

Fabrication of 3D Bio-scaffold by 3D printer, ES Nanofibers and FS laser

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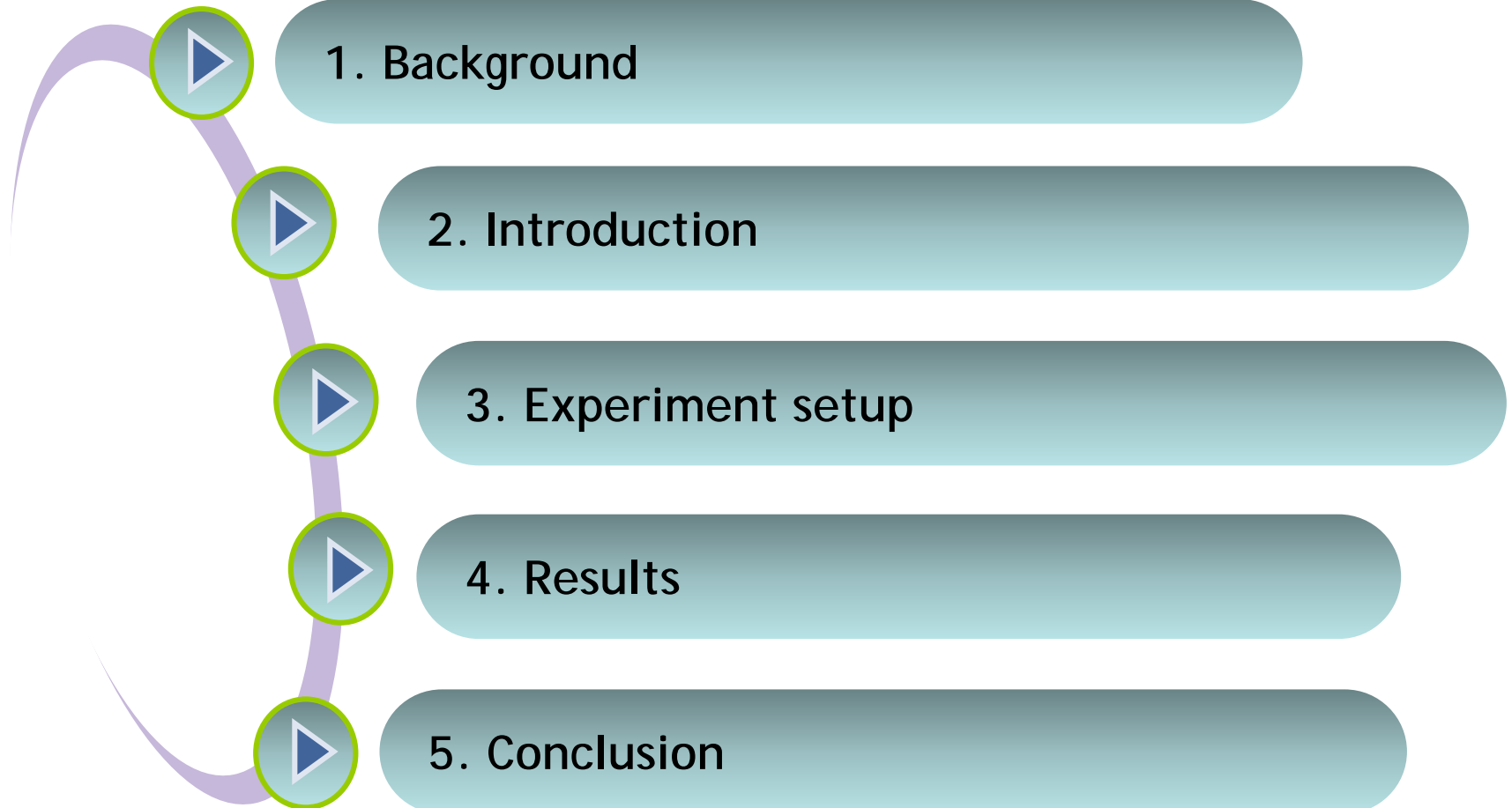
May 3rd, 2019

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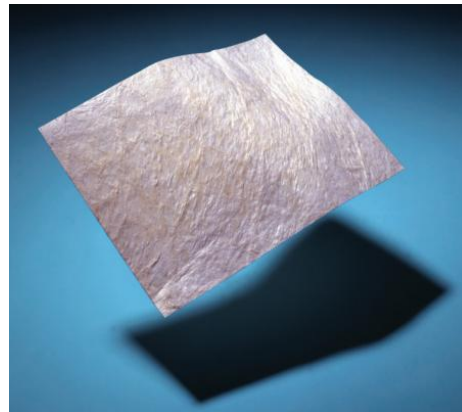
1. Background

Tissue engineering

- For the plastic and reconstructive surgery, biodegradable or biocompatible material tissue scaffolds are required for fast recovery
- For the design and fabrication, interdisciplinary knowledge is required such as materials science, biochemical and life science
- For the high surface area to volume ratio, nanofiber-based bio-scaffold has been used as one possible biocompatible bio-scaffold



OviTex Reinforced BioScaffold
(source: iData Research)



Injectable Biomaterial
(source: CorMatrix Cardiovascular)

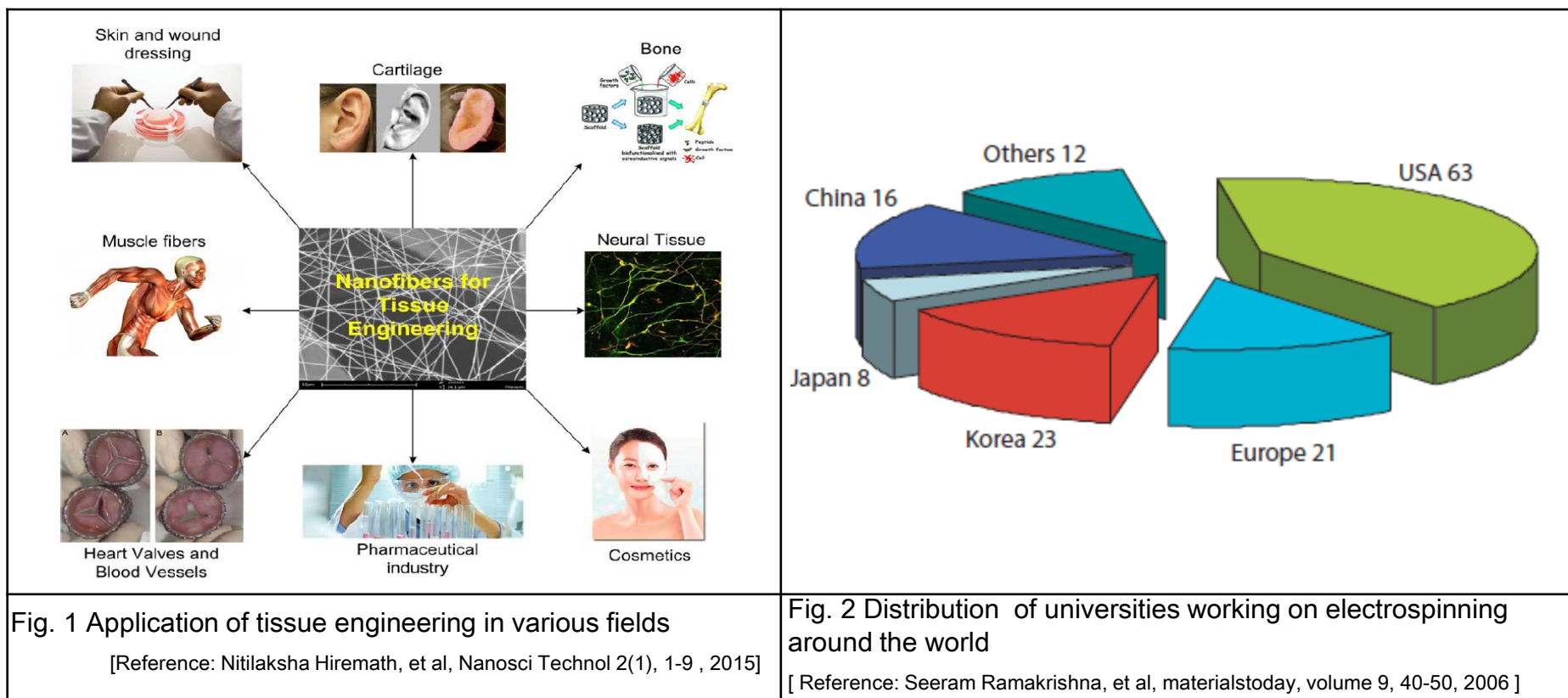


3D Prototyping, Cell Printing
(source: GESIM)

