Spray application of *in situ* cross-linkable multi-layered hydrogels for medical applications

医療用*in situ*架橋マルチレイヤーハイドロゲルのスプレー投与システム

医科学専攻修士課程修士論文発表会
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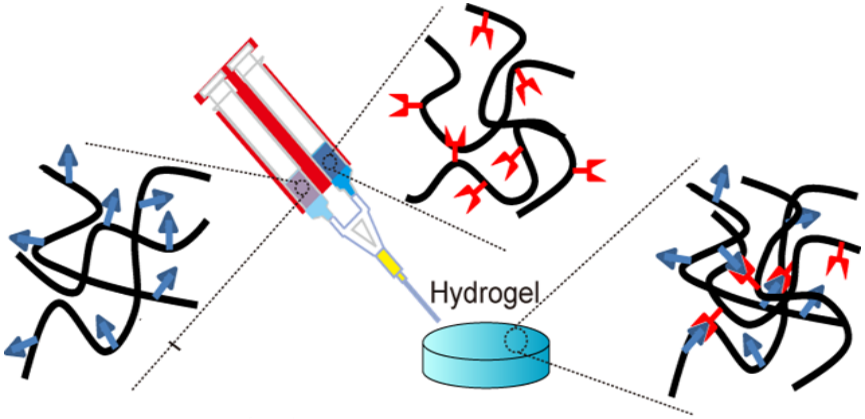
41-156214　Zheng Ying Grace

In situ cross-linkable hydrogels

- Gels formed by simple mixing of two different polymers

- Merits:
  - ✓ Cover complex tissue geometries
  - ✓ Used in both open & laparoscopic surgeries



- Used in various biomedical applications:
  1. Scaffolds for tissue engineering
  2. Clinical procedures
     - ➢ Hemostasis
     - ➢ Tissue adhesion prevention

Biomedical spray devices for clinical uses

- One method of delivery of *in situ* cross-linkable hydrogels
- Advantages:
  - ✓ Even delivery onto varying tissue surfaces
  - ✓ Less tissue handling

Fibrin sealants

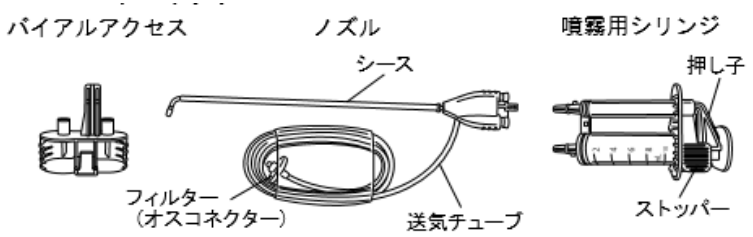
Bolheal®

Tisseel®

>50% of hemostats market share

Tissue adhesion barriers

Adspray®

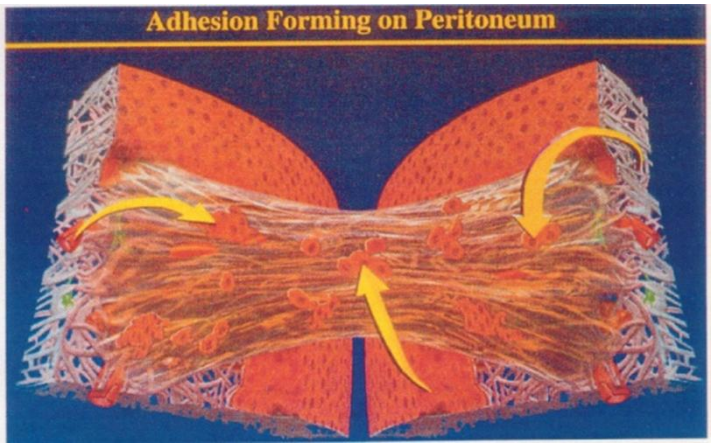


Few

Sprays for adhesion prevention remains largely unstudied

Tissue adhesion

- Abnormal fibrous bands attaching 2 adjacent body tissues
- Occurs after 54% of abdominal surgeries
- Problems such as pelvic pain etc.



G.S.Dizerega and J.D. Campeau: Human Reproduction Update 2001(7)547-555

Tissue adhesion barriers

- Physical separation of tissues
- Commercially available adhesion barriers have limitations:

Seprafilm®

http://www.colmed.in/interceed-adhesion-prevention/4350-interceed-adhesion-barrier-3-in-x-4-in-box-of-10.html

Interceed®

https://www.classactionlawsuithelp.com/seprafilm-class-action-lawsuit/

X Brittle

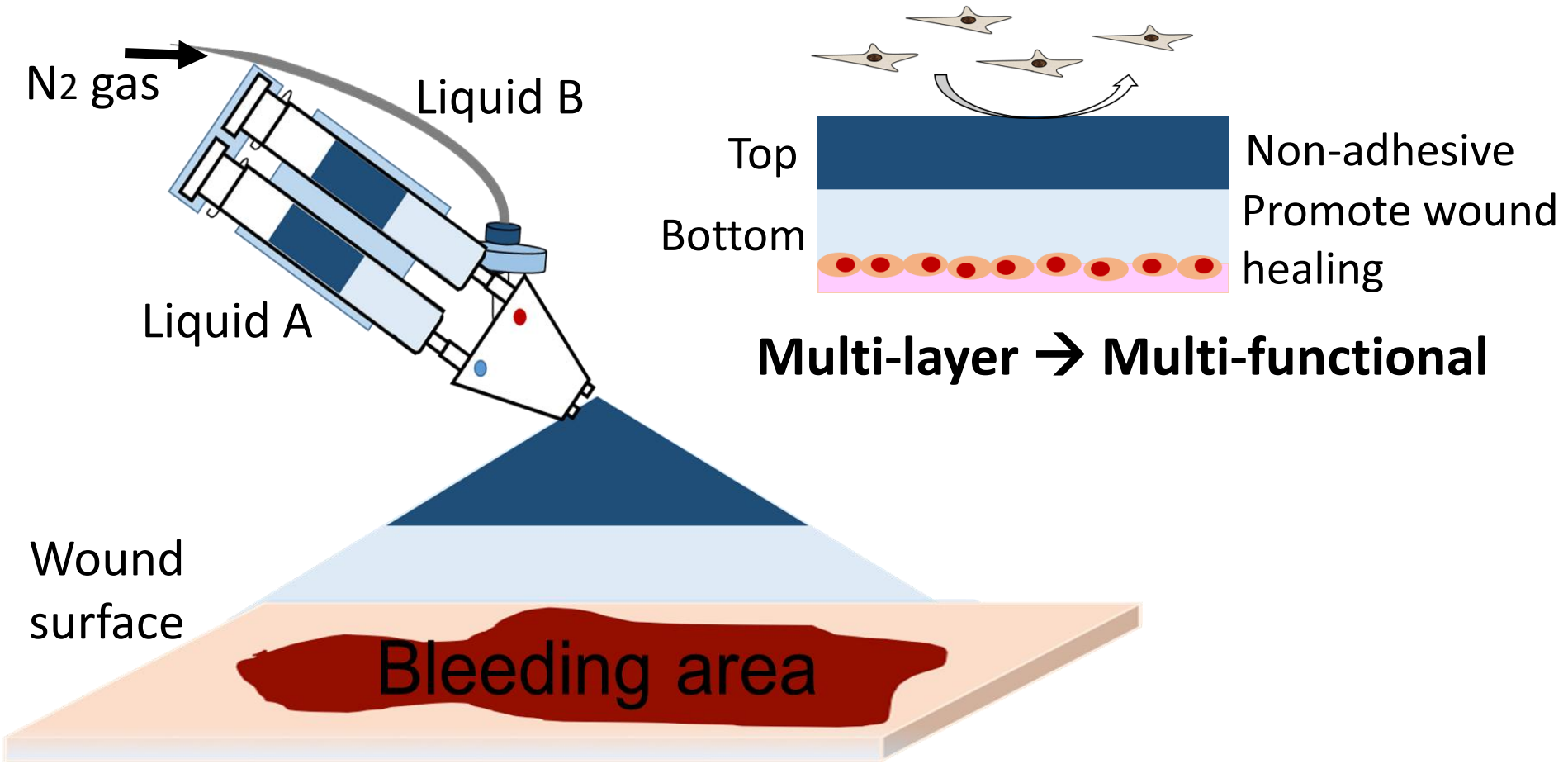
X Low efficacy in surgeries with heavy bleeding

