TWIns
Tokyo Women's Medical University – Waseda University
Joint Institution for Advanced Biomedical Sciences

Fabrication and properties of ultra-thin films (nanosheets) as innovative biomaterials

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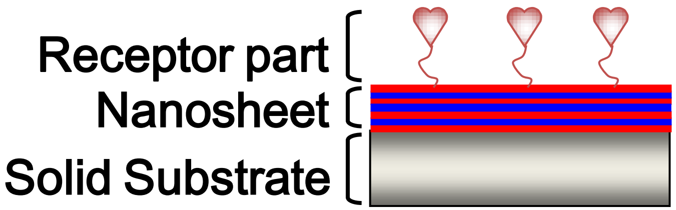
polymer chemistry, biomaterials, DDS, artificial red blood cells, artificial platelets, molecular assembling science and technology

Research background

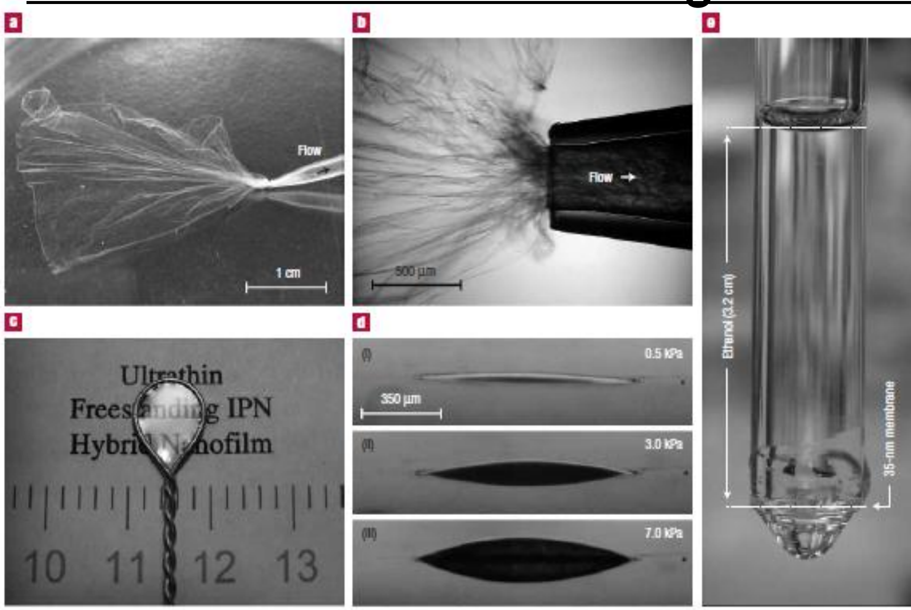
Researches on nanosheets fabricated in a bottom-up way from a solid substrate



Electric devices, Biosensors, Scaffolds for tissue engineering

(Platforms for surface modification)

Researches on free-standing nanosheets



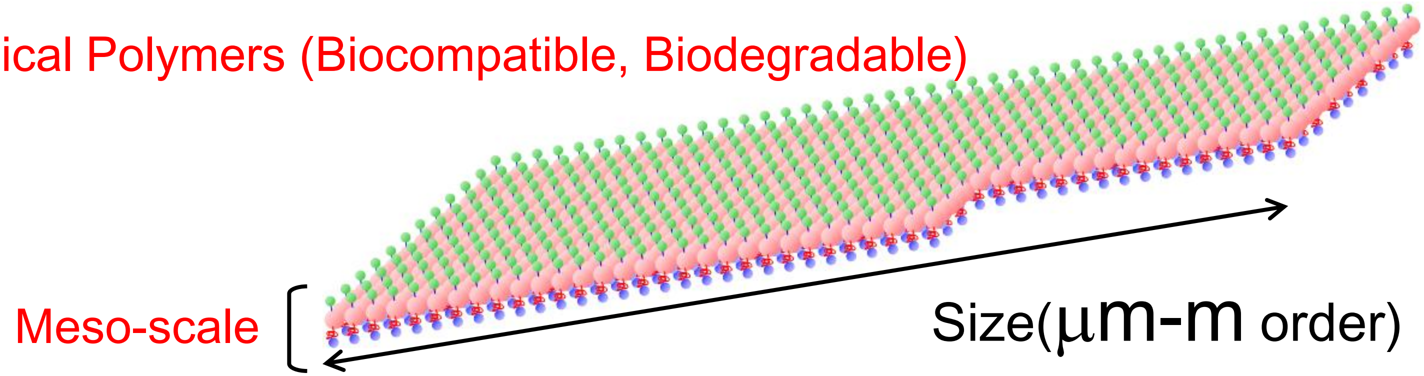
Kunitake *et. al.*, *Nature Mater*. **2006**, *5*, 494.

Development of novel nanomaterials

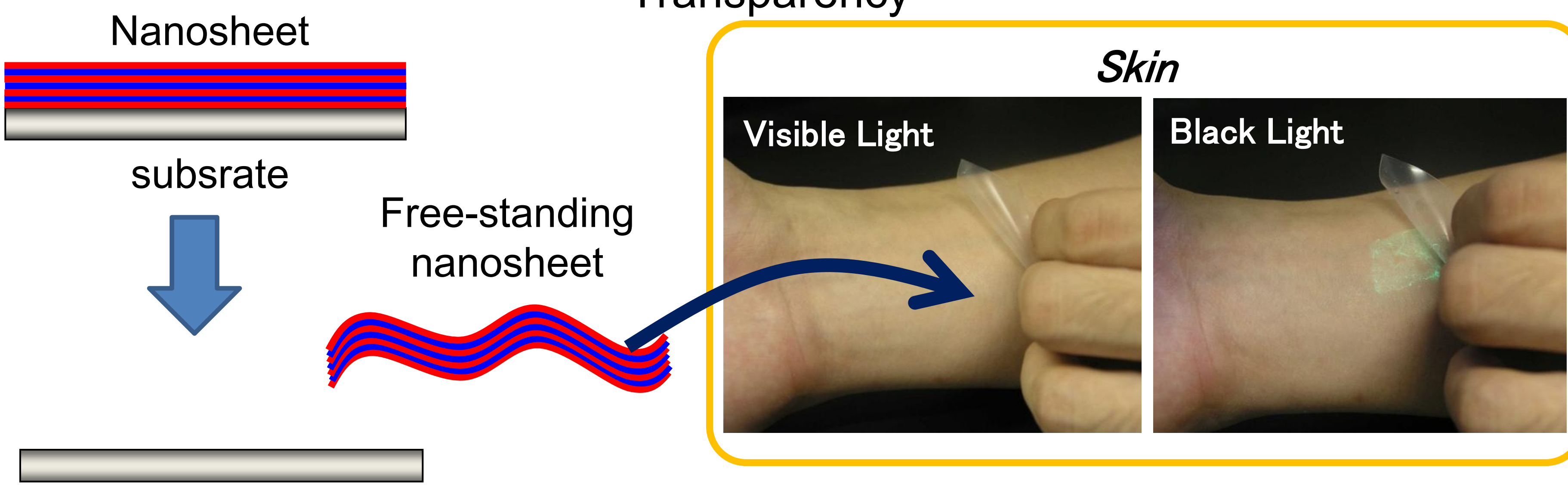
(Well-designed synthetic polymers and polymer network)

Free-standing nanosheets for biomedical applications

Medical Polymers (Biocompatible, Biodegradable)

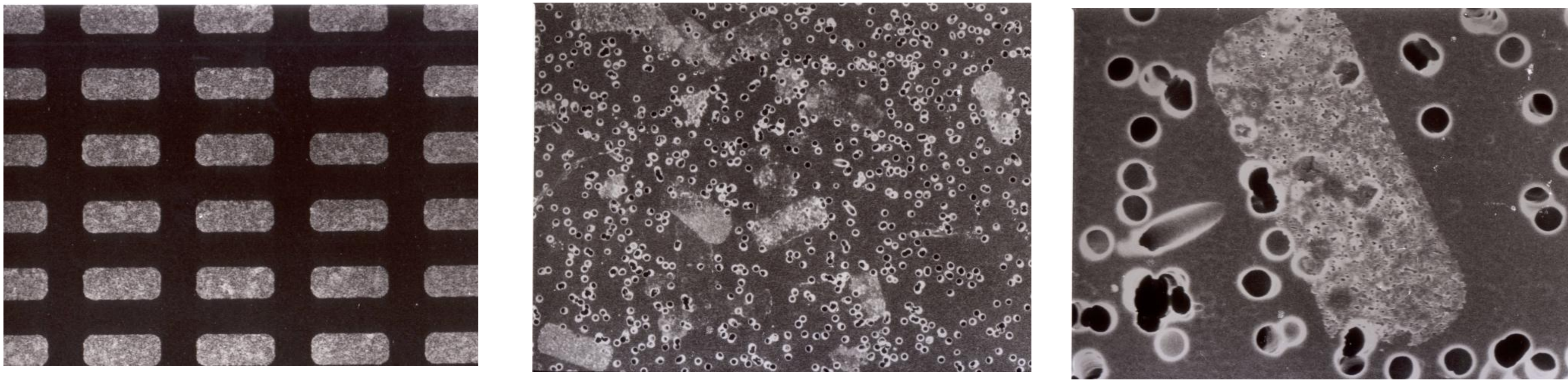


Huge aspect ratio (>10^6) · Small amount · High flexibility · Large contact area · Non-covalent adhesion · Heteromodification · Transparency



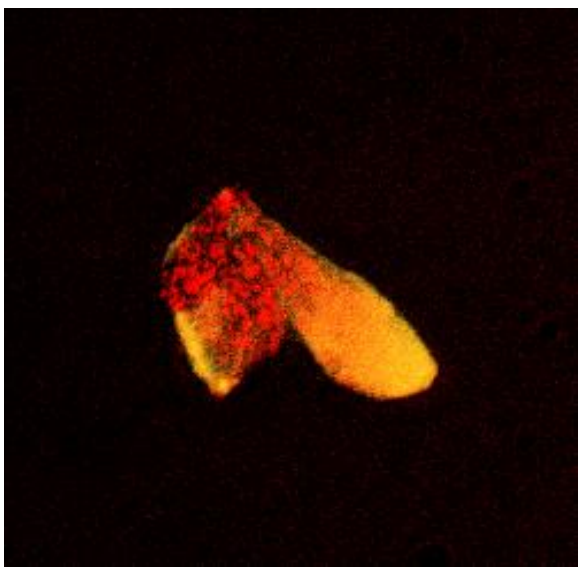
Adv. Mater. 2007, 19, 3549.

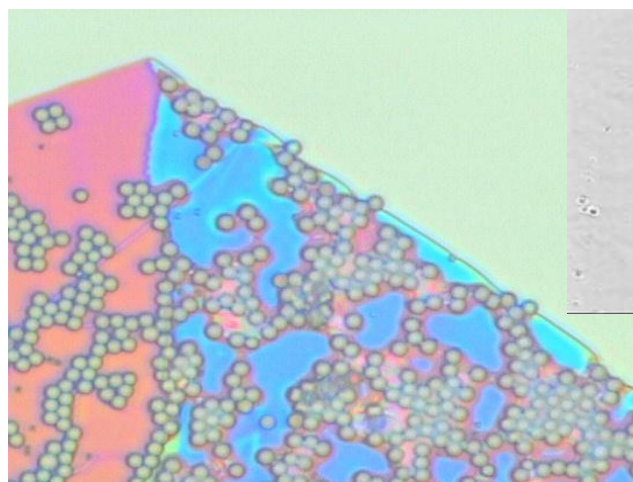
Development of various kinds of nanosheets

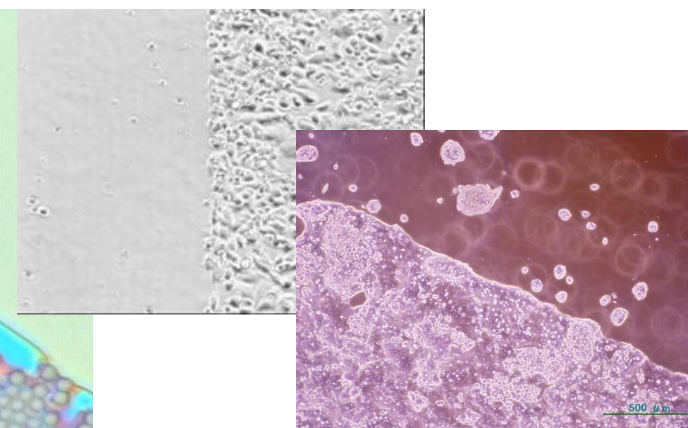


Micron-sized nanosheets with microtechnology and nanobeads technology

Colloid Surf. A-Physicochem. Eng. Asp., **318**, 184-190 (2008).







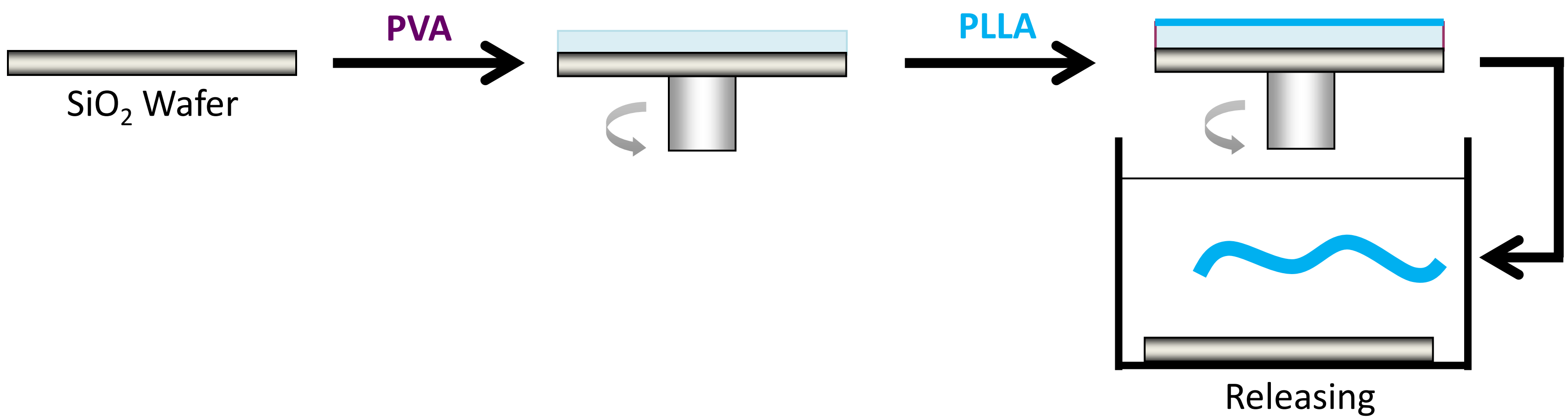
Cytophobic, cytophilic

Albumin nanosheet with heterosurfaces, huge nanosheets with microbeads

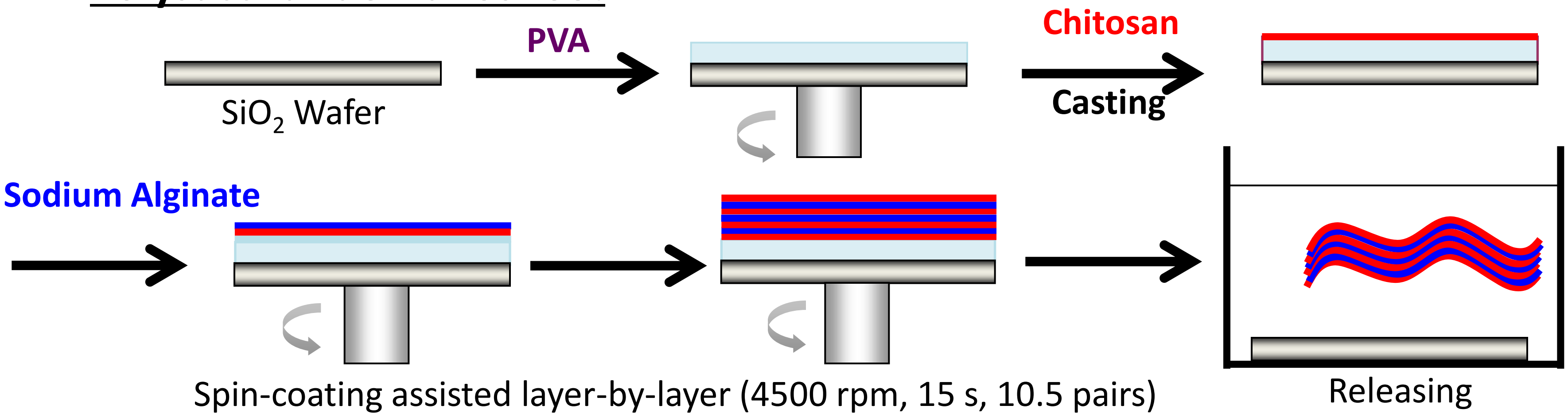
J. Biomed. Mater. Res. Part A, **89A**, 233–241 (2009).. *Coll. Surf. A-Phys. Eng. Aspects*, **334**, 28–33 (2009).

Preparation of the free-standing PLLA and polysaccharide nanosheets

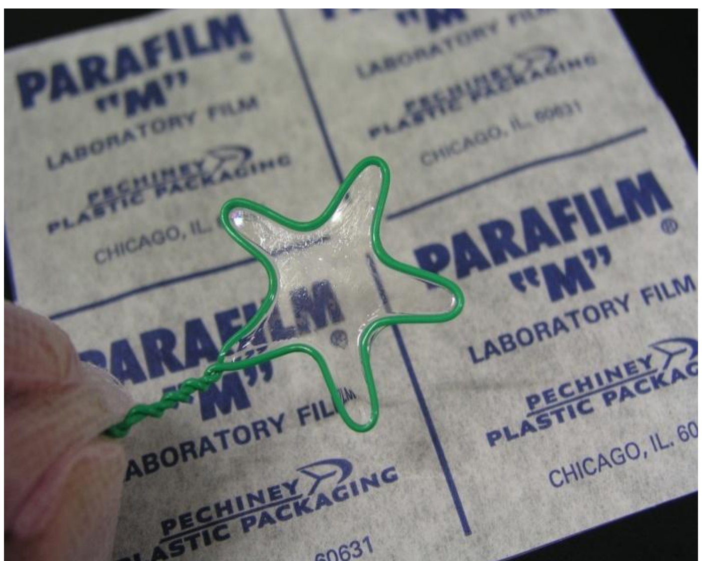
PLLA Nanosheet

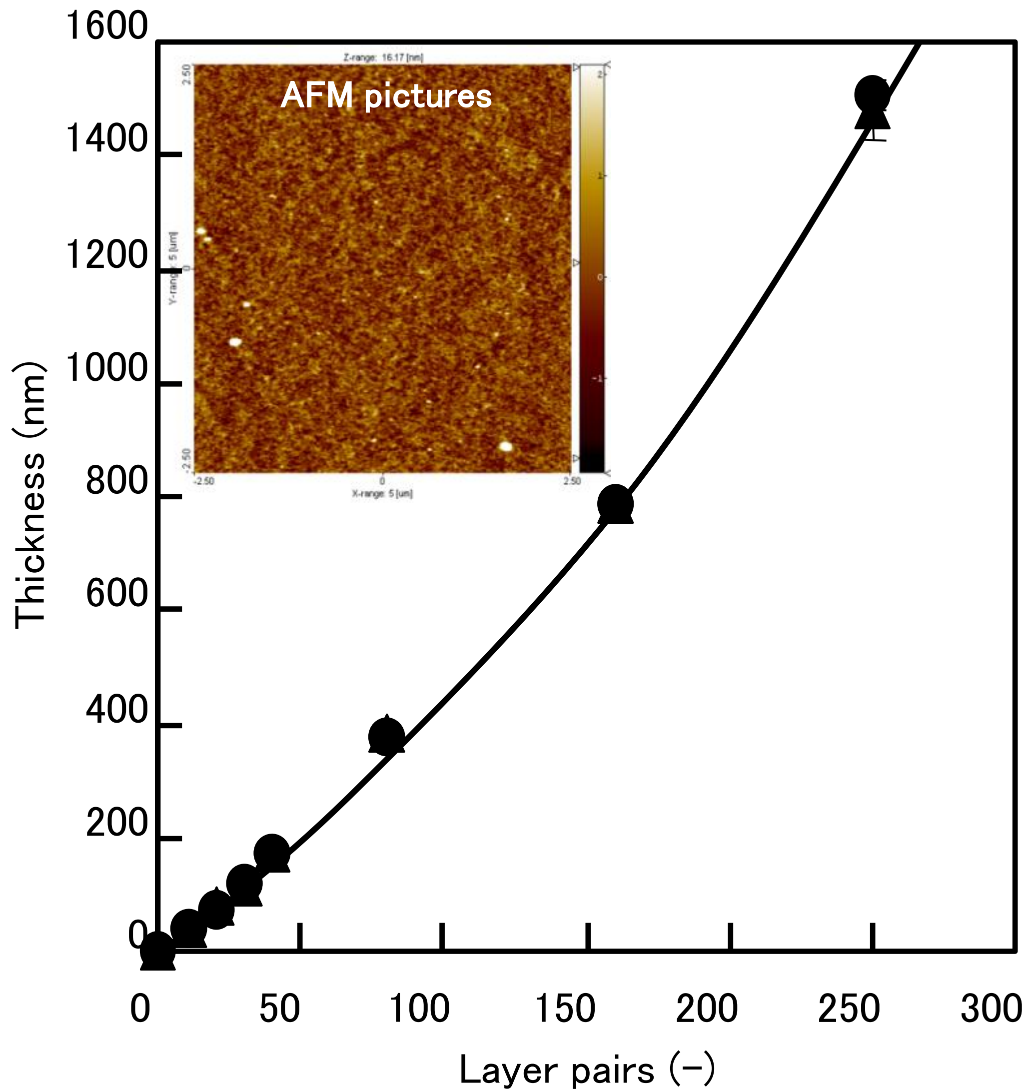


Polysaccharide Nanosheet



Control of the thickness of the polysaccharide nanosheet by LbL and scooped states