1. Background

Trend of nano fibers technology

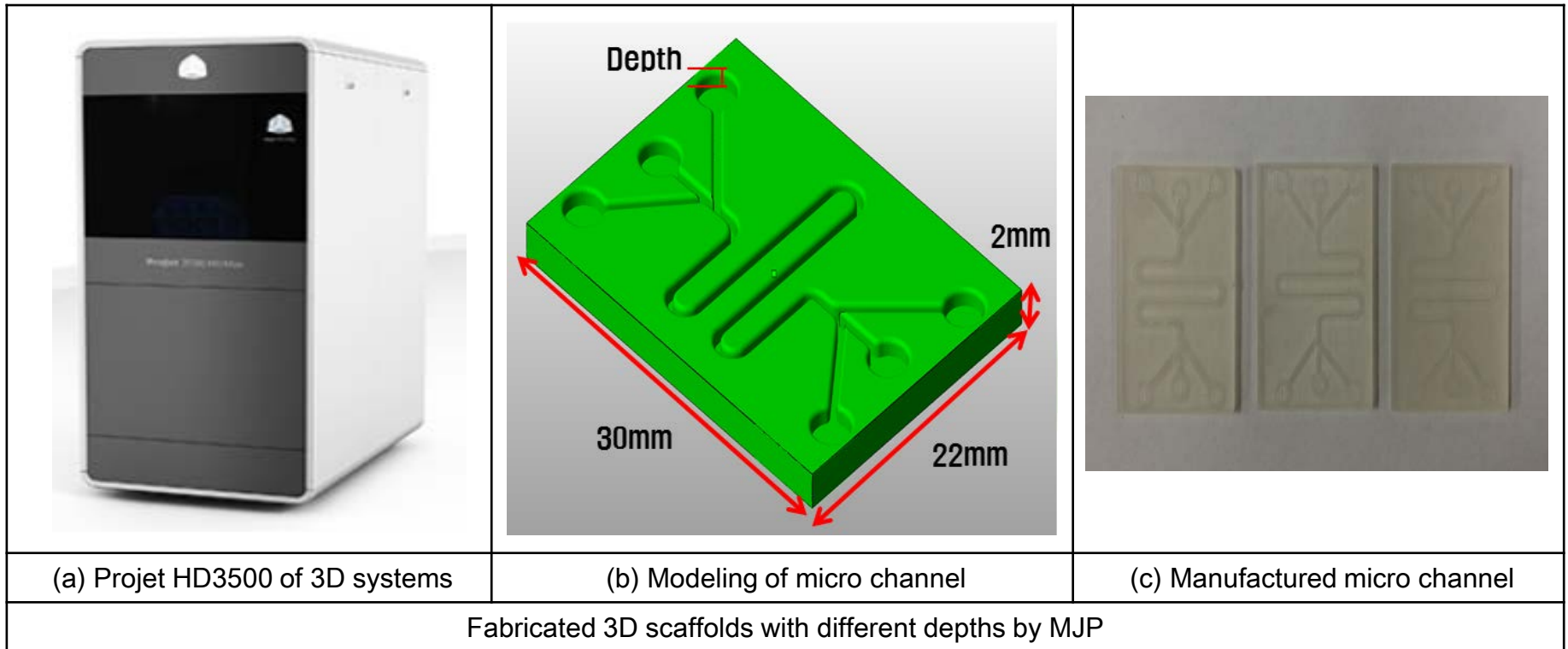
- Nanofibers by electrospinning has been studied by over 200 universities and research institutes
- Electrospinning is an effective way to fabricate nanoscale fibers for tissue engineering



2. Introduction

Prior research activities (3D printing)

- Fabricated 3D scaffolds support with different depths by MJP[Multi-Jet Printing]
- The resolution of system is 0.05 mm [3d systems Inc, Model: Projet HD3500]
- Depth of 0.3, 0.5, 1.0 mm for micro channel

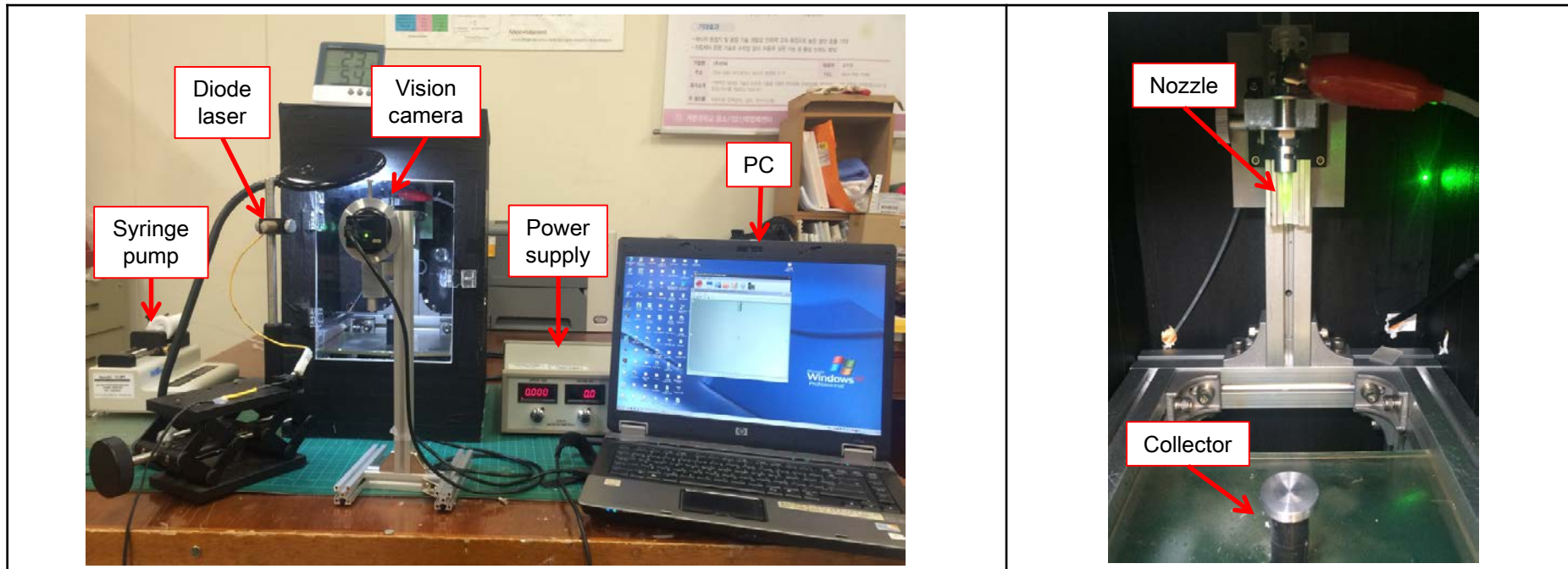


2. Introduction

Prior research activities (Electrospun Nanofibers)

- Experimental components: High voltage power supply, Syringe pump, Nozzle, Diode laser

▣ High voltage power supply	▣ Syringe pump	▣ Nozzle
<ul style="list-style-type: none">- Model: NNC-HV30- Max voltage: 30 [kV]	<ul style="list-style-type: none">- Model: KDS 100 CE- Linear travel: Min 0.00635 ~ Max 76.18 [cm³/hr]	<ul style="list-style-type: none">- Model: NNC-PN-32GA- I.D [Inner Diameter]: 0.1~0.25 [mm]

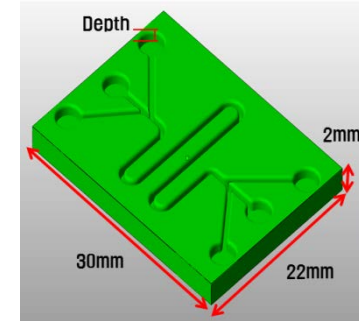


Experiment setup for electrospinning

2. Introduction

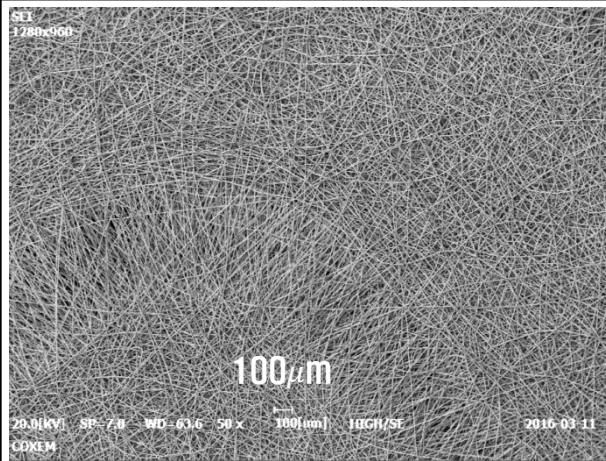
Prior research activities (3D Printing + Electrospun Nanofibers)