Limited efficacy of a single material

Need for improved material → Combination of various materials

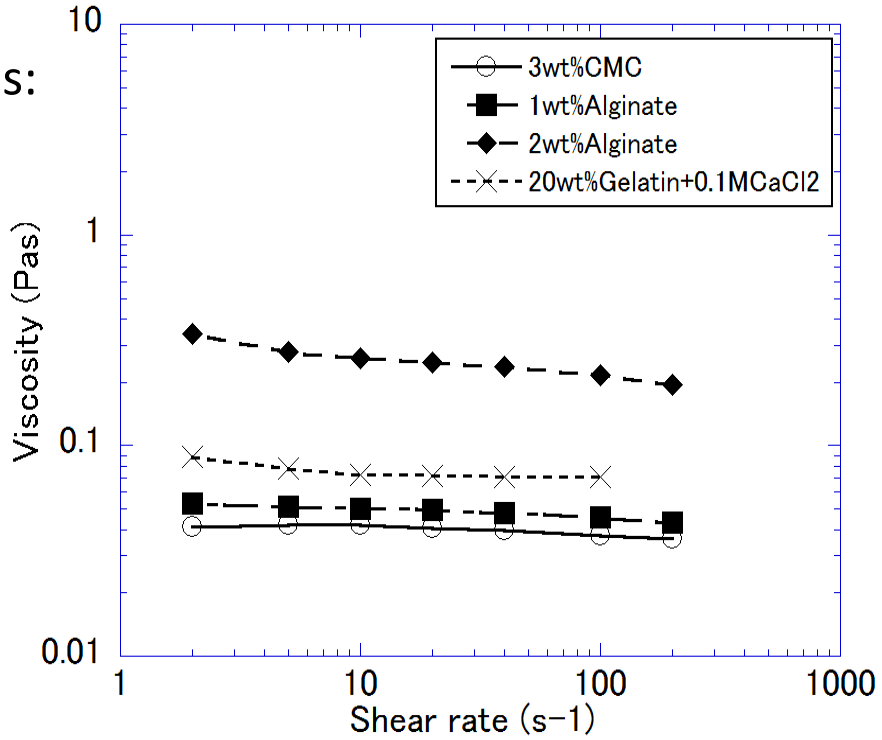
Objective

Spray application of multi-layered *in situ* cross-linkable hydrogels for tissue adhesion prevention



Materials

Viscosity of materials:



	Liquid A	Liquid B
Top (away from wound surface)	1wt% Alginate	3wt% Carbomethyl cellulose (CMC)+ 0.1M$CaCl_2$ **Alg-CMC**
Bottom (in contact with wound surface)	2wt% Alginate+ 1wt% Transglutaminase (TG)*	20wt% Gelatin+0.1M$CaCl_2$ **Alg-gelatin**

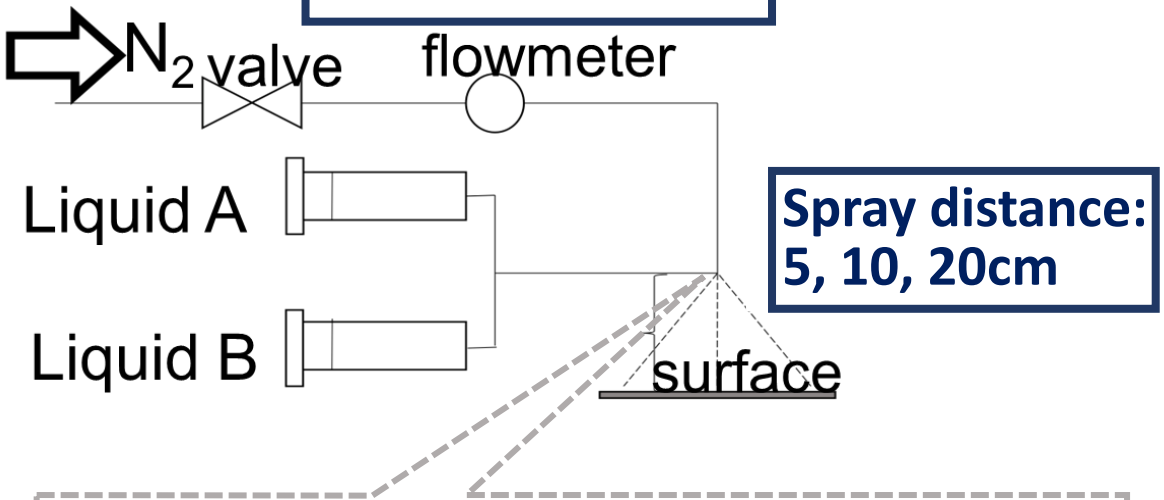
* [TG] was evaluated by fellow lab member Kikuchi E. 東京大学工学部卒論 (2016)

Experimental flow

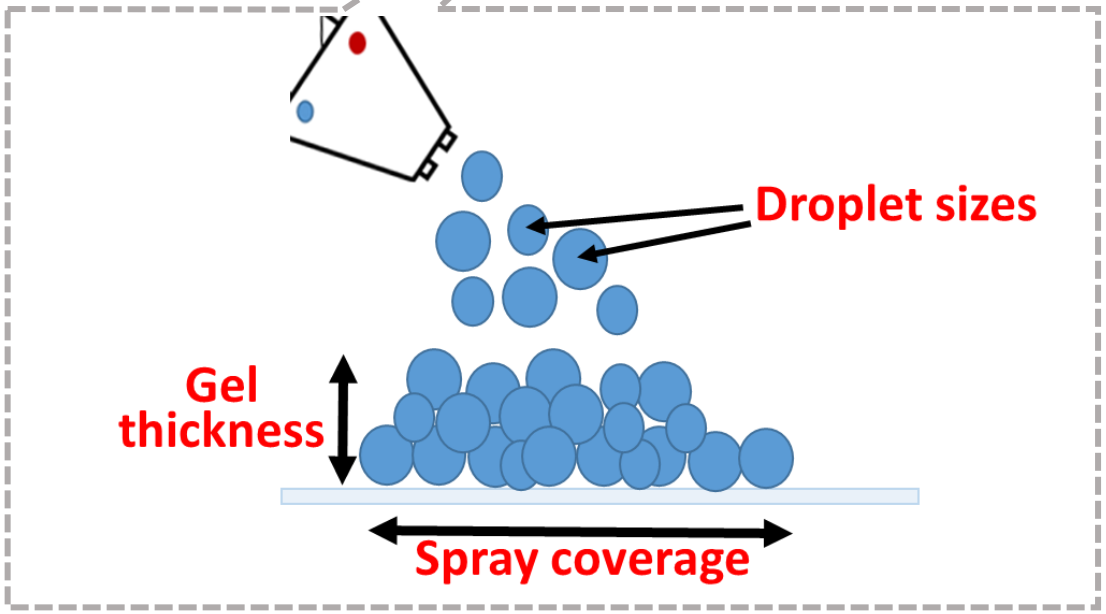
Spray characterization	• Spray coverage • Gel thickness • Droplet size	**Optimization of spray conditions**
In vitro analysis	• Cell viability • Cell migration	**Effect of spray conditions and materials on cells**
In vivo analysis	• Rat hepectatomy adhesion model	**Response to multi-layered hydrogels**

