AGH University of Science and Technology

Short Term Scientific Mission Project Report

September 18 – October 18

Krakow, Poland

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COST Action "European Network to connect research and innovation efforts on Advanced Smart Textiles" (CONTEXT) CA17107

12th call for Short Term Scientific Mission (STSM)

Under the title:

"Production and characterization of electrospun fibers and scaffolds for bone tissue engineering applications"

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Host Institution: Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Krakow, Poland

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STSM duration: September 18 – October 18, 2022

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Objective of the STSM

The STSM project is aimed at gaining knowledge and experience in the areas of electrospinning polymer solutions. This technology is one of the methods to produce reliable scaffolds for tissue regeneration and drug delivery mechanisms. On the other hand, it is also used to fabricate fiber films for water purification or harvesting. In general, it is a technique with several applications and tunable operating procedures. This STSM focused on the following:

- preparation of polymer solution,
- electrospinning of polymer fibers,
- characterization of electrospun fibers, and
- basics of cell culture studies on fibers.

Research planned during this STSM in the AGH University of Science and Technology, is strongly related to the objectives of WG1: Smart textiles for health and medical applications. The goal of the joint research is to produce and characterized cellulose acetate-based scaffolds prepared by electrospinning for tissue engineering applications. The polymer will be modified by graphene oxide particles.

Details of work carried out

The project started on September 19th by an official introduction to the team of Dr hab. inż. Urszula Stachewicz. We had an initial discussion on how we are going to follow through to the schedule. We agreed on updating the schedule and fast-tracking it so that we can use the expertise of the team to the fullest.

The polymer chosen for electrospinning is cellulose acetate. It is one of the preferred biomaterials for biocompatible applications. The team at AGH has had an experience in using it to produce fibers. To modify its properties, graphene oxide particles were used. These particles were produced at the home institution. They are synthesized from agricultural biomass, specifically ground coffee waste.

Electrospinning is a combination of the right polymers, environmental conditions and collection of fibers. The spinning parameters set up by the group in AGH helped in this regard. Thus, we moved the solubility test to the first week. The group was using a pre-developed parameter for electrospinning cellulose acetate. Thus, we used dimethylacetamide (DMAC) and acetone in 1:1 ration by volume as the chosen solvent.

Solution Preparation

We spun two concentrations of cellulose acetate, taken as a weight percentage of the total polymer solution. CA 19% stands for 19% cellulose acetate by weight of the solution, whereas CA 15% stands for 15% cellulose acetate by weight of the solution. After having good results in initial spinning, we continued with the modification of CA fibers with graphene oxide particles. We used two types of concentration of GO, per the weight of the polymer, for modifying the cellulose acetate fibers. GO 1% stands for 1% graphene oxide by weight of the cellulose acetate and GO 5% stands for 1% GO by weight of the cellulose acetate.

Electrospinning

Electrospinning took place in an EC–DIG (IME Technologies, Waalre, The Netherlands) apparatus. The electrospinning parameters were set for an optimal cellulose acetate film.

Characterization

Morphology, functional groups and mechanical properties were checked in the electrospinning lab. For these, scanning electron microscopy (Merlin Gemini II, Zeiss, Germany), FTIR spectra and tensile module with 1N load cell were used respectively. In order to supplement these, wettability test and thickness measurement of fibers were undertaken.

Cell culture studies

On the third week, we started cell culture studies (moved from the fourth week). We used MG-63 osteoblasts for checking cell viability and proliferation tests. Proliferation tests were carried out for 1, 3 and 7 days. The proper treatment of fibers; cell counting, seeding and incubation were undertaken under standard operating procedures. We characterized the cell growth using cell titer blue and used histogram to analyze the data. We also took SEM images to see the proliferation of cells on CA and CA/GO fibers against glass controls.

Results and discussion

In the initial try, we were able to successfully spin our polymer solution onto an aluminum foil wrapped rotating drum. The spun fibers were checked under table-top SEM for the formation of fibers. The first images indicated that fibers were formed and mostly free from beads. We wanted to investigate the incorporation of the GO particles in the cellulose acetate fibers. For this Merlin SEM was utilized. Figure 1 shows fibers with 5% GO addition and Figure 2 shows fibers with 1% GO addition. On both figures, it can be seen that the GO nanoparticles are incorporated in the CA fibers.

