**Original article**

# The in vitro research of bacterial invasion into prosthetic vascular grafts: Comparison of elastomer-sealed and gelatin-coated Dacron vascular grafts

Yuki Sasaki, MD

Division of Cardiovascular Surgery, Department of Surgery, School of Medicine, Faculty of Medicine, Toho University

**Address correspondence to:** Yuki Sasaki, MD

Division of Cardiovascular Surgery, Department of Surgery, School of Medicine, Faculty of Medicine, Toho University

6-11-1 Omori-nishi, Ota-ku, Tokyo, 143-8541 Japan

Telephone number: +81-3-3762-4151 (ext. 6540); FAX number: +81-3-3766-7810

E-mail address: yuki09091360542@yahoo.co.jp

**Running Title:** Research of bacterial invasion into grafts

**Key Words:** graft infection, bacterial invasion, elastomer-sealed Dacron vascular graft, gelatin-coated Dacron vascular graft, *Pseudomonas aeruginosa*

**Abstract**

***Purpose:*** To investigate the process of bacterial invasion from the surface to the inside of prosthetic vascular grafts.

***Methods:*** Elastomer-sealed (ESDVG) and gelatin-coated (GCDVG) Dacron vascular grafts were cut into 6-cm segments and placed in a U-shaped configuration in culture plates. Physiological saline was poured inside the grafts, and suspension of *Pseudomonas aeruginosa* was added to outside. Samples taken from insides of grafts at nine time points up to 60 hours were spread on agar and then bacterial colonies were analyzed. The grafts were also examined using scanning electron microscopy (SEM).

***Results:*** The contaminated vascular graft models of 18 ESDVG (group T) and 12 GCDVG (group G) were produced. Bacterial counts inside vascular grafts in both groups increased over time. Bacterial colonies were confirmed in all samples from group G by 30 hours, whereas bacteria appeared inside grafts in group T at various time points between 0 and 60 hours. Bacteria were undetectable in one model of group T throughout the study. SEM revealed that the elastomeric membrane in ESDVG was uneven.

***Conclusion:*** Bacterial invasion into vascular grafts should not always occur immediately after contamination. ESDVG may become more resistant to bacterial invasion as it has a thicker and evenly enriched elastomeric membrane.

## Introduction

Infection of a prosthetic vascular graft is an extremely serious complication.

Lethal systemic sepsis will result from bacterial invasion into vascular grafts if treatment is delayed. Thus, factors involved in the infectivity of prosthetic vascular grafts should be determined from bacterial adherence[1] and invasion. Regarding bacterial adherence to the vascular grafts[1] and antibiotic-coated grafts,[2,3] some manuscripts have been already published as *in vitro* and *in vivo*[4] study. The present study investigates bacterial invasion from the outer surface to the inside of vascular grafts using a new experimental system *in vitro* to determine relationships between time required for invasion and amount of bacteria inside the grafts. Graft fragments were also examined using scanning electron microscopy (SEM).

Besides, classical gelatin-coated Dacron vascular grafts (GCDVG) and new elastomer-sealed Dacron vascular grafts (ESDVG) were compared to determine the effects of bacterial invasion on different types of materials. Information about graft infectivity should help to treat this refractory complication and avoid implanted graft excision.

## Methods

The Committee for Biosafety and Biosecurity at Toho University School of Medicine (Tokyo, Japan) approved the study protocol.

Experiments involving virulent bacteria proceeded at a laboratory of the Department of Microbiology and Infectious Diseases (School of Medicine, Faculty of Medicine, Toho University, Tokyo, Japan) under the guidance of the appropriately qualified staff.

**Prosthetic vascular grafts**

Straight, 8-mm diameter ESDVG (Triplex®, Vascutek Terumo, Tokyo, Japan) and GCDVG (Gelweave®, Vascutek Terumo, Tokyo, Japan) were cut into 6-cm segments.

**Model bacterial strain and suspension**

The model bacterium was *Pseudomonas aeruginosa (P. aeruginosa)* strain PAO1 (ATCC 15692) because of its motility and ability to thrive in physiological saline.

*P. aeruginosa* frozen at -80°C was spread on LB agar and incubated for 18 hours at 35°C. Resulting bacterial colonies were transferred into 30 mL of LB broth with a sterile loop and incubated for 24 hours at 35°C. Thereafter, 3 mL of the LB broth containing *P. aeruginosa* was centrifuged at 8000 rpm for 5 min and then the sediment was resuspended in 30 mL of physiological saline and used as the bacterial suspension.

The bacterial concentration of the suspensions before experiments was confirmed to be approximately $1.0 \times 10^8$ colony forming units (CFU) /mL by spreading 10 μL of each properly diluted bacterial suspension on LB agar and counting the colonies manually after 18-hour

incubation at 35°C.

Bacterial suspensions were maintained by shaking at 160 rpm at 35°C until use.

**Study 1: Relationship between amount of time required for invasion and density of bacteria inside vascular grafts**

**Models of contaminated vascular grafts**

The 18 ESDVG (group T) and 12 GCDVG (group G) models of contaminated vascular grafts were produced as follows (Fig. 1). Prosthetic vascular grafts cut into 6-cm segments were placed in a U-shaped configuration in six-well culture plates (FALCON®, Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Sterile physiological saline (2 mL) was poured inside the grafts and bacterial suspensions (5 mL) were added to the area outside the grafts. The plates were covered and incubated at 35°C until the end of the study.

**Sampling and bacterial counting**

Samples (50 µL) collected from the inside of the grafts using micropipettes were appropriately diluted, spread on LB agar and then incubated at 35°C for 18 hours. Bacterial colonies were then manually counted.

Samples were collected every six hours for 36 hours, and then at 48 and 60 hours from the inside, and at 0, 24, 48 and 60 hours from outside the grafts. Bacterial counts are expressed as CFU/mL and were converted into log CFU/mL for statistical analysis. The limit of bacterial

detection was $2.0 \times 10^1$ CFU/mL.

**Statistical analysis**

Arithmetic data were expressed as means ± standard deviation and were statistically analyzed using JMP 9.0.3 (SAS Institute Inc., Cary, NC, USA). Bacterial counts inside and outside vascular grafts at each time point between two groups were evaluated using the Mann-Whitney U-test. The amount of elapsed time before bacteria appeared inside vascular grafts was determined from Kaplan-Meier curves and then values were compared between groups using the log-rank test. A P-value of $< 0.05$ was considered to indicate a statistically significant difference.

