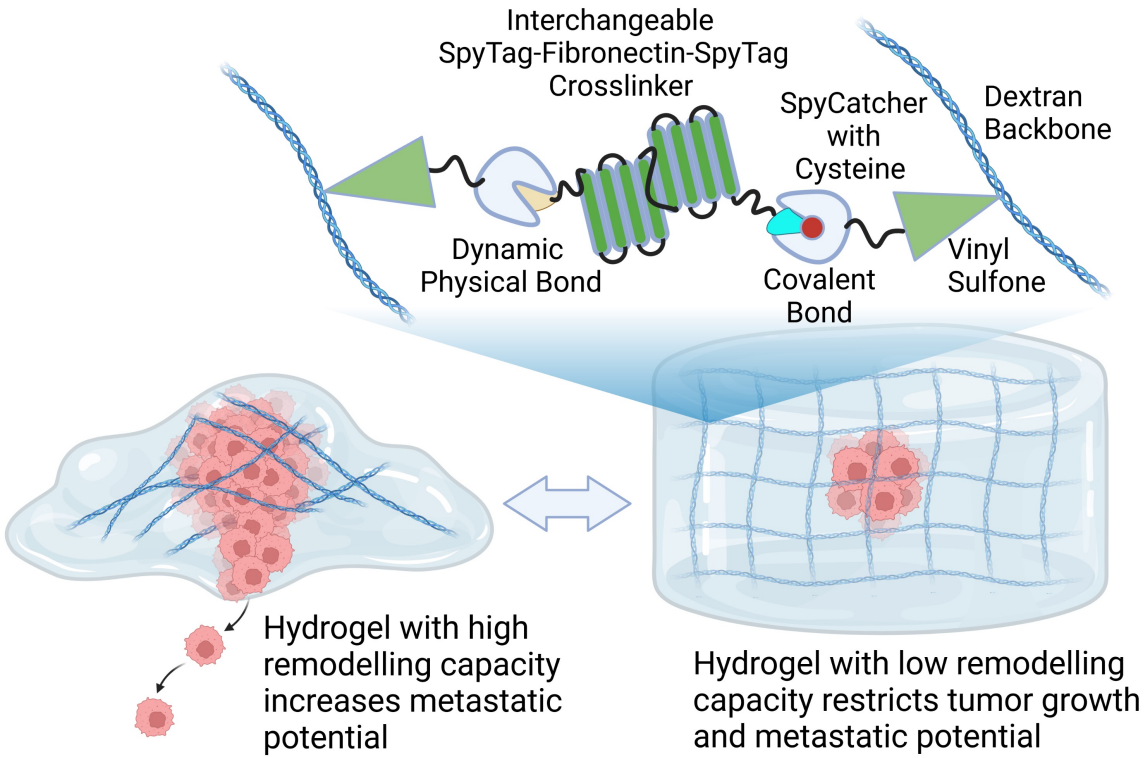


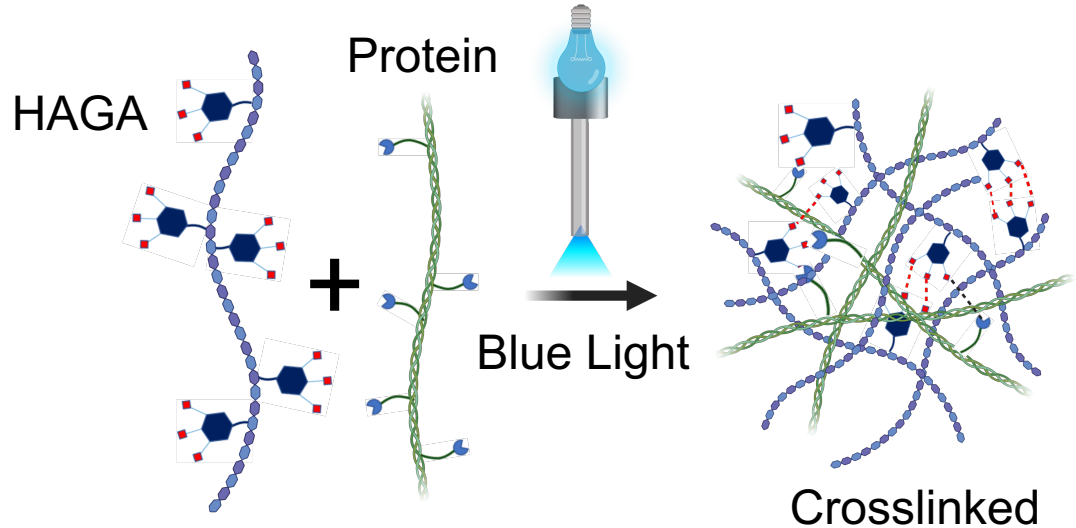
Figure made on Biorender.com

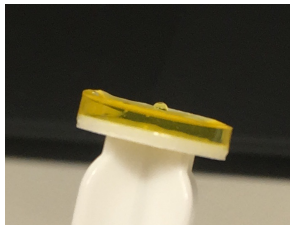
Our approach: Universal easy to use ‘Smart’ natural ECM functionalized hydrogels and next-gen hybrid synthetic protein-carbohydrate hydrogels

1. **Synthetic** ’Plug and Play’ Protein-Carbohydrate SpyCatcher/SpyTag Gels



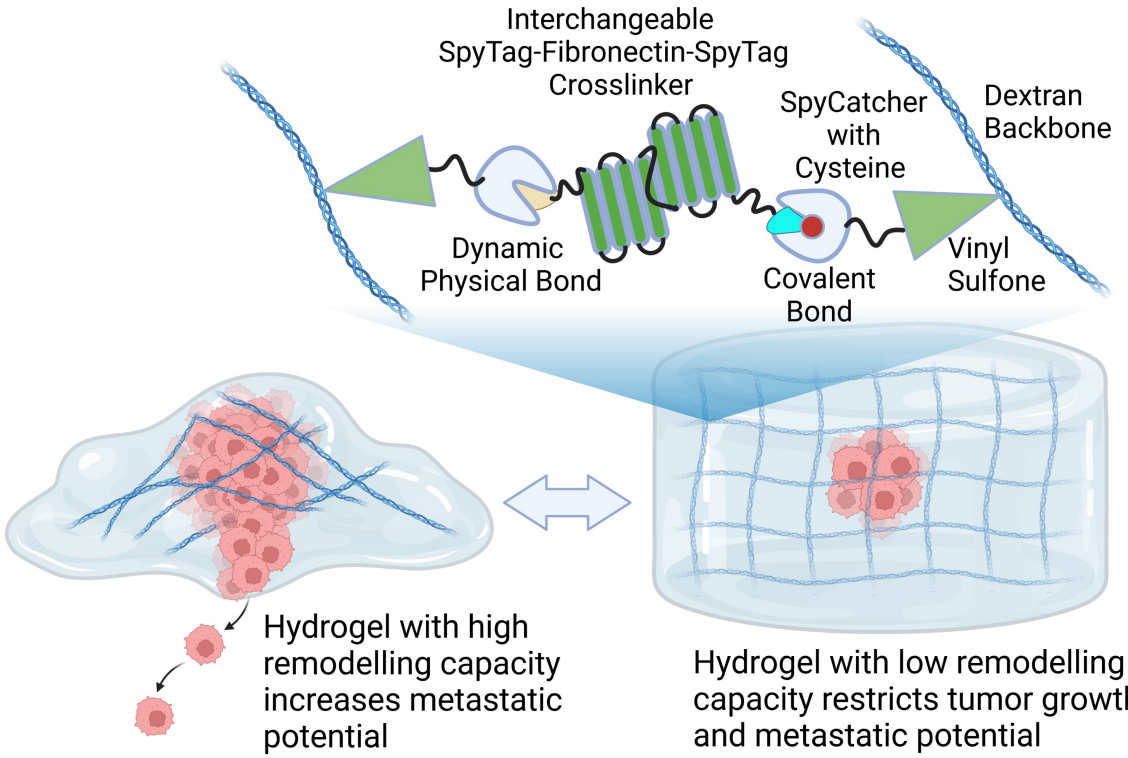
2. **Natural** Gallol Functionalized Hyaluronic Acid (HAGA)-Protein ECM mimicking Photocrosslinking Gels



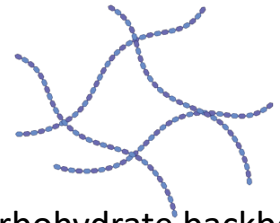


1. Synthetic Protein-Carbohydrate Hydrogels

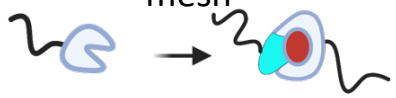
Synthetic 'Plug and Play' Protein-Carbohydrate SpyCatcher/SpyTag Gels