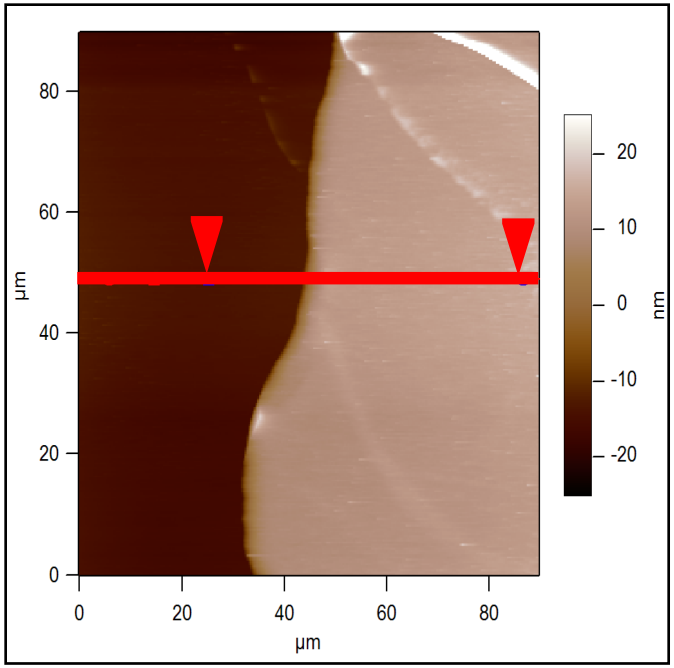


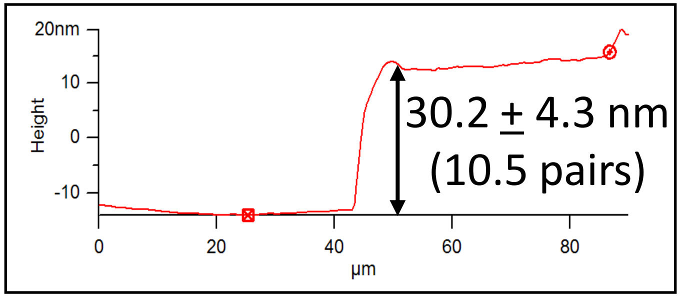


High transparency and smoothness

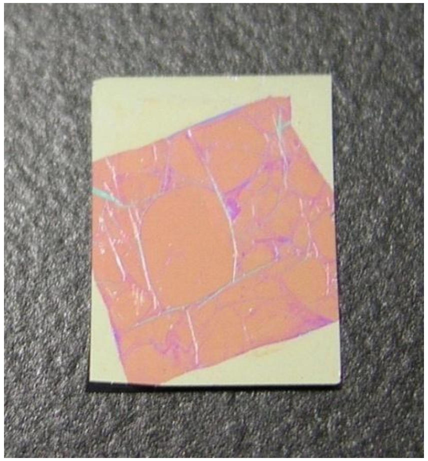
Polysaccharide nanosheets showing structural colors

Surface morphology scanned by AFM





Structural color change on SiO_2 substrate



Single : orange

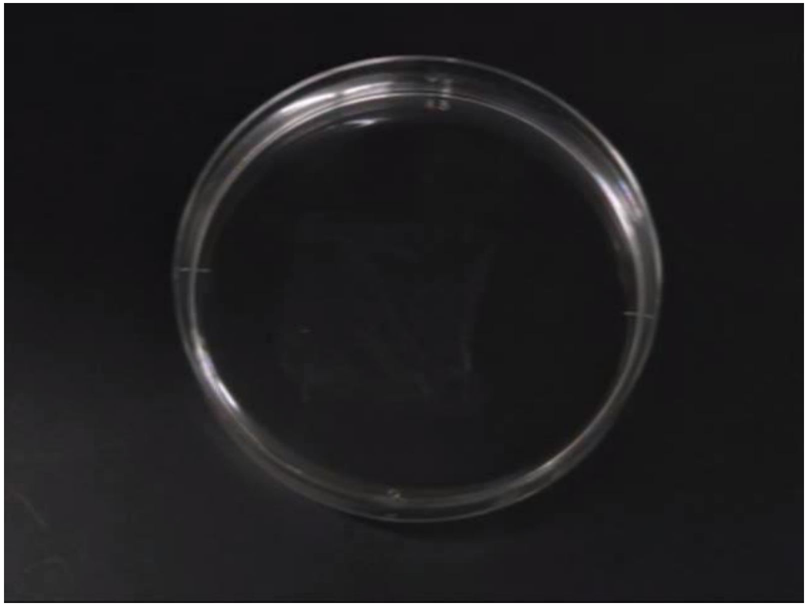
Double : blue

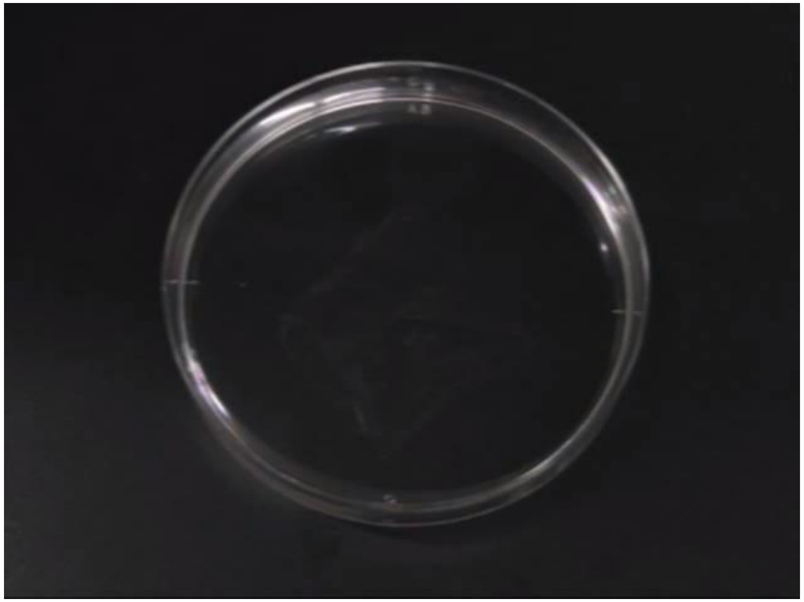
Triple : green

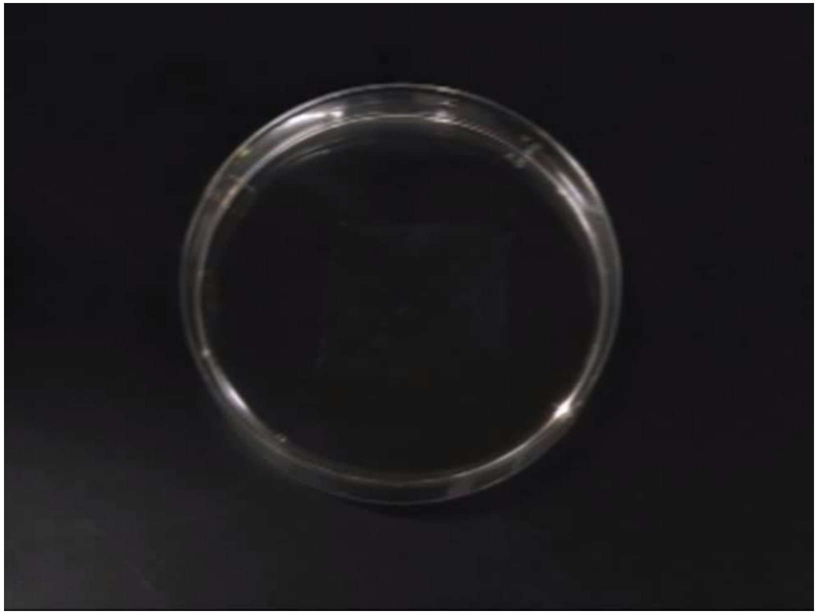
Thin film interference theory

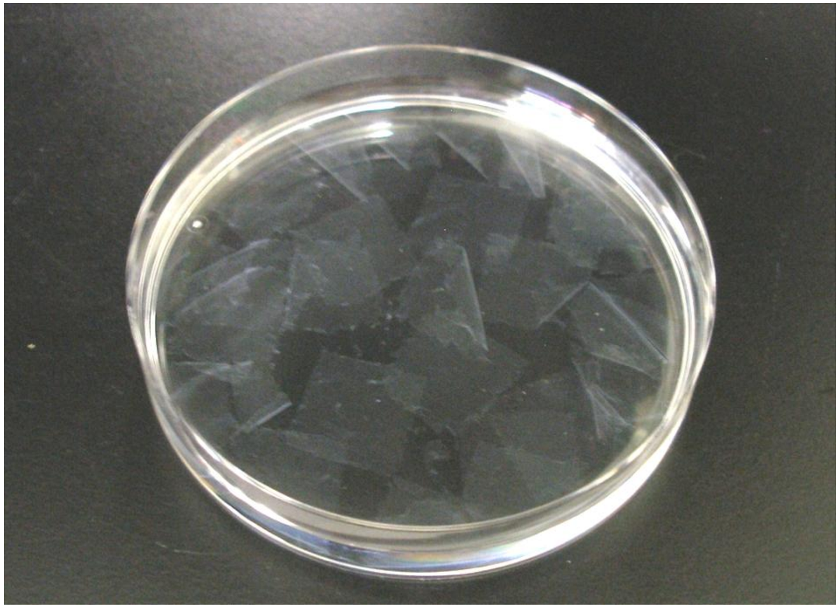
Colloids and Surface A: Physicochemical and Engineering Aspects, 334, 28-33 (2009).

Free-standing PLLA nanosheets (4 x 4 cm)



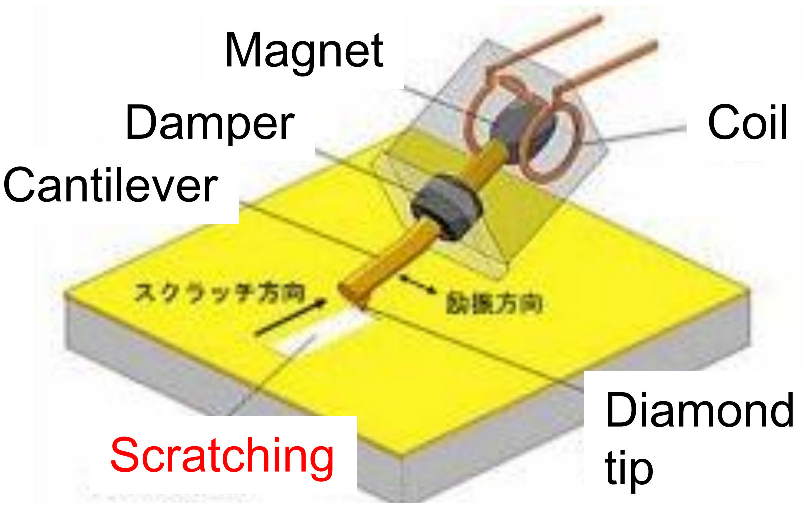






Evaluation of adhesive property of nanosheets

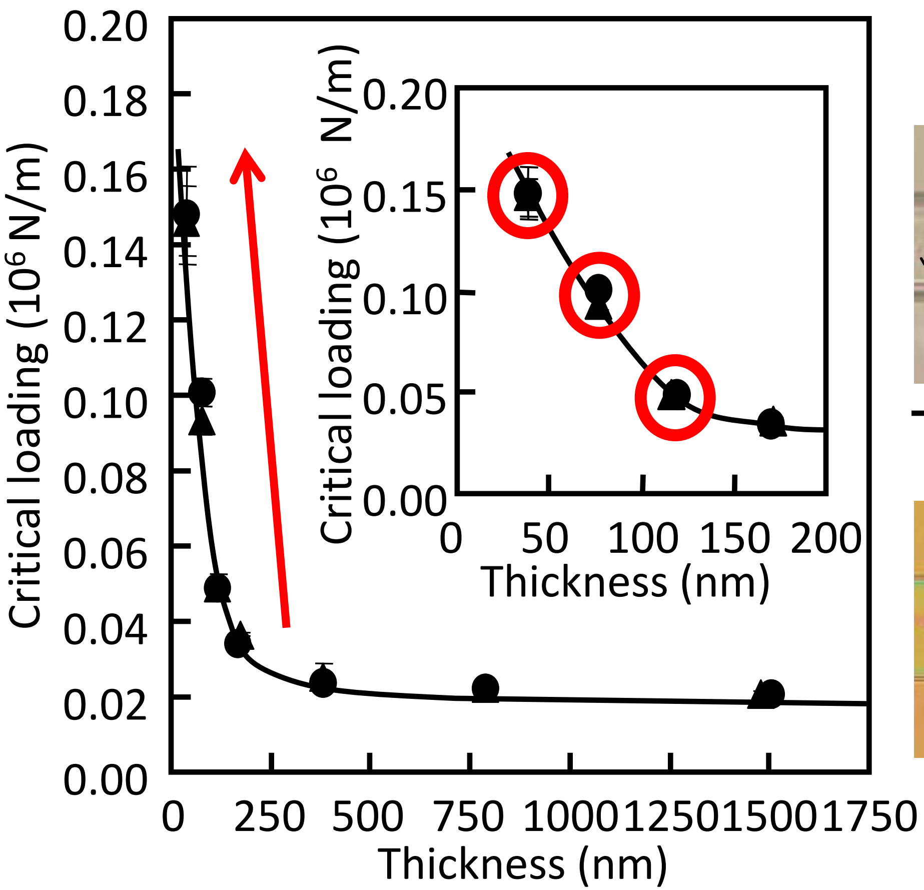
Scratch tester for thin films(Rhesca Co. Ltd.)

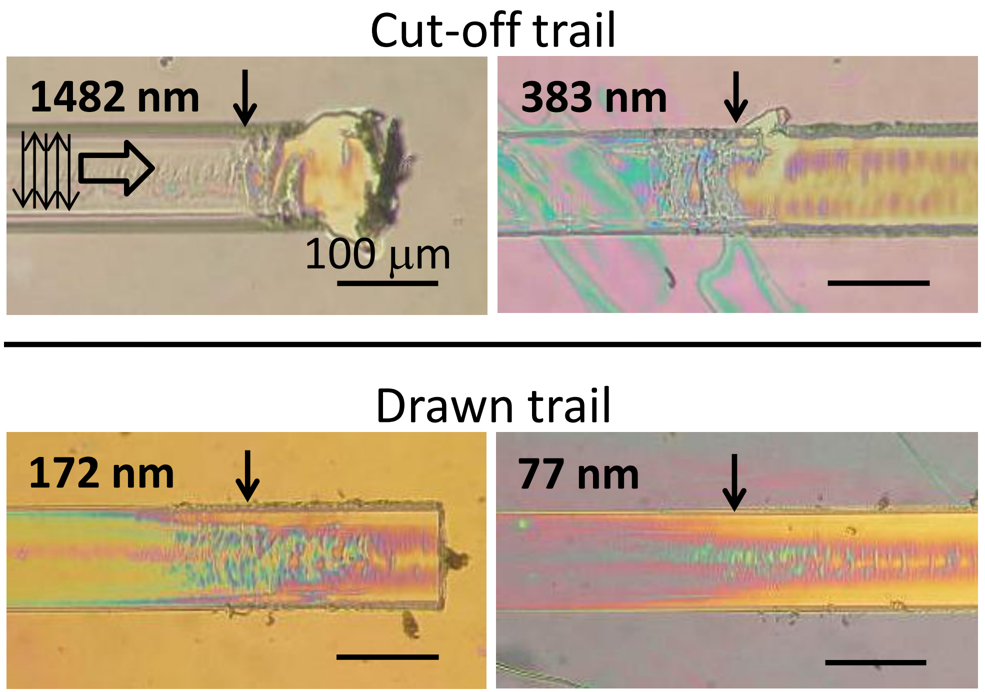


Microscratch detection part

Thin film on a substrate is scanned with a diamond tip by applying the increasing pressure. The signal of frictional vibration just after breaking of the nanosheet was detected as “critical loading”.

Adhesion strength of the polysaccharide nanosheet



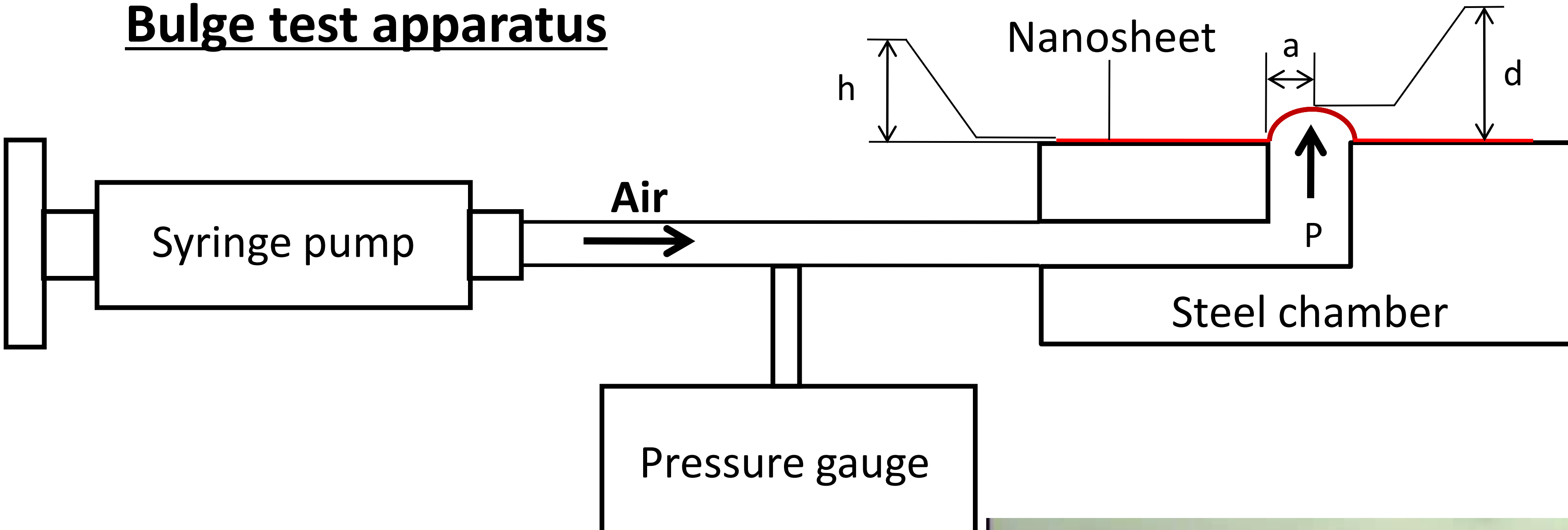


Adhesion strength of the nanosheet dramatically increases when the thickness becomes less than 200 nm with change of the trail.
This would be due to the lowering of the glass transition temperature.

Surgery, *148*, 48-58 (2010).

Evaluation of the mechanical strength of nanosheet

Bulge test apparatus



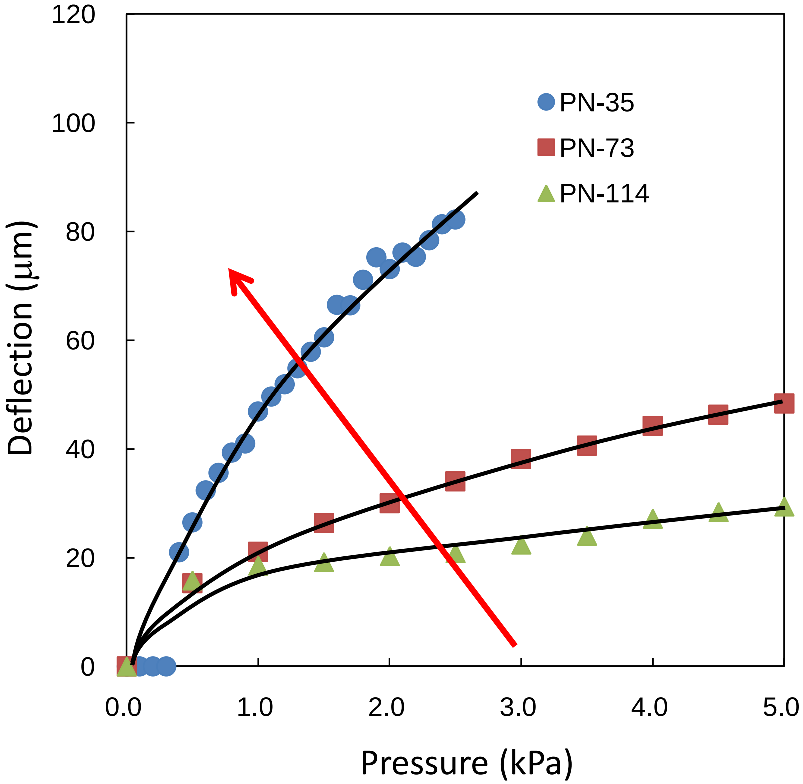
Strain: $s = (P \times a^2)/(4 \times h \times d)$

Stress: $e = (2 \times d^2)/(3 \times a^2)$

Elastic modulus: $E = s / e$

Tsukruk *et. al.*, *Adv. Funct. Mater.* 2005.