**Study 2: Observations of prosthetic vascular grafts by SEM**

An S-3500N Scanning Electron Microscope (Hitachi Ltd., Tokyo, Japan) was used to examine the structures of fresh ESDVG and GCDVG and determine the degrees of invasion of the graft walls at various angles after incubation for 60 hours during Study 1 in suspensions of *P. aeruginosa*.

**Sample processing for SEM**

Vascular grafts were fixed in 2.0% glutaraldehyde for 48 hours, rinsed in physiological saline, fixed with 2.0% osmium tetroxide for two hours, irrigated with distilled water and then

dehydrated in a graded series of 50% to 100% ethanol. They were then placed in tertiary-butyl alcohol and dried in an ES-2030 critical-point dryer (Hitachi Ltd., Tokyo, Japan). Dried grafts were trimmed, mounted onto SEM slabs at various angles and sputter-coated with platinum-vanadium for 80 seconds using an Ion Sputter E-1030 (Hitachi Ltd., Tokyo, Japan).

Graft structures and degrees of bacterial invasion were investigated as described above in long-axis and short-axis views.

**Results**

**Study 1.**

Bacteria started to invade the GCDVG from six hours post-immersion, and all samples from inside GCDVG had generated bacterial colonies by 30 hours. The average elapsed time until bacteria appeared inside the GCDVG was 15.5 ± 7.0 hours.

Bacteria were identified inside two ESDVG immediately after immersion, and in one model at 60 hours. Bacteria remained undetectable in another one model throughout the study. The average amount of time taken for bacteria to become detectable inside ESDVG was 22.0 ± 19.7 hours.

Bacterial counts inside the grafts increased over time in both groups. Bacterial counts outside (Table 1) and inside (Table 2) vascular grafts at each time point did not significantly differ

between groups ($P > 0.05$, Mann-Whitney U-test).

The amount of time that elapsed before bacteria appeared inside vascular grafts was determined from Kaplan-Meier curves (Fig. 2). Values did not significantly differ between the groups ($P > 0.05$, log-rank test).

**Study 2.**

Figure 3A and 3B shows short-axis SEM views of sterile GCDVG and ESDVG, respectively. The wall of the ESDVG comprised a central, low-porosity elastomeric membrane sandwiched between outer and inner layers of knitted Dacron.

Then, the elastomeric membrane was proved to be uneven on thickness and the thinnest portion was about 2 μm (Fig. 4A). Besides, some defects were evident in the membrane (Fig. 4B).

The uneven thickness and defects in the elastomeric membrane might have been associated with the amount of time required for the bacteria to invade ESDVG.

SEM views of grafts inoculated with *P. aeruginosa* for 60 hours revealed bacteria in gaps between GCDVG fibers (Fig. 5A) and in the elastomeric membrane of ESDVG (Fig. 5B).

**Discussion**

The model bacterial species in this study was *P. aeruginosa* because its motility and ability to

exist in physiological saline rendered it suitable for the study purpose. Although it is not a typical bacterium about vascular graft infection, the reports of the infected aortic aneurysm and prosthetic vascular graft infection caused by *P. aeruginosa* are often recognized.[5-7] Furthermore, the indications for both major aortic and peripheral vascular surgery have recently been extended to high-risk patients. Therefore, infection caused by *P. aeruginosa* is likely to increase and it has already become a serious issue, especially during vascular surgery.[8] Thus, vascular graft infection caused by this organism should be investigated.

The new ESDVG used in the present study has an unique structure comprising a central elastomeric membrane sandwiched between two layers of high-porosity knitted Dacron.[9,10] The elastomeric membrane consists of a highly flexible styrene polymer with a low-porosity material. The porosity of ESDVG is significantly lower than that of standard coated grafts, and thus prevents water leakage.[9] Accordingly, I postulated that the ESDVG would be more resistant to bacterial invasion than standard coated vascular grafts when exposed to direct external bacterial contamination.

Then, I selected gelatin-coated woven Dacron vascular grafts to compare ESDVG. In clinical setting, gelatin-coated woven Dacron grafts are used more frequently than knitted Dacron grafts due to enlargement of diameter during the postoperative course.[11] Coated knitted Dacron vascular grafts are not used in our institution even for surgery of the abdominal aorta. The

present study investigated vascular grafts that are actually applied in clinical medicine.

Although no statistically significant differences were identified between the two types of grafts, the amount of time that elapsed before bacteria appeared inside ESDVG varied between 0 and 60 hours and SEM confirmed thin areas and defects in the elastomeric membrane of sterile ESDVG. These features might have been associated with the immediate appearance of bacteria inside the ESDVG. Thinner areas and defects in the elastomeric membrane might have facilitated bacterial invasion. If so, this could be prevented by thickening and enriching the elastomeric membrane.

Then, no bacterium had been confirmed in samples of inside GCDVG at 0 hour. According to experimental data presented by Terumo Corporation (Tokyo, Japan) which simulates 14 days of *in vivo* hydrolysis, gelatin remaining rates on the grafts are respectively about 100%, 90%, 80%, 70%, and 5% on day 0, 2, 4, 7, and 14. Though my experiment is *in vitro* basic research, time and amount of bacterial invasion into vascular graft would be a little affected by hydrolysis rate of gelatin sealant.

The present *in vitro* results also indicate that bacterial invasion into vascular grafts is not always immediate even if the grafts are located in a highly contaminated area. Besides, bacterial counts inside vascular grafts should increase over time. Therefore, prosthetic vascular grafts implanted in infected wounds should be drained and irrigated as soon as possible to avoid systemic sepsis

and graft removal in the clinical setting. Recent clinical efforts to preserve infected prosthetic vascular grafts have progressed. Dosluoglu and colleagues argue that patients with exposed grafts after inguinal wound dehiscence without systemic sepsis can be treated by graft preservation therapy such as negative pressure wound therapy (NPWT).[12] Under probable contaminated conditions such as traumatic disease or infected aneurysms, using vascular grafts should be resistant to bacterial invasion and ensuring sufficient irrigation, a suction drain insertion around the vascular grafts, NPWT at wound dehiscence should also help to prevent graft infection and systemic sepsis.

The present study suggests that evenly thickening and enriching the elastomeric membrane of ESDVG may improve their ability to resist or suppress bacterial invasion.

Furthermore, this is the first investigation to examine the mechanism of bacterial invasion into prosthetic vascular grafts.

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**Conflict of interest statement**

Yuki Sasaki has no conflict of interest.

