Biomolecular Needling System for Medicals

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

Beyond the skin barrier

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Chair of Corporate Sponsored Research Division of Virological Medicine
Today, Topic

What is Micro Needles Patch?

Painless
0.06 mm
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

- 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

34G: 外径 (0.18 mm)
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

Various Types of Micro Needles for DDS

<table>
<thead>
<tr>
<th>Silicon</th>
<th>Metal</th>
<th>Polymer</th>
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<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
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</table>

Coated Micro needle (solid)

Hollow Micro needle

Micro Needles: Dissolving Micro needle (Biodegradable)
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, 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.

BNS (Bio molecular Needling System) Medicals @ BJ. Kim Lab., IIS, The University of Tokyo
**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](image)

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

![Diagram of MNs and drug transport](image)

- **No Pain, Patient-friendly**
- **Non-invasive**
- **Less space for storage**
- **No biohazardous waste**
- **No medical staff required**
Currently, Dissolving Microneedles

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)
J.D. Kim et al., J. Controlled Release (2013)
Conventional MN fabrication technology

1. 95% competitors

Micromolding

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


2. Others

DAB (Droplet-born Air Blowing)

Inkjet (Mushashi Eng.)

Raphas Ltd. Co. @ Korea

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

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

- Larger area of uniform microneedles with faster process time

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.

- 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

**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

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

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Biosensor MNP (Porous MN details)

Microparticle preparation using microfluidic technology

Micro molding

Fabricated PLA microparticles

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: 1.99-22.2 mM

Saliva: 0.008-1.77 mM

Sweat: 0.01-1.11 mM

Tears: 0.05-5 mM

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

→

Successfull Aging

Health

Beauty

Prevention, Remote medicine, Digital healthcare
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

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

<table>
<thead>
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<th>Input Needed</th>
<th>Connection</th>
<th>Continuous</th>
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<td>Skin Contact electrodes</td>
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<td>Electrical</td>
<td>Passive</td>
<td>Adhesive Electrodes</td>
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<td>Photoplethysmograph</td>
<td>Optical</td>
<td>Light</td>
<td>Adhesive Sensor</td>
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<td>Potentiometry</td>
<td>Wick</td>
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<td>Amperometry</td>
<td>Capillary Blood</td>
<td>Wick</td>
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<td>Nucleic Acid Amplification/Fluid Sample</td>
<td>Swab/Tissue Sample</td>
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<tr>
<td>Protien Markers</td>
<td>Eletrochemical/Optical</td>
<td>Swabbed Blood/Sweat/Urine/Sweat</td>
<td>Swab</td>
</tr>
</tbody>
</table>

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.
健康・疾患評価に用いられているマーカーとその分類

健康維持レベル
- 睡眠
- セロトニン
- メラトニン
- マーカー
  - LDL-C
  - HDL-C
  - Y-GTP
  - LDH

急性反応
- 食生活
  - ビタミン類
  - 葉酸
  - アルコール
  - 中性脂肪

慢性反応
- ストレス
  - コルチゾール
  - アドレナリン
  - 紫外線
- 薬物濃度
  - インスリン
  - 免疫抑制薬
  - ニコチン

疾患レベル
- 免疫反応
  - IL-2, 4, 6
  - ヒスタミン
  - TNF-α

特定疾患系
- ガン：TNF-α、アミノ酸インデックス
- 骨粗しょう症：エストロゲン

ペットの自宅健康診断
- 意思疎通が不可能な動物の健康状態を把握
Glucose sensor to extract the interstitial fluid (ISF)
It is estimated that 415 million people are living with diabetes in the world.

Diabetes is a leading cause of death and disability worldwide.
GOVERNMENT NEEDS

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

GROWING DANGER

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

Preparation
With adhesion tape

Attachment to the skin

Attach and analysis

Alert to the user

Release from the skin

Disposal
Results of fabrication

- Research object 1: porous MN
- Research object 2: PLGA biodegradable polymer
- Research object 3: Integration with paper-based sensor

MN side

Sensor side

Entire view of the device

Color development
Glucose analysis

BNS (Bio molecular Needling System) Medicals @ BJ. Kim Lab., IIS, The University of Tokyo
Glucose oxidase, peroxidase (2 enzymes) and TMB dye

- Capillary driven (毛細管力) - 35 nL/min.
- 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

**Device complexity**
- High
  - Sample: Blood (telemetry)
  - Metabolic heat confirmation
- Low
  - Sample: Sweat
  - Electric tattoo
  - Sample: Tear
  - MN-base electrochemical sensor
  - Smart contact lens
  - Sample: Urine
  - Only for diabetic patient