- No scaffold was observed at pattern depth of 0.3mm
- 3D patterns were observed from 0.5mm depth covered with nanofibers
- Coarse coverage was observed with 1.0mm of Pattern depth

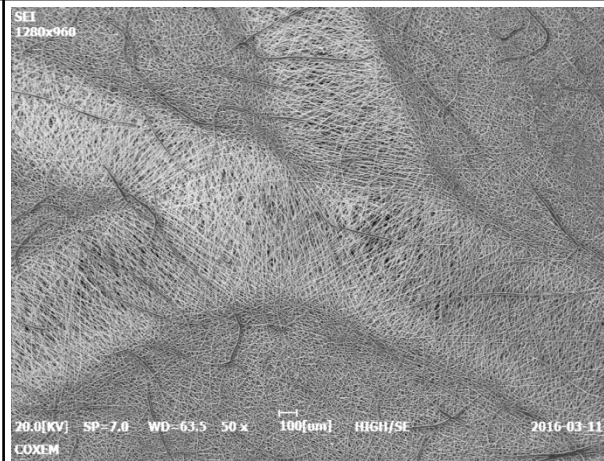


PCL 12wt%, Input voltage 24kV, Output velocity 0.4ml/h, TCD 100mm

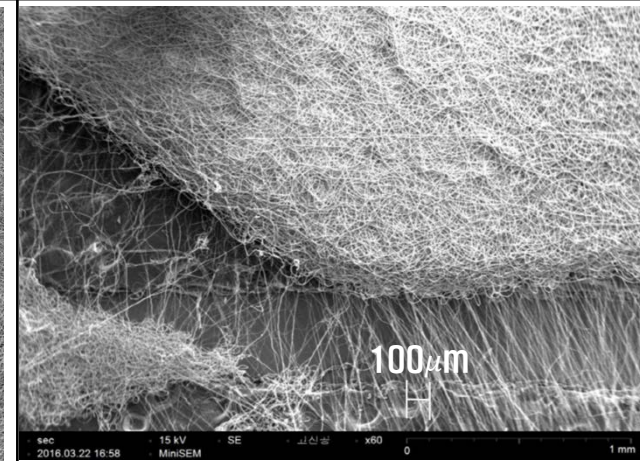
Depth = 0.3 mm



Depth = 0.5 mm



Depth = 1.0 mm

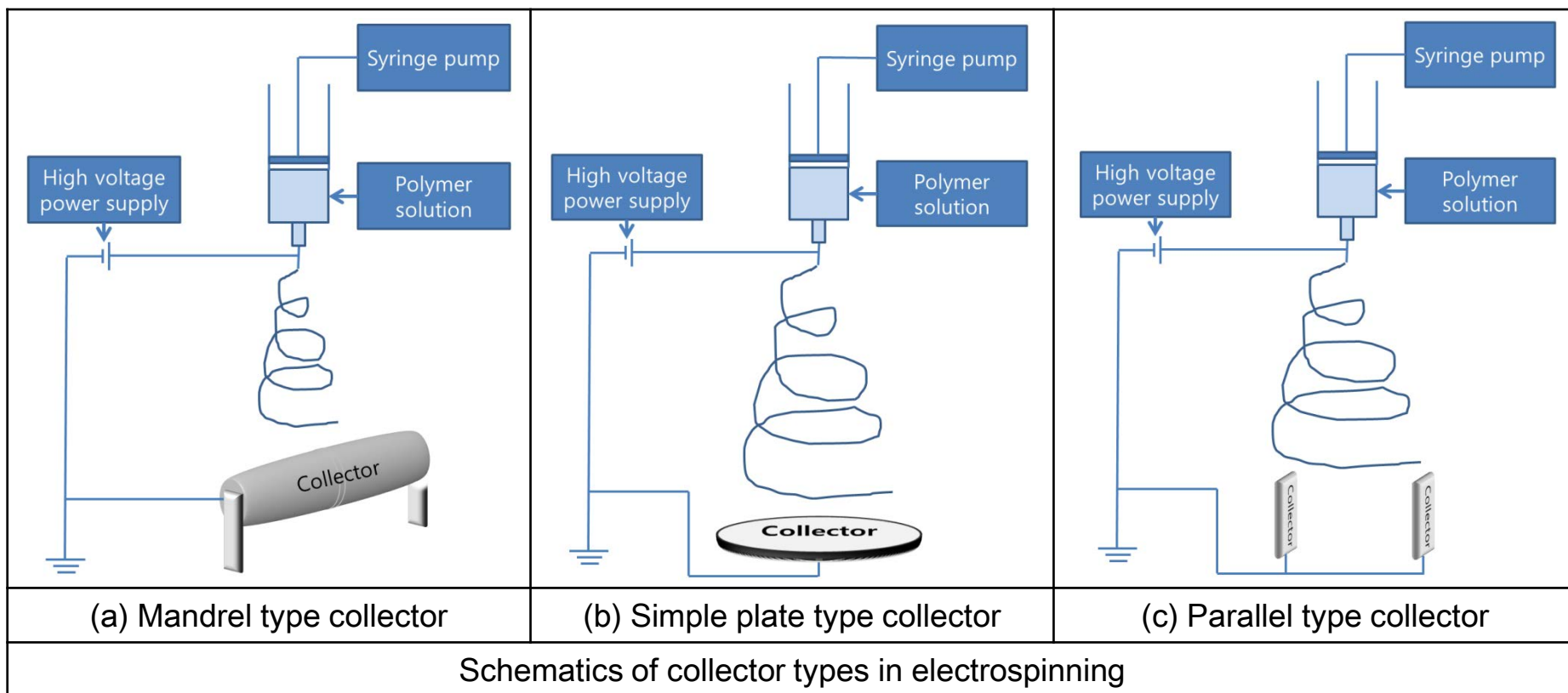


SEM images of 3D scaffold with Nanofibers

3. Experiment setup

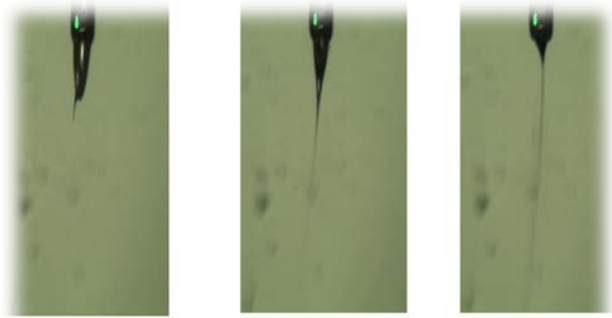
Collector designs for Electrospinning

- Collectors were designed for specific applications (flat, hollow or etc.)
- Net arrangement of nanofibers is different according to the kinds of collectors



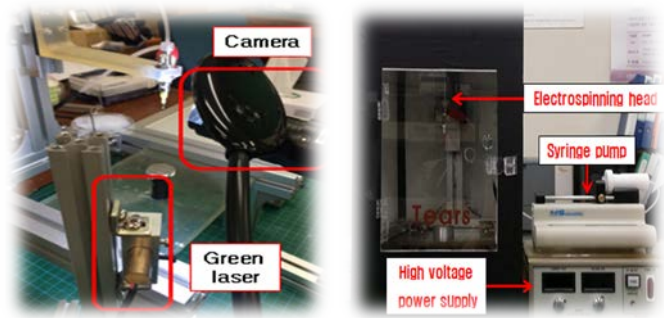
3. Experiment setup

Schematics of bioscaffold fabrication



< 8wt%, 2.0ml/h > < 8wt%, 4.0 ml/h > < 8wt%, 6.0ml/h >

(a) Nano nozzles



(b) Experiment setup

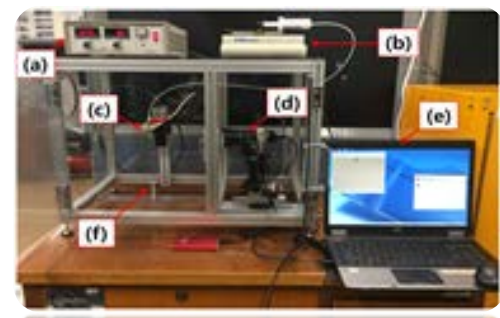


(c) Fabricated nanofibers

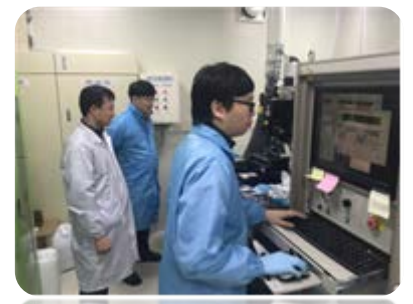
Equipment for bioscaffold fabrication



3D printer



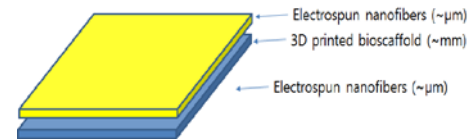
ES Nanofiber



Femtosecond laser

3. Experiment setup

Polymer preparation for Electrospinning



- Biodegradable solutions were produced based on the combinations of PCL/PLA/Collagen
- Electrospinning was performed based on the parameters of concentrations, applied voltages, flow rates and TCDs
- ① PLA(3D)+Collagen(coat) ② PLA(3D)+[PCL+Collagen](ES) ③ PLA(3D)+PCL(ES)+Collagen(coat)



