Beyond the skin barrier

Biomolecular Needling System for Medicals

Painless Transdermal Drug Delivery &
Self-testing Diagnostic Bio-sensors

マイクロニードルパッチ：DDSと予防医学

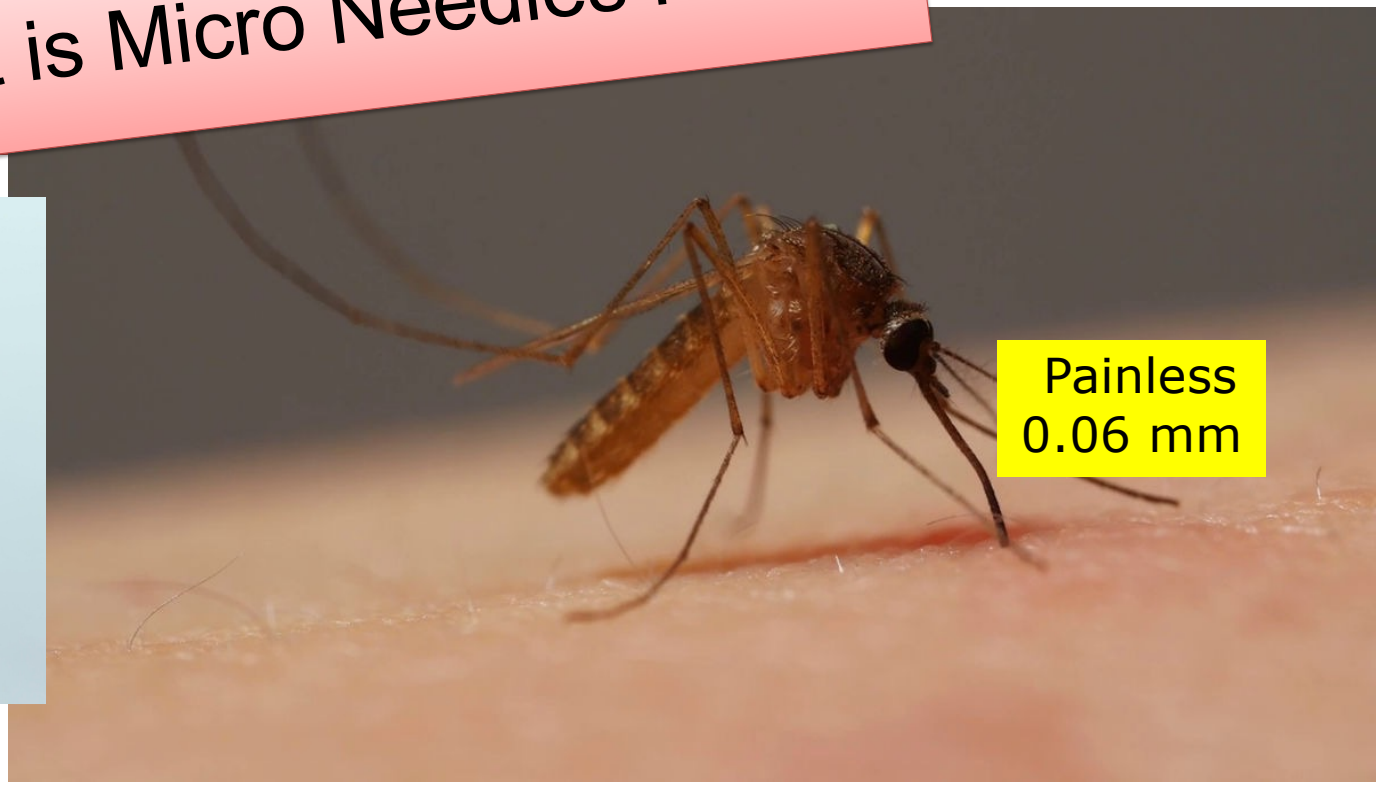
生体分解性マイクロニードルパッチを用いた
新規Drug Delivery System開発とバイオセンサーへの応用
:新型コロナ感染症の診断パッチ開発と新規ワクチンパッチの開発を目指して

Beomjoon KIM, Ph.D., Professor

Director of LIMMS/CNRS-IIS UMI 2820
Institute of Industrial Science, The University of Tokyo
Chair of Corporate Sponsored Research Division of Virological Medicine

Today, Topic

What is Micro Needles Patch?



INSTITUTE OF INDUSTRIAL SCIENCE
THE UNIVERSITY OF TOKYO

KIM Lab.

What is better for your Drug Delivery system?

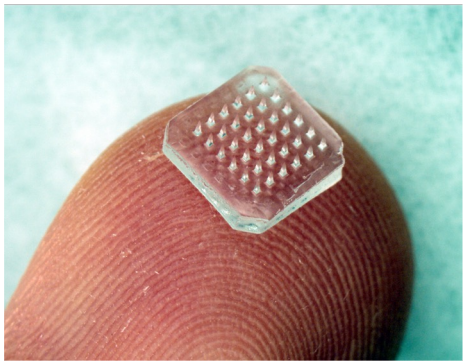
Hypodermic Injection

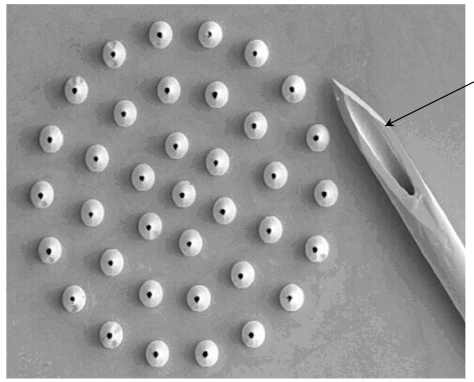
Oral Inoculation

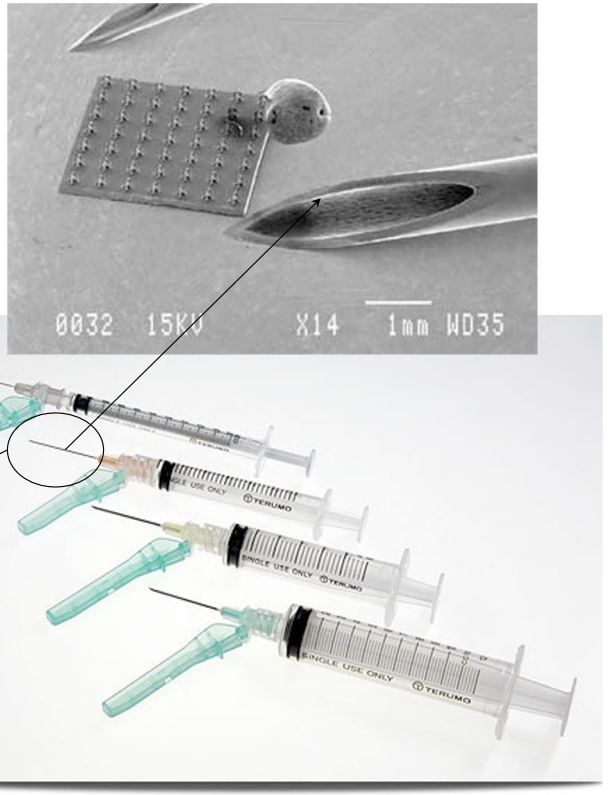
Drug Patch/ointment

マイクロニードル: 痛みのない針

34G:外径 (0.18 mm)





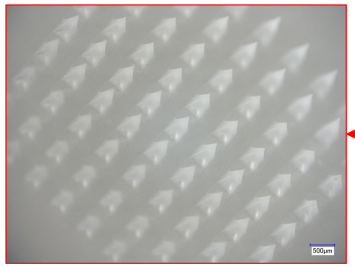


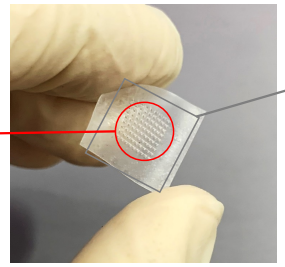
- 血管や神経を傷つけない針
- 痛みや出血を伴わずに生体内にアクセス可能
- 真皮層中の細胞間質液の採取への期待

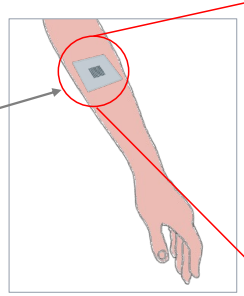
Microneedle Array

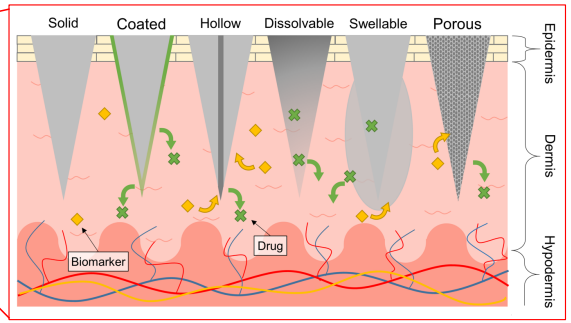
- **Microneedles (MNs), MN array**
  micrometer sized needles made of various biocompatible materials.
  > can create the pathways into epidermis or dermis layers to transport drug molecules.
  > minimally invasive, no medical professionals, convenient in storage as well as logistics

Representative MN array patch









Different types of MN array
- Solid
- **Coated**
- Hollow
- **Dissolvable**
- Swellable
- **Porous**

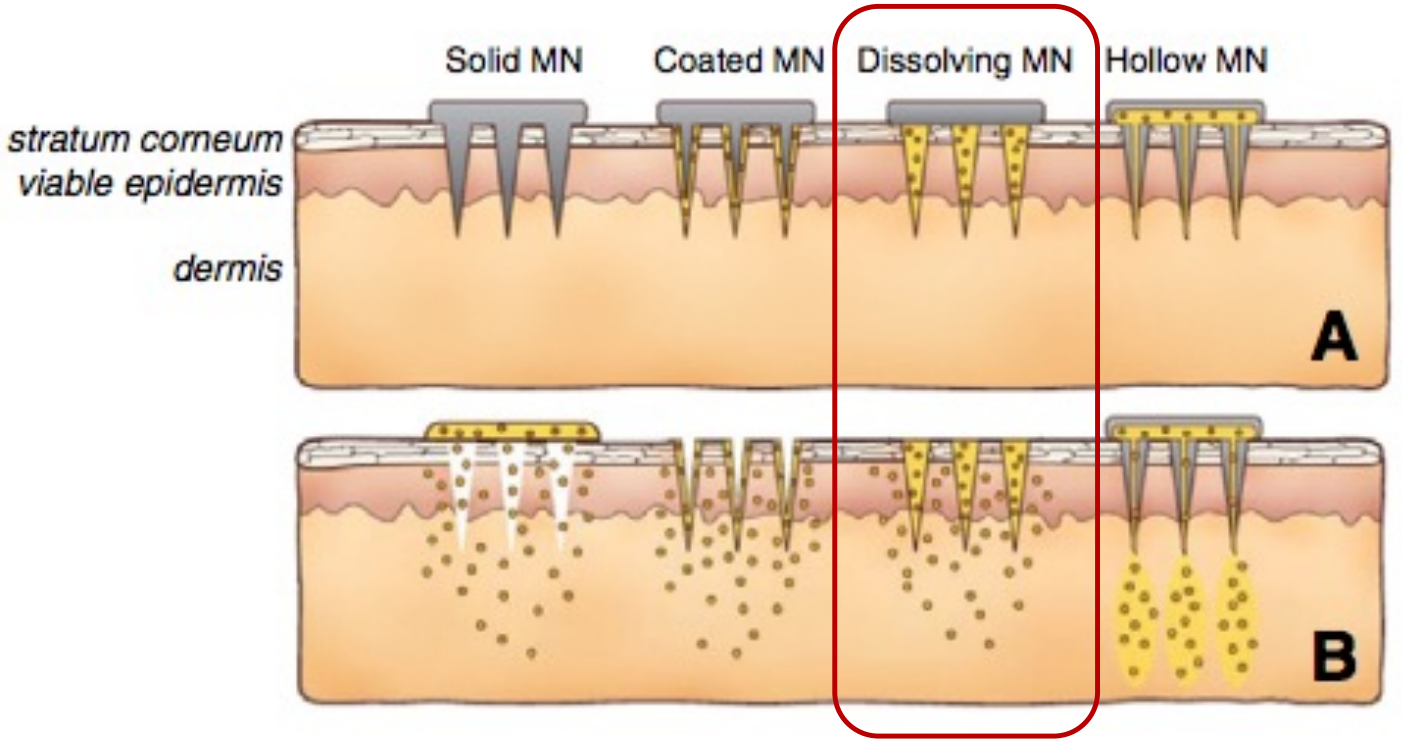
No Pain, Patient-friendly
Non-invasive

Less space
for storage

No biohazardous
waste

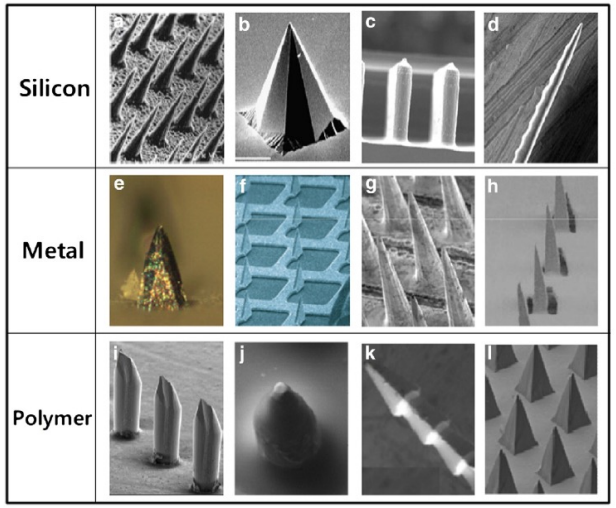
No medical staff
required

Conventional Micro Needles for Drug Delivery System

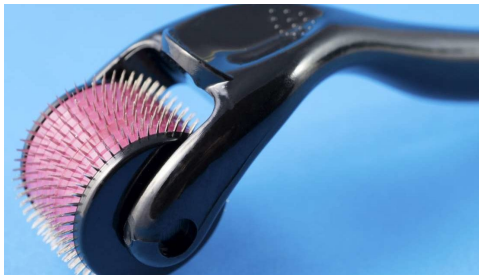


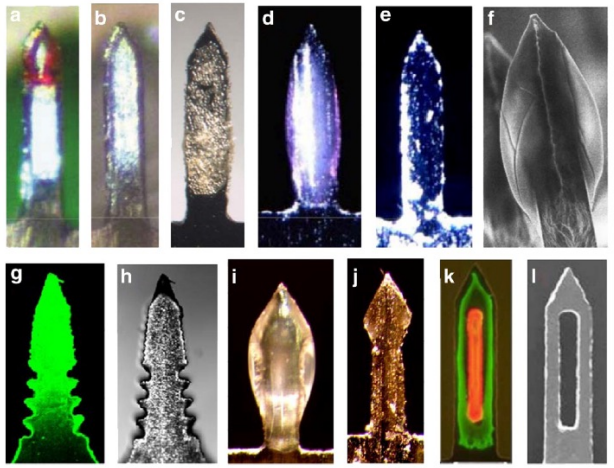
Refs: Y.C. Kim, *et al.*, ***Advanced Drug Delivery Reviews*** 64 (2012) 1547–1568

Various Types of Micro Needles for DDS

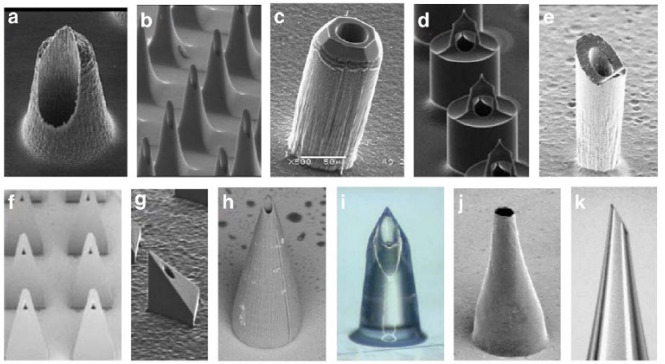


Solid Micro needle





Coated Micro needle (solid)

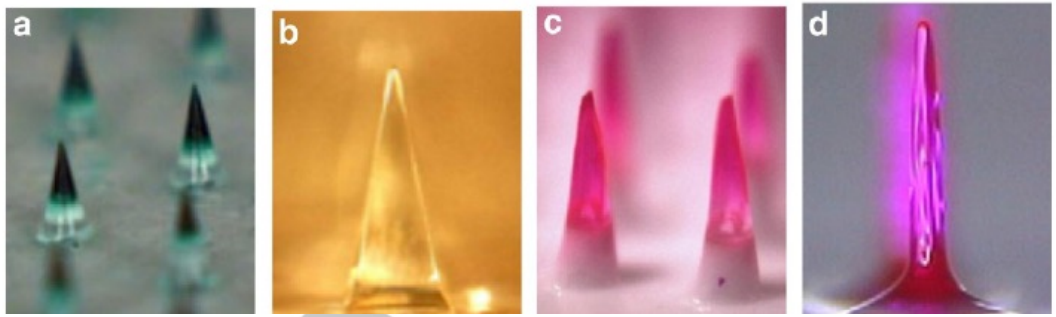


Hollow Micro needle

BNS MEDICALS

Refs: Y.C. Kim, *et al.*, ***Advanced Drug Delivery Reviews*** 64 (2012) 1547–1568

