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Design, Fabrication, and Testing of an Electrospinning Apparatus for the Deposition of PMMA Polymer for Biomedical Applications

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DESIGN, FABRICATION, AND TESTING OF AN ELECTROSPINNING APPARATUS FOR THE DEPOSITION OF PMMA POLYMER FOR FUTURE BIOMEDICAL APPLICATIONS

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Abstract

This paper describes the successful design and fabrication of a deposition system for synthesis and assembly of nanoscale and submicron sized fibers of poly(methylmethacrylate) (PMMA) polymer. To optimize the electrospinning deposition process, the distance between the needle and the electrically grounded substrate, the applied voltage, and the concentration of PMMA polymer in the solution were varied. PMMA fibers as small as 500 nanometers were observed using scanning electron microscopy (SEM). The chemical signature of PMMA was confirmed for best quality and retention of chemistry using Fourier Transformed Infrared spectroscopy (FT-IR). PMMA is a biocompatible polymer, and nanofibers of PMMA are key building blocks for scaffolds and other biomanufacturing applications, such as bioprinting for regenerative medicine and tissue engineering of synthetic organs (Mo, 2004).

1. Introduction

Nanomanufacturing is the science and engineering of advanced materials, their synthesis and manufacturing at submicron scales (National Nanotechnology Initiative, 2011). Metals, ceramics, polymers, semiconductors, and composites are the basic building blocks of bio-nanotechnology that are used in biomanufacturing. These materials vary not only in size but also in shape, such as fibers, rods, dots/particles, flakes, and other form factors. They are used to interface with biological and biomedical materials and components, including as organs, tissues, cells and macromolecules (e.g. DNA) (Schiffman, 2008). Thus there is a significant interest in synthesizing biocompatible materials, such as polymers, capable of interacting at submicron biointerfaces.

In the field of biomedicine today, biocompatibility in the research and development of advanced implants is a major problem (Balazsi, 2009). A challenge in engineering these implants is the ability to find materials and processing technology to create scaffolds that can mimic extracellular matrices, which promote implant-to-tissue compatibility (Balazsi, 2009). The substance ideally suited for scaffold creation is synthetic biomaterial, such as natural polymer, which can mimic the mechanical and biological properties of extracellular matrices. Thus biocompatible materials could be used to manufacture various biomedical constructs in order to realize advanced healthcare in the domain of nano-bio medicine and prosthetics.

Towards that goal, synthesizing uniform biocompatible materials with controlled dimension and chemistry is critical. Therefore an important research area is the design and development of an efficient and economically viable manufacturing process that will enable synthesis and deposit these materials at submicron scales and in a scaffold structure (Li, 2004). For example, in bio-printing, scaffolds are created from materials with various designs and chemistries that are then used as substrates, sometimes sacrificially, to allow the deposition of cells as basic building blocks for constructing the foundations of basic human tissues and organs. Nanofibers have the necessary morphological and dimensional requirements needed for scaffolds (Li, 2004), and they could also be used to embed and deliver other vital nanomaterials such as quantum dots for diagnostics and drugs for therapeutic applications (Li, 2004; Schiffman, 2008).

Based on this need, the research in this study had two objectives. The first was to design and fabricate an apparatus for reproducible synthesis of nanofibers manufactured from polymers deposited on a planar substrate using the electrospinning technique. Electrospinning, although not a new concept, has only been recently discovered as a new processing technique for fiber fabrication from organic polymers, especially on the micro to nano scale (Li, 2004). PMMA was selected as the polymer of choice from a wide range of biocompatible polymers, since PMMA can be used in biomedical applications, such as tissue engineering and wound dressing, and as supports or carriers for drug delivery (Mo, 2004; Wang 2010). In order to understand the deposition process, three critical parameters related to the deposition process were varied. The second objective was to validate the designed deposition apparatus by studying the size and chemistry of the deposited polymer for uniformity.

The research presented in this paper lays the foundation for the ultimate goal of scaffold creation for advanced biomedical manufacturing of implants, prosthetics, and functionalized delivery devices. The following text describes the deposition apparatus details, experimental process techniques, and analytical tools along with the deposition process mechanism. Results and discussion of conclusions are presented, along with suggestions for future research directions.

2. Materials and Methods

2.1 Design Criteria

Electrospinning is a drawing process that applies an external electric field to the charged jet of polymer molecules in a solution in order to segregate and deposit polymer fibers in solid form. Figure 1 shows the basic configuration of the electrospinning apparatus, which includes a polymer-injecting anode (syringe and needle), an injection pump, a solid fiber collection substrate cathode, a high voltage power supply (in the 0-30 kV range), and a polymer solution (polysol).

