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Abstract

Spider silk is becoming more useful for its desired properties as more information about the nanostructure is discovered. Due to the fact that this protein is nearly impossible to mass produce directly from the spider, the protein coding gene has been duplicated from the spider genome and inserted into the *E. coli*, goat, alfalfa, and silkworm genomes. This has allowed us to extract the produced protein from the transgenic hosts at a larger scale than the spiders offer. Spider silk is one of the strongest and most elastic fibers found in nature and these two characteristics, along with others, have very promising applications in many different areas. Areas including biomedical, automobile, military, and sports equipment all have products that could be benefitted by this spider silk. Considering the biomedical realm, there are encouraging results in the mechanical and chemical properties of the spider silk protein for tendon and ligament repair, tissue scaffolding, and also neural system regeneration. The aim of this project is to take the spider silk protein (M4) produced from the transgenic goat and spin it into nanofibers via electrospinning technique and analyze the properties of this fiber. Specifically, we will use FTIR (Fourier Transfer Infra Red) Spectroscopy, SEM (Scanning electro microscope) analysis, mechanical property analysis, as well as resistance testing at variable relative humidity levels (RH) to record the resistive behavior of the fiber as if it were in an actual neural system.

Introduction

With more data and knowledge continuously being discovered about this protein (M4), there are many applications that would benefit from this spider silk. The specific application that this project is focused on is the biomedical application of the protein. Because of the spidersilk's biocompatible character, cell growth is promoted by the spider silk and those cells are able to be successfully implanted into the body for the function of replacing or helping severed or damaged body structures regrow¹. With the nanofiber orientation of this spider silk, this project aims to gather data on the potential use of spider silk nanofibers in neural system regrowth. Specifically, this project will analyze the behavior of the spider silk materials in terms of electrical resistance as a function of time and relative humidity level. With these results, we will be able to compare the resistivity and complementary conductivity of the spider silk in dense environments.

