ABSTRACT

YOUNG, DENICE SHANETTE**.** Hyaluronic Acid-based Nanofibers via Electrospinning. *(Under the direction of Dr. Wendy E. Krause (TECS) and Dr. C.Maurice Balik (MSE)).*

Electrospinning is a novel technology that uses an electric field to form fibrous materials from a polymer solution. Unlike traditional spinning techniques, electrospinning can produce fibers, on the order of 100 nm, that can be utilized in applications where nanoscale fibers are necessary for specific applications, including tissue engineering and filtration. Outside of a smaller fiber diameter, electrospun nanofibers are also advantageous for biomedical applications because they have a larger surface area and pore size which promotes cell growth. A number of polymers have been electrospun successfully, including polyethylene (PEO) and polyvinyl chloride (PVC), which are two the most investigated electrospun materials. For the purpose of this study, hyaluronic acid (HA), a widely used biopolymer found in the extracellular matrix, was the chosen polymer to investigate the successful production of HA nanofibers for use in tissue engineering. Few studies have been conducted on electrospinning HA. Indeed, when this project was initiated, no investigations on electrospinning HA had been published. The goal of this research was to produce continuous fibrous strands of HA to be used as a mesh or scaffolding material. The high viscosity and surface tension of HA make it challenging to electrospin, as both are important parameters in successful production of nanofibers. To promote HA fiber formation by electrospinning, the effects of salt (NaCl), which is used to reduce the viscosity of aqueous HA solutions; molecular weight of the HA; and an additional biocompatible polymer (e.g., PEO) were investigated.

HYALURONIC ACID-BASED NANOFIBERS VIA ELECTROSPINNING

by

DENICE S. YOUNG

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APPROVED BY

Dr. C. Maurice Balik (Co-Chair of Advisory Committee)	Dr. Wendy E. Krause (Co-Chair of Advisory Committee)

Dr. Richard J. Spontak

BIOGRAPHY

Denice Shanette Young was born in Fayetteville, NC on February 23, 1982 to Franklin and Rebecca Young. After attending Pine Forest High School and becoming very interested in pursuing an engineering career in college, she enrolled at North Carolina State University as a student in Textile Engineering. Upon graduation in May 2004, Denice decided to take a summer internship with the Intel Corporation in Phoenix, AZ where she gained experience as a researcher in the area of Advanced Materials and Semiconductors. She thus decided to pursue a M.S. degree in Materials Science & Engineering in order to obtain a position in Research & Development. She enrolled at North Carolina State University in the Department of Materials Science & Engineering in August 2000 on a National Science Foundation GK-12 fellowship. Over the course of her graduate career she worked heavily with K-12 outreach in Wake County, NC at Southeast Raleigh High School and with the National Society of Black Engineers, serving as Region 2 Chairperson in 2005-2006. Upon graduating in May 2006, Denice will be entering industry as a Project Engineer for the Goodyear Tire & Rubber Company in Spring Hope, NC. Her career aspirations include work in research & development and the eventual promotion to Plant Manager.

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1.0 RESEARCH OBJECTIVES

1.1 Goals and Objectives

The objective of this research was to electrospin biopolymers into nanofibers. Traditional spinning techniques, i.e. wet spinning, gel spinning, etc., are not capable of producing fibers on the nanoscale. With this, the technique known as electrospinning is necessary to form nanofibers using a controlled electric field to pull fibers from an electrically charged polymer jet. There are hundreds of polymers that are electrospun for various applications, including polyethylene oxide (PEO), polyvinyl alcohol (PVA), polyethylene terephthalate (PET), nylon, and polyvinyl chloride (PVC). The polymer chosen for this research is that of hyaluronic acid or hyaluronan. Hyaluronic acid is a natural biopolymer ubiquitously distributed in the extracellular space, particularly in the extracellular matrix (ECM), but also found in synovial fluid of joints, the vitreous humor of eyes, and the scaffolding comprising cartilage [1,2].

As hyaluronic acid (HA) is known to have a high viscosity at relatively low concentrations [2], electrospinning the polymer is very challenging. However HA is an ideal biomaterial for drug-delivery, ophthalmology, and even dermatology as it is immuno-neutral and has excellent properties for these applications, including a high surface tension and viscosity [3]. Overcoming this high viscosity to successfully find the threshold between molecular weight, viscosity, and spinning parameters, are the challenges faced in fabricating nanofibers of HA for potential use in tissue engineering applications.

The successful development of biocompatible nanofibers for use in tissue engineering and bio-engineered material is critical to the advancement of medical textiles. Utilizing natural polymers, these fibers will allow access to new technology to treat diseases, burn victims, and provide materials for organ and tissue transplants. The U.S. Scientific Registry of Transplant Recipients reports that the number of Americans on the waiting list for organ transplants has doubled from 1995 to 2005 all due to the resources available in finding donors [4]. Since 1995, research in tissue and organ transplant has also doubled in order to find alternative means for meeting the high demands associated with transplants. One of the most researched areas is that of tissue engineering scaffolds [4]. However, a limited number of polymers and materials which can be utilized in such biomedical applications as the risk of thrombosis and rejection from the body are prevalent. The use of biodegradable and biocompatible polymers as tissue–engineering scaffolds is more attractive as the inflammatory response to the implant recedes after the scaffold is resorbed. The only potential drawback is the risk of triggering debris particle regeneration [3].

Through this study, the biodegradable polymer hyaluronic acid (HA) will be used in the fabrication of nanofibers thus providing adequate surface area, wide range of pore size, and a highly porous structure to allow and support cell, including cartilage (chondrocyte) cell growth at the nanoscale. In addition, the polymer blending with another biocompatible and successfully electrospun polymer of polyethylene oxide (PEO) will expand the opportunities for mesh and scaffold designs and functions.

The precise goals of the research for developing a polymer tissue engineering scaffold of HA through electrospinning are as follows:

- Determine the influence of molecular weight and sodium chloride (NaCl) concentration on the solution viscosity and ability to electrospin HA.
- Identify threshold for successful electrospinning of NaHA.
- Investigate the influence of blending HA with other electrospun polymers (i.e. PEO, PVA) in terms of solution viscosity and nanofiber production.
- Identify key parameters in electrospinning such as electric field, feed rate, concentration, and solvent evaporation rate.

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2.0 INTRODUCTION

2.1 Introduction to Fiber Technology

2.1.1 Definition of fibers

Fiber is a general term for a filament with a finite length that is at least 100 times its diameter (typically 0.10 to 0.13 mm). In most cases, fibers are prepared by drawing from a molten bath, spinning, or deposition on a substance.

2.1.2 Synthetic versus Natural

Fibers can be classified as natural or synthetic and either continuous or staple. Natural fibers, of course, can come from either animals or plants and probably the most well-known example of each are wool and cotton, respectively. Their chemical structure is polymer-based, in that a regular, repeat structure can be found in natural fibers. Synthetic fibers, too, are based on a regular polymeric structure. However, synthetic fibers are manufactured, or "synthesized," usually from petroleum products, but sometimes from coal or natural gas.

The term fiber is often used synonymously with filament. A filament is the smallest unit of a fibrous material. They are the basic units formed during drawing and spinning, which are gathered into strands of fibers to be fabricated into yarns and eventually fabrics. Filaments are of extreme length and very small diameter (usually less than 25 micrometers). Normally, filaments are not used individually but are bundled or twisted to form fibers. All natural fibers have a finite length associated with them, ranging from about 5 to 20 cm. This could be the length of the hair on a sheep (wool) or the length of a cotton filament in a cotton plant. In order to be woven into a fabric, these filaments must first be aligned together into a continuous strand, called yarn or thread. Spinning machines are used to accomplish this. The filaments are held together by van der Waal forces. The thread and yarn produced in this way contain occasional filaments which stick out away from the continuous strand; this is somewhat like branch groups on a polymer, albeit at a much larger scale. In any case, these filament branches help to provide the woven fabric with greater bulk and porosity and are associated with other positive aesthetic features of the fabric. Synthetic fibers, however, can be made into continuous filaments which are practically infinite in length. Even though the individual polymer molecules in the filament are 1000x longer than they are wide, they are still usually tiny fractions of millimeters in length. A “yarn” can be made of these filaments simply by bringing the filaments together continuously as the filaments are produced. This will not produce a yarn with filaments which occasionally stick out from the strand. In order to produce such a result, in some processes the continuous strands are chopped into strands of finite length, so that there is a somewhat closer match between the synthetic and the natural fibers. These chopped-up sections are called “staple” and the staple is brought together again in spinning machines which operate just like those used with natural fiber [1].

Another difference between natural and synthetic fibers is that the natural ones are usually curlier. Two techniques have been used to make the synthetic fibers and yarn curlier: one is to pass the yarn between two heated gears which can impart a permanent crimp to the yarn and the second is to produce the synthetic filaments from two different polymers passing out of a common die. The resulting filament will exhibit varying curliness, based on humidity conditions if the two polymers absorb water to differing degrees and if the absorption changes the shape of the filaments accordingly.

2.2 Traditional Fiber Formation Processes

Spinning has long been used as the process for the production of synthetic fibers. Presently, the world is consuming vast quantities of these fibers in the form of clothing, carpets, furniture upholstery, etc. and the demand is likely to increase in the future. Thus, spinning and fiber formation are processes of great industrial importance. The three basic spinning processes: (1) dry spinning, (2) wet spinning, and (3) melt spinning. All three steps involve the formation of continuous filament strands by forcing the material through circular dies, but melt spinning involves cooling of the subsequent strand to form the solid filament, whereas dry and wet-spinning involves removal of a solvent to form the solid filament.

In dry spinning, the solvent evaporates into a gas and in wet spinning the solvent is leached into a liquid bath. A mixture of polymer and volatile solvent is extruded through a capillary, the solvent is vaporized and the remaining polymer forms the synthetic fiber. This is a fairly costly process due to the extra costs for equipment to prepare the polymer-solvent mixture and for solvent recovery. In wet spinning, a polymer-nonvolatile solvent mixture is extruded through a spinneret into a coagulating solution, resulting in a fiber. Wet spinning is also fairly costly. Melt spinning, on the other hand, utilizes only the raw polymer. This raw polymer is melted and extruded through a spinneret to produce the fiber [1].

In addition to wet, dry, and melt spinning, two relatively newer spinning techniques include gel spinning and reaction spinning. In gel spinning, the primary mechanism of solidification is the gelling of the polymer solution by cooling to form a gel filament consisting of precipitated polymer and solvent. Reaction spinning utilizes both polymerization and spinning of the filaments simultaneously as one reactant is extruded into a bath containing another reactant [1].

2.2.1 Technology of the Spinneret

The one feature which is common to all the traditional spinning methods mentioned above is the step in which the very viscous liquid fiber forming material is forced under pressure through a spinneret. The spinneret serves as the actual capillary and is composed of a number of small holes, generally in the order of 0.1mm in diameter. This action involves two technical requirements: the use of high pressures behind the spinneret in order to force the viscous liquid through the holes at the required/set rate; the necessity for very efficient filtration of the liquid in order to prevent the blocking of the spinneret holes by solid impurities.

These conditions are particularly hard to meet in the case of melt spinning, where the liquid must be pumped, filtered and extruded at a temperature in the approximate range of 220ºC to 290ºC and at a viscosity of about 1000 poise. With regard to filtration, one is more or less limited to two filter media only, namely fine stainless steel gauze and sand layers of varying fineness. These are often used together. Glass and asbestos fiber filters are not successful, as these materials are too brittle, and they are apt to allow small bits of broken fiber to pass into the filtered polymer stream [2].

Research has also indicated that there is a lower limit to the spinneret hole size. It is evident that from the point of view of 'practical' spinners, larger holes require less pressure to produce a given rate of extrusion, and rates of extrusion are relatively slow. Filtration requirements would be minimal and consist only of removing such particles as would show in the finished product. Cleaning of the spinneret holes would also be greatly simplified. A further result of using large spinneret holes would be that by changing the take-up speed or extrusion speed, a large range of diameters, including filaments, might be spun from the same spinneret.

The main factor operating against the use of large spinnerets seems to be the belief of many that larger holes lead to more irregular filaments. The importance of this effect does not lie directly in the increase in irregularity, but in the additional fact that irregular filaments cannot be drawn at a sufficiently high draw ratio to give them acceptable properties such a tenacity and extensibility.

2.2.2 Difficulties in Traditional Spinning Processes

Among the different processes (dry, melt, wet, reaction, and gel) of producing synthetic fibers, melt spinning has been most extensively studied because it is relatively simple to treat, both theoretically and experimentally. However, a clear understanding of all the phenomena occurring in melt spinning is far from complete. The difficulty lies mainly in that although one may be primarily interested in the fiber-forming step where stretching and re-orientation of molecules occur, this step is governed to a large extent by the steps which precede it; namely the deformation of the polymer melt in the spinneret, and the relaxation of the stresses in the melt upon exiting the die [1].

Wet-spinning, used for cellulose, proteins, polyvinyl alcohol, polyacrylonitrile, and polyvinyl chloride, is the most complex spinning procedure. From the technological point of view the most important problems involve obtaining fibers of optimum morphological structure (very sensitive to the composition and conditions within the precipitation bath) and efficient recuperation of the individual components of the solvent and precipitating bath. The wet-spinning procedure, however, provides the possibility of obtaining fibers from materials which cannot be spun in other ways (proteins, cellulose, aromatic polyamides).

Dry spinning is the fiber formation process potentially emitting the largest amount of volatile organic compounds (VOCs) per pound of fiber produced. Air pollutant emissions include volatilized residual monomer, organic solvents, additives, and other organic compounds used in fiber processing. Unrecovered solvent constitutes the major substance. The largest amounts of unrecovered solvent are emitted from the fiber spinning step and drying the fiber. Other emission sources include dope preparation (dissolving polymer, blending the spinning solution, and filtering the dope), fiber processing (drawing, washing, and crimping), and solvent recovery [2, 3].

When a polymer melt exits from the spinneret, it swells and gives rise to a maximum thread diameter at a short distance from the spinneret face. Given a material, the exact location at which the maximum swelling occurs and the maximum thread diameter itself, depends on the deformation history of the melt in the spinneret holes.

Due to the technological importance of the extrudate swell behavior from the point of view of polymer processing, much attention has been given to seeking a better understanding of the cause(s) of extrudate swell in fiber spinning.

Traditional spinning methods are not capable of producing woven or nonwoven fibers on the order of 100nm. With this, the diameter of the fibers fabricated by traditional methods far exceeds that of the typical collagen fiber of the extracellular matrix (ECM) in the body (*ca.*50-500 nm) [4]. Thus, another technique must be used to produce fibers with a diameter on the order of 100 nm. This technique, as explained in more detail in Section 2.3 and Section 3.2, is known as electrospinning and is capable of producing nanofibers using electrical stimulation.

2.3 Fiber formation by Electrical Stimulation

In comparison to traditional spinning techniques, electrospinning is a straightforward, inexpensive, and unique method to produce novel fibers with diameter in the range of 100 nm or even less. Polymer solutions, liquid crystals, suspensions of solid particles and emulsions have been electrospun. The electric force results in an electrically charged jet of polymer solution outflowing from a droplet tip. After the jet flows away from the droplet in a nearly straight line, it bends into a complex path and other changes in shape occur, during which electrical forces stretch and thin it by very large ratios. After the solvent evaporates, a grounded collector plate will hold a mass of nanofibers. The process of electrospinning is explained in greater detail in Section 3.2.

2.4 Summary

This research investigates the fabrication of nanoscale fibers of hyaluronic acid (HA) using the technology of electrospinning. Hyaluronic acid is a naturally occurring polymer that is used in various biomedical applications and is important in joint lubrication [5]. Hyaluronic acid is an important polymer because it aids in cellular repair, keeps skin moist, and also helps to heal wounds faster. Non-woven nano-webs of hyaluronic acid have been produced using electrospinning and the webs are being tested in wound healing and compared to the Vaseline gauze present on the market [6]. These non-woven webs are fabricated solely through electrospinning as traditional fiber spinning technologies are unable to produce fibers on the nanoscale. Electrospun fibers of HA will provide the small diameter, small pore size, and large surface area needed for such applications. This project utilized electrospinning to fabricate HA-based nanofibers. HA's high solution viscosity presents a challenge in electrospinning. The influence of HA concentration and molecular weight, sodium chloride concentration, and blending HA with other polymers was studied as a function of nanofiber production, ability to electrospin, and solution viscosity.

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3.0 LITERATURE REVIEW

3.1 Nanotechnology and Nanofibers

The non-woven industry generally considers nanofibers as having a diameter of less than one micron, although the National Science Foundation (NSF) defines nanofibers as having at least one dimension of 100 nanometer (nm) or less [1]. The name derives from the nanometer, a scientific measurement unit representing a billionth of a meter, or three to four atoms wide.

Nanofibers are an exciting new class of materials used for several value added applications such as medical, filtration, barrier, wipes, personal care, composite, garments, insulation, and energy storage [2]. Special properties of nanofibers make them suitable for a wide range of applications from medical to consumer products and industrial to high-tech applications for aerospace, capacitors, transistors, drug delivery systems, battery separators, energy storage, fuel cells, and information technology [3,4].

Generally, polymeric nanofibers are produced by an electrospinning process (Figure 1). Electrospinning is a process that spins fibers of diameters ranging from 10 nm to several hundred nanometers. This method has been known since 1934 when the first patent on electrospinning was filed. Fiber properties depend on field uniformity, polymer viscosity, electric field strength and TCD (distance between the needle tip and collector) [4]. Scanning electron microscopy (SEM) allows us to explore the structure and morphology of electrospun nanofibers.

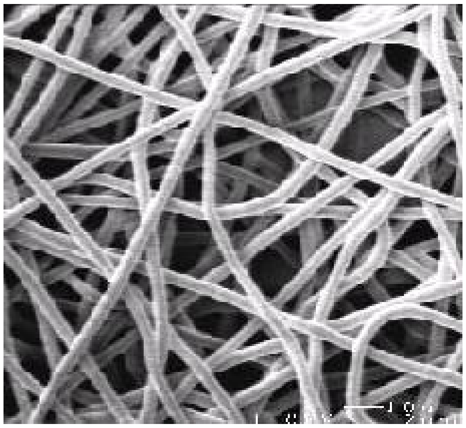

Figure 1: SEM image of PVA electrospun nanofibers.

Electrospinning is widely used to develop potential scaffolds for tissue engineering because the nanofiber surface provides large amount of surface area that is approximately *ca.*103 m^2/g [5]. This type of highly porous structure is necessary to allow cells to reach the center of the scaffold as well as pass through it to the other side. Fibers having nano-scale diameters resemble the diameter (*ca.*30 nm) of collagen fibers in the ECM [27]. Pore size is of great significance for tissue engineering, as cells are selective to certain range of pore sizes through which they can migrate and subsequently proliferate. High porosity of nanofiber scaffold can be expected similar to the porous structure of the cartilage in the physiological state.

As traditional spinning techniques are not capable of providing fibers on the nanoscale, electrospinning is critical for production of fibers on the order of 100 nm.

3.2 Electrospinning of Nanofibers

Electrospinning is not a new technology for polymer fiber production. It has been known since the 1930's; however, it did not gain significant industrial importance due to the low output of the process, inconsistent and low molecular orientation, poor mechanical properties and high diameter distribution of the electrospun fibers [6]. Although special needs of military, medical and filtration applications have stimulated recent studies and renewed interest in the process, quantitative technical and scientific information regarding process and product characterization are extremely limited [7].

The principle of electrospinning is to use an electric field to draw a charged polymer solution from an orifice to a collector. This creates a jet of solution from the orifice to the grounded collection device. The jet emerges at the base from the nozzle, which has a geometry of a cone (Taylor cone, see Figure 2). Then it travels to form a stretched jet of many fibers in the splaying region. But splaying is a misnomer as researchers have observed a rapidly-rotating spiral jet [2,8] which is indistinguishable from splaying phenomenon to the naked eye. The fibers are eventually collected on a grounded metal screen. In 1934, the process of spinning fibers this way was deemed electrospinning and was patented by Formhals [2,3], wherein an experimental setup was outlined for the production of polymer filaments using electrostatic force.