Spray characterization

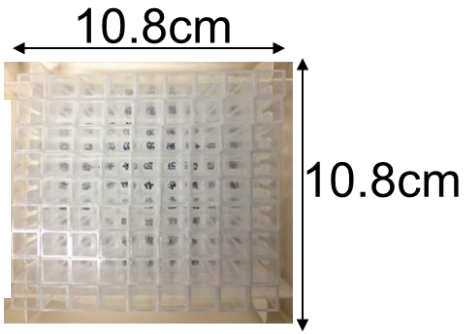
N_2 gas flow rate: 3, 5, 7L/min



Spray distance: 5, 10, 20cm

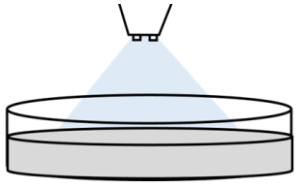


Spray coverage
Gel thickness



Homogeneity

Droplet sizes



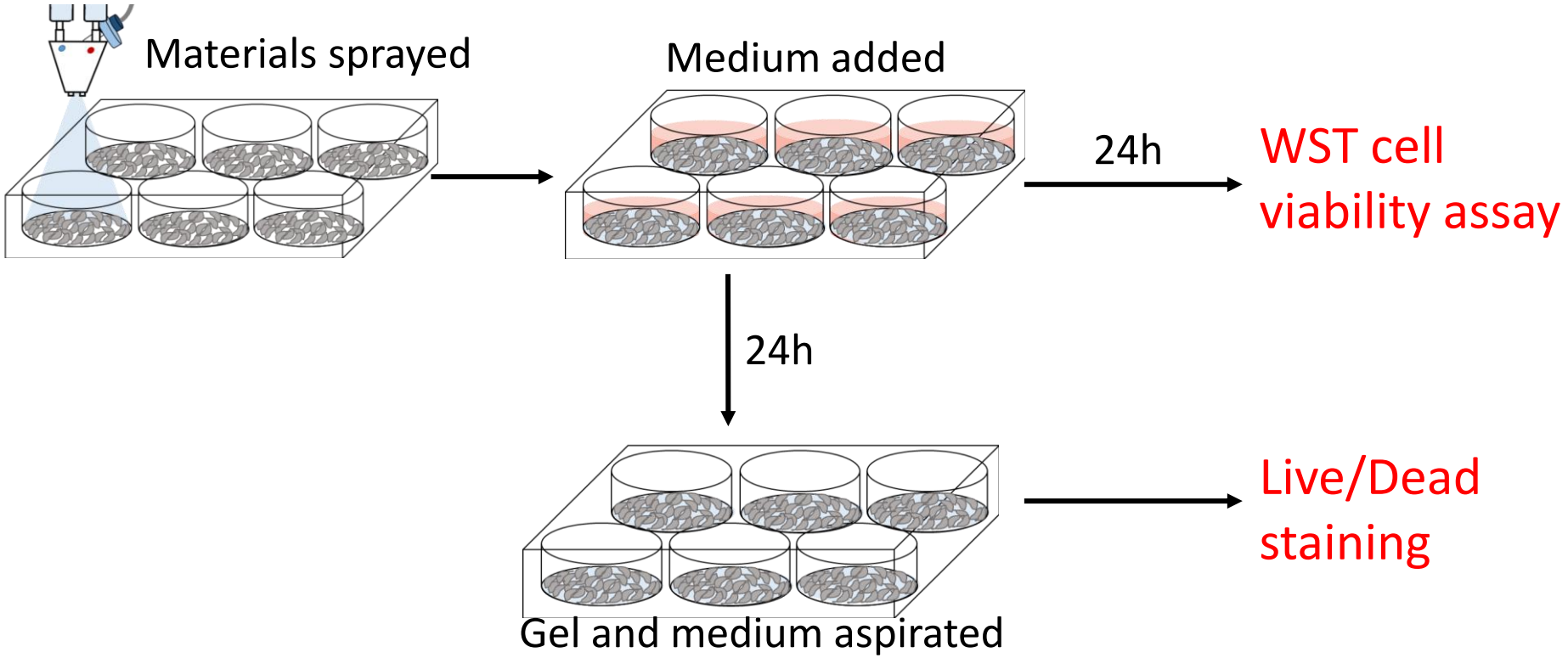
Φ= 200m
8ml of 0.1M $CaCl_2$

While stirring

Mixing efficiency

In vitro evaluation of cell damage by spraying

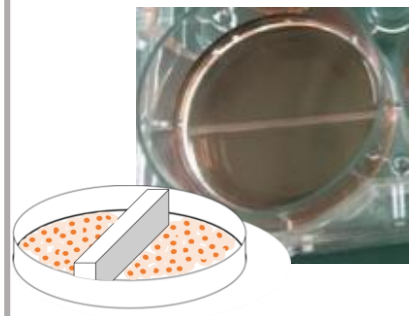
Cell types: NIH-3T3 fibroblasts, MeT-5A mesothelial cells
Gas flow rate: 3, 5, 7L/min
Spray distance: 5, 10, 20cm



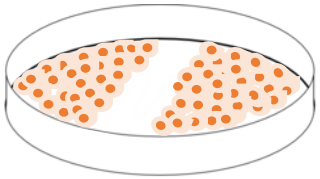
In vitro evaluation of cell migration

- To functionally differentiate the two layers

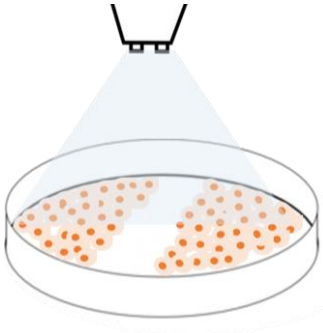
‘Scratch’ wound healing assay



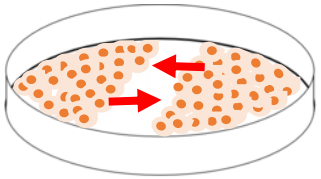
Silicon mold inserted before cell seeding



Mold removed

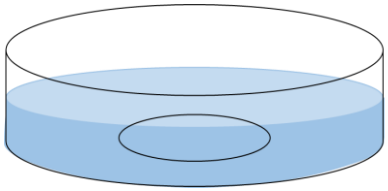


Materials sprayed

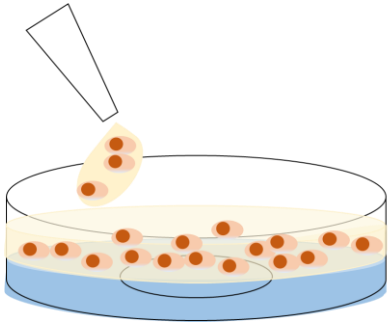


Wound closure observations

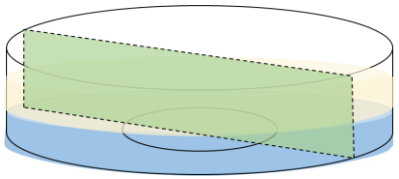
Cell penetration assay



Gels added via double syringes



Fluorescently labeled cells seeded onto gel

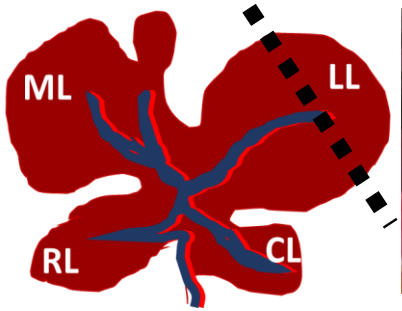


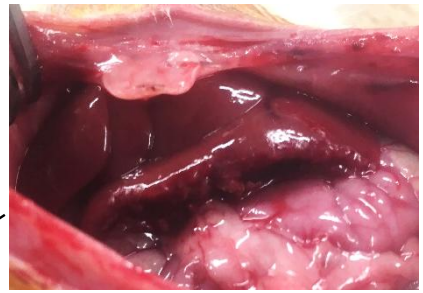
Observations of transverse gel sections

In vivo evaluation

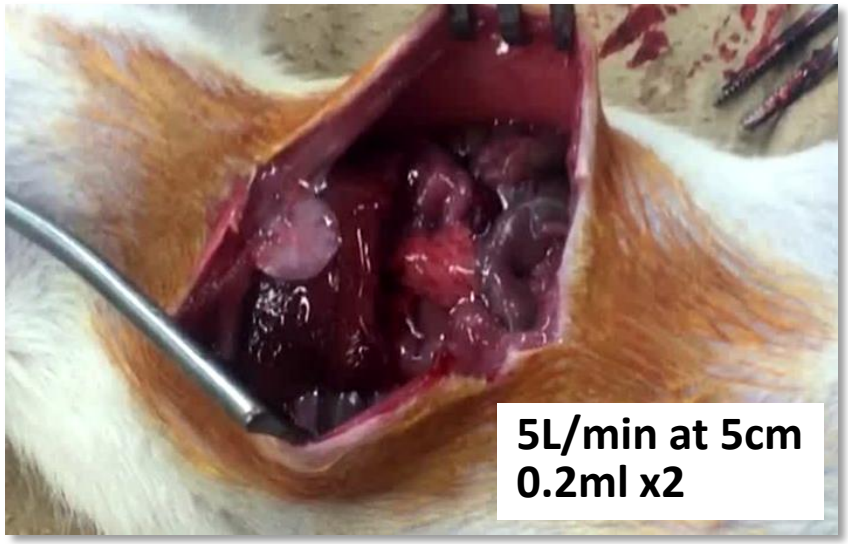
- Rat hepatectomy model

  - 8-11 week old SD rat ♂
  - **3cm** of left lateral lobe of liver was transected