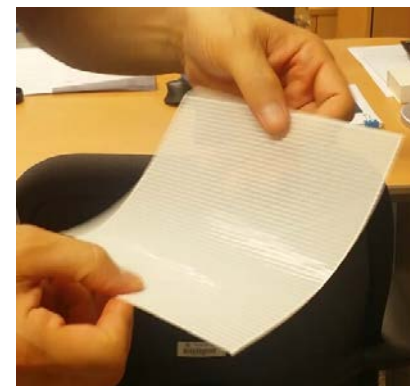
Dissolved polymers



Electrospinning process



3D printing process



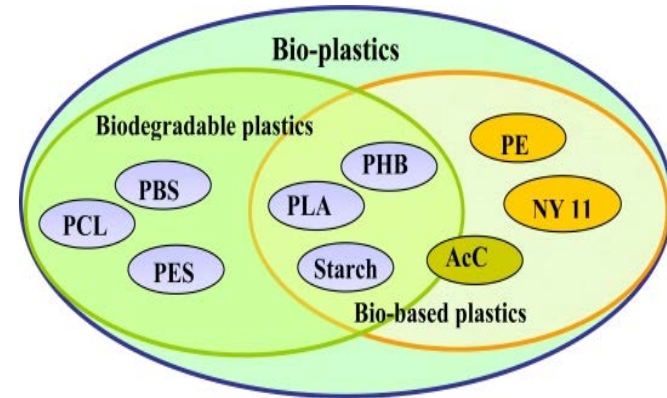
3D Printed PLA Bioscaffold

Experimental process of nanofibers

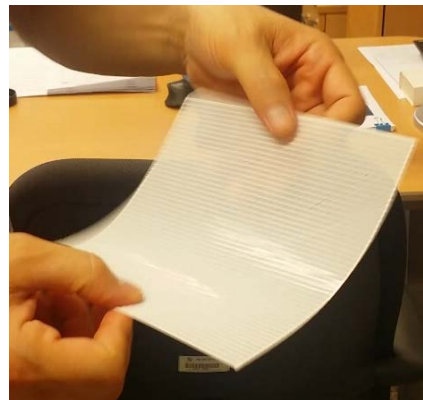
3. Experiment setup

PLA 3D printing

- 100 μm thick PLA scaffold was fabricated by FDM type 3D printer
- PLA is a biodegradable and bioactive thermoplastic aliphatic polyester derived from renewable resources, such as corn starch



FDM type 3D printer



Fabricated scaffold



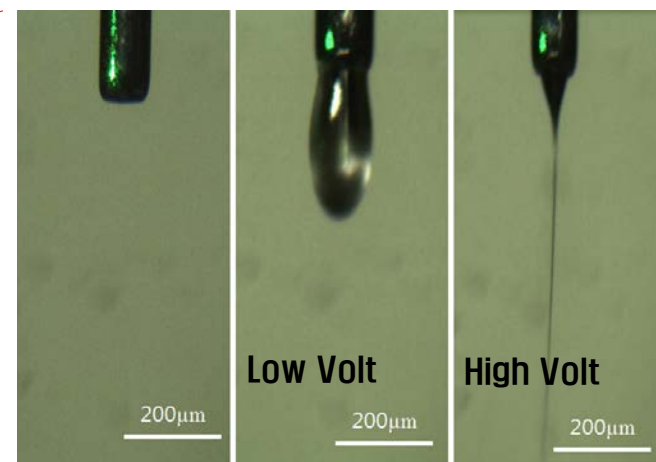
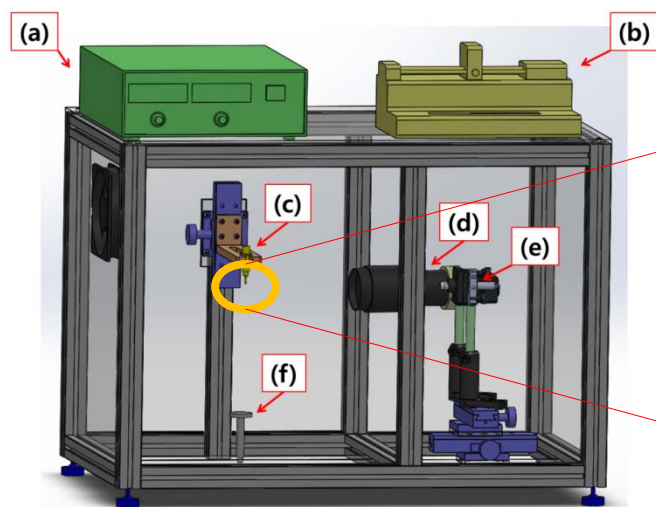
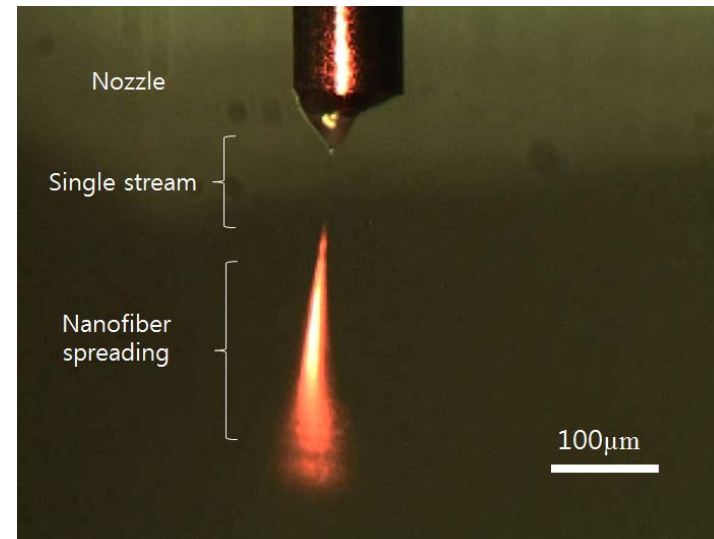
Optical image of PLA scaffold

3. Experiment setup

Nanofiber Electrospinning

- PCL was dissolved in different solutions
- PCL and Collagen were mixed and dissolved in solution

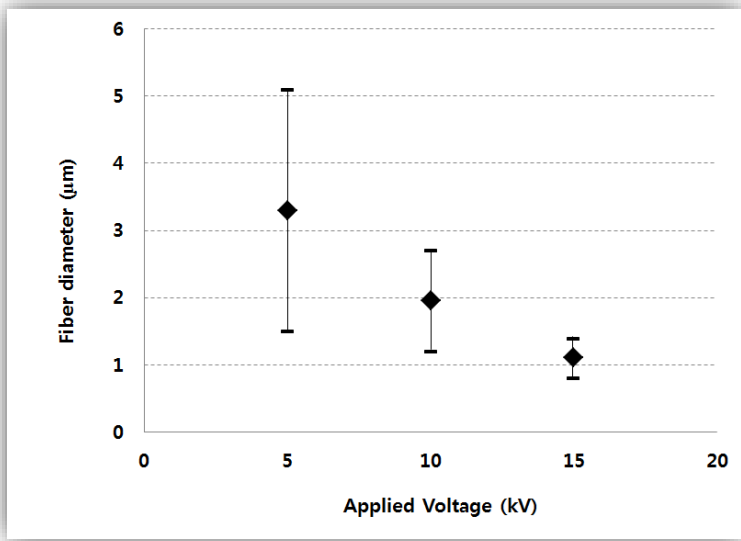
Parameters	Values
PCL concentration [wt%]	2, 4, 6, 8
Applied voltage [kV]	5, 10, 15
Flow rate [ml/hr]	1,2,4,6
TCD (Tip to collector distance) [mm]	90, 110, 130