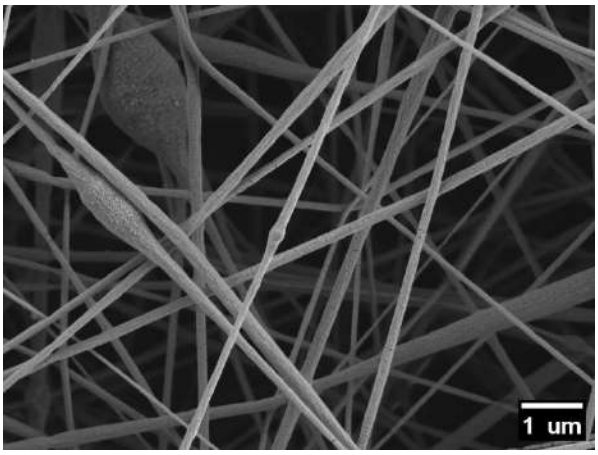

Figure 1: CA 15% GO 5%

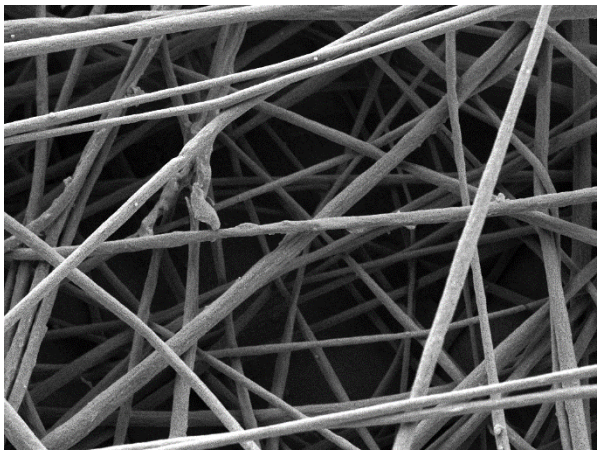

Figure 2: CA 15% GO 1%

In addition, FTIR was used to determine the GO particles incorporations. The FTIR spectra on Figure 3 shows a comparison between the functional groups of CA & GO powders, CA 19% fibers and GO containing CA nanofibers shows that an additional carbonyl group on the GO containing fibers. That shows that the CA fibers are integrated with GO particles.

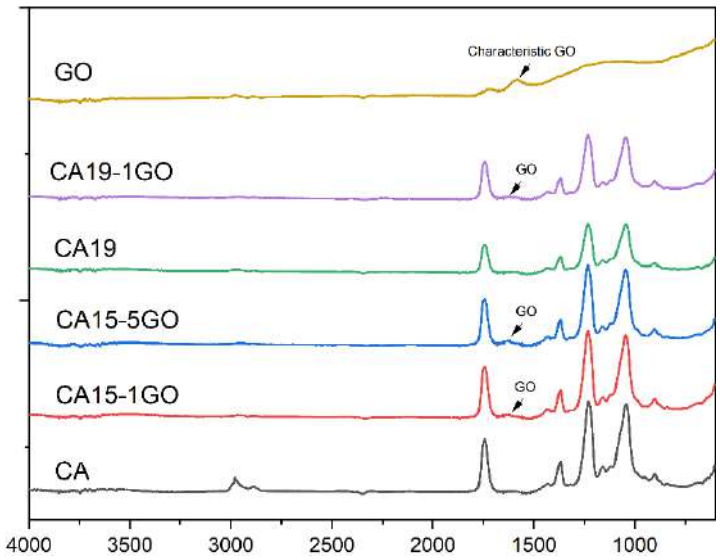

Figure 3: FTIR spectra of fibers, CA & GO powders

Mechanical Properties

Diameter of fibers were measured using high resolution desk microscope. And the results are shown on the histogram below. On average, fibers from CA 19% have 457 ± 157 nm, CA 15% have 323 ± 170 nm, CA 15% GO 1% have 405 ± 130 nm and CA 15% GO 5% have 329 ± 136 nm diameters.