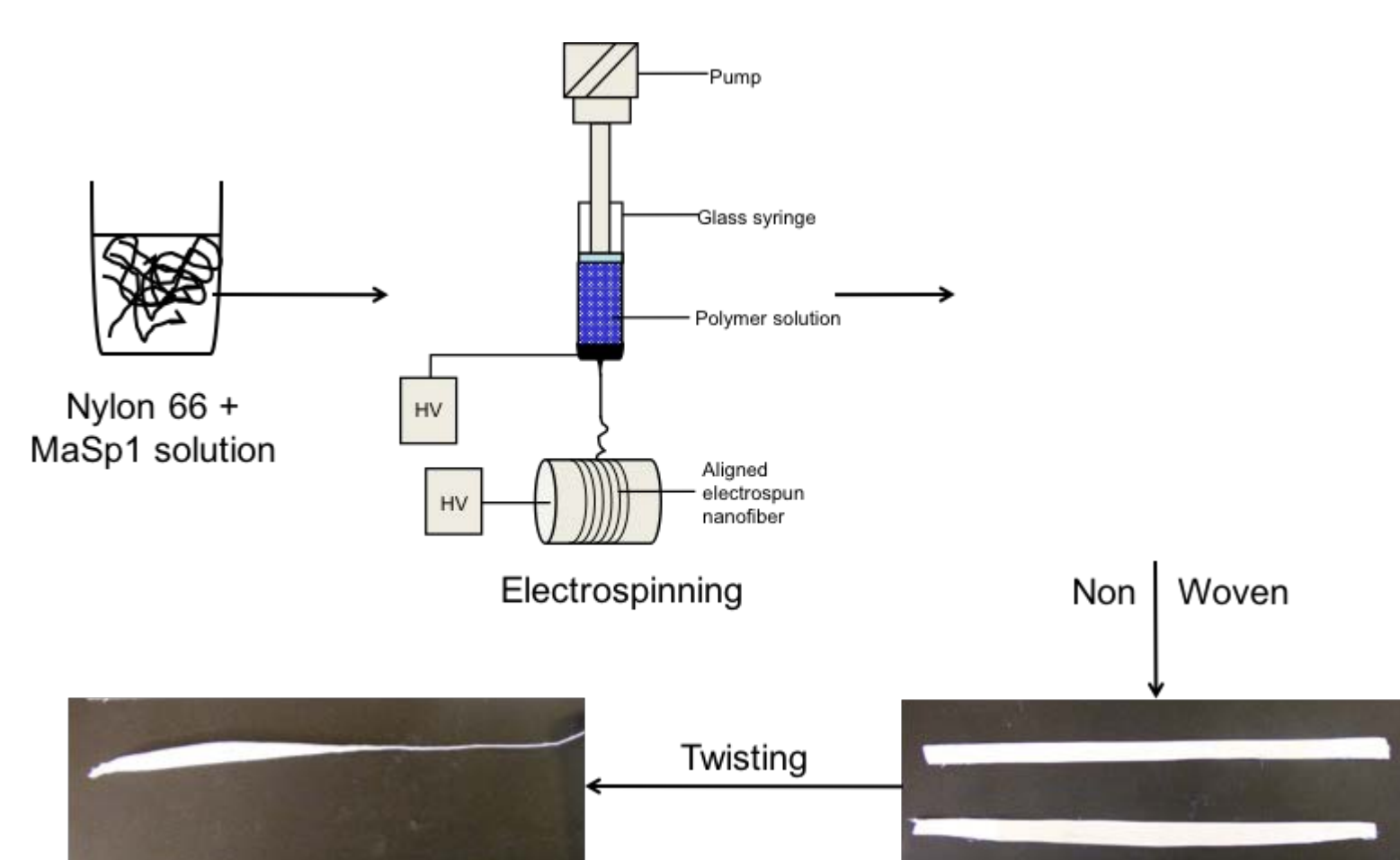


Figure 1: Electrospinning process

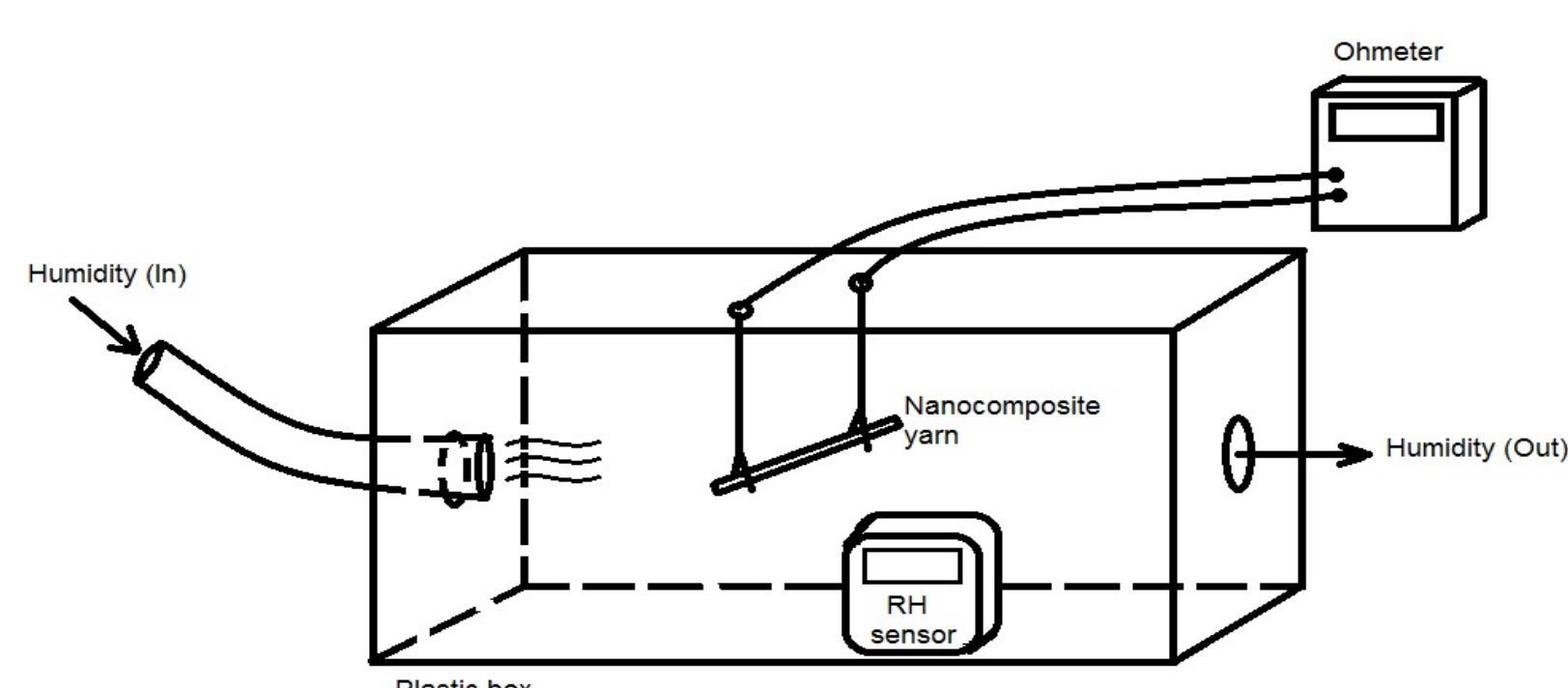


Figure 2: Humidity controlled environment

Experimental Procedures

1) The first procedure that we must do is isolate the protein from the goats milk. We retrieve the milk from the goat farm in frozen units. We then thaw the milk and centrifuge out the fat. From there the milk is combined with arginine in a TFF machine which mechanically filters the protein out of the defatted solution based on molecular size. The protein solution then is combined with ammonium sulfate, which precipitates the spider silk protein out of solution and is collected by centrifugation. The M4 protein pellet then is dehydrated and ready to be utilized.

2) We take .15g of the M4 protein and dissolve in with .75 ml of formic acid. We sonicate the solution to ensure it is homogenous. After this process 0.75 ml of HFIP and >.1ml of a surfactant is added which reduces the aggregation of the solution.