In the electrospinning process, a high voltage is used to create an electrically charged jet of polymer solution or melt, which dries or solidifies to leave a polymer fiber [4, 6]. One electrode is placed into the spinning solution/melt and the other attached to a collector. Electric field is subjected to the end of a capillary tube that contains the polymer fluid held by its surface tension. This induces a charge on the surface of the liquid. Mutual charge repulsion causes a force directly opposite to the surface tension [5]. As the intensity of the electric field is increased, the hemispherical surface of the fluid at the tip of the capillary tube elongates to form a conical shape known as the Taylor cone [9], seen in Figure 2. With increasing field, a critical value is attained when the repulsive electrostatic force overcomes the surface tension and a charged jet of fluid is ejected from the tip of the Taylor cone. The name “Taylor Cone” simply represents the conical shape formed at the needle tip (see Figure 2). This cone was described by Sir Geoffrey Ingram Taylor in 1964 [9] as a continuation of the work of Zeleny in 1917 [9] on the formation of a cone-jet of glycerine exposed to high electric fields. Several others continued researching this area, including Wilson & Taylor (1925), Nolan (1926), and Macky (1931) [9]. However it was Taylor who looked further into the reactions between droplets and electric fields.

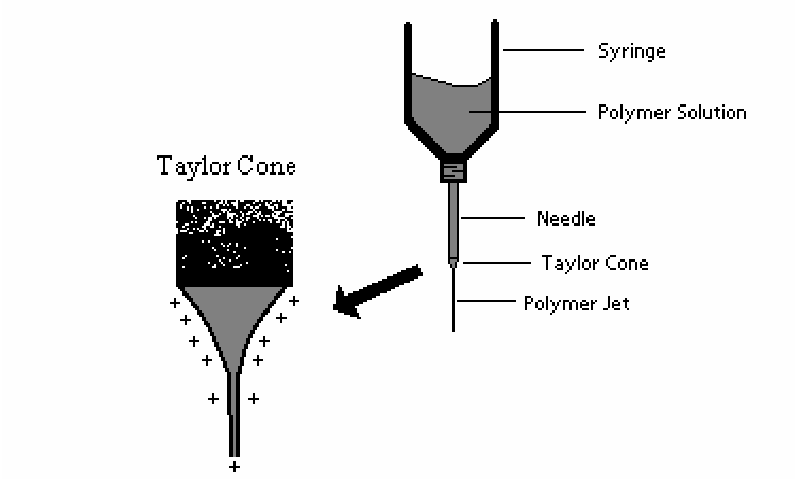

Figure 2: Illustration of Taylor Cone formation from the Syringe Needle Tip

Taylor's derivation is based on two assumptions: (1) that the surface of the cone is an equipotential surface and (2) that the cone exists in a steady state equilibrium. Once discharged and the Taylor cone activated, the polymer jet undergoes a whipping process [10] wherein the solvent evaporates, leaving behind a charged polymer fiber, which lays itself randomly on a grounded collecting metal screen. In the case of the melt the discharged jet solidifies when it travels in the air and is collected on the grounded metal screen [9]. An example of our experimental set up used for electrospinning is shown in Figure 3.

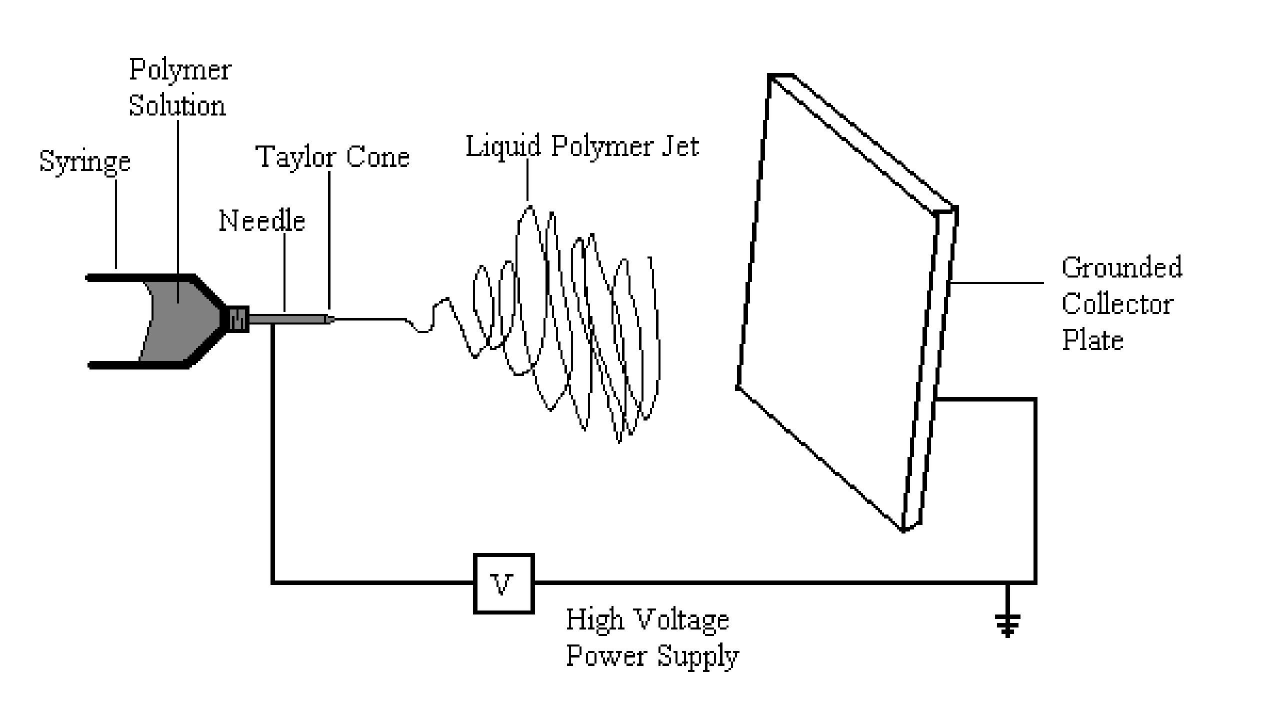

Figure 3. General Electrospinning schematic

The polymer solution or melt is contained in a syringe. A metering pump attached to the plunger of the syringe generates a constant pressure and flow of the fluid through the pipette. The driving force is provided by a high voltage source that is attached to the needle. The high voltage source can generate up to 30 kV, and the setup can be run on either positive or negative polarity. Adjusting the flow of the fluid and the magnitude of the electric field controls the spinning rate.

Using electrical forces alone, the electrospinning process can produce fibers with nanometer diameters. Because of their small diameters, electrospun fibers have larger surface-to-volume ratios, which enable them to absorb more liquids than do fibers having large diameters.

3.3 Polymer Selection in Electrospinning

Hundreds of polymers have been electrospun to produce nanofibers for various applications. Table 1 lists a few polymer solvent systems that are widely used in electrospinning [11]. Polymers such as polyvinyl alcohol (PVA) are widely used in tissue engineering and drug delivery. There are many more polymers that have not been successfully electrospun based on their properties and the parameters involved in electrospinning. Properties of the polymer in solution are very important in both the electrospinning process and the end application of the electrospun nanofibers. Concentration and solvent type directly impact the properties of the solution. Highly concentrated polymer solutions are very difficult to electrospin as viscosity is an essential parameter in electrospinning. Polymer solvent is also pertinent to electrospinning as the polymer must be dissolved in a suitable solvent and spun from solution. Solvent type is also important when generating nanofibers. Nanofibers in the range of 10 to 2000 nm diameter can be achieved by choosing the appropriate polymer solvent system [6]. Table 1 lists some polymer solvent systems used in electrospinning. Depending on the system, applications may vary.

Table 1. Polymer solvent systems for electrospinning [11]

POLYMER	SOLVENTS
Nylon 6 and nylon 66	Formic Acid
Polyacrylonitrile	Dimethyl formaldehyde
Polyethylene Terephthalate	Trifluoroacetic acid/Dimethyl chloride
Polyvinyl Alcohol	Water
Polystyrene	DMF/Toluene
Nylon-6-co-polyamide	Formic acid
Polybenzimidazole	Dimethyl acetamide
Polyramide	Sulfuric acid
Polyimides	Phenol

In order for electrospun polymers to be utilized for tissue engineering applications, biocompatibility and the non-release of harmful by-products (i.e. toxicity) are two necessities for electrospun biopolymers. For the sake of this research, hyaluronic acid (hyaluronan) is the polymer of choice based on its commercial availability, polymer properties, and influence in the medical industry.

3.4 Hyaluronan

Hyaluronan is a naturally occurring biopolymer used in medical applications ranging from cataract surgery and post-surgical adhesion prevention to hydrophilic coatings [12]. A unique biopolymer, hyaluronan is one of a number of polysaccharides that occur in the body's mucous membranes and are known as mucopolysaccharides. It was first isolated from the vitreous body of the eye in 1934 by Karl Meyer, who called it hyaluronic acid [12]. The term hyaluronan is attributed to Endre Balazs, who coined it to encompass the different forms the molecule can take—for example, the acid form, hyaluronic acid, and the salts, such as sodium hyaluronate, which form at physiological pH of 3 [3].

Over the years, quite a lot is known about the appearance of the hyaluronan molecule; its behavior; its occurrence in different tissues and body fluids; the manner in which it is synthesized by the cells, metabolized, and cleared from the body; and the nature of some of the functions it performs. Hyaluronan and related polysaccharides are called glycosaminoglycans [13-14]. These substances are made up largely of repeating disaccharide units containing a derivative of an aminosugar. The most abundant glycosaminoglycans in the body are chondroitin sulfates; others are keratin sulfate, heparin and heparin sulfate, and dermatan sulfate. Figure 4 shows the disaccharide unit of hyaluronan, consisting of alternating glucuronic acid and N-acetylglucosamine units, which are repeated over and over to form long chains [15]. Each repeating disaccharide unit has one carboxylate group, four hydroxyl groups, and an acetamido group.

Hyaluronan differs from the other major glycosaminoglycans in that it does not have sulfate groups [15].

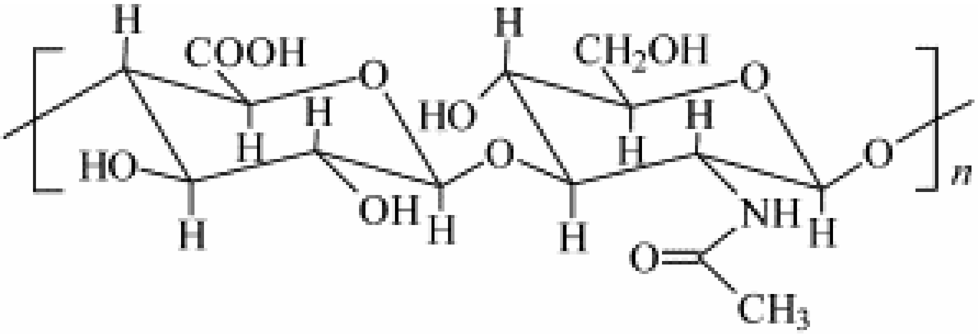

Figure 4. Molecular Structure of Hyaluronic Acid [15]

In the body, hyaluronan is synthesized by many types of cells and extruded into the extracellular space where it interacts with the other constituents of the extracellular matrix to create the supportive and protective structure around the cells. It is present as a constituent in all body fluids and tissues and is found in higher concentrations in the vitreous humor of the eye and the synovial fluid in the joints. In mammals, the highest reported concentration is found in the umbilical cord [15].

Hyaluronan is also a major contributor to wound healing. This polymer plays a significant role in the inflammation, granulation, and remodeling phases that the body undergoes, as seen in Table 3:

Table 2: Hyaluronic Acid and the Wound Healing Process [4]

Wound-Healing Phase	Contributing Role of HA
Inflammation	Activation of macrophages and neutrophils
	Moderation of inflammation
Granulation	Cellular differentiation
	Cellular Proliferation
	Cellular Migration
Remodeling	Reduced Scar Formation

Polymer Characteristics

Hyaluronan possesses a unique set of characteristics: its solutions manifest very unusual rheological properties and are exceedingly lubricious, and it is very hydrophilic.

- *Rheological Properties*. In solution, the hyaluronan polymer chain takes on the form of an expanded, random coil. These chains entangle with each other at very low concentrations, which may contribute to the unusual rheological properties. At higher concentrations, solutions have an extremely high but shear-dependent viscosity [4].
- *Lubricity.* The extraordinary rheological properties of hyaluronan solutions make them ideal as lubricants. There is evidence that hyaluronan separates most tissue surfaces that slide along each other. Solutions of hyaluronan are extremely lubricious and have been shown to reduce postoperative adhesion formation following abdominal and orthopedic surgery [4].
- *Hydrophilicity.* As mentioned, the polymer in solution assumes a stiffened helical configuration, which can be attributed to hydrogen bonding between the hydroxyl groups along the chain. As a result, a coil structure is formed that traps approximately 1000 times its weight in water [4].
- *Commercial Availability.* The classical sources for the isolation of hyaluronan have been either from rooster combs, mammalian tissues, or from certain strains of cultured bacteria. At one time, the material was isolated from human umbilical cords collected in hospitals. One company, Pharmacia AB (Uppsala, Sweden), developed a special strain of roosters with very luxuriant combs, from which the compound was isolated. More recently, submerged cell-culture techniques using

certain strains of streptococci have been developed to produce hyaluronan. The commercially available material comes in molecular weights ranging from less than 1 million to as high as 8 million [4,6].

  - There are a large number of hyaluronan producers around the world. Biomatrix Inc. (Ridgefield, NJ), a U.S. company, operates a plant that produces hyaluronan from mammalian sources in Canada. Anika (Woburn, MA), Genzyme Corp. (Framingham, MA), and Lifecore Biomedical (Chaska, MN) are other domestic suppliers. Pharmacia produces hyaluronan in Sweden, Fidia Advanced Biopolymers (Brindisi) in Italy, Bio-Technology General Corp. (Iselin, NJ) in Israel, and a number of companies, including Kibun Food Chemifa Co. and Seikagaku Corp. (both Tokyo), in Japan [16].

- *Challenges.* As a widely used polymer throughout various applications, hyaluronic acid does present some disadvantages and challenges when dealing with electrospinning the polymer. Its high viscosity and surface tension at relatively low concentrations are indeed ideal in the medical industry for ophthalmology, tissue engineering, and drug delivery. However, viscosity is an important parameter in electrospinning and to achieve successful spinning, a low viscosity and low concentration are needed. In addition, the short residence time of HA due to biodegradability limits the possibility to widen its range of biomedical application. To overcome this problem, many methods may be adopted including: chemically modifying HA by crosslink or coupling reactions and most effective, by blending HA with other polymers [17,18].

3.5 Electrospinning versus Electroblowing of Nanofibers

Many researchers have been successful in electrospinning nanofibers from polymers including PVA, PVP, and PEO. To our knowledge, no published work reports the successful electrospinning of hylauronic acid nanofibers. However, one team in particular, led by Chu, et al.[16], has successfully fabricated nanofibers of HA utilizing a technique similar to electrospinning known as "electroblowing." Figure 4 is an illustration of the electrospinning apparatus used by Chu, et al. [16]. The apparatus is identical to typical electrospinning devices with the addition of a blowing machine, which is equipped with an air temperature and flowrate controller, air distributor, and steam system. Using this additional system did result in the fabrication of HA nanofibers; however, several key parameters were modified to stabilize the process. The effects of various experimental parameters, such as air-blowing rate, HA concentration, feeding rate of HA solution, applied electric field, and type of collector on the performance of blowing-assisted electro-spinning of HA solution were major areas of investigation.

In their first report [16], the group successfully produced HA nanofibers with electo-blowing assisted procedures, as illustrated in Figure 5. The nonwoven mat HA nanofibers were characterized using scanning electron microscopy (SEM) and morphology characterization techniques to observe the porosity and fiber morphology of the electrospun nanofibers and verify the presence of beads and scales on the fibers.

After testing several solutions through numerous trials, the researchers concluded the following parameters as optimal conditions for the process:

Solution Concentration: 1.3-1.5 w/v%
Solution Viscosity: 3-30 Pa.s
Flow Rate: 20 to 60 μL/min.

From these parameters, the researchers concluded that the effect on polymer concentration by blending, solvent evaporation by the addition of ethanol, and solution feed rate all improved the quality of HA fibers. Consequently, these improvements were deemed inadequate as they were not able to be replicated over a series of experimental trials. To overcome this issue, the electro-blowing assistance was introduced and from further testing, the researchers found that at a blowing rate of 57°C at a 70 ft^3/hr. flow rate, the nanofiber production was of high quality and consistency.

In their second publication [19], two methods to fabricate water-resistant HA nanofibrous membranes without the use of reactive chemical agents were demonstrated with the exposure of HA membranes in hydrochloric acid (HCl) vapor, followed by a freezing treatment at -20°C for 20-40 days; and the immersion of HA membranes in an acidic mixture of ethanol/HCl/H_2O at 4°C for 1-2 days. Although both methods could produce hydrophilic, substantially water-resistant HA nanofibrous membranes (the treated membranes could keep their shape intact in neutral water at 25°C for about 1 week), the immersion method was shown to be more versatile and effective. Characterization techniques utilized in this study included IR spectroscopy (for cross-linking mechanisms) and rheological studies (for viscosity measurements of the solutions). IR spectroscopy was used to investigate this 'cross-linking' mechanism in the solid HA membrane. The researchers concluded that after a freezing time of 8 hours, the

HA solution was similar to a gel with increases in hydrogen bonding. Therefore, the decrease in water solubility was perhaps due to the increase in hydrogen bonding throughout the HA membranes after the freezing technique.

Both studies from Chu and co-workers [16,19] provided assistance in investigating the threshold parameters in electrospinning HA. Both studies utilize an acid, ethanol, and ethanol/HCl/H_2O, as the solvent for the HA solutions. In addition, electro-blowing post-treatment freezing techniques were utilized to provide consistent and high quality HA nanofibers as pure electro-spinning was deemed incapable of doing so.

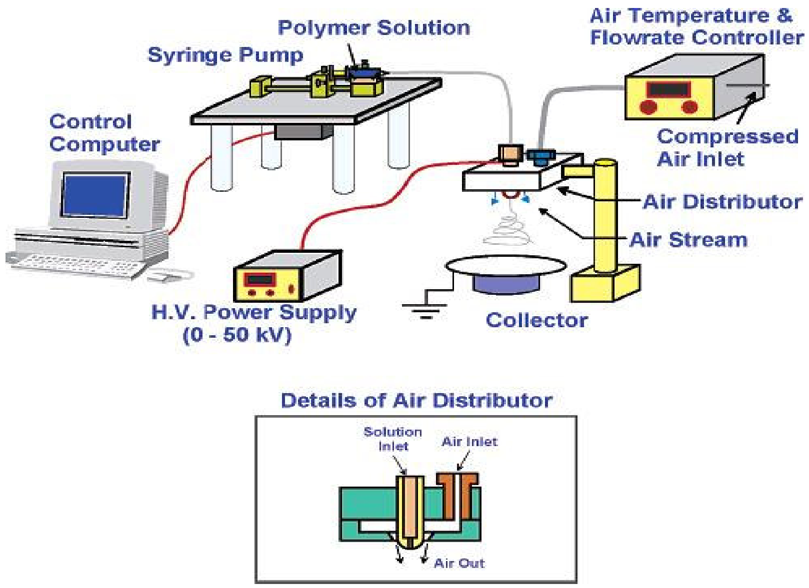

Figure 5: Schematic of Stony Brook Electroblowing Apparatus [16]

3.6 Electrospinning Parameters

3.6.1 Process Parameters

In electrospinning, process parameters are those constraints directly linked to the apparatus itself and can be identified from the measurements controlled by the various devices throughout the process. The devices contributing to the electrospinning set-up are a high voltage power supply, syringe pump, and collector. Unlike previous research in electrospinning HA, no other assisted techniques were utilized. The power supply used throughout this experimentation has the capacity to output up to 60,000 volts and as mentioned earlier, electric field strength is a critical process parameter in electrospinning. The syringe pump serves as a metering device in both securing the capillary/syringe in which the liquid polymer is contained and generates a constant pressure and flow of the fluid through the syringe needle. Flow rate is thus another process parameter in the system. The collector in the system is a grounded metal plate that is mobile in supplying a tip-to-collector distance for collecting the spun fibers as they travel from the needle tip to the collector. Tip to collector distance, varied by the plate distance from the needle, is another important parameter as it is responsible for providing a collector surface for spun fibers and enough distance between the tip and collector for solvent evaporation. Other process parameters include, temperature, humidity, and air velocity in the hood in which the apparatus is contained.