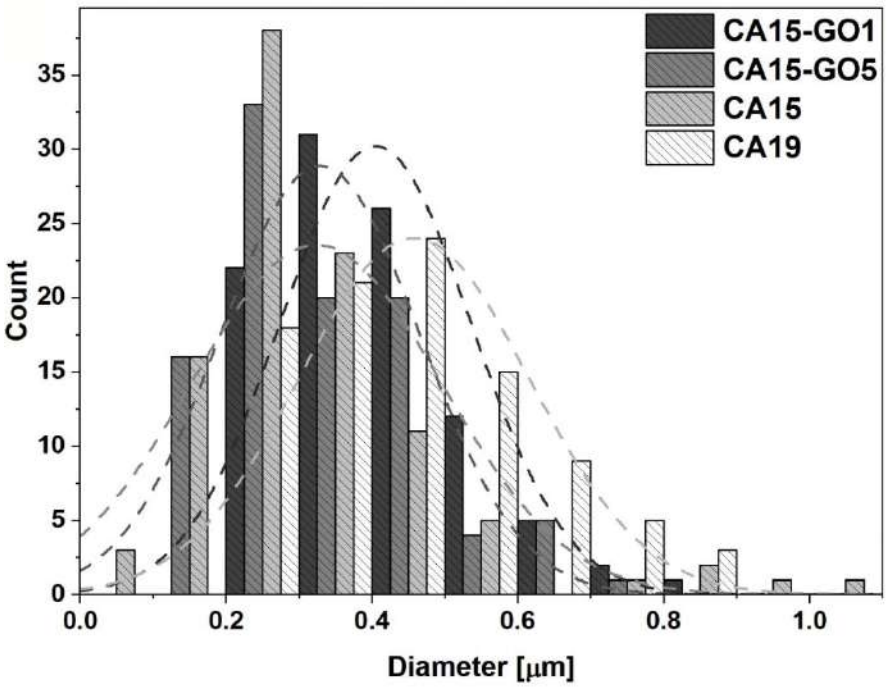

Figure 4: histogram of electrospun fiber diameters

Wettability test was undertaken to see the effect of the fibers on hydrophobicity. Water droplets were released from a tip of a standard 21-gauge syringe to the fiber mats on aluminum foil background. The water contact angles were then measured using ImageJ software. The results are shown on Figure 5 below. Cellulose acetate is a hydrophobic polymer. This can be shown by the high contact angle on Figure 5a. But upon addition of graphene oxide particles, this hydrophobicity is seen to decrease.

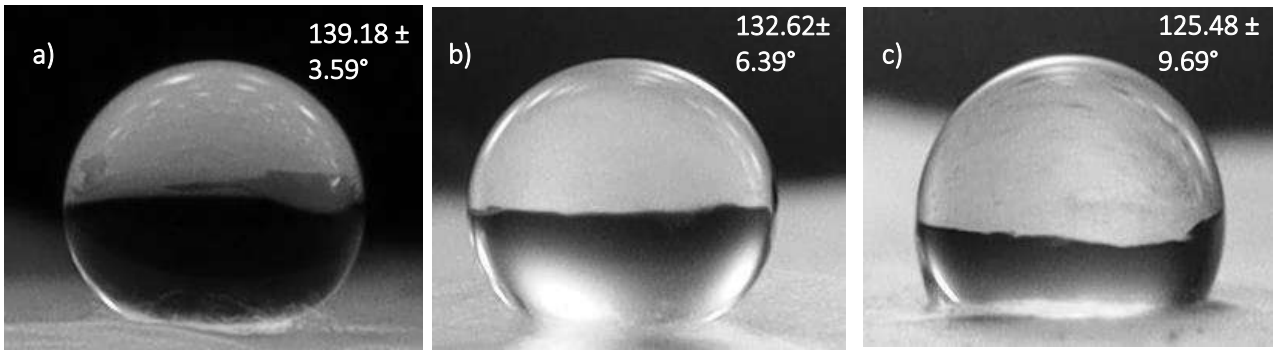

Figure 5: droplet images and contact angles for, a) CA 19%, b) CA 15% GO 1%, and c) CA 15% GO 5%

The mechanical strength of the fibers was measured by electrospinning fibers on paper and later laser-cutting into desired sizes. The fibers were carefully removed from the paper and attached to the clamps of the tensile module. 1N load cell was used to apply the load and the load-elongation curve was obtained until the fibers broke. These curves were analyzed and stress-strain graph was developed using Origin software. The following figure shows the stress-strain graphs of fibers produced from CA alone, and CA modified with GO. As can be observed, the fibers containing 5% GO show a higher stress value compared to the fibers with only CA. On the other hand, the fibers containing 1% GO have an elastic nature and have the highest strain.