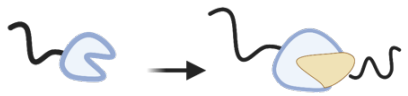
A **Legend**



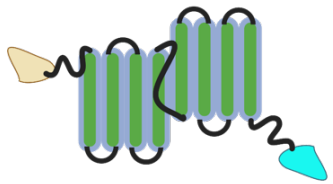
Carbohydrate backbone mesh



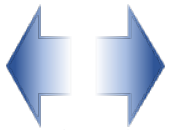
Covalent bond formation



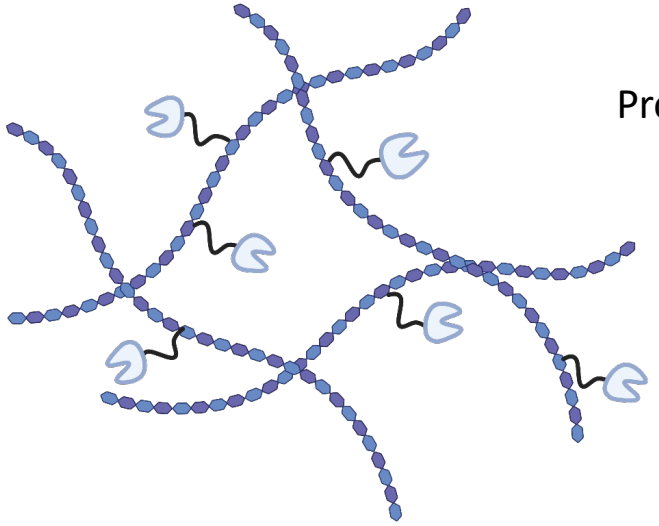
Non-covalent interaction



Recombinant Protein Crosslinker

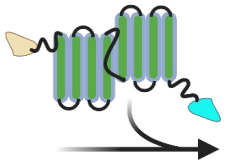


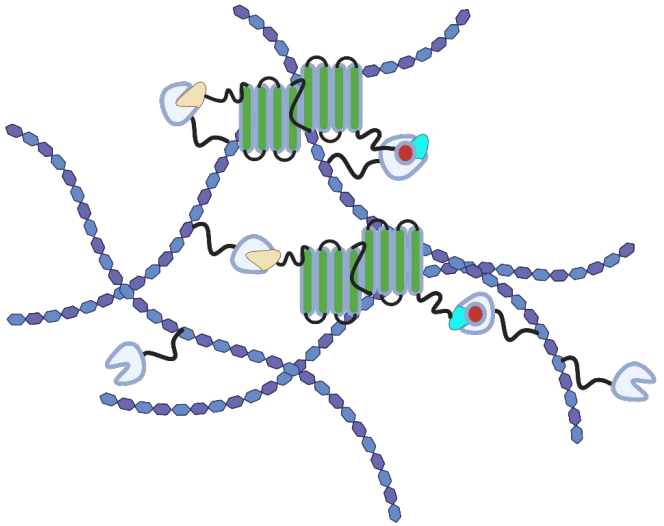
Deformation Stress on hydrogel



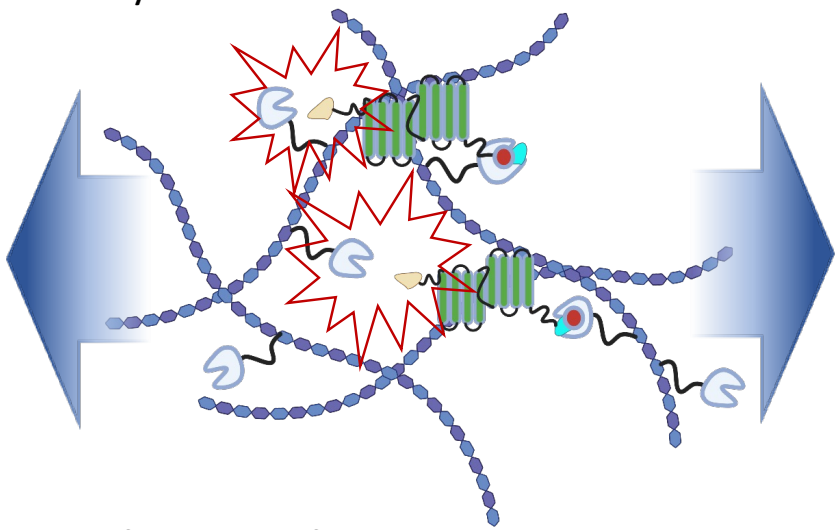
1. Protein Catcher Functionalized Dextran or Hyaluronic Acid

Recombinant Protein Crosslinker

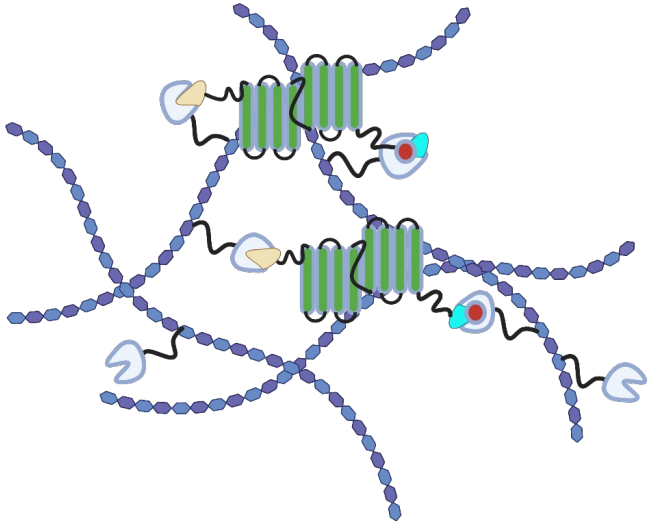




2. Crosslinked hydrogel network

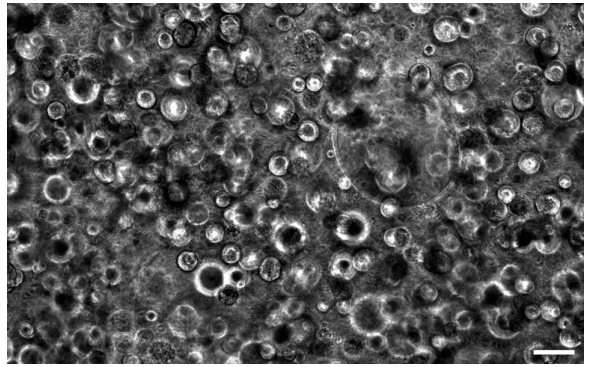


3. Deformation from external or cellular forces breaks non-covalent interactions

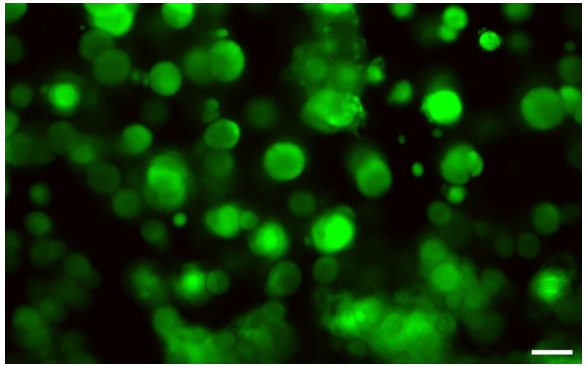


4. Relaxation leads to self-healing of non-covalent interactions, restoring hydrogel mechanics

Initial optimization steps so far...

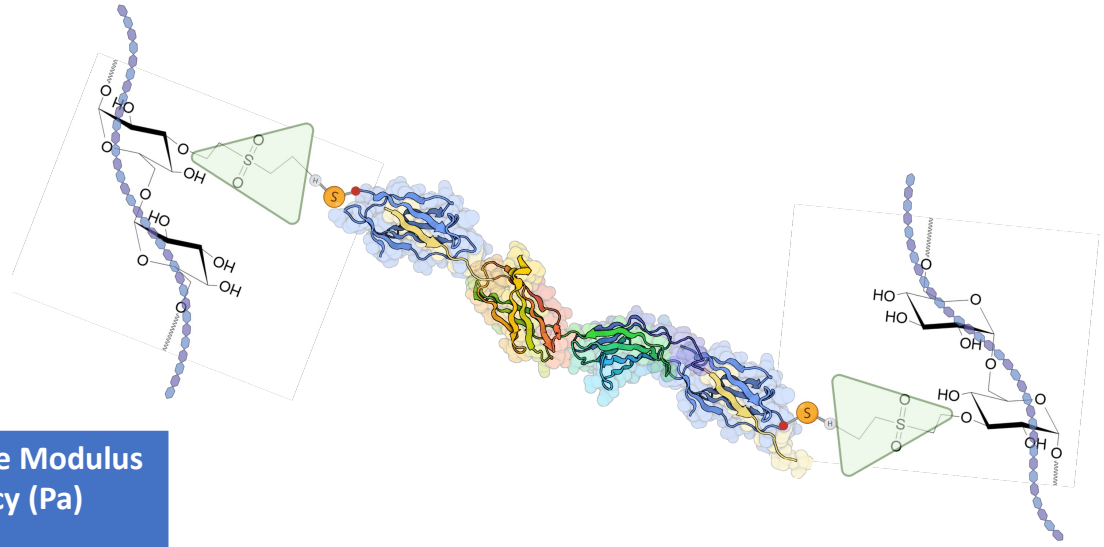


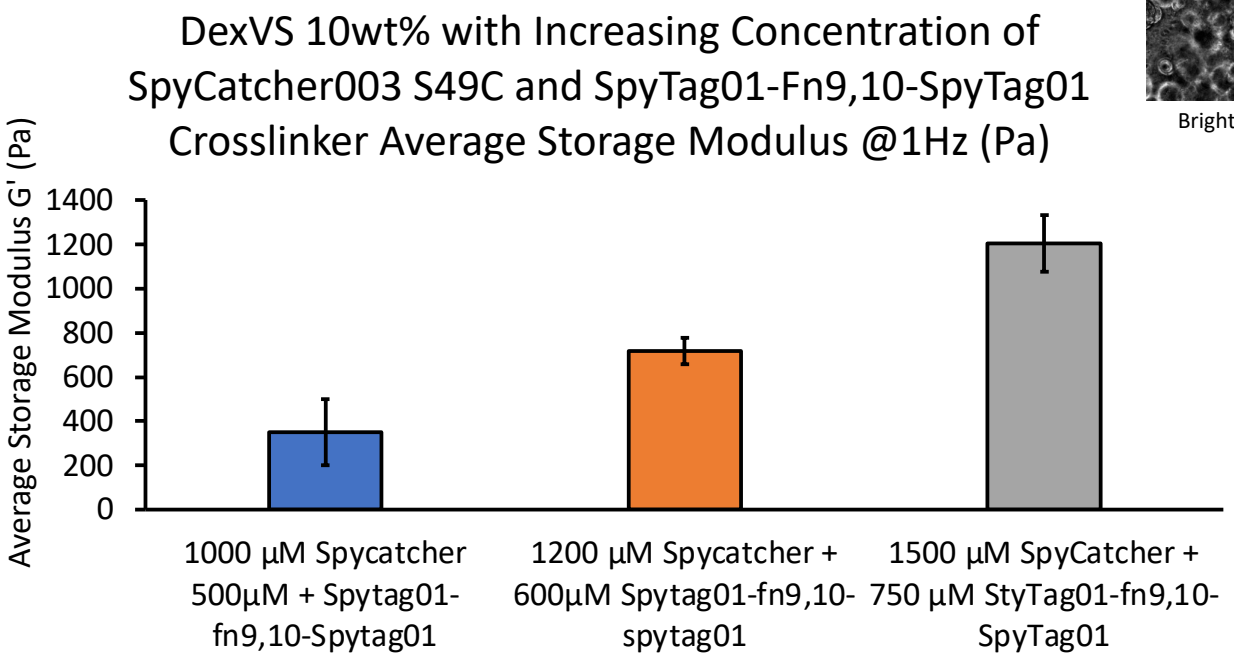
Brightfield



Calcein AM/EtBR (Live/Dead)