Micro Needles : Dissolving Micro needle (Biodegradable)



active compounds

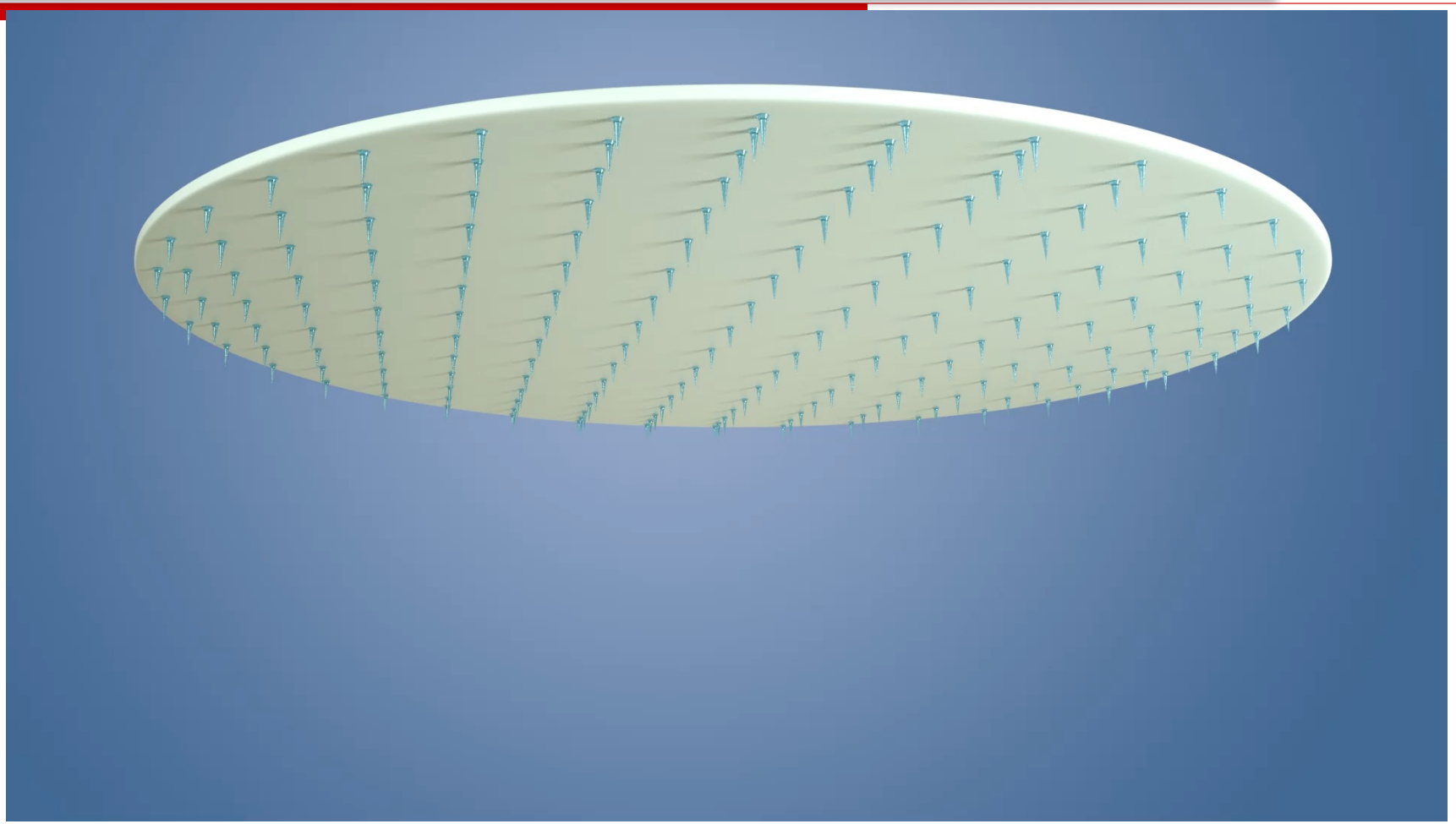
- Chemical compounds, functional natural extracts
- Functional proteins, peptides, hormones, DNA
- Nanoparticles, liposomes, and etc.
- Applicable to biopharmaceuticals such as vaccines, insulin, and etc.

backbone materials

- Water soluble polymers: CMC, HA, PVP, PVA, etc.
- Non-water soluble polymers: Chitosan, PLA, PLGA, etc.
- Water-oil emulsions: polymer + water + oil

Existing conventional Dissoluble Micro Needle Patch for DDS

生体分解性マイクロニードルを用いたドラッグデリバリーシステムの革命と予防医学の実現



100 % transdermal local delivery of high molecule API (Active Pharmaceutical Ingredient)

Commercialization Dissolving Micro needle

EGF (Epidermal Growth Factor,上皮成長因子)
and Hyaluronic Acid
+ Argireline (Acetylhexapeptide-3)

Cosmetic

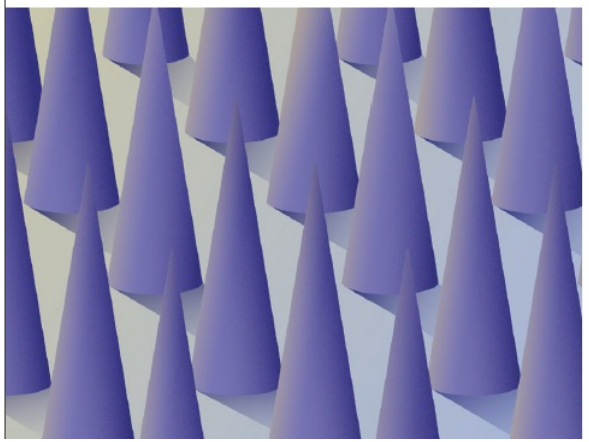
Prof. Stanley Cohen
Nobel prize in Physiology &
Medicine to discover ***EGF*** (1986)

Experts highlight advances with the potential to revolutionize industry, healthcare and society

MEDICINE

1 **Microneedles for Painless Injections and Tests**

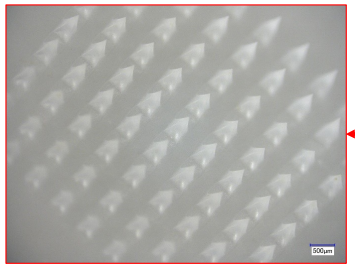
Fewer trips to medical labs make care more accessible

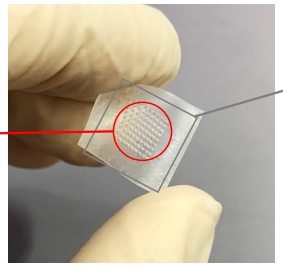


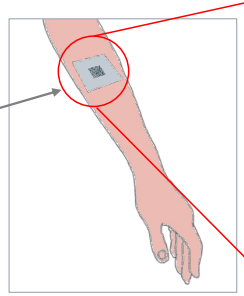
Microneedle Array

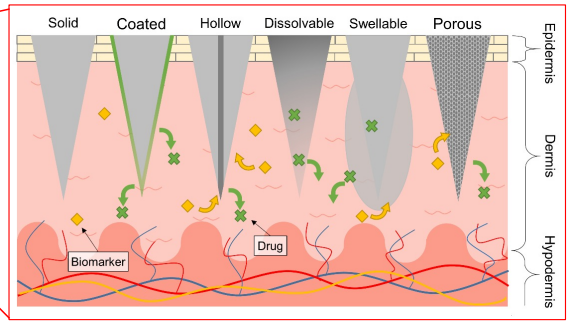
- **Microneedles (MNs), MN array, Microneedle patch (MNP)**
  micrometer sized needles made of various biocompatible materials.
  > can create the pathways into epidermis or dermis layers to transport drug molecules.
  > minimally invasive, no medical professionals, convenient in storage as well as logistics

Representative MNP









Different types of MNs
- Solid
- **Coated**
- Hollow
- **Dissolvable**
- Swellable
- **Porous**

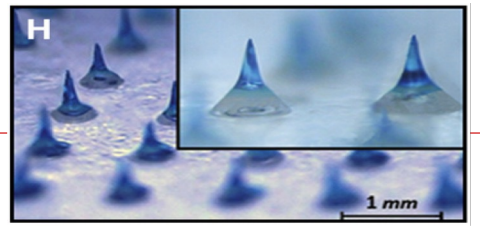
No Pain, Patient-friendly
Non-invasive

Less space
for storage

No biohazardous
waste

No medical staff
required

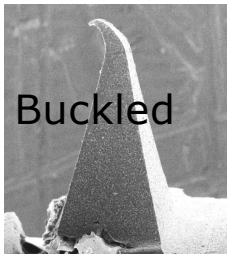
Currently, Dissolving Microneedles

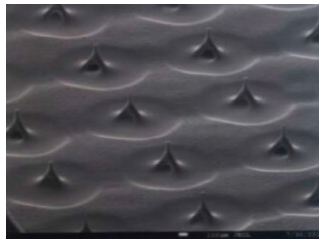


J.D. Kim et al., J. Controlled Release (2013)

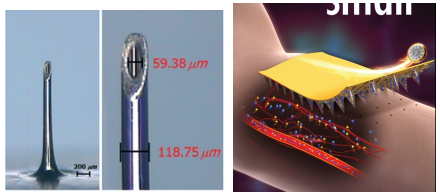
Problems

- Commercialized only in Cosmetic, skin trouble care products
  Still very few in DDS (acne care, influ vaccination, research levels for medical applications)
- Minimally invasive manner – still **inevitable pain**
- Limitation of Low-cost, Mass fabrication of microneedle with arbitrary shapes, various dimensions





- Recently, only few works about ISF extraction sensor applications

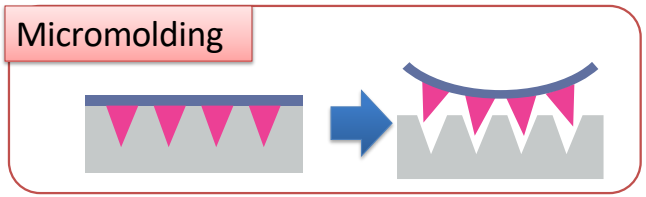


C. G. Li et al., Lab on a Chip (2017)
J. Zhu et al., Small (2020)

Conventional MN fabrication technology

1. 95% competitors

Micromolding



Difficulties

- Filling in mold- vacuum, centrifuge
- Drying in mold- long time, Heating or UV
- Detach from mold- surface treatment, cleaning

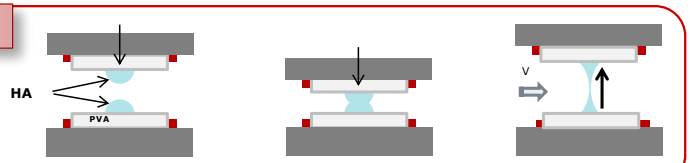
3M Corium TheraJect FUJIFILM

Korean Companies: SNvia Co., Ltd., Dermajet, Small Lab., Endoderma, Karatica Co., Ltd , QuadMedicine..

2. Others

DAB(Droplet- born Air Blowing)

Inkjet (Mushashi Eng.)



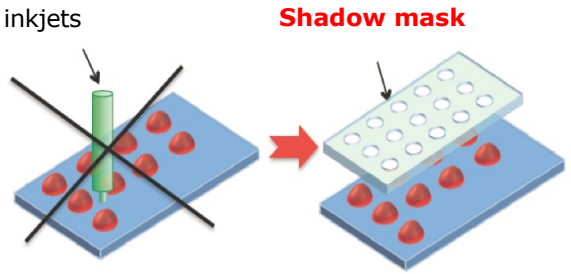
Raphas Ltd. Co. @Korea

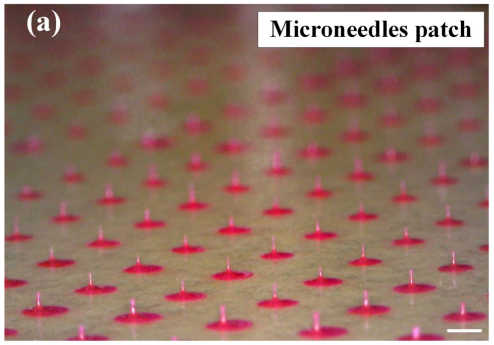
(Prof. H. Jung, Journal of Controlled Release., 170, 2013)

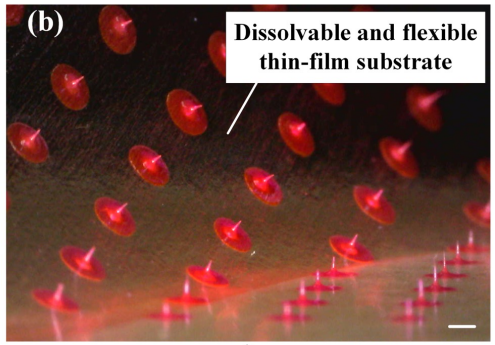
- Limitation of API components
- Difficult to fabricate various shapes/lengths of needles

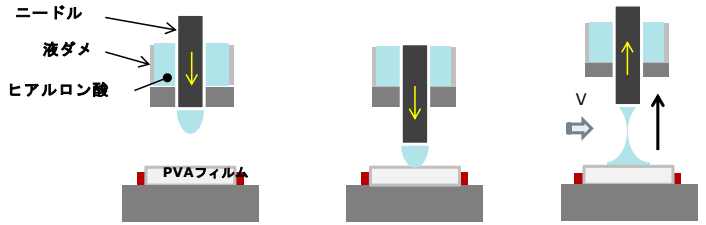
We Can improve

- **Larger area of uniform microneedles with faster process time**









Direct Micro needle type
Dispensor usage or micro stamping

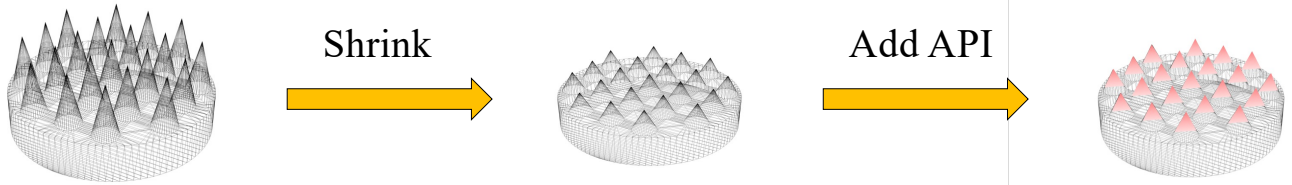
Patents by Raphas. Co., Ltd. (collaboration with UTokyo, BJ Kim one of inventors)

- WO2017/200213 “METHOD FOR MANUFACTURING MICRONEEDLE” (PCT/KR2017/004058)
- (韓国)特許10-2016-0061903号 出願日2016年5月20日, 2017年12月29日登録) 発明名称:マイクロニードル製造方法
- (韓国)特許10-2016-0061909号 出願日2016年5月20日, 2017年9月14日登録) 発明名称:マイクロニードル製造用粘性物質供給装置

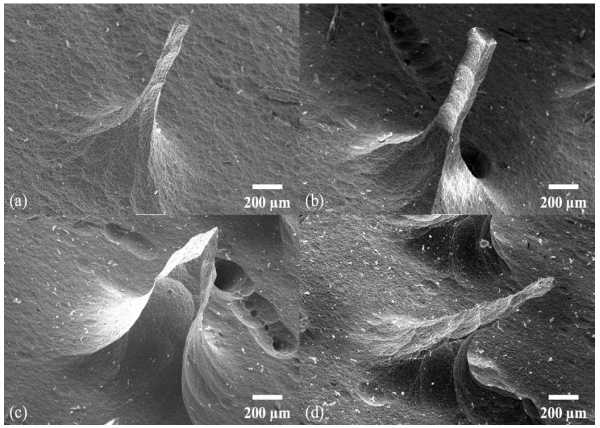
New fabrication methods by Kim Lab.

特許PCT/JP2018/035899

- Use the 3D printing to easily get the batch fabrication of MN array
- Make the dimension of the 3D printed needle shrink to micro-scale
- Active Pharmaceutical Ingredient (API) with MN for drug delivery



Stronger Biodegradable Microneedles with Coated various drugs

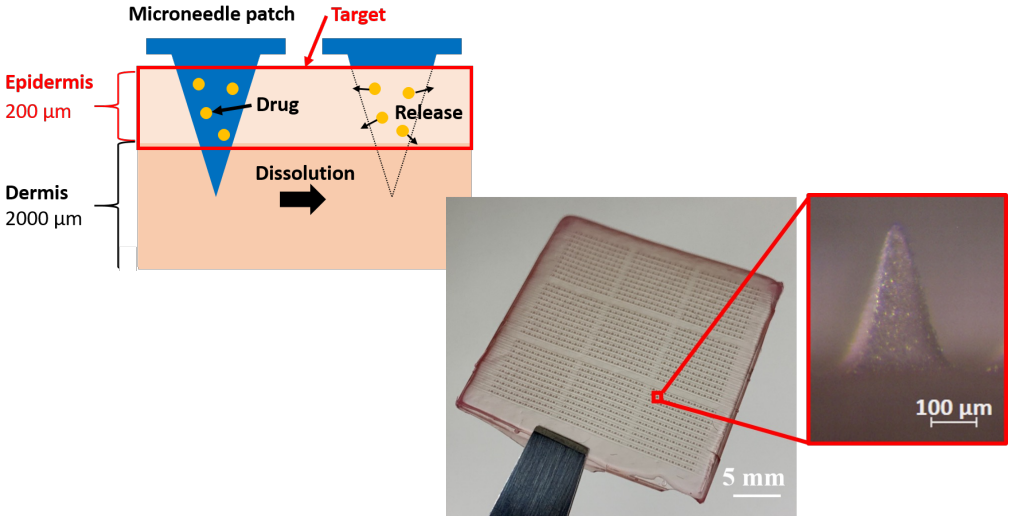


- Design of arbitrary shapes of needles
- Various lengths of needles
- Various materials & APIs loading

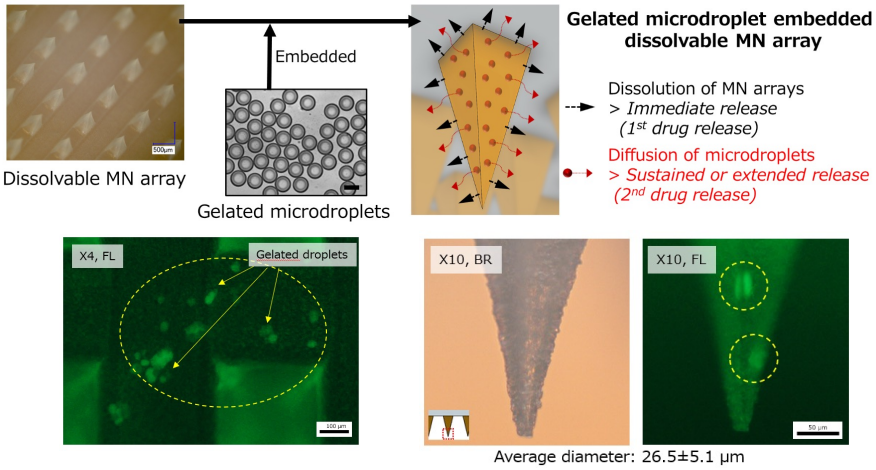
Microsystems & Nanoengineering 7:58 (2021)

Drug Delivery MNP (poke, and deliver)