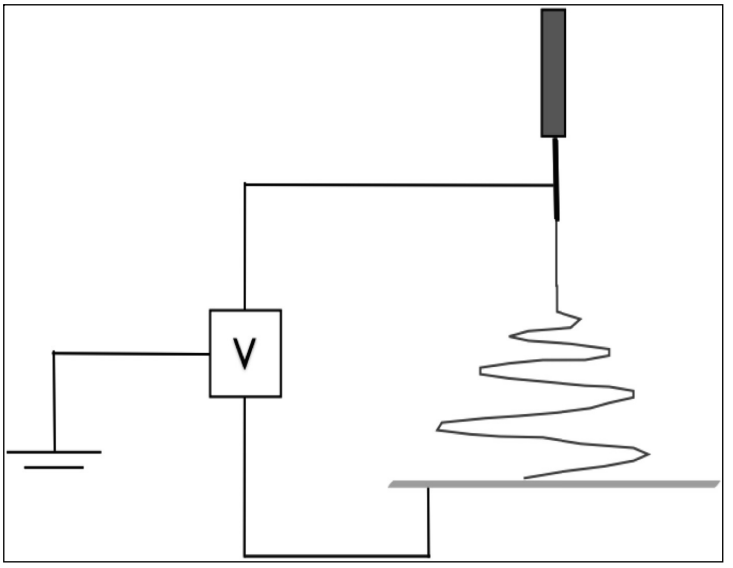


Figure 1. Illustration depicting basic equipment and design characteristics of an electrospinning deposition system.

The electrospinning process can be categorized into three stages: (A) formation of a stable jet of polymer molecules, (B) formation of a "whipping" unstable jet, and (C) formation of nanofibers through elongation, evaporation, and finally deposition (Figure 2) (Electrospinning: Map, 2011). During stage (A), both syringe pump and power supply are started and voltage is increased to around 5 kV (below the threshold voltage for polysol ejection). The voltage charges the drop forming at the tip of the needle, and the induced charges are evenly distributed. Electrostatic forces distort the drop into a specialized cone on the tip of the needle, called a Taylor Cone (Taylor, 1964). The geometry of the cone is governed by the ratio of solution surface tension to the forces of electrostatic repulsion and **E** field strength (Ramakrishna, 2005; Taylor, 1964). Also during stage (A), as the voltage is increased beyond the threshold voltage (~6kV), the strength of **E** field and electrostatic repulsions in the polysol increase and overcome the opposing force and the solution's surface tension, finally resulting in the forced ejection of a stable liquid jet from the needle (Ramakrishna, 2005). During stage (B), as the voltage is increased beyond the threshold voltage (>6kV) to near 11 kV, the stable jet destabilizes (Ramakrishna, 2005). This region is known as 'the region of jet instability,' and the bending instability results in formation of a "whipping jet." As shown in Figure 2, stage (B) is the boundary between the stable and whipping jet (Rutledge, 2001).

During stage (C), nanofibers are formed through the elongation, evaporation, and deposition of the whipping jet. Finally, the fibers are attracted toward the grounded substrate due to the attraction of opposite charges and are deposited onto the substrate (Ramakrishna, 2005). Although much of the solvent will evaporate as the jet whips through the air, some will evaporate upon deposition.

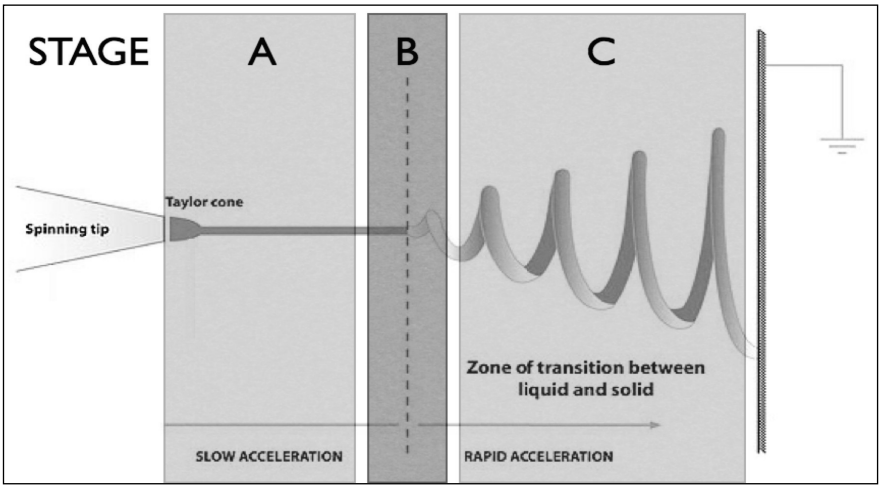


Figure 2. Illustration depicting the movement of the polymer solution fluid jet as a stage-by-stage process during electrospinning (Electrospinning: Map, 2011).

