

Gel strength and gelation kinetics as parameters in the development and quality control of hydrogels for medical products

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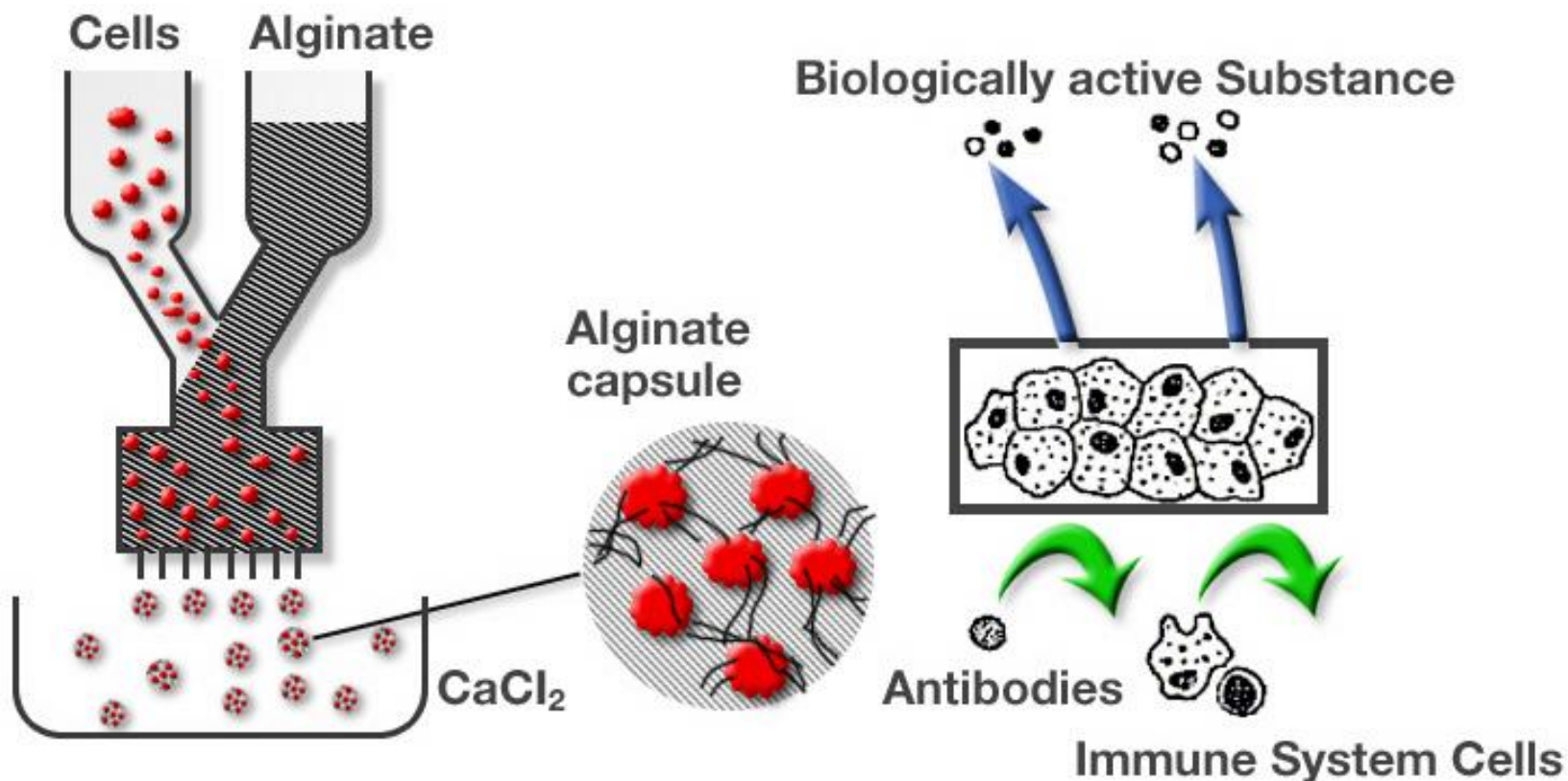
ASTM Scaffolds Workshop, 21 May 2013, Indianapolis IN

Disclosure

- The authors are employed by FMC BioPolymer AS.
- NovaMatrix, a business unit of FMC BioPolymer, manufactures, characterizes, documents, and sells ultrapure alginate, hyaluronan, fucoidan, and water-soluble chitosan salts for biomedical applications.
- Alginate-based ionically cross-linked gel formulations are enabling technologies