- **Objective**
  Deliver active pharmaceutical ingredients (APIs) into skin in a non-invasive & effective way
  >> Innovate & Substitute conventional drug delivery using MNP technology
- **Keywords: vaccine, sustained drug release, dissolvable MN, droplet-embedded MN**
- **Current targets:** COVID-19 & other vaccines, antibiotics, sclerosis



COVID-19 Vaccine delivery MNP using vaccinia virus vector

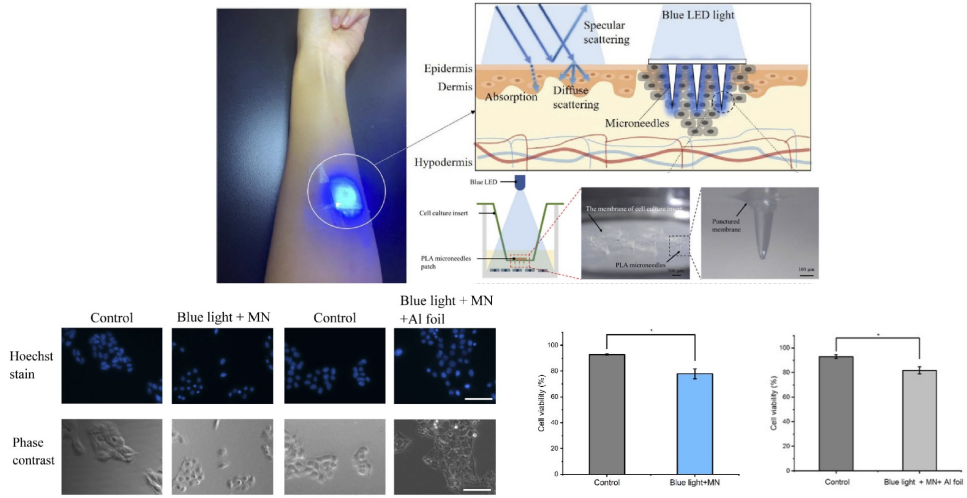


MNP with HA microdroplets embedded[2]

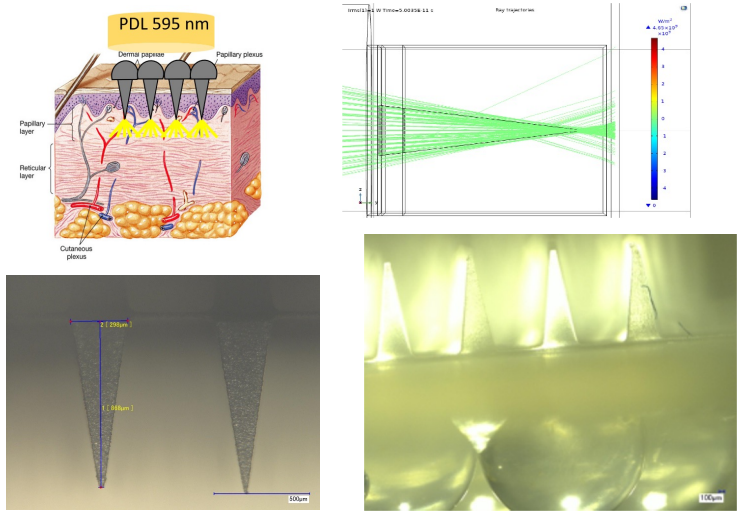
[1] The 13th Symposium on Micro-Nano Science and Technology, 15P2-PN-5 (2022), [2] JSPE Spring Meeting, G103 (2021)

Light (Optical) Therapy MNP (poke, and irridiate)

- **Objective**
  Develop MN array to treat skin-related diseases directly >> Realize fast, simple, and low-cost treatment / Establish novel light therapy (Photodynamic Therapy, PDT) using MNP
- **Keywords: light/optical therapy, photodynamic therapy (PDT), skin diseases**
- **Current targets:** melanoma, acne, telangiectasia, hair removal, and so on



Optical MNP to treat melanoma[1]



Optical MNP to treat telangiectasia[2]

[1] Biomedical Optics Express, Vol.13, Issue 2 (2022), [2] JSPE Spring Meeting, G0101 (2021)

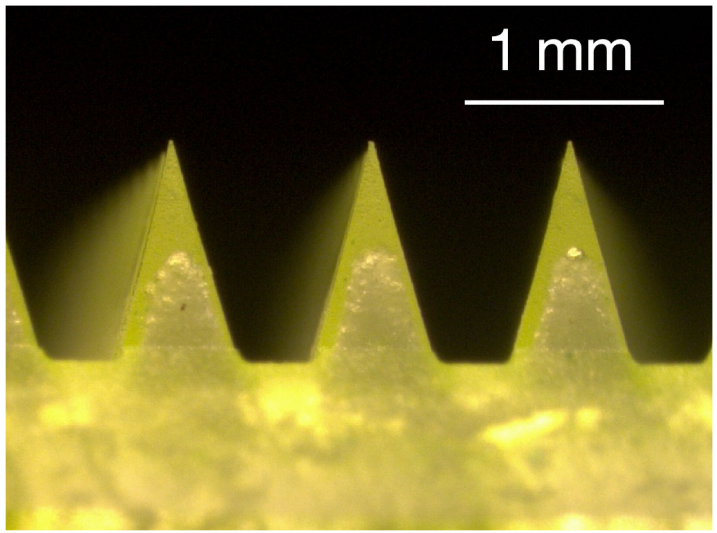
東京大学生産技術研究所
Institute of Industrial Science, The University of Tokyo

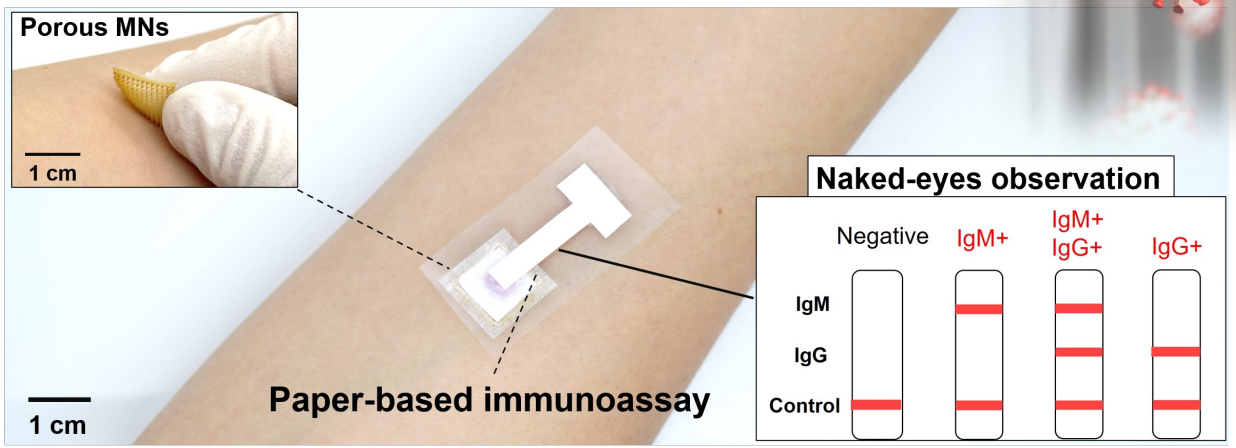
KIM Lab.

Main Topic

Porous Microneedles@B.J. Kim Lab.

Sensor – sampling by “Porous Needles”

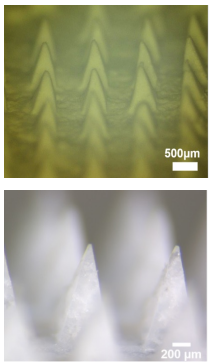




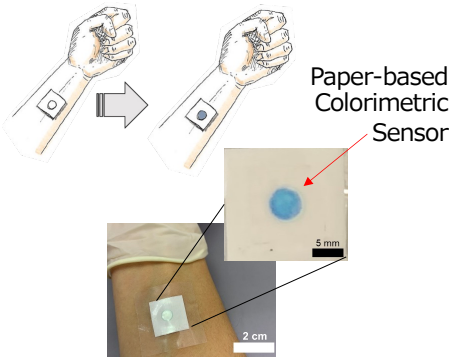
Scientific Reports, *12, 10693 (2022). https://doi.org/10.1038/s41598-022-14725-6*

Biosensor MNP (poke, extract, and analyze)

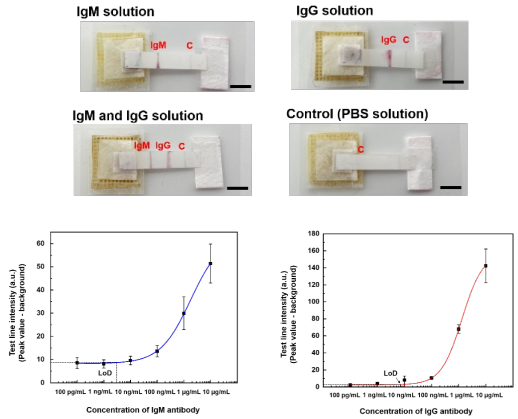
- **Objective**
  Develop MNP to extract interstitial fluid (ISF) & analyze ISF for sensing & monitoring the change of body functions >> Realize fast & simple diagnosis on site as healthcare device
- **Keywords: porous MN, capillary action, interstitial fluid (ISF), colorimetric sensing**
- **Current targets:** glucose, antibodies, cholesterol, cortisol, hormones, and so on



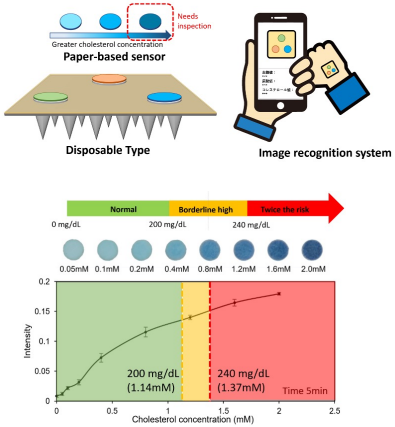
Porous MNP
(PLGA or PLA)



Glucose Sensing[1]



Sensing COVID-19
antibodies & concentration[2]

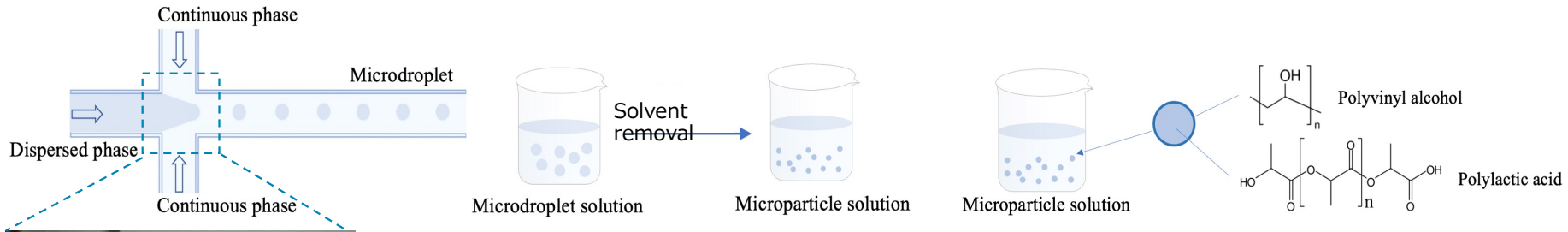


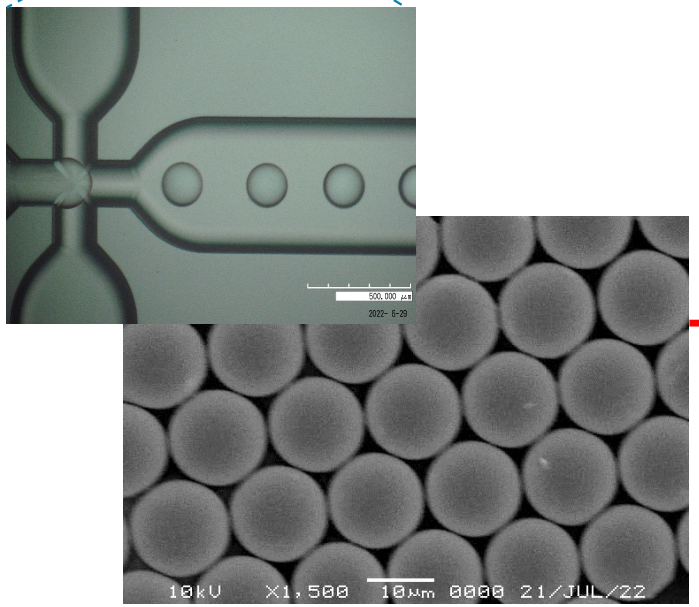
Sensing Cholesterol
Level[3]

[1] Medical Devices and Sensors, Vol.3, Issue 4, e10109 (2020), [2] Scientific Reports, Vol.12, 10693 (2022), [3] JSPE Autumn Meeting, G31 (2021)

Biosensor MNP (Porous MN details)

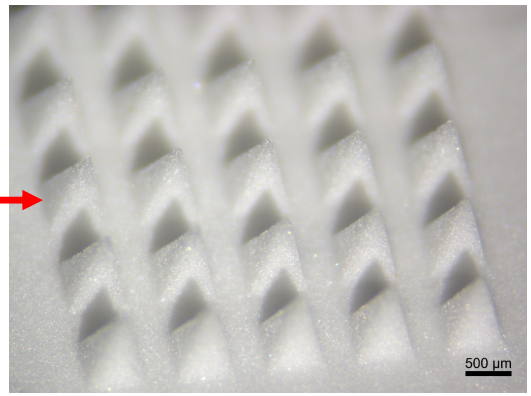
Microparticle preparation using microfluidic technology

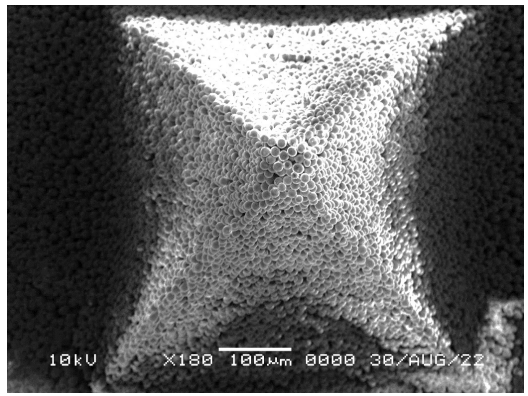




Fabricated PLA microparticles

Micro molding

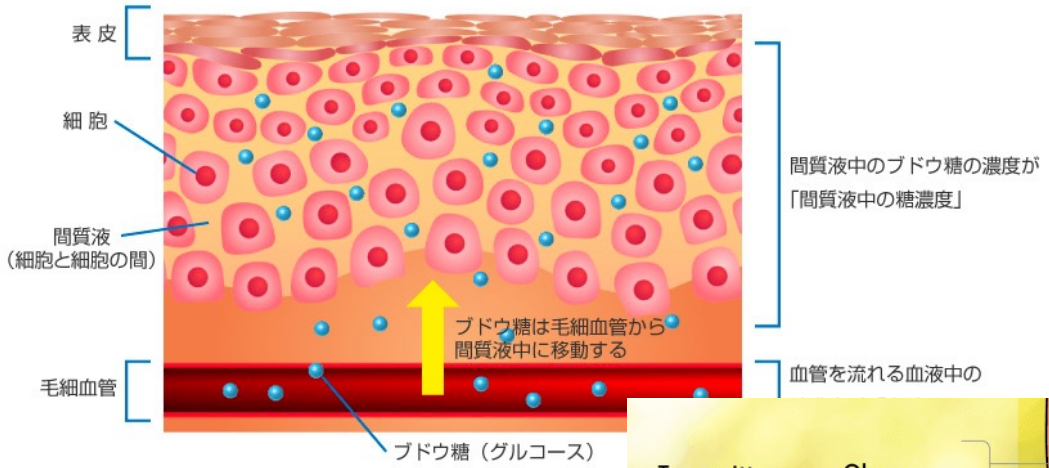


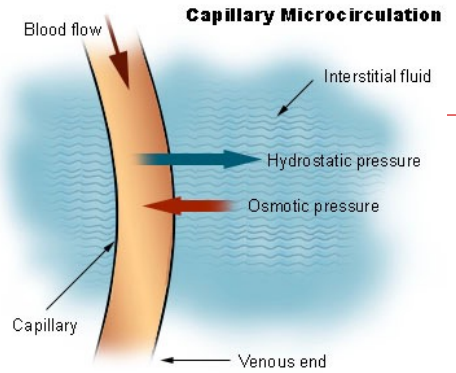


PLA porous MNP composed of bonded microparticles

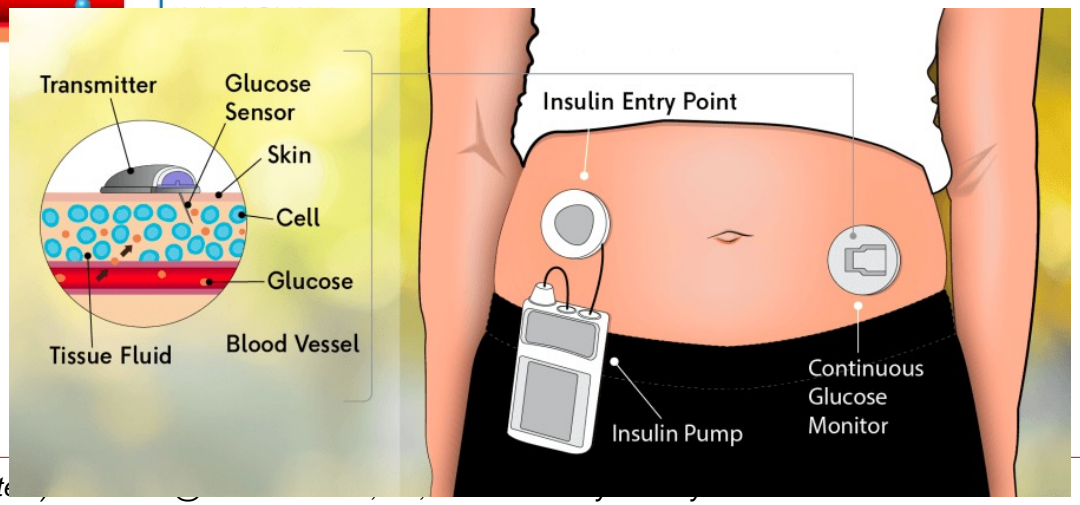
ISF (Interstitial fluid) 間質液

CGM (Continuous Glucose Monitoring)
FGM (Flash Glucose Monitoring)





Concentration range of Glucose
blood plasma: 2- 40 mM
ISF : 1.99-22.2mM
Saliva : 0.008-1.77mM
Sweat : 0.01-1.11mM
Tears : 0.05-5mM



血漿とは違う local 情報を得られる

Our Mission

- We believe that prevention is better than cure.
- Preventive solutions should be widely accessible, convenient, and accurate.