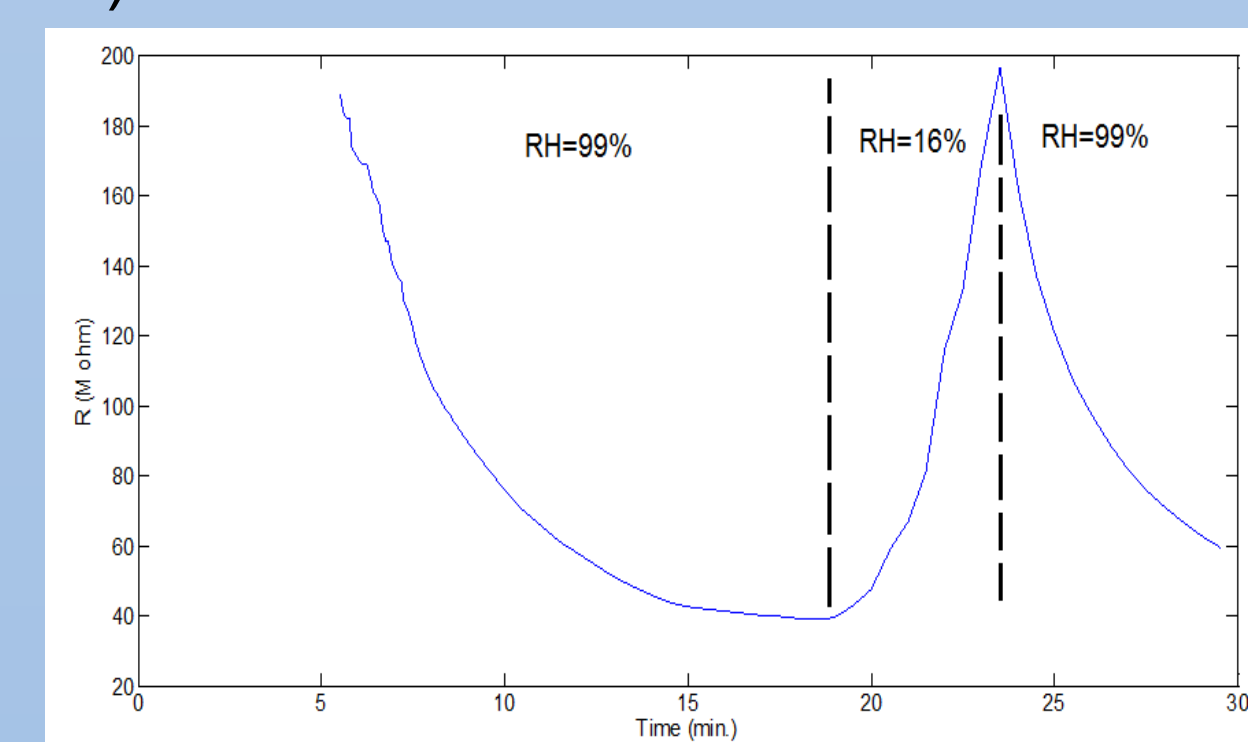
3) This solution is then undergoes the electrospinning process as described in **Figure 1**. Specifically, what is happening is the prepared solution is suspended above a metallic cylinder in a syringe that has a automatic controlled flow rate of .5ml/hour. The solution is then pushed through a charged needle, with the presence of an oppositely charged cylinder rotating at 1500 rpms located 10cm beneath this needle, there is a 25 KV potential difference which creates a strong electric field that pulls the spider silk protein out of solution that is formed into a nanofiber mat-like structure on the metallic cylinder. This process runs for approximately one hour. The generated nanofiber mat is twisted into yarn strips.