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**Table 1. Average bacterial counts outside vascular grafts.**

| Bacterial counts (log CFU/mL) | | | |
|---|---|---|---|
| Elapsed time (h)* | Group G | Group T | |
| 0 | 7.0 ± 0.4 | 7.2 ± 0.6 | NS |
| 24 | 7.5 ± 0.3 | 7.3 ± 0.4 | NS |
| 48 | 7.5 ± 0.3 | 7.6 ± 0.3 | NS |
| 60 | 7.7 ± 0.2 | 7.7 ± 0.4 | NS |

Bacterial counts are expressed as means ± standard deviation. *Hours after establishing models of contaminated vascular grafts.

Mann-Whitney U-test of data at each time point revealed no significant differences (NS) between group T (ESDVG group) and group G (GCDVG group).

**Table 2. Average bacterial counts inside vascular grafts.**

| Bacterial counts (log CFU/mL) | | | |
|---|---|---|---|
| Elapsed time (h)* | Group G | Group T | |
| 0 | 0 | 0.1 ± 0.4 | NS |
| 6 | 0.3 ± 0.6 | 1.2 ± 1.5 | NS |
| 12 | 1.0 ± 1.1 | 1.2 ± 1.7 | NS |
| 18 | 2.6 ± 1.5 | 1.8 ± 2.0 | NS |
| 24 | 3.6 ± 1.4 | 2.4 ± 2.3 | NS |
| 30 | 4.4 ± 0.9 | 3.4 ± 2.3 | NS |
| 36 | 4.6 ± 1.1 | 3.8 ± 2.4 | NS |
| 48 | 4.9 ± 1.0 | 4.1 ± 2.5 | NS |
| 60 | 5.1 ± 1.0 | 4.5 ± 2.5 | NS |

Bacterial counts are expressed as means ± standard deviation. *Hours after establishing models of contaminated vascular grafts.

Mann-Whitney U-test of data at each time point revealed no significant differences (NS) between group T (ESDVG group) and group G (GCDVG group).

**Figure legends**

Fig. 1. Establishment of contaminated vascular graft models.

Fig. 2. Kaplan-Meier curve (Study 1).

Vertical axis shows ratio of models in which bacteria were undetectable inside vascular grafts. The horizontal axis shows elapsed time. Bacteria were detected inside grafts in all models of group G (GCDVG group) by 30 hours. Bacteria were undetectable throughout the study in one model from group T (ESDVG group). However, log-rank test did not reveal any significant differences between the two groups.

Fig. 3B. Scanning electron microscopy finding of sterile elastomer-sealed Dacron vascular graft.

Short-axis view of elastomer-sealed Dacron vascular graft shows unique three-layer structure comprising a central elastomeric membrane sandwiched between layers of knitted Dacron (magnification, ×50).

Fig. 4A. Scanning electron microscopy findings of sterile elastomer-sealed Dacron vascular grafts.

Short-axis views at ×300 (left) and ×200 (right) magnification show uneven thickness of elastomeric membrane. Arrows: a, 50 μm; b, 150 μm; c, 2 μm; d, 300 μm.

Fig. 4B. Scanning electron microscopy findings of sterile elastomer-sealed Dacron vascular

grafts.

Short-(left) and long-(right) axis views at magnification of ×200 and ×300, respectively, show defects in elastomeric membrane (arrows).

Fig. 5A. Scanning electron microscopy views of gelatin-coated Dacron vascular graft after immersion in *Pseudomonas aeruginosa* suspension for 60 hours.

There are long-axis views of the fragment of gelatin-coated Dacron vascular graft at magnification of ×270 (left) and ×1000 (right). Many bacteria are evident in gaps between graft fibers at ×1000 magnification (arrows).

Fig. 5B. Scanning electron microscopy views of elastomer-sealed Dacron vascular graft after immersion in *Pseudomonas aeruginosa* suspension for 60 hours.

There are short-axis views of elastomeric membrane in fragment of elastomer-sealed Dacron vascular graft at magnification of ×1500 (left) and ×4000 (right). Bacteria are evident in elastomeric membrane (arrows).