The old

- Alginate microbeads for cell encapsulation



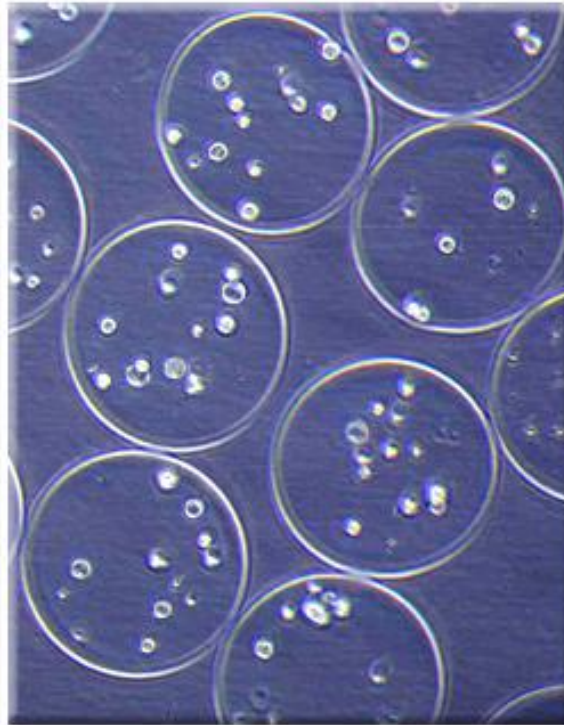
Applications using cell encapsulation

DISEASE	PRODUCER CELLS	SUBSTANCE SECRETED
Cancer	Genetically manipulated cells	Angiostatin, endostatin, cytokines
Cancer	Hybridoma	Mab's
Diabetes	Islets of Langerhans	Insulin
Parkinson's	Adrenal chromaffin cells	Dopamine
Chronic pain	Multiple cell types	Endorphin, enkephalin
Hypocalcemia	Parathyroid cells	PTH
Liver failure	Hepatocytes	Artificial Organ
Tissue repair	Chondrocytes	Tissue regeneration