- Preventive medicine
- Regenerative medicine

⇩

Successful Aging

Health

Beauty

Measuring “People”

Clinical grade Accuracy critical

- Patient monitoring, diagnosis & therapy
- CT, X-ray, MRI, Ultrasound, Terahertz imaging
- Vital sign monitors, disease state, fatigue, hydration, etc.
- Implantable-neural implants, pacemakers

Occupational

- Factory or harsh conditions

Extreme Performance

- Military, Public Safety/Homeland Security, Professional Athletics
- Mentally & Physical demanding settings

Wearable sensors

Wellness/Fitness

- General personal use (information only)
- Non-critical relative accuracy

Hierarchy of accuracy

Wearable sensors

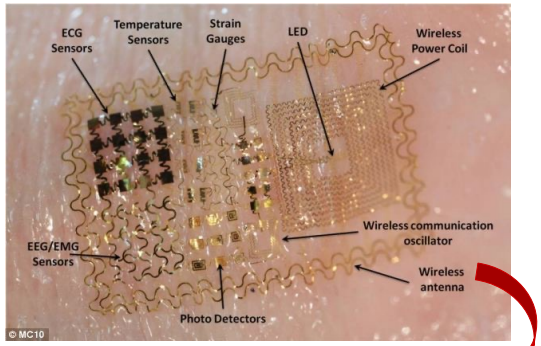
Motion-Tracking sensors

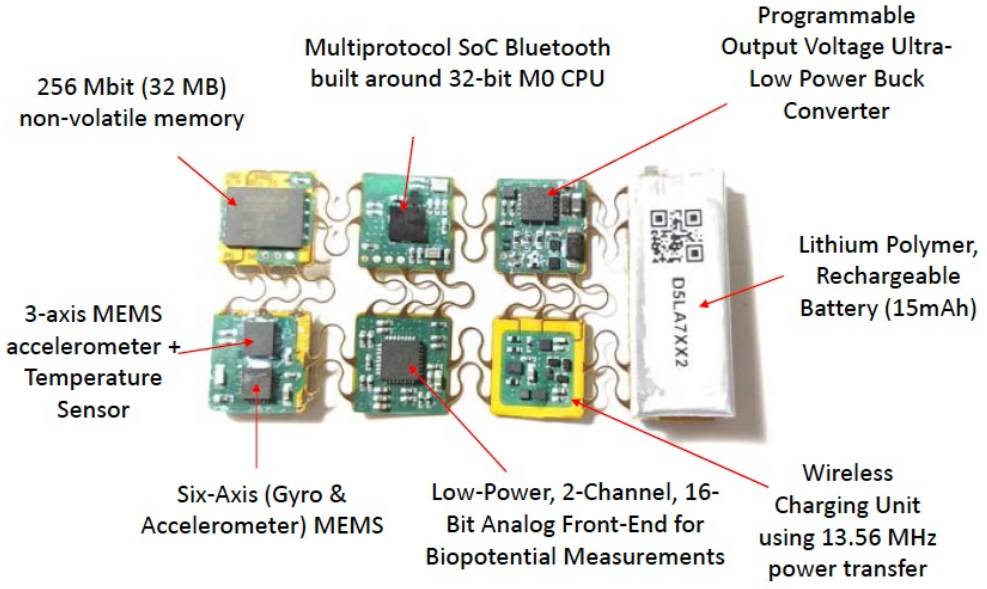
- Accelerometer, Gyro, Magnetometer
- GNSS(GPS, Galileo, Beidou, GLONASS)

Bodily Function sensors

- Heart rate, Pulse Oximetry
- Temperature
- Chemical/electrical: RF communication

@MC10 BioStamp





Bio markers -> Fluidic Biomarkers for smart bandages

	Measurement	Input Needed	Connection	Continuous
Temperature	Electrical	Voltage	Skin Contact Electrodes	Yes
Electrocradiogram	Electrical	Passive	Adhesive Electrodes	Yes
Photoplethysmograph	Optical	Light	Adhesive Sensor	Yes
Electrolytes	Potentiometry	Wicked Sweat/Blood	Wick	Yes
Blood Gasses	Amperometry	Capillary Blood	Microneedle	Yes
DNA Markers	DNA	Nucleic Acid Amplification/Fluid Sample	Swab/Tissue Sample	No
Protien Markers	Eletrochemical/Optical	Swabbed Blood/Sweat/Urine/Sweat	Swab	Maybe

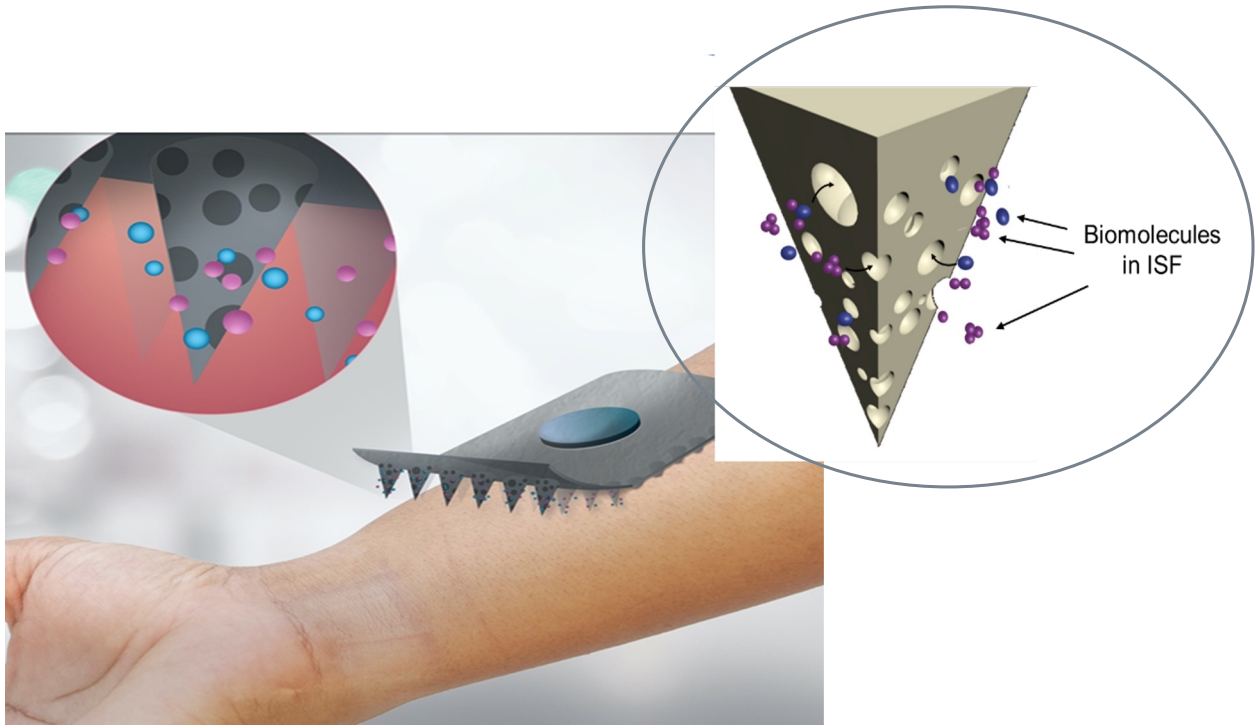
Biomarkers examples

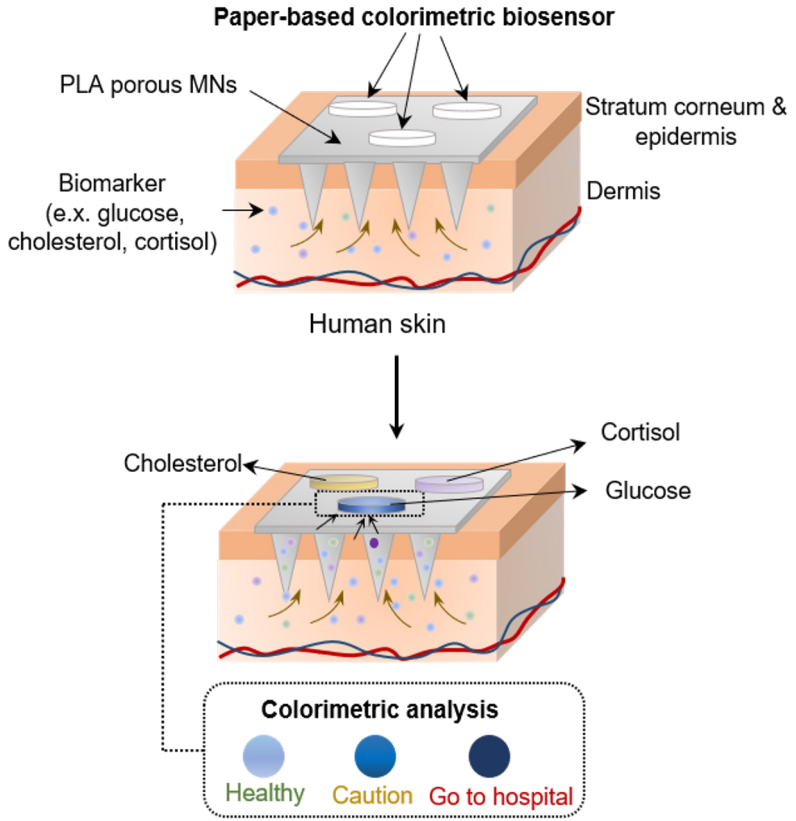
- Neuropeptides (NpY, Orexin A..), Catecholamines
- Cytokines, Corticosteroids
- PSMA/Antigens
- Glucono Lactone (glucose oxidase)
- Saccharide (boronic acid)

Good Point-of-Care Testing
For real diagnostics

Our Solution

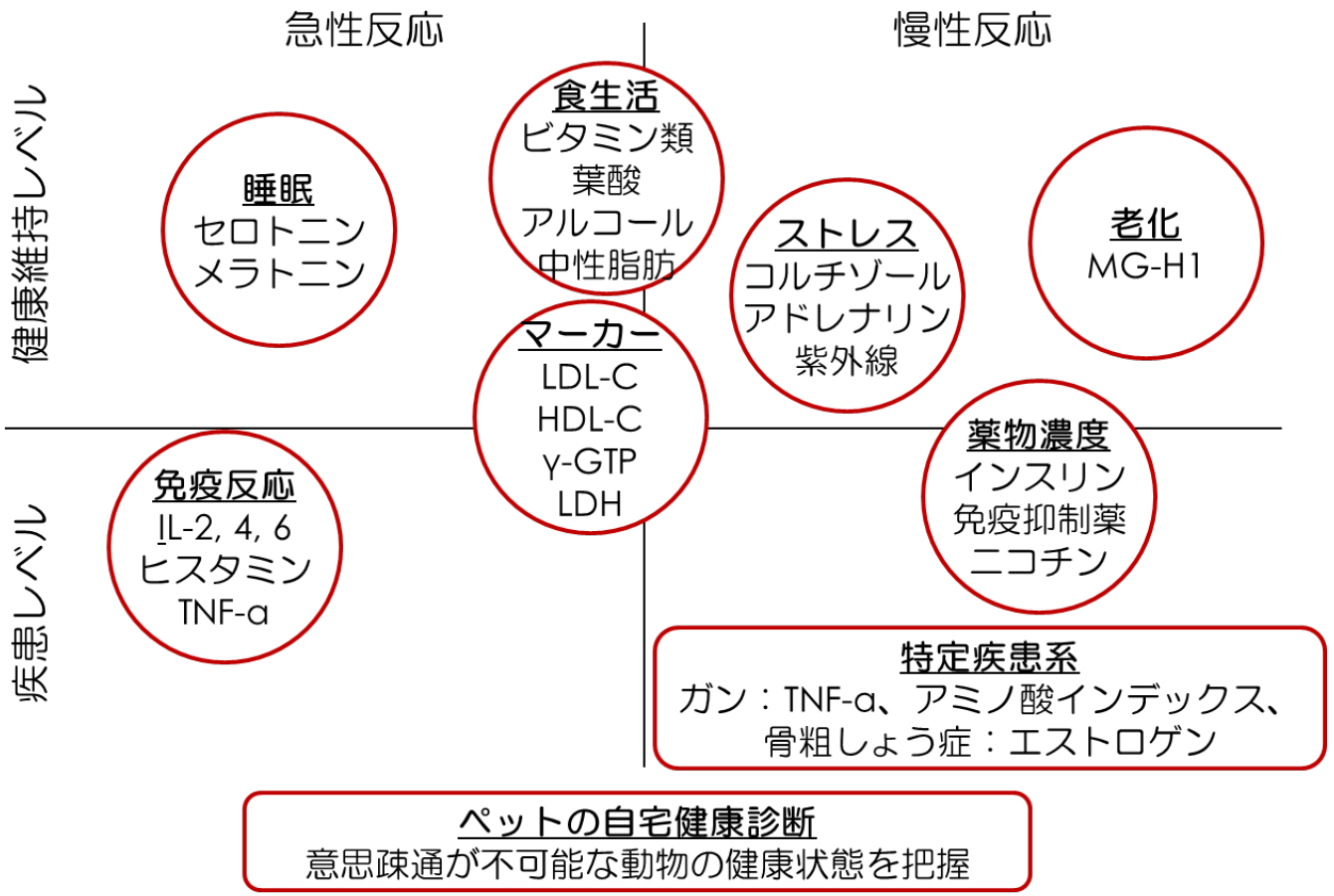
We make self-monitoring sensors for everyone.





BNS MEDICALS

健康・疾患評価に用いられているマーカーとその分類



Glucose sensor

to extract the interstitial fluid (ISF)

日経産業新聞　2020年（令和2年）8月31日（月曜日）

貼るシートで血糖値測定

東大、糖尿病予備軍を発見

東京大学生産技術研究所の金範埈教授らのチームは、微小な針を並べてシート状にした血糖値センサーを開発した。肌に貼り付けると体液が針を通して染み出し、その体液中のグルコースの濃度を測定する。皮膚に傷をつけず貼るだけで手軽に測定できる。まだ症状のない糖尿病の予備軍の人の発見などに活用できる可能性がある。今後は人での試験を進め、実用化につなげる予定だ。

糖尿病は悪化すると動脈硬化や失明などを引き起こす。2016年の国民健康・栄養調査によれば患者数は約1000万人とされ、予備軍も同数いるとみられている。未発症の予備軍を早期に見つけ、生活習慣の改善の指導につなげれば、患者数を減らせると期待される。

糖尿病の指標の血糖値は、一般に健康診断の血液検査で確認する。健診回数は限られている上、注射針を刺す際の痛みなども課題だ。糖尿病患者には、自分で血糖値を測定できるキットもあるが、医師の処方や扱うための訓練なども必要。低コストで血糖値を確認できる装置があれば、自分が糖尿病予備軍にあたるか簡単に調べられる。

金教授らは、化粧品用途の微小な針の作製技術を応用し、針をシート状にして、センサーと合体させた。痛みを感じないように針の長さは0・8ミリほどと短くした。材料には乳酸グリコール酸共重合体と呼ばれる体に蓄積しない高分子で作り、折れた際の安全性を高めた。

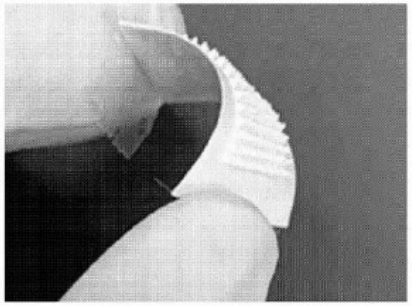
針のないシートの反対側には、グルコースの濃度を色の濃さで示せるセンサーをつけた。シートを強く押さなくても肌に刺さるよう、針の間隔を工夫して痛みを与えにくくした。

針は高分子と塩とを適切な割合で混ぜて固め、水につけて塩を溶かした。硬いがスポンジのような空洞を持つ構造にした。シートを皮膚に貼ると、表皮の体液が毛細管現象で針の空洞部分を伝い、センサーに到達する。表皮の体液は血液と関係しており、体液のグルコース濃度から血糖値を推定できるという。

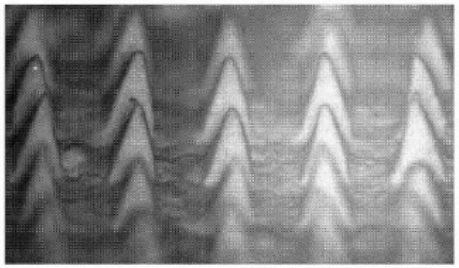
開発したシートを皮膚に似せたゲルに貼ってみた。ゲル内部のグルコース濃度を測れることを確認した。グルコース濃度を測るのに必要な液量を数秒で集められたという。

今後は人に貼って効果を調べる臨床研究を実施する予定。企業などと協力して早期の実用化を目指す。

血中成分の測定では、心筋梗塞などのリスクを高めるコレステロールや、男性ホルモンのテストステロンなども健康状態の判断に役立つ。センサー部を変えればこれらの物質も測定できる。自宅で測定できる健康管理グッズとしてほかの分野での応用も目指すという。（尾崎達也）



針を紙のシートに並べ、皮膚に貼り付けやすくした



開発した針には微細な空洞があり、染み出す体液を吸収する仕組みだ（写真はいずれも金教授提供）

談
家に問う

初の大規模患者調査始動

農地

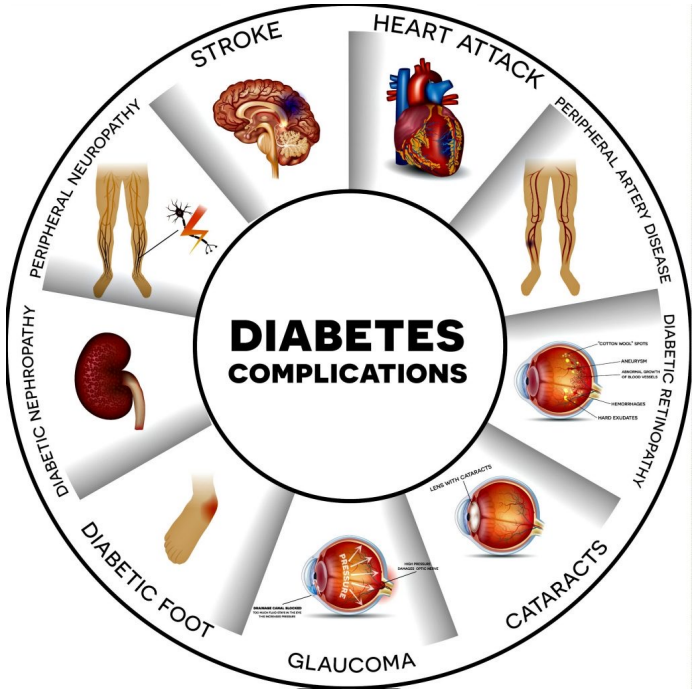
深紫

老年医学会、コロナ備え「人生会議」を

新型コロナウイルスに高齢者が感染すると、周囲と

既に動物実験に

Diabetes Mellitus



1 in 11

It is estimated that 415 million people are living with diabetes in the world

Diabetes is a leading cause of death and disability worldwide.