Fig.1

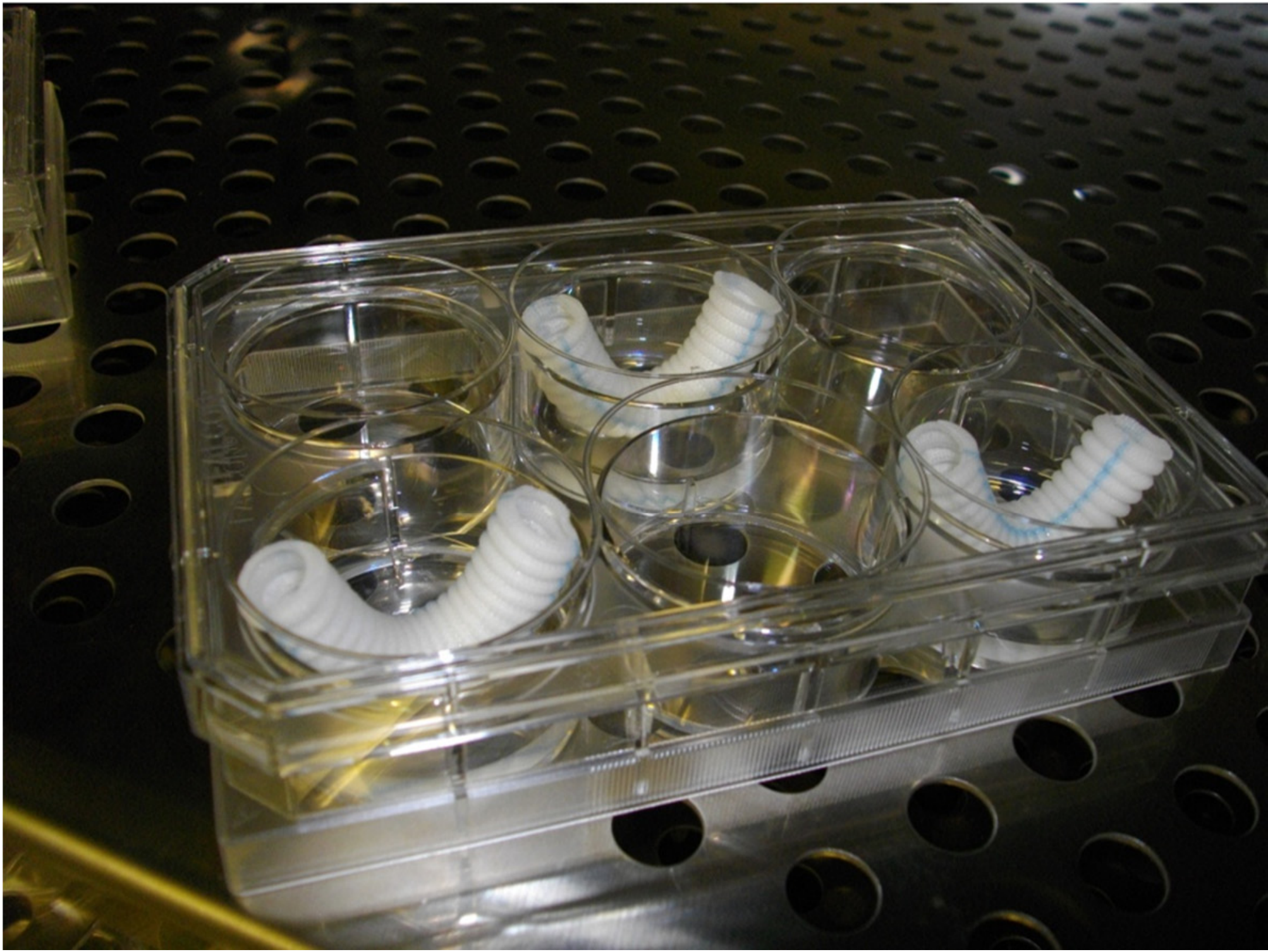


Fig.2

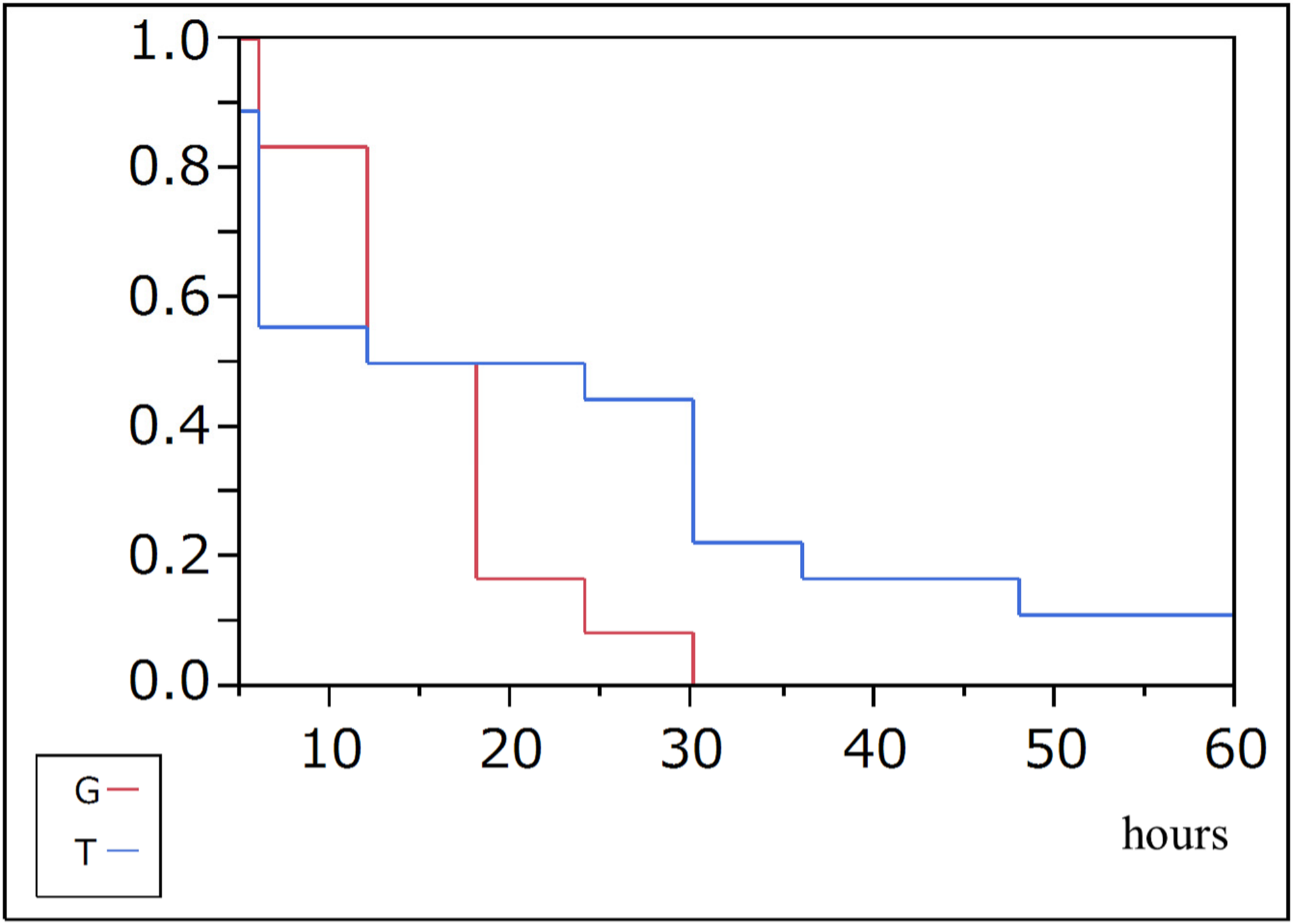