Pressure-Deflection and Stress-Strain curves (ϕ: 1 mm) of polysaccharide nanosheets



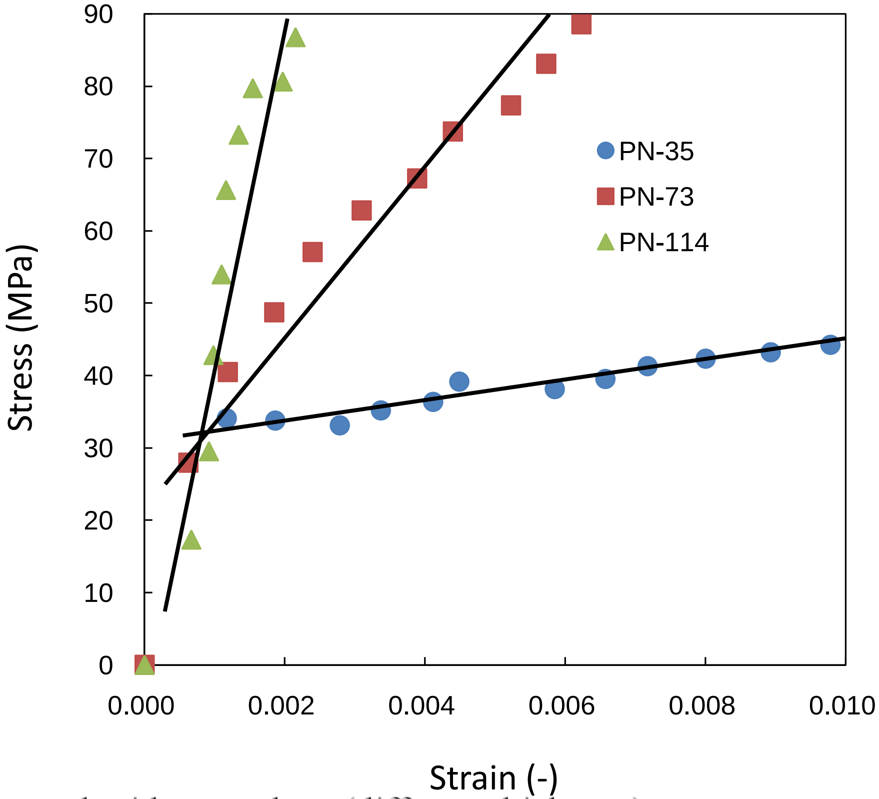
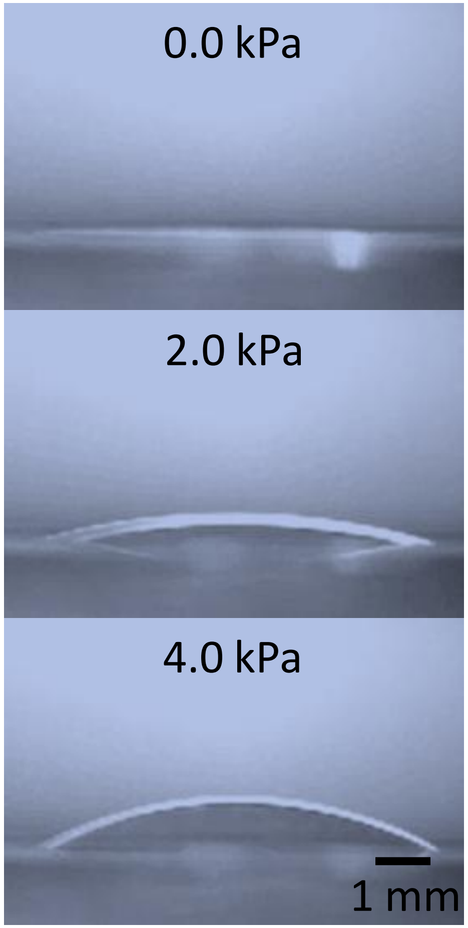


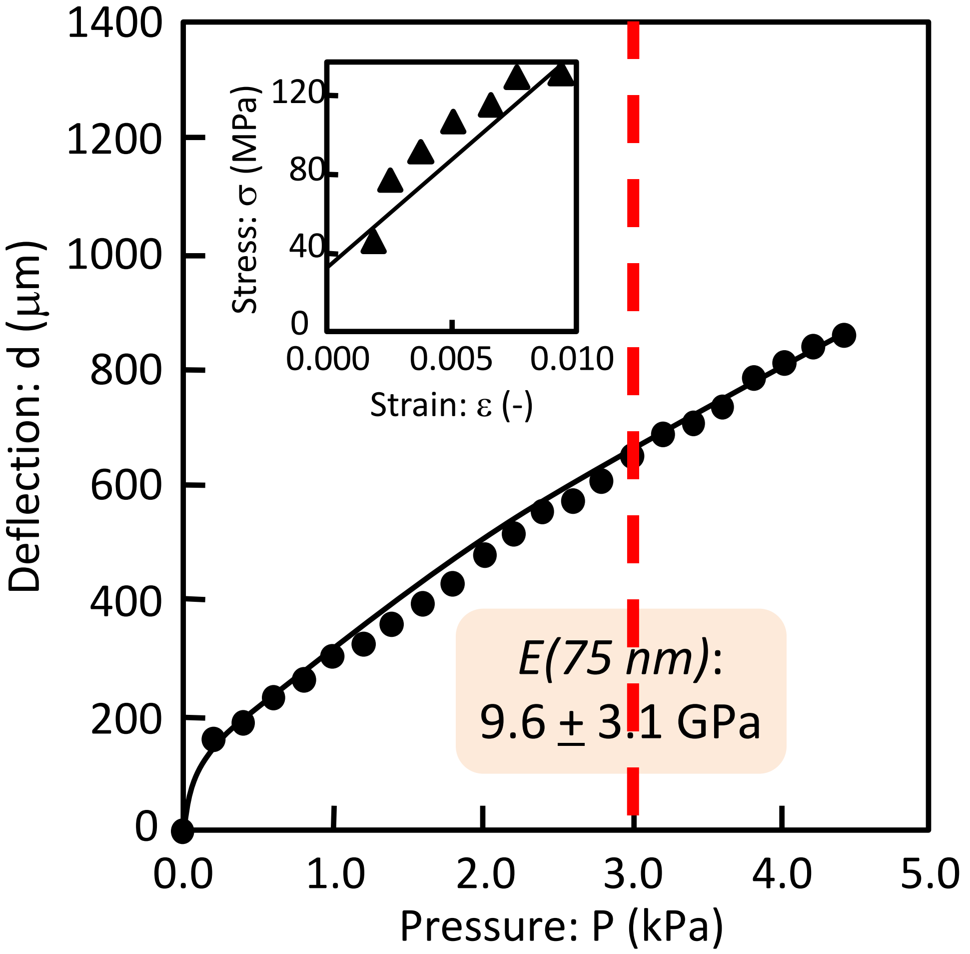
Table 1 Mechanical property of polysaccharide nanosheet (different thickness)

hole diameter (mm)	Thickness (nm)	ultimate tensile strength: σ (MPa)	ultimate tensile elongation: ε (%)	elastic modulus: *E* (GPa)
1	35	51	3.2	1.1 ± 0.4
1	75	123	6.0	8.1 ± 2.5
1	114	110	3.3	32.6 ± 18.9

Adv. Func. Mater., 19, 2560-2568 (2009).

Pressure-Deflection curve (ϕ: 6mm) of the nanosheets

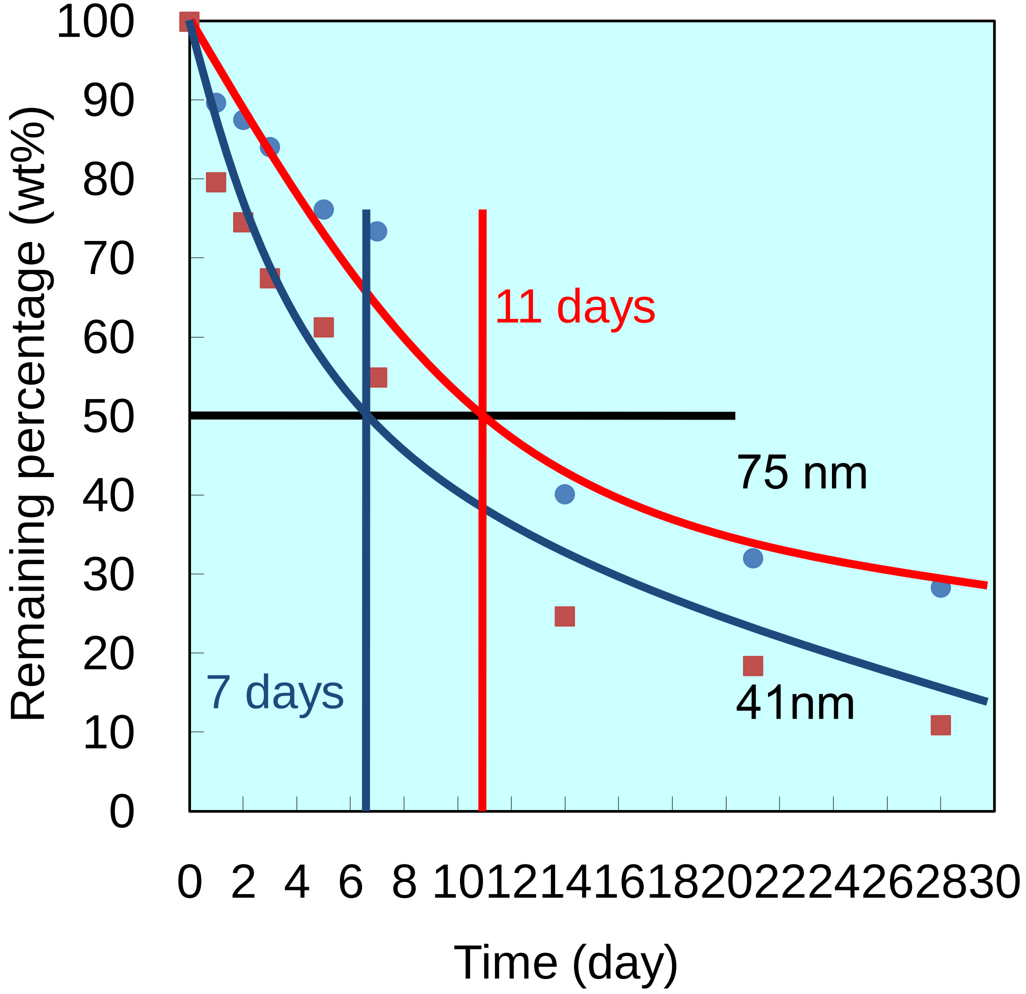




Red line indicates that the nanosheet is well tolerated at the air pressure of 3kPa (30 cmH_2O), corresponding to the normal respiratory pressure.

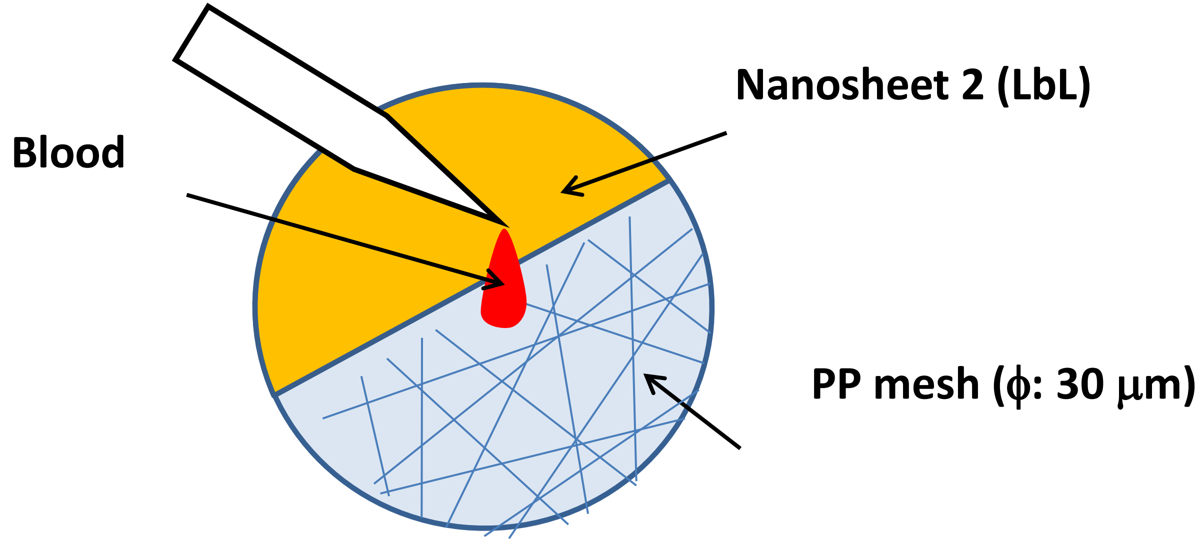
Degradation of the polysaccharide nanosheets with different thicknesses

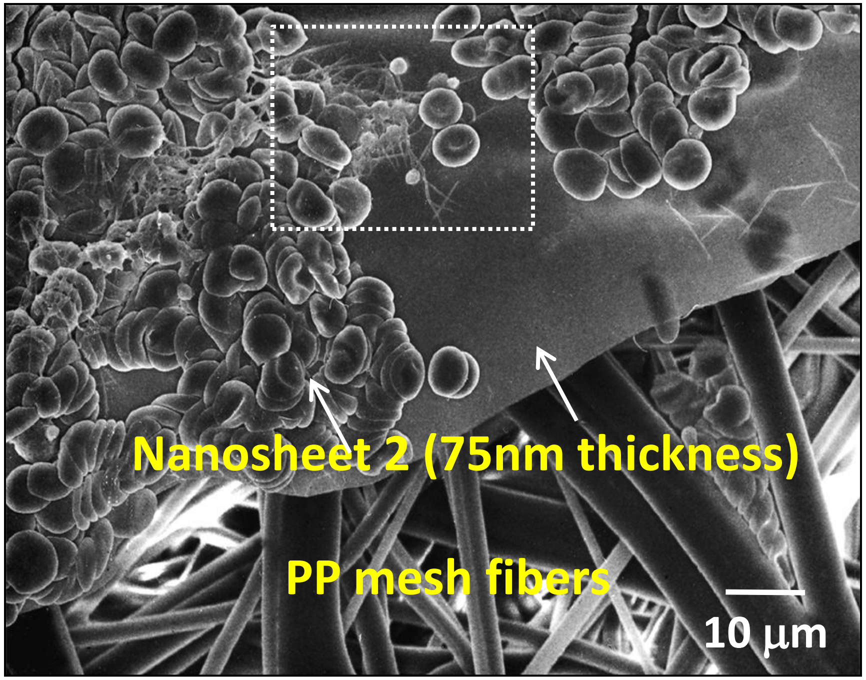
PBS, 37°C

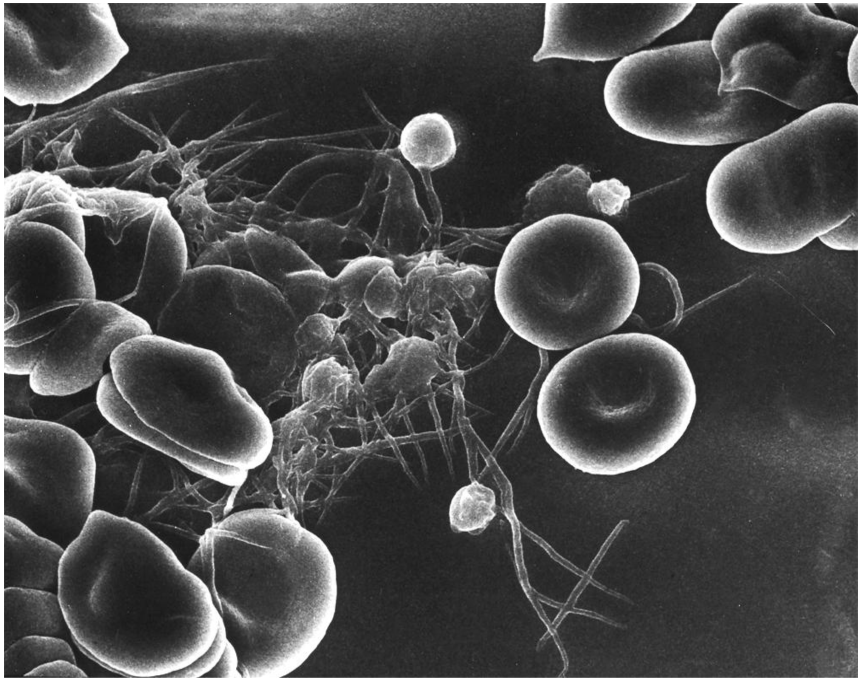


$T_{1/2}$ (41 nm): 7 days, $T_{1/2}$ (75 nm): 11 days

Blood compatibility test

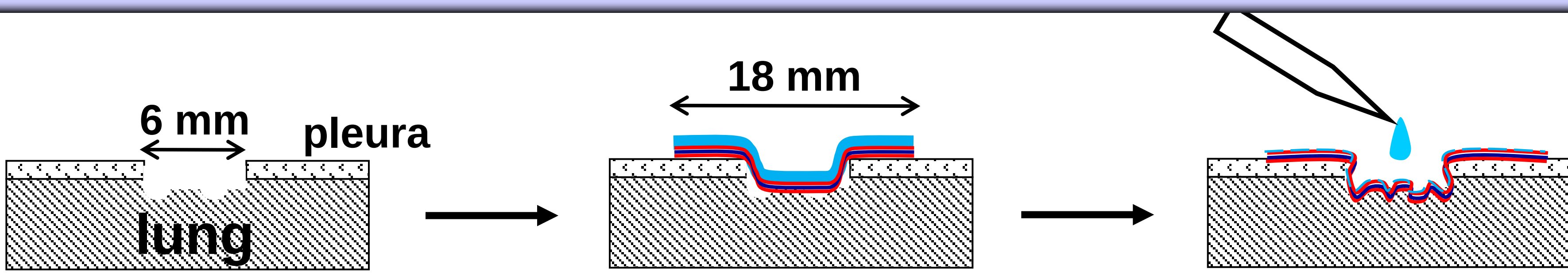




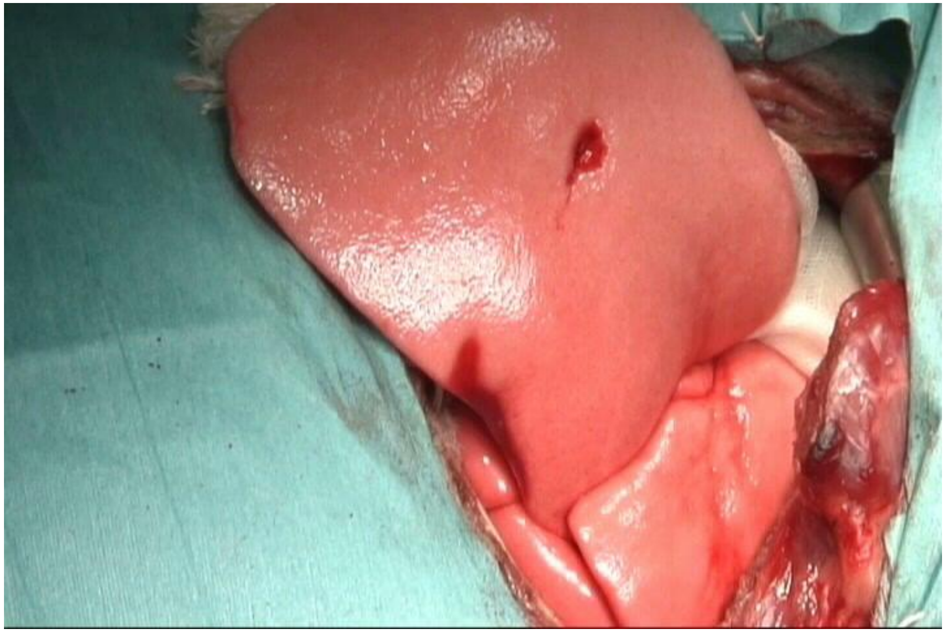


Adv. Func. Mater., 19, 2560-2568 (2009).

Sealing effect of the polysaccharide nanosheet in a lung perforation model (canine)



Tissue-defect by punch → Sealed with the (nanosheet + PVA) film → Nanosheet adhered after dissolution of PVA



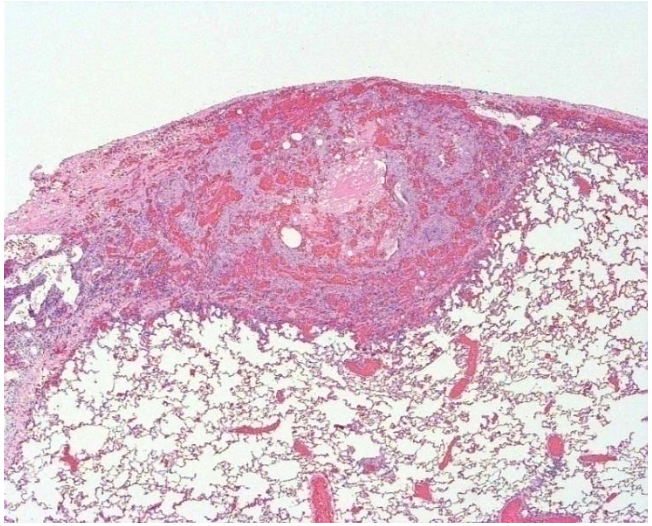
Operation for a lung-defected beagle

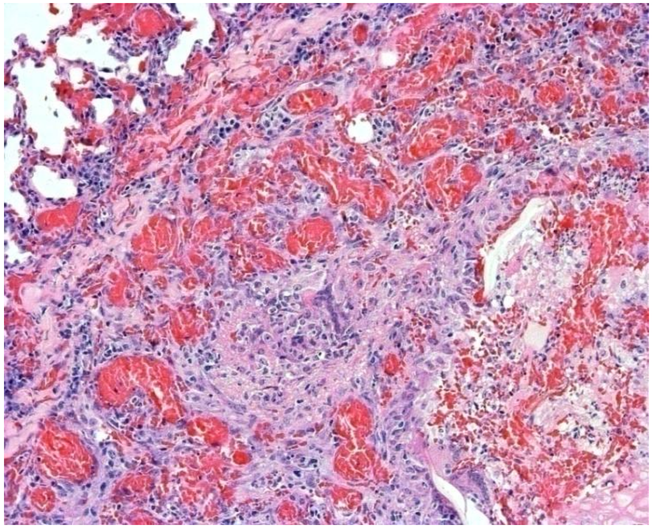
after operation

Adv. Func. Mater., 19, 2560-2568 (2009).