4. Results

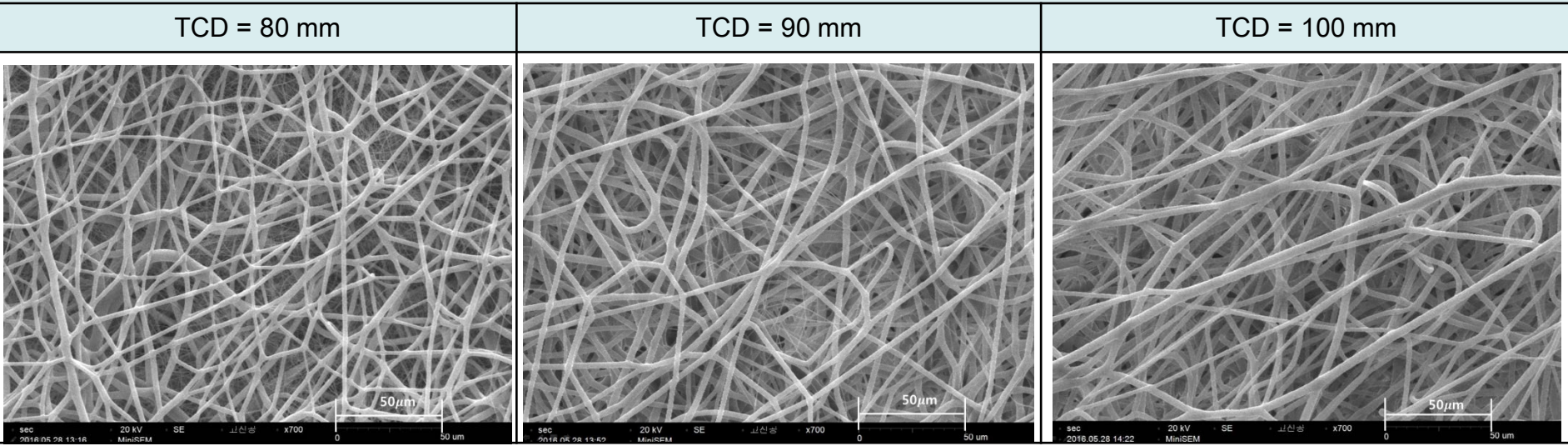
Nanofiber Electrospinning

- ① PLA(3D)+Collagen(coat)
- ② PLA(3D)+[PCL+Collagen](ES)
- ③ PLA(3D)+PCL(ES)+Collagen(coat)



Distribution of average nanofibers per various applied voltage

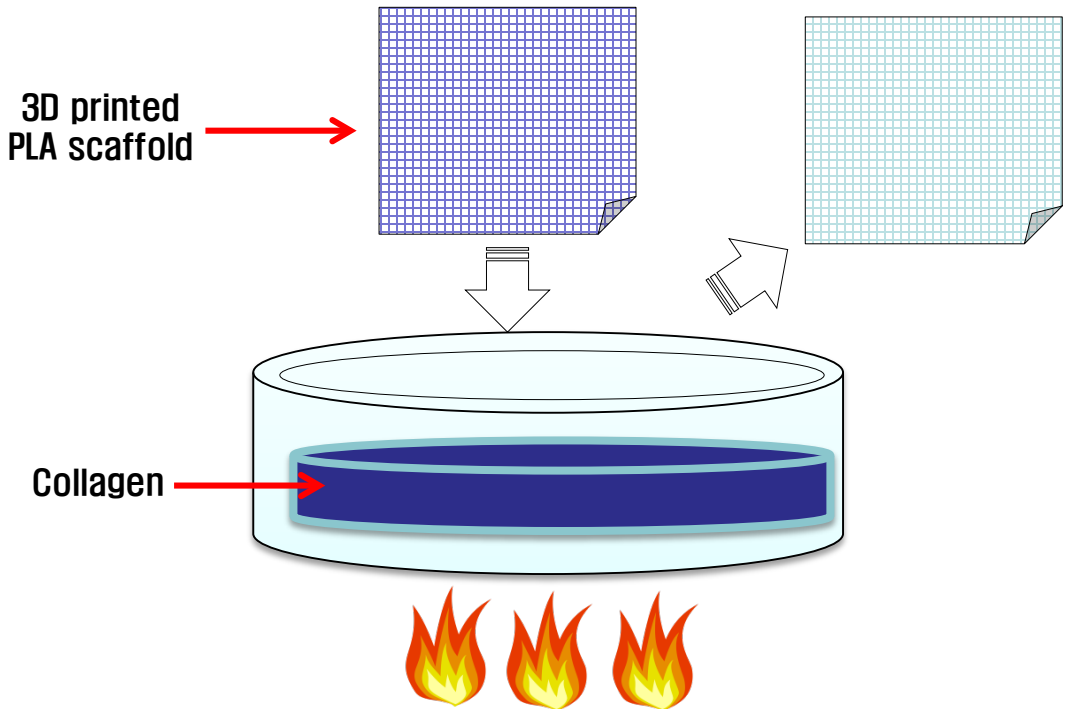
Weight ratio 8wt%, Input voltage 24kV, Output velocity 0.10ml/h



4. Results

Nanofiber Electrospinning: ① PLA(3D)+Collagen(coat)

- To increase the cell proliferation, collagen was coated on the surface of PLA
- Meshed bioscaffold was helpful to hold liquid collagen coating



OM of collagen coated PLA

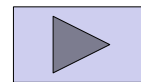
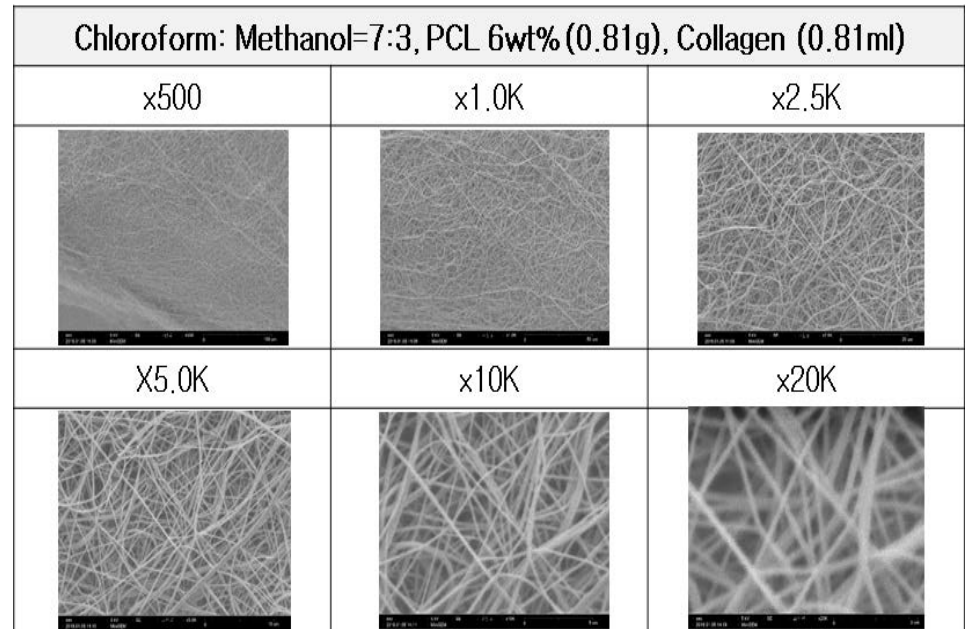
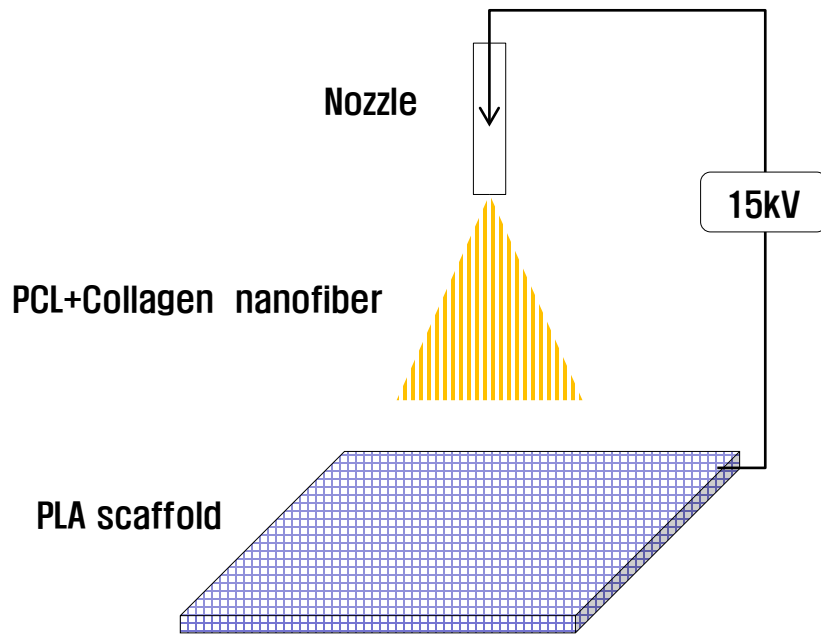


Cell proliferation by collagen coated PLA

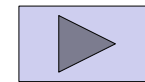
4. Results

Nanofiber Electrospinning: ②PLA(3D)+[PCL+Collagen](ES)

- 6 wt% PCL was mixed with 6 wt% collagen
- The mixture was dissolved in a solution of chloroform and methanol in a ratio of 7: 3
- A small amount of NaCl was also added to the solution for fiber size homogeneity.