2.2 Fabrication of Apparatus

Using these process design criteria, the following apparatus was designed, fabricated, assembled, and tested. Figure 3 shows the final set up, which evolved over three generations during the process of designing and testing over one year. The apparatus included:

(1) a syringe pump (CHEMYX Fusion 100 Classic Syringe Pump; two syringe capacity – 0.5 µl to 60 ml) that was used to inject the polysol at a prescribed feed rate (Ramakrishna, 2005);

(2) a syringe (McMaster-Carr – 5cc) with a highly electrically charged metal needle (McMaster-Carr – 0.5 mm orifice diameter), which was used to contain and inject the polysol and was mounted on the described syringe pump;

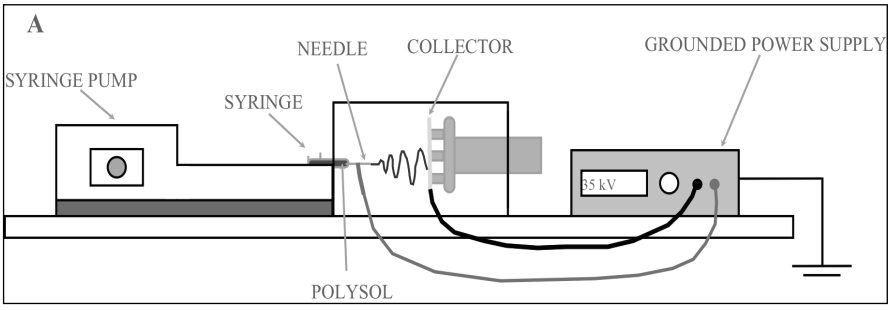
(3) an electrically conducting and grounded hardened aluminum substrate (McMaster Carr - .001 in diameter) as a collector material;

(4) a 0-30kV voltage supply fabricated by the author (power supplied by purchased Ultravolt A Series +30kVdc, 4W) that uses a spring clip to supply voltage to the needle.

One challenge was the design and fabrication of a high voltage supply capable of supplying at least 15-30 kV. To maintain economic viability, a high voltage supply was fabricated on the bench top instead of purchasing an expensive unit. The circuit and component parts were first designed, then assembled, and finally tested for reliability. The high voltage supply proved to be reliable and was successfully used to carry out this research.

In this arrangement of the apparatus, when high voltage is applied to the system, the drop of polysol at the tip of the needle will become electrically charged and the charge will spread across the surface of the drop. As a result, the polymer molecules will experience two types of electrostatic forces: the electrostatic repulsion between the induced surface charges and the Coulombic force exerted by the applied **E** field. Due to these electrostatic forces the polysol drop will morph into the Taylor cone (Ramakrishna, 2005). Once the strength of the **E** field has increased beyond the threshold voltage (>6kV), that force will overcome the surface tension of the polymer solution, forcing the ejection of a polysol jet from the needle tip and depositing onto the substrate due to electrostatic attraction, resulting in the formation of macro, micro, and most importantly, nano sized fibers.

As mentioned earlier, one potential process application of this proposed research (for the ultimate goal of biomanufacturing tissues and organs) includes the 3-dimensional structural patterning of the substrate material in order to create the highly mesh-like structure of a scaffold through fiber deposition. But first, the deposition process and contributing processing parameters affecting fiber morphology and dimension had to be understood. To study this further, the following experiments were executed.