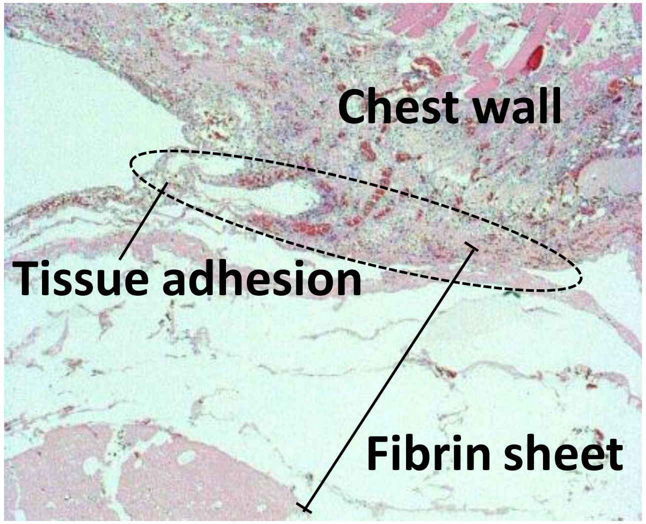
Histological sections of perforated lesion (after 1 week)

Nanosheet (Growth of fibroblast)



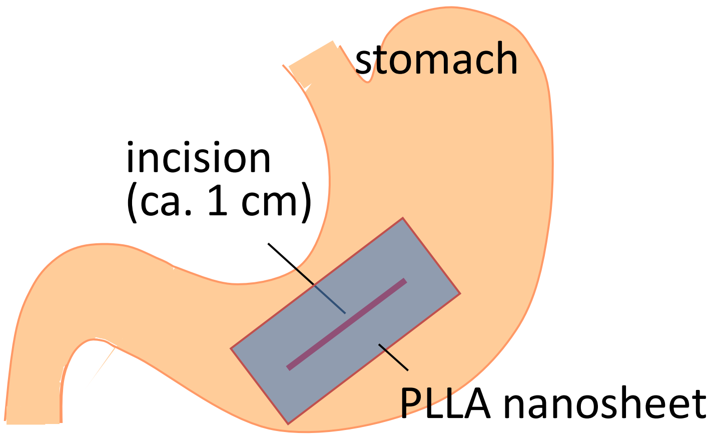


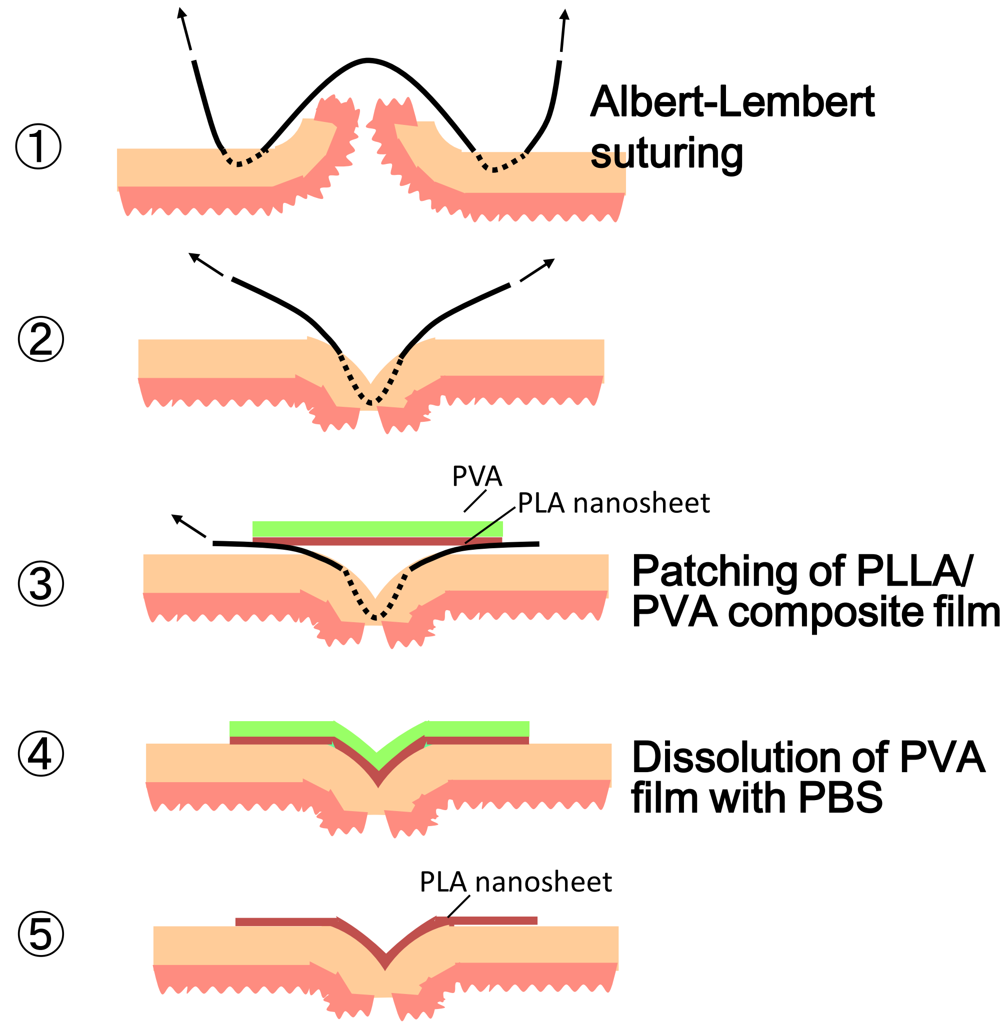
Fibrin sheet (post-surgical adhesion)



Fibrin sheet is used as a conventional treatment of pleural injury/ defect
But causes pleural adhesion.

Evaluation of PLLA nanosheet patching to the incision site of mouse stomach

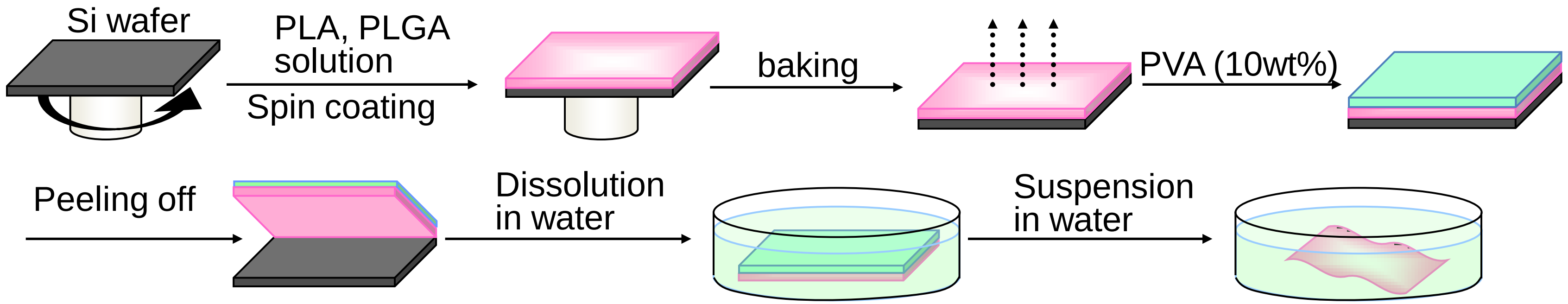


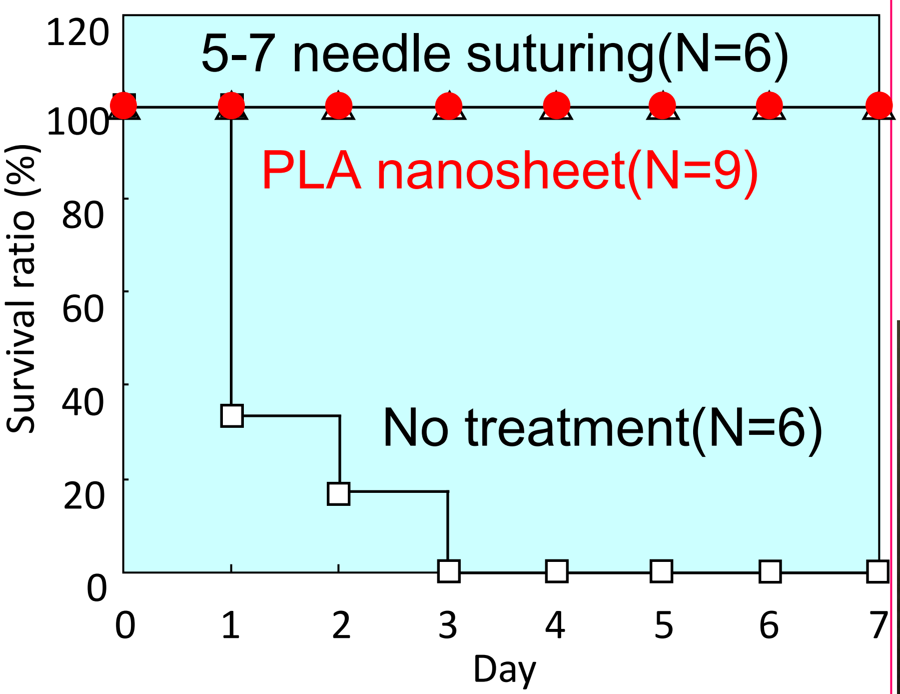


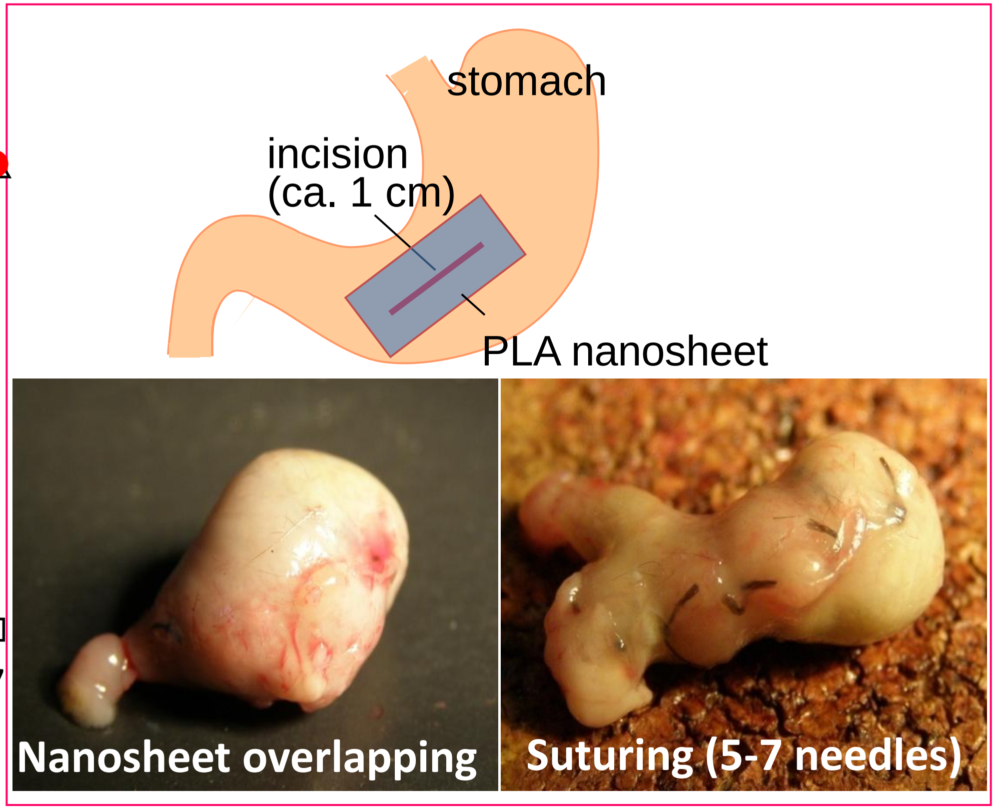
Positive control: 5-6 needle suturing
Negative control: no suturing

Adv. Mater., 21, 4388-4392 (2009).

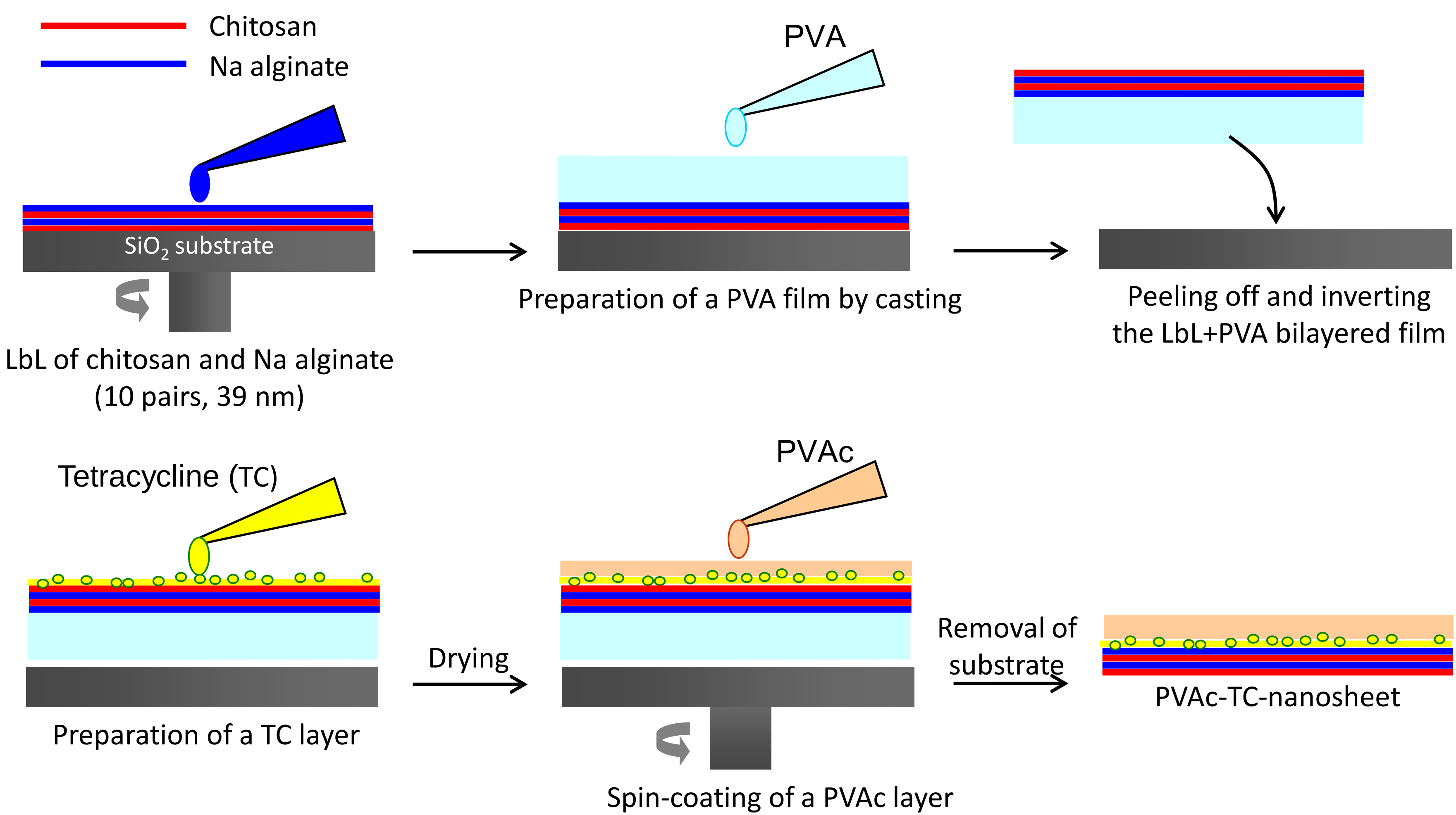
Overlapping of PLLA nanosheet on an incision of stomach and repair (mouse)







Antibiotic-loaded nanosheets for the treatment of gastrointestinal tissue defects



Biomaterials, 31, 6269-6278(2010).

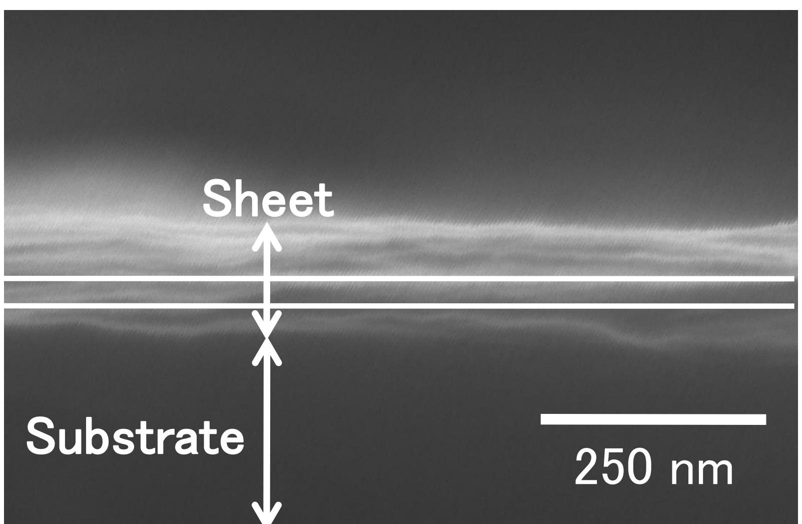
Structure and function of antibiotic-loaded nanosheets

Entry	Thickness (nm)	TC (mg/cm^2)	ZOI (mm)
PVAc–TC–nanosheet	177±9	6.2±0.5	7.0±1.7
TC–nanosheet	69 ±6	5.6±0.3	7.0±1.6
PVAc–nanosheet	142 ±4	0	0