10x magnification, 100 µm scale bar, Day 7
10wt% DexVS with 1200 µM SpyCatcher and 600µM Spytag-fn9,10-Spytag crosslinker
HCT116 Colorectal cancer cells at 1million cells/mL in DMEM with 10%FBS

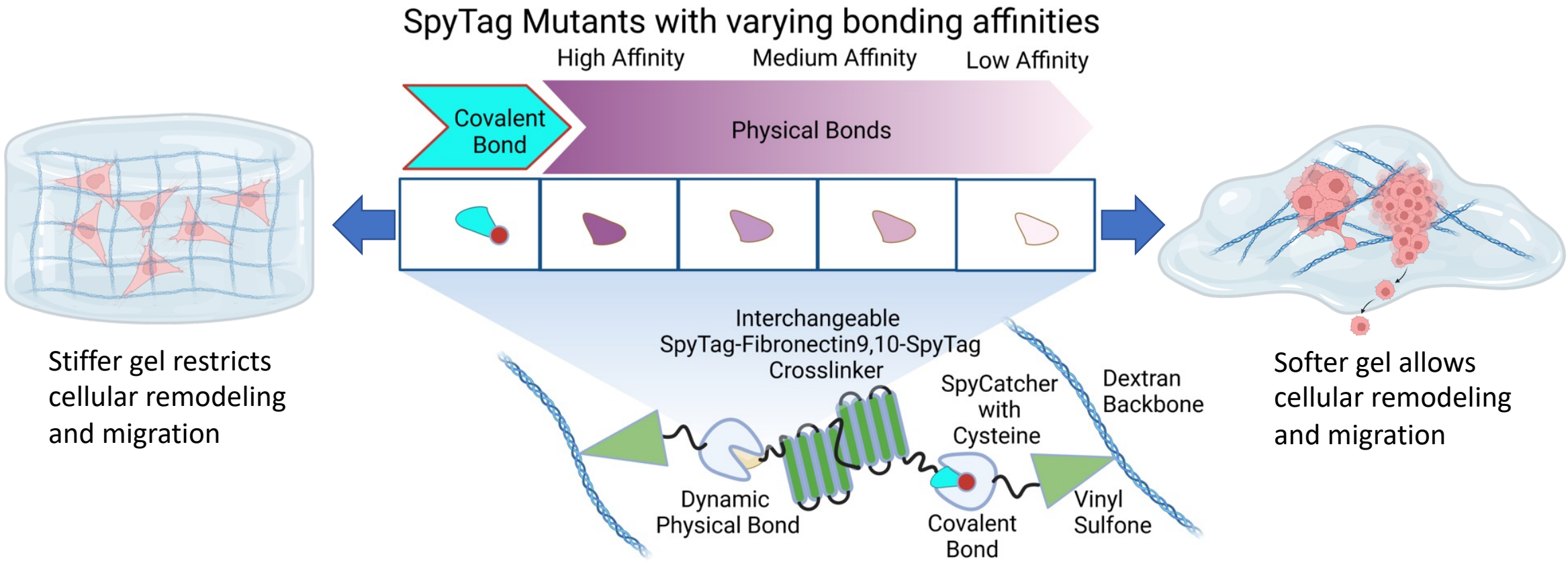




DexVS wt%	Spycatcher003 s49C concentration micromole (µMol)	SpyTag01-Fn9,10-SpyTag01 Crosslinker Concentration (µMol)	Average Storage Modulus @1Hz Frequency (Pa)
10	1000	500	350
10	1200	600	719
10	1500	750	1205

Evans et al, in preparation

Next steps with the synthetic system:



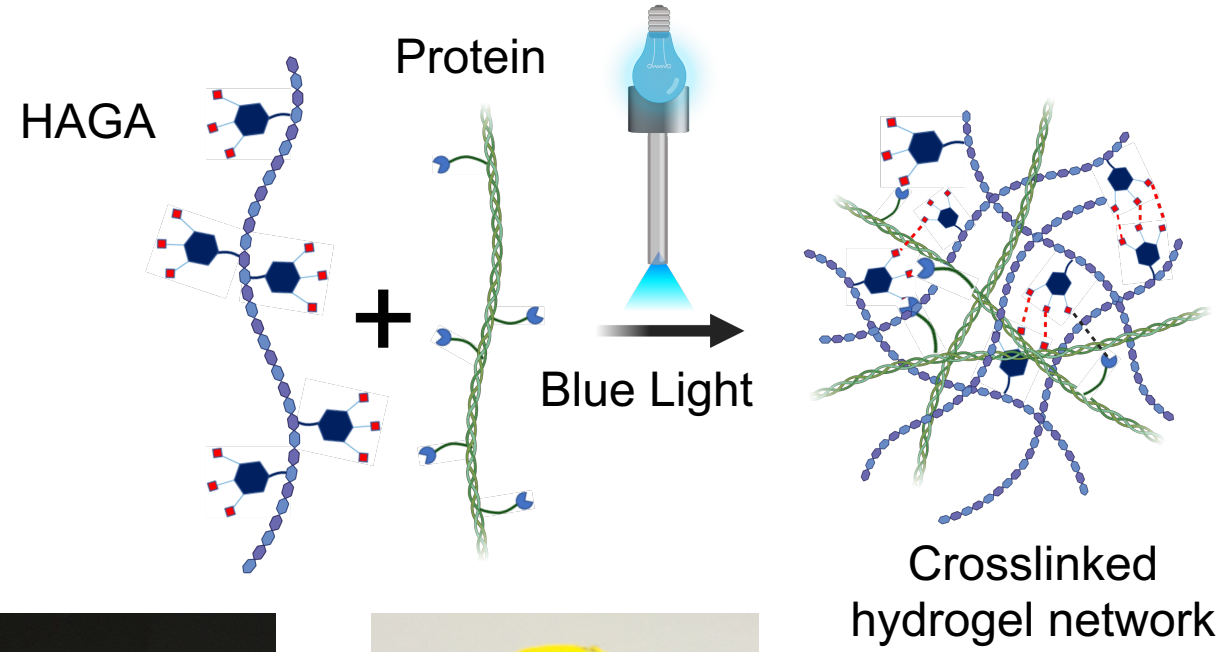
Stiffer gel restricts cellular remodeling and migration

Softer gel allows cellular remodeling and migration

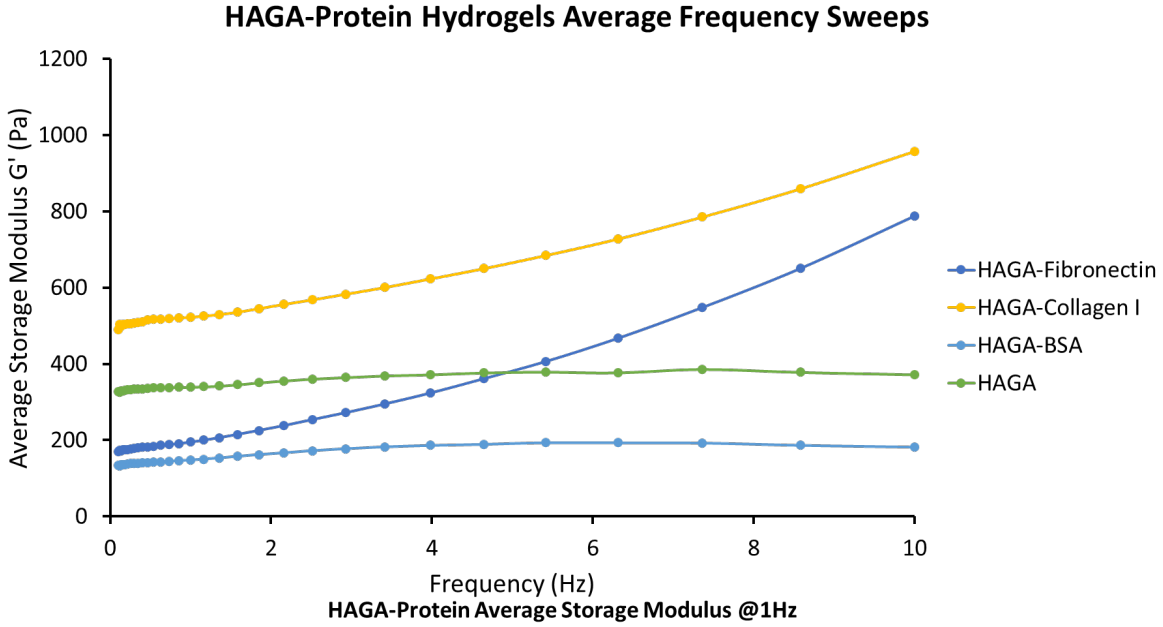
- Explore cellular remodeling of the matrix in the tumor microenvironment
  - Our aim is to build materials that can be reorganized by cells in very specific controlled ways.
- **Out of the box** use for these hydrogels: Ballistics testing with hypersonic projectiles with Ben Goult's Lab
- Add to our repertoire of available custom crosslinker proteins for specific purposes, like LEGOs!