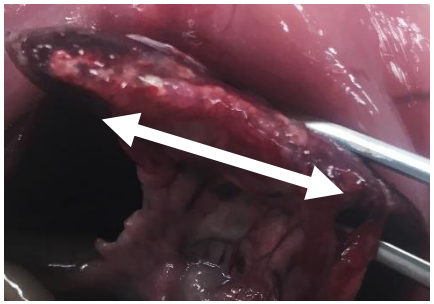


- Control
- Alg-CMC
- Alg-gelatin
- Double layer



Evaluation after 1 week

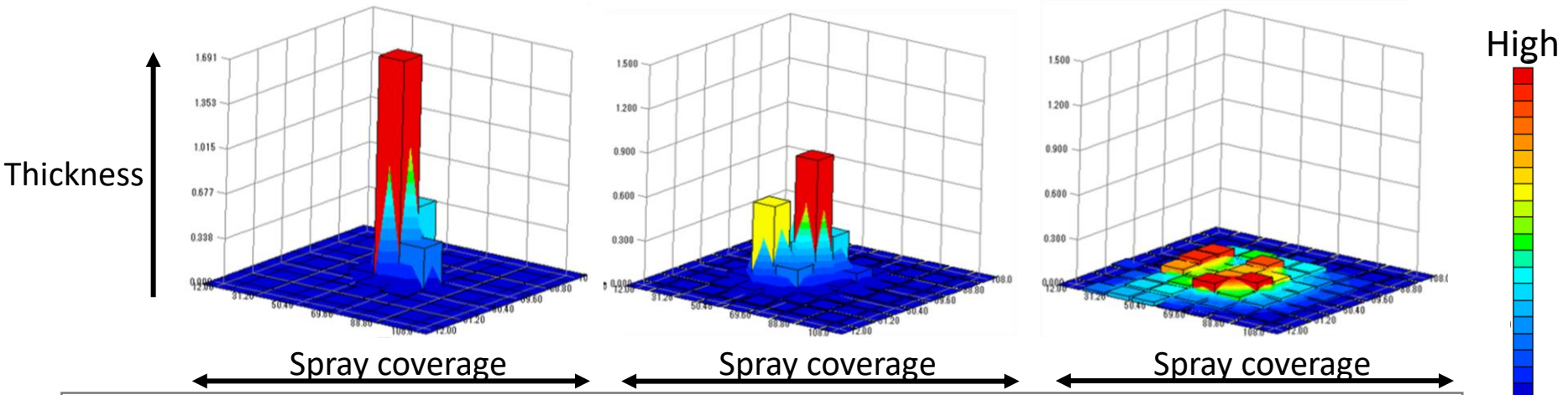
EXTENT



GRADE

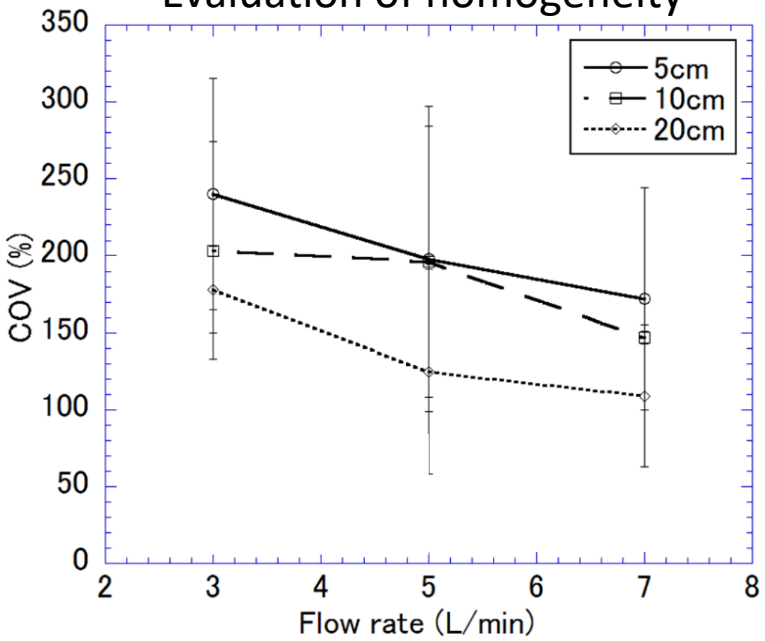
Grade 0: No adhesion
Grade 1: Spontaneously separating adhesion
Grade 2: Adhesions separated by blunt dissection
Grade 3: Adhesions separated by sharp dissection

Evaluation of homogeneity



Spray distance	5cm	10cm	20cm
Gas flow rate	3L/min	5L/min	7L/min
Recovery rate	96.4%	83.3%	78.7%

Evaluation of homogeneity

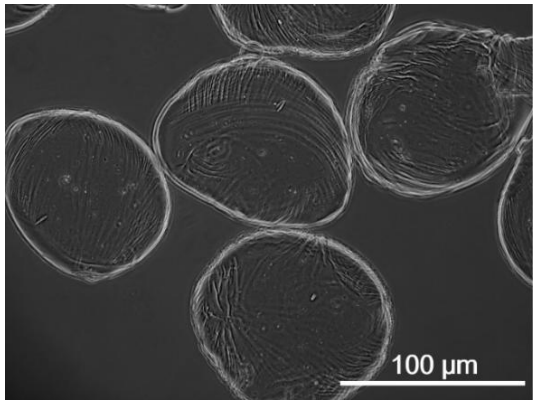


- Coefficient of variation (COV) = $\frac{Standard\ deviation\ (\sigma)}{Mean\ (\mu)}$ of gel thickness

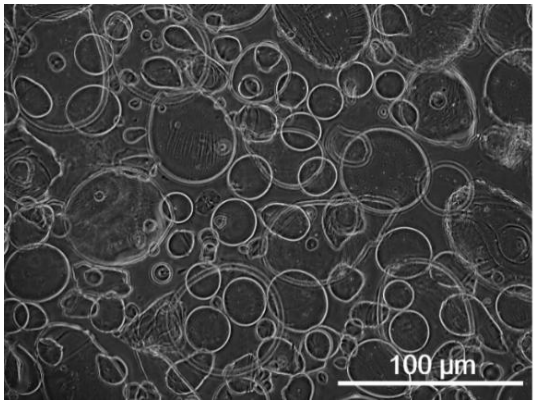
The higher the gas flow rate and spray distance, the higher the homogeneity

Droplet size measurements

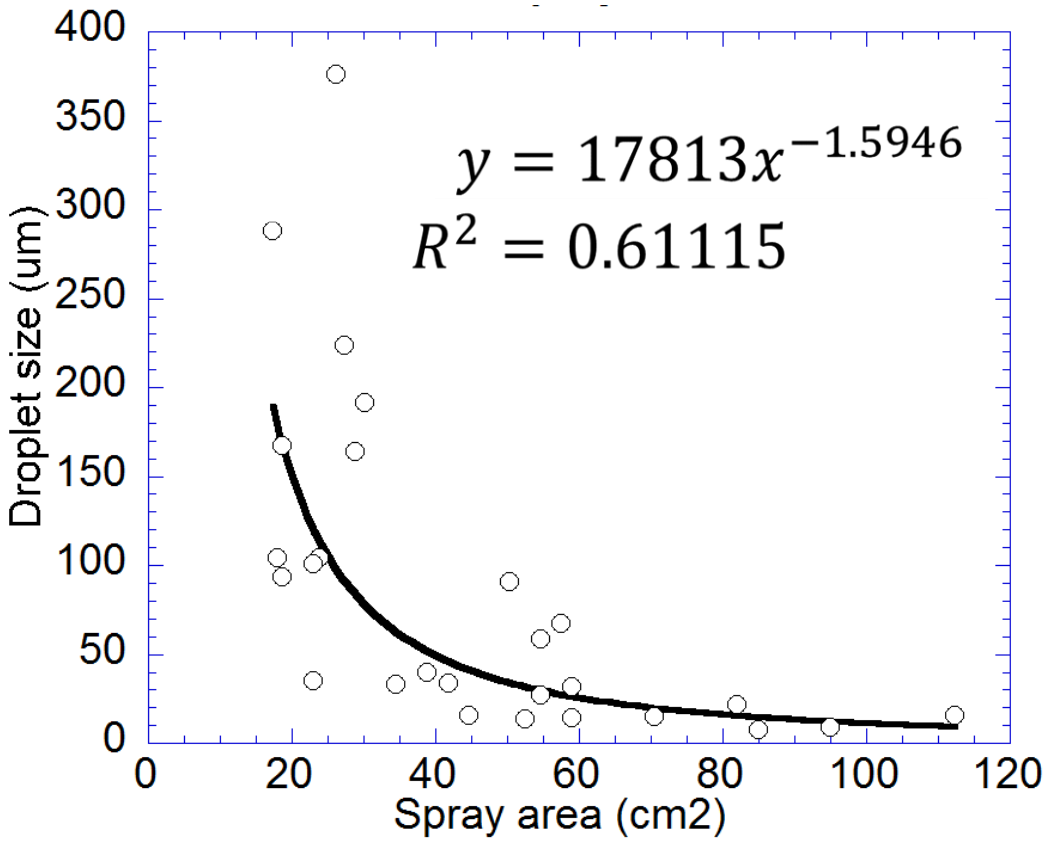
Spray distance: 5cm
Gas flow rate: 3L/min