4) These nanofiber yarns are analyzed using scanning electron microscopy (SEM). The chemical structures of the formed nanofibers is analyzed through FTIR spectroscopy. The mechanical properties of the spider silk properties are tested using an MTS system (Synergie 100). The effects of humidity on the mechanical properties of the yarn are measured by using different samples of the same electro spun yarn at different humidity levels.

5) Additional characterization of the electrical resistance of the spider silk nanofibers is done by methods illustrated in **Figure 2**. Specifically, the spider silk is suspended in a controlled environment. Each side of the yarn is attached to a metal clamp that is connected to leads which run back to a commercial multimeter. The controlled environment is then exposed to a certain relative humidity level and the spider silk's resistance is recorded as a function of time and humidity. The resistance value is generally recorded for 30 minutes per experiment. RH levels of 50%, 60%, 70%, 80%, and 85% were tested.

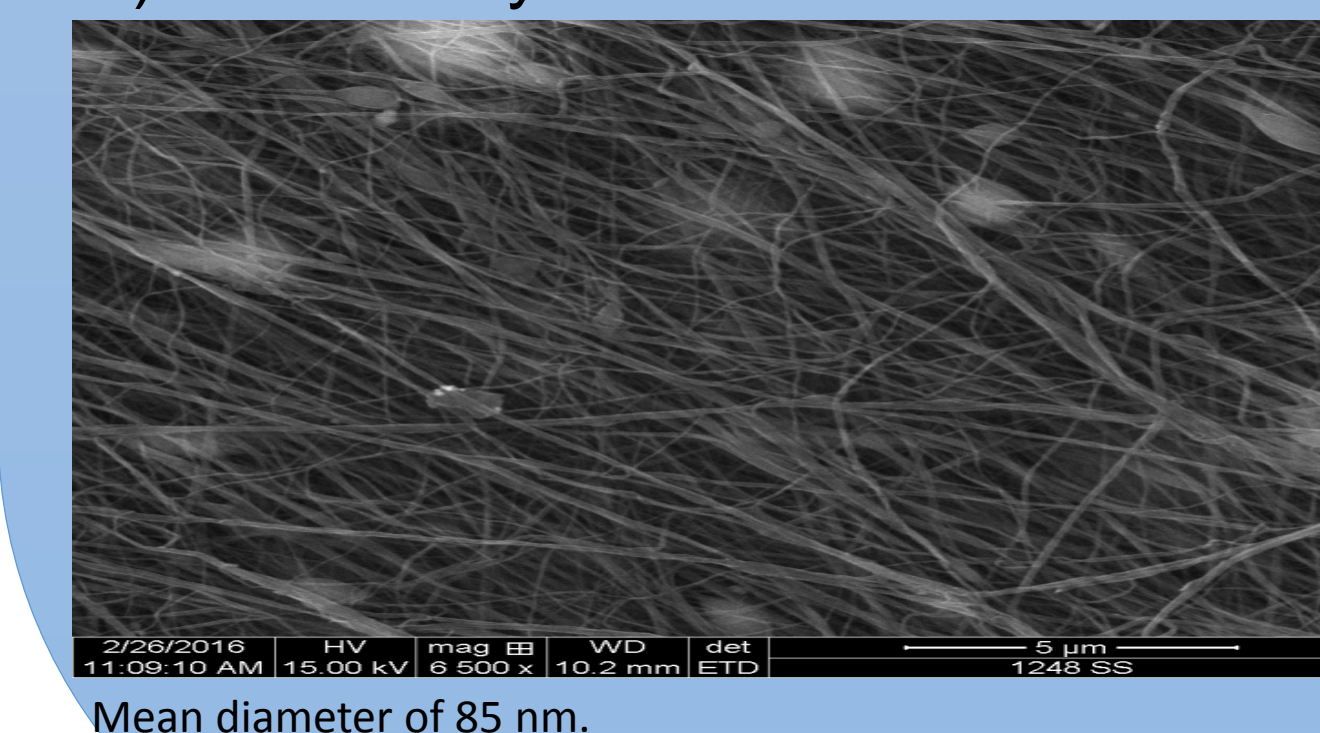
Results and Discussion

1) Resistance vs. Time at different RH



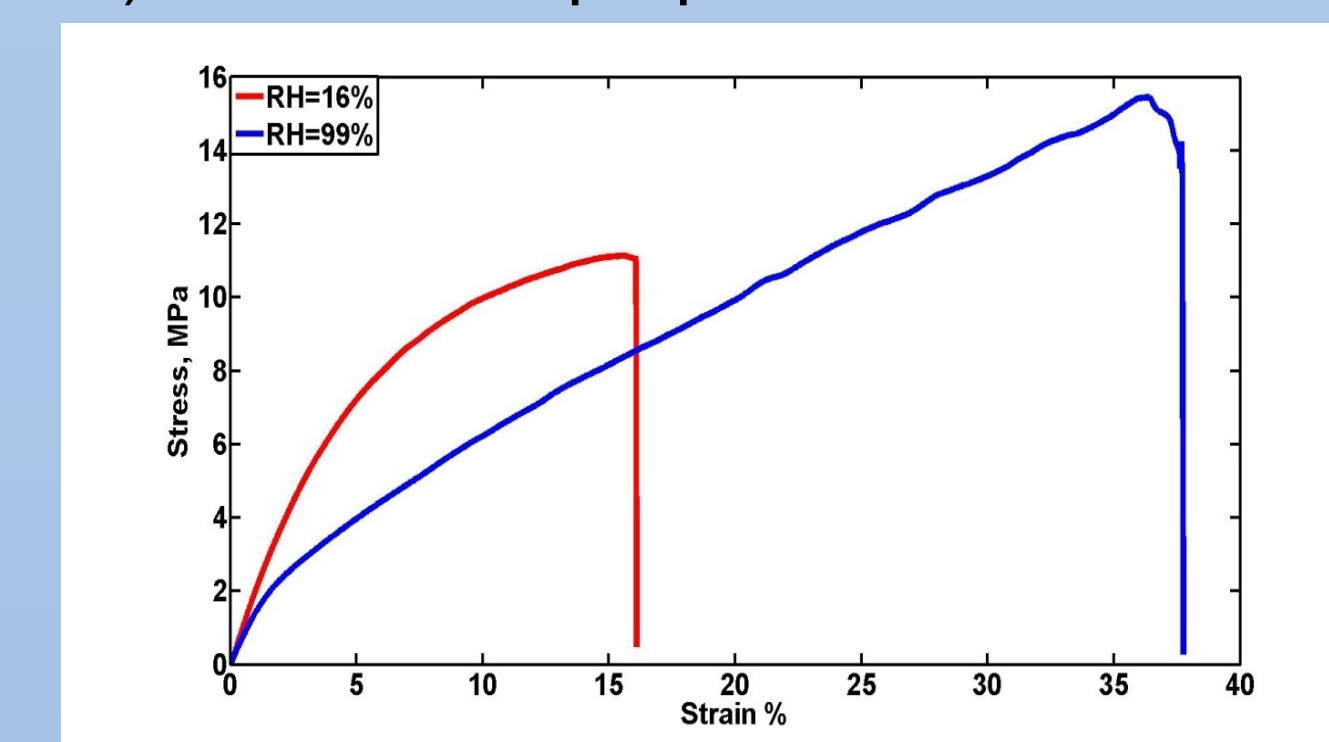
In this graph of cyclic resistance, we observed that the resistance during the first five minutes was above the 200 mega ohm level making us unable to record the value with the multimeter we were using. After five minutes, the resistance values were then in the measuring range of the multimeter and we observed an exponential-like decay of the resistance of the spider silk nanofiber. As the environment then returns to normal relative humidity of 16% the resistance follows the trend by elevation of resistance. The humidity is then returned at the 99% level, and the resistance, as hypothesized drops again.