GROWING DANGER

Type 2 diabetes increasing in every country every year.
78,000 children develop type 1 diabetes every year

GOVERNMENT NEEDS

Diabetes caused at least 465 billion USD in healthcare
expenditures in 2011.
(11% total healthcare expenditures in adults)

More Problems

LIMITED DIAGNOSTIC SOLUTIONS

Current devices are expensive and obtrusive for pre-diabetes and diabetes patients. The blood collecting process is painful, requires administration, and nobody has the time for it these days.

80% ARE NOT AWARE

Approximately 88 million American adults—more than 1 in 3—have **pre-diabetes**. Of those with pre-diabetes, more than 80% don’t know they have it.

80% CAN'T AFFORD

Nearly 80% of people with diabetes live in low and middle-income countries. Current mass glucose monitoring solutions are expensive for governments .

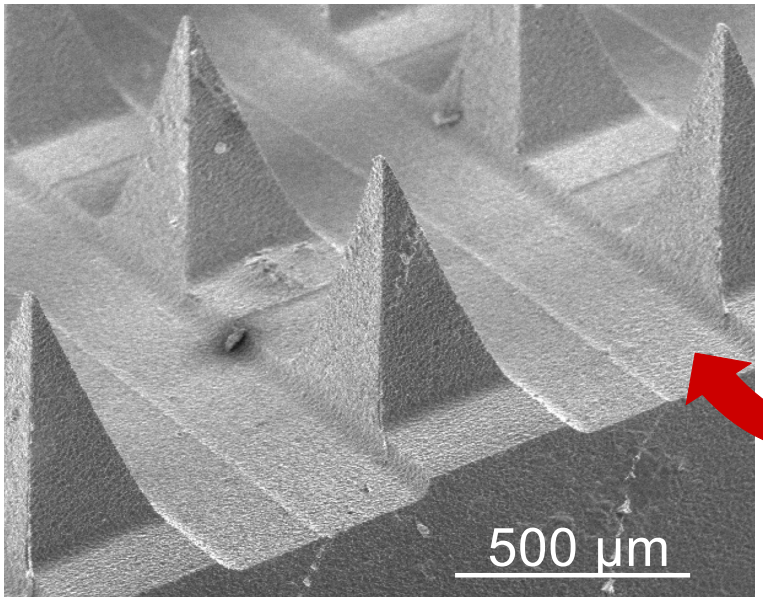
Why Now?

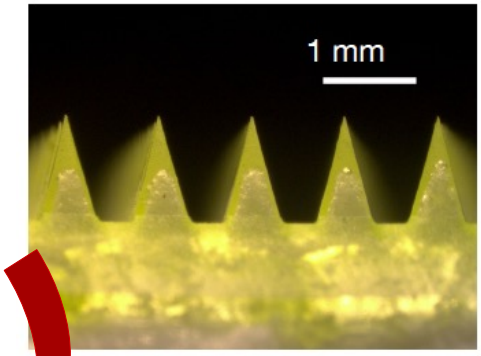
ACT BEFORE IT'S TOO LATE

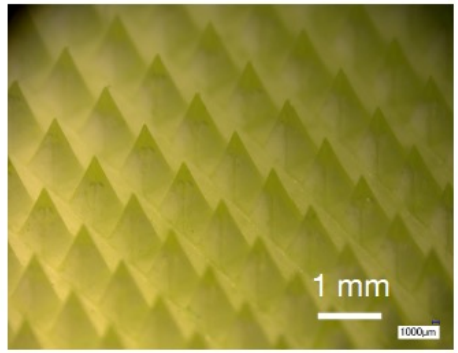
Worldwide diabetes can be treated and its consequences avoided or delayed with diet, physical activity, and medical treatments but most importantly: Regular Diagnostics.

key tech: Biodegradable, interconnected Porous Microneedles for Sensing

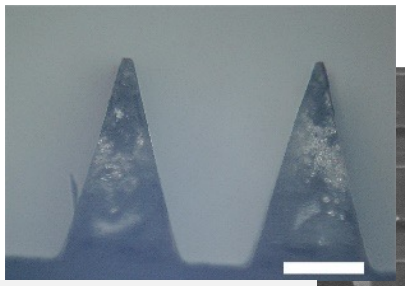
Innovation- Bio sensor patch:
Micro Lateral Flow chip to interface **porous MNs**

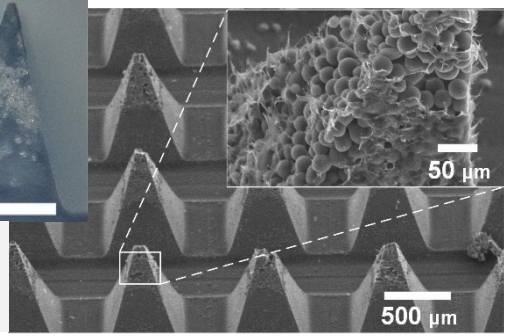






SEM picture of PDMS Porous MNs coated with HA





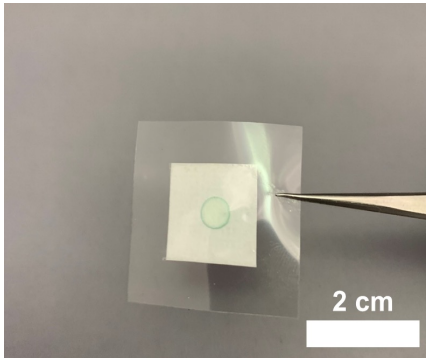
Biodegradable sphere(emulsion)-type

WO2019/176126 PCT/JP2018/020224
米国、欧州、韓国、日本、中国へ移行

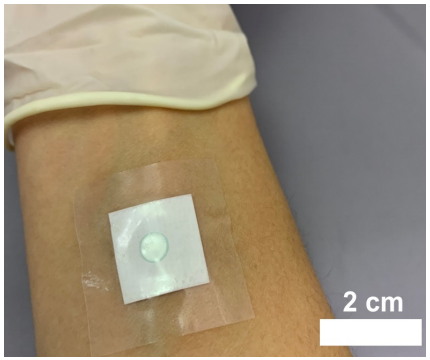
Application of device

Preparation

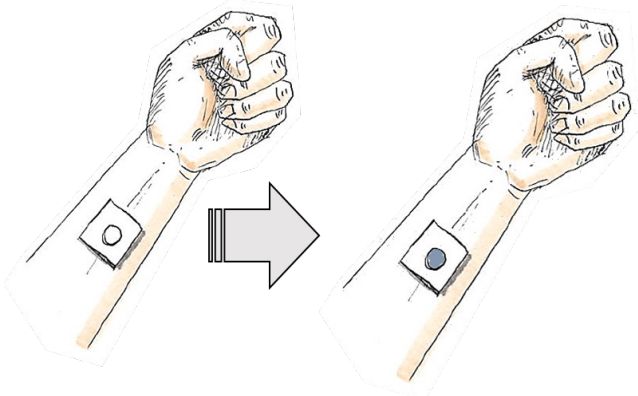
With adhesion tape



Attachment to the skin



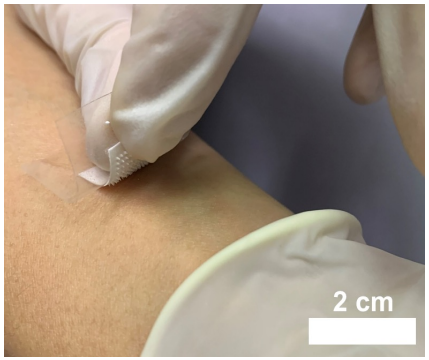
Attach and analysis



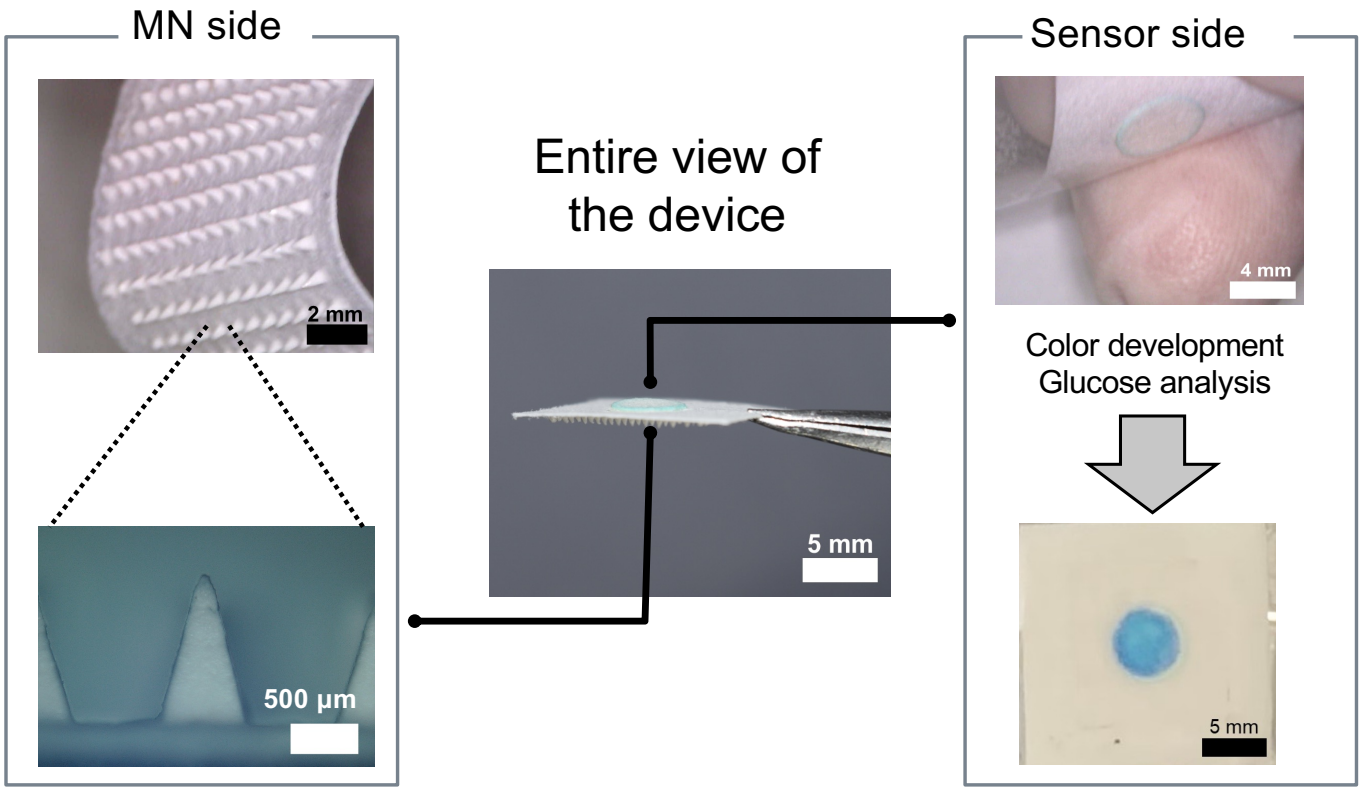
Alert to the user

Disposal

Release from the skin



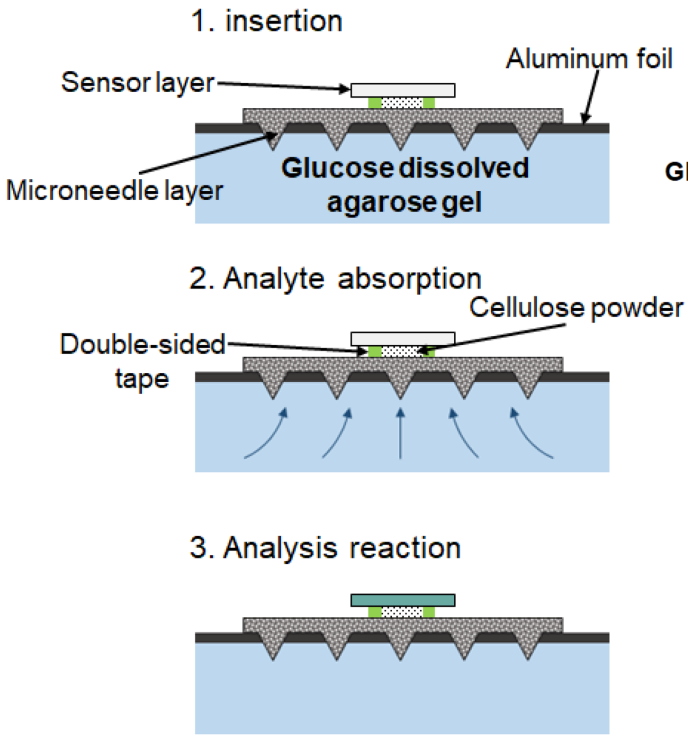
Results of fabrication



Research object 1 : porous MN
Research object 2 : PLGA biodegradable polymer

Research object 3 : Integration with paper-based sensor

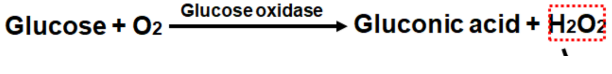
(a)

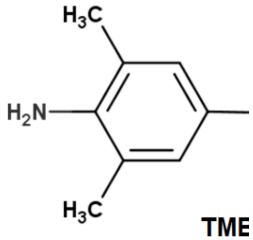


(b)

既に動物実験に

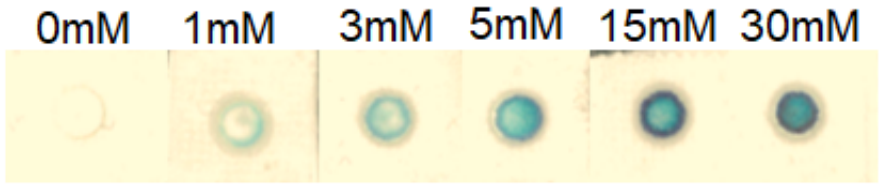
Glucose oxidase, peroxidase (2 enzyme) and TMB dye

