4-5-2016

Molecular Combing of DNA Nanofibers and Comparison to Electrospinning

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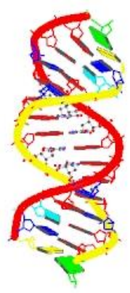
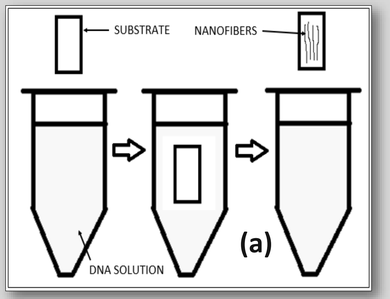


Introduction

- **Goal** - Create uniform, continuous DNA nanofibers (NFs) with molecular combing techniques and compare them to electrospun DNA NFs.
- **Molecular combing** - Process by which molecules are stretched by tension forces in a receding meniscus.[1]
- **Electrospinning** - process by which nanofibers are formed from a solution or melt that is pumped through a high voltage electric field, forming a fiber jet.[2]

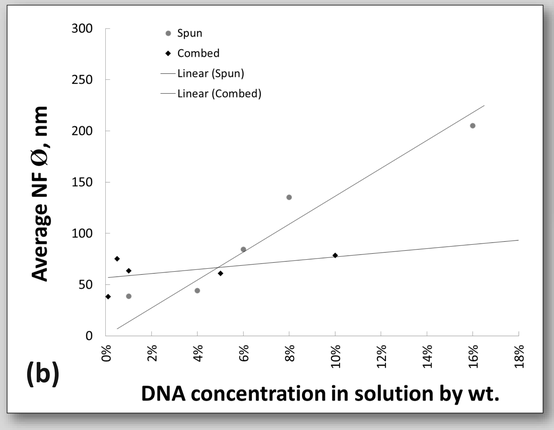
Materials / Methods

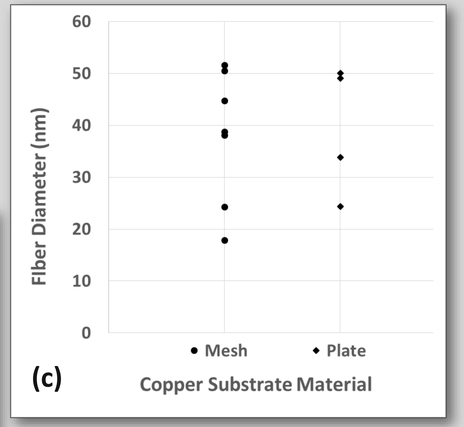
- **Materials**
  - Salmon testes sodium salt DNA, double stranded, 2000 base pairs
  - Black Teflon, Purple Teflon, polyolefin - Dahlar® Release Bag 125, Copper mesh and sheet substrates
  - Fluka Buffer Solution pH 4.0 (20ºC) (Sigma-Aldrich catalog # 33643), Fluka Buffer Solution pH 7.0 (20ºC) (Sigma-Aldrich catalog # 33646)
- **Combing Procedure**
  - Immerse substrate into centrifuge tube filled with 1 mL DNA solution
  - Leave substrate in solution for 5 minutes to allow molecules to bind to substrate
  - Remove substrate at an average speed of 500 µm/s
  - Sputter coat for 10 seconds and image using Quanta 200 environmental SEM on HV mode, 10kV beam
- **Parametric Studies**
  - Substrate material – 1% concentration; black Teflon, purple Teflon, polyolefin substrates
  - pH – 1% concentration; polyolefin substrates; pH 4, 4.5, 5, 5.5, 6, 6.5, 7
  - Hydrophobicity – 1% concentration; pH 5.5; $AgNO_3$/HDFT modified copper substrates
  - Concentration – pH 5.5, polyolefin substrates, 0.1%, 0.5%, 1%, 5%, 10% concentrations (by weight)



(a) Schematic of combing procedure showing substrate, solution and process

(b) Average diameter vs concentration for electrospun and combed NFs





(c) Average diameters for fibers on copper substrates

Comparison of combed fibers (left) to electrospun fibers (right).[5]

Results / Discussion

- SEM imaging showed that the release film was the best substrate out of the three, because it was the only out of the three that had fibers formed on it.
- It was expected that DNA NFs would be most uniform if the DNA strands bonded denatured partial denaturation, to allow only the extremities to bind to the substrate. Denaturation is the process by which DNA strands separate due to the application of a stressor.[3] It was predicted that partial denaturation would occur within a small pH range 5.5-6.[4] However, non-uniform NFs were formed at all pH levels tested. This is believed to be caused by the inconsistency of the combing speed and direction, and the lack of hydrophobicity of the substrate.
- It was expected that a hydrophobic substrate would create NFs that were uniform and straight. This was proven true with the modified copper substrates. Fibers formed were straight, unbranched, and had constant diameters ranging from 10-80 nm. Lengths ranged from 1-5 µm. But, NF formation was non-directional and the fiber density per area was much lower than with the release film. This made obtaining large measurement samples difficult. So, the release film was used to analyze the effects of concentration.
- Similar to electrospinning a correlation between increasing solution concentration and increasing diameter was found with combed fibers. The combed fibers were tapered away from the meniscus. The taper appeared to be linear. Fiber lengths were measured between 5 and 25 µm with no apparent trend. Fiber branching showed a decreasing trend as concentration was increased.

Conclusion

- Experiment showed that DNA molecules tend to self assemble into fibers with molecular combing, but that Electrospinning is a much more effective, controllable, and repeatable method of NF manufacturing.
- The combed NF diameter range was smaller (~20-200 nm) compared to the range for spun fibers which ranged from a few nanometers to several micrometers.[2]
- Combed NFs were much shorter (lengths less than 25 µm). While spun NFs are uniform, continuous, and more oriented directionally
- Combed fibers were branched except for those on the hydrophobic copper substrates.
- Experiment should be repeated with new substrates that are both hydrophobic, and support higher fiber per area density. (ex: silanized glass slides)[1] Also, a mechanical device should be used to create a consistent combing speed and direction.

References & Acknowledgements

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5. I thank Kaspars Maleckis for providing data and SEM images of electrospun DNA NF bundle.
6. Thank you to Dr. Yuris Dzenis and Kaspars Maleckis for mentoring me throughout this past year. Thank you to Kaspars for introducing me to methods and equipment used in our lab and for providing feedback and criticism on my reports and experimental methods. Also, thank you to the UCARE program for funding this research experience.