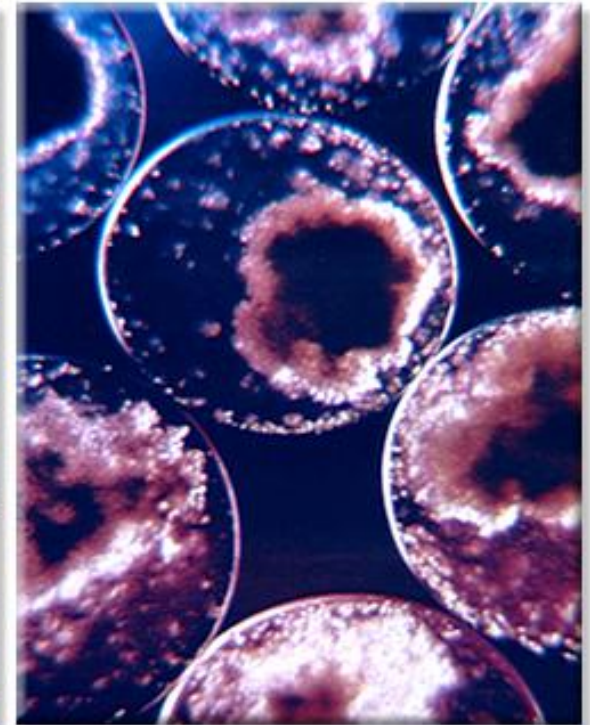
Encapsulated cells



Mouse 3T3



HEK 293

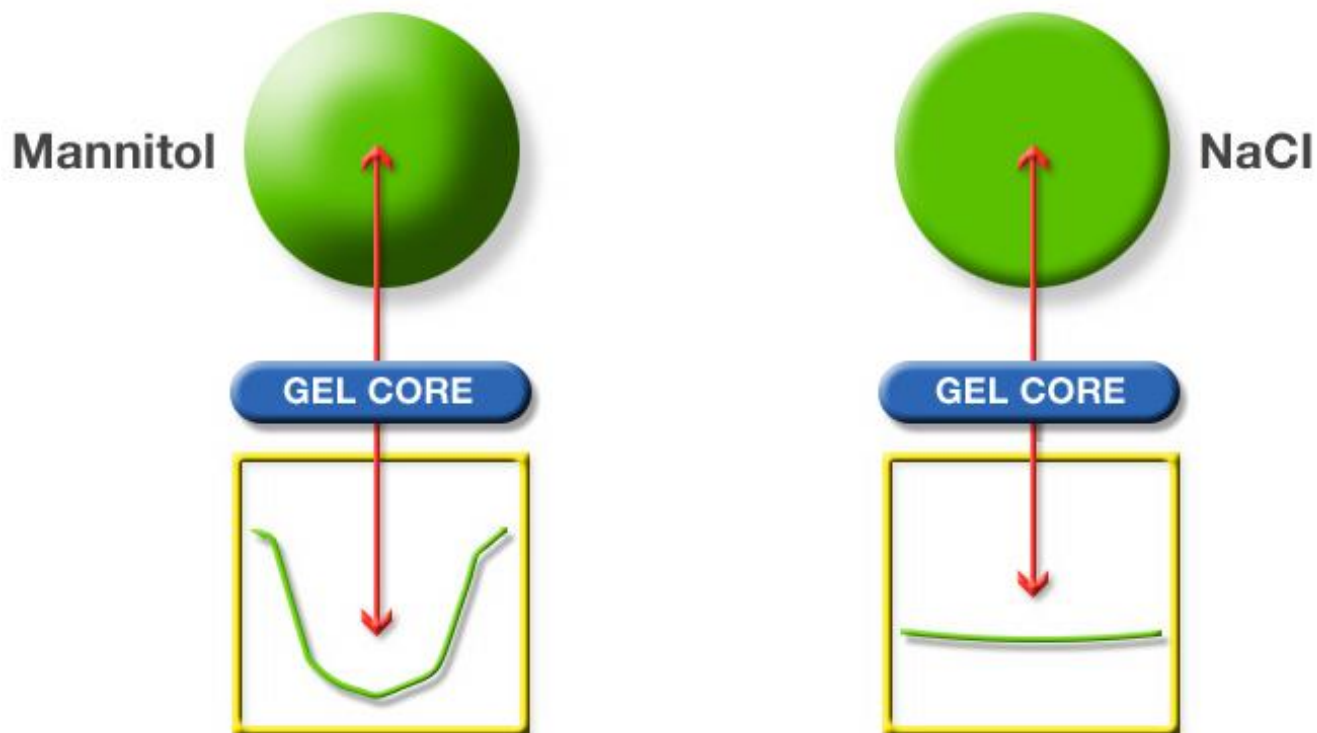


Human Islets

Inhomogeneous vs homogeneous gelling

INHOMOGENEOUS GEL

HOMOGENEOUS GEL

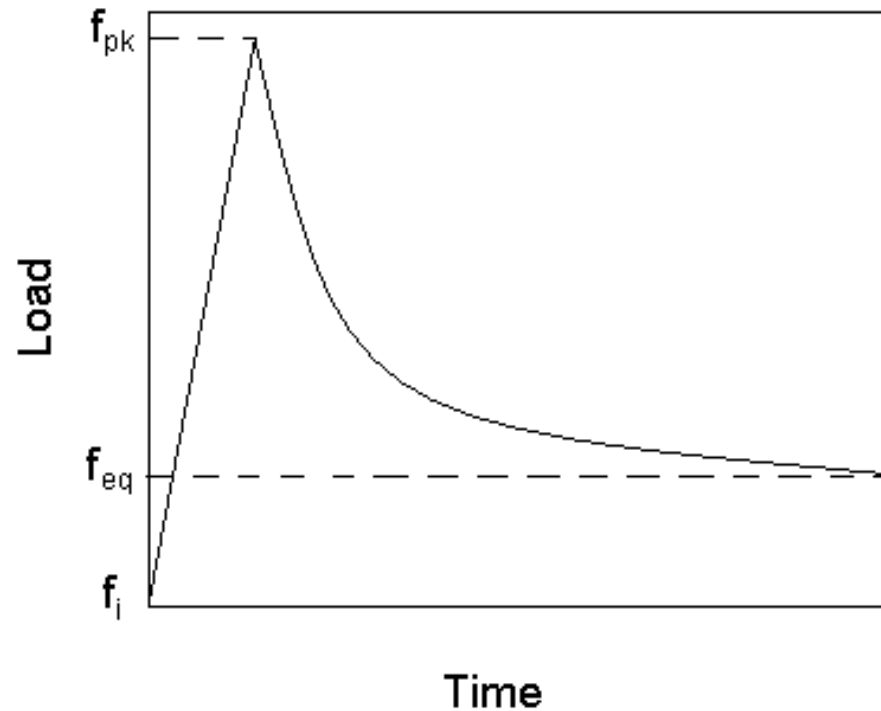


Alginate Concentration Gradient

Strength and stability

- **High strength/stability factors**
 - High G-content
 - Average G-block content about 15
 - Not MW-dependent above certain value
 - High affinity cations (Sr^{2+} , Ba^{2+} , Ca^{2+})
 - Coating with polycation
- **Low strength/stability factors**
 - Chelating compounds (phosphate, citrate, lactate)
 - Non-gelling ions (ratio non-gelling/gelling)
 - Swelling properties