3.6.2 Solution Parameters

In electrospinning hyaluronic acid, there are several key solution parameters that must be accounted for to achieve successful spinning. As stated previously, HA is known to have a high viscosity and surface tension. High values of these two parameters make successful electrospinning extremely challenging. The key solution parameters investigated throughout this research include solution concentration, viscosity, pH, solvent evaporation rate, and molecular weight. To ensure successful electrospinning, it is critical that the viscosity of the HA solutions be controlled. Sodium chloride (NaCl) and polyethylene oxide (PEO) were used as viscosity modifiers. PEO is another biocompatible, non-toxic, and non-mutagenic polymer that is capable of producing electrospun nanofibers. Over fifty studies have been published illustrating the potential applications, successful manipulations, and properties of electrospun PEO fibers. The possible blending of PEO and HA has not previously been reported in electrospinning.

3.7 Applications for Nanofibers

Nanofibers exhibit special properties mainly due to their extremely high surface to weight ratio compared to conventional nonwovens. Low density, large surface area to mass, high pore volume, and tight pore size make the nanofiber non-woven mats appropriate for a wide range of applications from filtration to drug delivery. In comparison to an average human hair fiber, which is about 50-150 μm, electrospun fibers are on the order of 100 nm. Those nanofibers used in industrial applications are at a diameter of 350 nm or less.

Typically, controlled fiber materials can yield products such as braided and woven structures, conductive fibers, filters, and fiber reinforced support. Braided and woven nano- & micro- structures can be utilized in applications such as tissue engineering, the aerospace industry, and military purpose [20]. The use of electrospun fibers at critical places in advanced composites to improve crack resistance is also another promising area [20]. Filtration and tissue engineering are currently the two major areas for nanofiber utilization.

3.7.1 Filtration

Nanofibers utilized as filters and filtration systems have been a growing field for over a decade [20]. Research is growing in this area as scientists attempt to develop nanofibers potentially capable of filtering out viruses, bacteria and hazardous nano-particles. However, in the case of thin webs, the mechanical properties are poor when not used in conjunction with a substrate material capable of being processed into a filtration medium.

Regardless of the weak points involved with nanofibers in filtration, the area continues to grow as the potential for nanofibers webs is endless. New research is evolving for the development of a high-tech, nanoscale fiber spinning process capable of producing nanofibers for high-performance filtration of contaminants, biological agents and hazardous but very small particles [10]. These novel filtration systems are expected to be capable of providing protection against such hazardous substances as toxic mold and infectious agents, including severe acute respiratory syndrome (SARS) and various strains of influenza [21].

3.7.2 Tissue Engineering Scaffolds

In terms of tissue engineering, an ideal scaffold for tissue engineering should be compatible to the tissue environment. It must not initiate any antigenic or toxic reactions in the body. The scaffold should be mechanically strong enough in order to maintain itself inside the body at physiological state. The scaffold should show rapid adherence to the wound area when implanted. They should either be biodegradable and completely excreted from the body eventually after regeneration of the new tissue, or they should be bio-stable and able to resist wear and tear. The small wear particles from the non-degradable polymer may act as toxic foreign element to the surrounding tissue. Over all, for being available to the patient of all economic classes, the cost of the scaffold has to be low. Finally, an indefinite shelf life and easy, inexpensive storage is expected from an ideal synthetic scaffold [2].

Nanofibers are highly promising and effective as their high surface area and porosity provide the biological functionality necessary for in vivo stability and maintenance [12]. When selecting polymers for use in tissue engineering scaffolds, biological function is of high importance as the polymer must be biocompatible with cells in the body. Biological interactions in the body, the extracellular matrix (ECM), and cell growth and regulation also aid in mechanical support. Both hyaluronic acid and PEO (secondary), the polymers of choice throughout this research, are biocompatible, non-mutagenic, and non-carcinogenic, providing healthy interactions in the body [16,19]. As a natural non-immunogenic polymer, HA plays a vital role in wound-healing processes, is a component in the extracellular matrix, and is environmentally sustainable; thus making it a great candidate as a tissue engineering scaffold matrix. It is also facilitates growth and movement of fibroblasts as well as controlling hydration [22]. Ideally, once fabricated, the HA nanofibers would serve as a mesh capable of encapsulating cells.

Figure 6 is a depiction of how nanofibers are utilized to grow cells on the scaffold/mesh or deliver materials to various systems throughout the body. This HYAFF® scaffold serves as a 3-dimensional matrix for growing the cells that comprise human skin [23]. The HYAFF® scaffold is a recently developed hyaluronic-acid based biodegradable polymer, which has been shown to provide successful cell scaffold for tissue-engineered repair. This new scaffold has been patented by Johnson and Johnson as the answer to a clinical need for biocompatible and biodegradable structural matrices that facilitate tissue infiltration to repair/regenerate diseased or damaged tissue. Previous attempts have only used a number of naturally occurring, as well as synthetic biodegradable materials as scaffolds in the tissue repair process.

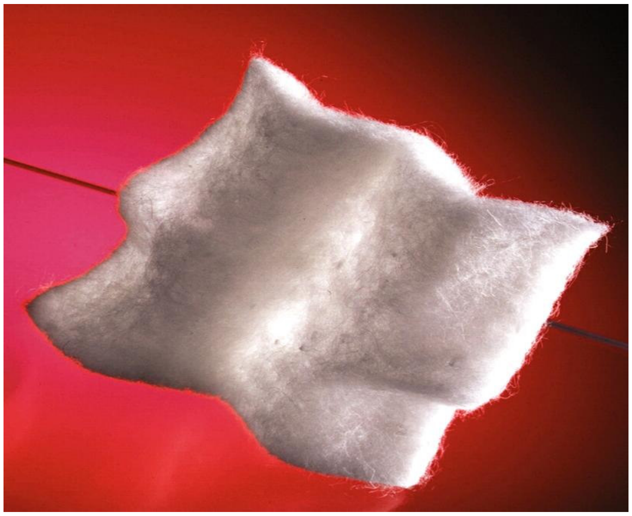

Figure 6: HYAFF® 3-dimensional Matrix [24]

After the matrix, as seen in Figure 6 is formed, researchers have found even more opportunities for both cell adhesion and cell growth to take place, as seen in Figure 7. These cells may mimic many bodily functions, for example dermal tissue growth [24].

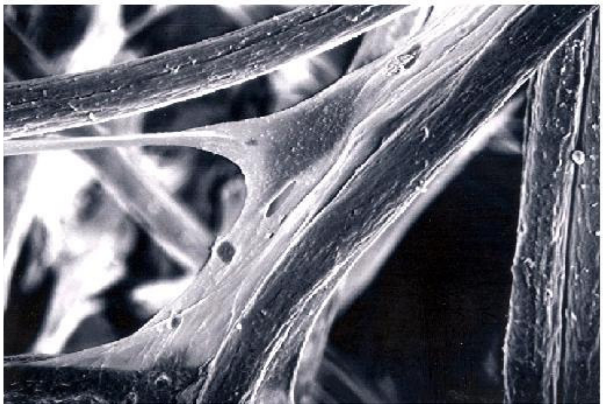

Figure 7: Illustration of Cell Growth on PEO Nanofibers [24]

Upon cell growth, the matrix is fabricated into a film covering that operates as actual skin. This film (as shown in Figure 8) may be utilized both in vivo and ex vivo for wound treatment, skin lacerations, ulcers, and other bodily wounds. The advantage of such a treatment is the minimization of scar tissue and cell adhesion between the wound and adjacent surfaces in the body while also using drug delivery techniques.

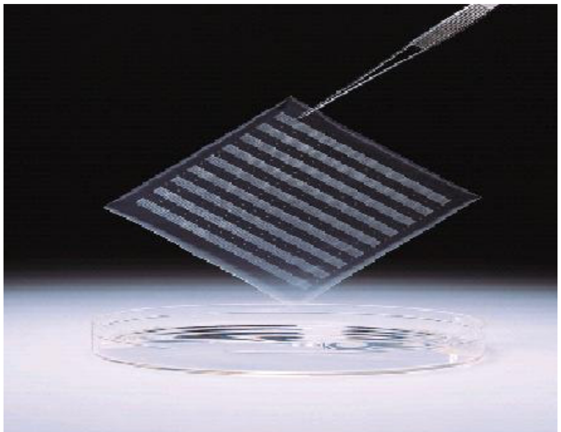

Figure 8: Skin formed from nanofiber mesh [23]

Conductive nano-fibers can be used in microchip applications and throughout the semi-conductor industry. Filters composed of nano-fibrous materials are also beneficial for medical and space assembly clean rooms. Fiber reinforced supports are ideal for very thin film applications and biodegradable wound dressings. With electrospun nanofibers small pore sizes of electrospun fibers make them suitable candidates for military and civilian filtration applications. They may eventually find application in composite materials as reinforcements. As the field of tissue engineering grows, nanofibers continue to find use in this new technology [25,15].

3.7.3 Challenges with Nanofibers

The process of making nanofibers is quite expensive compared to conventional fibers due to low production rate and high cost of technology. In addition the vapors emitting from electrospinning solution while forming the web need to be recovered or disposed of in an environmentally friendly manner. This involves additional equipment and cost. The fineness of fiber and evaporated vapor also raises much concern over possible health hazards due to inhalation of fibers. Thus the challenges faced can be summarized as:

- Economics (processing and production costs)
- Health hazards (processing)
- Solvent vapor
- Packaging/shipping handling

As nanofibers continue to provide exceptional qualities and a range of applications, research continues in order to find a balance between cost and efficiency [15].

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4.0 EXPERIMENTAL

4.1 Materials

Three types of hyaluronic acid were utilized in this study. In preliminary trials, HA with a molecular weight of 1.5 million was obtained from HyluMed®, item number 4876-05. For the remainder of the investigation, two types of HA were used: high molecular weight HA (MW=1 million from the distributor FMC Biopolymer ACS in Norway) and low molecular weight HA (MW=680,000 from Hyaluronan Inc). Polyethylene oxide, PEO (MW=900,000) and polyvinyl alcohol (MW = 126,000) were obtained from Scientific Polymer Products. All of the HA samples were in the form of a sodium salt (sodium hyaluronate) and all polymers were used as received. Deionized, distilled water was exclusively used as a solvent and all other reagents (e.g. sodium chloride) were analytical grade or better, obtained from Sigma-Aldrich and used as received.

Samples were made by dissolving the HA powder with deionized water solutions and NaCl solutions. The NaCl solutions were comprised of analytical grade NaCl crystals of different concentrations (i.e. 1M and 0.5M) as a dilution technique to possibly decrease the overall solution viscosity, thus solution concentration, of the HA solutions, see Chapter 5. In addition, blended solutions of HA:PEO were made from HA powder and PEO powder both dissolved in deionized water. The total weight of the polymers was used to calculate the solution concentration as described in Chapter 5.

4.2 Rheological Measurements

HA solutions were measured as a function of polymer concentration and salt. The rheological behavior was measured using an ATS Rheosystems Stresstech HR rheometer at 25ºC. The instrument was utilized with a parallel plate fixture at a gap distance of 0.4

mm for all samples. The zero shear rate viscosity (η_o) was determined from the Newtonian plateau for each sample.

4.2.1 Electrospinning

The actual electrospinning apparatus used for this research was constructed to accommodate several different polymer solutions, including PVA, PEO with carbon nanotubes, and Chitosan. The device was constructed with the intent of using a horizontal arrangement using two parallel plates to better control the electric field. Figure 10 is a Solidworks® drawing of the plate, stand, and base of the apparatus. The wooden base is comprised of a sliding slot allowing for the collector distance to be varied between samples. The height of the plate on the stand may also be varied as a vertical sliding slot was installed on the stand to aid in any height changes to the plates during electrospinning.

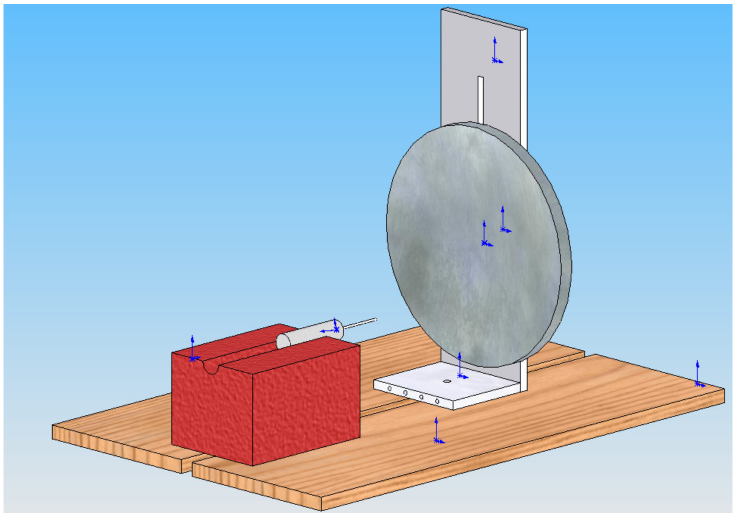

Figure 9: Plate and Stand Design of Electrospinning Apparatus

The electrospinning apparatus used for this study is illustrated in Figure 11 below. In particular, flow rate is controlled using a digitally controlled syringe pump which delivers fluid through Becton Dickinson syringes. The syringes encapsulate metal syringe needles of gage 16 and 18 in moving the fluid from the syringe to be exposed to the electric field between the two plates. The syringe pump was obtained from New Era Pump Systems, model NE 500. Syringes (10mL luer-lock) and needles (16 and 20-gage with 2” blunt-tip) were obtained from Sigma-Aldrich, product numbers Z192171 and Z192562 respectively. Regulated DC voltages, up to 60 kV, was applied using a High Voltage Research power supply. Lastly, electric field measurements are simplified by implementing a parallel-plate electrospinning design, as shown in Figure 10; although the experiments could be executed with or without the top plate in place. By adjusting the protrusion of the needle tip from the upper plate, we can vary the electric field curvature near the needle, independent of other parameters. Since electric fields and potentials are obtained by dividing the force and potential energy by the charge, they are measured in units of *N/C* and *J/C* respectively. But a "Joule per Coulomb" (*J/C*) is also known as a *volt* (*V*), and the electric potential is thus often referred to as the *voltage*. The electric field can therefore also be quoted in units of volts per meter, since *V/m = N/C*.

Typical operating regimes are flow rates between 0.05 and 2 ml/min, voltages between 20 and 40 kV, and tip-to-collector distance (TCD) of 9 to 15 cm. The parallel plates are 20mm disks covered with commercial aluminum foil serving as the actual collector. The two plates are comprised of a 1-1/2” piece of Lexan® material that is covered by a ½” piece of aluminum covering. Lexan® was used as it has high resistance to corrosives and is easily machinable. The two plates were wrapped after each trial with commercial aluminum foil, which served as the collector for the electrospun fibers. The stands were cut from ½” slabs of Delrin®. Delrin® material was used for the plates as it is cost-efficient, aesthetically appealing for the assembly, and non-conductive for the electrospinning process.

Figure 10: Digital Image of Electrospinning Apparatus

4.2 Characterization Techniques

Quantitative analysis of the electrospinning process falls into these categories: solution properties, operating parameters, and fiber characterization.

4.3 Solution properties

The relevant fluid properties are density, viscosity, surface tension, pH, conductivity, permittivity, and visco-elasticity. Of these, viscosity, pH, and conductivity appear to play the greatest role in the electrospinning of dilute solutions. For all the modeling studies, the solutions used were confirmed to be shear-thinning for the range of wall shear rates expected to occur at the needle tip. A StressTech HR rheometer from Reologica Instruments and ATS Systems was used to investigate the rheological properties, mainly viscosity vs. shear rate, of the polymer solutions. With the assistance of Rheologica Rheo-Explorer 5.0 software, data looking at the viscosity, shear rate, and variations in each amongst solutions was obtained. See Section 5.0 “Data & Analysis.”

4.3.1 Rheology Measurements

Rheology can be defined as the science of flow and deformation of materials. To determine the consistency of a material both its viscosity and elasticity parameters must be studied; the viscosity of a material is related to its resistance to flow, while the elasticity is related to its degree of structure. A rheometer measures the rheological properties as a function of rate or frequency of deformation; for example, a rheometer imposes a shear flow on a system and it measures the resulting stresses or, it could impose a shearing stress and measure the resulting shearing rate. Different flow geometries exist in order to impose a shearing flow: sliding plates, concentric cylinders, cone and plate, parallel disks, capillary, slit flow and axial annulus flow. The flow geometry used for this research was that of parallel plates (see Figure 12) due to the known high solution viscosities of the samples. In addition, the ability to “squeeze” samples, like the ones being studied, into a conical shape is very difficult; while with parallel plates, both viscous and non-viscous samples may be easily loaded, and data may be obtained within minutes of preparing the sample. Parallel plates are also recommended for time dependent studies, like stress relaxation.

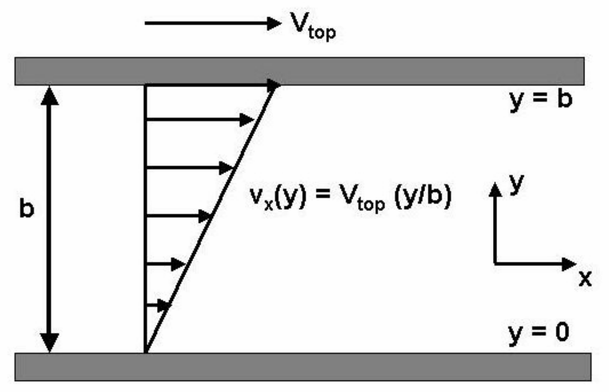

Figure 11: Schematic parallel plates

Before continuing talking about shear stress, shear strain and shear rate one should know what happens in a system under shear. Consider a small cubical volume of material. Under the action of forces that produces shear stress, the shape shifts to a parallelogram. Figure 12 again shows such a volume at rest and immediately following application of force. The change in shape has two components, elastic deformation E and slippage S. The elastic deformation is accompanied by storage of elastic energy within the structure of the material, while the slippage is associated with a continuous input of viscous energy. When the force is removed, the deformed material undergoes a partial recovery of shape as the elastic energy is recovered; the shape change due to slippage is permanent. Thus, in steady flow the displacement component S continues to increase and measurements of the non time-varying force and velocity provide no information about the elastic energy component. In a time-varying flow, however, the elastic energy component also varies with time and may be either increasing or decreasing, while the viscous energy is always increasing. Consequently, the relation between the time-varying force and velocity reflects both the elastic and viscous properties of the material.

Shear stress, σ, can be defined as the force that a flowing liquid exerts on a surface, per unit area of that surface, in the direction parallel to the flow. Based on this, shear viscosity can be defined as:

$$\eta = \sigma/\gamma \text{ (Equation 4)}$$

where viscosity (η) is the ratio of shear stress to shear rate. Shear rate may be defined as the rate of change of shear stress or the velocity gradient (as seen in Figure 12) perpendicular to the direction of shear flow (dv/dx). Shear rate is measured in units of 1/s or s^{-1}. The units of viscosity are typically measured in Pascal-Seconds (Pa.s) or Poise (P).

4.3.2 Operating parameters

The relevant operating parameters are flow rate, electric field strength, and electric current flow between the needle tip and collector. The volumetric flow rate is closely controlled through the use of a syringe pump. Field strength may be varied by changing either the applied voltage or the distance over which the voltage drop to ground occurs. Both variables were studied. Thinning of the jet depends principally on the field strength; however, the development of instabilities in the jet requires sufficient distance of travel for the instabilities to grow in amplitude. The total electric current has the ability to provide an indirect measure of the total surface charge density on the jet. Surface charge density, in turn, is believed to play a major role in determining jet stability. Jet current is measured by monitoring the voltage drop across a resistor between the collector and ground, and correcting for any displacement current between the parallel plates.