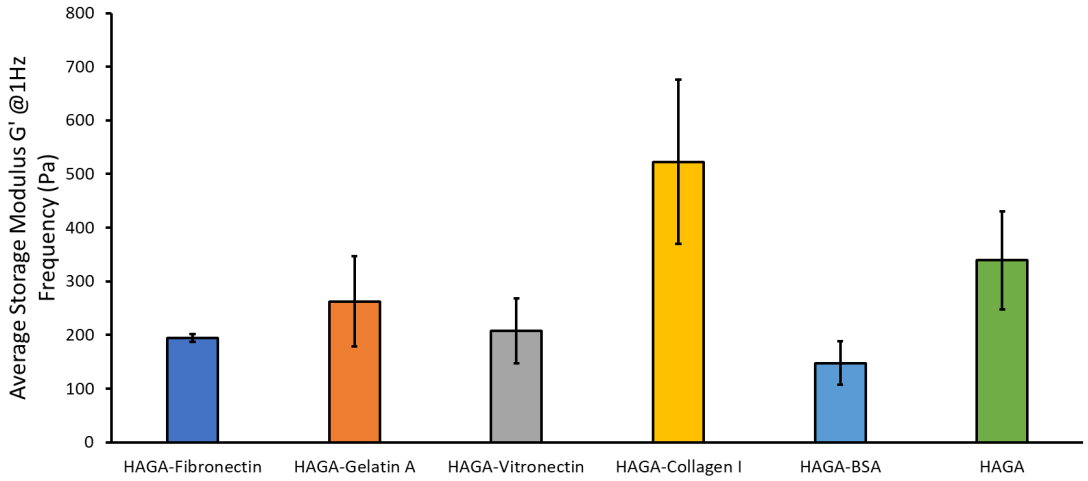
2. Natural ECM functionalized photocrosslinking Hydrogels

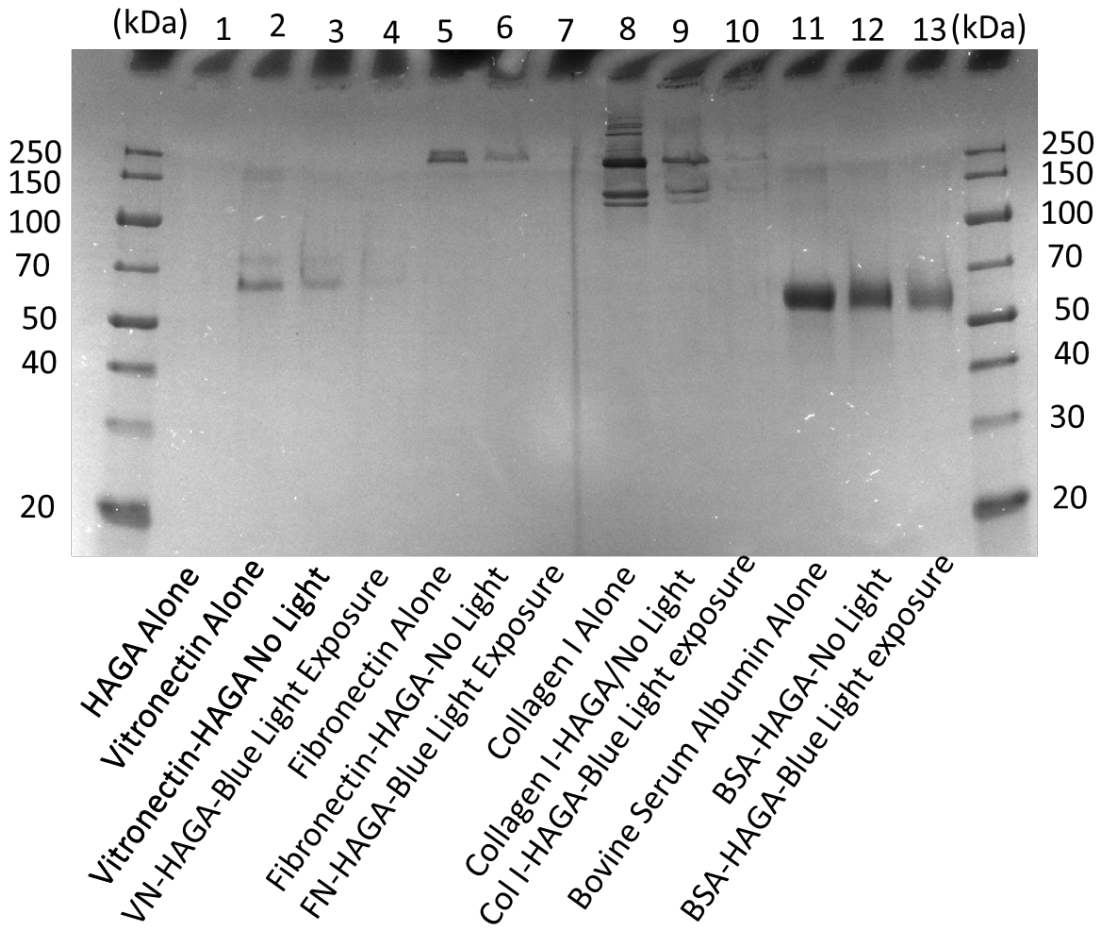
Natural Gallol Functionalized Hyaluronic Acid (HAGA)-Protein ECM mimicking Photocrosslinking Gels



HAGA-ECM hydrogels as photoactivated multifunctional bioconjugation system to all types of biomolecules: Protein, Peptides, DNA, RNA etc.

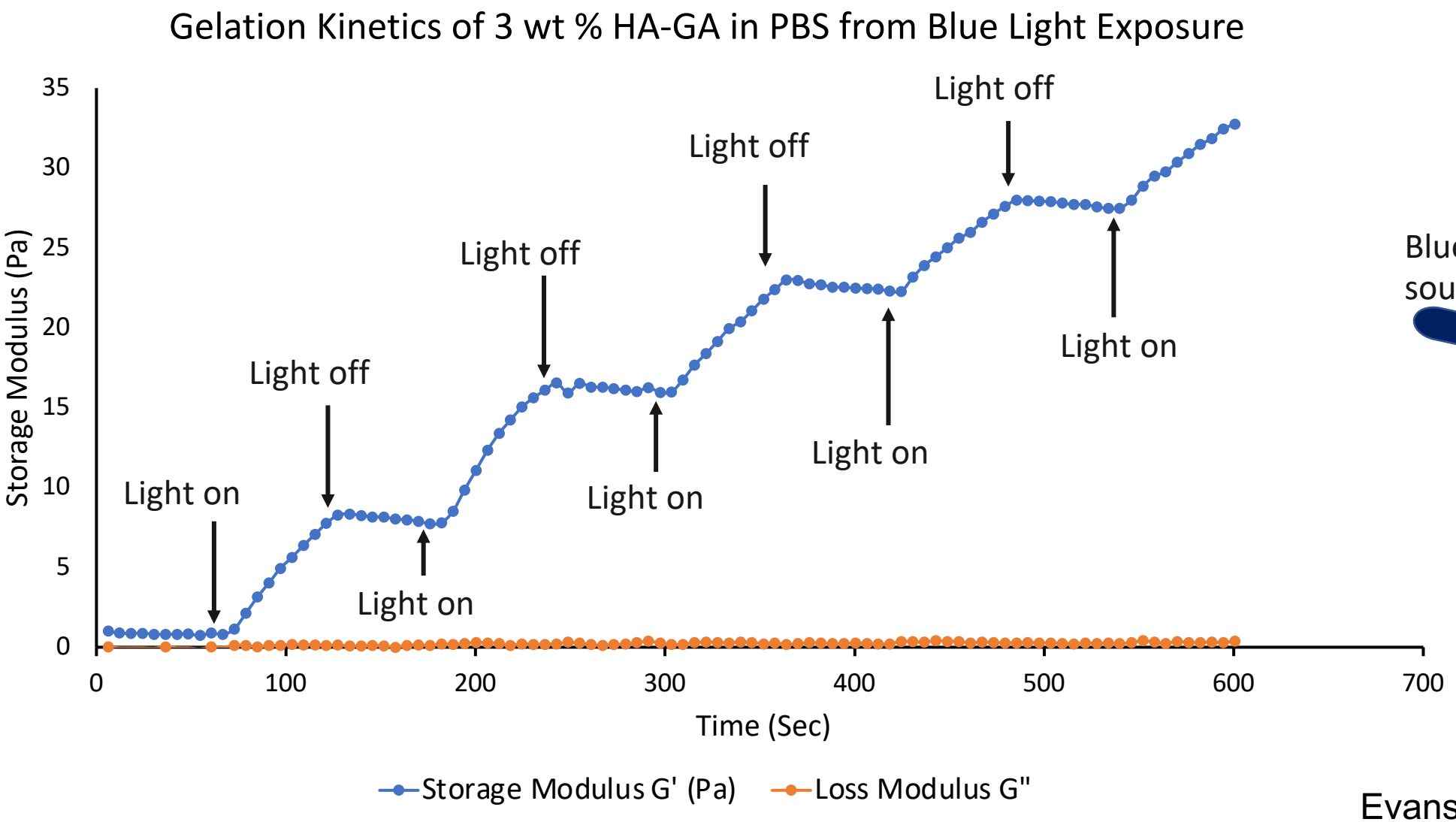


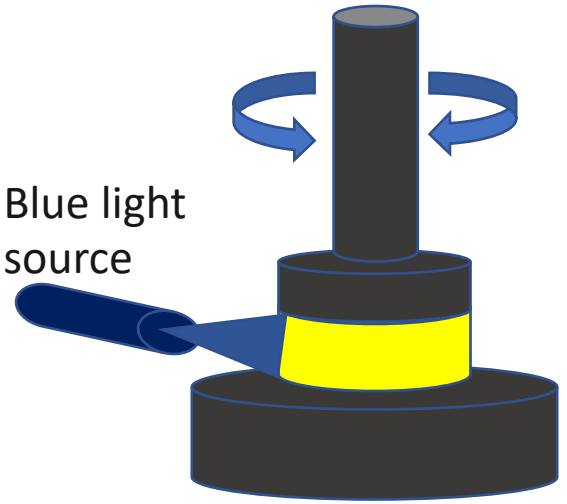




Evans et al, in preparation

Gelation and reaction kinetics estimation





Example of Experiment setup on Rheometer with Oscillation at 1Hz.