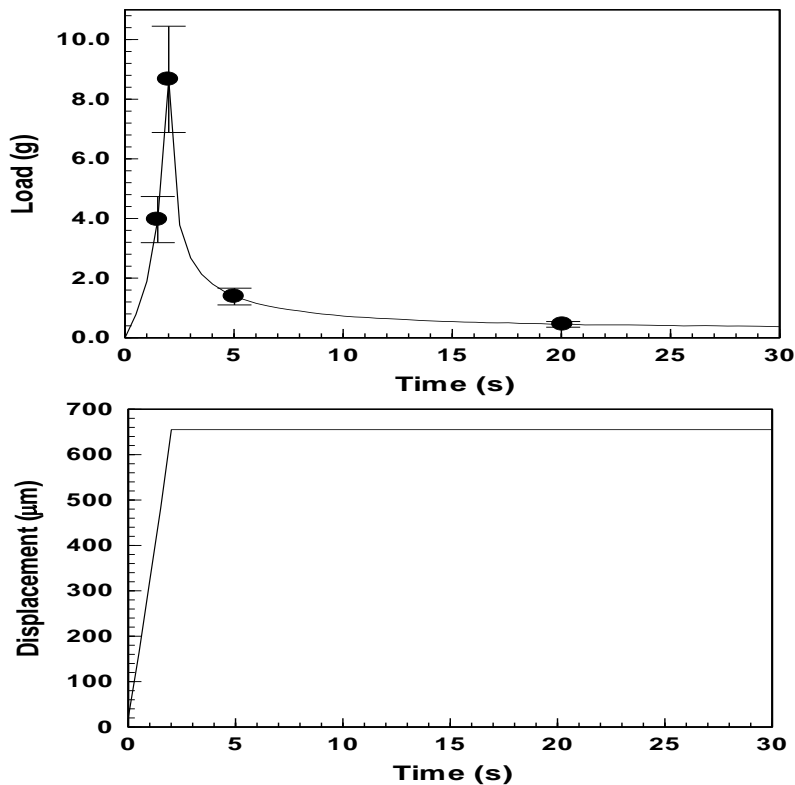
Alginate hydrogel bead characterization



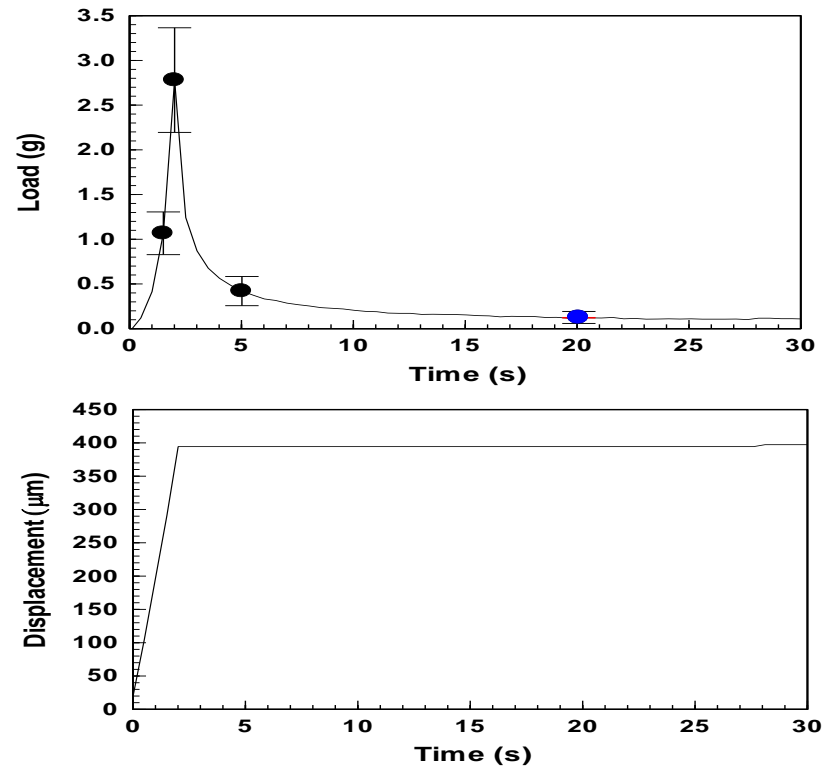
Typical behavior of a viscoelastic material under the stress relaxation test. f_i is the initial load, f_{eq} is the load or stress at equilibrium, and f_{pk} is the peakload

Stress relaxation testing

LVG Samples in Calcium Chloride



LVM Samples in Calcium Chloride



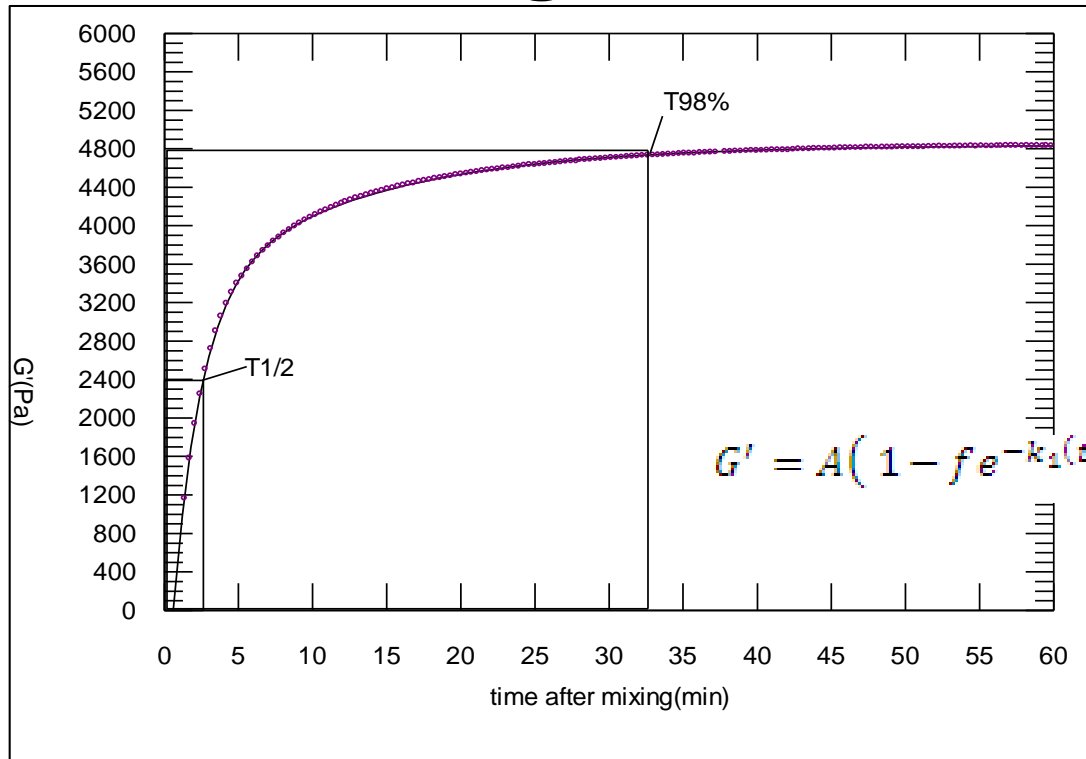
Stress relaxation test for samples in CaCl_2 , a) Mean force, b) Mean displacement