4.3.3 Fiber characterization

Fiber diameter distributions are measured using scanning electron microscopy (SEM). Samples of non-woven webs were coated with gold palladium using a Denton Vacuum Desk II sputtering machine and observed using a JEOL, Inc. Field Emission model J600F scanning electron microscope, operating at 5 kV. Fiber diameters were sampled by measuring the width of those fibers intersected by a straight line drawn at random across the image

4.3.3.1 Scanning Electron Microscopy (SEM)

All scanning electron microscopes consist of a column which generates a beam of electrons, a specimen chamber where the electron interacts with the sample, detectors to monitor the different signals that result from the electron beam/sample interaction and a viewing system that builds an image from the detector signal. The electron column consists of an electron gun and two or more electron lenses. The electron gun, which could be either a thermionic or a field emission source, is responsible for the production of electrons and the acceleration of these, to a range of 1 – 40 keV. These electrons, coming out of the gun, produce a beam with large diameter. Before continuing, it is important to know that the final beam diameter, also known as the spot size, plus the amount of current in the final probe, limits the image resolution in the SEM; that is why operators have to play with different parameters, until the “right combination” of parameters is found, in order to obtain high quality images. Electron lenses are used to reduce the diameter of the beam coming out of the gun, placing a small focused electron beam on the specimen. This results in an interaction between the beam and the near surface region of the specimen up to certain depth (1-2 μm) and generates signals used to form an image. The deflection system is responsible for scanning the beam along a line and then displacing the line position for the next scan so that a rectangular raster is generated on both the specimen and the viewing screen. Two pairs of deflection coils are responsible for the raster of the beam. Magnification is defined as the ratio between the linear size of the viewing screen to the linear size of the raster on the sample. High magnifications are obtained when a smaller raster width appears larger when displayed on the viewing screen. The two signals most often used to produce images are secondary electrons (SE) and backscattered electrons (BSE); these are collected by an Everhart – Thornley (E – T) detector.

4.4 Preliminary Results

To verify the functionality of the electrospinning apparatus (as described in Section 4.1), PEO and PVA were electrospun into nanofiber, non woven mats, as illustrated in Figures 13 and 15. First, the two polymer solutions were electrospun to find the threshold for successful electrospinning. Second, the electrospun samples were compared to reported PEO electrospun nanofibers to evaluate the fiber properties. Fiber diameter ranged from 100 nm to 250 nm for both the PEO and PVA nanofiobers, which is very similar to recent reports. In initial experiments, 2 wt% PEO and PVA solutions were produced from 2 grams of 900,000 MW PEO and 146,000 MW PVA with 100 mL of distilled water, respectively. Next, 5 wt% PEO or PVA solutions were electrospun (5 grams of PEO or PVA). All images reveal smooth, bead-free, and continuous fiber strands of polymer PEO and PVA. These images were used to verify the critical parameters needed for fabricating fibers of the various polymers, as summarized for PVA in Table 5.

Table 3. DOE for PVA Electrospinning

Concentration	Flow Rate	Voltage	Tip-to-Collector Distance	Double Plate	Fibers?	Film	Particles
5 wt%	0.5 mL/min	20 kV	20 inches	Yes	Yes	No	Yes
2 wt%	0.5 mL/min	24 kV	12 inches	No	No	Yes	No

The PVA solution was able to fabricate nanofibers at a concentration of 5 wt% with an average zero shear rate viscosity of 12000 cp, as shown in Figure 13.

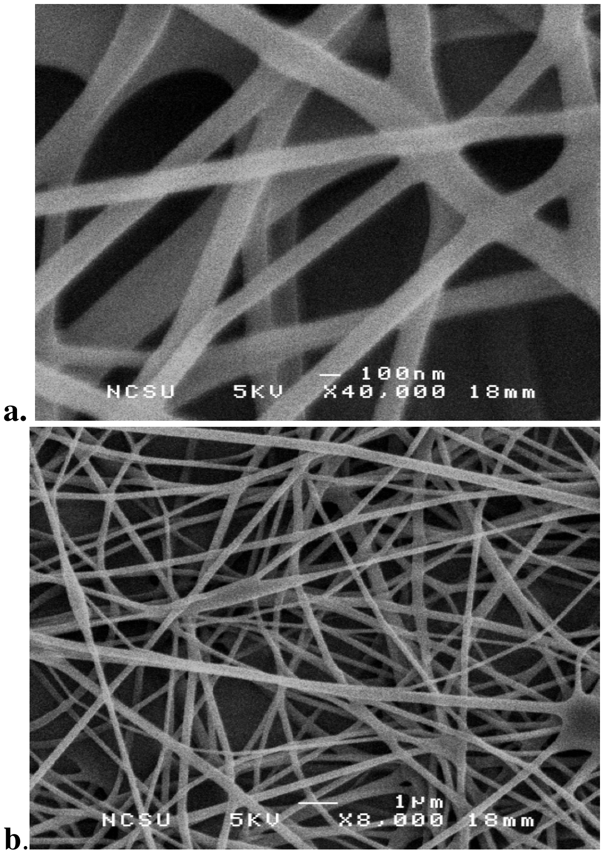

Figure 12: SEM image of 5wt% PVA.
a. High Magnification (40,000x) b. Low Magnification (8,000x)

The PEO nanofibers, as shown in Figure 13, were fabricated from a PEO solution consisting of distilled water and 900,000 MW PEO. The process parameters varied slightly from the PVA solutions as shown in Table 4.

Table 4. DOE for PEO Electrospinning

Concentration	Flow Rate	Voltage	Tip-to-Collector Distance	Double Plate	Fibers?	Film	Particles
5 wt%	0.5 mL/min	20 kV	20 inches	Yes	Yes	Yes	Yes
2 wt%	0.5 mL/min	24 kV	12 inches	No	Yes	No	No

The PEO solution was able to produce nano-scale fibers at the above process parameters and concentrations of both 5 wt% and 2 wt%. The fibers produced are ideal in that they have a uniform fiber diameter, as shown in Figure 13 below and are smooth along the surface showing no signs of bead or particle formation.

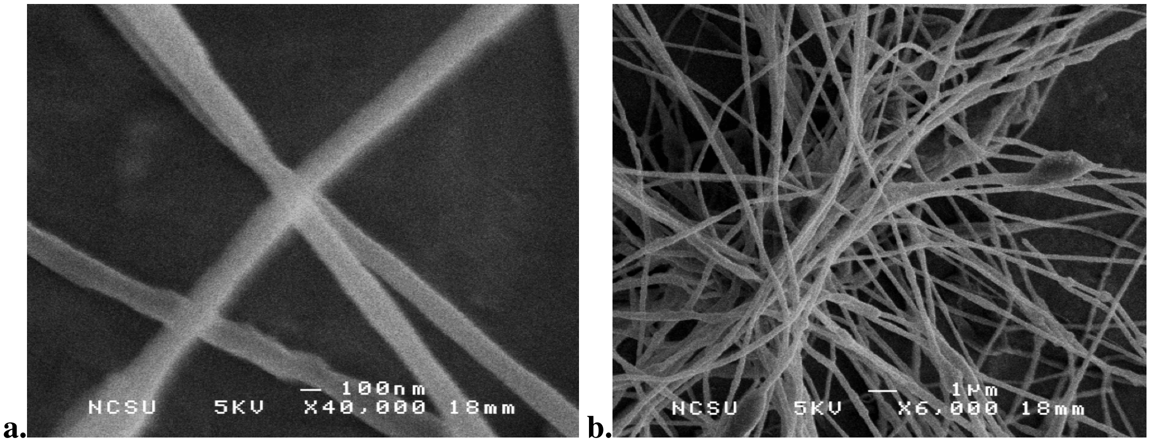

Figure 13: SEM image of 5wt% PEO nanofibers
a. High Magnification (40,000x) b. Low Magnification (8,000x)

Rheology measurements also reveal that the viscosity of the electrospun solutions is around 1810 cp for the 2 wt% solution and 1940 cp for the 5 wt% solution. These parameters, for both polymer solutions, were selected based on past electrospinning research parameters through experimentation in altering the parameters slightly to adjust for factors such as the variations in assembly design, operator, and polymer properties (i.e. molecular weight and viscosity). Through experimentation, the functionality of the electrospinning assembly along with the thresholds for fiber fabrication amongst the polymer solutions were verified. Following parameters from previous research in order to fabricate PEO and PVA fibers and then verifying the fiber diameter actually being on the –nano scale allowed HA electrospinning to commence based on successful fiber production and process variables.

5.0 DATA & ANALYSIS

The goal of this research is to produce biopolymer nanofibers of hyaluronic acid via electrospinning. The challenges associated with hyaluronic acid include its high solution viscosity and surface tension which are both parameters within the electrospinning process that contribute heavily to successful fiber formation. In order to reduce the viscosity of the polymer, two different methods were used: addition of sodium chloride (NaCl) and blending with biocompatible polymers that readily electrospin, such as polyethylene oxide (PEO) and polyvinyl alcohol (PVA). Both polymers, PEO and PVA, are highly capable of successful electrospinning for the production of nanofibers. Indeed, after our electrospinning apparatus was developed and assembled (refer to Section 4.2.1), PEO and PVA were electrospun to verify the functionality of our device. Once the successful operation of the electrospinning apparatus was established, the ability to form HA nanofibers from solutions of HA at various concentrations and molecular weight, with and with out NaCl, and with and with out PEO or PVA were investigated.

5.1 Design of Experiment

A Design-of-Experiment, see Chapter 10 Appendix A, was designed to include all process and solution parameters to verify the threshold for successful electrospinning. However, at each of the four concentrations (2 wt%, 1.5 wt%, 1 wt%, 0.5 wt%), there was no fiber production. Instead, electro-spraying of the polymer solution took place at the end of the needle tip. This electro-spraying phenomenon results in a film-like glaze over the collector surface, resulting in neither fiber nor particle production. At voltages exceeding 20 kv, there was significant spraying of the polymer but no fiber production based on all parameters throughout the process. The spraying is believed to have been a combination of the viscous solution interacting with the high voltage field but the polymer was unable to overcome such a high solution viscosity in order to pull fibrous materials from the end of the Taylor cone.

With this, the addition of salt and blending was definitely seen as a necessity in order to reduce the solution viscosity for fiber formation. After each of the HA solutions were made and electrospun with no success the HA solutions with sodium chloride (NaCl) and blended solutions of HA-PEO and HA-PVA were prepared to determine if a reduction in solution viscosity would take place and thus aid in fiber production through electrospinning. Blending mechanisms allowed for an overall decrease in solution viscosity while still providing optimal fiber properties for application use.

5.2. Processing effects on the performance of electrospinning

5.2.1 Effect of HA concentration

Polymer concentration has been known as one of the key parameters in electrospinning because it determines the solution viscosity, the polymer chain entanglements that are essential for successful operation and the amount of solvent that must be removed in the electrospinning process. The solution concentration also plays an important role in blowing-assisted electrospinning. SEM results of electro-spun HA mats prepared over a narrow concentration range from 1.0 wt% to 2.0 wt%, appeared to be an optimal concentration range for electro-spinning of HA at the chosen conditions. At higher solution concentrations and a high molecular weight (MW=1 million), the electric force could not overcome the high viscosity and the surface tension of the fluid, resulting in the failure to produce a stable jet stream. The initial concentration used for the HA samples, was at 2 wt%. This 2 wt% designation allowed for incremental decreases in viscosity of such a high electrospinning concentration for HA along with blending opportunities with other biocompatible polymers. Previous studies reveal successful fabrication of HA nanofibers with assisted electrospinning at a concentration range of 0.5-1.3 w/v%, as mentioned in Chapter 3. To clearly evaluate the role of concentration range, in terms of sodium salts and polymer blending, the higher weight percent (that is a concentration of 2 wt%) was utilized to identify variations in successful electrospinning based on polymer concentration and viscosity. As HA is known to have a high viscosity at low concentrations and with viscosity being a significant parameter in electrospinning, NaCl was added to HA solutions to observe the effect of NaCl on solution viscosity.

5.2.1.1 Electrospinning with HA

The viscosity measurement data for the 1 wt%, 1.5 wt%, and 2 wt% samples is shown in Figure 14. At a concentration of 2 wt%, the HA solution did exhibit very high viscosity, as compared with the previously electrospun PEO and PVA solutions. Looking at the zero-shear rate viscosity, the 2 wt% HA solutions was approximately 100,000 cp, which was difficult to electrospin, thus providing that this sample was too viscous for electrospinning. A significant decrease in viscosity with lower concentrations at 1.5 wt% and 1.0 wt% HA solutions are observed. Table 5 reveals the average zero shear rate viscosity measurements for the pure HA solutions at 2 wt%, 1.5 wt%, and 1.0 wt%.

Table 5: Zero Shear Rate Viscosity Measurements for Pure HA Solutions

Concentration (wt%)	Zero Shear Rate Viscosity (cp)
2.0 wt%	100,000 cp
1.5 wt%	23,080 cp
1.0 wt%	4,400 cp

From the data in Table 5, the concentration maximum for pure HA solutions to be electrospun was chosen at 100,000 cp based on observations of the samples overtime, including the increased viscous nature of HA at high concentrations, and previous studies. As mentioned earlier, at this concentration, the 2 wt% solution has an average zero shear rate viscosity of 100,000 cP, which is outside of the threshold for solution viscosity in successful electrospinning. Therefore, in order to electrospin this sample, the viscosity must be reduced by means of dilution, salt addition, or polymer blending.

At relatively low solution concentrations and low molecular weight (MW=680,000), there is a reduction in solution viscosity. In Figure 14 the shear viscosity of high and low MW HA reveal that the viscosity of the low MW and low concentrated HA solutions are 10^4 cp and below.

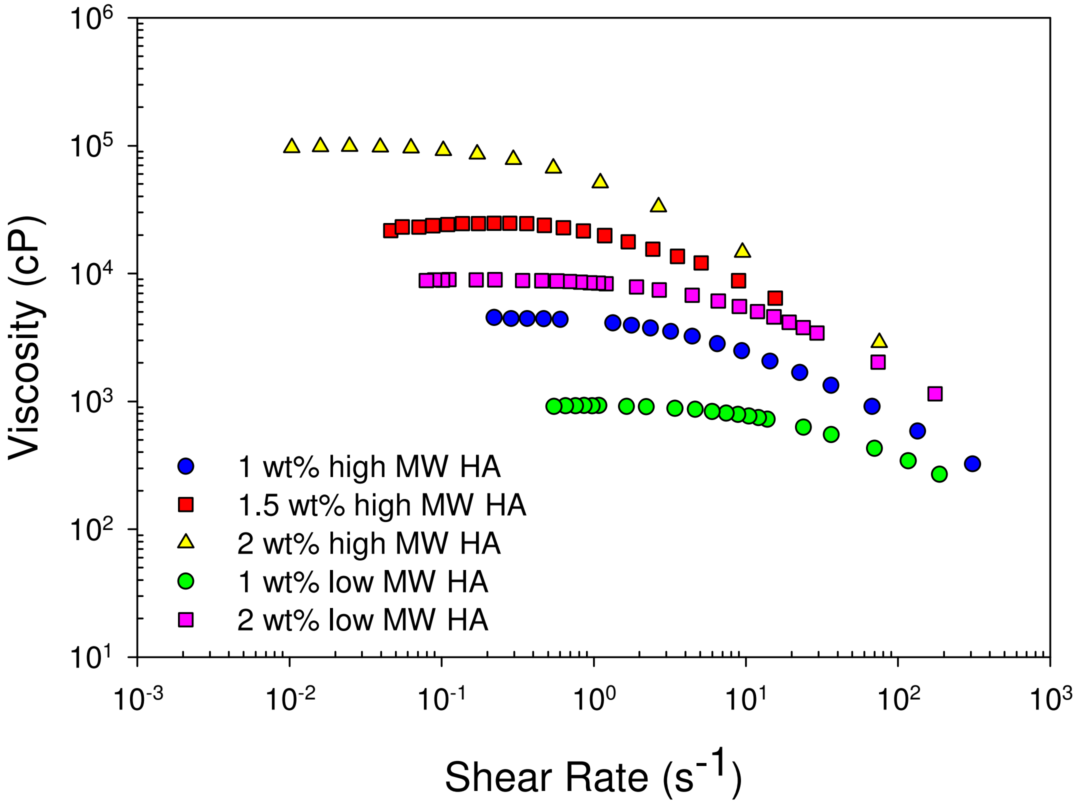

Figure 14: Shear viscosity of HA solutions with various concentrations

As seen in Figure 15, lower molecular weight HA is less viscous than that of high MW HA. However, the electrospinning results from the lower molecular weight HA samples do not produce nanofibers as the polymer chains are not sufficiently entangled to form fibrous morphology. Figure 17 below reveals the SEM images for the 1.5 wt% HA solutions (MW=680,000). The images reveal a film-like porous structure with no fiber production.

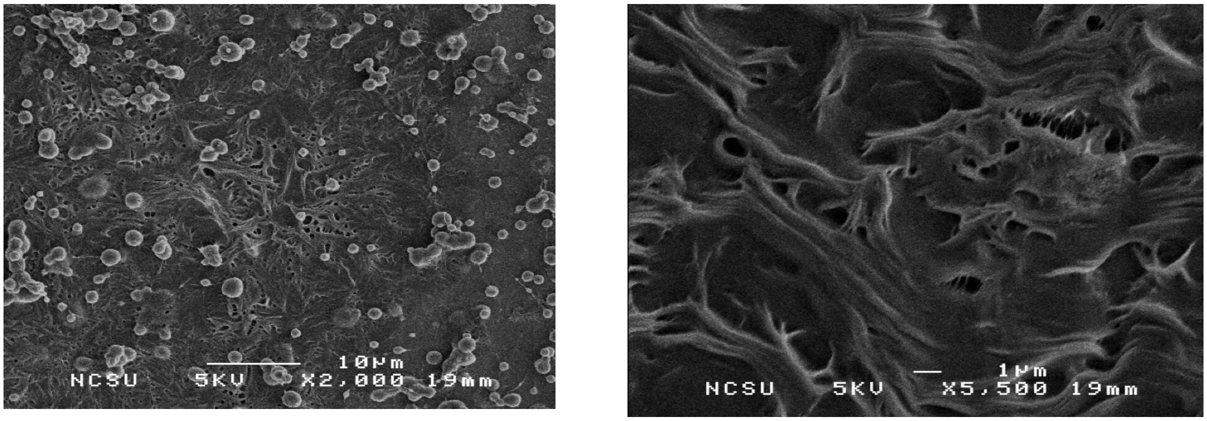

Figure 15: SEM image of 1.5 wt% HA Solutions
a. Magnification (2000x) b. Magnification (5500x)

5.2.1.2 Electrospinning HA with NaCl

To probe deeper into the influence of solution viscosity on the electrospinning of HA nanofibers, HA with NaCl was made using a 1M NaCl solution and a 0.5M NaCl solution. The whole purpose of the addition of the salt is to decrease the shear viscosity, thus placing it in a range that may be electrospun. From Figure 16, we see that even starting at a relatively low concentration of 2 wt%, that HA displays a highly viscous nature, around 10^5 cp. The 2 wt% HA solutions containing salt (1M NaCl) do exhibit a decrease in viscosity to 2×10^4 cp thus proving that the overall solution concentration may be decreased by the addition of salt.

Figure 16 again illustrates how the presence of NaCl in the polymer solution does reduce the solution viscosity of the HA solution by approximately one-third. A solution of 1M NaCl with 2 wt% HA and a solution of 0.5M NaCl with 2 wt% HA were made to evaluate the solution viscosity as a result of dilution for each of the two solutions. From the addition of the NaCl solutions, the viscosity of the 2 wt% HA, which was unable to produce fibers through electrospinning, was reduced by 70% of the original solution viscosity.