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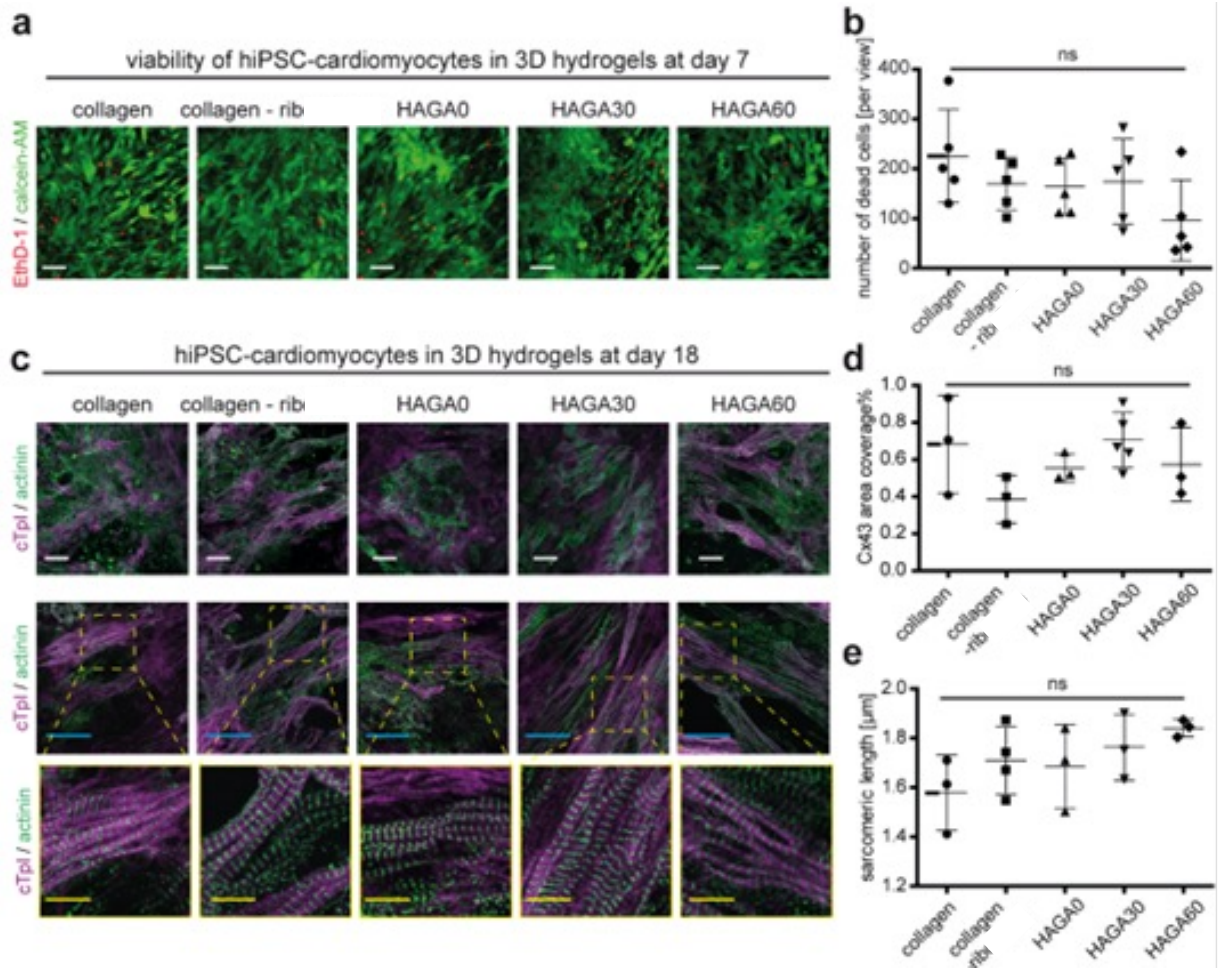
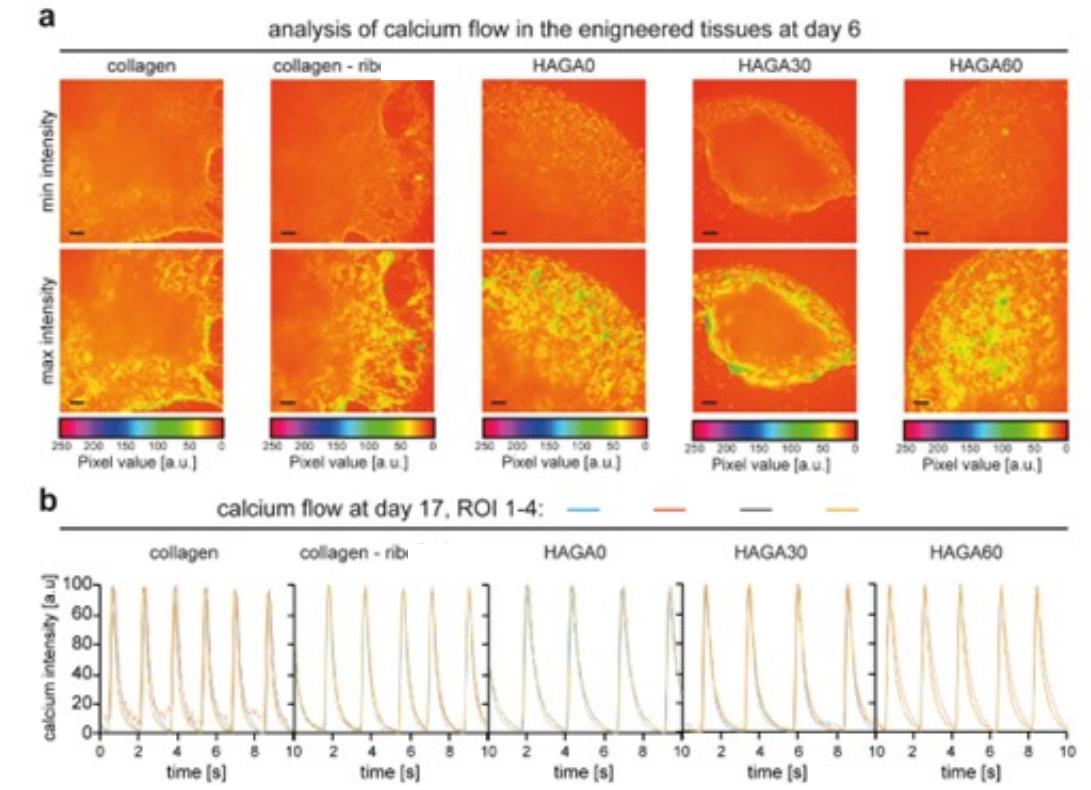
Our natural hydrogel materials are useful for tissue engineering, 3D bioprinting:



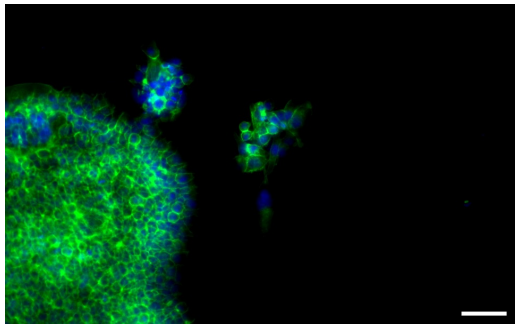


Roshanbinfar, Evans et al, in preparation

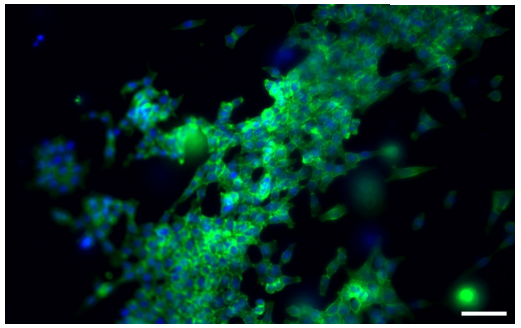
Tampere University

Tumor microenvironment recapitulation

HCT116 in HAGA Alone

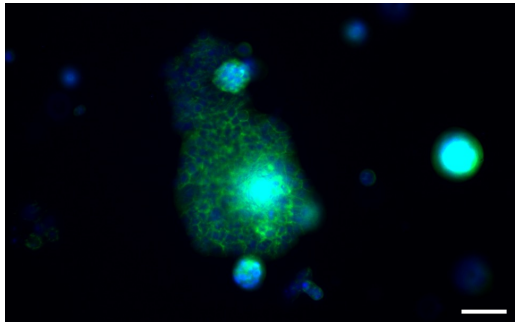


HCT116 in HAGA - RatTail 3D Collagen I

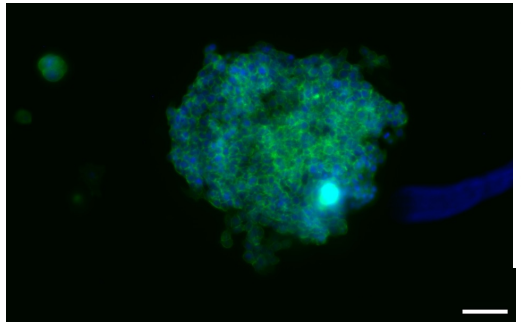


Day 9 20x images with F-Actin Phalloidin 488 in Green, DAPI Nuclear Stain in Blue (Scale Bar = 100 µm)