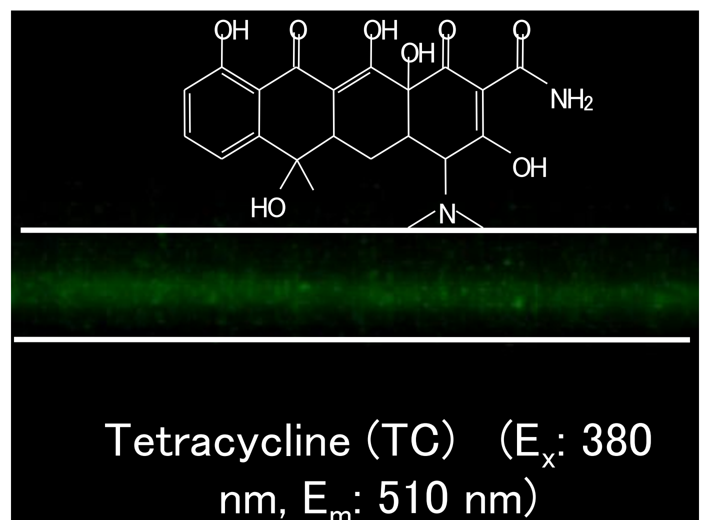
ZOI: zone of inhibition

SEM

TC

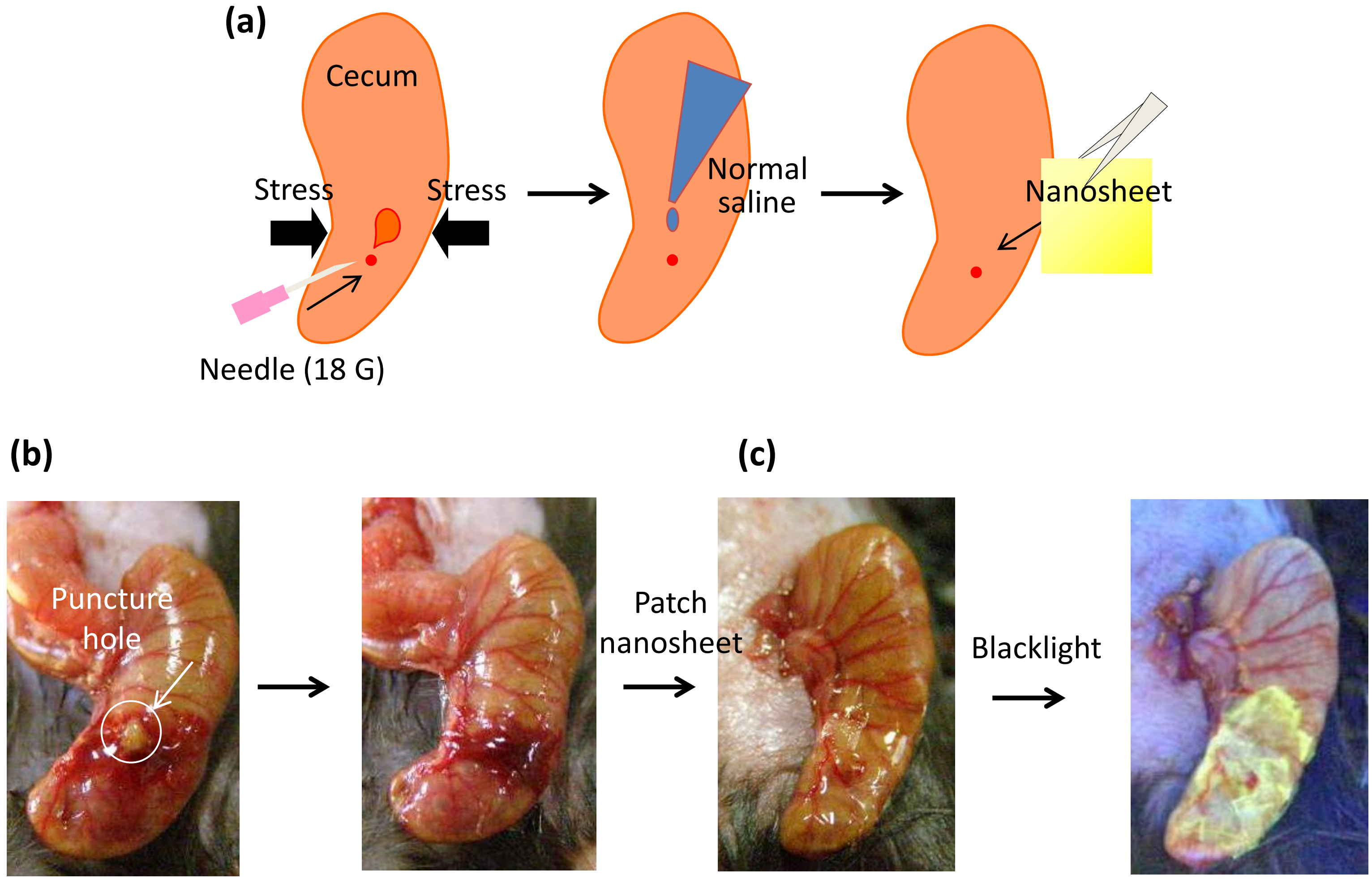


Confocul microscopy



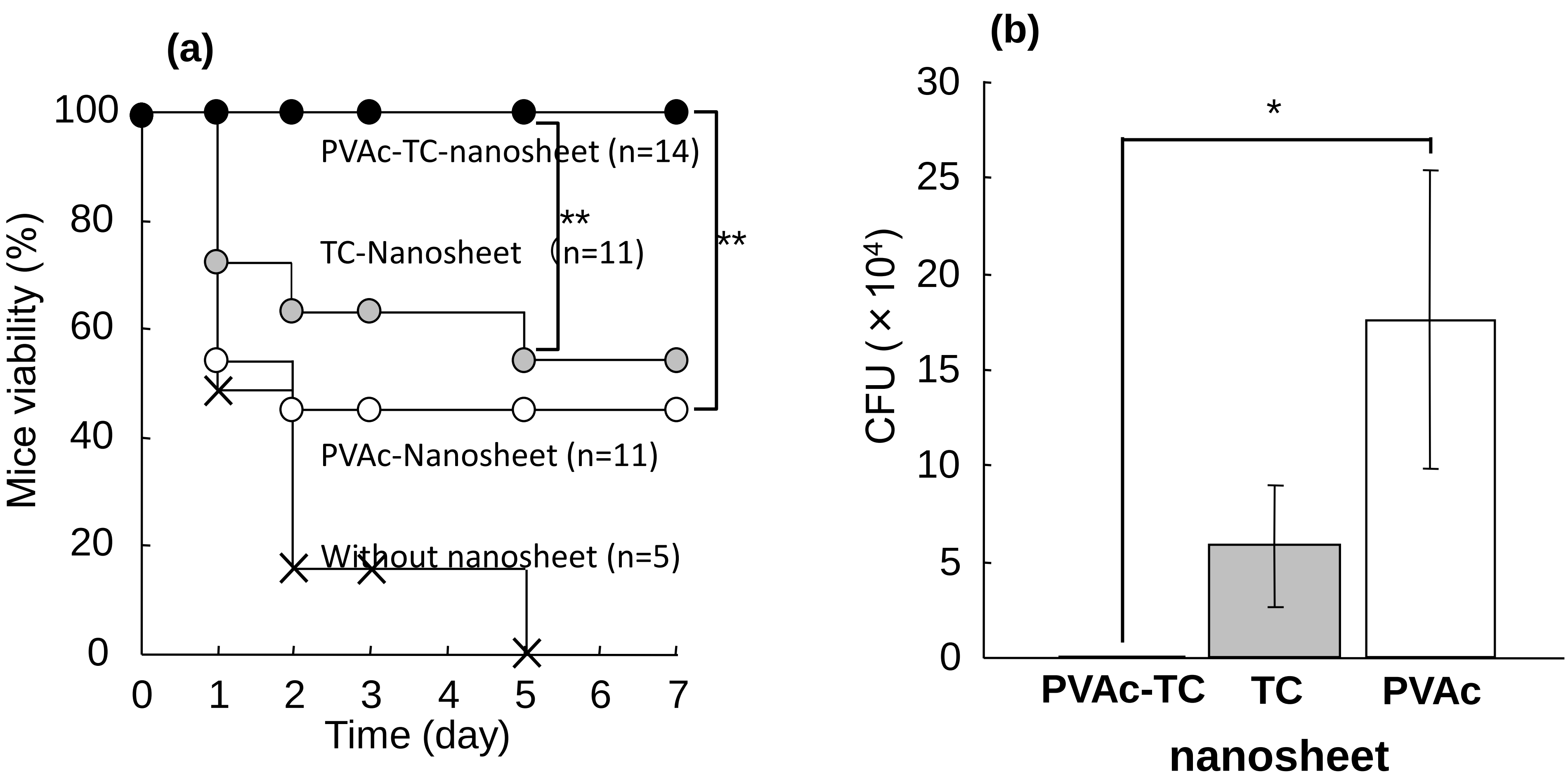
PVAc:100nm
TC:30 nm
NS:40 nm

Murine cecal puncture model :
(a) schematic representation
(b) macroscopic images
(c) location of TC under black light

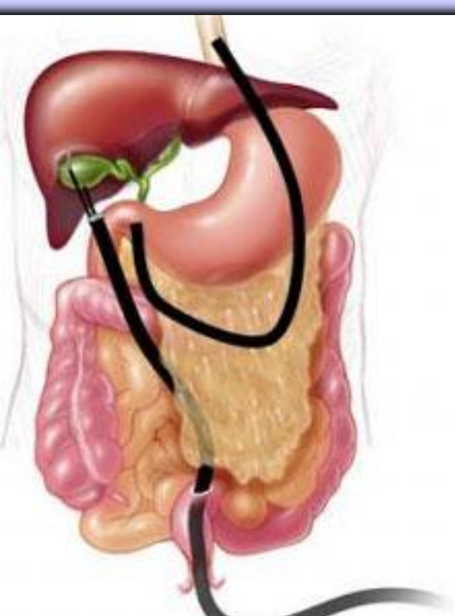


Biomaterials, *31*, 6269-6278 (2010).

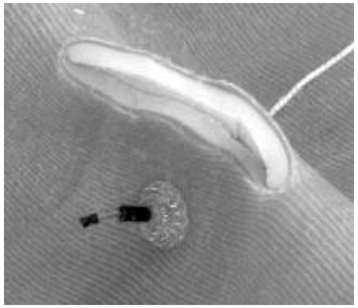
Effects of nanosheets until 7 days after operation, (a) murine survival, (b) the number of bacteria in the intraperitoneal lavage

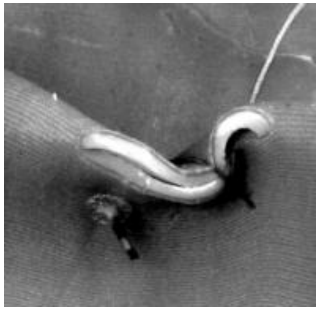


Iron oxide nanoparticles-loaded nanosheet for endoluminal surgery (with SSSA@IIT)

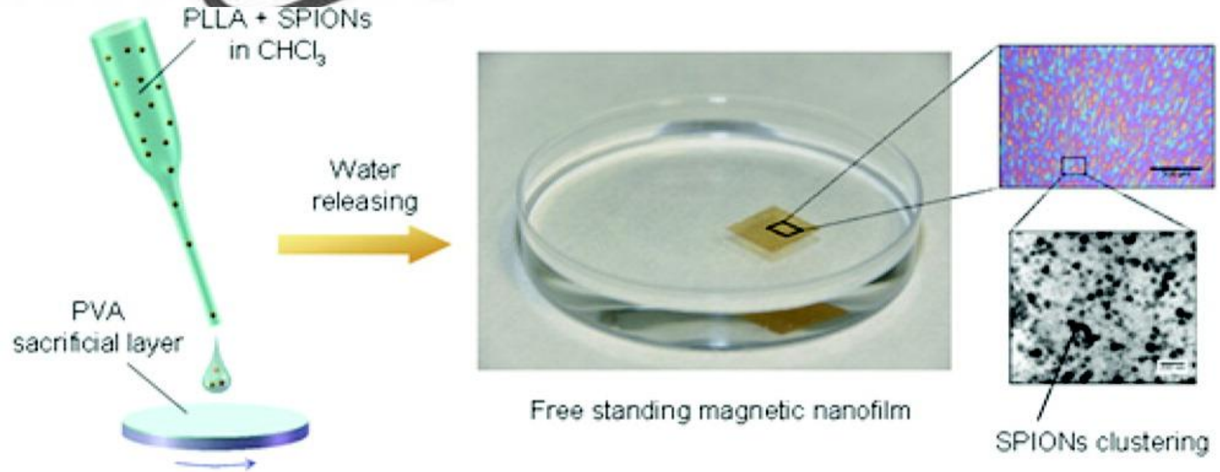


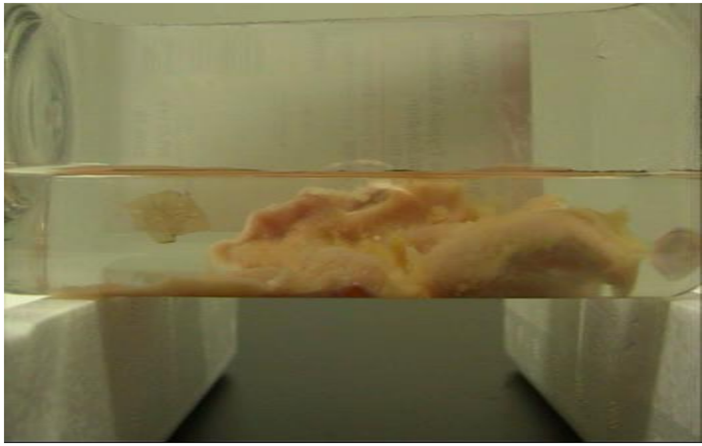
NOTES (Natural Orifice Transluminal Endoscopic Surgery), access to the target organs through holes made in stomach/ vagina /lung wall, etc.





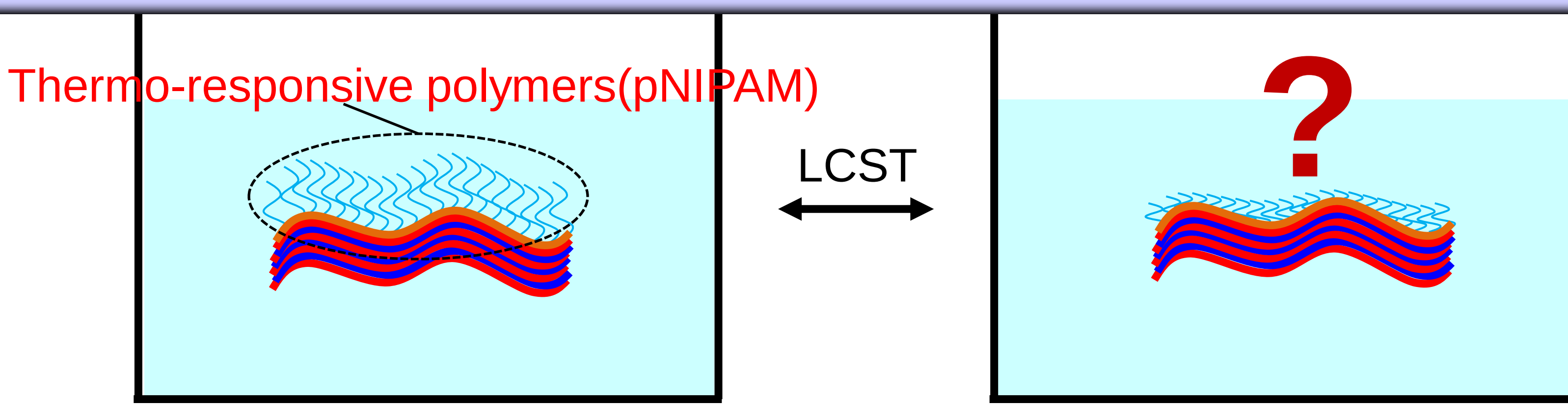
Non invasive, flexible, efficient methods for hole closing are requested because current techniques show many limitations

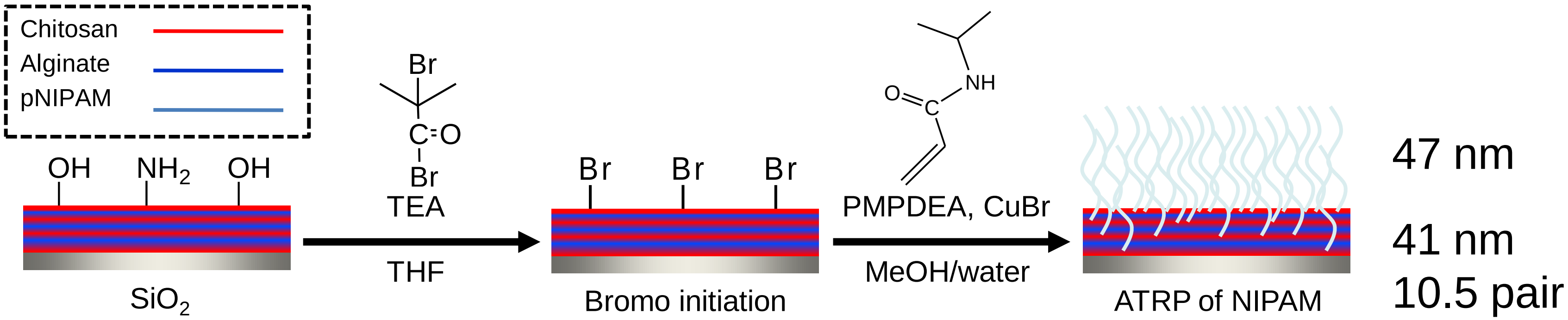




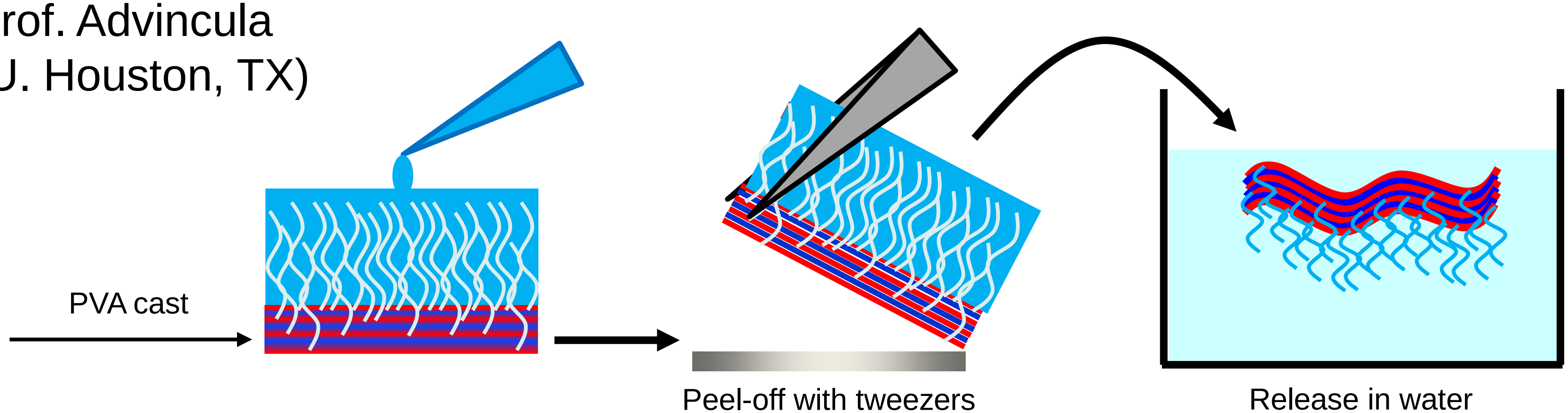
Silvia et al., *Langmuir*, *27*, 5589–5595 (2011).

Hydrodynamic transformation of a free-standing nanosheet Induced by a thermo-responsive surface

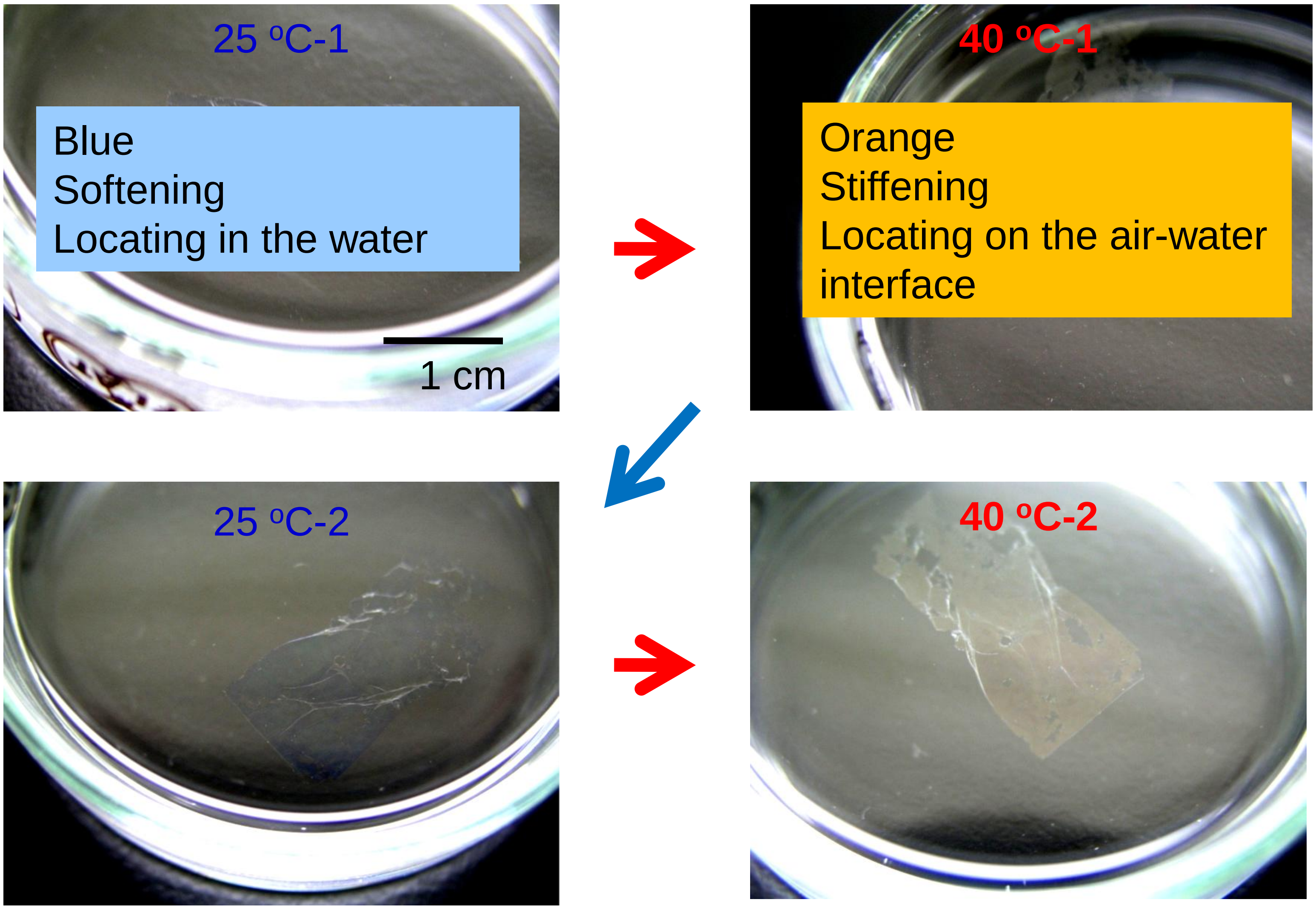




Prof. Advincula
(U. Houston, TX)

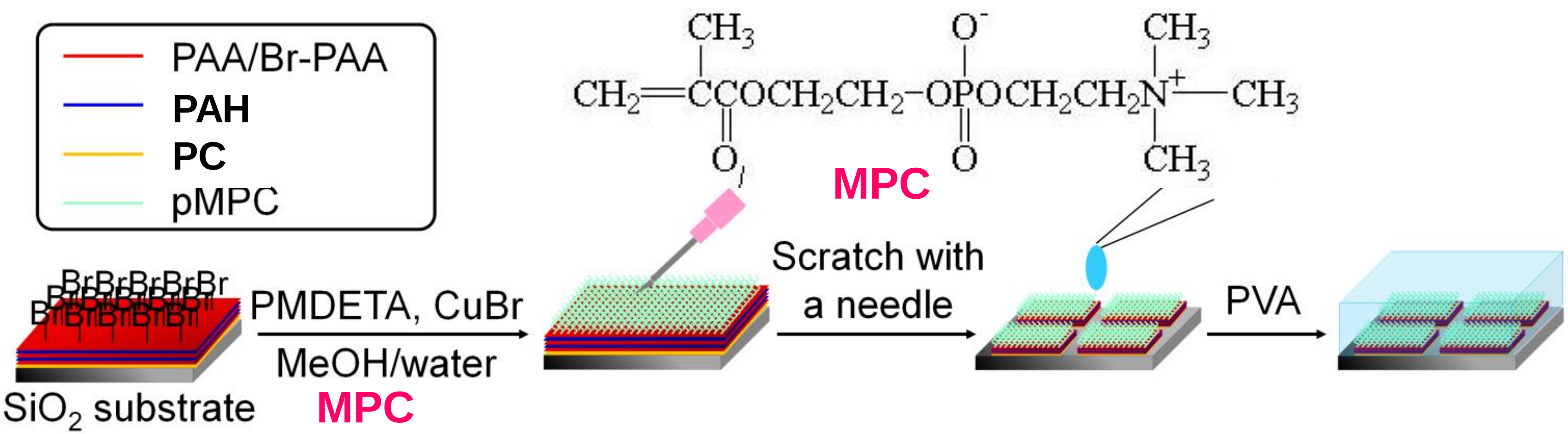


Reversible conversion of the color, property, and location of the nanosheet by temperature change

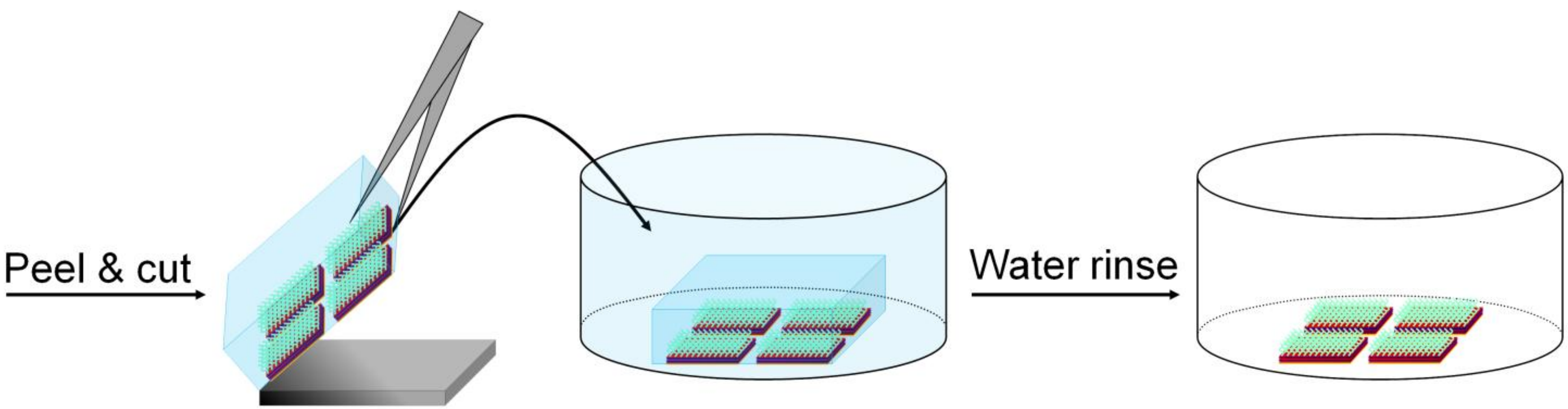


ACS Appl. Mater. Inter., 1, 1404-1413 (2009).