Low voltage

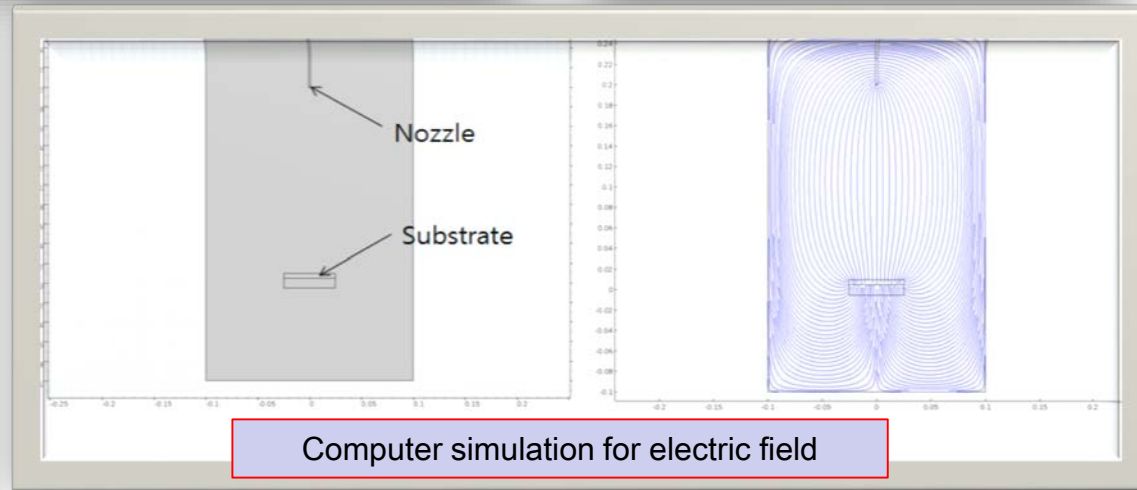
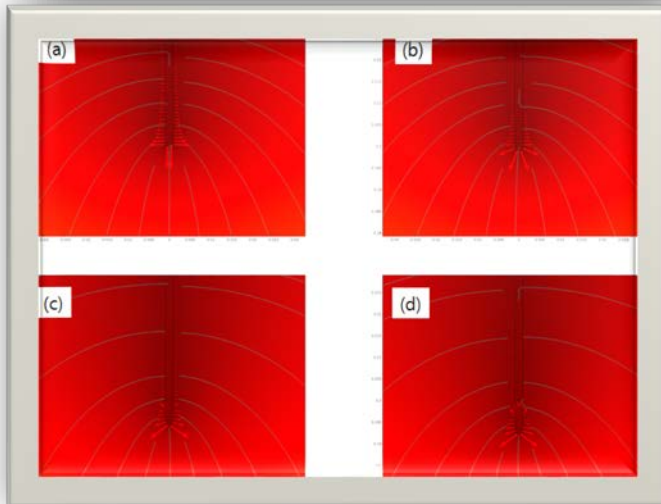
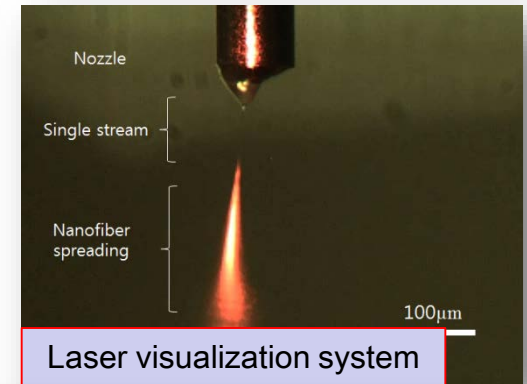
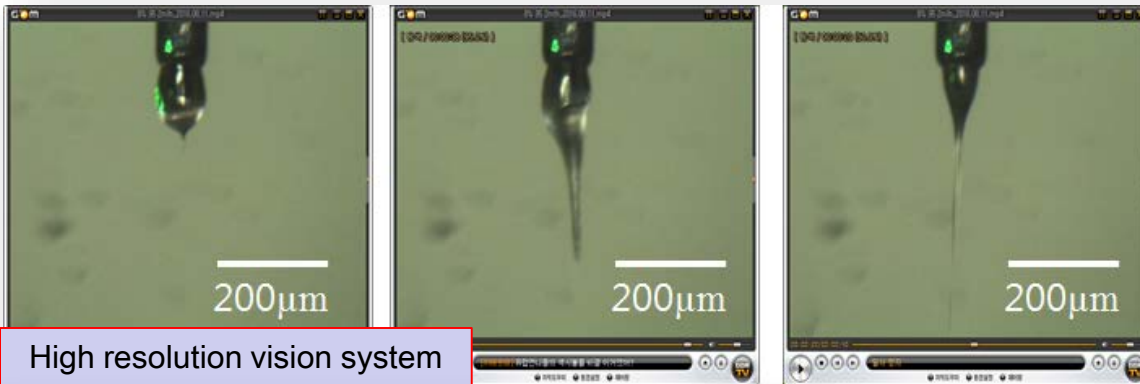


High voltage

4. Results

Nanofiber Electrospinning: ②PLA(3D)+[PCL+Collagen](ES)

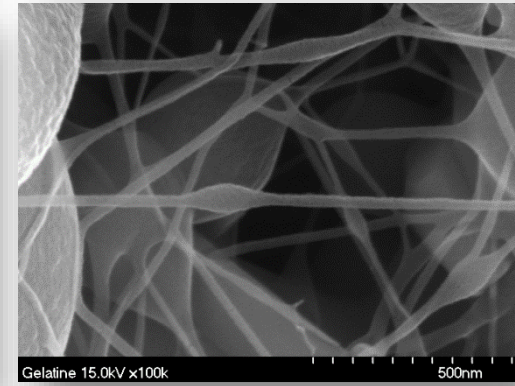
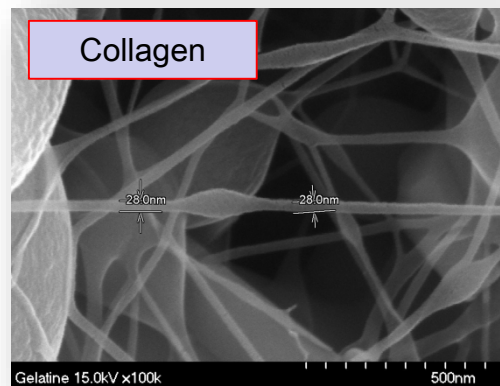
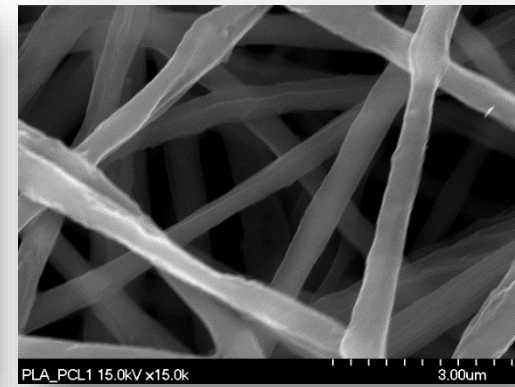
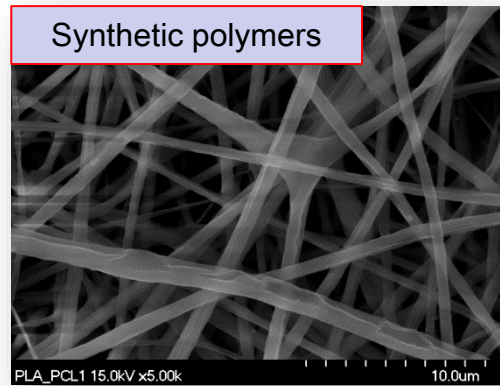
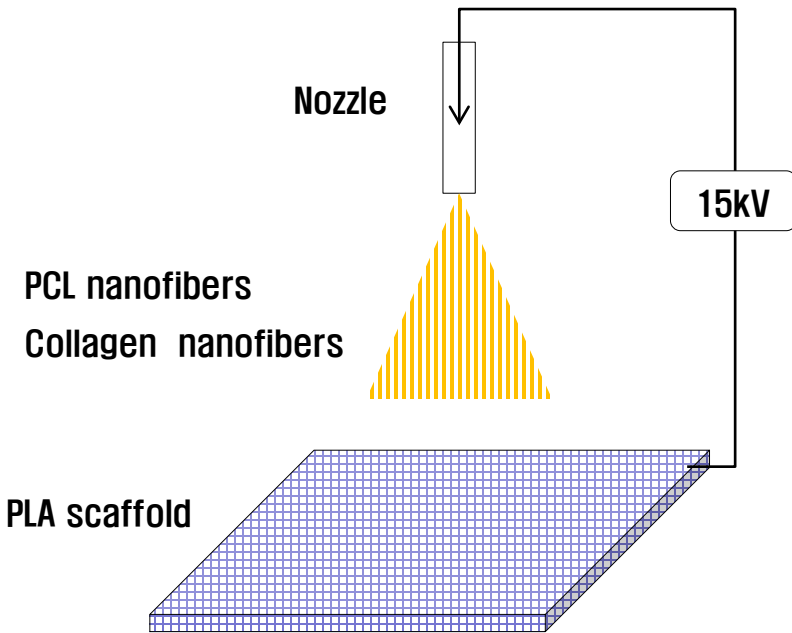
- High resolution camera and laser visualization system was used for real time monitoring
- Fiber size homogeneity or uniformity can be controlled
- Computer simulation was performed to understand the droplet to fiber development