Spray distance: 10cm
Gas flow rate: 7L/min



Material: 0.5, 1, 2, 3wt% Alginate

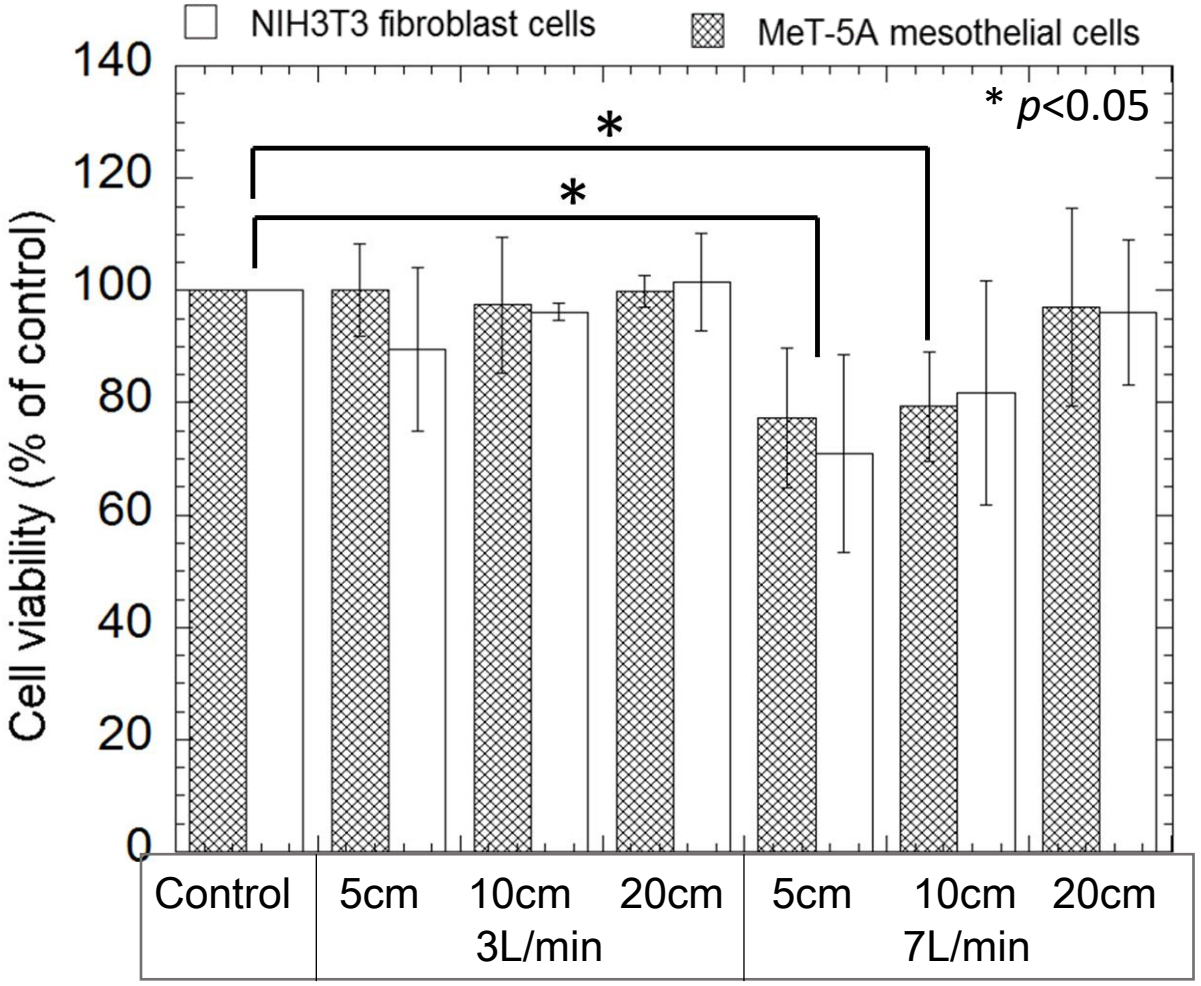


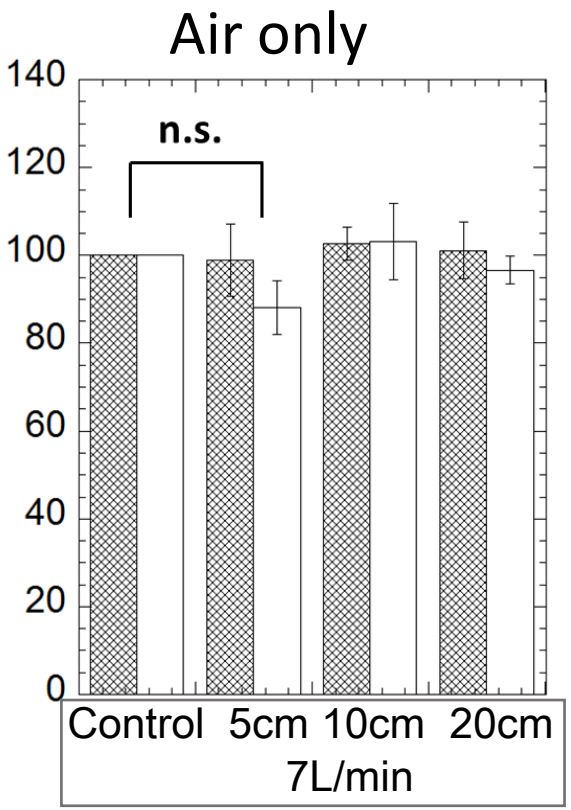
Smaller droplets with larger spray coverage

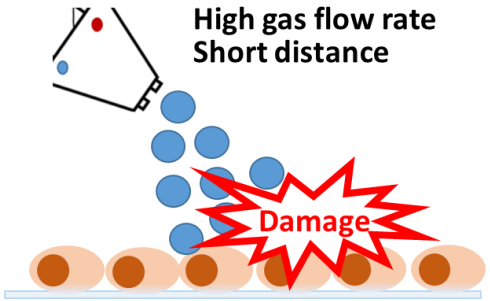
High gas flow rates
High spray distances
→ **High homogeneity**
High mixing efficiency of gels