**Commercialized device**
- CGMs
- SMBG kit

**Our device**
- Sample: ISF

**Invasion**
- Low
  - Sample: ISF
- High
  - Concentration range of Glucose
    - Blood plasma: 2-40 mM
    - ISF: 1.99-22.2 mM
    - Saliva: 0.008-1.77 mM
    - Sweat: 0.01-1.11 mM
    - Tears: 0.05-5 mM

**For non-diabetic patient**

BNS (Bio molecular Needling System) Medicals @ BJ. Kim Lab., IIS, The University of Tokyo
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 compared to other sensors.
Continuous 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

Open capillary pump

Inlet port

Microneedle

Si substrate

Glucose sensor in assay chamber

特許PCT/JP2018/020224

Biomedical Microdevices, 21, Vol.28 (2019)

BNS (Bio molecular Needling System) Medicals @ BJ. Kim Lab., IIS, The University of Tokyo
Porous Needle -> 3D Micro Fractal Pipettes for liquid sample handling

Capillary force

Comparison between fractal pyramid and solid pyramid

Two-photon 3D Lithography

Fabricated structure

500 μm

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
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)

1. Collect blood sample
2. Add blood sample to sample well
3. Place 2-3 drops of buffer in sample well
4. Read results after 15 minutes

→ 採血

■ 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
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

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 蘭 (東京大学 生産技術研究所 教授)

2. 発表のポイント:

・皮膚内の流体から、新型コロナウイルスに対する抗体（IgMおよびIgG）を検出しようという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捕体の応用を組み合わせ、既存の検出キットと同等以上の性能を示し、これまでにないパッチ型の抗原検出デバイスを開発した。

・パッチ型抗原検出デバイスは小型かつ簡便で、皮膚に貼ることができる。検体を取得しやすく、得られる結果に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 蘭 教授、大学院工学系研究科 精密工学専攻 博士課程3年の頃 藻・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を代えて、皮膚に貼るだけで抗原検出ができる、多孔質マイクロニードル（空）とイムノプロマトアッセイ（I、2）を組み合わせた新しいパッチ型抗原検出デバイス（図1）。Porous MicroNeedle and

https://www.iis.u-tokyo.ac.jp/ja/news/3908/
SARS-CoV-2 & Diagnosis

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

• Antibody detection → Determine infection stages & Measure antibody level
  - Blood sampling

- Real-time reverse transcription polymerase chain reaction (RT-PCR)
- SARS-CoV-2 antigen lateral-flow immunoassay (LFIA) test
- SARS-CoV-2 Abs LFIA test
- Enzyme-linked immunosorbent assay (ELISA)
- Paper-based electrochemical biosensor

[Safiabadi Tali, S. H., 2021]
[Dhamad, A. E., 2020]
Porous MNs for SARS-CoV-2 diagnosis

- 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.

By Leilei BAO (Ph.D.) et al.
Scientific Reports, Vol.12, 10693 (2022)
Antibody detection via interstitial fluid (ISF)

- ISF sampling

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

Human skin

- Stratum corneum (15-20 µm)
- Epidermis (130-180 µm)
- Dermis (2000 µm)
- Hypodermis

[M. Leone, 2017]
PLA Porous MNs

Fabrication using single emulsion method

Schematic diagram of fabrication process

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)

After Drying

With heat treatment

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<th>170 °C</th>
<th>180 °C</th>
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<tr>
<td>Cross-section</td>
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</table>

SEM images of PLA porous MNs
**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

**Interpretation of results**

- **Negative**
- **IgM positive**
  - Recent infection with COVID-19 (~1 week)
- **IgM/IgG positive**
- **IgG positive**
  - Recent infection with COVID-19 (1–3 weeks)
  - Previous infection with COVID-19

**Capture bioreceptor**

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

- **Human Anti-SARS-CoV-2 IgM**
- **COVID-19 antigen-conjugated AuNPs**
- **Human Anti-SARS-CoV-2 IgG**
- **Rabbit-IgG-conjugated AuNPs (for Control check)**
Evaluation of lateral-flow PMNIA

Anti-SARS-CoV-2 IgM & IgG detection

(a) IgM positive
(b) IgG positive
(c) IgM & IgG positive
(d) Negative

- 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: 3 ng/mL

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

LoD: 7 ng/mL
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

- **Sample: Nasopharyngeal/throat swab**
  - Medical technicians + PCR equipment
  - LFIA for antigen detection

- **Sample: Nasopharyngeal swab/saliva**
  - LFIA for antibody detection

- **Sample: ISF**
  - Antibody detection

**This study**
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!