The new

➤ Self-gelling alginate

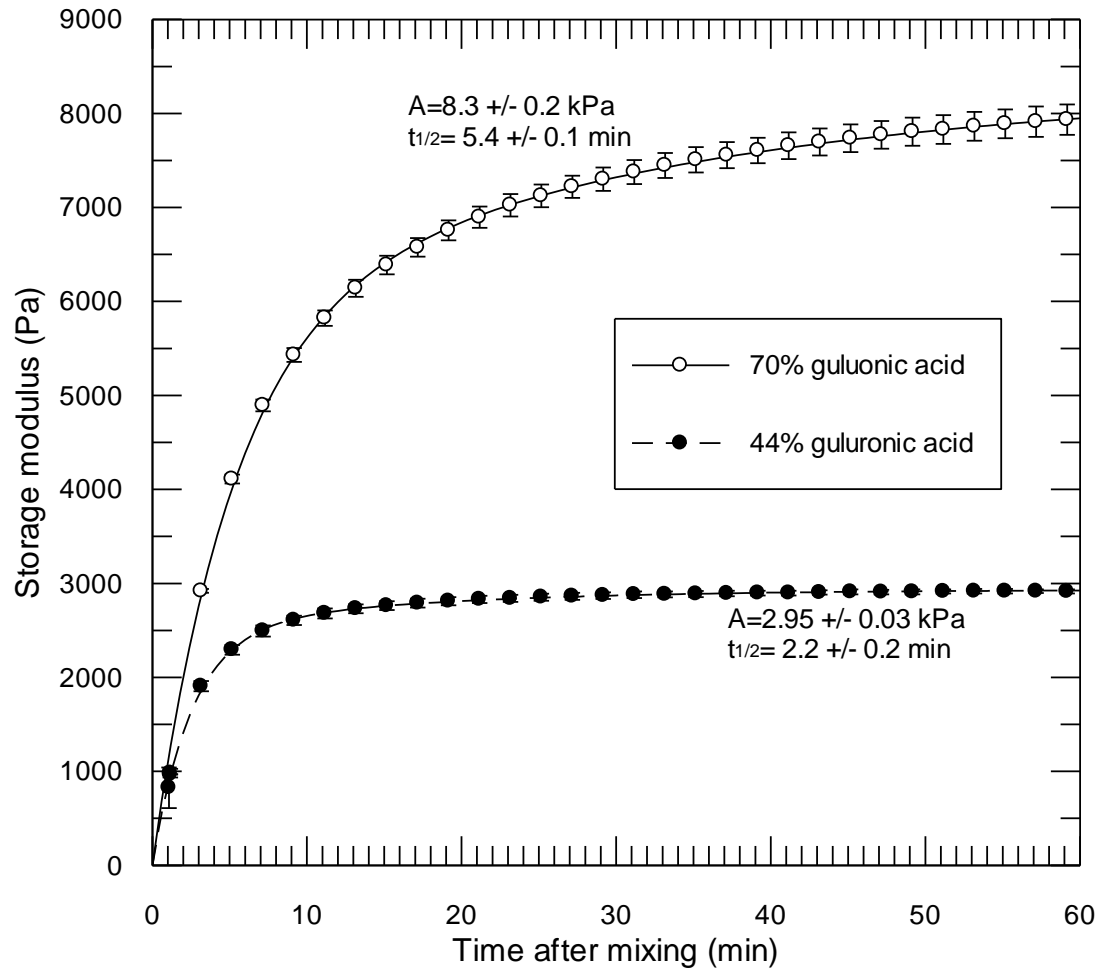


Rheology measurements of self-gelling alginate

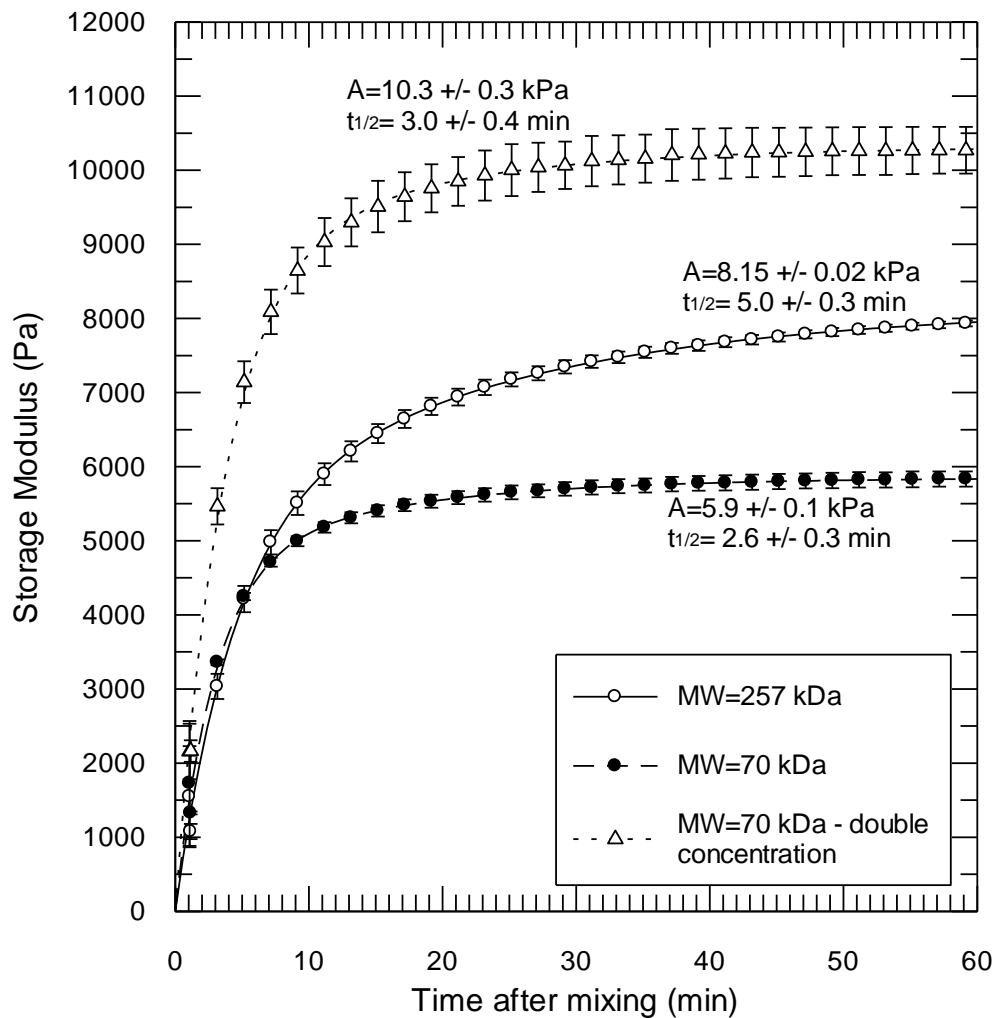


Parameter	Description
FG	Final Gel strength given as Elastic Modulus at completed gelling
FGT1/2	Time (minutes) to obtain 50% of Final gel elastic modulus(G')