In vitro analysis: WST assay

- Alg-CMC at varying spray parameters

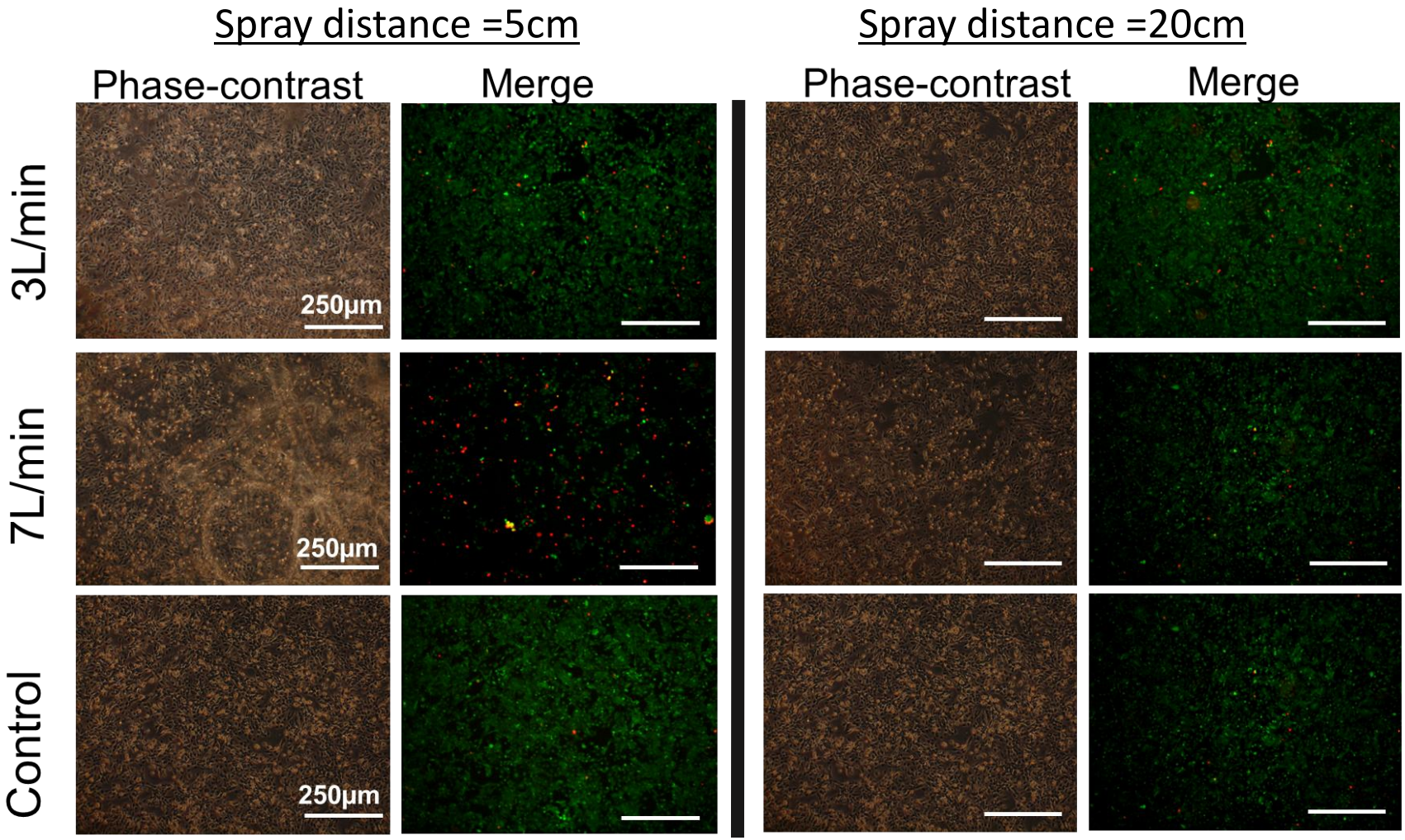






Materials sprayed at high gas flow rate and short distances caused significant cell death

In vitro analysis: Live/Dead Staining

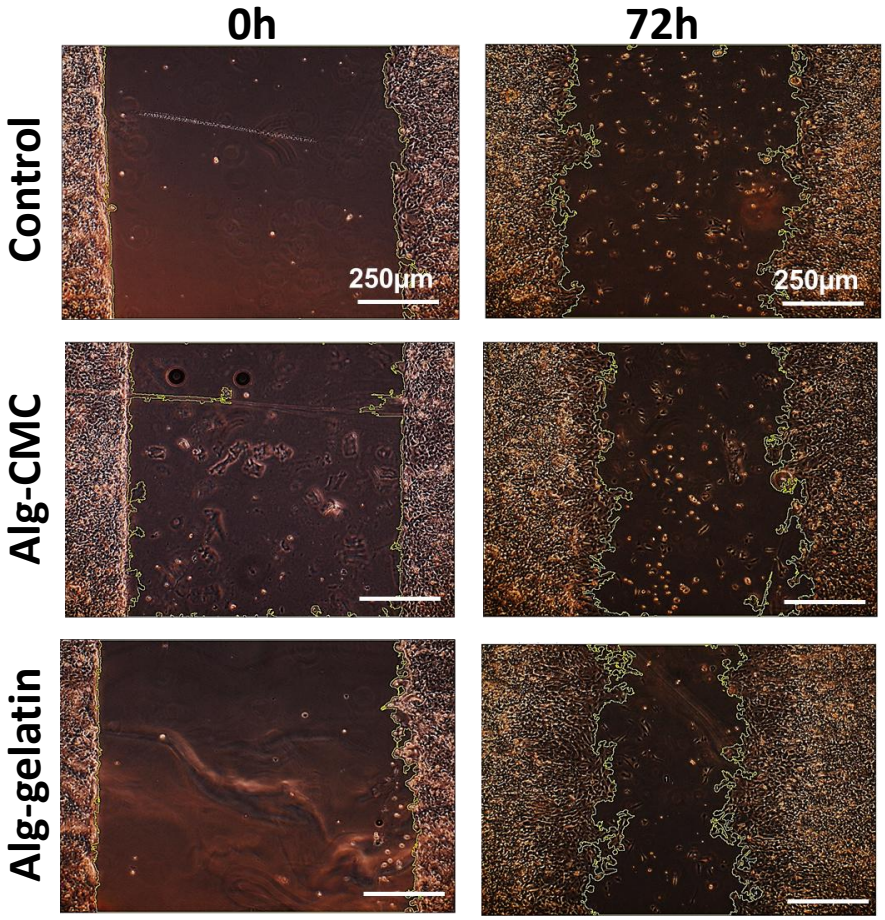


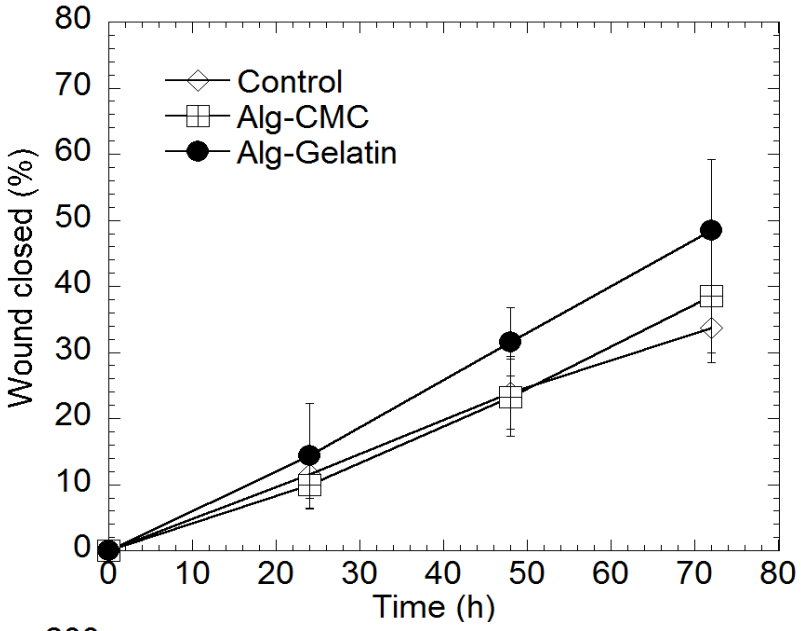
Green= Live cells **Red**= Dead cells

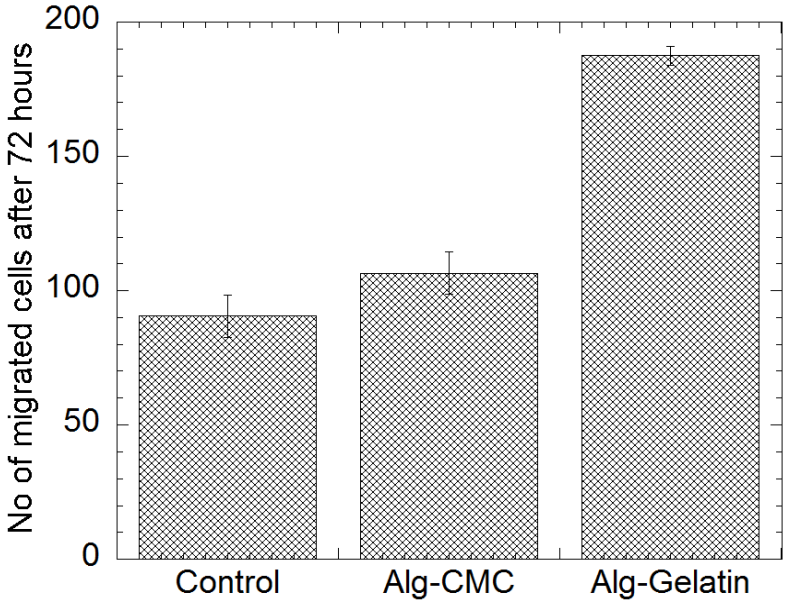
Materials sprayed at high gas flow rate and short distances caused cell detachment and cell death of remaining attached cells