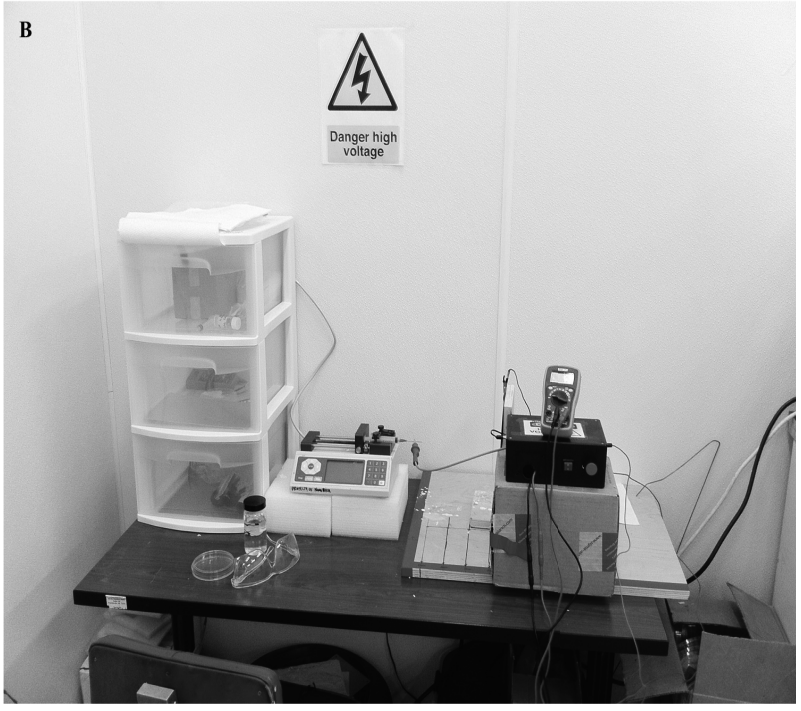


Figure 3. (A) Illustration depicting the design schematic of the electrospinning deposition system, pre-fabrication. (B) Photograph of the newly fabricated electrospinning deposition system at NanoMech, Inc..

2.3 Materials

Poly(methylmethacrylate) (PMMA) (Sigma-Aldrich, M_w = 120,000), was selected as the polymer for potential future biomedical research and applications (Wang, 2010). In order to inject the polymer as a solution, it first had to be solvated. N,N-dimethylformamide (DMF) (Sigma-Aldrich) was recommended by Ramakrishna (2005) as the solvent that was chemically able to solvate the PMMA polymer. Different concentrations of the PMMA/DMF polysol were created for this study. One significant challenge that the author overcame through the course of this experimentation was the correct preparation method for varying concentrations of polysol. Different concentrations of polysol were needed for the study. Both PMMA polymer and DMF solvent were not completely pure; therefore different stoichiometry calculations had to be computed. A polysol for a specific concentration, using the values for the stoichiometrically predetermined amounts of PMMA to DMF, was created by first adding the PMMA to the DMF. It is important to note that a homogeneous solution of polymer is required for electrospinning due to the ability of a solution to carry a uniform electric charge across its surface versus the inconsistent charge a mixture would carry (Ramakrishna, 2005). Through experimentation, it was discovered that the best polysol preparation method to insure the complete solvation of the PMMA in the mixture required the mixture to be stirred for fifteen minutes and then left for at least eight hours in a dark cool place.

2.4 Process Parameters

In order to fully understand the behavior of the newly fabricated apparatus and to examine the process control over fiber diameter that is necessary for biomedical applications, key processing parameters were varied. These parameters included: (1) distance between the polymer injection tip and the collection substrate (cm); (2) the applied voltage (kV); and (3) the concentration of PMMA in the PMMA/DMF solution (% (PMMA) by weight). Table 1 shows the process parameters (and their variation range) experimentally studied.

2.5 Characterization and Analytical Techniques

To explore the effect of these key parameters, the morphology, size and chemistry of the deposited polymeric material were studied. Optical analysis along with scanning electron microscopy (SEM; Nova NanoSEM, FEI) were used for morphological and micro/nano dimensional analysis. Fourier Transformed Infrared spectroscopy (FT-IR; NETZSCH) was applied to analyze polymer solution before deposition and deposited fiber web samples.

2.6 Challenges

The test apparatus was fabricated with an iterated approach in three 'generations' as previously described. However, the fabrication and assembly of the components was a major challenge, and it took six months for iterative optimization to realize an apparatus that was capable of being used for the necessary research. Key challenges were confronted due to the complexity of optimization involved in fabricating a apparatus that could conduct reproducible experiments and produce reliable data for the study. These challenges included: design of the electrospinning apparatus components, materials, fabrication of individual components, assembly of the apparatus and its optimization through three generations, reliability testing, experimental matrix design, and ultimately testing of the technique using the final system.

Table 1. Conditions and effects of various parameters on resulting morphologies.