Fig.3A

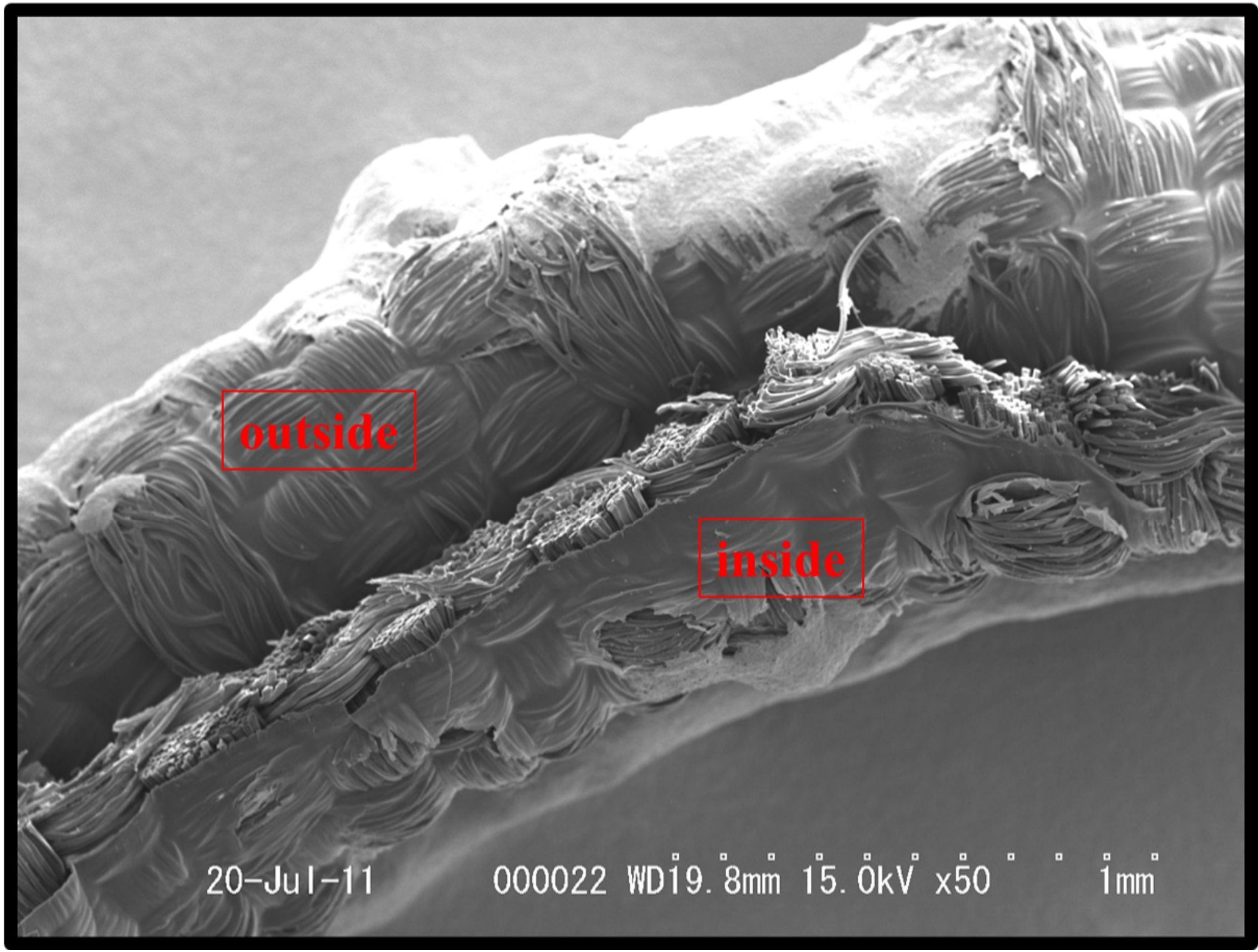


Fig.3B