4. Results

➤ Nanofiber Electrospinning: ③ PLA(3D)+PCL(ES)+Collagen(coat)

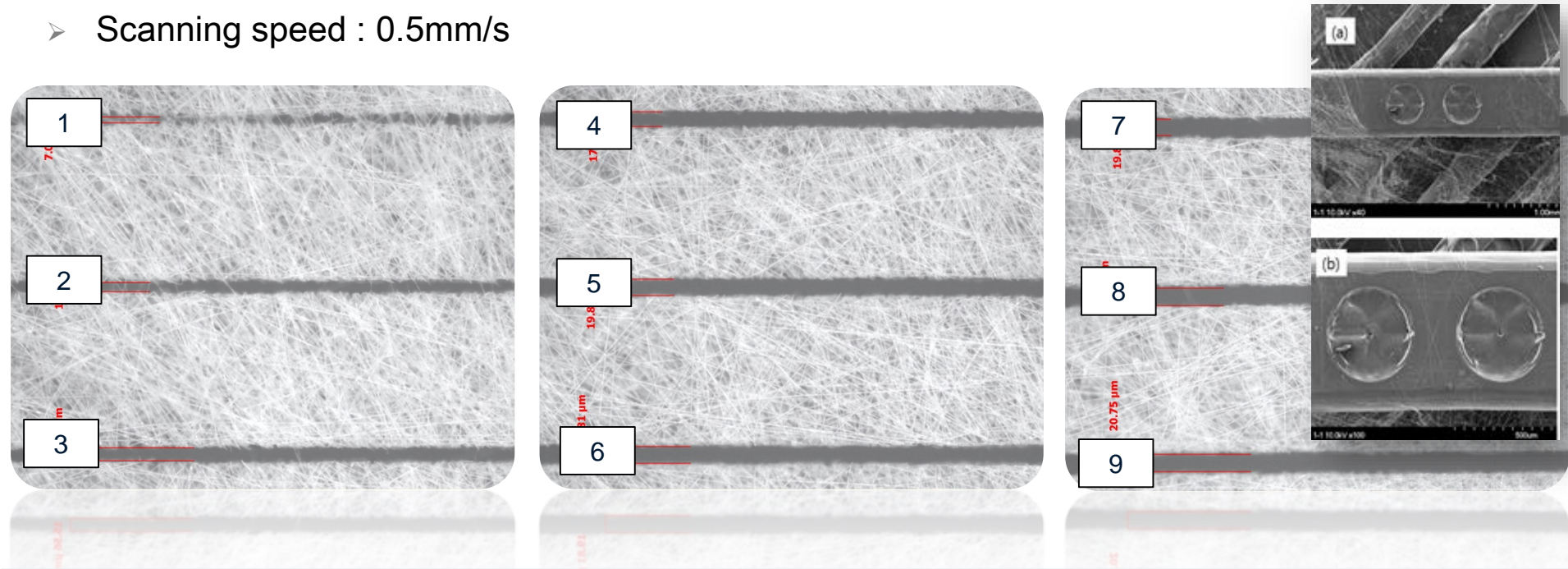
- Similar experiment as Exp.#2
- PCL electrospinning and Collagen electrospinning (Physical layering)



4. Results

Laser micro machining for cell growth (Femtosecond laser)

- PPR :100 kHz, Wavelength: 1030 nm, Pulse duration: 250 fs, Objective lens: 5x
- Scanning speed : 0.5mm/s

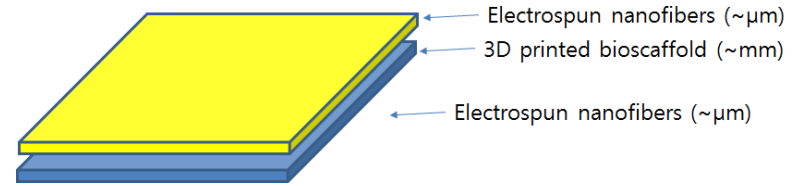


	1	2	3	4	5	6	7	8	9
Ablation width	7 μm	11 μm	16 μm	17 μm	20 μm	20 μm	20 μm	21 μm	21 μm
Power	140 mW	180 mW	230 mW	280 mW	340 mW	410 mW	490 mW	560 mW	650 mW
Pulse energy	1.4 μJ	1.8 μJ	2.3 μJ	2.8 μJ	3.4 μJ	4.1 μJ	4.9 μJ	5.6 μJ	6.5 μJ

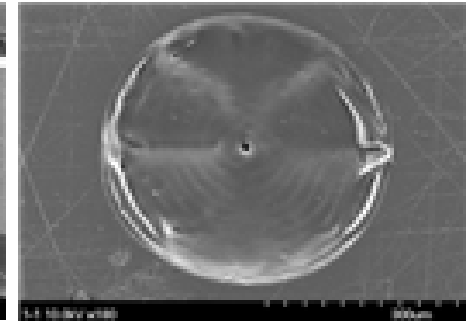
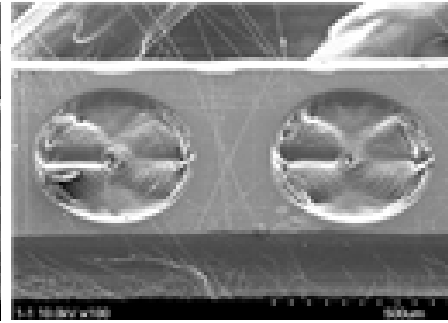
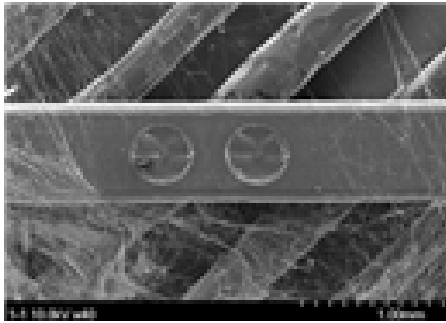
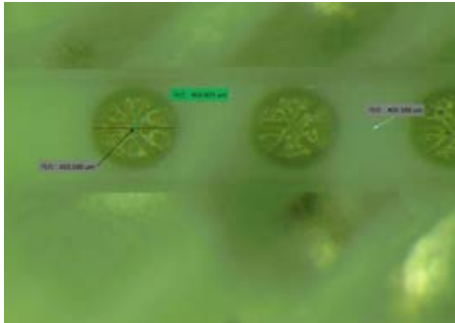
4. Results