Test	Collection Distance (cm)	Voltage (kV)	Polymer Concentration (wt%)	Morphology
1	10	10.68	5	Polymer beads
2	10	18.23	5	Smaller polymer beads
3	15	10.45	30	Macro to micron sized fibers
4	**15**	**15.03**	**20**	**Nanofibers**
5	15	20.22	30	Micron sized fibers
6	15	15.42	40	Macro sized fibers
7	20	15.35	30	Micron sized Fibers

3. Results

Voltage (V) supplied, distance (D) between the syringe tip and the grounded substrate, and the concentration (C) of PMMA polymer solution were each varied to identify the primary parameters required to deposit nanofibers during fabrication of the newly designed apparatus. It became apparent that this method of experimentation was unique in its ground-up methodological approach to fabrication of a "lab-top" electrospinning apparatus. In various experiments (see Table 1 for the most relevant cross-section of those experiments), V and D had a major effect on the morphology and dimension of the deposited polymer mesh, although C had a key secondary effect. This was most likely due to the viscosity of the injected PMMA polysol. A more thorough discussion of the results is presented later in this paper.

3.1 Morphological and Dimensional Analysis: SEM and visual observation

Figure 4(A) shows the image of the deposited web (Test 1 and 2 from Table 1). Smaller values of D and C, irrespective of V, yielded a thin polymer deposit or glob. Figure 5(A) shows the SEM micrographs of the deposits. At a higher magnification, it is observed that the deposits contained meso and micro scale beads of polymer without a mesh-like fiber matrix structure.

Figure 4(B) shows an image of the dense collection of fine PMMA fiber web deposited under the conditions of Test 4 (Table 1). Analysis of this mesh using SEM, seen in Figure 5(B), shows the presence of fibers with a diameter of 500 nm or less dispersed densely and uniformly in the fiber matrix. This fiber size is ideal for entrapping nanoparticles; i.e. drug-functionalized hydroxyapatite (HA), a natural ceramic that contributes to the strength and stability of human bones, which can improve the biocompatibility of scaffolds for biomedical implants such as advanced prosthetics (Balazsi 2009). Figure 4(C) shows the image of the web deposited under Test 7 (Table 1), where a uniform and dense mesh of fiber was observed. High magnification SEM, Figure 5(C), showed PMMA fibers of dimensions tens of microns in size. The comparison among this data demonstrates that there is a close dependency among the V, D and C parameters.

Figure 6 shows the SEM micrographs for the four conditions. To study this dependency further, D was held constant (at 15 cm) and the V and C parameters were varied (Tests 3 through 6 shown respectively in Figures 6(A) through 6(D)). Between Tests 3 and 5, D and C were held constant and V was varied. It was observed that a lower V yielded micro to macro sized PMMA fibers, whereas a higher V yielded only micro sized fibers. Between Tests 4 and 6, D and V were held constant and C was varied. It was observed that lower C yielded micro sized fibers, whereas a higher C yielded macro sized fibers. These experimental conditions were tested for repeatability and showed good reproducibility to deposit nano and micro sized PMMA fibers.

3.2 Chemical Analysis: FT-IR Spectroscopy

Chemical analysis and confirmation of the retention of the chemistry of PMMA polymer, before and after the electrospinning deposition process, is important to ensure that the electrostatic force interactions do not chemically dissociate the polymer chains or alter the polymer molecules. Assurance of the chemical structure is key for ensuring the retention of the biocompatibility property of PMMA polymer, which is the key for future biomedical applications (Mo, 2004; Wang, 2010).

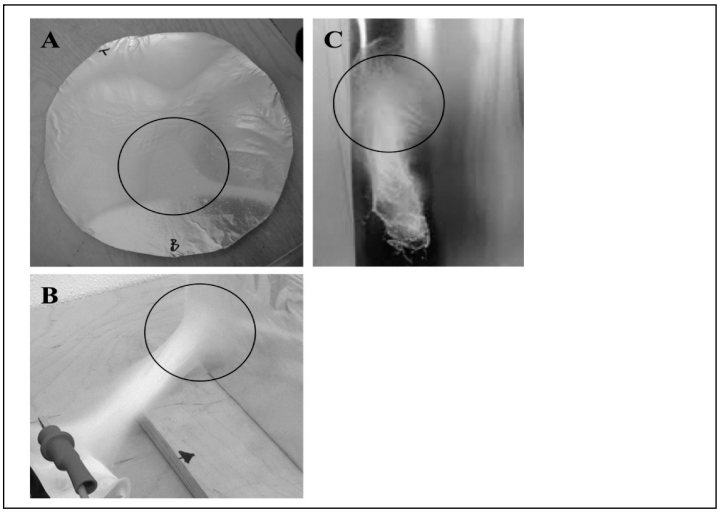


Figure 4. (A) Photograph of Test 1 and 2 from Table-1 (deposited polymer circled). (B) Photograph of Test 4 from Table-1 (depositd PMMA polymer nanofiber web matrix circled). (C) Photograph of Test 7 from Table-1 (deposited PMMA polymer micro fiber web matrix circled).