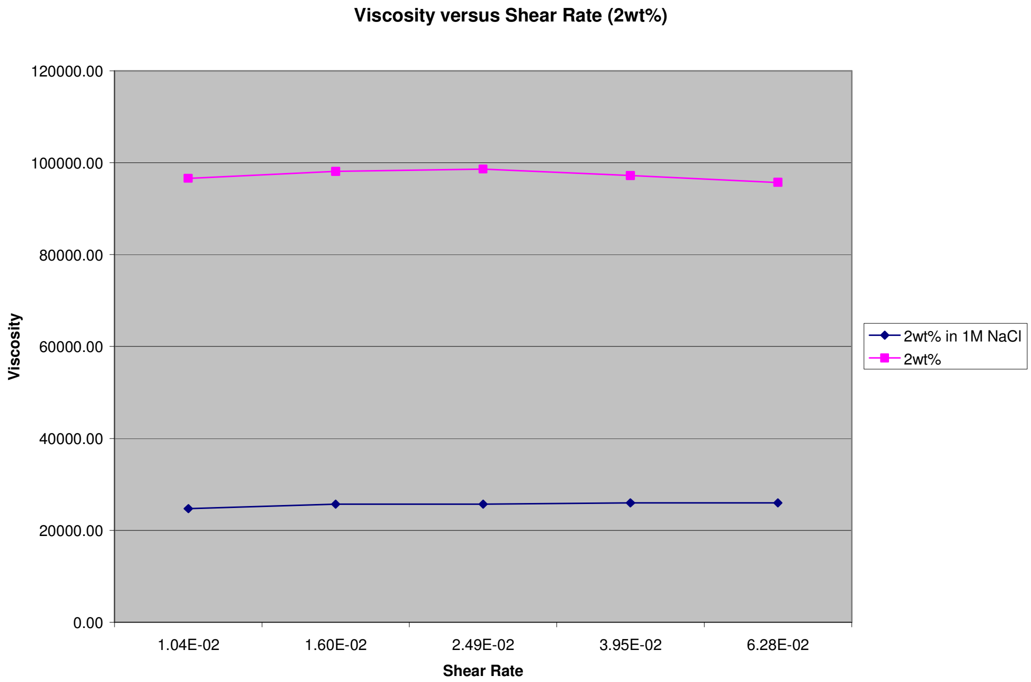

Figure 16: Viscosity (cP) versus Shear Rate (s^{-1}) of 2wt% HA

The new viscosity of the 2 wt% with 1M NaCl was at an average of 2607 cp. Figure 17 below compares 2 wt% HA with 2 wt% HA diluted with 1M NaCl and thus verifies the dilution capability of the NaCl solution. The 1M NaCl solution did present an overall decrease in solution viscosity, as shown.

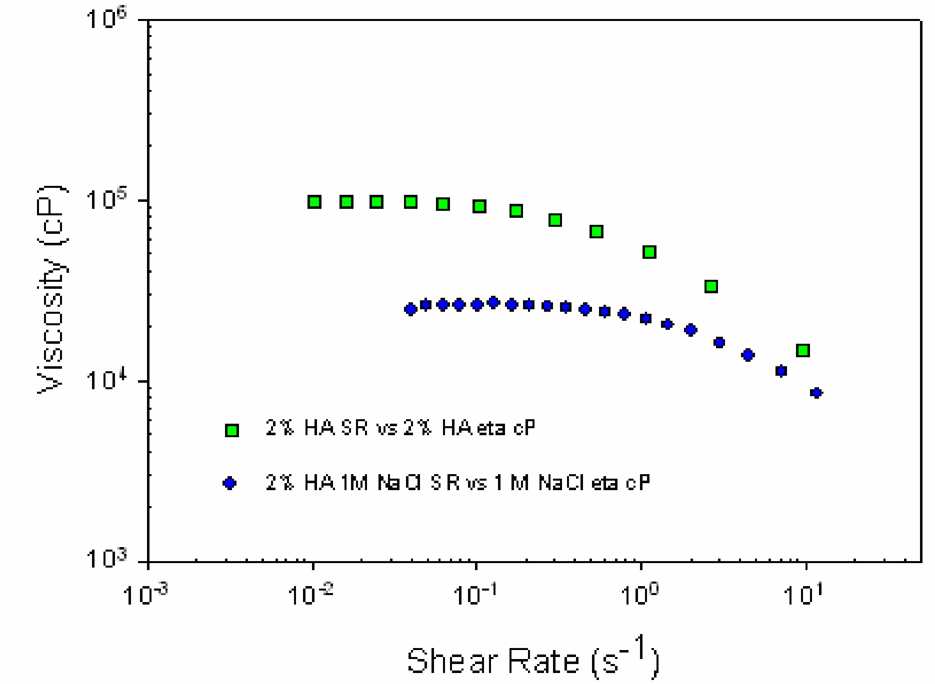

Figure 17: Viscosity versus Shear Rate of 2wt% HA at various concentrations.
Concentrations include pure HA, HA with NaCl (0.5M), and HA with NaCl (1M)

Figure 18 reveals the SEM images for this electrospun solution. The images, very different from those in Figure 15, reveal a nano-porous material with a film-like consistency. The morphology of the particles/material shown in Figure 19 is quite interesting in that there is no fiber formation and a unique shape and composition. The particles seem to be of a crystallized shape as the edges are very sharp and distinct. The electrospinning parameters measured for these samples were as follows:

Feed Rate: 0.01 mL/min
Tip-to-Collector: 12cm
Voltage: 30kV
Notes: Jet formed with fibrous material exiting syringe but as shown no fiber formation present, only that of small particles.

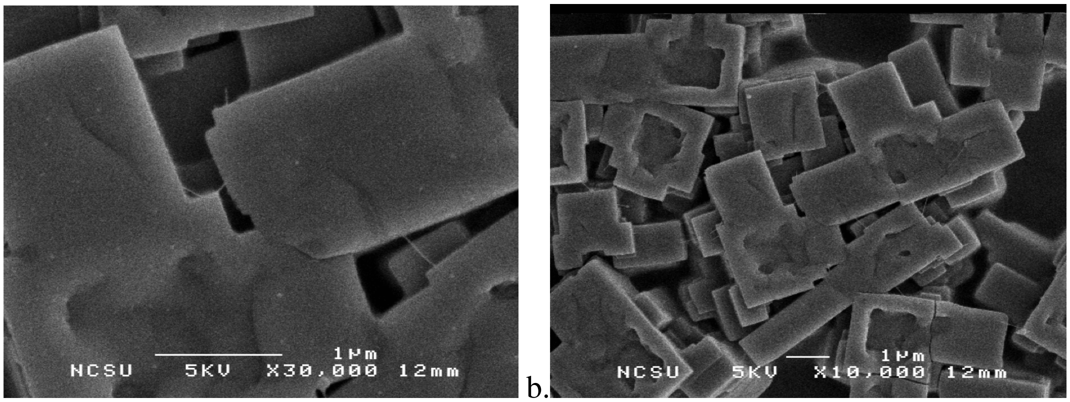

Figure 18: SEM image of 2 wt% HA Solutions (diluted with 1M NaCl)
a. High Magnification (30,000x) b. Low Magnification (10,000x)

The viscosity of this solution was around 4.0×10^5 cp and the pH was 4. Solution pH seems to be a strong indicator of the solution properties. At a lower pH (below pH=4), the solutions resembled a gel-like material, very viscous, and requires a higher voltage and feed rate in electrospinning. This gel-like consistency may be a direct correlation to solution viscosity and concentration. With a pH above 4, which was measured at the blended HA solutions, the solution is less viscous and uses a lower voltage and feed rate. These blended solutions were visibly less viscous than the pure HA solutions and HA with NaCl solutions and thus required modifications of the electrospinning parameters to occur in order for successful spinning.

To better understand the morphology of the images in Figure 18, non-electrospun HA with 1M NaCl was collected and photographed using the SEM to compare the polymer characteristics before and after electrospinning. Figure 19 reveals the images for the non-electrospun 2 wt% HA with 1M NaCl and illustrates that some phenomenon is occurring as the HA takes on a distinct particle shape when electrospun. For these samples, the blended HA solution was not electrospun but placed on the collector surface to view the morphology of the polymer solution after the evaporation of the solvent. The unique morphology of the samples in Figure 19 does not take on any shape but represents a film-like covering. Thus, the concentration of 2 wt% HA with 1M NaCl does cause a unique structural change in the solution prior to electrospinning.

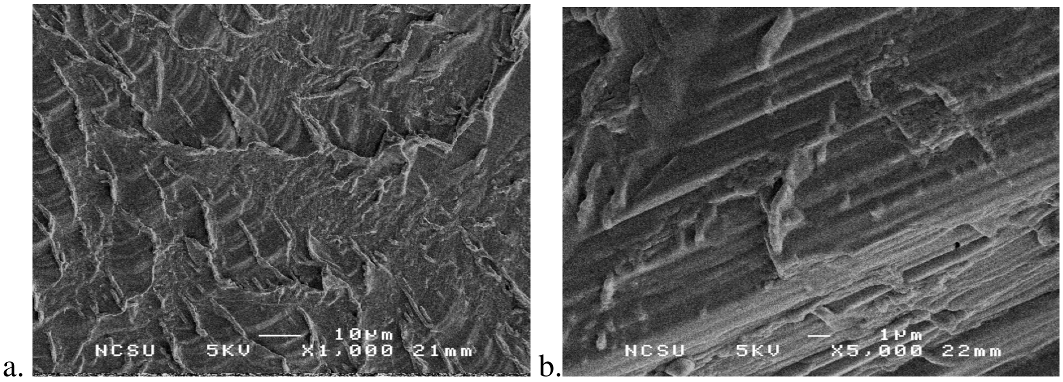

Figure 19: SEM image of non-electrospun 2 wt% HA Solutions (diluted with 1M NaCl)
a. Magnification (1000x) b. Magnification (5000x)

As mentioned earlier, 2 wt% HA with 0.5M NaCl was also electrospun. The rheology data in Figure 16 reveals yet again a decrease in solution viscosity. However, as seen in the SEM images in Figure 20, no fiber production took place in the electrospinning process. The difference seen with this sample was with the actual process parameters. Although, no continuous polymer jet was formed, electrospraying occurred as with the 1M NaCl solutions causing the formation of block-like particles. The electric field range and flow rate were increased in order to accommodate this polymer as it was more viscous.

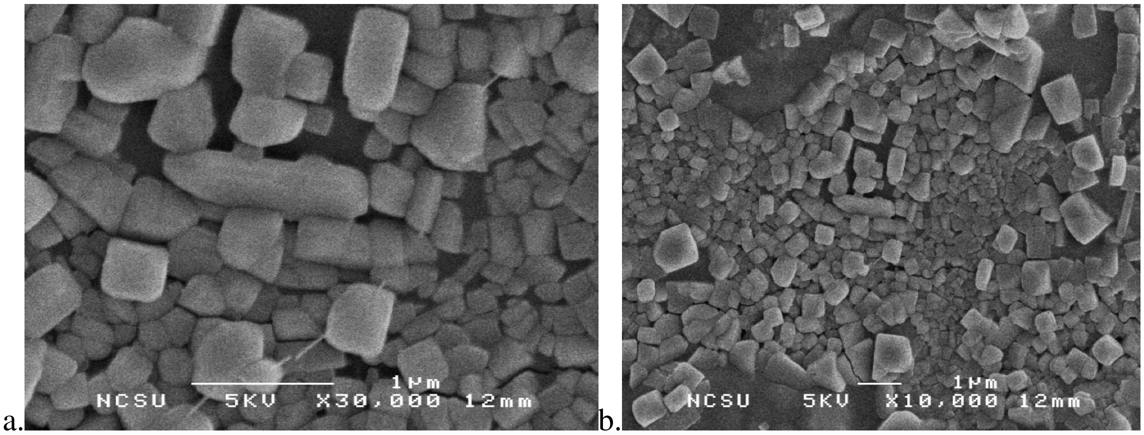

Figure 20: SEM image of 2wt% HA Solutions (diluted with 0.5M NaCl)
a. High Magnification (30,000x) b. Low Magnification (10,000x)

5.2.1.3 Electrospinning HA with Blends

As the HA solution revealed viscosity measurements that were extremely high for such a low concentration solution, blending the polymer with another polymer was the second mechanism utilized in decreasing the solution viscosity. The polymers selected for blending were PEO and PVA, both previously electrospun with at a medium-range viscosity. Viscosity measurements of PEO are shown in Figure 21 and compared to pure HA solutions, is a lot lower providing that the opportunity to blend the two polymers together may have a distinct effect on the solution properties.

Blending of HA and PEO, was done by using the formula for weight percent with the combined powder forms of HA and PEO as the solute and distilled water as the solvent. The MW of the PEO was at 900,000 and the HA at 1 million. Five different blend solutions were created at various ratios of HA:PEO, including 80:20, 70:30, 60:40, and 50:50 ratios of HA:PEO. The total polymer concentration of each solution was at 1.5 wt%, providing that 1.5g of total polymer (the ratios indicate the amount of HA:PEO) was used to produce the solutions. After electrospinning each of the four solutions at the parameters listed in the DOE (Appendix A), it was discovered that in order for electrospinning of the blended solutions to occur, the concentration of HA:PEO would at least need to contain 30% PEO. To verify this measurement, blends of HA and PEO were formulated at ratios of 75:25 (HA:PEO) and 85:15 (HA:PEO) to compare the results of these electrospun samples, in which there was no fiber or film production, with those containing at least 30% PEO. SEM images obtained for the blends revealed that the 1wt% concentrations of ratios 60/40 HA-PEO and 70/30 HA-PEO did successfully fabricate nanofibers, as seen in figures 22 and 23. Rheology data in Figure 21 indicates that blending the HA with PEO attributes a decrease in viscosity from that of HA alone.

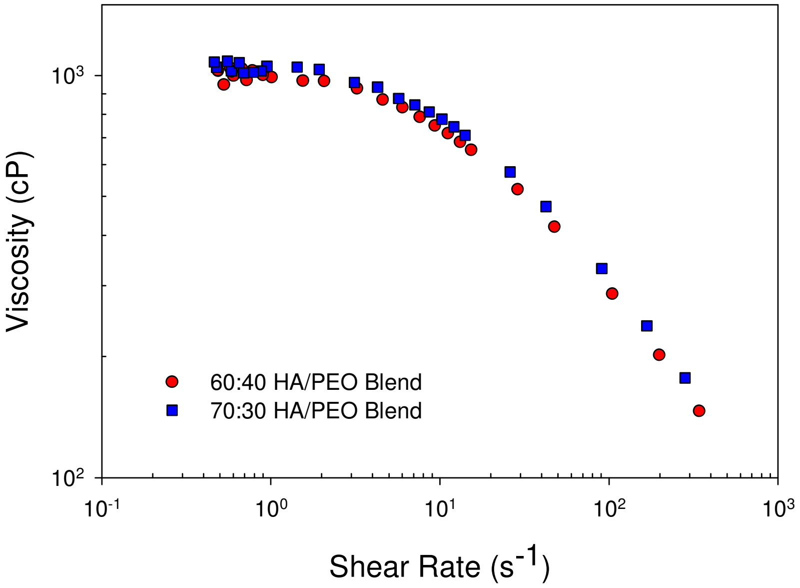

Figure 21: Rheology of HA:PEO Blended Solutions Versus Pure PEO

SEM images revealed that the blended HA-PEO solutions produce fibers with beaded structures. This bead formation was first thought to mean that that the tip-to-collector distance (TCD) may be interfering with the solvent evaporation rate. To verify this, the TCD was increased from 12 cm to 20 cm and then to 30cm. However, there is no significant difference in bead formation with an increased TCD. There is also not a huge difference between the two blended concentrations of HA and PEO (i.e. 70:30 and 60:40 HA:PEO solutions) as both produced beads at a high rate. The 70%-30% HA-

PEO blend (see Figure 22) and the 60%-40% HA-PEO blend (see Figure 23) exhibit similar fiber morphology. These ultra-thin fibers are continuous in fiber length and contain no scales.

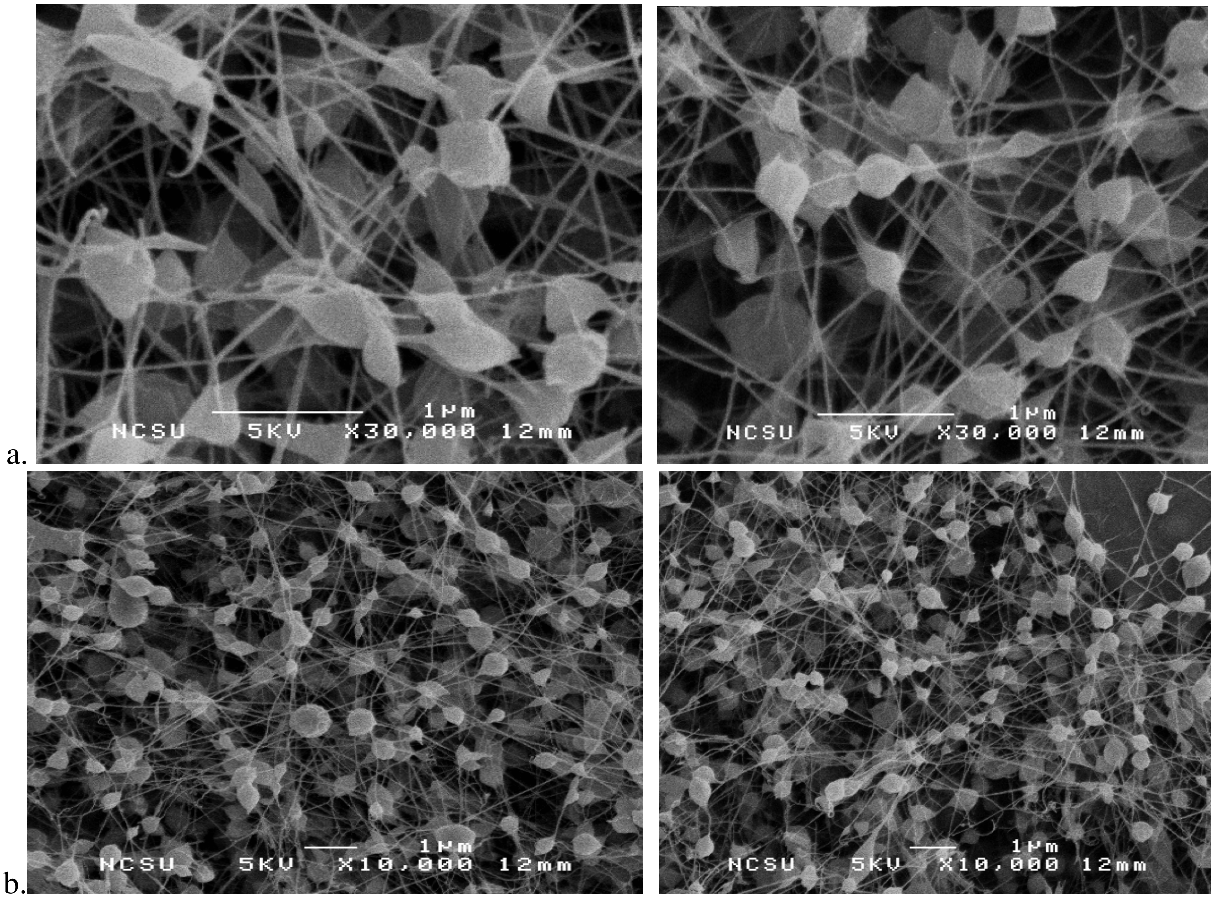

Figure 22: SEM image of 1 wt% HA:PEO (70:30) Solutions

a. High Magnification (30,000x) b. Low Magnification (10,000x)

Electrospinning Parameters: Feed Rate: 0.015 mL/min, Tip-to-Collector: 12cm, Voltage: 16kV

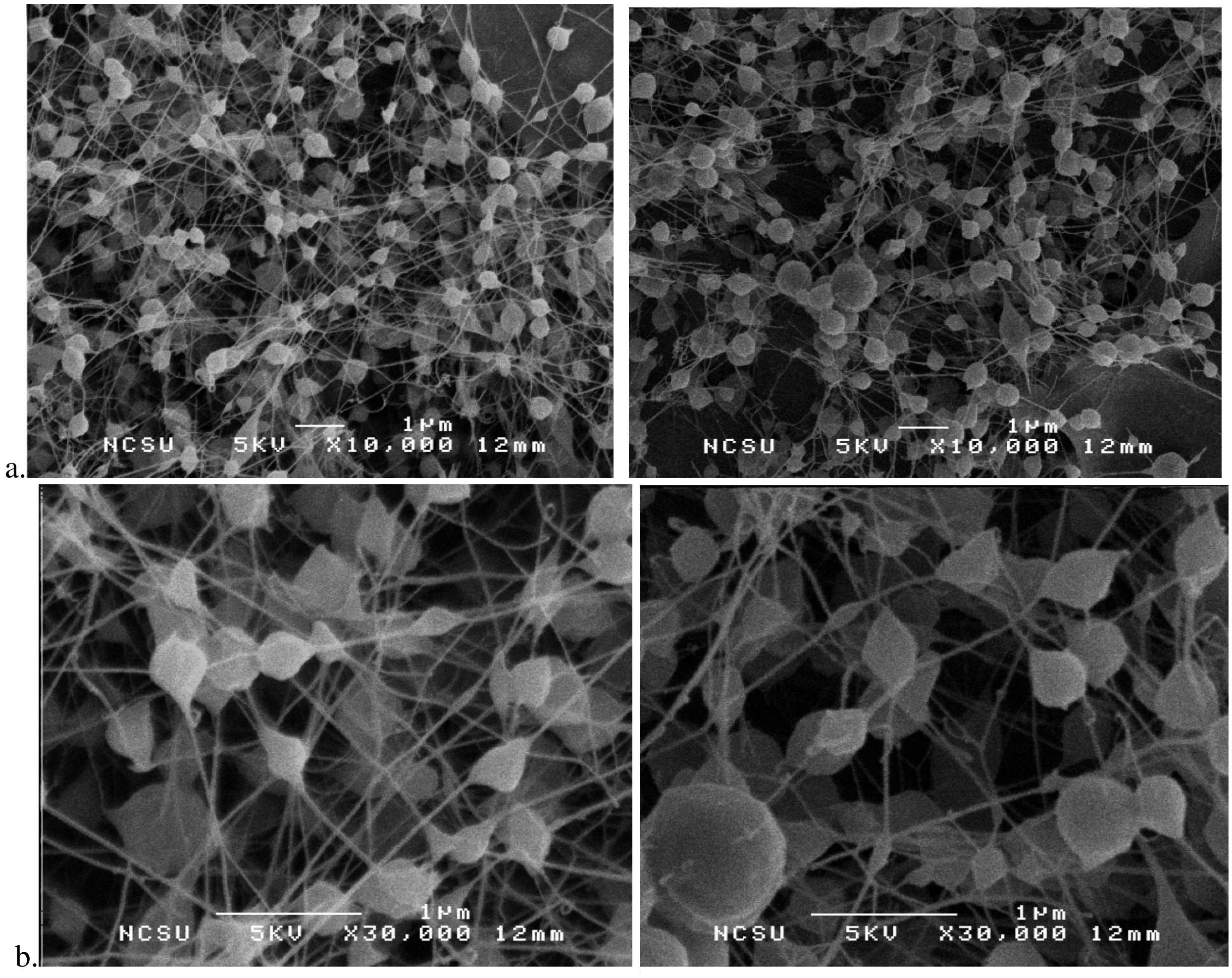

Figure 23: SEM image of 1 wt% HA:PEO (60:40) Solutions
a.)Low Magnification (10,000x) b.)High Magnification(30,000x)
Electrospinning Parameters: Feed Rate: 0.015 mL/min, Tip-to-Collector: 12cm, Voltage: 16kV

Blending also took place with the HA (MW=1million) and PVA (MW=126,000). Blending was done using the same procedure as the PEO blends using D.I. water as the solvent and various ratios or HA and PVA powder at an overall solution concentration of 1wt%. The difficulty arose with trying to dissolve both polymers. At no point did the polymers completely dissolve as the solutions were extremely cloudy and the PVA was still visible. After heating the solutions and using magnetic stirrers to dissolve the polymers completely, the solutions remained the same and thus no electrospinning was able to performed. The objective in blending HA with PVA was to see if the same results could be produced as with the HA-PEO blends to fabricate beaded nanofibers.