FGT98%	Time (minutes) to achieve 98% of Final gel elastic modulus(G')

Effect of alginate composition

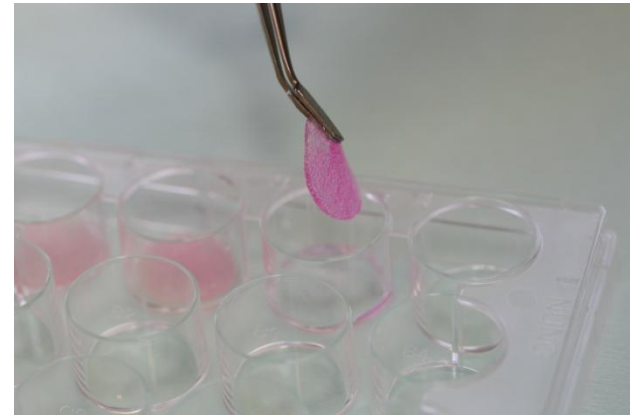
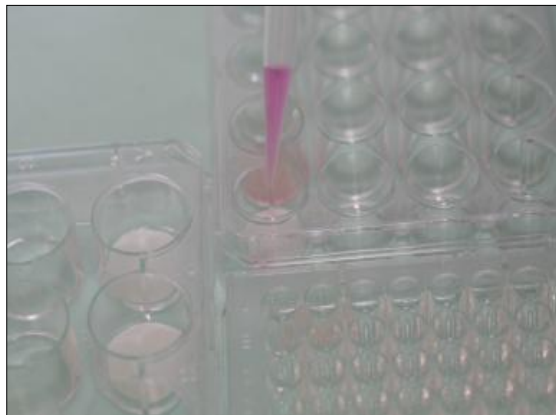
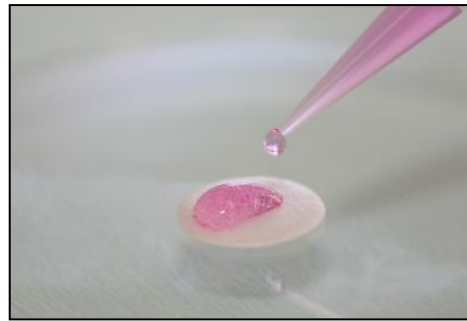
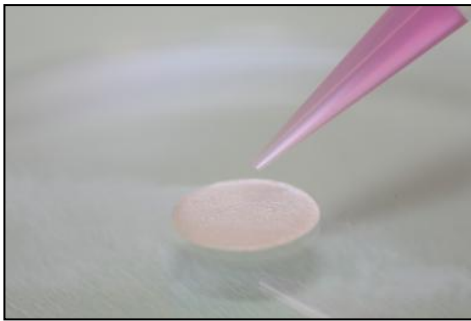