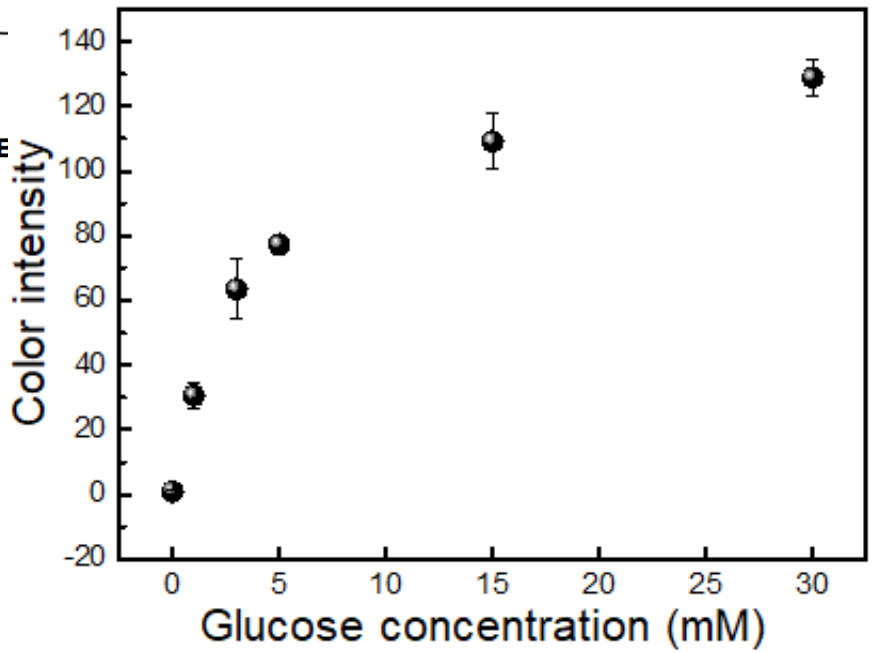




TME

37

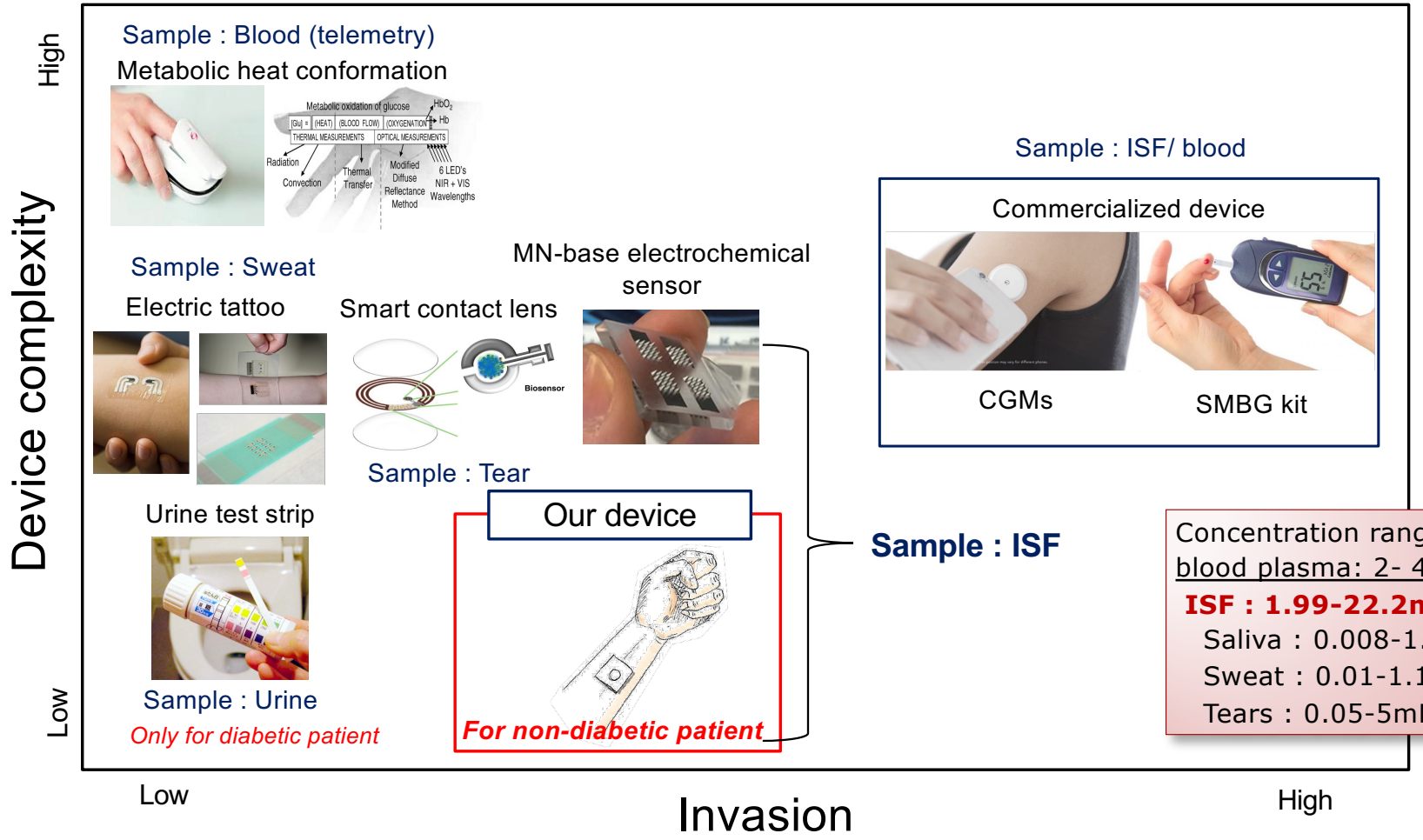




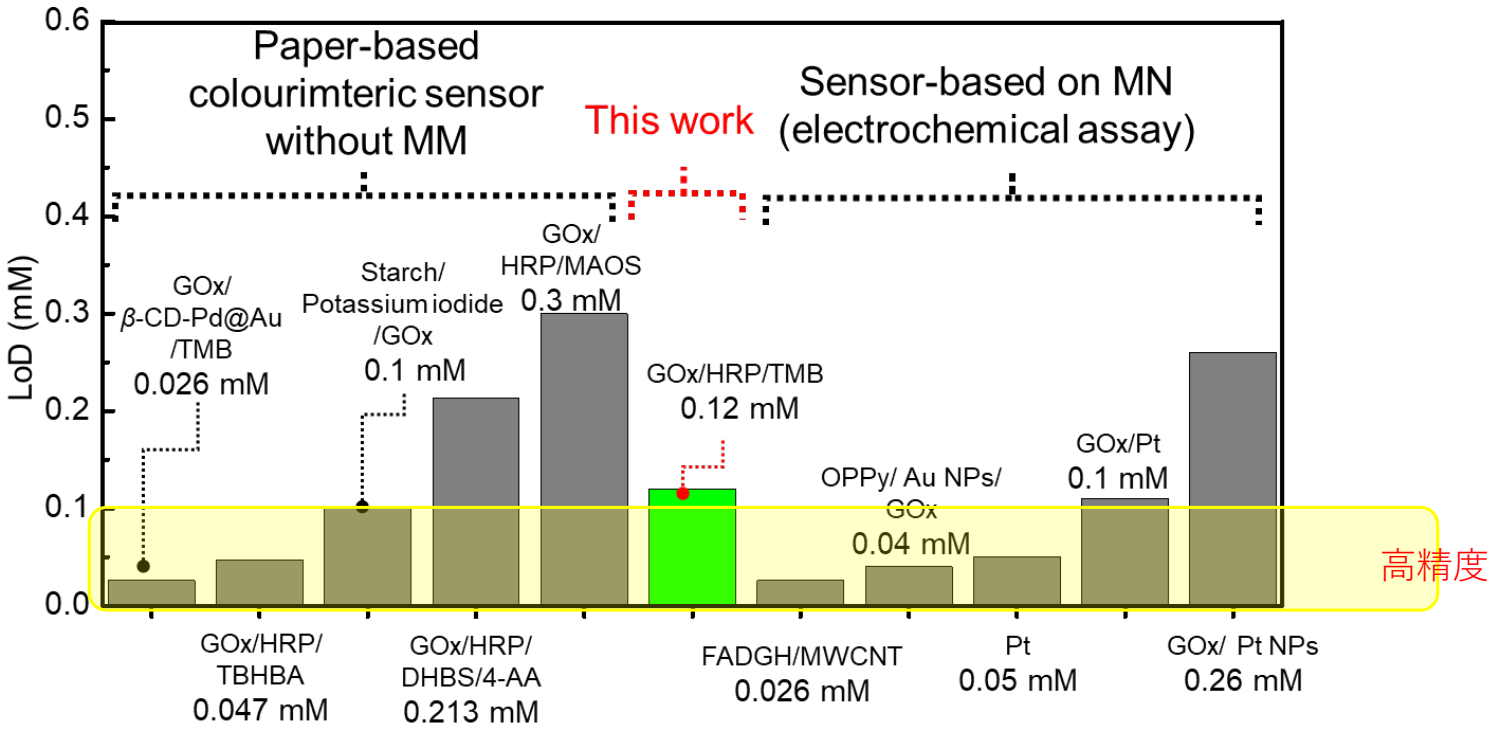
- Capillary driven(毛細管力)- 35 nL /min./一本
- Within 2 min.
- Non-diabetic individual < 6.9 mM

Medical Devices & Sensors **2020** *(July)*
Collaboration with Minami Lab.

Comparisons with previous study on glucose monitoring



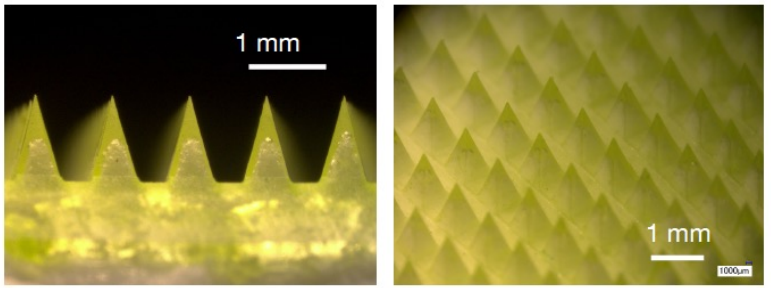
Comparison of with previously developed glucose sensor

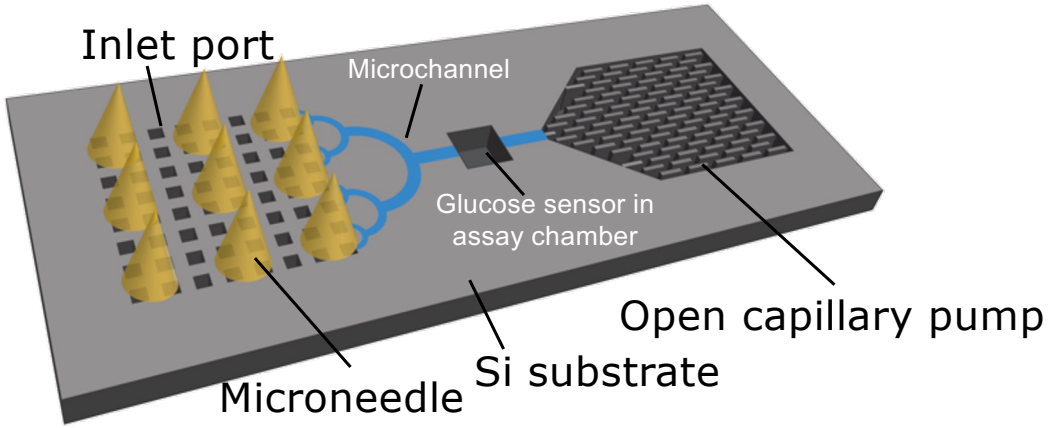


- The fabricated and applied sensor in this work has a satisfying LoD compared to previous research
- The device proposed in this work has as an advantage in usability compare to other sensor

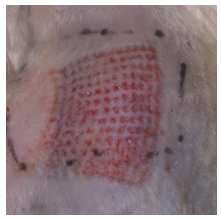
Continous Glucose sensor patch:Microfluidic chip to interface **porous MNs**

- Requirements
  - Flat surface to support the porous MNs
  - Inlet ports to extract the interstitial fluid (ISF)
  - Microchannels to transport the fluid

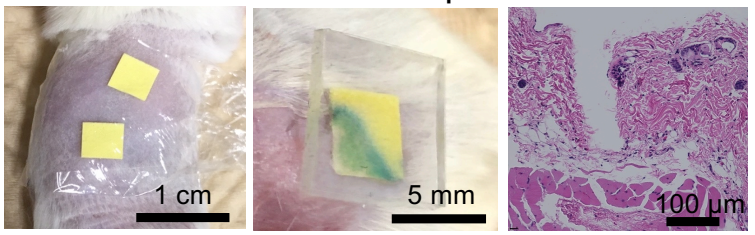




In vivo-animal test



Punctuated pores



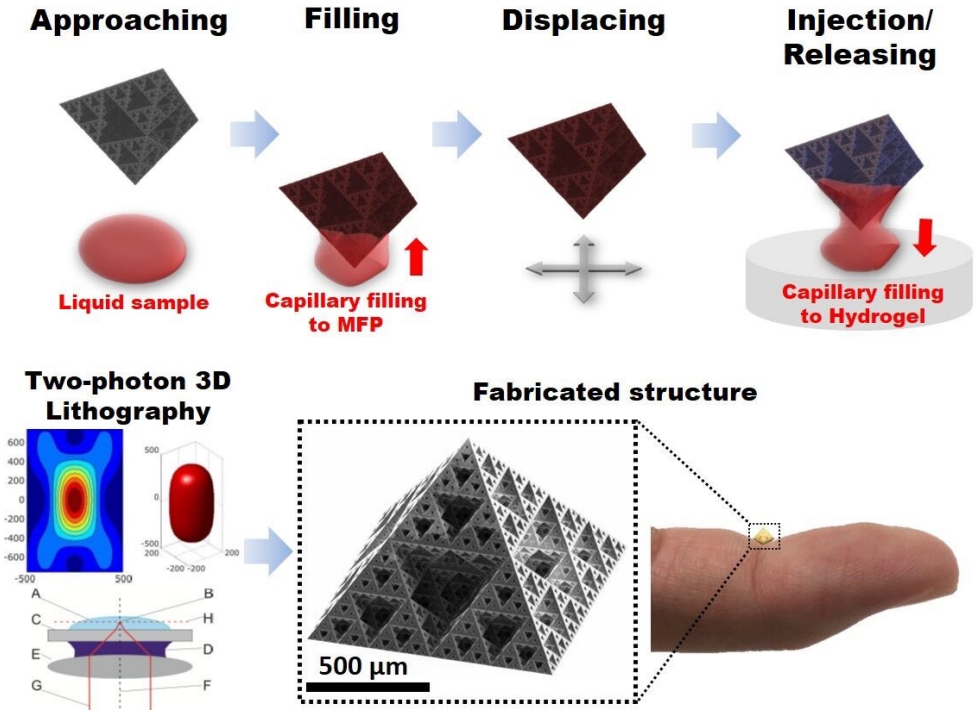
Negative control | Positive control | Incised skin

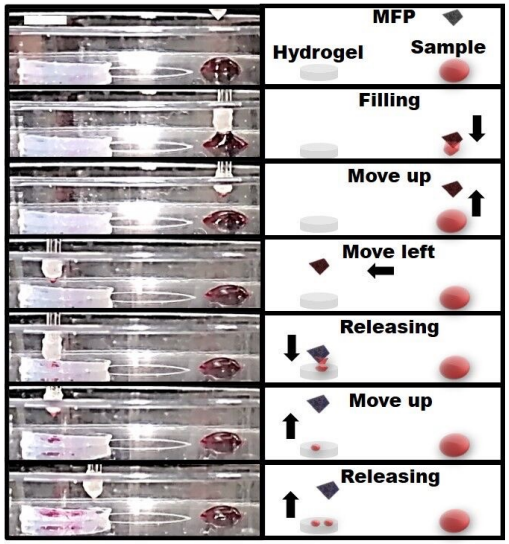
特許PCT/JP2018/020224

Biomedical Microdevices, 21, Vol.28 (2019)

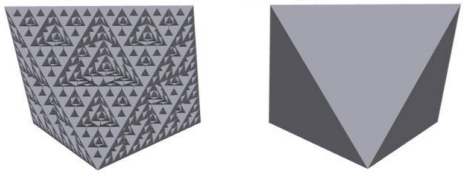
Porous Needle -> 3D Micro Fractal Pipettes for liquid sample handling

Capillary force





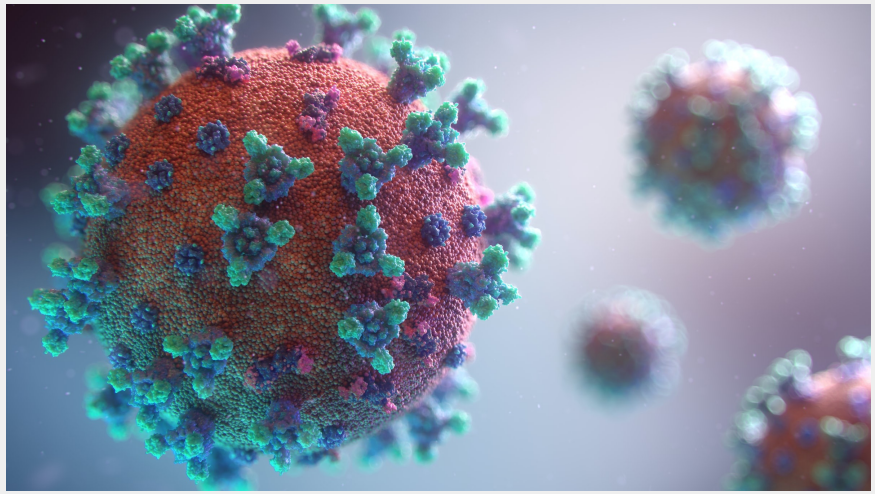
Comparison between fractal pyramid and solid pyramid



	Fractal Pyramid
Surface area	> 8.5x
Volume/Mass	< 0.3x
Absorbability	> 28x
Porosity	78% (185nL)
Inlet opening	76% (1.32mm^2)

Review of Scientific Instruments, 91, 086104, 2020
https://doi.org/10.1063/5.0018456

Porous Microneedles



Diagnosing COVID-19

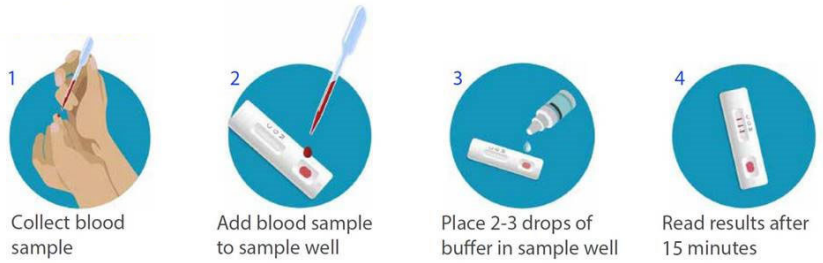
Tool for Painless, Rapid Detection

Scientific Reports, 10.1038/s41598-022-14725-6, 2022

BNS MEDICALS

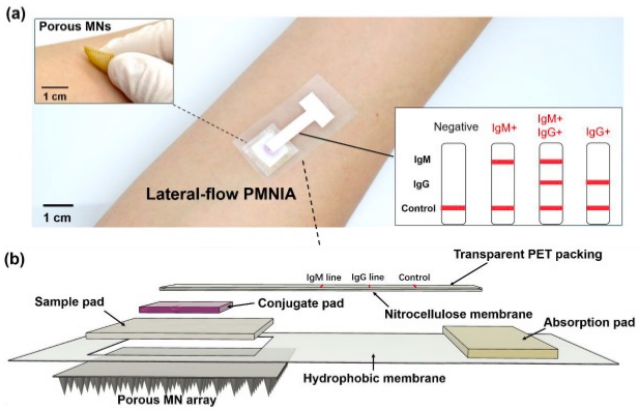
2．マイクロニードルパッチの展開:
新型コロナウイルス抗体検査パッチ

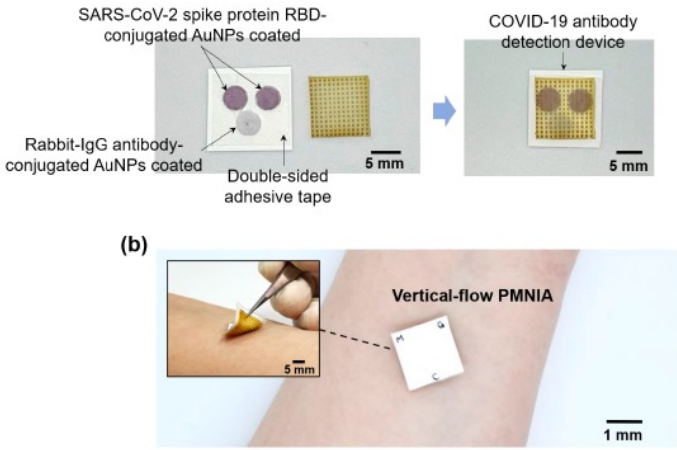
従来の検査キット
(ラテラルフロー)



採血

マイクロニードル抗体検査





- 従来の検査デバイスをパッチ内に実装（動物実験成功）
- 測定原理は免疫学的検定→将来の感染症にも有効

BNS MEDICALS

2021.3.23 TV 朝日
2021.3.24 TBS

日本経済新聞 記事利用について

東大、新型コロナウイルス感染症の無痛・迅速診断パッチを開発

2022/7/1 18:01 | 日本経済新聞 電子版

発表日:2022年07月01日

新型コロナウイルス感染症の無痛・迅速診断パッチの開発

――マイクロニードルを用いた、貼るだけの抗体検出へ――

1.発表者：

金 範●（◇）（東京大学 生産技術研究所 教授）

鮑 蕾蕾（東京大学 大学院工学系研究科 精密工学専攻 博士課程3年）

◇教授名の正式表記は添付の関連資料を参照

2. 発表のポイント：

◆皮膚内の体液から、新型コロナウイルスに対する抗体（IgMおよびIgG）を検出しうることを初めて示した。また、皮膚内で分解する多孔質マイクロニードルの作製方法を新たに開発した。