In vitro analysis: 'scratch' assay

- Observations of 'scratch' wound closure





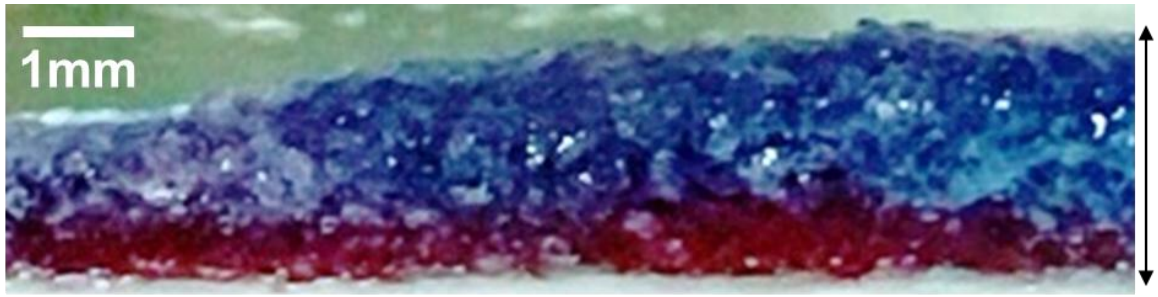


Alg-gelatin promoted horizontal cell migration

Fabrication of multi-layered hydrogels

- Different colored food dyes added to materials before spraying
- Transverse section of gel:

Top layer: Alg-CMC



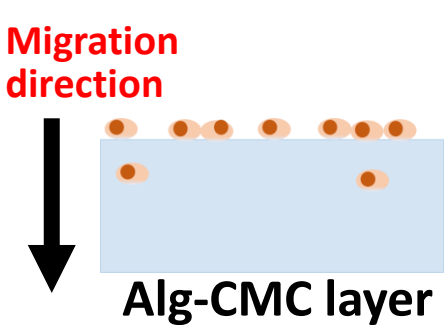
Bottom layer: Alg-gelatin

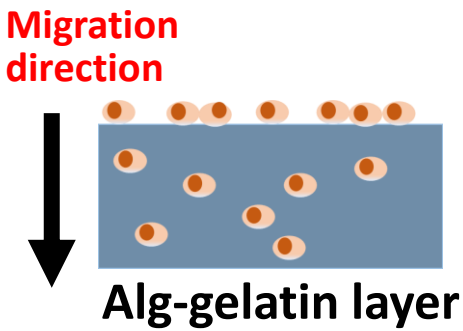
(Spray distance: 10cm
Gas flow rate: 5L/min)

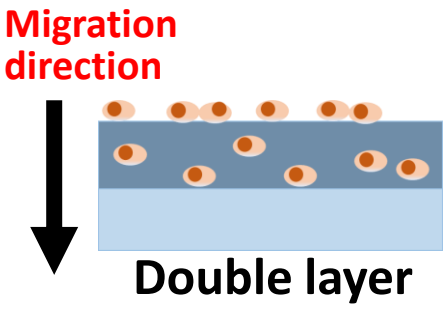
Multi-layered hydrogel was successfully fabricated

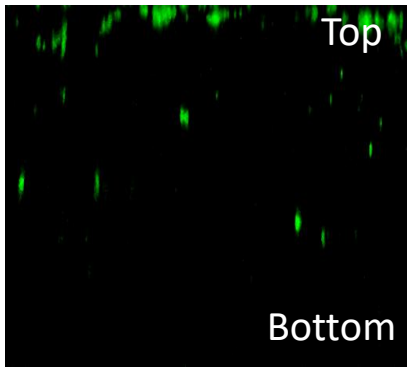
In vitro analysis: cell penetration assay