5.2.2 Effect of solution feeding rate

The feeding rate of solution during electrospinning is another factor affecting the fabrication process including electro-spinnability. In this study, 1.0wt% HA-PEO at various ratios of HA-PEO solution were electro-spun by using different fluid feeding rates in order to elucidate their effects on the process. By using SEM to evaluate the performance of the final sample, the optimal feeding rate was from 0.25 to 0.50 mL/min. Above 0.50 mL/min, an unstable jet was developed abruptly. Thus, in our current study, the optimum feeding rate was set to be 0.25 mL/min, in order to maintain the jet stability and to increase the production efficiency. In a typical electro-spinning operation, there is a narrow window of feeding rate for successful processing because the consumption of solution being electro-spun has to be limited to within a certain range in order to maintain the Taylor cone at the spinneret. When the solution-feeding rate is lower than this range, the Taylor cone can be broken. When the solution-feeding rate is higher than this range, the extra solution will drip out, which can interfere with the electrospinning process. For our electrospinning trials, the variation of solution concentration, through dilution, was the critical parameter contributing to successful nanofiber production, as solution viscosity was the most difficult property to overcome in the process. As mentioned earlier, the influence of concentration has two major effects on the spinning process: (1) an increase in the evaporation rate and (2) an enhancement in the effective fiber pulling force. We believe that in the chosen study to demonstrate the broadened capability of electrospinning HA, the concentration of the polymer solution is one of the dominant factors because an increase in the solution-feeding rate is needed in order to maintain the

Taylor cone, as a lower solution-feeding rate will result in the solidification of the solution during spinning. In looking at the SEM images for the two ratios of 1wt% HA-PEO, there was not a significant change in fiber morphology observed between the 60%-40% HA-PEO and 70%-30% HA-PEO.

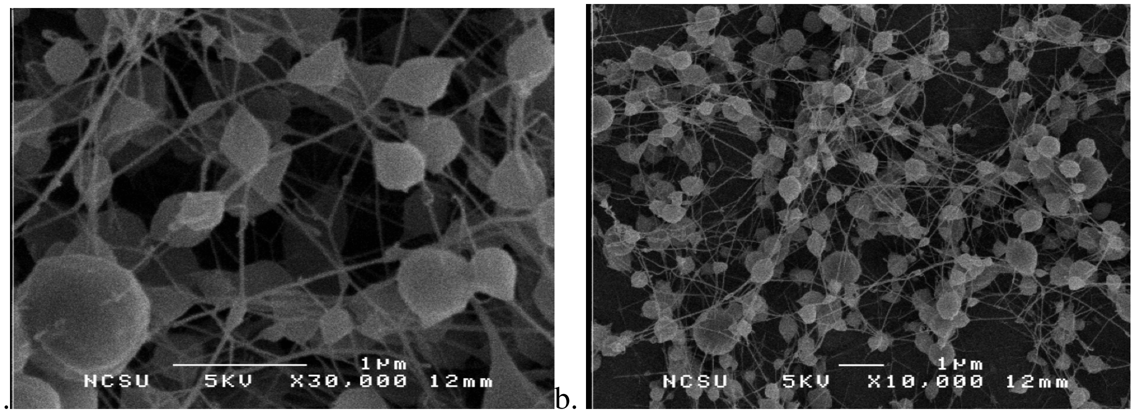

Figure 24: SEM image of 1 wt% HA:PEO (60:40) Solutions
a. High Magnification (30,000x) b. Low Magnification (10,000x)

Solution feed rate is another electrospinning parameter that also plays a significant role on the final fiber diameter of the electrospun blended solutions. As the zero shear rate solution viscosity for these blended solutions was proven to be lower than the viscosity of both the pure HA and HA with NaCl solutions, feed rate also decreased to maintain stability in the process. From Figure 25, there is an increase in fiber diameter as the amount of HA in the solution decreases. This can be attributed to the already higher solution viscosity of HA overpowering the relatively low solution viscosity of PEO, thus the total solution viscosity increasing with HA concentration. Fiber diameter was measured using the SEM images of the nanofibers and using measuring tools within the SEM software package to measure the diameter across the fibers, along various points.

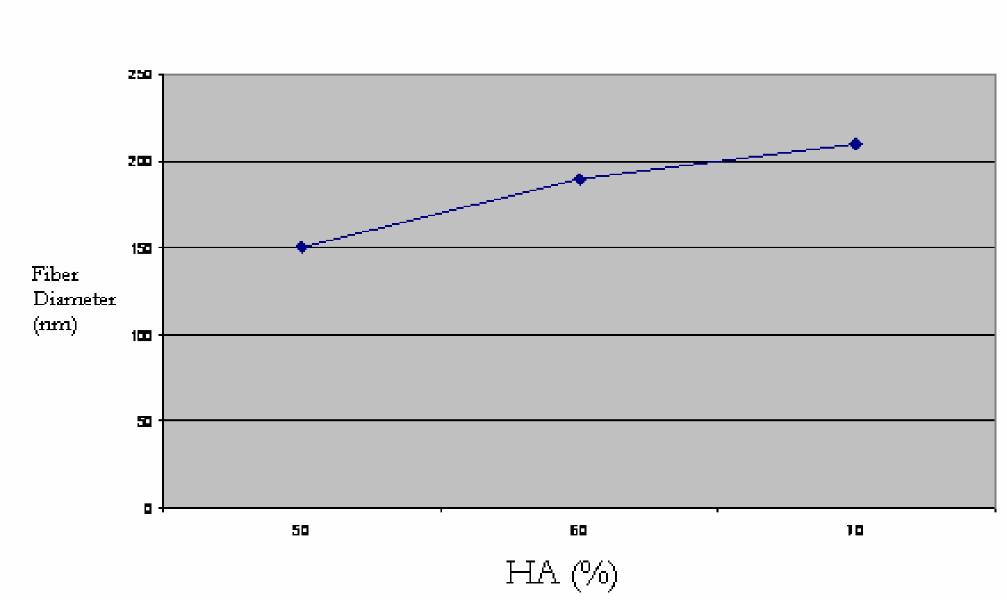

Figure 25: Effect of Blend Concentration on Fiber Diameter

5.2.3 Effect of electric field

The applied electric field has also been known to be one of the most important factors influencing the electro-spinning process. We demonstrated that even with 30 kV of applied voltage, the HA solution could still not be electrospun. Although HA has been electrospun with the assistance of air, as described in Chapter 3 in Chu's experiment, the high viscosity of the HA solution still challenged the electrospinning process. However, the reduction in solution viscosity by the addition of NaCl and blending with PEO aids in the ability of the electric field to overcome the surface tension and viscosity of the fiber and thus adding an extra pulling force. Results for the effect of electric field on the blowing-assisted electro-spinning performance for HA solution reveal that too low of an electric field does not overcome the surface tension of the polymer. The electric force could not overcome the surface tension until the applied electric potential reached beyond 10 kV for the HA-PEO solutions. The jet became stabilized at 12 kV and remained stabilized until 20 kV. Above 20 kV, the electrospinning process became unstable and electro-spraying occurred resulting in more film-like nanostructures instead of beaded nanofibers, as seen

in Figure 26.

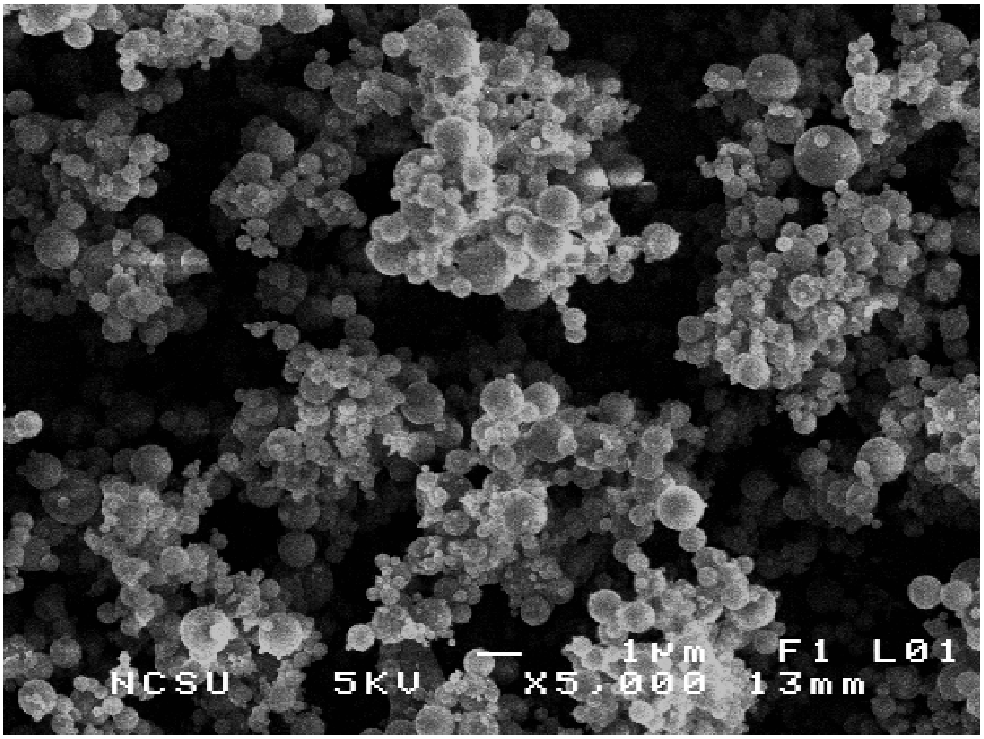

Figure 26: SEM image of 1 wt% HA:PEO (60:40) Solutions
High Magnification (30,000x)
Electrospinning Parameters: Feed Rate: 0.015 mL/min, Tip-to-Collector: 10cm, Voltage: 25kv

5.3 Additional Experimentation

As mentioned previously in this section, electrospinning was successful in nanofiber production with the HA-PEO blended solutions at 60%HA-40%PEO and 70%HA-30%PEO. With this, further experimentation was done to look at the use of a different collector material (Seprafilm®) and the composition of the beads on the nanofibers. In these trials, the previously used aluminum collector was covered with the film as the surface for nanofiber collection. The combination of electrospun nanofibers with Seprafilm® will aid in further in vivo tissue engineering applications, as discussed in the next section.

Since both HA-PEO blended solutions produced similar SEM images of beaded nanofibers with a solution viscosity in the range of 1000cP, this additional experimentation was carried out utilizing the 60%HA-40%PEO solutions.

5.3.1 Electrospinning on Seprafilm®

In addition to electrospinning the HA-PEO on the aluminum collector, the ability to change collectors for different applications was also studied. Seprafilm adhesion barrier (Seprafilm®) has been proven to prevent adhesion formation after abdominal and pelvic surgery. The biomedical company, Genzyme, manufactures a polysaccharide film called Seprafilm™ for use as a surgical adhesion barrier. The material is comprised of chemically modified sugars, some of which occur naturally in the human body. This technology combined with electrospinning and hyaluronic acid presents a unique opportunity to fabricate a scaffold or porous covering to be used in vivo for tissue engineering.

Electrospinning was successful in the fact that the polymer jet was formed between the needle tip and collector under the same conditions as listed previously for 60%HA-40%PEO blends. After electrospinning the solutions, the samples were viewed under SEM and revealed the following phenomenon in Figure 26, which is similar to the 1.5 wt% electrospun HA solutions.

The difference in the Seprafilm® collector could be contributing factors to the lack of nanofiber production as the film contained an adhesive-like surface characteristic; whereas the aluminum was smooth. As the polymer jet reached the collector, solvent evaporation was more difficult, even with increased distance between the tip and collector, and dripping of the polymer solution was apparent on the film. This is very similar to the action seen during the 1.5 wt% pure HA trials where nanofiber production was unsuccessful. In other research, Seprafilm® has been used as the collector in electrospinning both PEO and PEO-Chitosan blends. Here, the difference is that both polymers have a relatively lower solution concentration than HA.

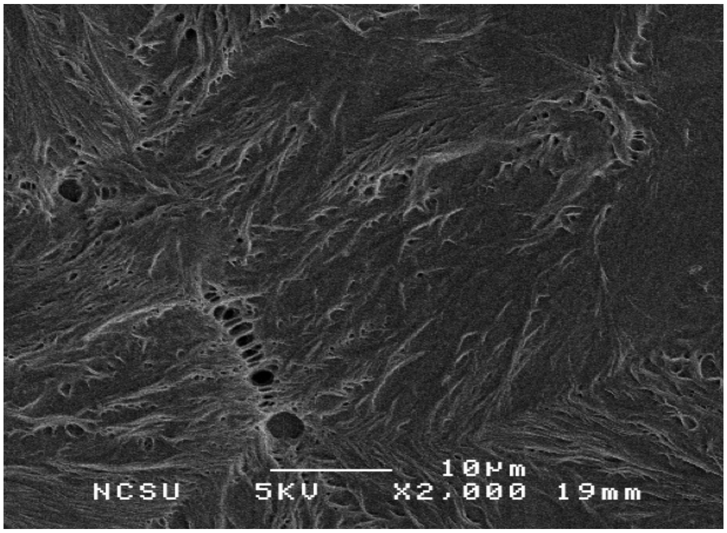

Figure 27: SEM Image of Electrospun HA-PEO (60:40 blend) on Seprafilm®

5.3.2 Energy Dispersive X-Ray Spectroscopy (EDS) Analysis

After using SEM to capture images of the electrospun HA-PEO nanofibers, the resulting images provided evidence that the presence of beads on the fiber surface indicates that at some point in the electrospinning process, the instability of the polymer jet is directly related to the presence of the beaded structures. If stable, the jet would allow for the production of continuous, bead-free electrospun nanofibers, as the electric field would be capable of overcoming the solution viscosity of the polymer solution. In understanding this phenomenon, the composition of the beads was studied using Energy Dispersive X-ray Spectroscopy or EDS). EDS can provide rapid qualitative, or with adequate standards, quantitative analysis of elemental composition with a sampling depth of 1-2 µm. EDS allows elemental analysis with the SEM. Qualitative and quantitative analysis of the elements Sodium to Uranium but excluding Lanthanides, Actinides and gases down to levels of ~0.1 wt%, and from an area of ~1µm in diameter. Ideally, this technology was believed to identify the composition of the beaded structures to see if the blended solutions were producing PEO nanofibers with HA beads or a truly blended array of HA:PEO beaded fibers.

The samples that were studied included the 60%-40% HA-PEO solutions. The collector was aluminum foil and the experimentation was carried out using the same SEM device as in previous sections. The only difference was that with the EDS analysis, it was very difficult to isolate one bead or a cluster of beads to examine the bead composition. Even more difficult, the technicians, once able to isolate one bead/cluster, found it difficult to read any elements from the samples except Aluminum (Al). However, with aluminum foil serving as the collector, it was concluded that the aluminum and nano-scale sized beads were preventing the device from identifying the composition of the beaded nanofibers. Figure 28 represents the SEM image used for EDS and the areas in which EDS was used to identify the fiber/bead composition.

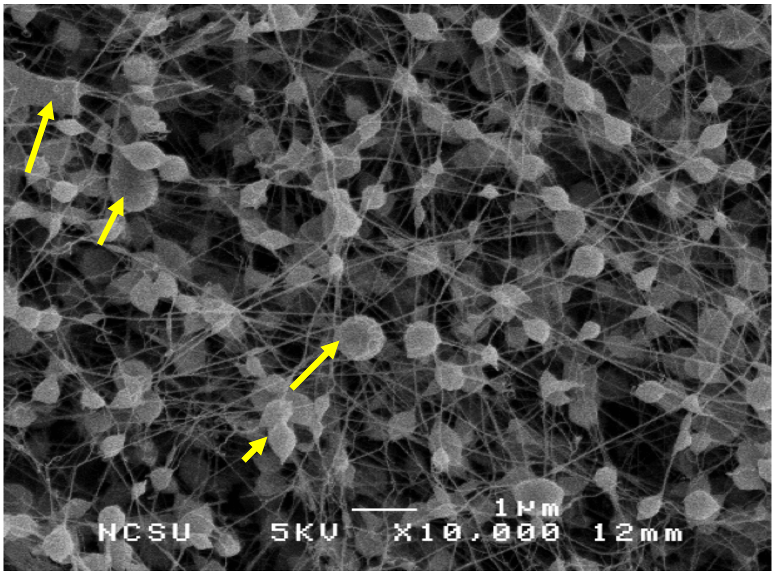

Figure 28: SEM Image of Electrospun HA-PEO (60:40 blend) for EDS Analysis

5.4 Summary of Results

Polymer concentration, thus solution viscosity, plays perhaps one of the most important roles in electrospinning. When the solution concentration is low, especially when it is near but below the overlap concentration (the overlap concentration of typical HA from synovial joints is about 0.135 w/v % [19]), the likelihood of intermolecular entanglement among the individual polymer molecular chains, although present, is relatively low. Under this condition, when the solvent is removed, particles are usually produced due to the fluctuations of concentration (in dilute solutions, the process turns into electrospraying). In the case of blended HA solutions, a fiber-and-bead morphology is found.

On the other hand, if the polymer concentration is too high, the electric field may not be able to overcome the high viscosity of the solution, and thus a continuous jet stream from the polymer solution is not obtained and fiber production fails. Therefore, one can argue that electrospinning is only successful in a small concentration range, over which the polymer solution has sufficient entanglement concentrations with weak resistance to form a polymer jet from the fluid droplet at the spinneret, i.e., the solution with a relatively weak surface tension. These requirements represent, to a certain extent, a contradiction, because a solution with a high degree of intermolecular entanglement is normally associated with high viscosity and high surface tension. Thus, electro-spinning is a process that can encompass only a limited range of solution viscosity and surface tension, especially when the applied electrical field strength is limited.