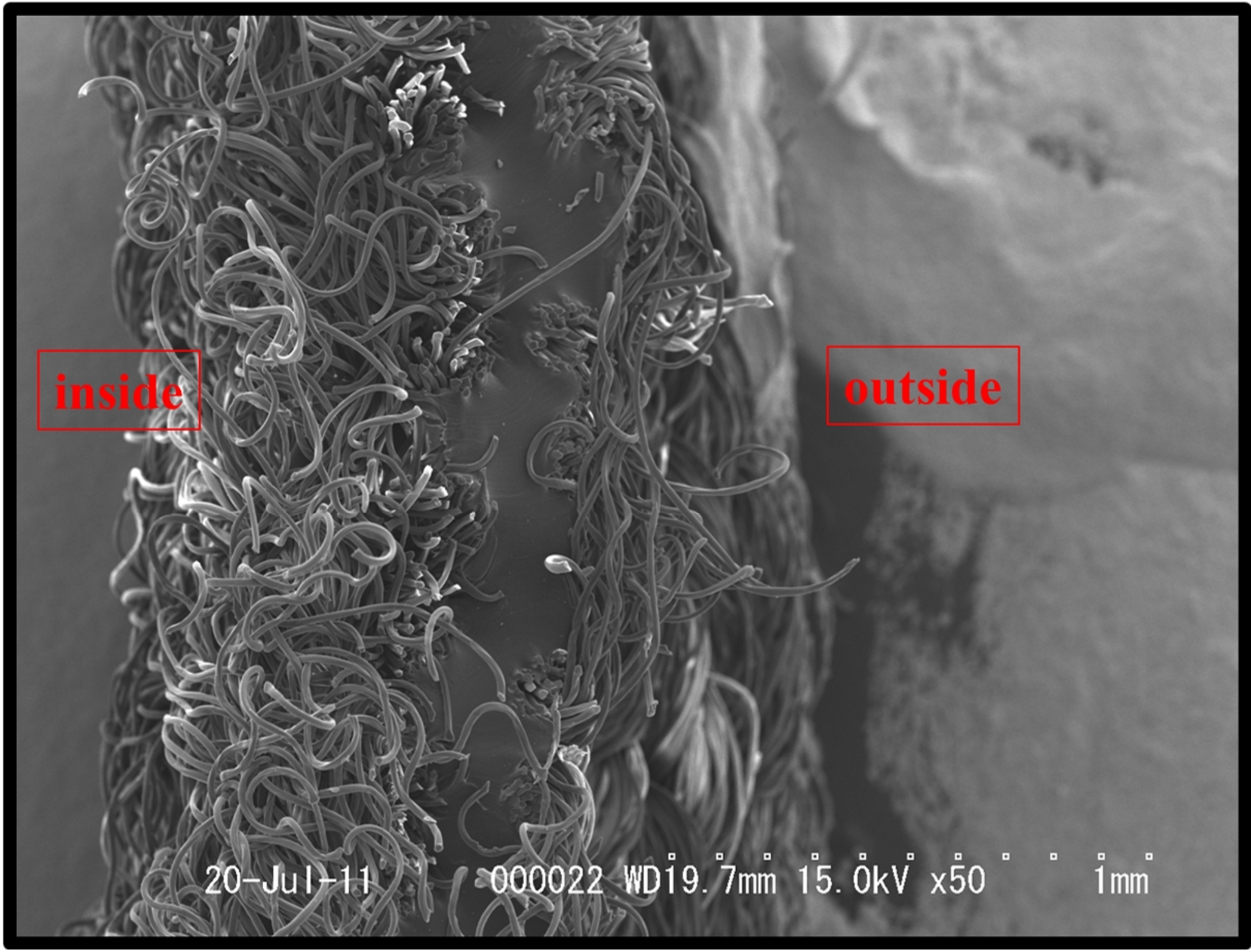
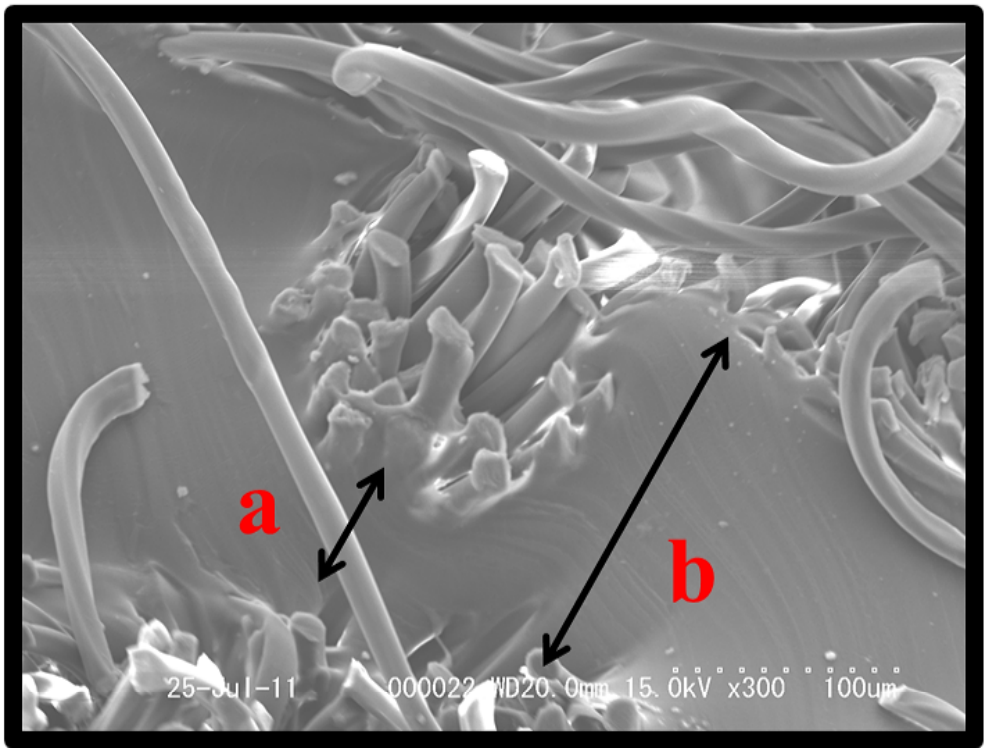