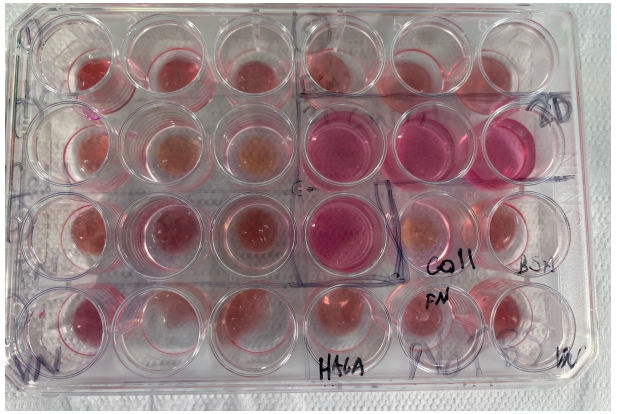
HCT116 in HAGA - BSA

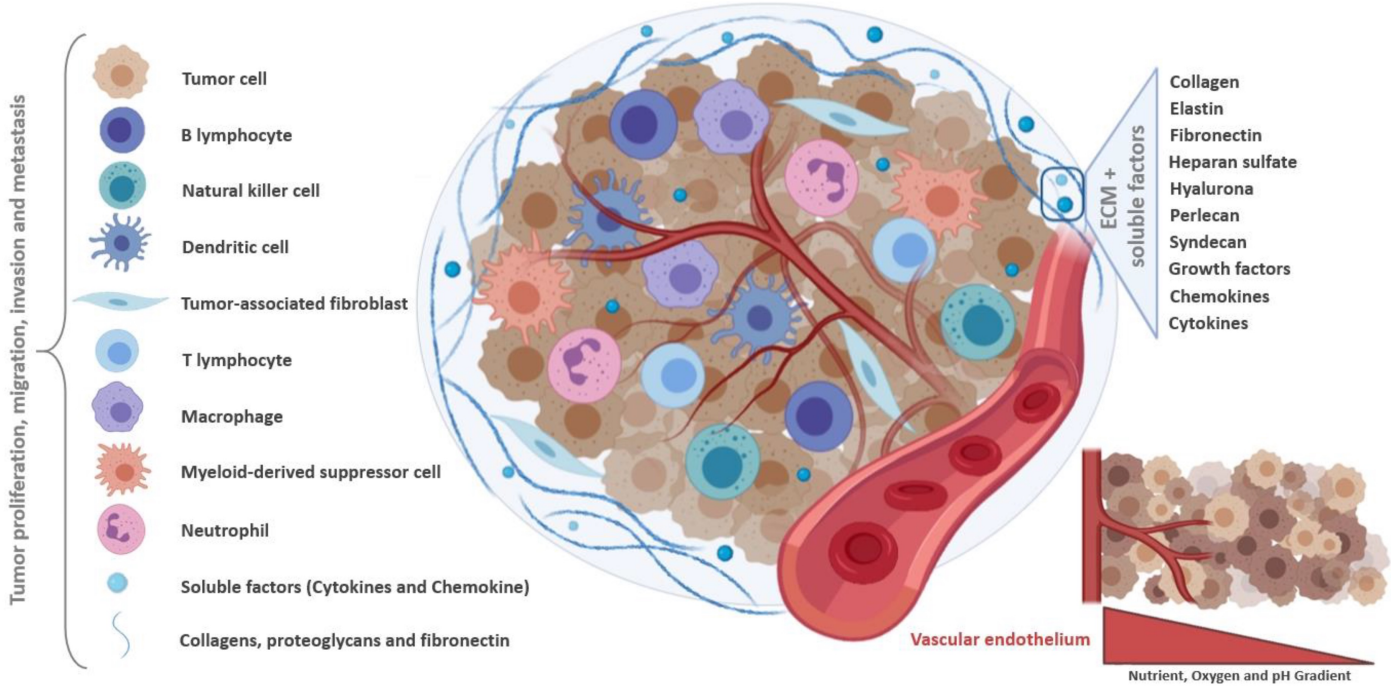


HCT116 HAGA - Fibronectin



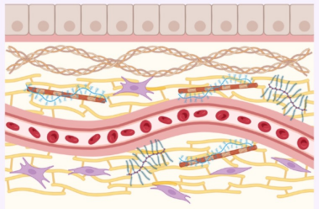
Evans et al, in preparation





https://facellitate.com/the-importance-of-the-tumor-microenvironment-in-cancer-research/

Structural Component



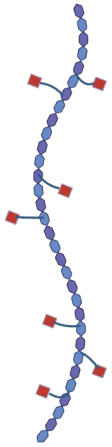
Extracellular Matrix with collagen, fibroblasts and abnormal vasculature

Other important ECM components:

- Laminin
- Fibronectin
- Other proteoglycans and glycosaminoglycans

Cancers **2023**, *15*(17), 4376

Possibilities for high-throughput in vitro drug screening for personalized cancer therapeutics

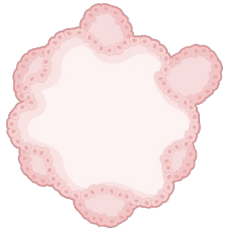


Chemically modified **hyaluronic acid (HA)** or other glycosaminoglycan or polysaccharide

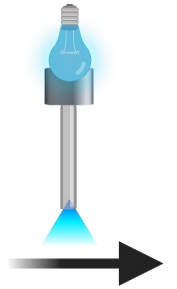
+

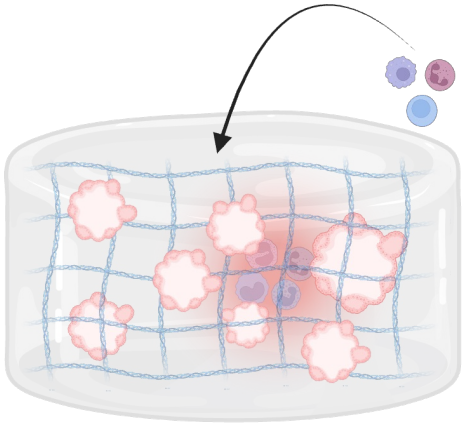
Matrigel or custom **ECM proteins** matching target tissue type

+

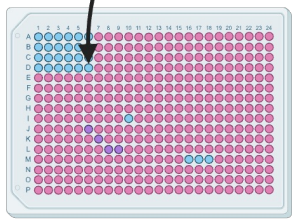


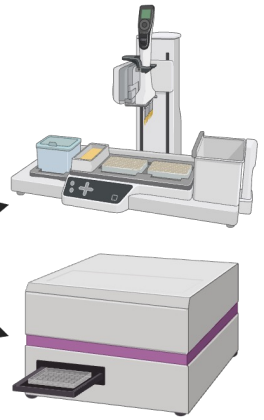
Patient derived cells, cancer organoids, (intestinal, pancreatic, glioblastoma etcetera)



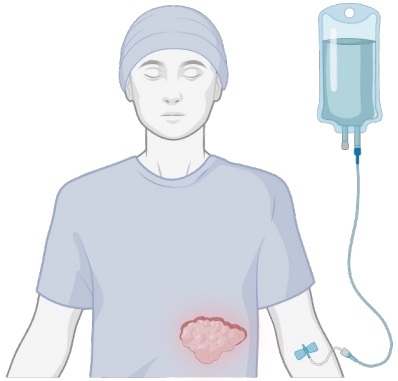


In vitro patient specific tumor microenvironment models





High-throughput personalized chemotherapy screening



Personalized chemotherapy regimen targeted to their specific tumor type

(In collaboration with Toni Seppälä)

Neural network modelling

Contents lists available at ScienceDirect

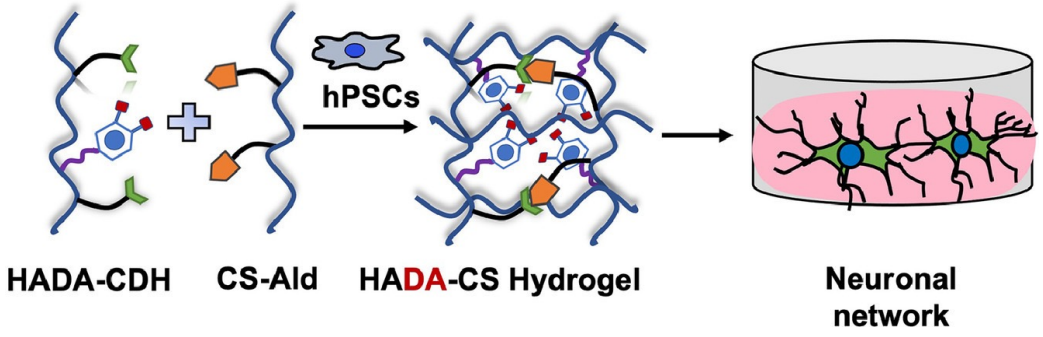
Acta Biomaterialia

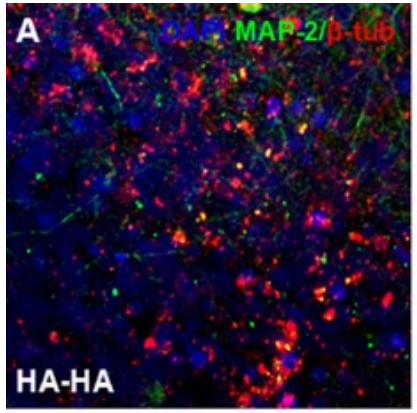
journal homepage: www.elsevier.com/locate/actbio

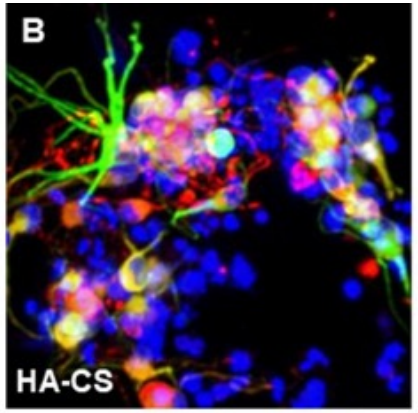
Full length article

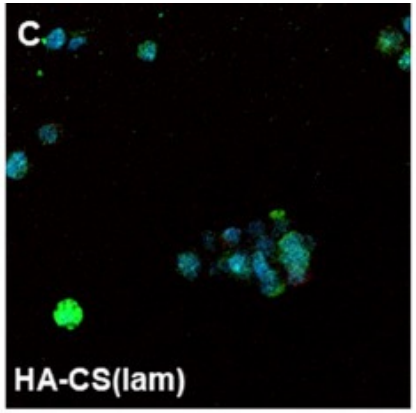
Bidirectional cell-matrix interaction dictates neuronal network formation in a brain-mimetic 3D scaffold

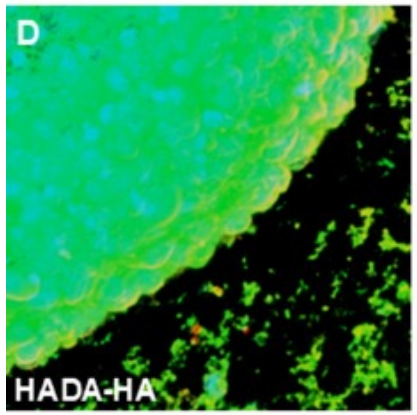
Sumanta Samanta[a], Laura Ylä-Outinen[b,c], Vignesh Kumar Rangasami[a], Susanna Narkilahti[b], Oommen P. Oommen[a,*]