Convenient method for surface modification by patching a free-standing anti-biofouling nanosheet



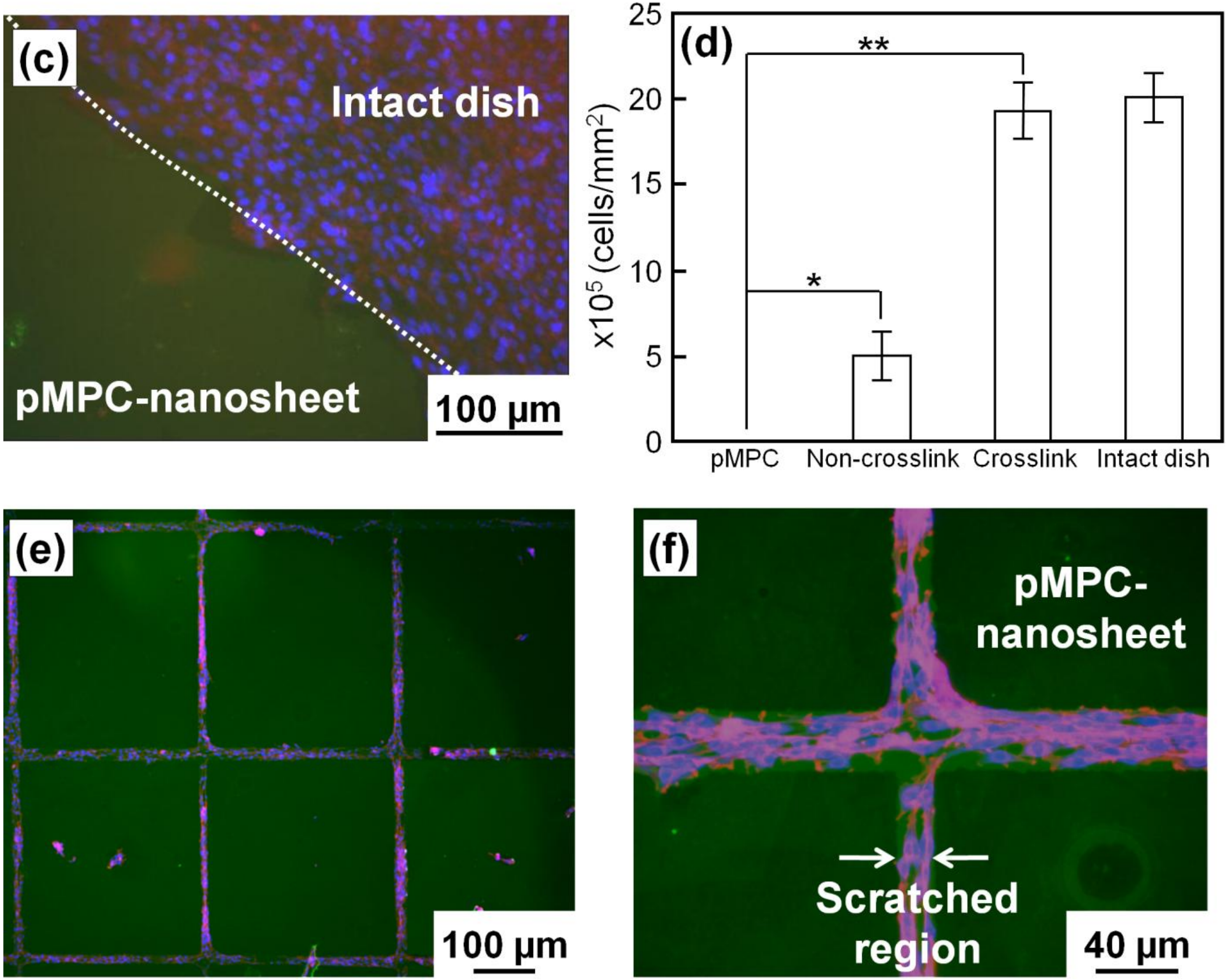
pMPC brush prepared on the nanosheet by ATRP, and peeled by the PVA film



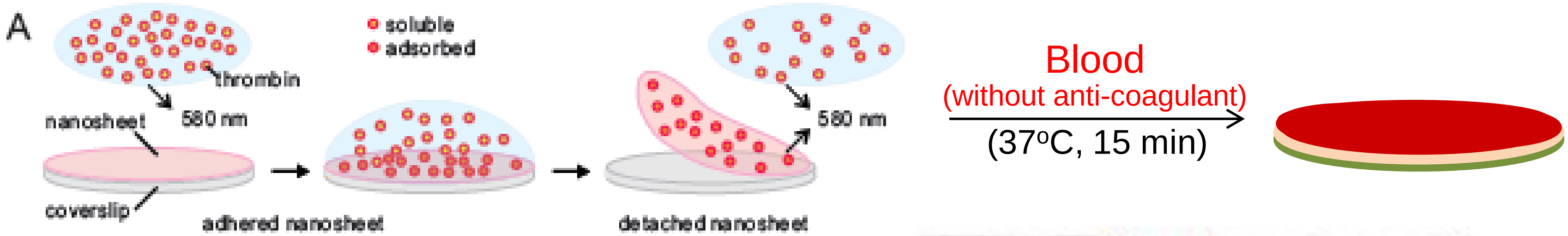
pMPC-nanosheet patched on a dish surface by dissolution of the PVA film

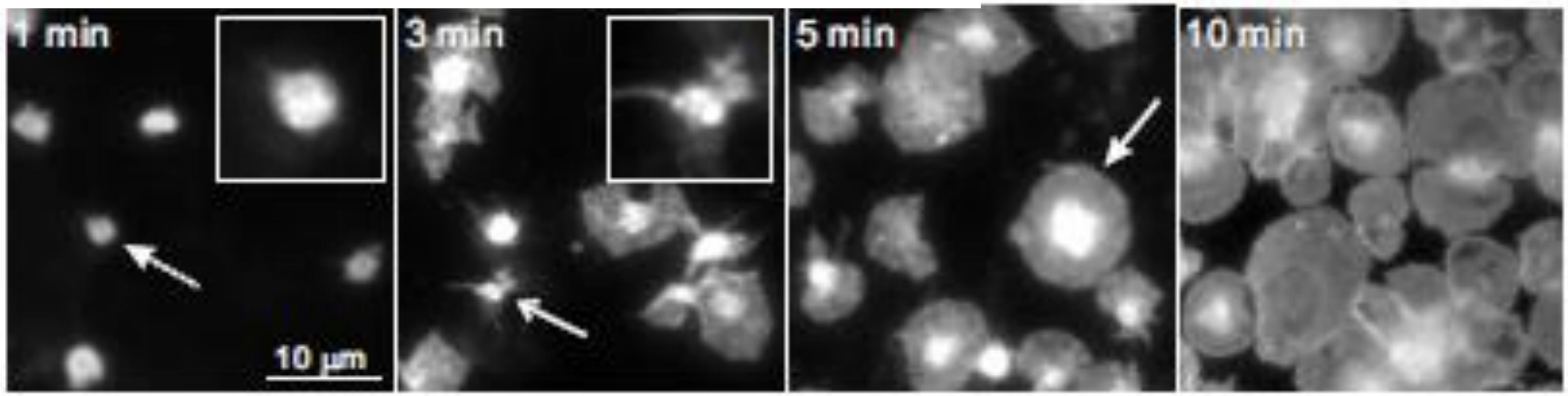
J. Mater. Chem. (2011) in press

Fluorescent microscopic images of NIH-3T3 cells at the interface between the FITC-labeled pMPC-nanosheets and the intact cell culture dish after 72-hrs culture

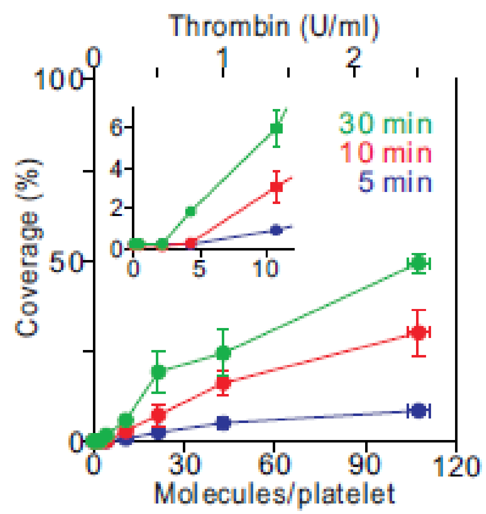


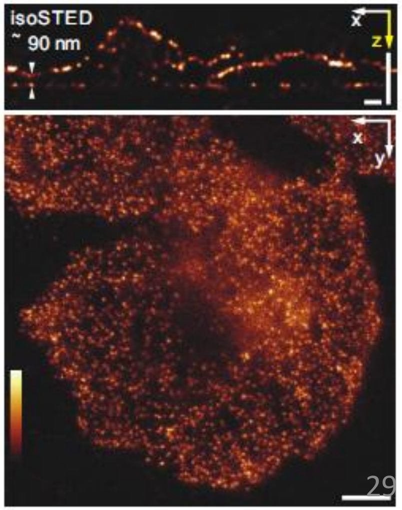
Few immobilized thrombins are sufficient for platelet spreading (with LIMES, U. Bonn)





A few immobilizing Thr molecules on the nanosheet readily activate platelets. This number is more than 1000-fold lower than expected from experiments in solution !!

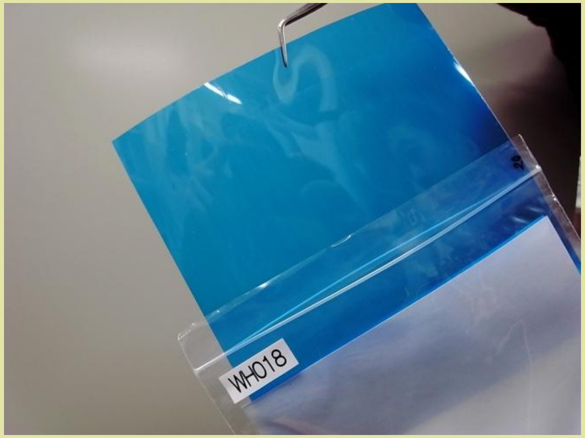




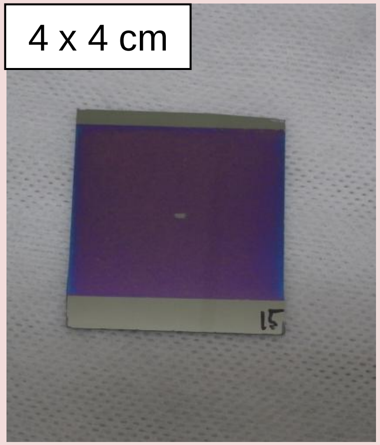
Biophys. J. 100,1855- 1863 (2011).

Manufacturing of polysaccharide nanosheets in an industrial scale

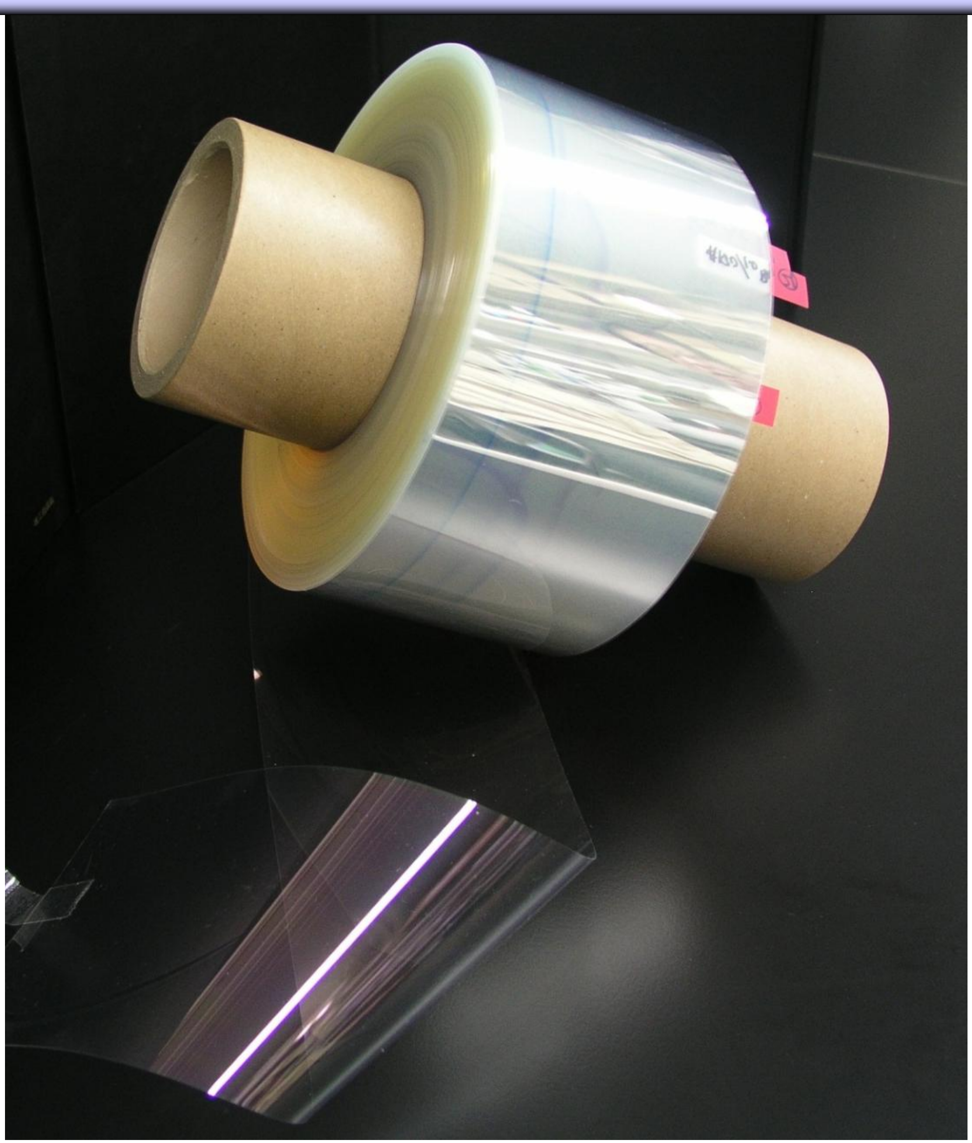
Dipping

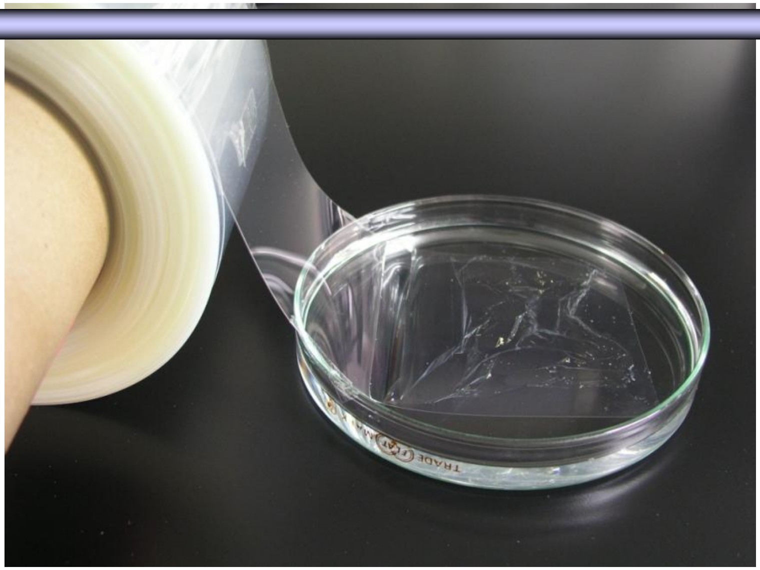


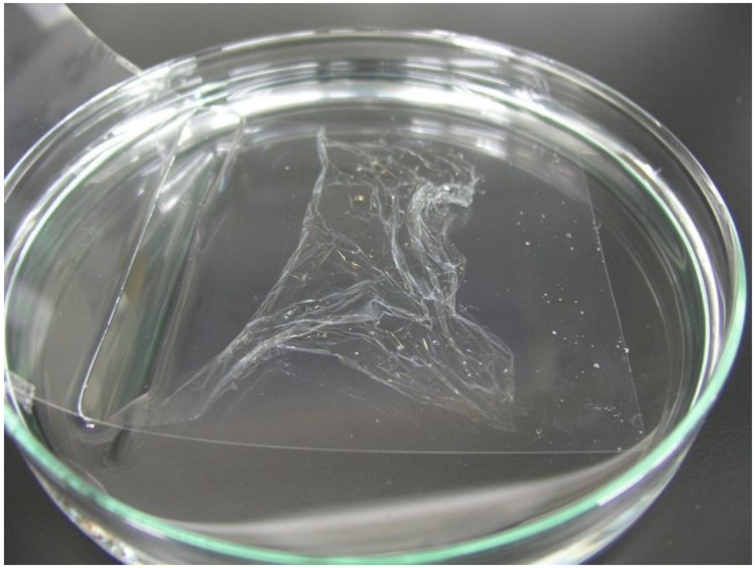
Spray-Coating



Manufacturing of PLLA nanosheets in an industrial scale



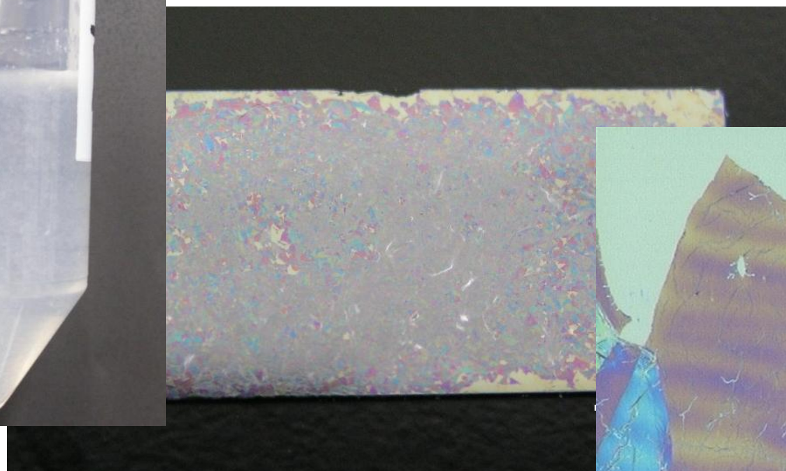
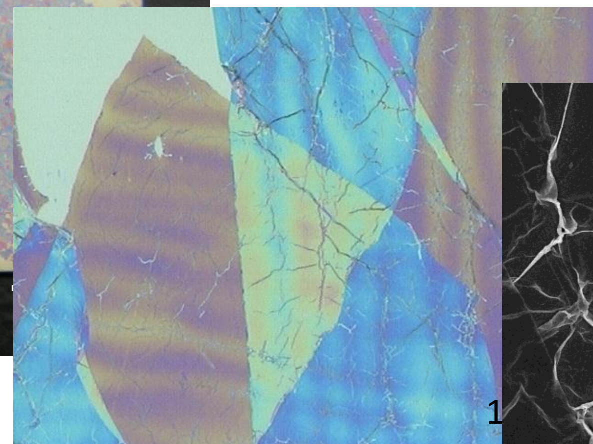
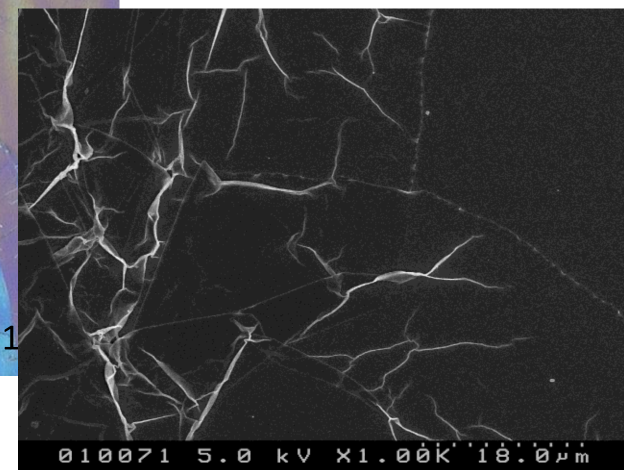


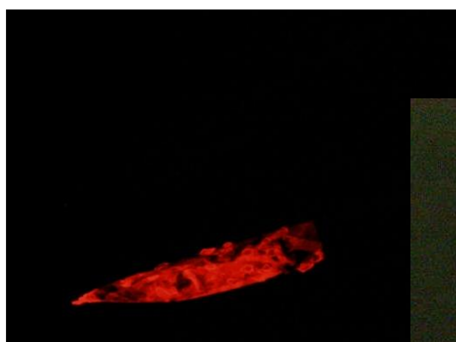


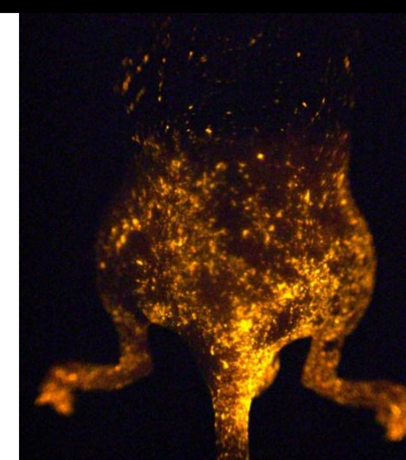
10 cm x 200 m x 60 nm as a free-standing state

Reconstruction of nanosheets from fragments

Casting from a fragmented nanosheets suspension



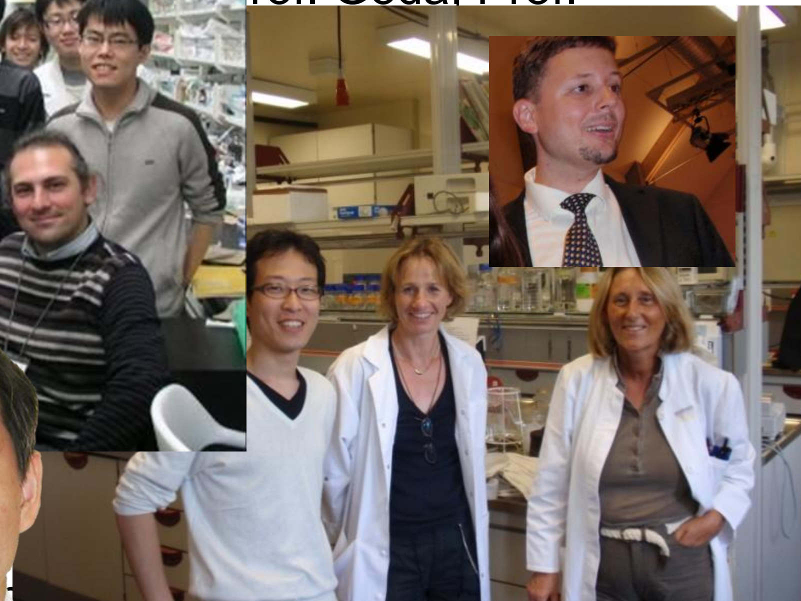
Dipping in a fragmented nanosheets suspension and drawing

Various researches and applications

- Nanoparticles(Ag, Au, Fe_2O_3)-loaded nanosheet as a new functional materials.
- Drug-loaded nanosheets for periodontal, dermatological, ophthalmological, and otorhinolaryngological applications.
- Polymorphic structures such as tube-like, bag-like, chip-like, ribbon-like, porous structures by various methods such as spray-coating, printing, phase-separation and removing, molding methods.
- Other applications such as cosmetics, foods, optoelectronics, catalysts, environmental materials.
- Basic polymer physics such as crystallinity, thermal properties, permeability, surface properties between substrate-side and air-side.