In this study, the solutions prepared from the 1 million and 680,000 molecular weight HA sample did not show a concentration range where the polymer jet stream could consistently maintain its stability. This indicates that there is no suitable concentration window for the HA solution to meet all of the requirements of producing nanofibers by electro-spinning (we have tested the concentration range from 0.5 wt% to 2 wt%). Despite this finding, the variation of concentration could affect the fiber formation capability. Although no nanofiber structure was formed at 2.0 wt%, electrospinning started to produce nanofiber structures at concentrations of 1 wt% HA-PEO. Although fibers were formed only at blended lower concentrations of HA-PEO due to perhaps an increase in intermolecular entanglement, there was an upper concentration limit (at least 30% PEO) for the blends below which electro-spinning could not be carried out. This has been explained earlier. In the chosen electro-spinning conditions, the applied electric field was 20 kV, which was close to the practical limit for operation (higher electric potentials usually produced sparks or shorted out the other equipment). At this potential (20 kV), the electrical field could not overcome the high viscosity and the surface tension of the HA blended solutions at concentrations higher than 1 wt% nor solutions containing less than 30% PEO. Thus, the optimal concentration range for the production of nanofibers by electrospinning of the chosen HA solution was at 1 wt% total polymer of HA and PEO, a very specific window. As the stability of the jet formation was poor even in the "optimal" concentration range (or the viscosity range), the electrospinning results from this solution were unsuccessful.

For HA, the solution viscosity often exceeds the operating viscosity range of the instrumentation for electrospinning. Thus, it was necessary to lower the solution viscosity while still maintaining a high level of polymer concentration. This is because at low polymer concentrations, an excess amount of solvent must be removed during the relatively short time period between the jet fluid stream leaving the spinneret and the fiber reaching the ground, as mentioned earlier in the discussion on solvent evaporation rate. With a relatively high amount of solvent, the electrospinning process, even if it is operational, favors particle formation. HA particles have a unique morphology. Therefore, a highly concentrated solution with a proper viscosity is essential for the successful fiber formation in the electro-spinning process. We find that the blending of polymers with solutions of NaCl can reduce the solution viscosity and thus place the solution in a viscosity range where successful electrospinning can take place.

6.0 CONCLUSIONS

In this study, the effects of various experimental parameters, including solution concentration, feeding rate of solution, and electric field on the electrospinning performance of hyaluronic acid (HA) were investigated. To improve the electrospinning process, several new schemes were tested, including the control of HA concentration and viscosity by making 1M NaCl with 2 wt% HA and 0.5M NaCl with 2 wt% HA. These schemes, however, were not sufficiently effective to overcome the high viscosity of HA solution at relatively low concentrations. Only with the blending of HA with PEO, were we able to produce nanosized HA-PEO fibers with uniform diameters in the range of 190 nm - 500 nm.

Overall, electrospinning HA was difficult in producing bead-free, continuous nanofibers, due to the physical properties of the polymer. The molecular weight of pure HA was high at one million. The unusually high viscosity and surface tension of HA were thought to be the key factors that hinder the electrospinning of HA solution. Additionally, the strong water retention ability of HA may have lead to fusion of nanofibers electrospun on the collector due to the insufficient evaporation of the solvents in electrospinning. The fabrication of HA into nanofibrous non-woven membranes from aqueous solution was successfully carried out only after the blending of the polymer with polyethylene oxide (PEO).

Rheological measurements showed that the viscosity changed significantly after the polyethylene oxide (PEO) and NaCl were added into the HA solution. The dilution and blending of NaCl and PEO, respectively, did result in an overall decrease in solution viscosity. However, even with the dilution, the HA-NaCl solution viscosity was still relatively high to produce nanofibers, as was the case with the pure HA solutions.

Scanning electron microscopy (SEM) results revealed that the blended HA:PEO solutions did fabricate nanofibers, with the presence of beaded structures and nanoparticles of HA with NaCl. The blending of the two polymers provided an even lower solution viscosity than the NaCl diluted solutions. A concentration of 1wt% HA-PEO total polymer formed the optimum conditions for the fabrication of HA-PEO nanofibers. The ratios of HA-PEO producing these nanofibers was at 70% HA/30% PEO and 60% HA/40% PEO.

7.0 FUTURE WORK

The HA-PEO nanofibers obtained from electrospinning contained beaded structures. The blended HA-PEO solutions had a significantly lower viscosity than both the HA and HA-NaCl solutions. This kind of nanostructure could have many potential clinical applications, especially in the field of artificial skin. Also, HA-PEO nanofibers at different HA/PEO compositions obtained by electrospinning are expected to have different cell proliferation properties and biodegradability, and have potential applications in wound dressings, such as gauzes, tissue engineering scaffolds, and drug delivery.

Specifically, tissue engineering requires the utilization of a porous biodegradable scaffold to replicate the natural ECM, which serves to organize cells in space, to provide them with environmental signals and to direct site-specific cellular regulation. HA-based hydrogels are ideal materials for soft-tissue engineering because of their unique rheological properties and complete biocompatibility. The successful fabrication of HA-PEO nanofibers may be used for cell attachment, growth, and migration.

These nanofiber-based scaffolds may then be characterized by a wide range of pore size distribution, high porosity, and high surface area-to-volume ratio, which are favorable parameters for cell attachment, growth, and proliferation. The structures could also provide effective mechanical properties suitable for soft tissue, such as skin or cartilage.

This study provides a basis for future optimization of electrospun nanofibrous scaffold for tissue-engineering applications. Applying this structure as a substitute for a specific tissue, such as cartilage, will be a focus of our future investigations. In addition, identifying the beaded structures on the beaded nanofibers will determine whether or not the HA can be electrospun into bead-free fibers. The production of bead-free fibers is the ultimate goal, possibly with the use of a different solvent, such as ethanol. Further investigation of EDS and other characterization tools will determine if we are forming blended fibers with beads.

Other areas of focus include, investigating different collector devices and methods to extract the electrospun nanofibers from the collector, varying the process parameters to obtain ultra-thin nanofibers, and utilizing other chemicals/solvents to dilute the solution for a possible reduction in the HA solution viscosity.

8.0 FELLOWSHIP EFFORTS

8.1 Fellowship Overview

Throughout my graduate career, I have had the pleasure of serving as a graduate fellow for the Recognizing Accelerated Math Potential in Underrepresented People (RAMP-UP) program. The RAMP-UP program is designed to promote rigorous mathematics using inquiry techniques and curriculum integration with other subjects. It aligns the teaching of math across all participating grade levels and will include university students working alongside teachers. Two partner high schools will work with NC State's College of Engineering and College of Education to offer an "introduction to engineering course" that will transfer to NC State. According to NC State Chancellor, Mary Anne Fox, "This program helps support the vital outreach missions of NC State's colleges of Engineering and Education, and strengthens our important work with Wake County schools to provide all students an exciting opportunity for life and work in this new century."

With the help of a $500,000 grant from the GE Foundation, North Carolina State University launched a joint effort with Wake County Public Schools to strengthen the math skills of middle and high school students and increase their opportunities to pursue careers like engineering. The grant was given the name Recognizing Accelerated Math Potential in Under-Represented People (RAMP-UP). By increasing the number of under-represented students in higher level math classes, the program seeks to raise interest and performance in math by the targeted groups, giving them the educational foundation necessary to study engineering or other math-based fields in college. The program is designed to promote rigorous mathematics taught using inquiry techniques and curriculum integration with other subjects. It will align the teaching of math across all participating grade levels and will include university students working alongside teachers. Two partner high schools will work with NC State's College of Engineering and College of Education to offer an "introduction to engineering course" that will transfer to NC State.

RAMP-UP Fellow Responsibilities

As a National Science Foundation GK-12 fellow from 2004-2006, I was given the opportunity to perform outreach with two schools in Wake County, NC. The program of which is funded by NSF is deemed RAMP-UP (Recognizing Accelerated Math Potential in Underrepresented People) and is a partnership between North Carolina State University, Shaw University, Wake County Schools, and the GE Foundation. With five NSF graduate students, 28 undergraduate students from NCSU and Shaw in STEM (Science, Technology, Engineering, or Mathematics) and education fields, and 3 program directors, the program was designed with the intent to provide supplemental instruction in math to reinforce math concepts and skills as a way to increase the number of students taking Algebra 1 by the eighth grade, Calculus by the 12th grade, and entering a university/college to pursue a STEM curriculum. Wake County has contributed efforts in allowing students in the program from NC State University to teach and tutor in math, science, and student electives at five elementary schools (Washington Elementary, A.B. Combs Elementary, Bugg Elementary, Fuller Elementary, and Dillard Elementary), two middle schools (Carnage Middle School and Centennial Middle School), and one high school (Southeast Raleigh Magnet High School).

In my first year as a graduate fellow during 2005-2006, I worked with two schools: Washington Elementary and Southeast Raleigh Magnet High School. At

Washington I had the opportunity to manage two undergraduate students (Mr. Clyde Gholston, sophomore in Mathematics at Shaw University and Ms. Heidi Bunn, a sophomore in Chemical Engineering at NC State University) providing tutoring and mentoring to students in grades 3-5. The goal of working with this nationally renowned elementary school was to find innovative ways to reinforce fundamental skills in math, including multiplication tables, division rules, basic counting, etc. From student developed activities to group and after-school tutoring sessions, our team was able to provide the students with creative techniques to help them better understand daily class lessons and past knowledge. For example, Mr. Gholston was able to start an after-school tutoring program that targeted African-American students who seemed noticeably confused with the math being taught in their classroom. With the addition of these after-school sessions, we noticed a significant increase in the performance and behavior in these students with one-on-one attention and a mentor who was able to cater his teaching style to the learning styles of his pupils. Research proves that most humans are visual learners and learn from hands-on activities. Surveying the teachers at all of our partner schools has verified this observation for our local students. However, at all levels of primary and secondary education, most teachers prepare and execute lessons through auditory means. This makes it difficult to improve student performance in the classroom when the classroom style of teaching is reverse from the majority of the student populations' learning style. The opportunity for improvement within RAMP-UP is to allow teachers to teach to a mass group of students, usually between 23-28, while having an outside representative reinforce taught topics through supplemental instruction, that is group tutoring, hands-on lessons, and one-on-one tutoring.

8.2.1 Washington Elementary

At Washington Elementary, supplemental hands-on instruction has proven to be a strong force in improving not only student performance but the actual behavior of the students who became confused in class and thus chose not to do the assigned work. For these students, utilizing the RAMP-UP fellows became a means to gain understanding by asking questions in a smaller more relaxed setting while also providing a role model figure. Role modeling and mentoring have proven to be two tools that have a direct impact on performance and self-confidence in education, family life, and even the workplace. For this program to provide this for students at an early age, approximately 7-12 years old, means that there is a strong chance for an upward trend in increased performance and self-confidence for retention in students after the program ends, which could potentially be at the end of the year or length of the grant (5 years).

8.2.2 Southeast Raleigh Magnet High School

Southeast Raleigh Magnet High School (SRMHS) is one of twelve high schools in Wake County and the only magnet program offered to students in grades 9-12 in the county. The school was designed as a leadership and technical enhancement tool in allowing students to better their technical skills and leadership qualities. During 2005-2006, my role at the school was to again manage three undergraduate students and also co-teach the introductory engineering course offered to students in the Engineering Career Focused Learning Community (CFLC). SRMHS currently has seven CFLC’s in Engineering Technologies, Biotechnology/Medicine, Information Technologies, Digital Arts, Global Connections, Education, and Law & Human Services.

With RAMP-UP, I have had the opportunity to work firsthand with students in the Engineering Technologies CFLC in two courses: Introduction to Engineering (Fall 2004) and Introduction to Engineering Design (Fall 2005 and Spring 2006). Career-focused learning communities provide *relevance* to the rigorous coursework available to students. Students may select a career focus and then benefit from elective courses, career experiences, and extra-curricular activities which center on that interest. The mission of Career Focused Learning Communities (CFLCs) is to create communities of learners who work together academically and through clubs and activities as they prepare for the ever-so-growing job market of our society. CFLC's, as deemed by the school's guidance center:

- *were created through a federal grant funding Smaller Learning Communities, ways for students to feel connected within large schools*
- *create communities of learners through their participation in CFLC courses, activities and affiliated clubs*
- *provide workforce, two-year college and four-year college pathways*
- *provide challenging course sequences and relevant, sequenced career experiences*
- *prepare students for top colleges and job opportunities*

The Engineering Career Focused Learning Community (CFLC) program is designed for highly motivated students interested in post secondary studies in the field of engineering. Through the program's directed course of study students will be introduced to the concepts of mathematics, science, and technology that are the foundations of an engineering curriculum. The Engineering CFLC exposes students to the different disciplines of engineering through a combination of courses, extracurricular experiences, guest speakers, and internship opportunities.

The cornerstone of the Engineering CFLC is the sequence of courses developed by a national program deemed Project Lead the Way (PLTW). PLTW partners in public schools, colleges and universities and the private sector. The project has developed a four year sequence of courses that when combined with college preparatory mathematics and science, introduces students to the scope, rigor and discipline of engineering and engineering technology. Students participating in PLTW courses are better prepared for college engineering programs. PLTW was launched at SRMHS in the Fall of 2005 as a recommendation from myself and the Project Director of RAMP-UP. We both sat on the administrative committee to work with Wake County in providing over $100,000 in computers, software, and other classroom resources to the successful implementation of this program. In the summer of 2005, I also became a PLTW certified instructor for the first of the 4 PLTW classes to be implemented at SRMHS, which is Introduction to Engineering Design (IED). After attending a 2-week training course at the University of South Florida in Tampa, FL, I was certified after receiving passing scores on written and performance based modeling examinations and effective teaching presentations. As the program continues to grow at SRMHS, which is a span of four years, I will continue to sit on this committee to ensure proper organization and execution of the classes as outlined by the national curriculum.

In addition to working with Engineering CFLC, I worked heavily with students in the math course Algebra 1 Part A and Algebra 1 Part B (Spring 2005). At the beginning and end of each semester working with the students at SRHMS, which is on a semester block academic schedule, we were able to both survey and monitor student progress in their coursework. In the subsequent sections, data obtained from student scores and comparison schools was obtained to measure the impact of RAMP-UP and the interaction of the fellows on the academic progress of the students.

8.3 Data & Results

The data obtained in this section measures the improvements in various areas found from the program's impact on SRHMS. Data from Washington Elementary was not obtained because of limited availability of scores and the low level of interaction at the school inside the classroom.

In 2004-2005, the data in Tables 6-8 reveals that in comparison with another class of Algebra I students at a similar Wake County school with comparable demographics, more students from SRMHS were enrolled in Algebra 1. Each of the percentages listed, gives a value of the number of students enrolled in Algebra 1 based on the student population taking math. This is a direct reflection on why programs such as RAMP-UP are needed. Although more students are enrolled in Algebra 1 in grades 9-12, few enroll in higher level math after Algebra 1 that will put them on the track to pursue a Science, Technology, Engineering, or Mathematics (STEM) field in the future.

Table 6: SRMHS students enrolled in Algebra 1 in 2004-2005 by count and overall percentage based on math-enrolled student population.

Took ALG 1 in 2004-05

	Counts		**Percents**	
	Yes	**No**	**Yes**	**No**
RAMP UP	101	58	64%	36%
Comparison	84	75	53%	47%

Table 7: SRMHS students enrolled in Algebra 1 in 2004-2005 broken up by grade-level

Took ALG 1 in 2004-05 by Grade

	Count			
	9th	10th	11th	12th
RAMP UP	66	27	8	0
Comparison	64	18	2	0

Table 8: Percentage of SRMHS students enrolled in Algebra 1 in 2004-2005 based on grade level.

	Percent			
	9th	10th	11th	12th
RAMP UP	73%	59%	67%	0%
Comparison	71%	39%	17%	0%

A. <u>Results: 9th Grade</u>

Data from the 9th grade students reveals that the number of Level 3 students at SRMHS, which are the students that the program strives to increase (Level 2 -> Level 3) is higher than at a comparison school, as shown in Figure 29.

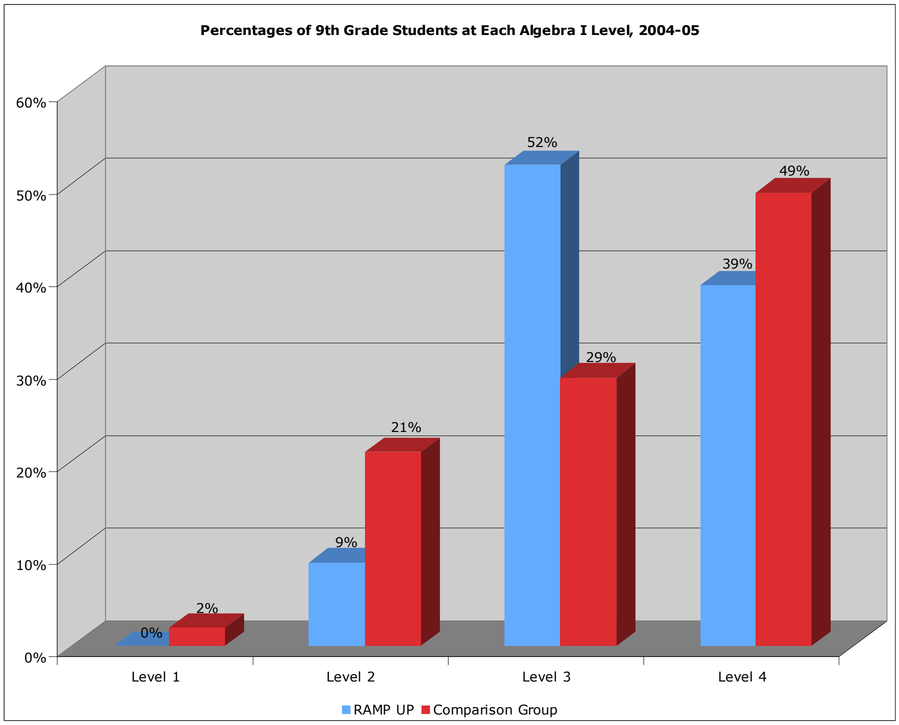

Figure 29: Graphical Representation of Algebra I Levels for SRMHS RAMP UP Students and Comparison Students

In terms of 9th grade EOC scores of RAMP-UP student versus a comparison group, Figure 30 shows that the number of students who took Algebra I in 8th grade (prior to entering high school) was lower at SRMHS RAMP-UP group. However, after taking

Algebra 1 in 9th grade at SRMHS, these students did not receive a drop in grade level mastery on the EOC. More comparison group students showed a decline in Algebra 1 scores, even though they took the course prior. In addition, the number of below grade level students in RAMP-UP on the 8th grade Math EOG and Algebra 1 EOC is around 9%. For comparison school students, there is a significant increase in the number of below grade level Algebra 1 students by approximately 300%. This data reveals that the RAMP-UP program is providing students with constant reinforcement and foundation skills needed to succeed in math. Those students not receiving this supplemental instruction show a decline in test scores, perhaps because they are not proficient in the course matter even though they have previously seen a majority of the course work. Thus, RAMP-UP students seem to show more mastery of the subject matter once being exposed to the program.