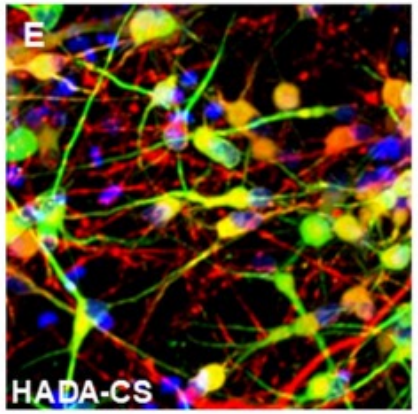


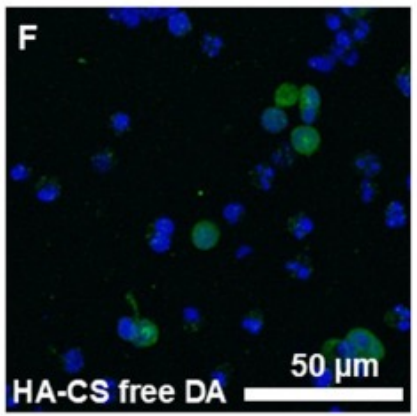


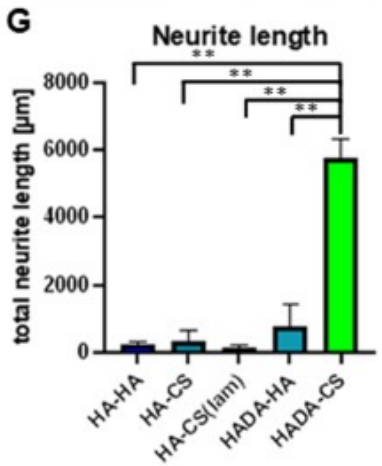


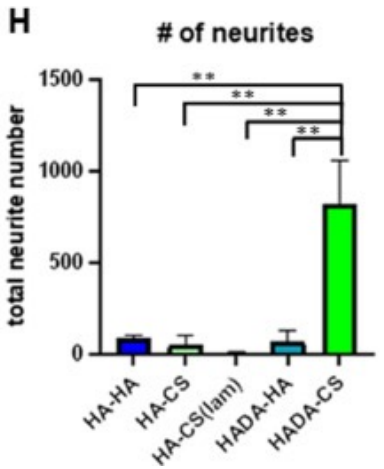


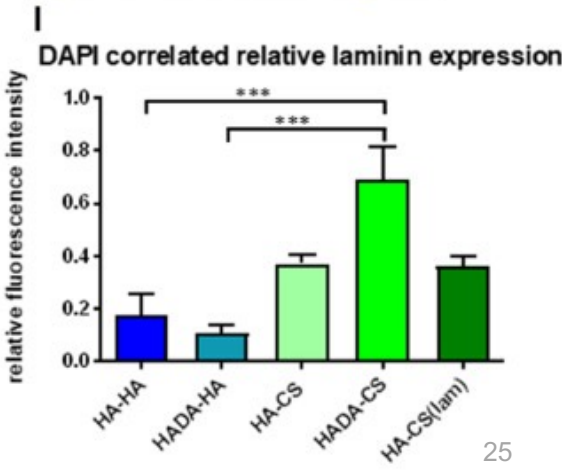












Immune system modeling and immune response manipulation

Contents lists available at ScienceDirect

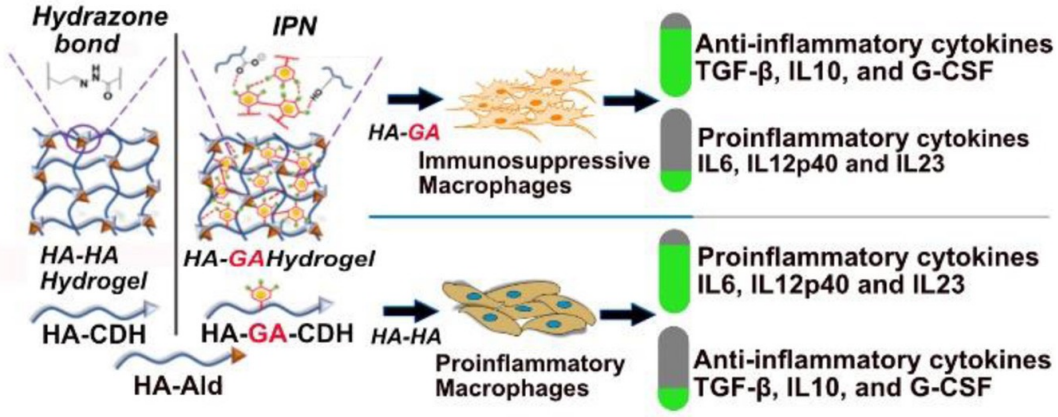
Acta Biomaterialia

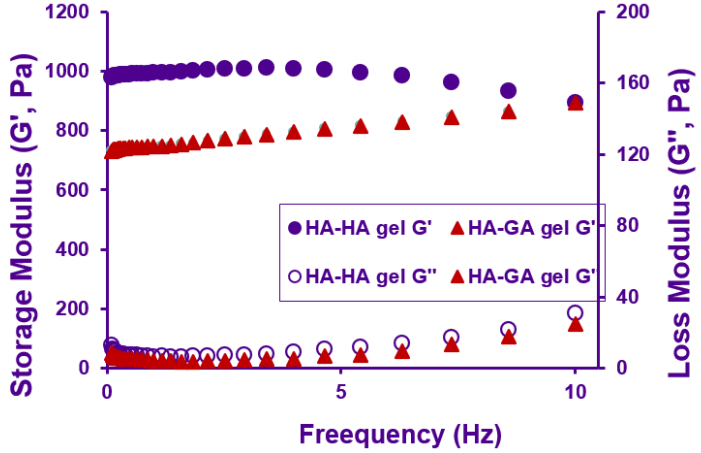
journal homepage: www.elsevier.com/locate/actbio

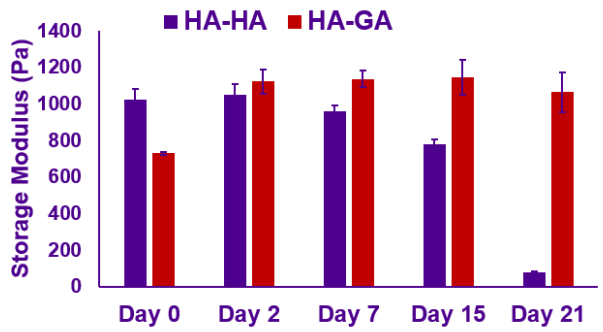
Full length article

Interpenetrating gallol functionalized tissue adhesive hyaluronic acid hydrogel polarizes macrophages to an immunosuppressive phenotype

Sumanta Samanta[a,1], Vignesh K. Rangasami[a,b,1], Heela Sarlus[c], Jay R.K. Samal[a,d], Austin D. Evans[a], Vijay S. Parihar[a], Oommen P. Varghese[b], Robert A. Harris[c], Oommen P. Oommen[a,*]







Future projects under development in our labs using our materials

- Cancer matrix mechanobiology and matrix remodeling studies in 3D with different hydrogel compositions
- 3D on chip platforms for hydrogel to vascular interface studies, eg. cancer metastasis studies, neural network engineering
- In situ hydrogel cancer immunotherapy and vaccine hubs
- Immune system modelling and tumor-immune crosstalk
- Glioblastoma, prostate, and colorectal tumor microenvironment models with patient derived organoids
- Nanoparticle hydrogel systems for drug delivery and targeted therapeutics

Summary: Why hydrogels?

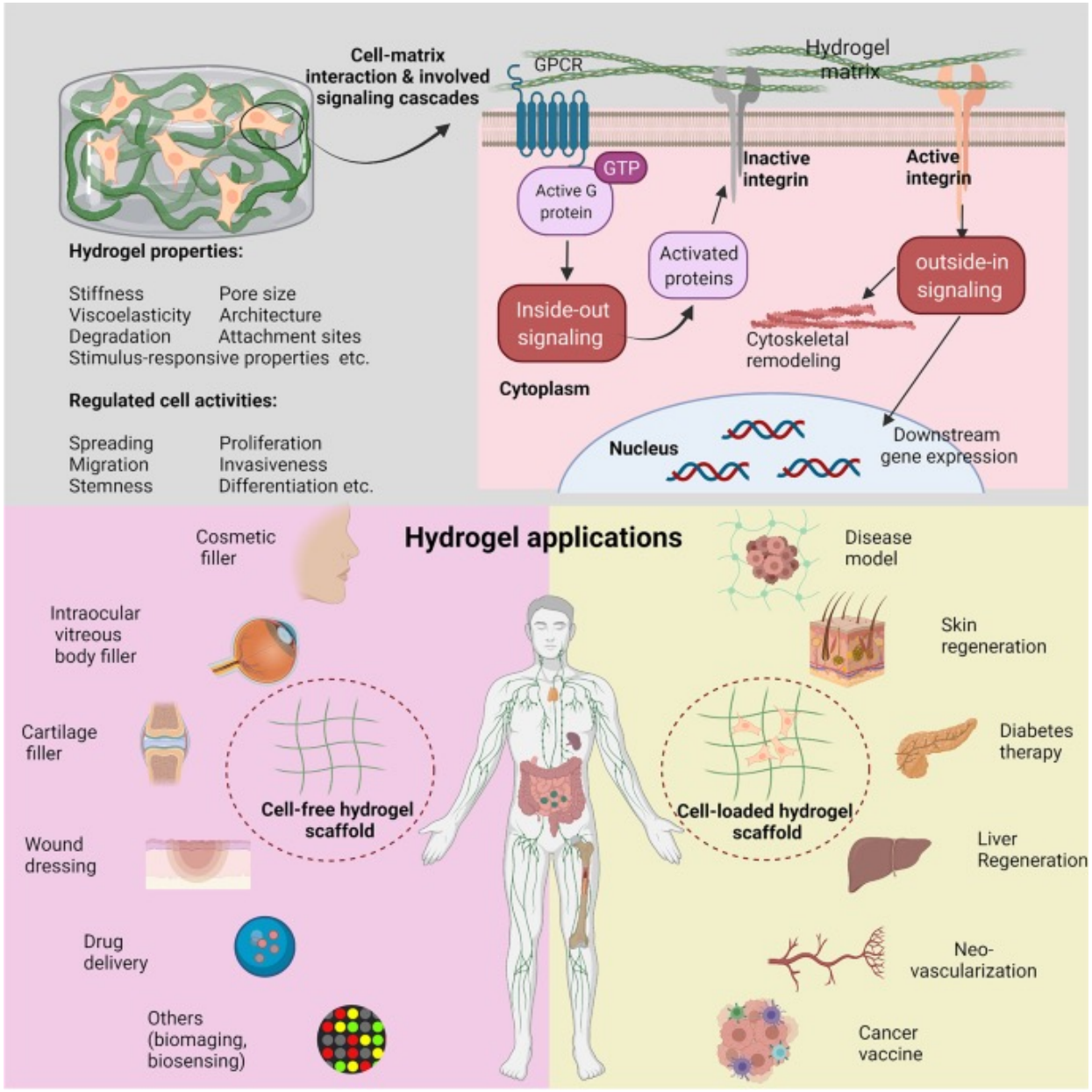
Biomedical applications: tissue engineering/regenerative medicine, cell culture systems, and drug delivery/therapeutics