➤ Femtosecond laser machining for seeding well drilling

3D printed PLA scaffold



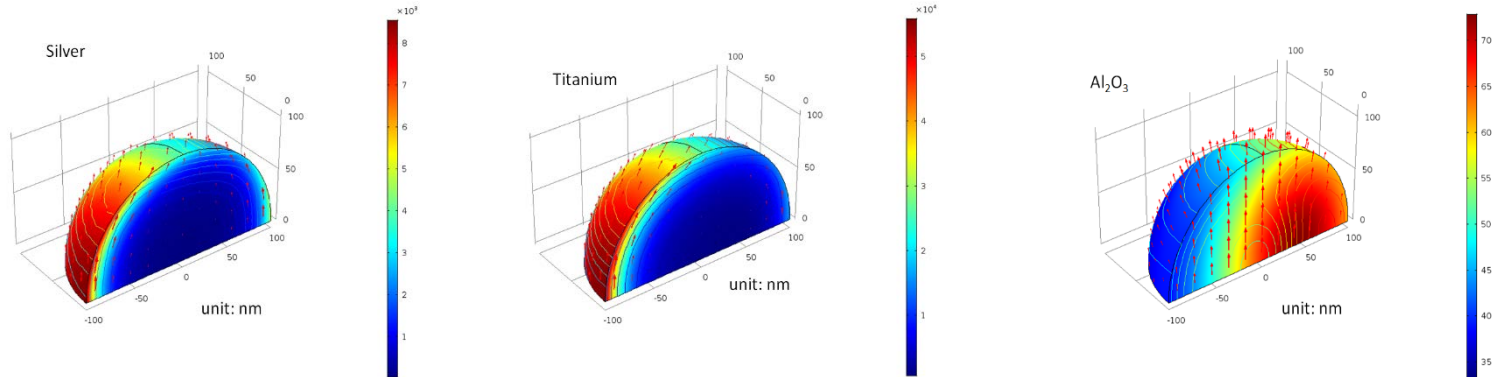
Femtosecond laser drilling



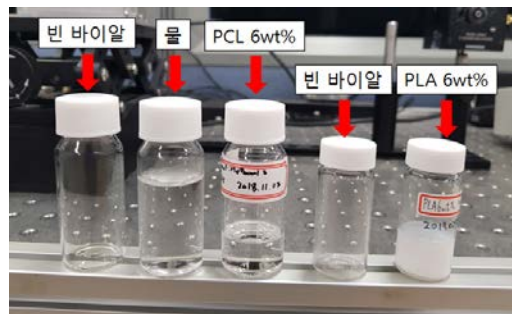
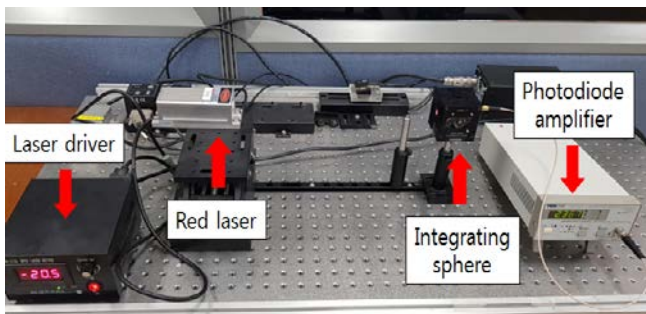
4. Results

Analysis of laser beam scattering in liquid state polymers

Laser beam scattering on particles



Laser beam scattering on particles in liquid

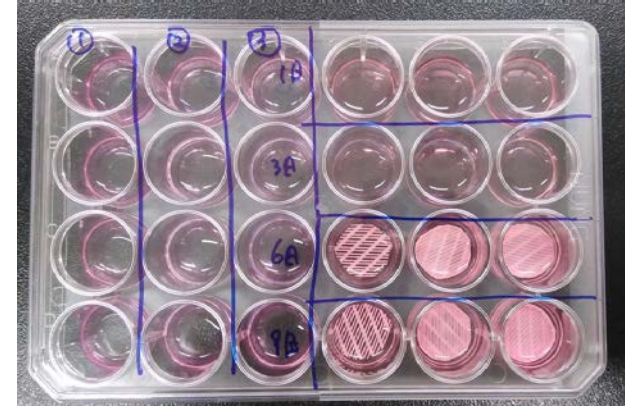


실험용액	빈 바이알 (μW)	용액 투과 측정값 (μW)	투과율 (%)
물	1.918	0.319	16.63
Chl 7: Meth 3, PCL 6wt%	1.918	0.136	7.09
Chl 7: Meth 3, PLA 6wt%	1.797	0.102	5.68

4. Results

Fibroblast viability assay

- Human skin fibroblast (Hs68 cells)
- MTS assay



① PLA (3D)
collagen coating



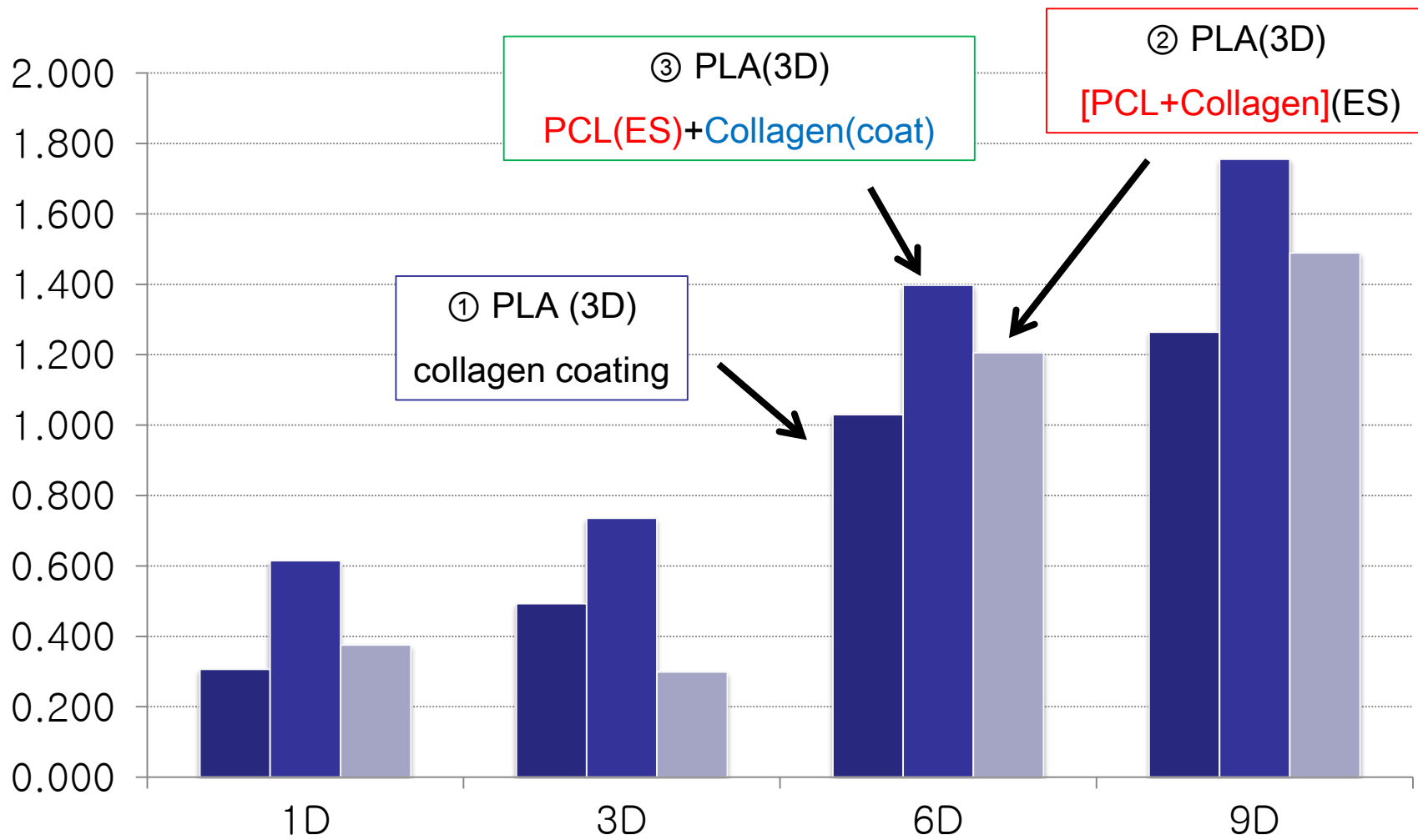
② PLA(3D)
[PCL+Collagen](ES)



③ PLA(3D)
PCL(ES)+Collagen(ES)

4. Results

MTT Assay results



5. Conclusion

- A hybrid bio-scaffold combined electrospinning technology and 3D printing technology for the higher mechanical strength and better cell growing environment.
- ① PLA(3D)+Collagen(coat), ② PLA(3D)+[PCL+Collagen](ES), ③ PLA(3D)+PCL(ES)+Collagen(ES) were compared
- Average sizes of nanofibers were 3.2 μ m, 2 μ m and 1.2 μ m at 5kV, 10kV and 15kV of electric voltage, respectively.
- PLA(3D)+PCL(ES)+Collagen (coat) resulted in the highest cell viability
- In vitro cell infiltration evaluation, extracellular matrix deposition, quantification of scaffold contraction, real-time PCR, VEGF, PDGF, bFGF, TIMP-1, MMP-1, collagen I, fibronectin mRNA level, are planned as a future work



Thank you