3) SEM Analysis



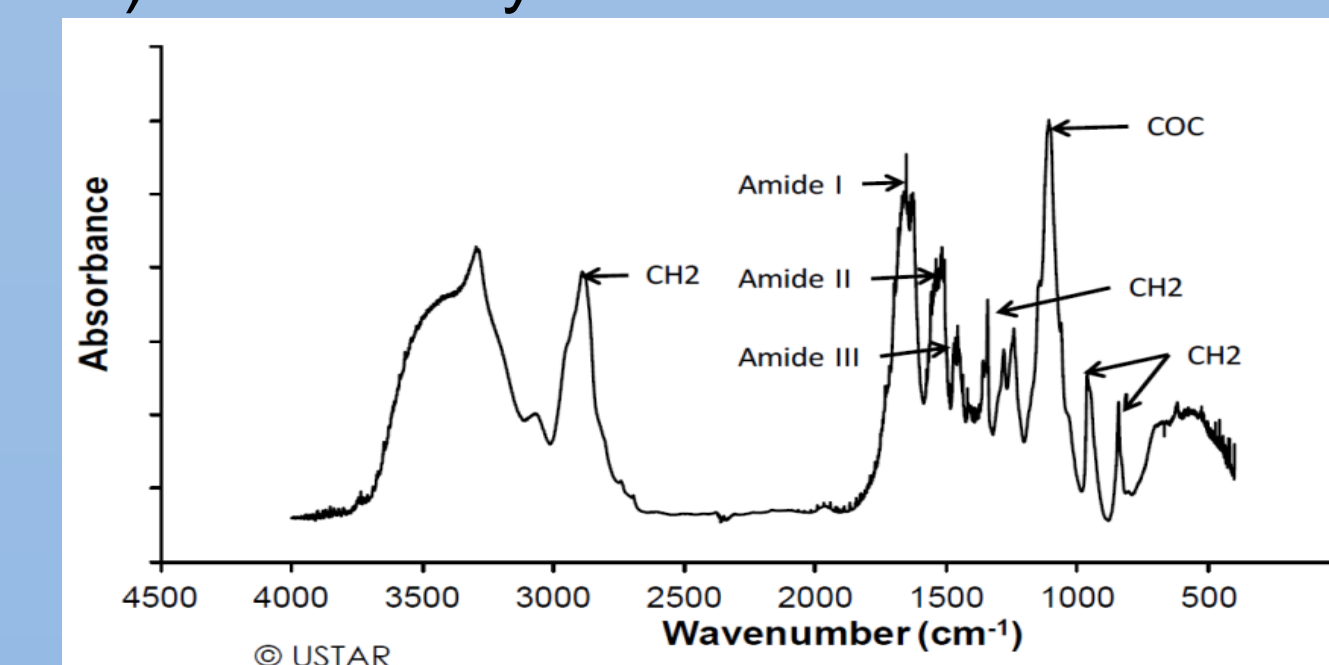
Mean diameter of 85 nm.

2) Mechanical properties of the fibers



In this graph we have compared the stress strain graph of the spider silk nanofiber in normal humidity (16%) and in extreme humidity (99%). We observe that the elastic modulus of the normal humidity is higher than the extreme humidity. Roughly, the elastic module of the normal humidity is 1.4 MPa/% strain and the extreme humidity is .4 MPa/% strain. This being true, the elastic deformity of the extreme humidity sample is approximately seven times greater than the normal humidity. The breaking point of the extreme humidity fiber is an additional 20% past the breaking point of the normal humidity fiber. The Stress endured by either graph is approximately a difference of 4 MPa.

4) FTIR Analysis



Formed Beta Sheets and multiple functional groups present.

Conclusion and Future Work

We have successfully studied electrospun spider silk nanofibers. We found that its resistance is dramatically decreasing over time at high RH with a cyclic effect of the resistance in the absence/presence of wet environment.

We are planning on studying the piezoelectric effect of the electrospun spider silk nanofibers within different environmental conditions (RH, Temp). Also, we are targeting to study the impact of some conductive additives *in-situ* such as carbon nanotubes (CNTs) on the performance of the generated spider silk nanofibers.

References

¹Anna Rising, Mona Widhe, Jan Johansson, Spider silk proteins: recent advances in recombinant production, structure-function relationships and biomedical applications. Cellular and molecular life science: 68:169-184