Effect of molar mass

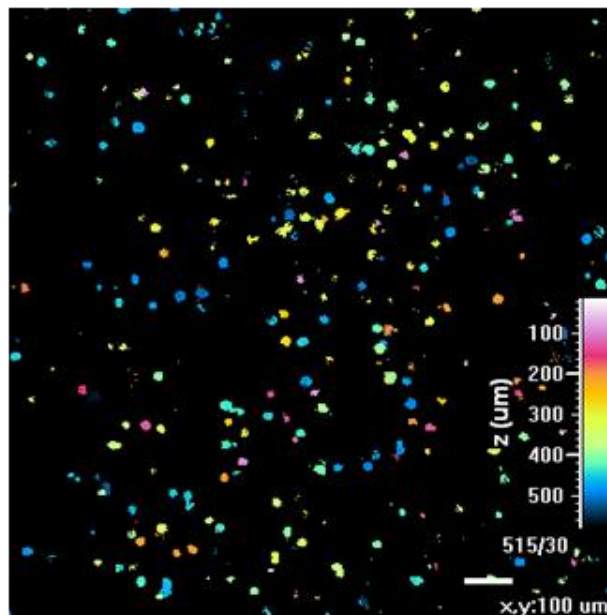
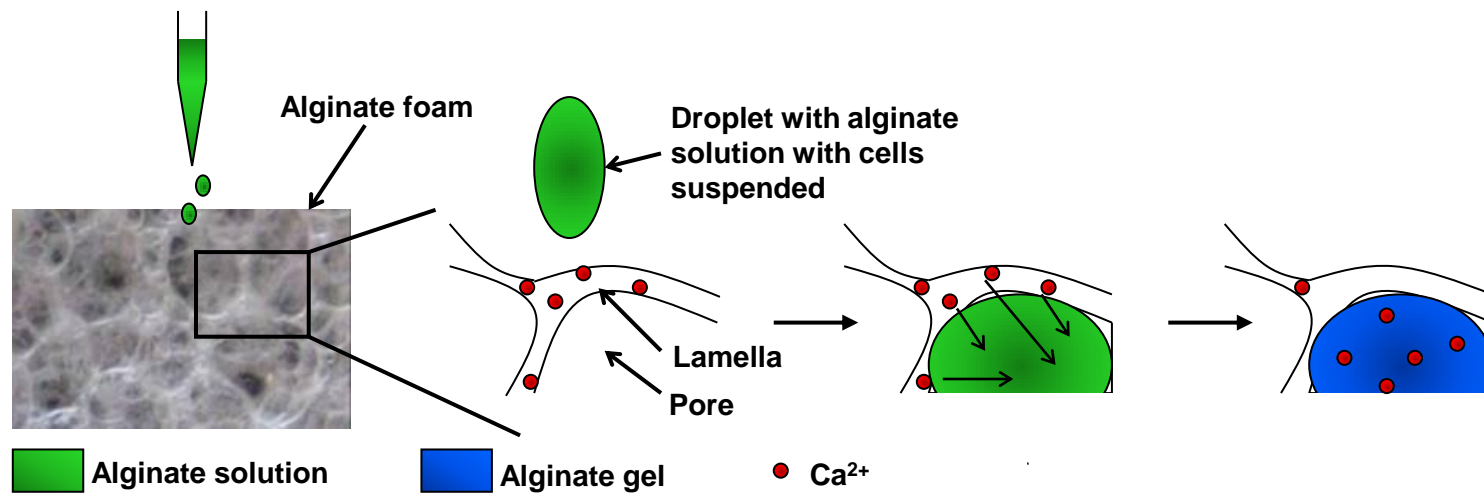


The future

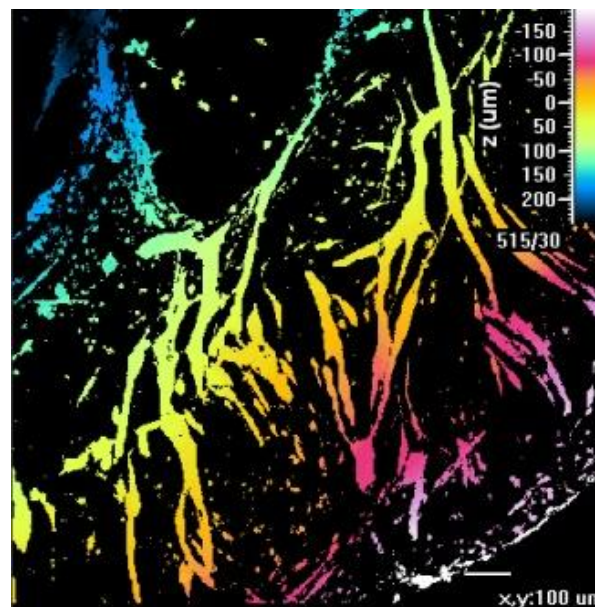
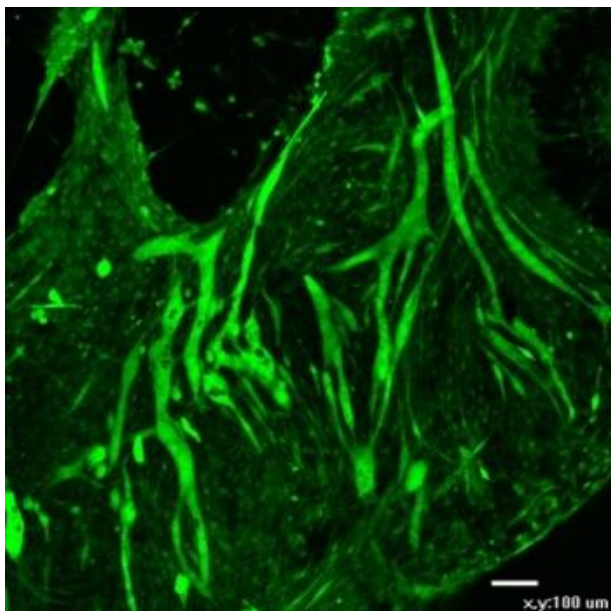
➤ Alginate foam scaffolds



Alginate foam as a cell scaffold



Cell/tissue growth in alginate scaffolds



Multinucleated myotubes – C2C12 mouse myoblasts (Day 60)