◆開発した多孔質マイクロニードルと抗原抗体反応を組み合わせ、既存の検出キットと同等以上の感度を示す、これまでにないパッチ型の抗体検出デバイスを開発した。

◆パッチ型抗体検出デバイスは小型かつ低侵襲（無痛）で、皮膚に貼るだけで使用でき、将来的にはさまざまな感染症の迅速なスクリーニングへの応用が期待される。

3. 発表概要：

東京大学 生産技術研究所の金 範● 教授、大学院工学系研究科 精密工学専攻 博士課程3年の鮑 蕾蕾 大学院生らの研究グループは、従来の注射針を用いた採血に代えて、皮膚に貼るだけで抗体検出ができる、多孔質マイクロニードル（注1）とイムノクロマトアッセイ（注2）を組み合わせた新しいパッチ型抗体検出デバイス（図1、Porous MicroNeedle and

https://www.iis.u-tokyo.ac.jp/ja/news/3908/

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Anti-SARS-CoV-2 IgM/IgG antibodies detection using a patch sensor containing porous microneedles and a paper-based immunoassay

Leilei Bao, Jongho Park, Boyu Qin & Beomjoon Kim✉

Infectious diseases are among the leading causes of mortality worldwide. A new coronavirus named severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) was identified in Wuhan, China in 2019, and the World Health Organization (WHO) declared its outbreak, coronavirus disease 2019 (COVID-19), as a global pandemic in 2020. COVID-19 can spread quickly from person to person. One of the most challenging issues is to identify the infected individuals and prevent potential spread of SARS-CoV-2. Recently, anti-SARS-CoV-2 immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody tests using immunochromatographic methods have been used as a complement to current detection methods and have provided information of the approximate course of COVID-19 infection. However, blood sampling causes pain and poses risks of infection at the needle puncture site. In this study, a novel patch sensor integrating porous microneedles and an immunochromatographic assay (PMNIA) was developed for the rapid detection of anti-SARS-CoV-2 IgM/IgG in dermal interstitial fluid (ISF), which is a rich source of protein biomarkers, such as antibodies. Biodegradable porous microneedles (MNs) made of polylactic acid were fabricated to extract ISF from human skin by capillary effect. The extracted ISF was vertically transported and flowed into the affixed immunoassay biosensor, where specific antibodies could be detected colorimetrically on-site. Anti-SARS-CoV-2 IgM/IgG antibodies were simultaneously detected within 3 min in vitro. Moreover, the limit of detection of anti-SARS-CoV-2 IgM and IgG concentrations was as low as 3 and 7 ng/mL, respectively. The developed device integrating porous MNs and immunochromatographic biosensors is expected to enable minimally invasive, simple, and rapid anti-SARS-CoV-2 IgM/IgG antibody testing. Furthermore, the compact size of the MN and biosensor-integrated device is advantageous for its widespread use. The proposed device has great potential for rapid screening of various infectious diseases in addition to COVID-19 as an effective complementary method with other diagnostic tests.

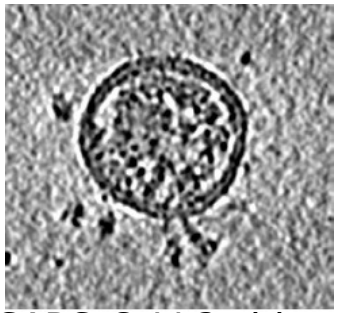
At the end of 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified, which causes coronavirus disease 2019 (COVID-19). It spread worldwide within 3 months owing to its high infectivity[1,2]. In March 2020, the World Health Organization (WHO) announced the COVID-19 outbreak as a global pandemic[3]. A COVID-19 infection spreads quickly from person to person and its symptoms include fatigue, cough, fever, dyspnea, anosmia, and ageusia; more severe symptoms include respiratory insufficiency, which can be life-threatening[4,5]. Furthermore, the rate of asymptomatic infections is reported as 16–38%, which brings difficulties in identifying all the individuals with SARS-CoV-2 infected[6]. COVID-19 vaccines are effective in reducing infection risk and virus transmission; however, the proportion of the population fully vaccinated against COVID-19 remains less than 10% in several low-income countries[7,8]. Therefore, one of the current global challenges is to identify both symptomatic and asymptomatic patients as soon as prevent potential spread of SARS-CoV-2.

Currently, real-time reverse transcription polymerase chain reaction (RT-PCR) is the predominant detection method and remains the gold standard for COVID-19 diagnosis[9]. However, there are certain drawbacks

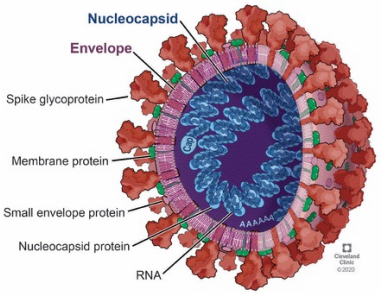
Institute of Industrial Science, The Univeristy of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan. ✉email: bjoonkim@iis.u-tokyo.ac.jp

Nature "Scientific Reports", 10.1038/s41598-022-14725-6, 2022

SARS-CoV-2 & Diagnosis



SARS-CoV-2 virion
[Ke, Z, 2020]



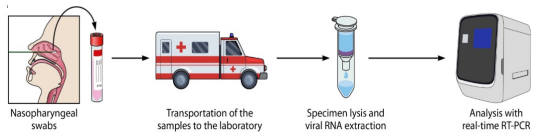
A New coronavirus was identified in Dec, 2019:
Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2)

- High Transmission rate
- Severe symptoms
- High asymptomatic rate

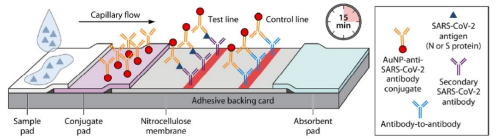
→ ***Global challenge***
Diagnose & quarantine positive viral carriers

Current COVID-19 diagnostics

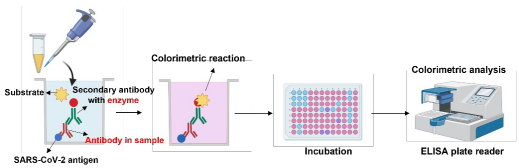
- Viral detection (RNA, antigen) → Presence of virus
  - *Samples from Respiratory secretion*
  - Real-time reverse transcription polymerase chain reaction (RT-PCR)
  - SARS-CoV-2 antigen lateral-flow immunoassay (LFIA) test



[Safiabadi Tali, S. H.,2021]



- Antibody detection → Determine infection stages & Measure antibody level
  - ***Blood sampling***
  - SARS-CoV-2 Abs LFIA test
  - Enzyme-linked immunosorbent assay (ELISA)
  - Paper-based electrochemical biosensor



[Dhamad, A. E.,2020]

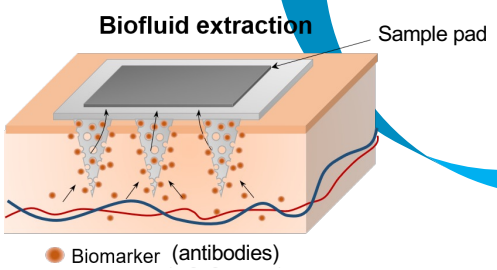
Porous MNs for SARS-CoV-2 diagnosis

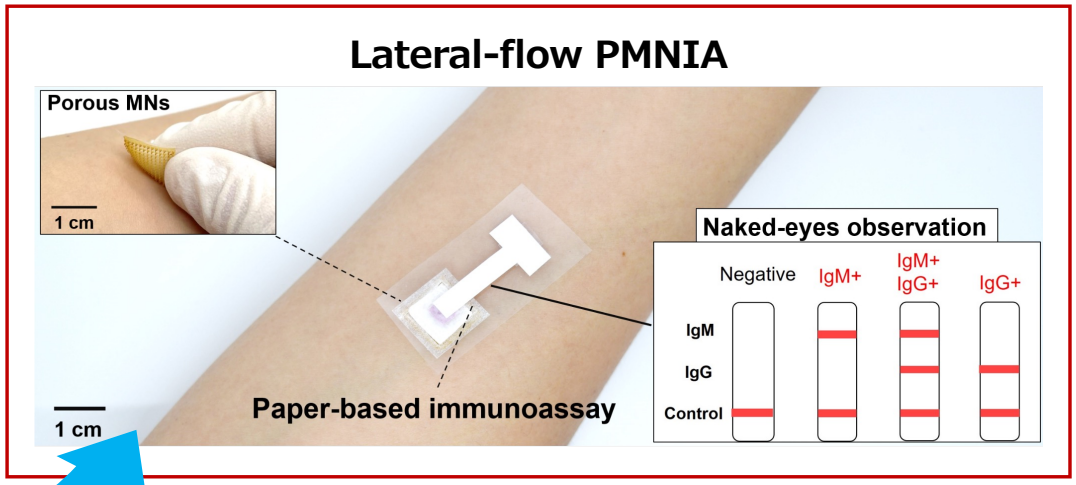
By Leilei BAO (Ph.D.) et al.
Scientific Reports, Vol.12, 10693 (2022)

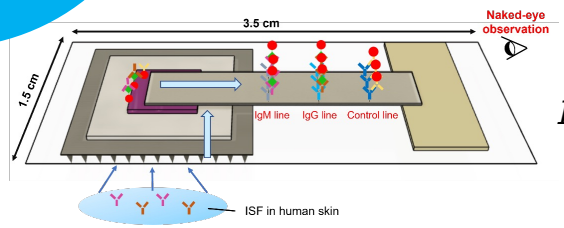
- Developed a portable and self-applied device for minimally-invasive & rapid detection of anti-SARS-CoV-2 IgM and IgG in dermal ISF.
- Integrated immunochromatographic assay for target Abs detection using porous MNs for painless ISF extraction.

Porous MNs (self-admin. & minimally invasive)

ISF extraction (antibodies' sampling)





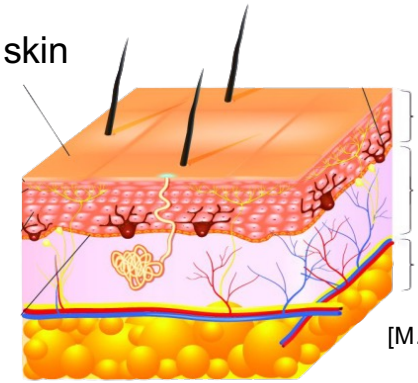


Lateral-flow Immunochromatographic Assay (detection & quantification)

Antibody detection via interstitial fluid (ISF)

- **ISF sampling**

Human skin



[M. Leone, 2017]

Dermal ISF is formed by blood transcapillary filtration and primarily located in epidermis and dermis layer

Abundant biomarkers in skin ISF: proteins (e.g. **human antibodies**) [M. Eigenmann, 2017]

Anti-SARS-CoV-2 IgG level in blood (Target IgM level is similar to IgG)

- Onset of symptoms: **331 ng/mL–25.7 μg/mL** [Ibarrondo, F. J., 2020]
- Convalescent serum: **7 ng/mL–2100 ng/mL** [Tan, X., 2019]

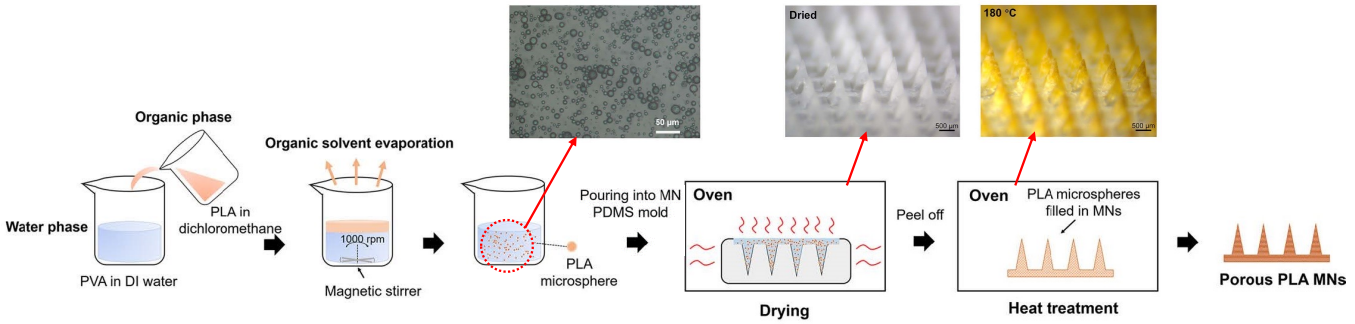
15–25% antibody level in ISF [J.Heikenfeld, 2019]

Anti-SARS-CoV-2 IgM/IgG level in ISF: 1 ng/mL–6.4 μg/mL

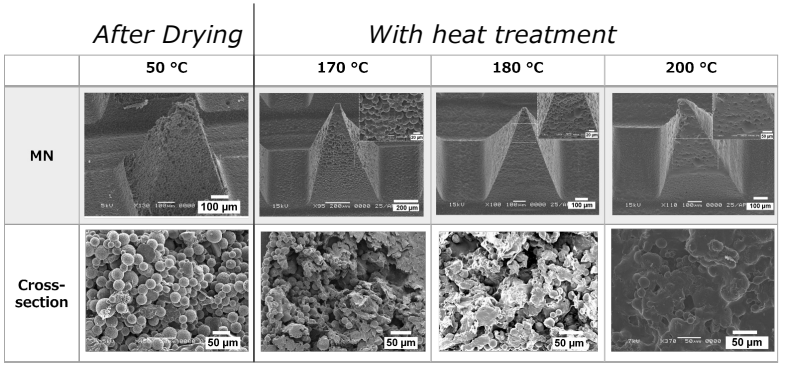
Target: anti-SARS-CoV-2 IgM and IgG in ISF

PLA Porous MNs

Fabrication using single emulsion method

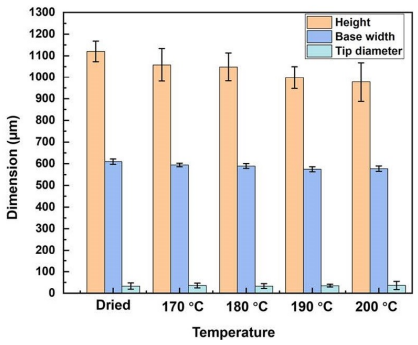


Schematic diagram of fabrication process

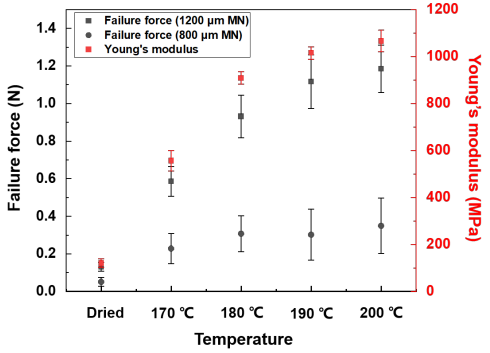


SEM images of PLA porous MNs

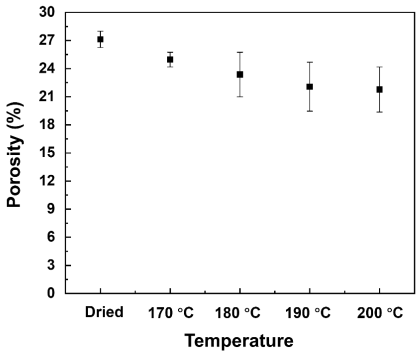
Evaluation results of PLA porous MNs



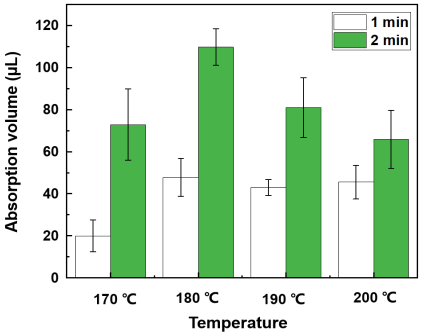
Dimensions of porous PLA MNs after heat treatment



Failure force & calculated Young's modulus



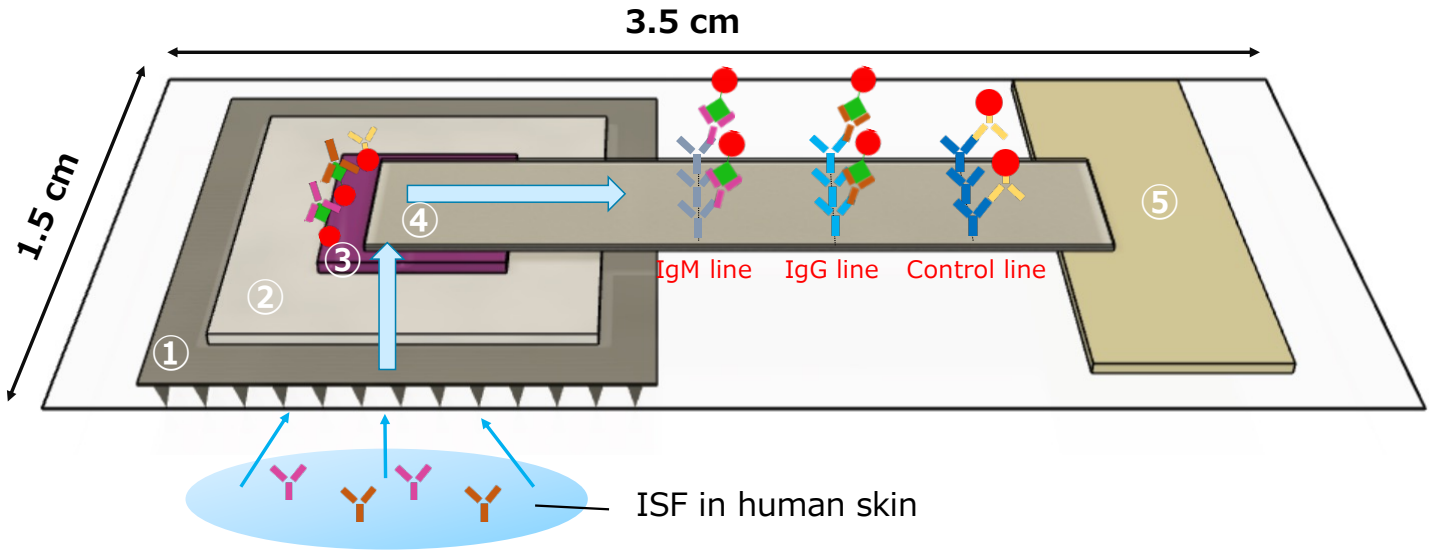
Porosity of porous MNs by water imbibition method