Acknowledgments

(Waseda Univ.) Prof. Osa … rof. Goda, Prof.



C. Advinc…

…ter” Project …

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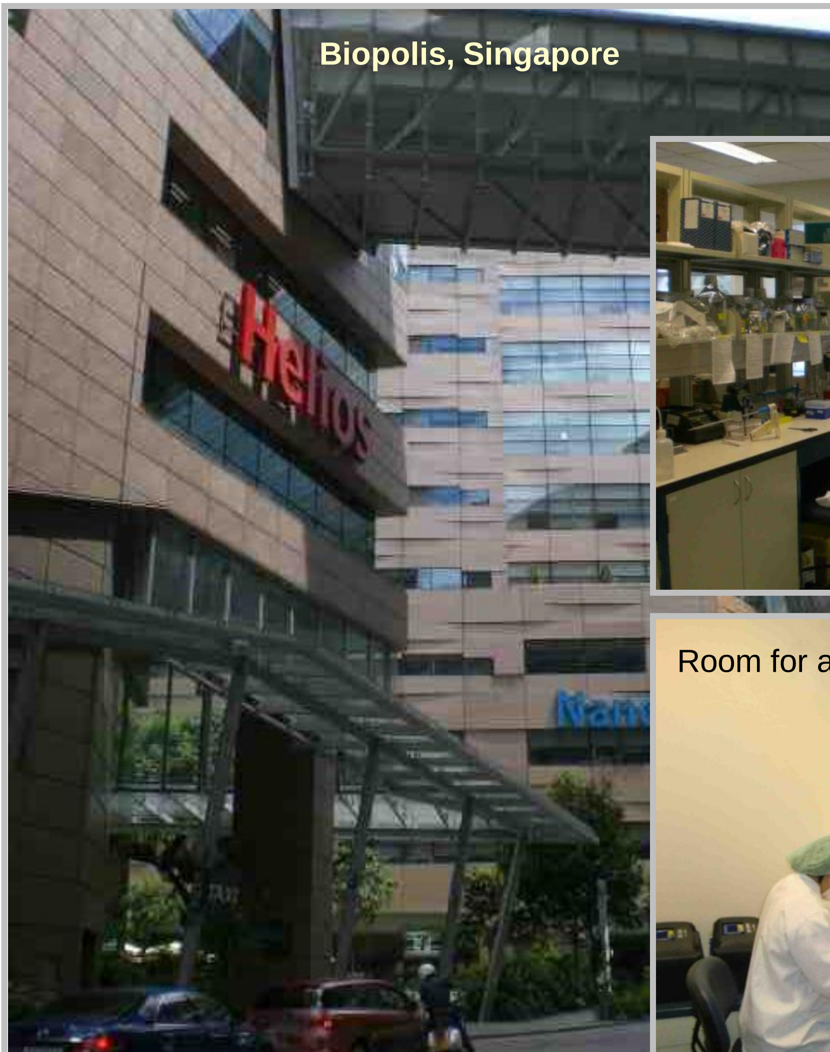
… Program fro…

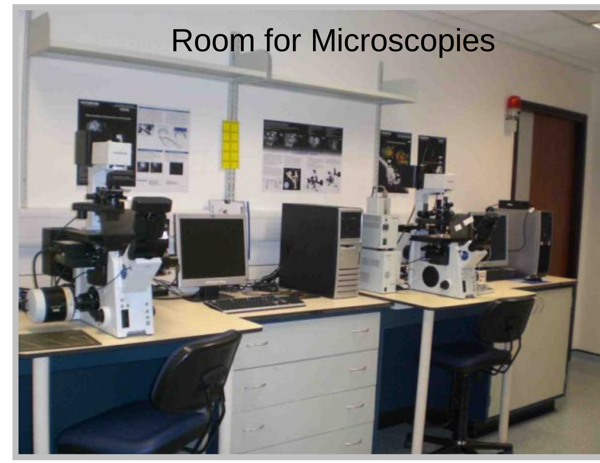
Medical College

WASEDA BIOSCIENCE RESEARCH INSTITUTE IN SINGAPORE



Biopolis, Singapore

Open labo



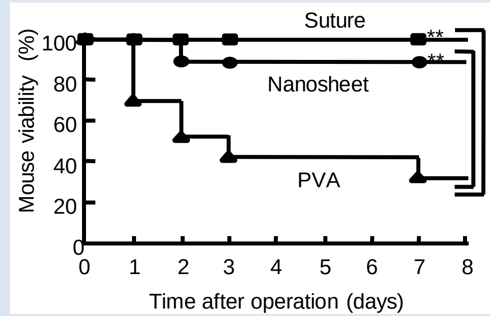
Room for Microscopies

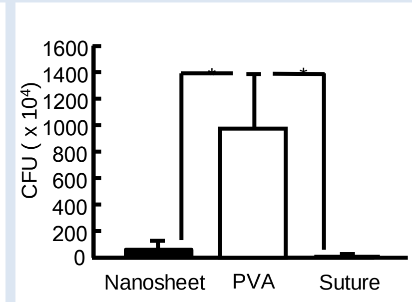
Room for animal study

Clean room for nanoparticle preparation

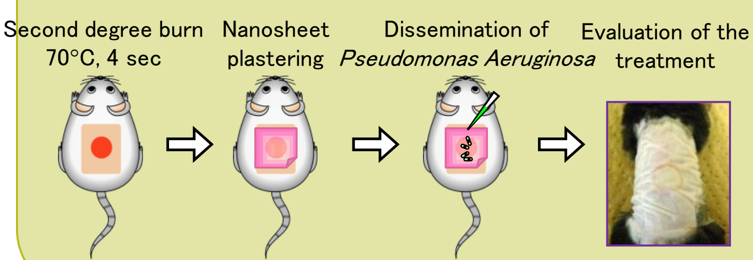
Evaluation of nanosheets as wound healing materials

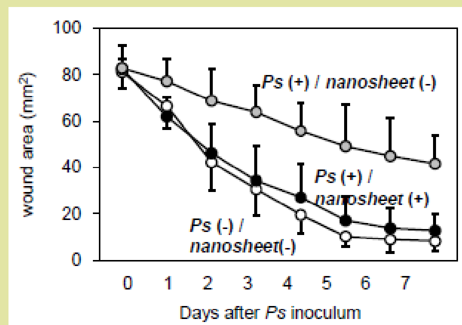
Overlapping treatment of nanosheets of the perforated cecum (Surgery 2010;148:48-58)

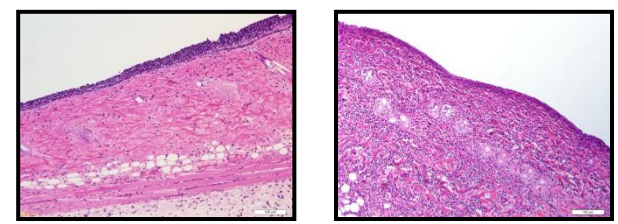




Plastering treatment of nanosheets for the mouse burn skin

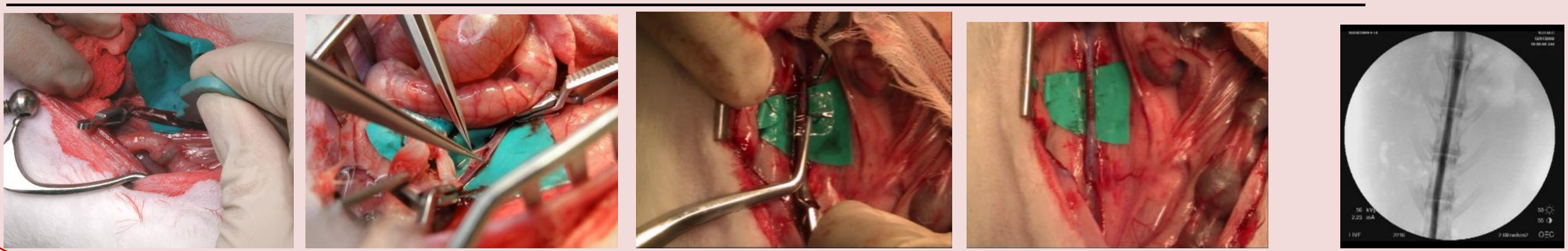




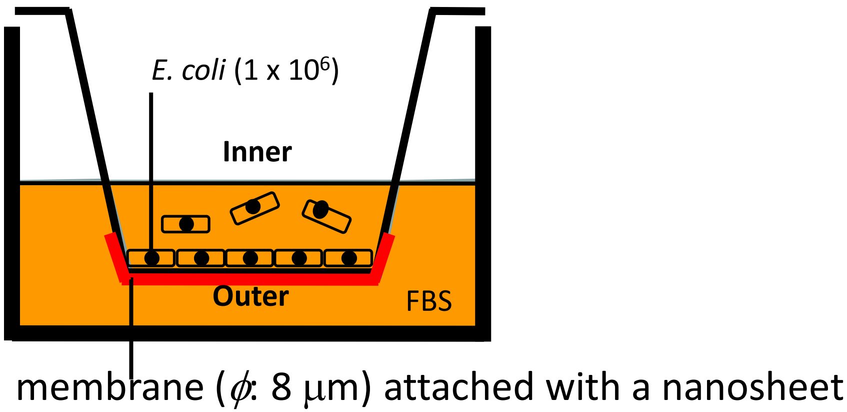


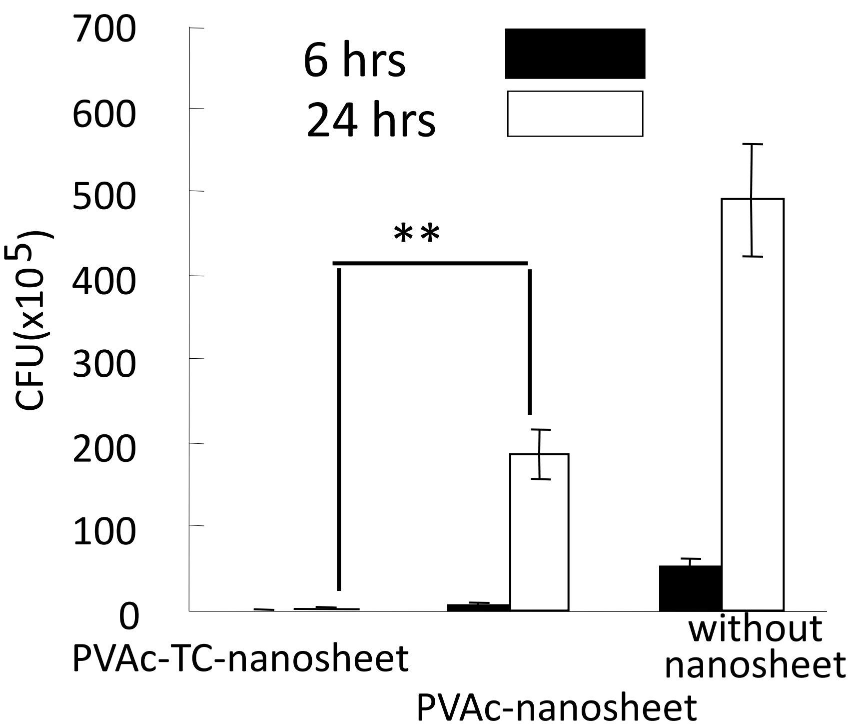
Ps **(+) / nanosheet (+)** ***Ps*** **(+) / nanosheet (-)**

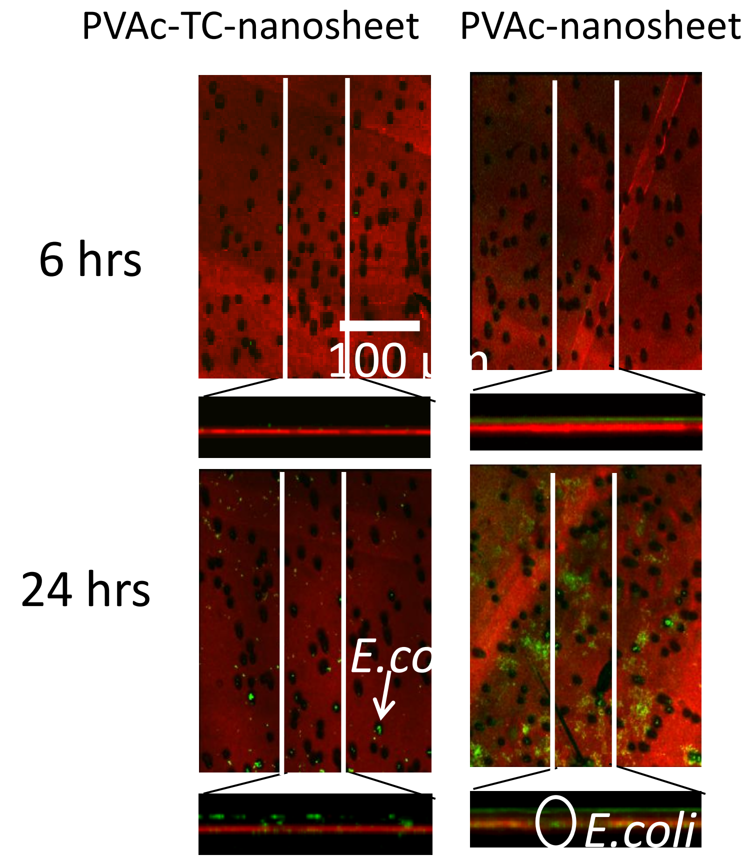
Hemostatic treatment of incision wound of the inferior vena cava



Antimicrobial permeability test using a transmembrane assay

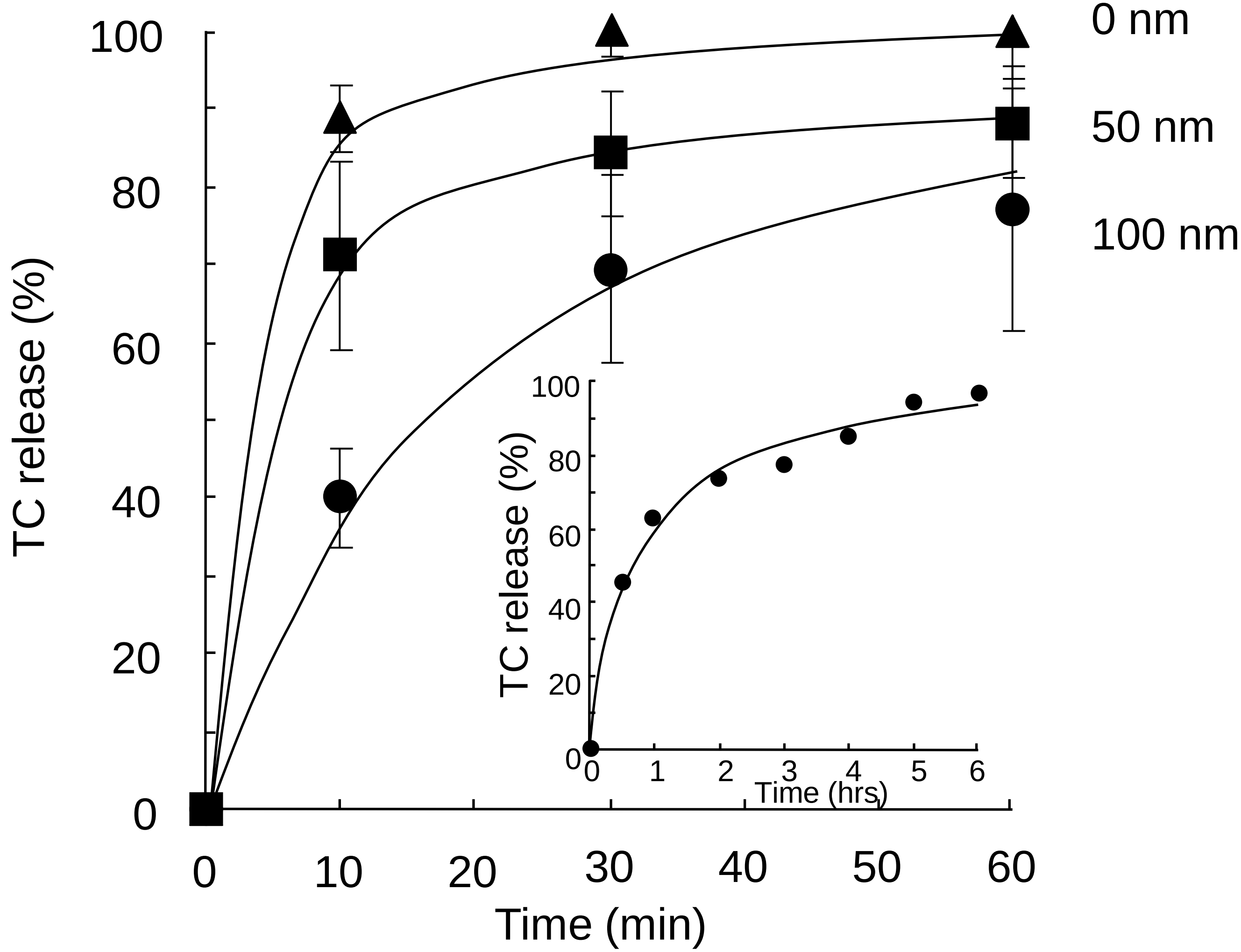




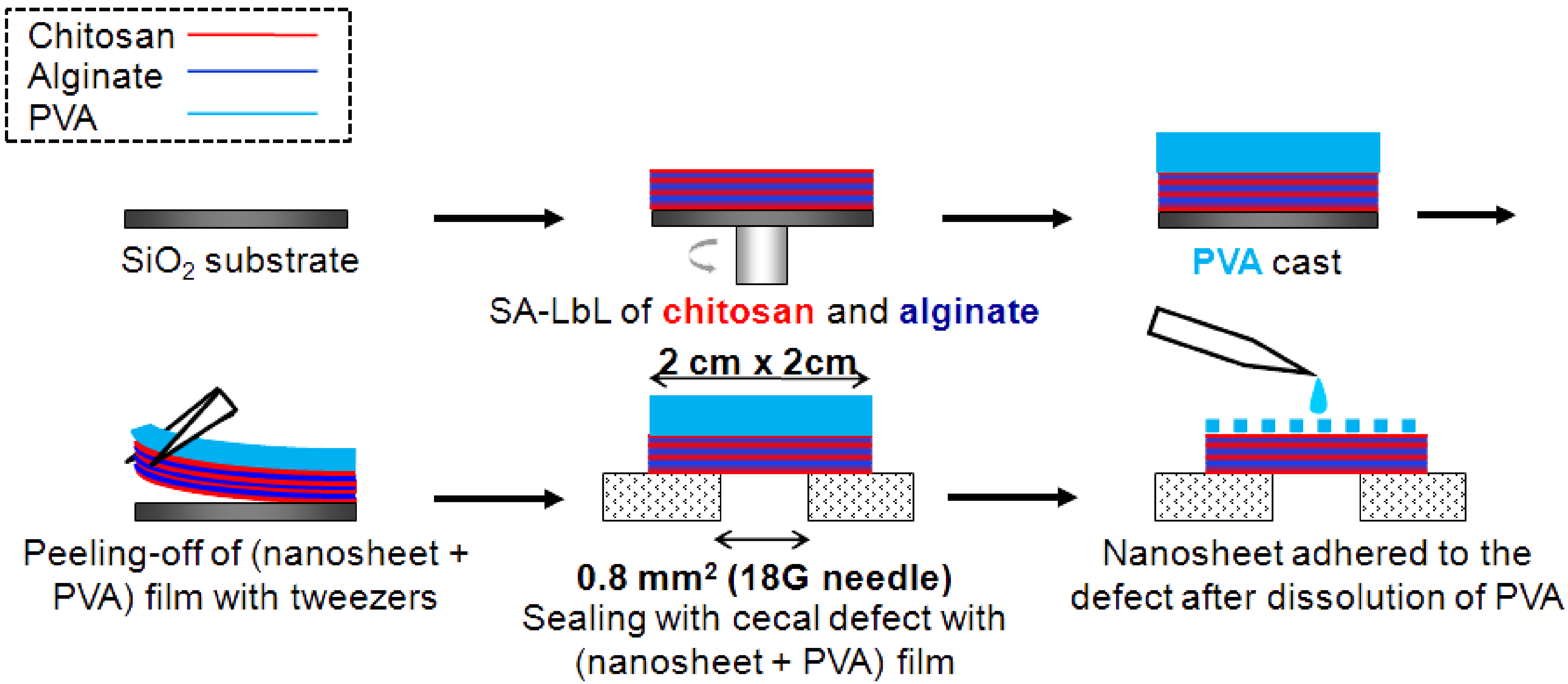


E. Coli were penetrating through the nanosheet without TC and such invasion was shut off by the TC layer.

TC release from the PVAc-TC-nanosheet with different PVAc thickness under physiological condition (37^{o}C, pH7.4)