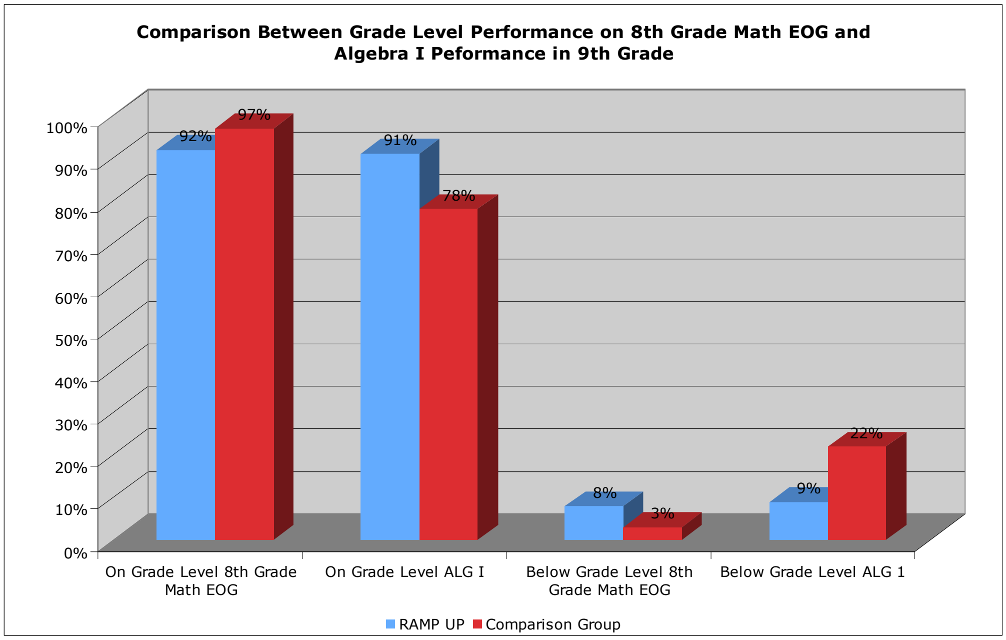

Figure 30: Graphical Representation of EOG Versus EOC Algebra 1 Scores

As previously stated, the RAMP-UP program targets minority students in the ethnicity categories of African-American, Pacific-Islander, Asian, and Hispanic while also putting focus on gender to include women. In breaking down ethnicity and gender data, Table 9 and Figure 31 reveal that the only group heavily influenced by the program was that of African-American males. As current research shows that only 45% of African-American males graduate from high school who are proficient in math up to Algebra 1, those African-American males in RAMP-UP are at Level 3 and Level 4 by percentages of 52% and 39% respectively. Other groups, as shown in the charts, showed no significant difference in participation in the program as correlated to student performance. In fact, some groups were so low in significance that these groups were not included in Table 10 but can be viewed in Figure 32.

Table 9: Algebra I Performance for 9th Graders by Group, Ethnicity, and Gender

Algebra I Results by Ethnicity and Gender

	Level 1	Level 2	Level 3	Level 4	N
RAMP UP Black Female	0%	5%	68%	26%	19
Comparison Group Black Female	0%	13%	50%	38%	16
RAMP UP Black Male	0%	9%	52%	39%	4
Comparison Group Black Male	0%	22%	39%	39%	23
RAMP UP White Female	0%	0%	50%	50%	6
Comparison Group White Female	0%	14%	0%	86%	7
RAMP UP White Male	0%	7%	36%	57%	14
Comparison Group White Male	7%	29%	7%	57%	14

while significant difference was observed for race and group, no interaction effect was observed between group and race or group and gender

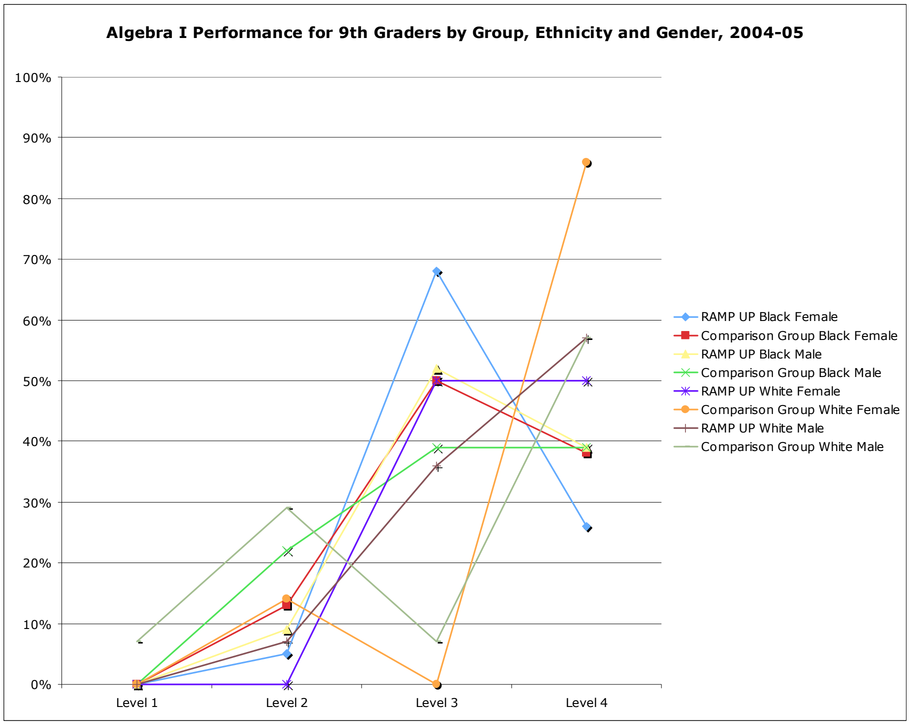

Figure 31: Graphical Representation of Algebra I Performance for 9th Graders by Group, Ethnicity, and Gender

Table 10: Miscellaneous Student Characteristics not included in Figure 32

Results for "Other" Students	Level 1	Level 2	Level 3	Level 4	N
RAMP UP Other Female	0%	100%	0%	0%	1
RAMP UP Other Male	0%	33%	33%	33%	3
Comparison Group Other Female	0%	100%	0%	0%	1
Comparison Group Other Male	0%	0%	0%	100%	2

B. Results: 10th grade

In regards to the percentage of 10th grade students at the Level 3 and Level 4 levels, there was no significant group difference, as shown in Figure 32. However, one can conclude that the majority of RAMP-UP students are in Levels 3 or above compared to the comparison group which has 30% of its students in Level 2. Neither group had students in Level 1, which is impressive for a 2nd-year program of this nature and demonstrates a shift in performance for the students.

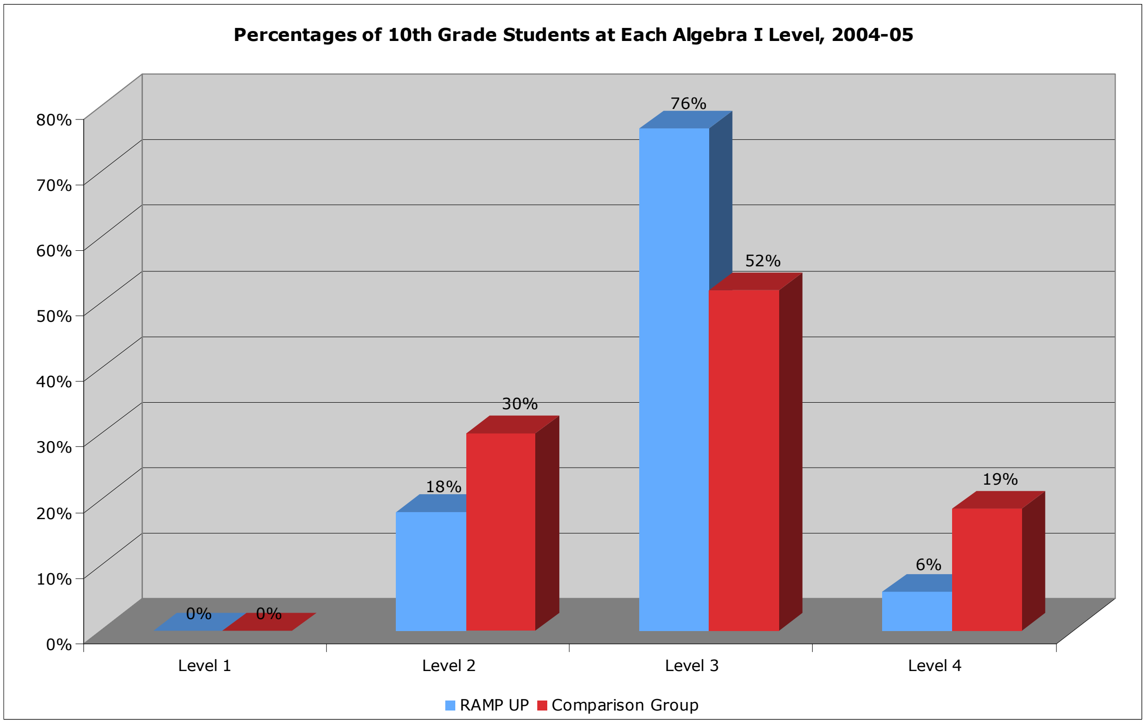

Figure 32: Graphical Representation of 2004-2005 Percentages of 10th Grade Students at Each Algebra 1 Level

Unlike the 9th grade data, in terms of ethnicity and gender, no significant data was measured to support the notion of increased performance in the minority groups. The reason for this stems from the fact that not enough white students were found in this sample to calculate meaningful differences between students of different races; data are provided for descriptive purposes only. No main effects were observed for group or gender, and no interactions, as seen in Figure 33.

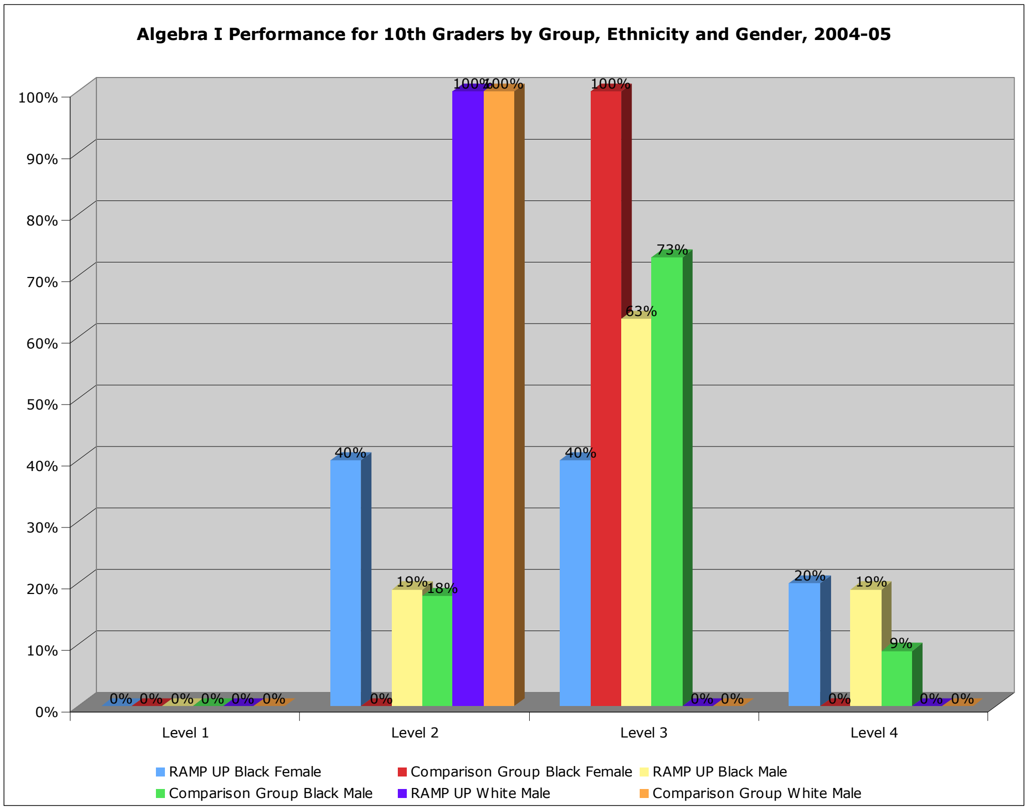

Figure 33: Graphical Representation of 2004-2005 Algebra 1 Performance for 10th Grade Students by Group, Ethnicity, and Gender

C. Teaching & Math Nights

Teaching opportunities at SRMHS were virtually endless from 2004-2006 in the Engineering CFLC to include the courses Introduction to Engineering Design, which I co-taught with the Project Director of RAMP-UP, and they included Introduction to

Engineering Design, (IED). IED is the first course offered at SRMHS in the Project Lead the Way (PLTW) course sequence. IED is a project based pre-engineering course which challenges students to solve problems and communicate ideas just like an engineer would. Students will use Inventor, which is Autodesk´s 3-D design package. Project Lead The Way Inc. (PLTW) is a national program forming partnerships among Public Schools, Higher Education Institutions and the Private Sector to increase the quantity and quality of engineers and engineering technologists graduating from our education system. PLTW has a course sequence of 6 courses with 2 more under development. Introduction to Engineering Design, Principles of Engineering and Digital Electronics are the introductory courses. Computer Integrated Manufacturing, Civil Engineering and Architecture, Engineering Design and Development are the subsequent courses in the PLTW curriculum.

Below are actual projects and designs that I assigned to the students for classwork using the Inventor software and PLTW curriculum.

D. Innovation Designs (Organizer Projects)

In this assignment, students were tasked as designers in a company that produces organizers for every purpose. Their specific task was to come up with a new design for a desktop organizer. The constraints were as follows:

Constraints:
The organizer:
    Must be no bigger than 20” x 20” x 10”
    Must use 3/16” material
    Must have at least 4 different parts (fasteners do not count as a part).
    Must have at least 1 moving part.
    Must be easily manufactured.

Figure 34 is an image of a cereal spool organizer designed by a student in the class, Matt Gossett.

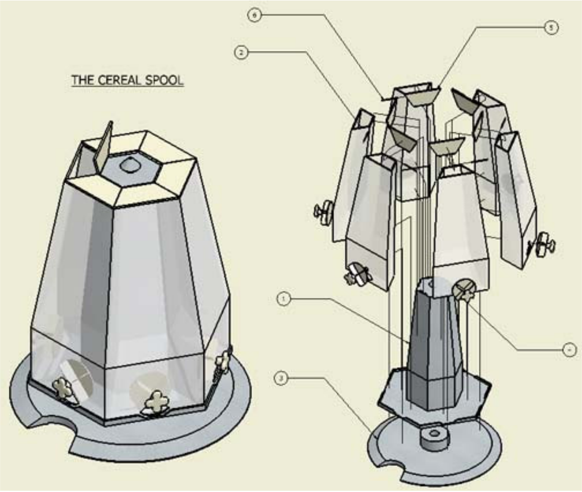

Figure 34: Matt Gossett's The Cereal Spool

E. Reverse Engineering Projects:

The purpose of this exercise was for the students to reverse engineer an object made up of several moveable parts. They were charged with finding a physical object and given access to an assortment of measurement tools. First, they had to determine which tool(s) to use and take all required measurements. Then, they used these measurements and draw a freehand sketch and place the dimensions on it. Once the sketch and measurements were taken and recorded, an assembly model and 2D working drawing from the model were generated using the Inventor software, as seen below in figures 35 and 36.

Figure 35: Brandon Shaw's Mechanical Pencil

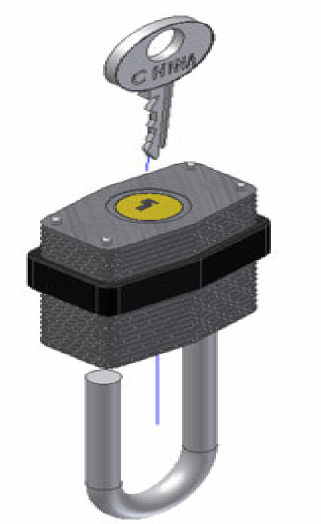

Figure 36: Jef Farmer's Lock

F. Rim Project

In this project, the students served as a member of a design team assigned to design a new set of alloy Wheels for a car. They first had to research the size and number of holes on a standard rim in order to produce a functional bolt pattern and countersink size. Using the geometric requirements below, the students developed designs as seen in Figure 37.

Geometric Requirements:
Rim diameter = 15" - 18"
Rim width = 8"
Bolt holes = .5" Diameter around a 7" Diameter circle.
Fillet radius for inside and outside corners = .5"
Interior design can be anything within good taste.

Figure 37: Rim Design by Kelly Justice

H. Miscellaneous Classroom Images:

Figure 38: Kyle (center) building, Sarah and Kevin (left to right) painting their puzzle cubes

Figure 39: Jaqueline Wright receiving assistance with classwork in modeling project.

Figure 40: Kindergarten students at Willow Springs Elementary receive instruction at September 2005 Math Night.

8.4 Conclusions

Overall, my participation in RAMP-UP has played a significant role in my professional and academic development with personal gains streaming from leadership enhancement, project management, strategic planning, and improved communication. From working with my undergraduate fellows to the students and teachers at SRMHS, I have gained a tremendous respect and admiration for teaching our youth as K-12 outreach is perhaps one of most hidden wonders in academia.

From my work with SRMHS from Fall 2004 to Spring 2006, the following conclusions can be noted as successes reflecting the time and effort put in at the school with the students and teachers:

- Fall 2005: 5 minority SRMHS students entered engineering schools across NC as a direct influence from the program:
    - Charnell Sutton: UNC-Chapel Hill, Biomedical Engineering
    - Shereese Banks: NC A&T State University, Computer Science
    - John Milner: NC A&T State University, Computer Science
    - Kenny Hayes: NC State University, Civil Engineering
    - Will Jones: UNC-Charlotte, Mechanical Engineering
- Algebra 1 Course:
    - 9th Grade:
        - RAMP-UP students outscore comparison school on Algebra 1 EOC test
        - RAMP-UP students outnumber Level 3 students in Algebra 1 as compared with comparison school
        - Increase in African-American males at Level 3 and Level 4 status
        - Statistics show RAM-UP students who took Algebra 1 by 8th grade show improved scoring in Algebra 1 when taken in high school. Comparison students who decline in test scores after Algebra 1 enrollment following middle school.
    - 10th Grade:
        - RAMP-UP students outscore comparison school on Algebra 1 EOC test
- Engineering CFLC
    - Introduced the Engineering Club as the extra-curricular club activity for the engineering CFLC
    - Co-taught the Introduction to Engineering course
    - Instrumental in the planning and execution of Project Lead the Way launching at SRMHS in Fall 2005
    - Certified Project Lead the Way instructor in Introduction to Engineering Design(Summer 2005- University of South Florida)

There are still a vast number of opportunities to improve upon at SRMHS along with the other 7 partner Wake County schools participating in RAMP-UP. My involvement has been an instrumental role in my graduate success and I hope to continue outreach work with my future employer to continue to strengthen the pipeline of success and close the drift between students and math on the K-12 level.

9.0 REFERENCES

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10.0 APPENDIX

10.0 APPENDIX

A. DOE of Experimental Trials

The table below includes all trials of the HA/NACl/PEO electrospinning where a viable Taylor cone and jet were formed. The categories include the sample number, concentration (in wt%), MW of the total solution, blending (i.e. HA alone, HA with NaCl, HA with PEO, or HA with PVA), electrospinning success (i.e. yes (nanofibers produced), no (no nanofibers produced)), and viscosity (zero shear rate viscosity obtained from rheometer measurements).

Sample	Concentration	MW	HA/NaCl/PEO	Electrospin?
1	2 wt%	3 million	HA	no fibers
2	2 wt%	3 million	HA/NaCl	no fibers
3	2 wt%	1 million	HA	no fibers
4	1.5 wt%	1 million	HA	no fibers
5	1.0 wt%	1 million	HA	no fibers
6	2 wt%	1 million	HA/NaCl	no fibers
7	1.5 wt%	1 million	HA/NaCl	no fibers
8	1.0 wt%	1 million	HA/NaCl	no fibers
9	0.5 wt%	1 million	HA/NaCl	no fibers
10	2 wt%	1 million	HA/NaCl	no fibers
11	1.0 wt% (70:30)	1 million HA; 900,000 PEO	HA/PEO	fibers w/beads
12	1.0 wt% (60:40)	1 million HA; 900,000 PEO	HA/PEO	no fibers
13	1.0 wt% (50:50)	1 million HA; 900,000 PEO	HA/PEO	fibers w/beads
14	1.0 wt% (70:30)	1 million HA; 126k PVA	HA/PVA	no fibers
15	1.0 wt% (60:40)	1 million HA; 126k PVA	HA/PVA	no fibers
16	1.0 wt% (50:50)	1 million HA; 126k PVA	HA/PVA	no fibers
17	1 wt%	600,000	HA	no fibers
18	1.5 wt%	600,000	HA	no fibers
19	2.0 wt%	600,000	HA	no fibers

B. Electrospinning Parameters

The following electrospinning parameters represent the conditions identified as the optimal conditions for the various concentrations of HA, although not all produced nanofibers.

HA Solutions
Feed Rate: 0.5 mL/min
Tip-to-Collector: 12cm
Voltage: 25kV

HA-NaCl (1M) Solutions
Feed Rate: 0.15 mL/min
Tip-to-Collector: 12cm
Voltage: 25kV

HA-NaCl (0.5M) Solutions
Feed Rate: 0.25 mL/min
Tip-to-Collector: 12cm
Voltage: 30kV

HA-PEO Solutions
Feed Rate: 0.115 mL/min
Tip-to-Collector: 12cm
Voltage: 15kV