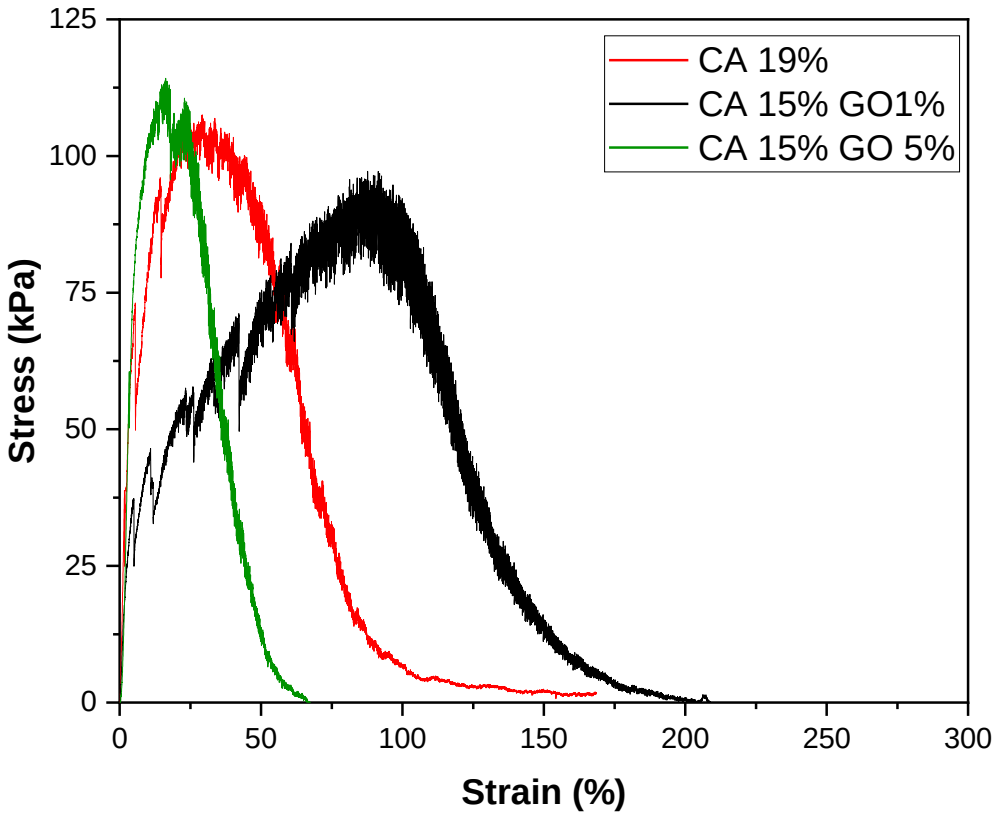

Figure 6: Tensile stress-strain graphs of electrospun fibers

Cell culture studies

The biocompatibility of the fibers was checked by using standard cell proliferation test. Fresh MG 63 cells were treated with culture media containing DMEM supplemented with 10% FBS, 2% antibiotics (penicillin/streptomycin), 1% amino acids, and 1% L-glutamine. They were [placed on glass slides containing where the fibers were electrospun. They were then incubated for 1, 3, and 7 days for the respective proliferation tests. The results of CellTiter-Blue Assay are described on the figure below. Glass slides were used as controls.