Alginate scaffold product testing

Properties	Internal Specification	External Specification
Cell distribution	> 300 μm at day 1	Conforms
Cell Viability	> 70% at day 15 (live/dead)	Conforms
Cell Proliferation	Should have doubled in number in 14 days	Conforms
Absorption volume	12-well: 200 \pm 20 μl 24-well: 100 \pm 10 μl 96-well: 10 \pm 2 μl	N/A
CaCO ₃ residues	Score \geq 3	N/A
Air bubbles	Score \geq 3	N/A
Sterility	No growth, 20 kGy	No growth, 20 kGy

PRODUCT RELEASE REPORT

Product name: NovaMatrix®-3D cell culture kit
 Batch no: BU-
 Generic name: NovaMatrix®-3D kit
 Storage conditions: 2 - 8 °C
 Packaging material: Glass vial and plastic multi-well plate
 Manufacturing site: FMC BioPolymer AS d/b/a NovaMatrix

<u>Test</u>	<u>Result</u>	<u>Specification</u>	<u>Method</u>
<u>Freeze-dried alginate vial</u>			
Apparent viscosity	52	40 – 80 mPas	AM-056
<u>Alginate foam</u>			
Cell distribution	Pass	> 300 µm at day 1	401007
Cell viability	Pass	> 80 % at day 15	401007
Cell proliferation	Pass	minimum 1 population doubling in 14 days	401007
Sterility	No growth	No growth	401010

AM: FMC BioPolymer AS / NovaMatrix Standard Method

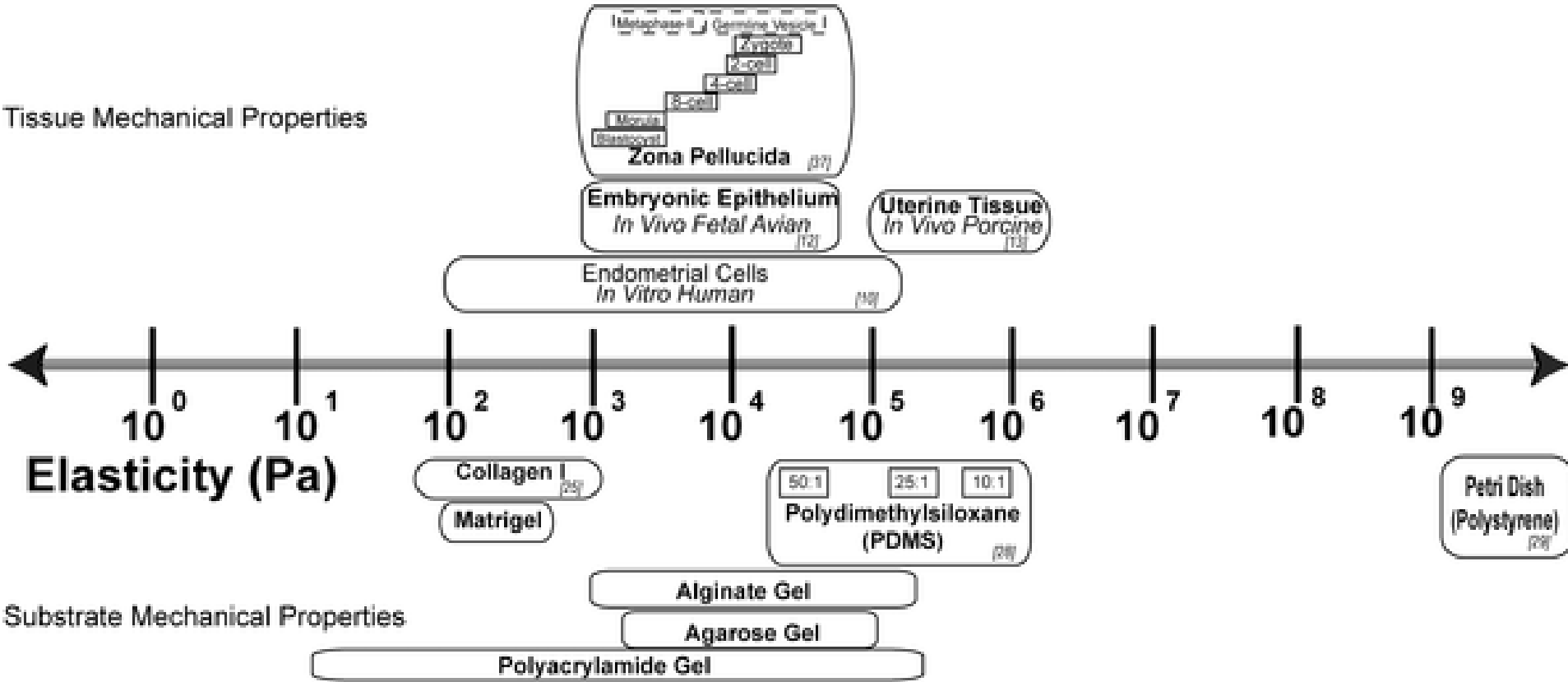
Date

Date

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 Scientific Director

Elasticity of some tissue compared with materials used in TE



Kolahi KS, Donjacour A, Liu X, Lin W, et al. (2012) Effect of Substrate Stiffness on Early Mouse Embryo Development. PLoS ONE 7(7): e41717. doi:10.1371/journal.pone.0041717

<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0041717>

Conclusions

- Hydrogels are already in clinical trials
- Application-specific product specifications
- BUT no universal testing methodology