Absorption evaluation (1% (w/v) agarose gel, for 1 min & 2 min)

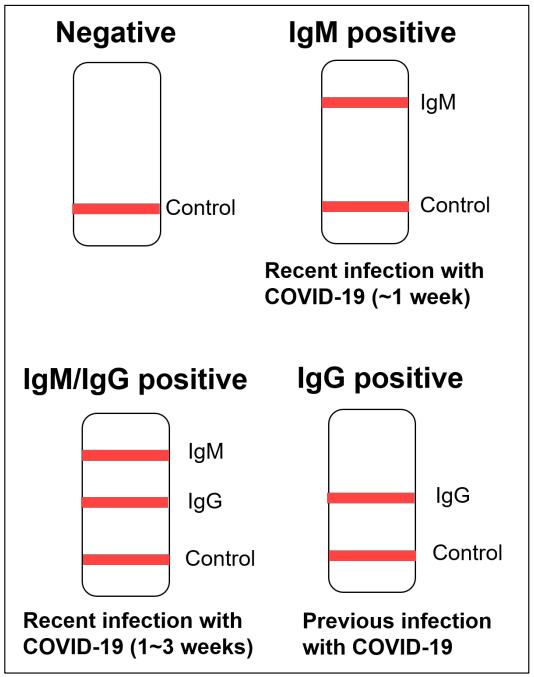
Porous Microneedles & immuNochromatographIc Assay

Working principle of lateral-flow PMNIA



① Porous MNs	② Sample pad	③ Conjugate pad	④ NC membrane	⑤ Absorption pad
				Excess AuNPs

Interpretation of results



Human Anti-SARS-CoV-2 IgM
Human Anti-SARS-CoV-2 IgG
COVID-19 antigen-conjugated AuNPs
Rabbit-IgG-conjugated AuNPs (for Control check)

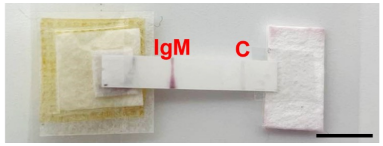
Capture bioreceptor

Mouse anti-human IgM antibody
Mouse anti-human IgG antibody
Goat anti-rabbit IgG antibody

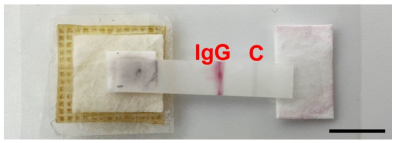
Evaluation of lateral-flow PMNIA

Anti-SARS-CoV-2 IgM & IgG detection

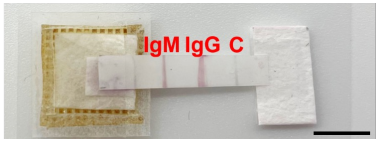
(a) IgM positive



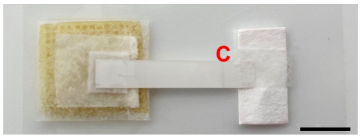
(b) IgG positive



(c) IgM & IgG positive



(d) Negative



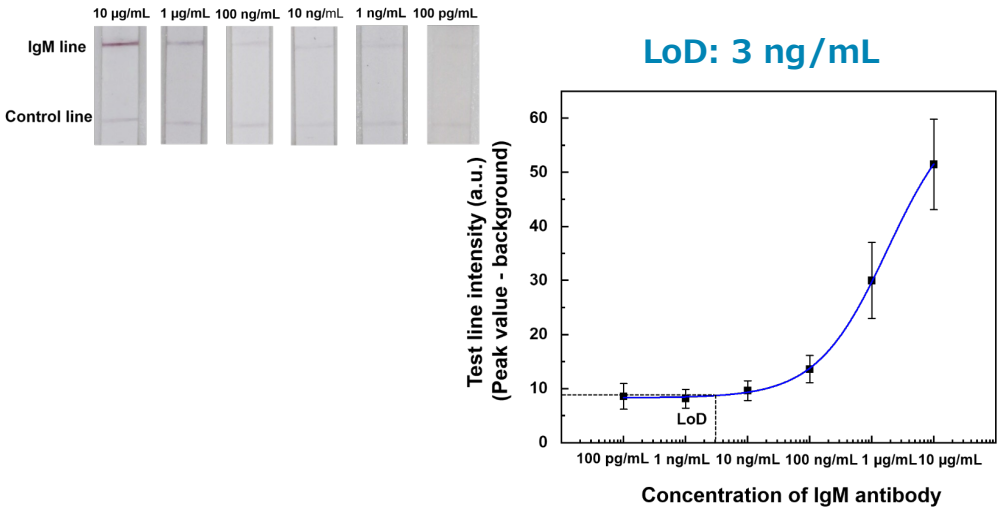
* Scale bar: 5 mm

a. 5 μg/mL anti-SARS-CoV-2 IgM antibody; **b**. 5 μg/mL anti-SARS-CoV-2 IgG antibody;

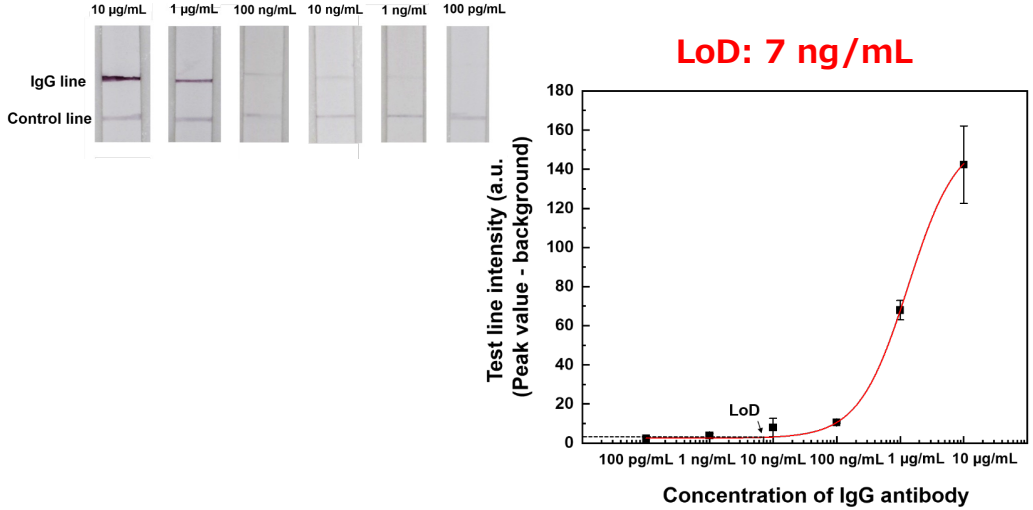
c. mixture of 5 μg/mL anti-SARS-CoV-2 IgM and IgG antibody solution; **d**. PBS solution

Limit of Detection (LoD)

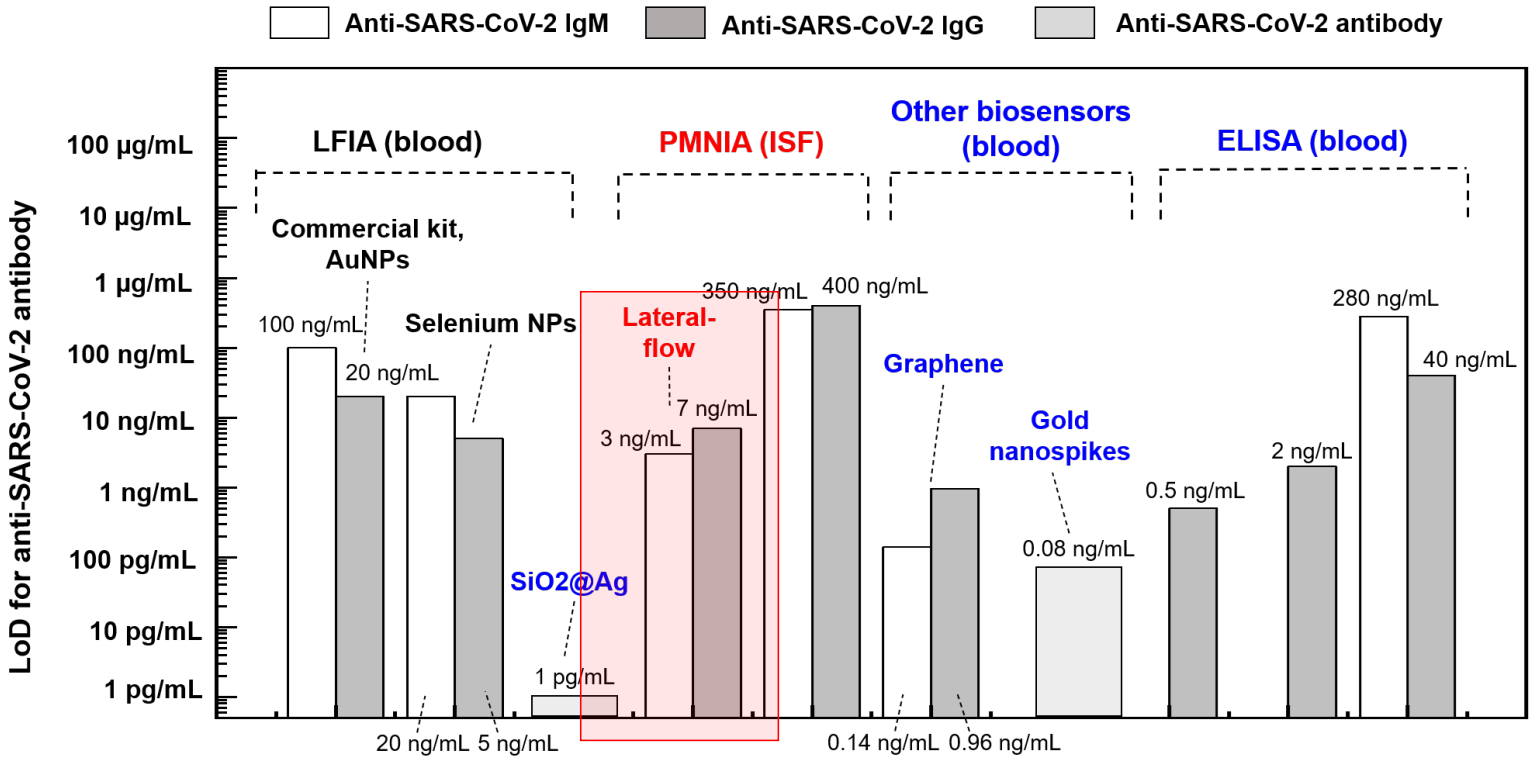
LoD for anti-SARS-CoV-2 IgM (n=3)



LoD for anti-SARS-CoV-2 IgG (n=3)



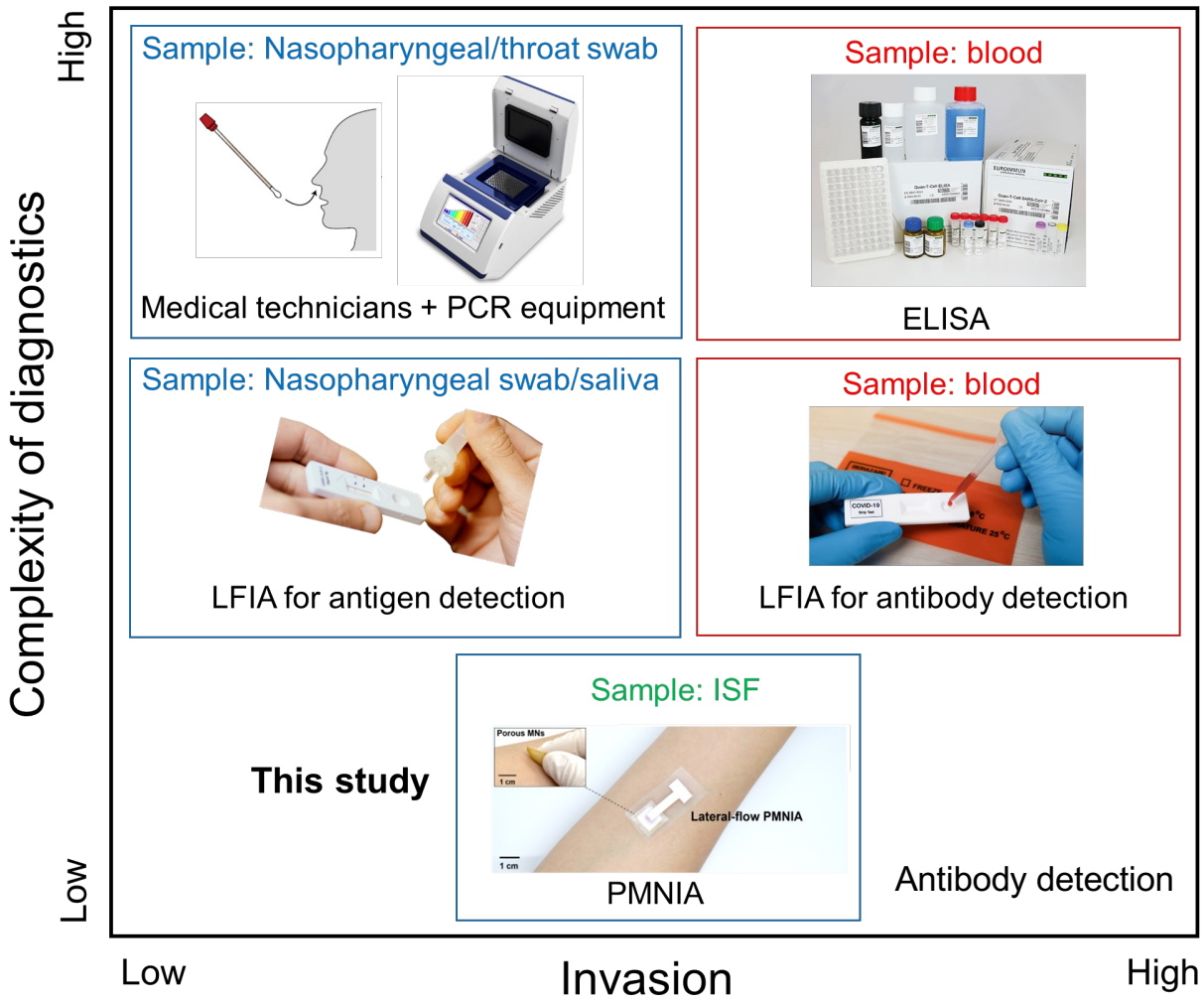
Comparison with previous researches (LoD)



Comparable or higher sensitivity than currently available commercial kit

>> Demonstrated that the proposed lateral-flow PMNIA can be a promising device for painless detection of SARS-CoV-2-specific antibody in ISF

Comparison of complexity of diagnostics

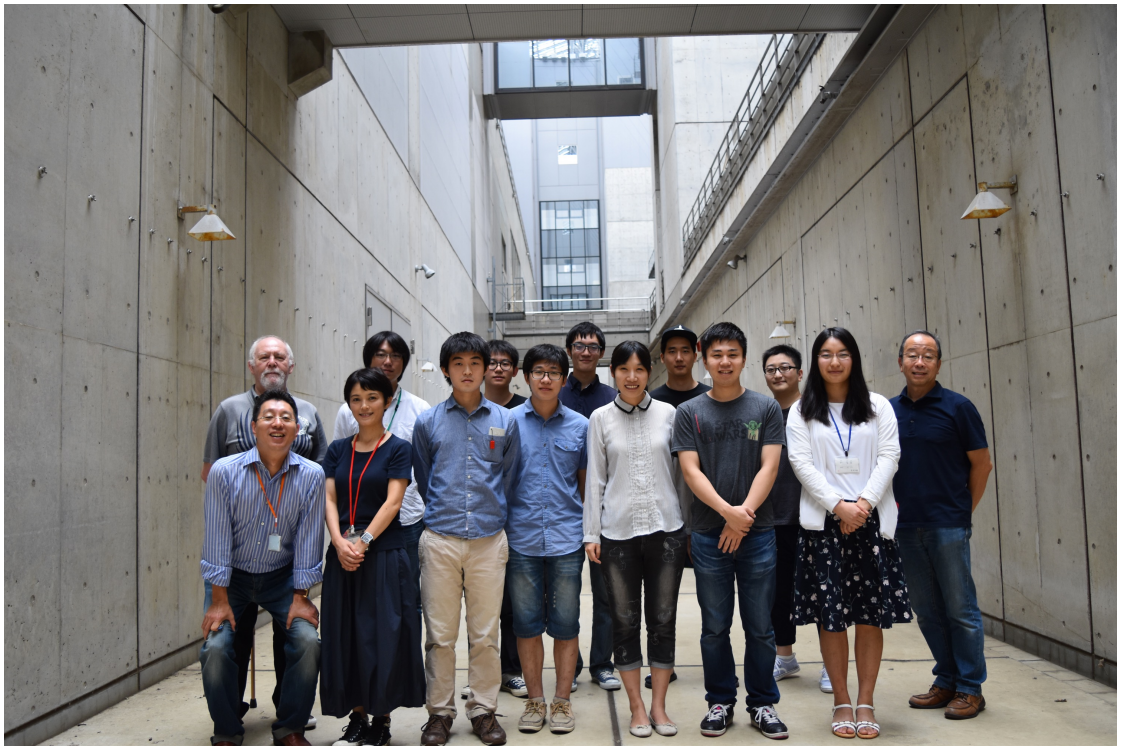


Summary

- Assessment and characteristics of proposed lateral-flow and vertical-flow PMNIAs
- Antibody detection results of proposed PMNIAs can be observed by naked-eyes rapidly
- LoD for anti-SARS-CoV-2 IgM and IgG was measured and lateral-flow PMNIA revealed high sensitivity compared with commercial LFIA kits

The proposed lateral-flow PMNIA is a prospective diagnostic tool to painlessly detect SARS-CoV-2-specific antibody in ISF and obtain information regarding the infection stage

Thank you for your attention!



Please, click here!

Appreciate Dr. Anthony W. Coleman@ Lyon
Prof. Maruoka @国立国際医療センター
Supported by SEIKEN Special TENKAIKENKYU
JSPS科研費、国立国際医療センター、東大病院、
AmtixBio（株）、SUNROI(株)，Lintec（株），
Premier Wellness Science(株)，CG bio社，etc…