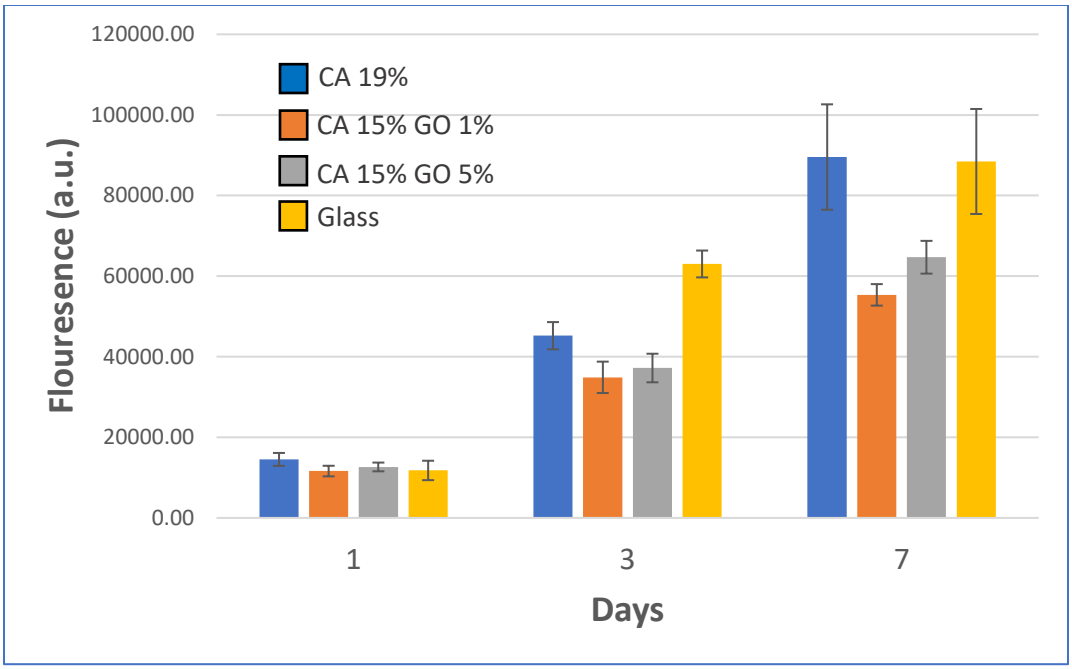

Figure 7: cell proliferation results for 1. 3. and 7 days of cell growth

As can be seen on the figure, the cellulose acetate fibers create a conducive environment for cell growth. Followed by the CA fibers with 5% GO addition. We can see that the addition of GO is not toxic for the growth of cells rather promotes their proliferation.

For SEM imaging, the cells were fixed with 2.5% glutaraldehyde for 1 h at 4°C. Afterward, they were washed three times with PBS and dehydrated in series of ethanol. The following images were seen under SEM.

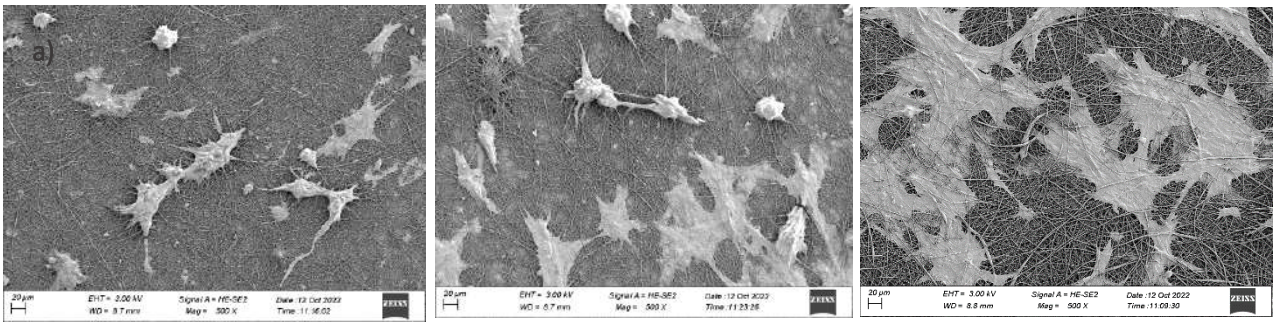

Figure 8: SEM images of cell growth on scaffolds produced from a) CA 15% GO 1%, b) CA 15% GO 5%, and c) CA 19%

As can be seen, the cells on the pristine CA scaffolds tend to spread onto the surface much better than that with the fibers containing GO. 5% GO addition also proved to be conducive for the growth of the cells.

Summary and further collaborations

Within the one month stay at the faculty of metals engineering and Industrial computer Science, I, together with the electrospinning team was able to synthesize and characterize scaffolds from cellulose acetate and cellulose acetate modified with graphene oxide. These scaffolds were checked for their material properties and biocompatibility. What we have found was promising. We saw improved mechanical properties (strength and ductility) when the cellulose acetate fibers were modified with graphene oxide. In addition, the graphene oxide particles are non-toxic and can be used as scaffold biomaterials. Overall all, we can conclude that we were successfully able to incorporate GO particles in CA fibers in a polymer solution and the scaffold produced from this solution improved the mechanical properties of the CA fibers while being biocompatible.

AT the end, we discussed about further collaborations between the Center of Polymer Systems at TBU and the Faculty of Metals Engineering and Industrial computer Science at AGH. The fields of biomaterials and electrospinning for scaffolds could be very much linked through researches such as this STSM. Thus, we agreed on writing proposal for other biomaterials and further applications through electrospinning in the coming months.

Below is a picture showing Dr inż. Piotr Szewczyk (left, AGH), Dr hab. inż. Urszula Stachewicz (middle, AGH) and myself.