Fig.4A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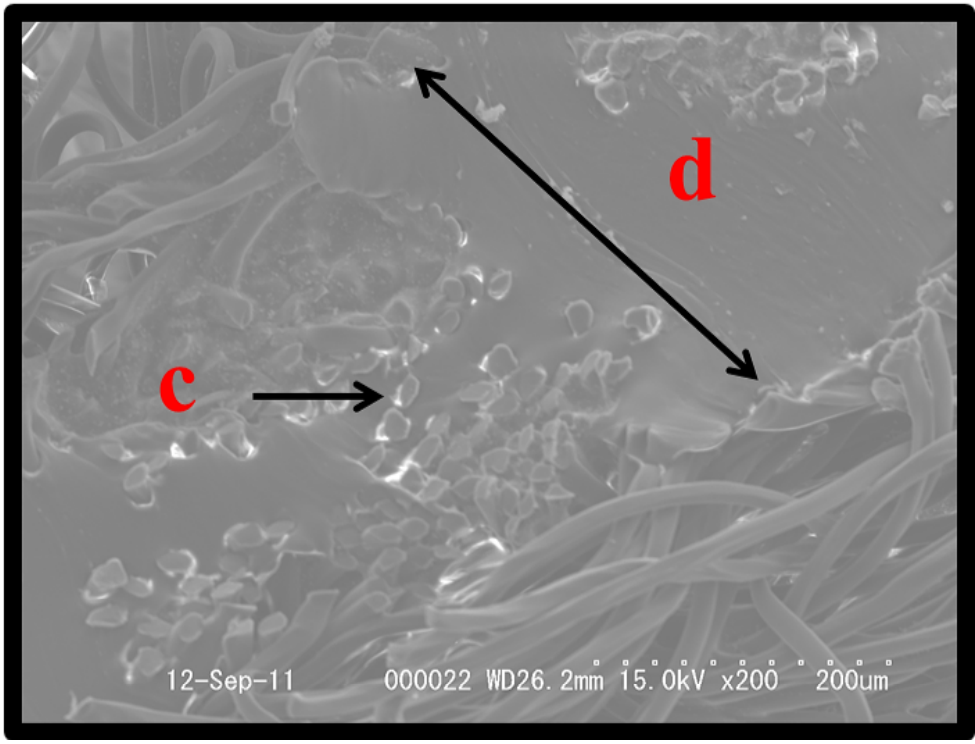
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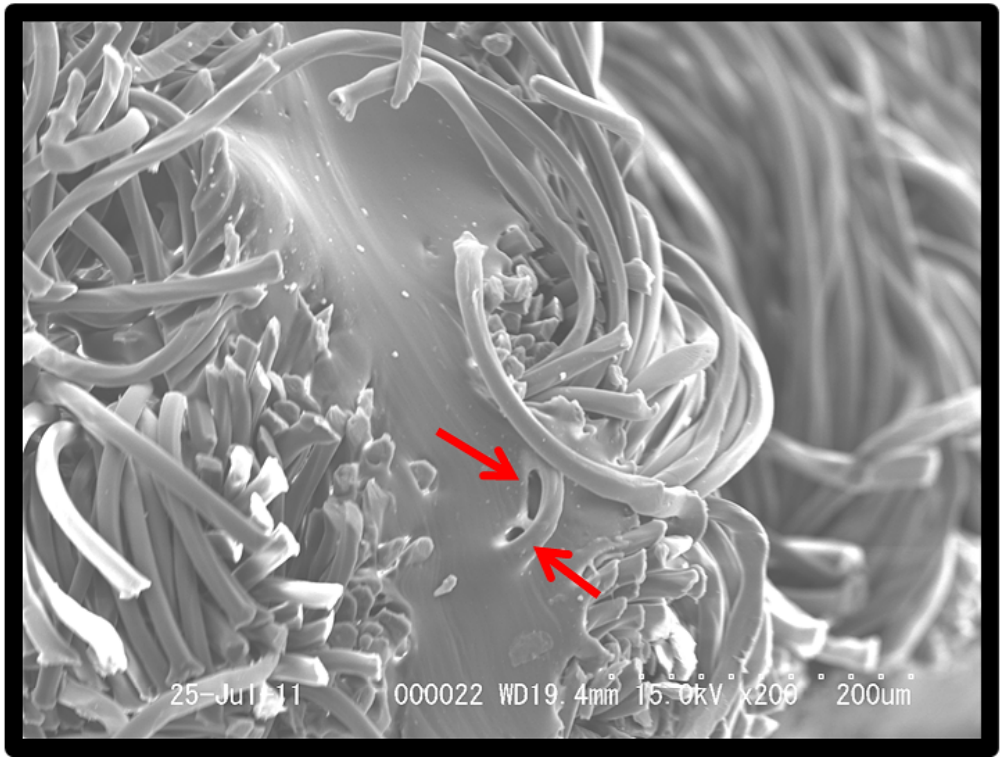
Fig.4B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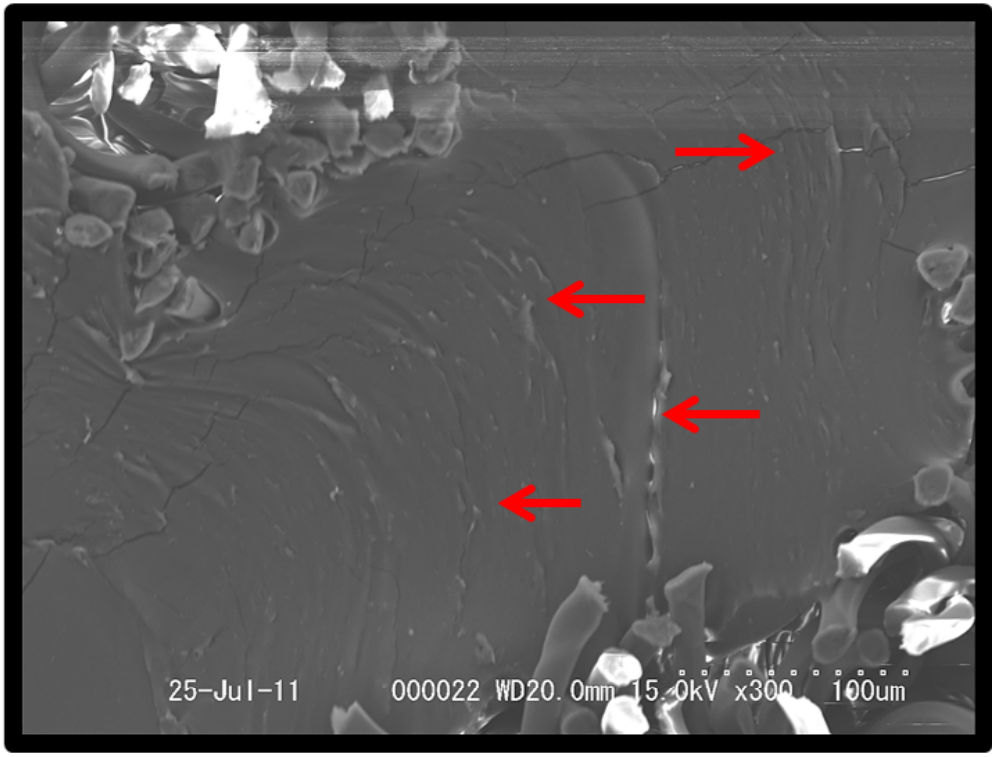
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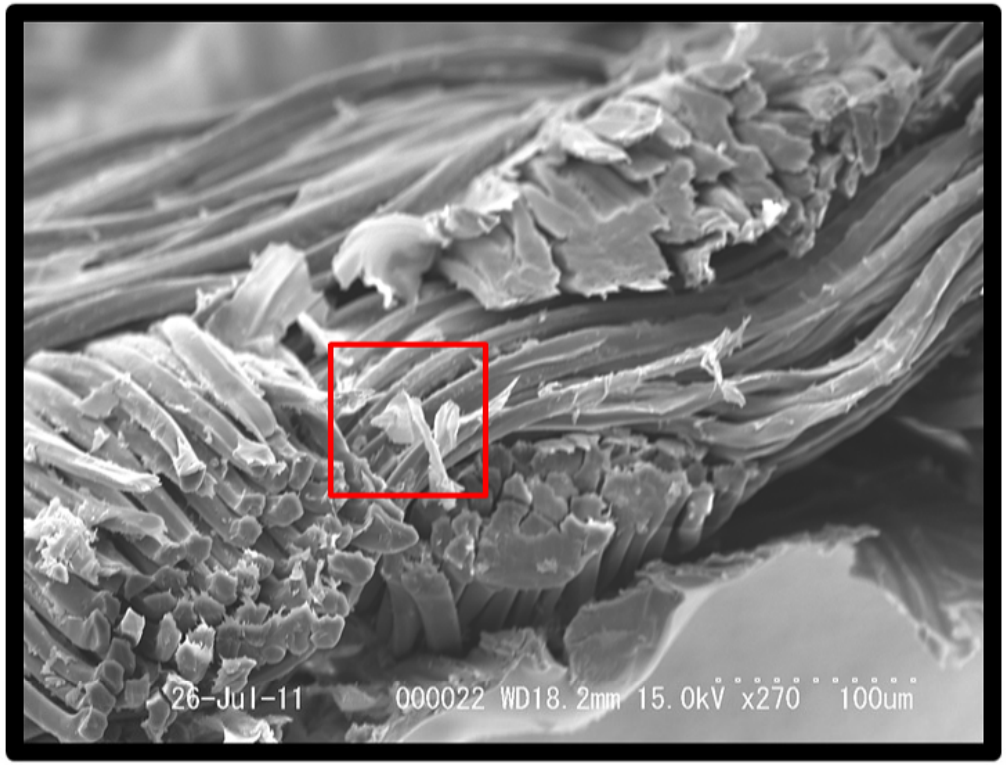