Advantages:

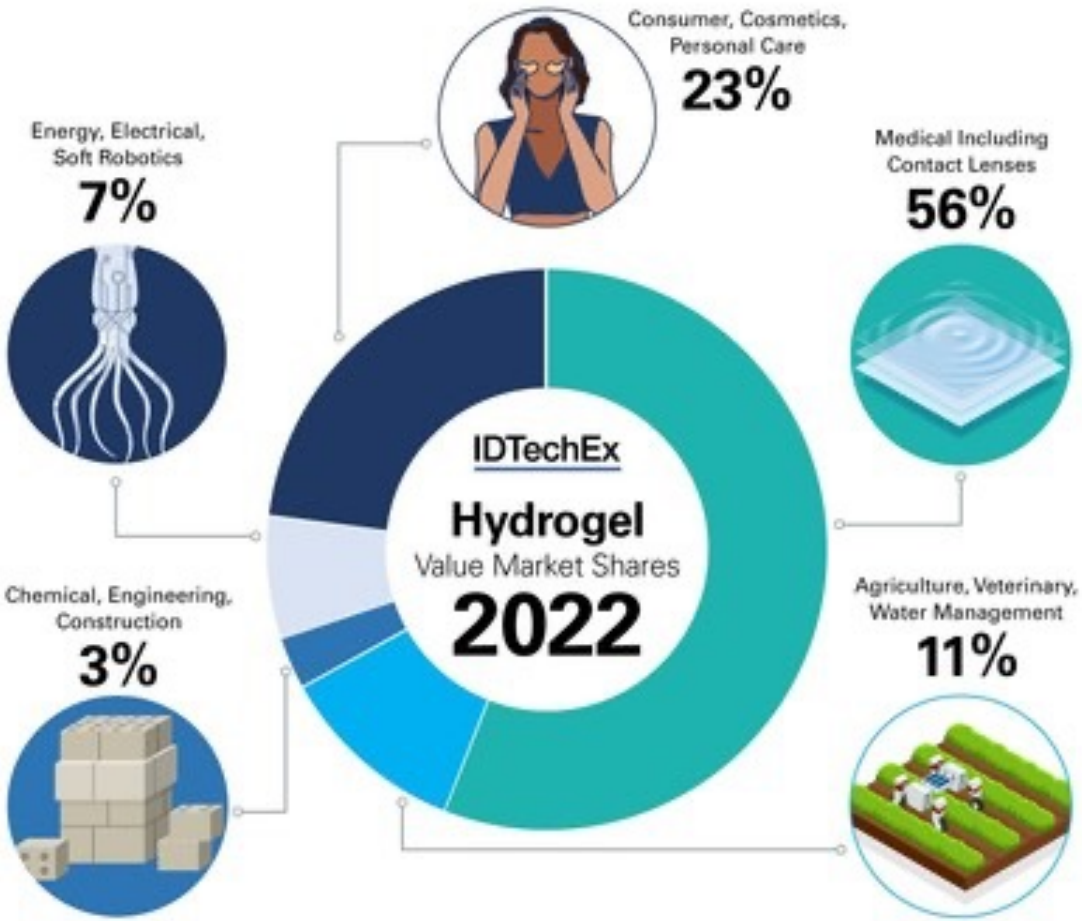
-Protective environment for cells and other substances (ie drugs, proteins, peptides, RNA…)
- Timed and controlled release or uptake of nutrients and growth factors
- Good transport properties
- Biocompatible
- Injectable and occasionally 3D printable
- Easy to modify and crafted without animal derived materials

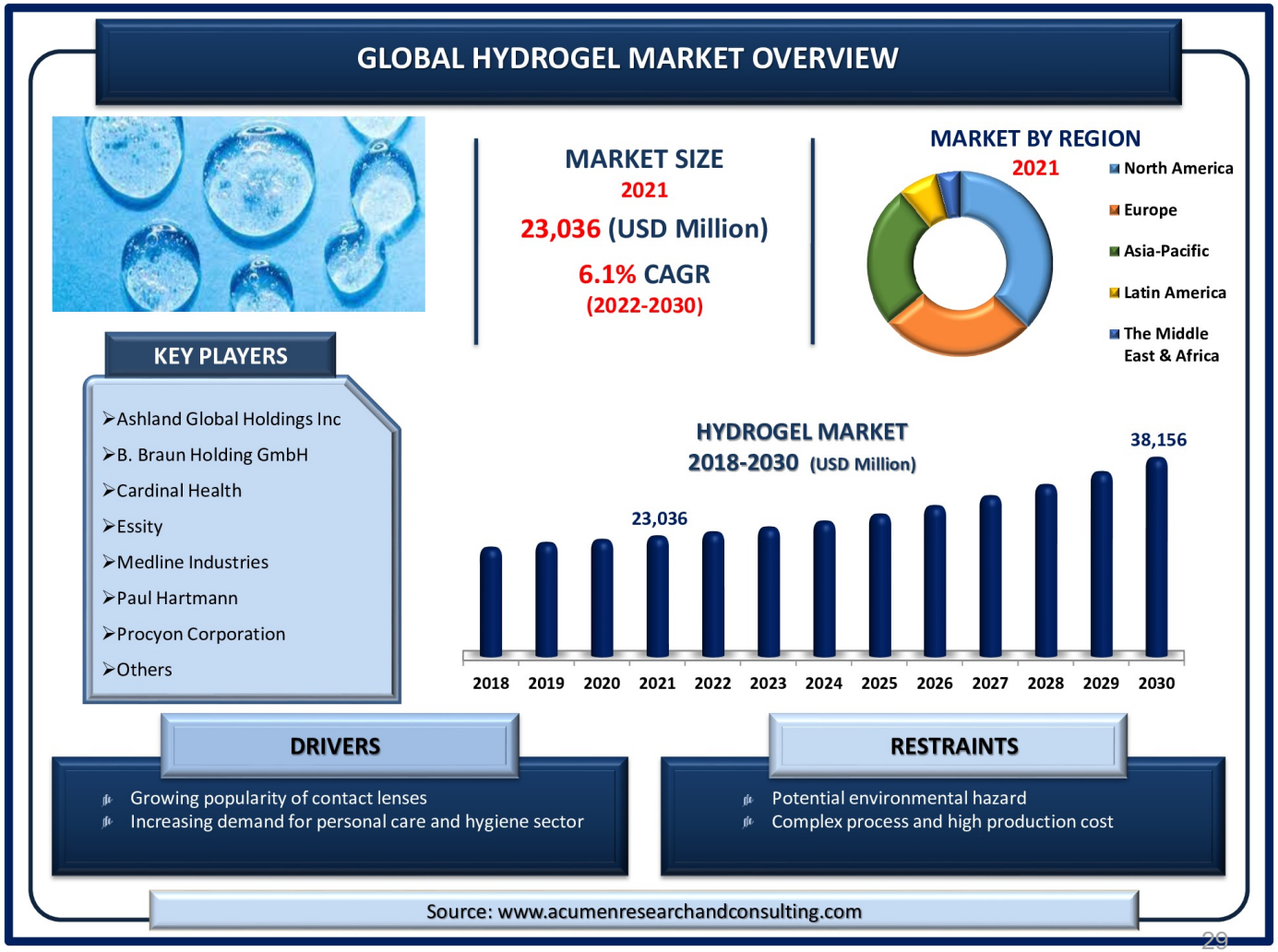
Disadvantages:

- Low mechanical strength relative to natural tissues
- Difficult to handle without proper training
- Difficult to sterilize
- Low physiological complexity, but getting there



Hydrogel conclusions and current relevant real-world demand, not only in biomedical!





Tampere University

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Thank You!

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Lab Team and Collaborators

- Bioengineering and Nanomedicine group:

- Protein Dynamics Group:

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Research Council of Finland

FINNISH NATIONAL AGENCY FOR EDUCATION

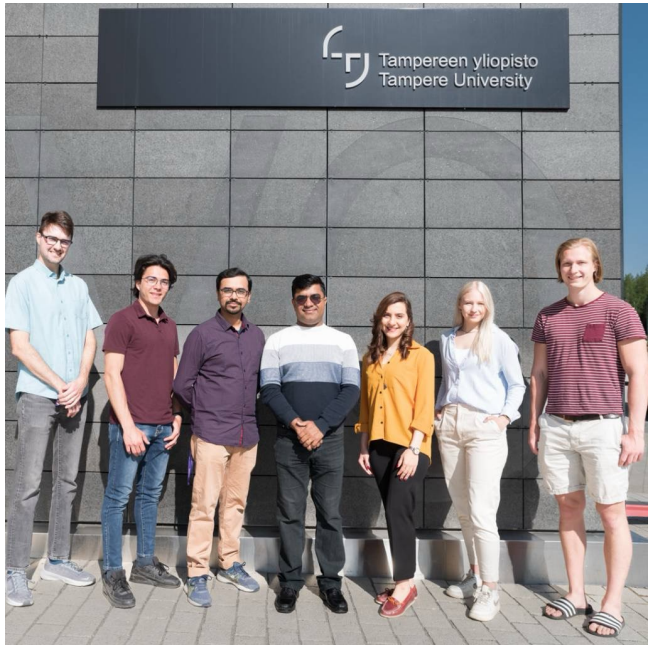
TEKNIIKAN EDISTÄMISSÄÄTIÖ

MARIE CURIE ACTIONS

JUSELIUS SIGRID SIFTELSE

Syöpäsäätiö Cancer Foundation Finland

Tampere University



Email: austindonnelly.evans@tuni.fi

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