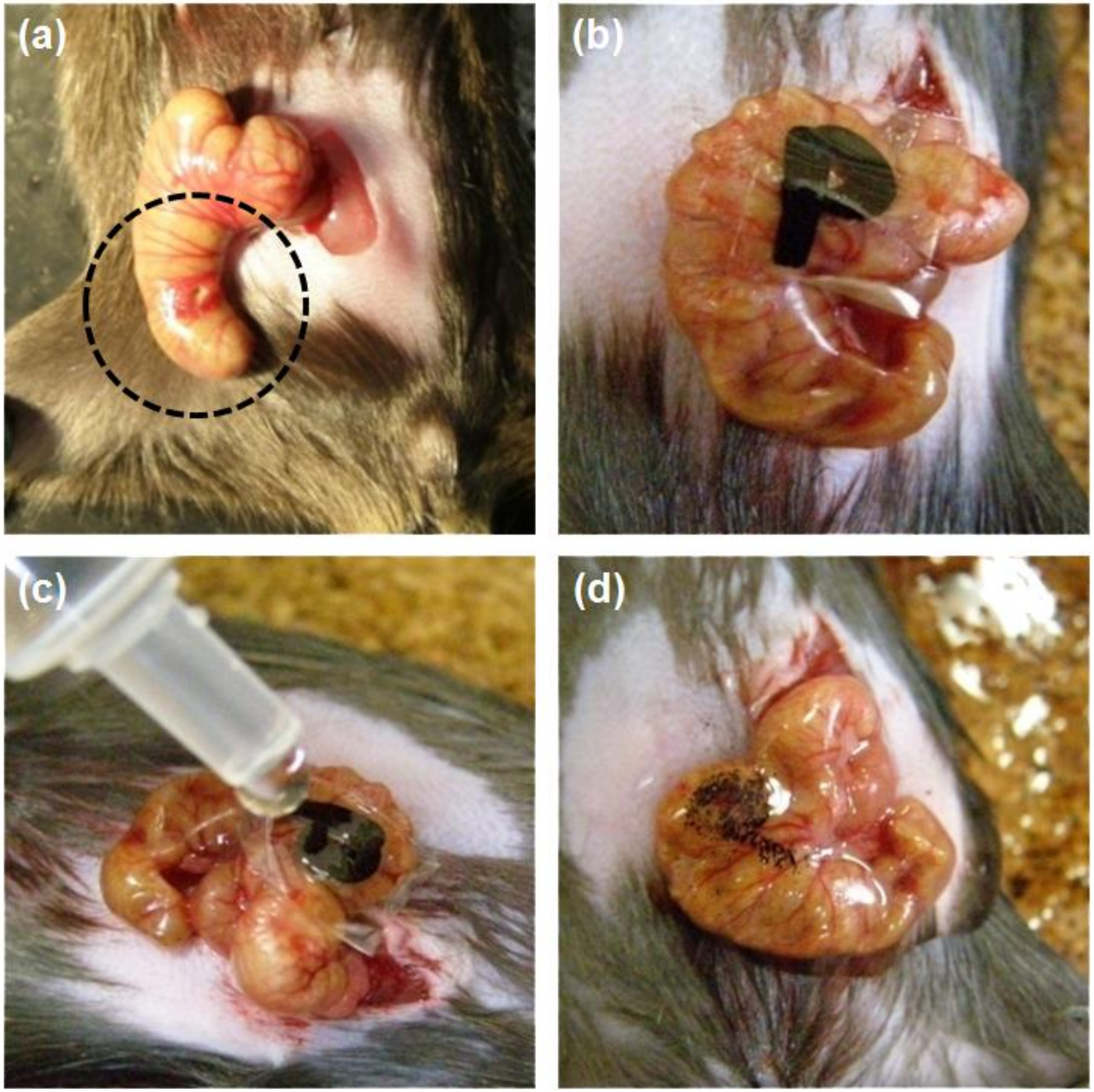


Fabrication procedure for a polysaccharide nanosheet supported by a PVA film and its application for a cecal defect caused by needle puncture.

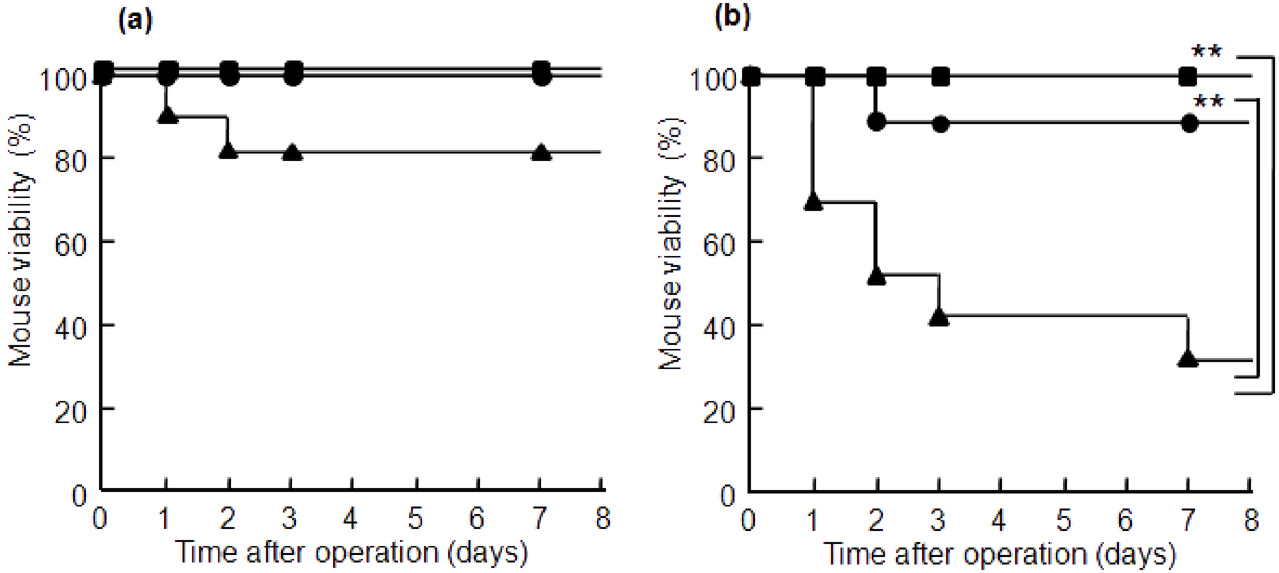


Surgery, *148*, 48-58 (2010).

Application of the polysaccharide nanosheet to a murine cecal puncture model



Time-course study of murine survival after sealing the puncture holes with nanosheet samples compared with suturing.

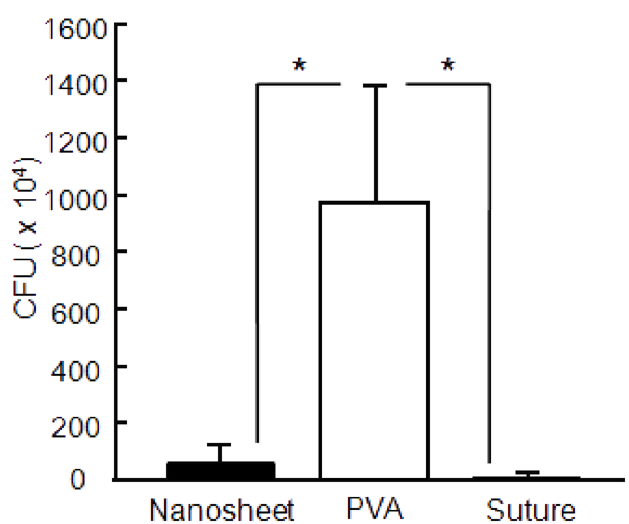


suture(square).
nanosheet(circle)

PVA film (triangle)

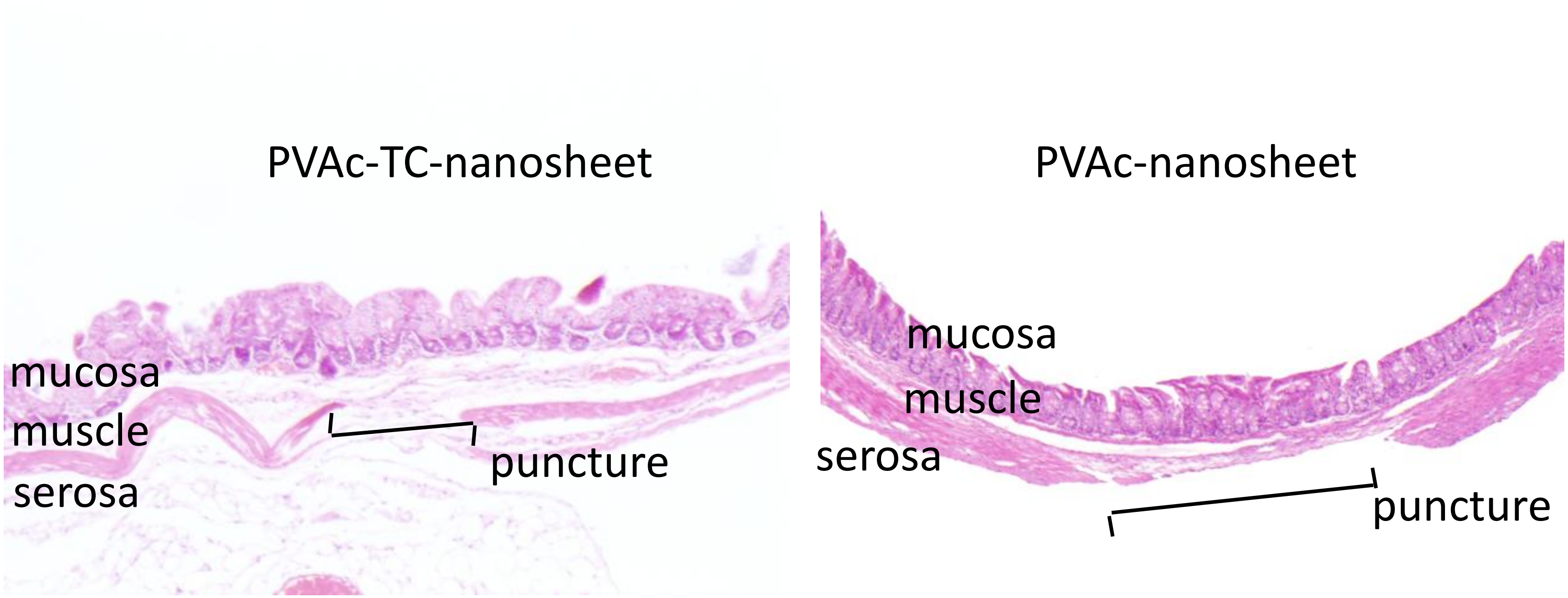
0.5 mm^2 puncture hole

0.8 mm^2 puncture hole



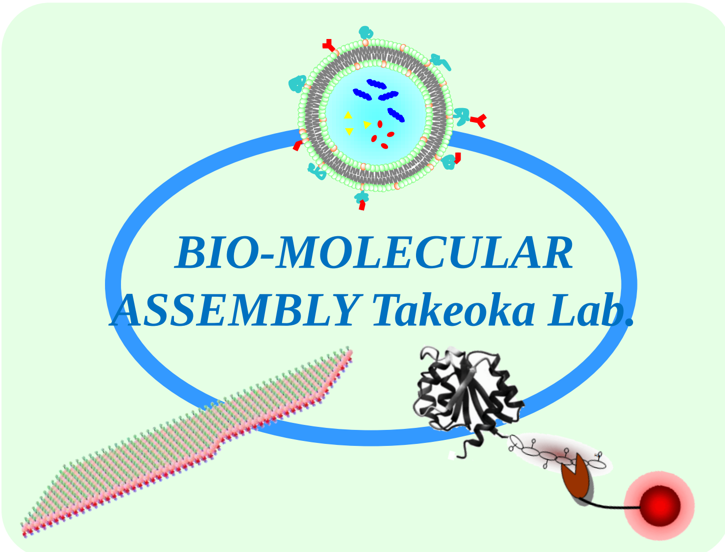
The number of intraperitoneal bacterium one day after the treatment using a nanosheet for the 0.8 mm^2 hole.

Histological sections of cecal punctured lesion after treatment with nanosheet samples (2 weeks)

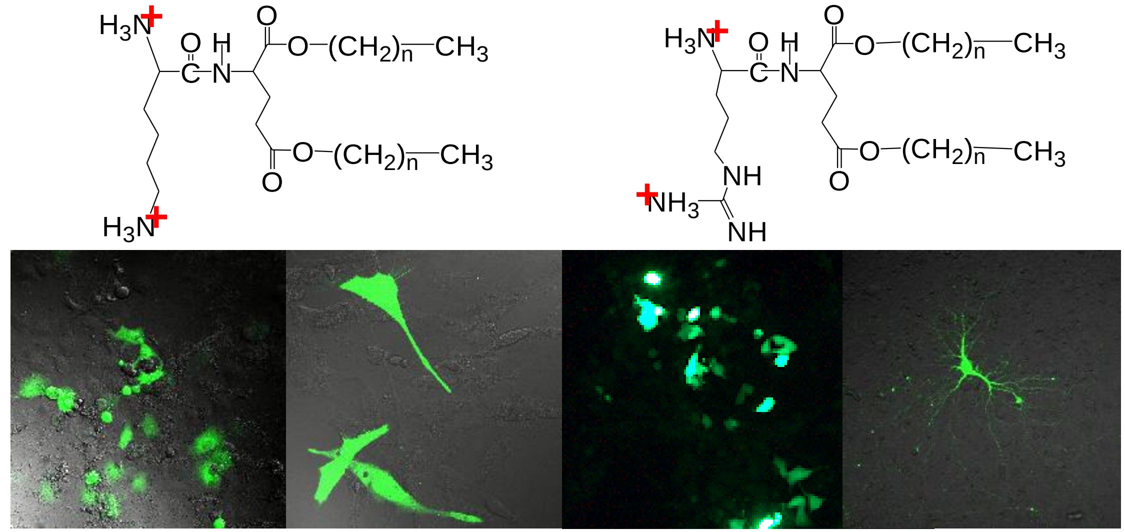


Instead of the typical accumulation/growth of fibroblasts around the site of injury, lipocytes or fibroblasts specifically bridged the tissue-defect site without any associated inflammatory reactions and tissue adhesion, resulting in almost complete regeneration of the mucosal defect.

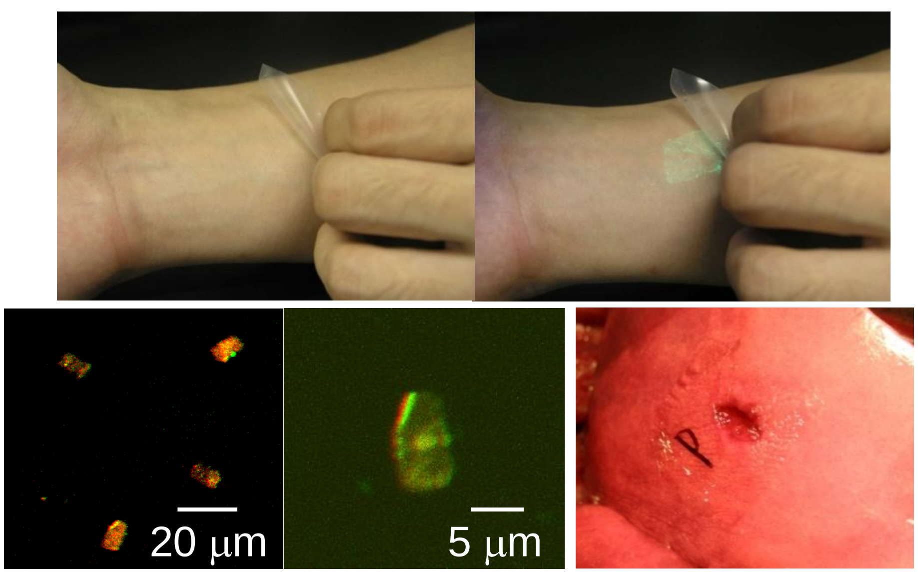
Introduction of Bio-molecular Assembly Science Labo.



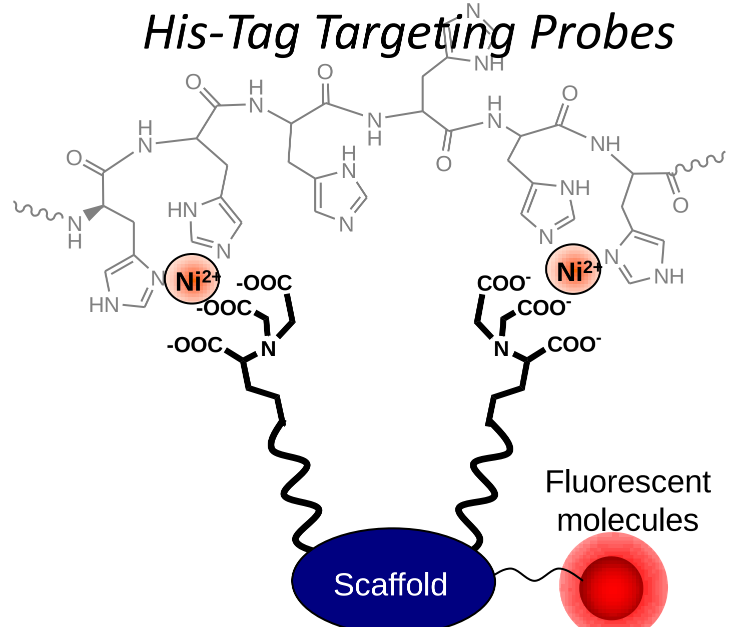
Gene Transfection by Cationic Liposomes



Nanosheets: Ultra-thin Dressing Material

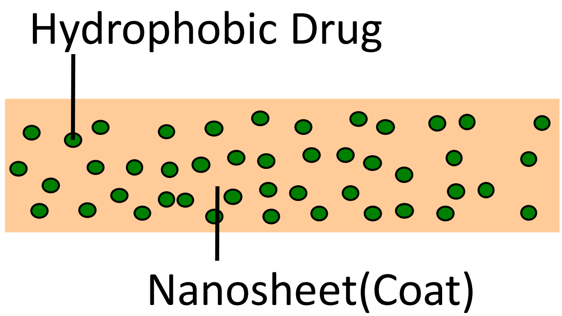


His-Tag Targeting Probes



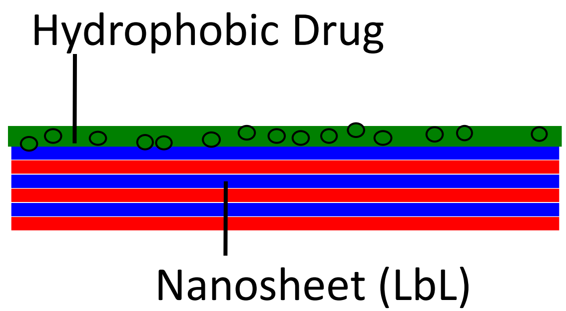
Drug-loading nanosheets

Nanosheet(coating)



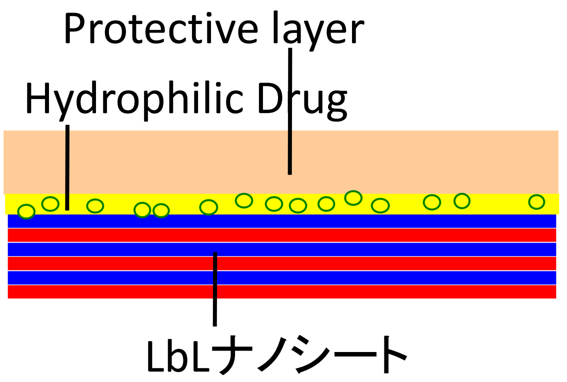
Hydrophobic (drug) +
Hydrophobic (sheet)

Nanosheet(LbL)



Hydrophobic (drug) +
Hydrophilic (sheet)

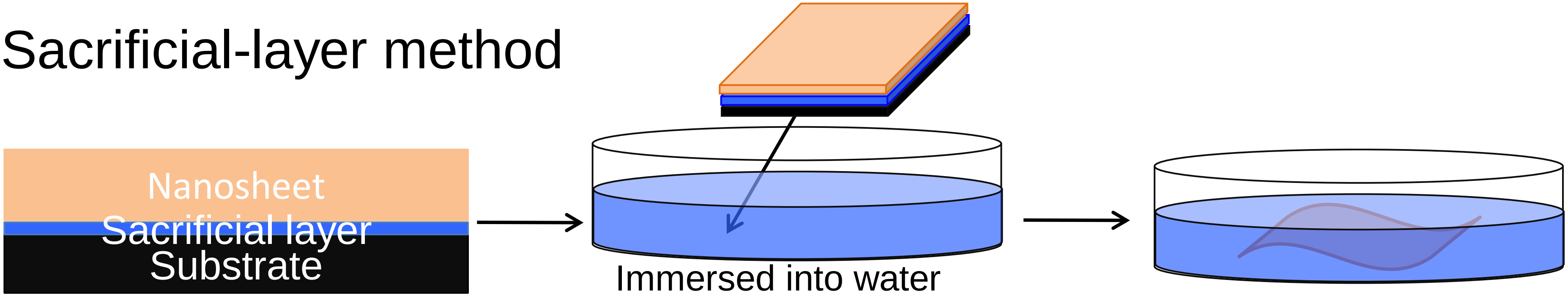
Nanosheet(hybrid)



Hydrophobic (barrier) +
Hydrophilic (drug) +
Hydrophilic (sheet) +

Methods to prepare free-standing nanosheets

Sacrificial-layer method



Supporting-layer method