Fig.5A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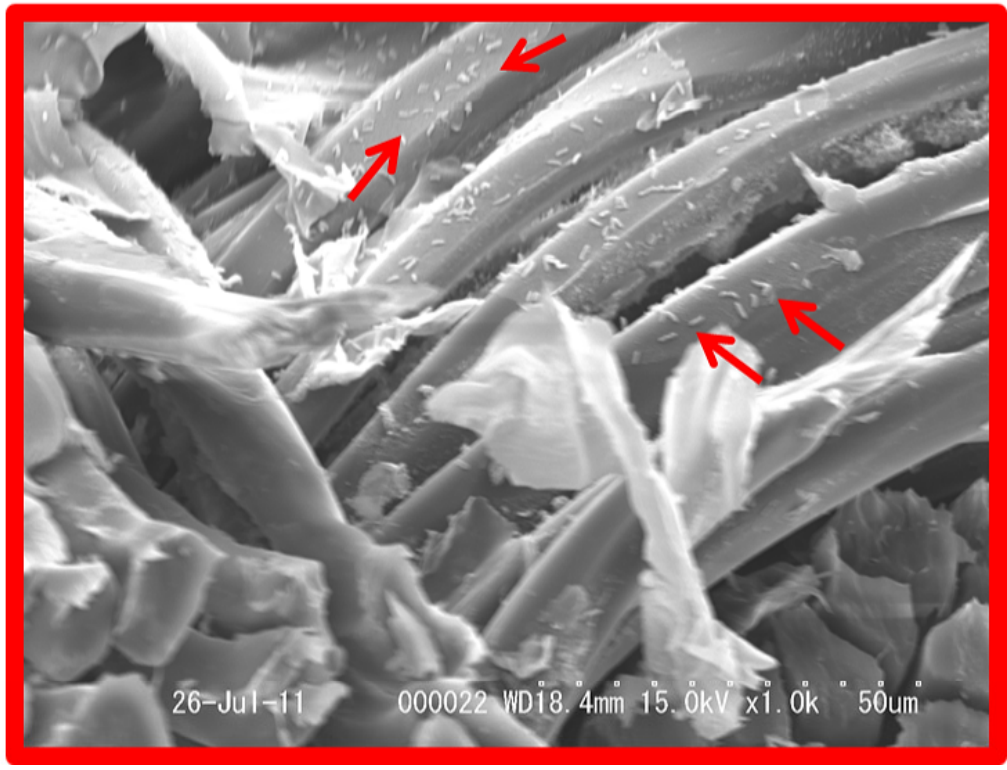
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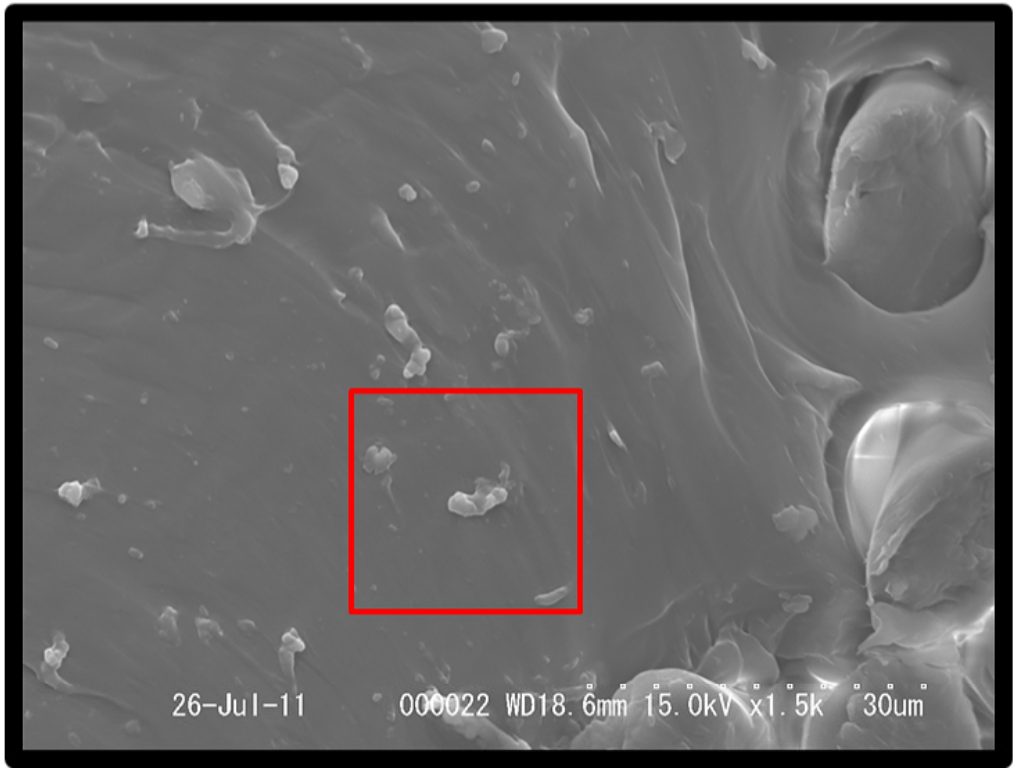
Fig.5B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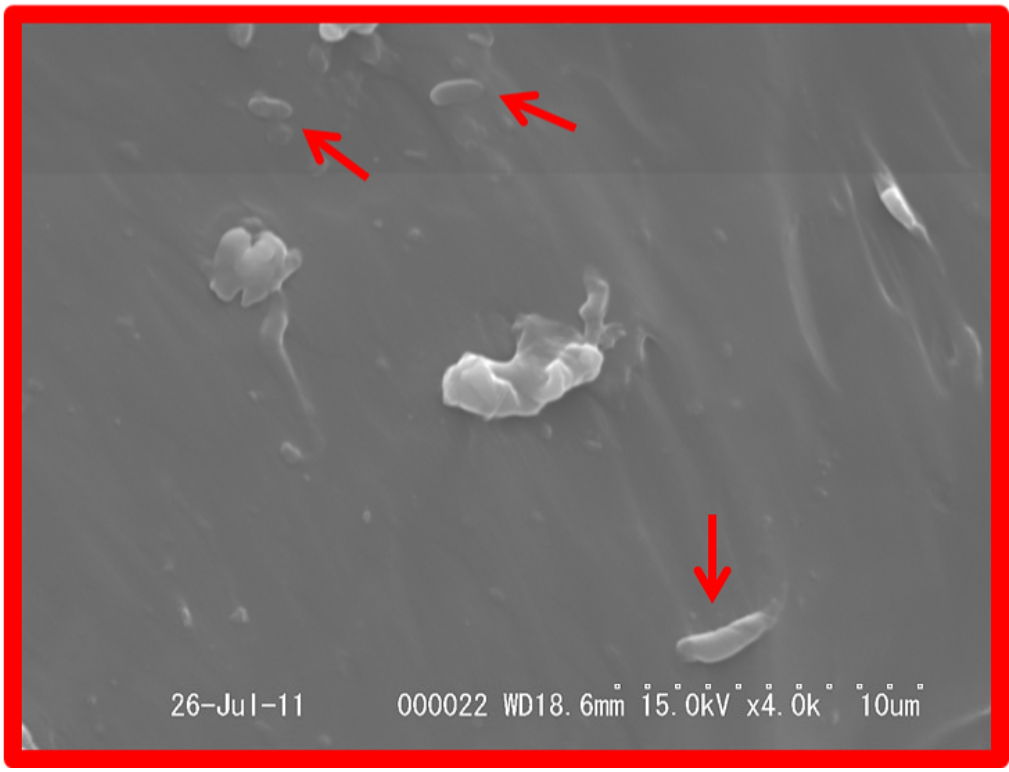
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