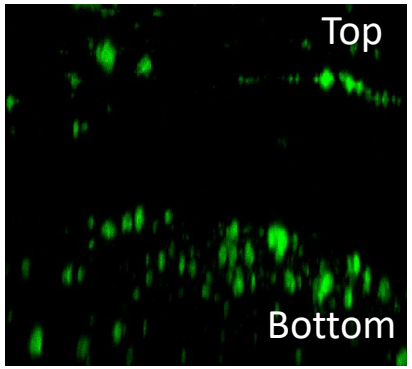
- Transverse gel sections after 24h

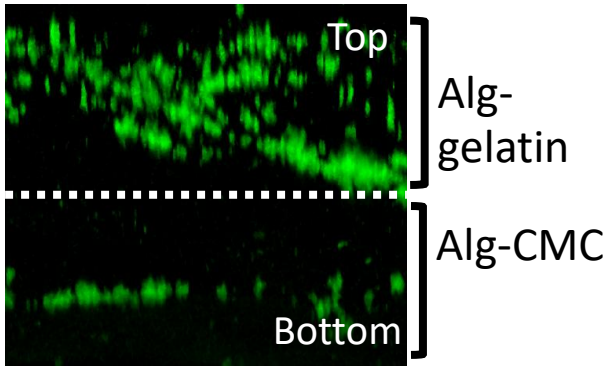




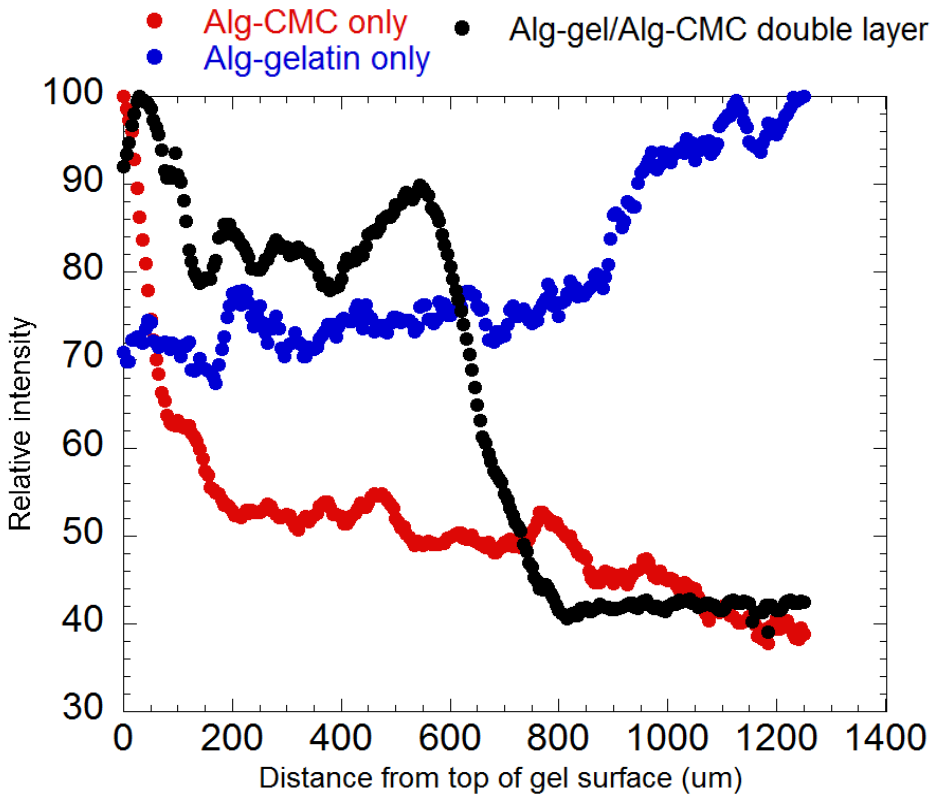








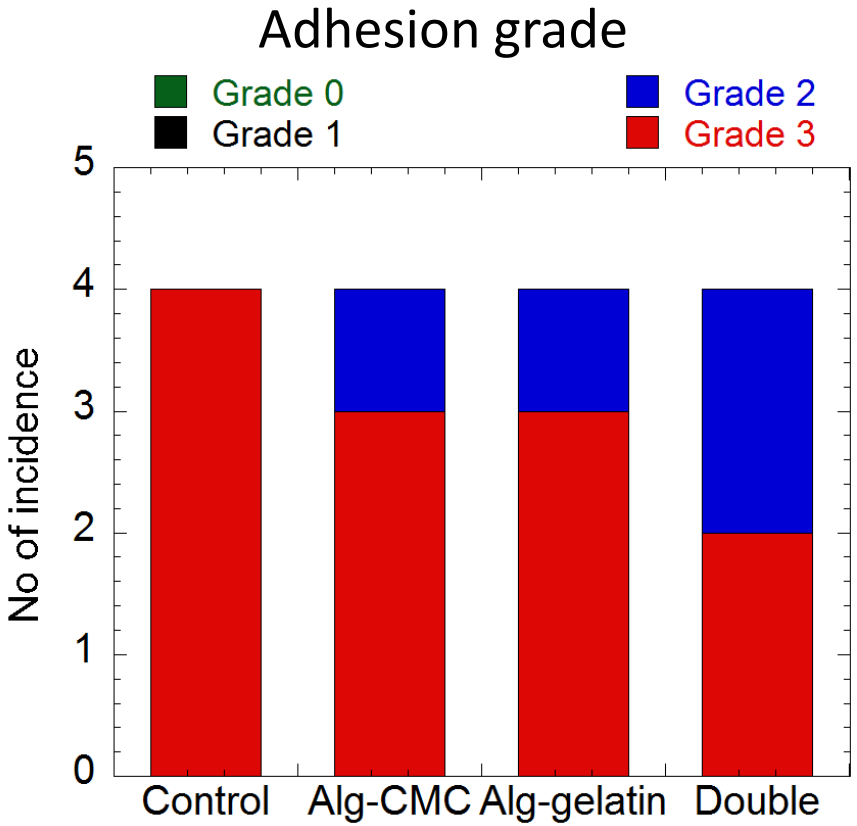
- Fluorescence intensity

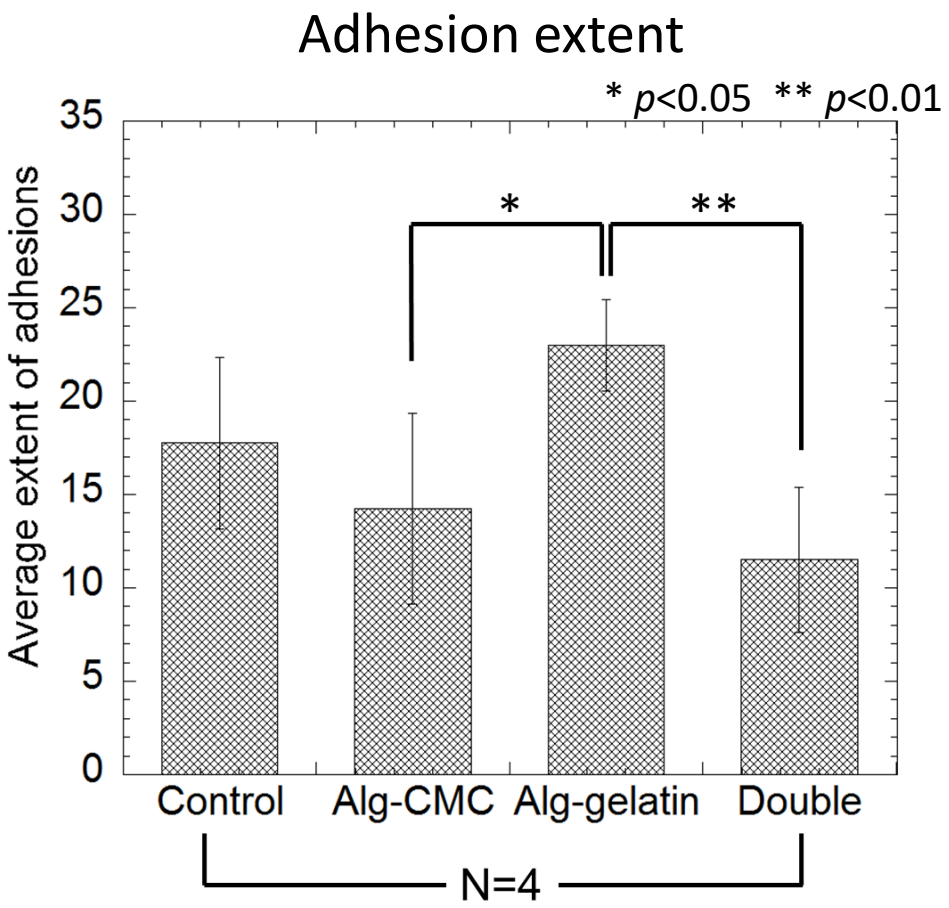


Cells migrated quickly through Alg-gelatin, not Alg-CMC

In vivo analysis

- Cut liver surface-greater omentum (離断面と大網)

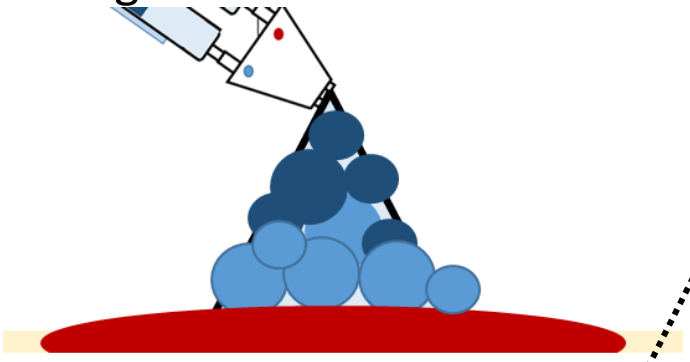




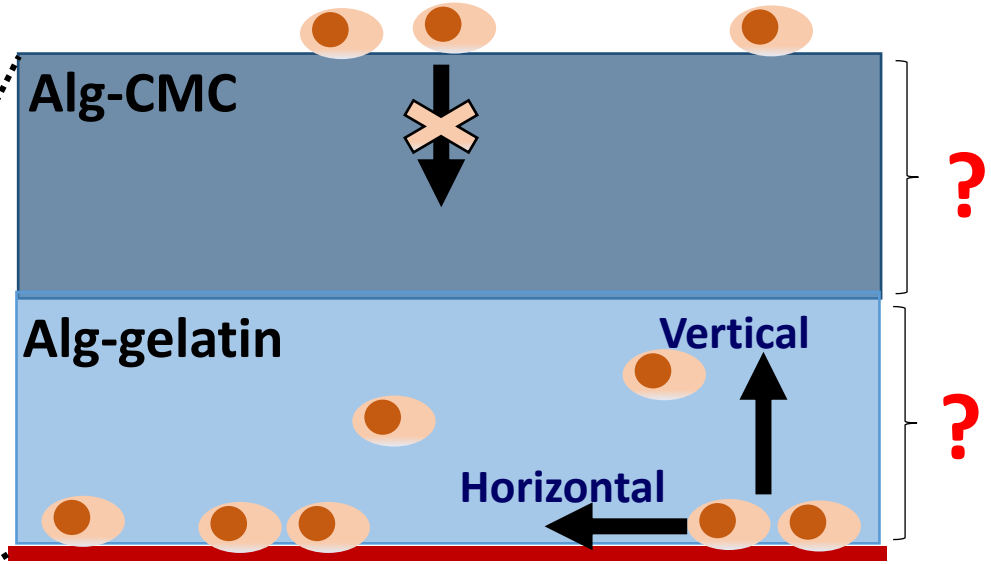
- **Alg-gelatin single layer worsened adhesion extent**
- **Double layer improved adhesion extent**
- **No significant difference between control and double layer**

Discussion

- At LOW gas flow rate:

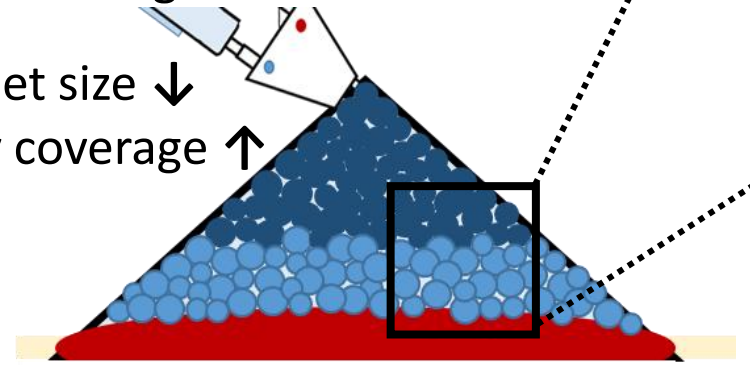


✓ Reduced adhesion formation



- At HIGH gas flow rate:

Droplet size ↓
Spray coverage ↑



✓ Promoted wound healing

X Worsened adhesion formation

Mixing efficiency Homogeneity ⟷ **Trade-off** ⟷ **Cellular damage**

Precise control of thickness of each layer is needed

Conclusions

- Spray distribution and droplet sizes were measured
- Impact of spray conditions on cell viability was evaluated
- The wound healing property, and the anti-adhesive property of the respective hydrogel layers were shown
- Multi-layered hydrogel was successfully fabricated
- Efficacy of the multi-layered hydrogel delivered by spraying was evaluated using the rat hepatectomy model

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