Figure 7(A) shows the FT-IR spectrum for the PMMA/DMF polysol before deposition, and Figure 7(B) for Test 4 during the nanofiber deposition. In order to prepare the samples for FT-IR analysis, they were first diluted in acetone before being added to the KBr disk for analysis. Both FT-IR spectral graphs match one-to-one, showing the retention of the PMMA polymer chemistry as a polysol before deposition and as a fiber mesh after deposition. Also, most of the molecular absorption peaks for the PMMA polysol and fiber FT-IR spectra match the standard data for PMMA (Kinusaga, 2011). The FT-IR spectra, for both the polysol and the deposited fiber, indicate the details of functional groups present in PMMA. A sharp intense peak at 1731 cm^{-1} is due to the presence of the ester carbonyl group stretching vibration. The broad peak, ranging from 1260-1000 cm^{-1}, can be explained by the C-O (ester bond) stretching vibration. The broad band from 950-650 cm^{-1} is due to the bending of the C-H bond. The broad peak ranging from 3100-2900 cm^{-1} is due to the presence of stretching vibration. The band at 3000 cm^{-1} is assigned to CH_3 stretch vibration. As stated above, these signature peaks for PMMA remained unaltered between the polysol sample and the fiber web (Test 4). Although initially unknown and discovered empirically through this research, the retention of PMMA chemistry does indeed occur after electrospinning.

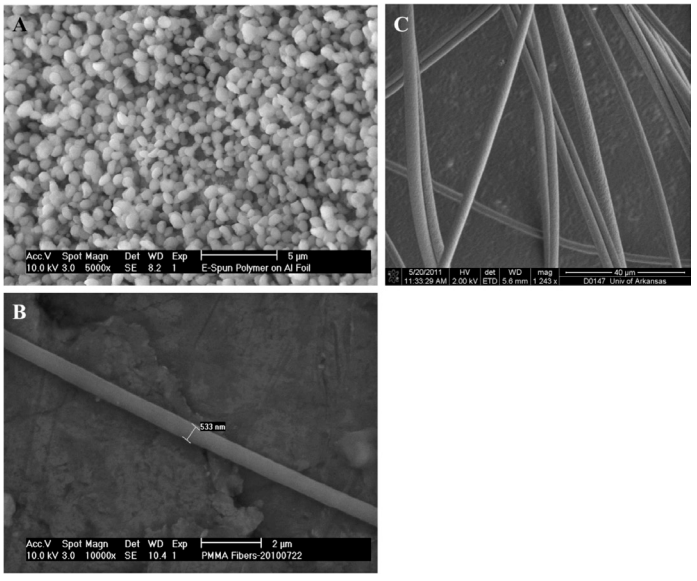


Figure 5. (A) SEM micrograph of Test 1 and 2 from Table-1. (B) SEM micrograph of Test 4 from Table-1 (with fiber diameter measured). (C) SEM micrograph of Test 7 from Table-1.

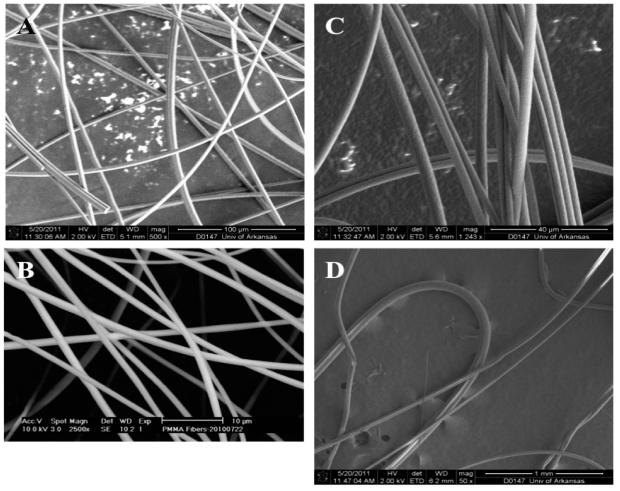


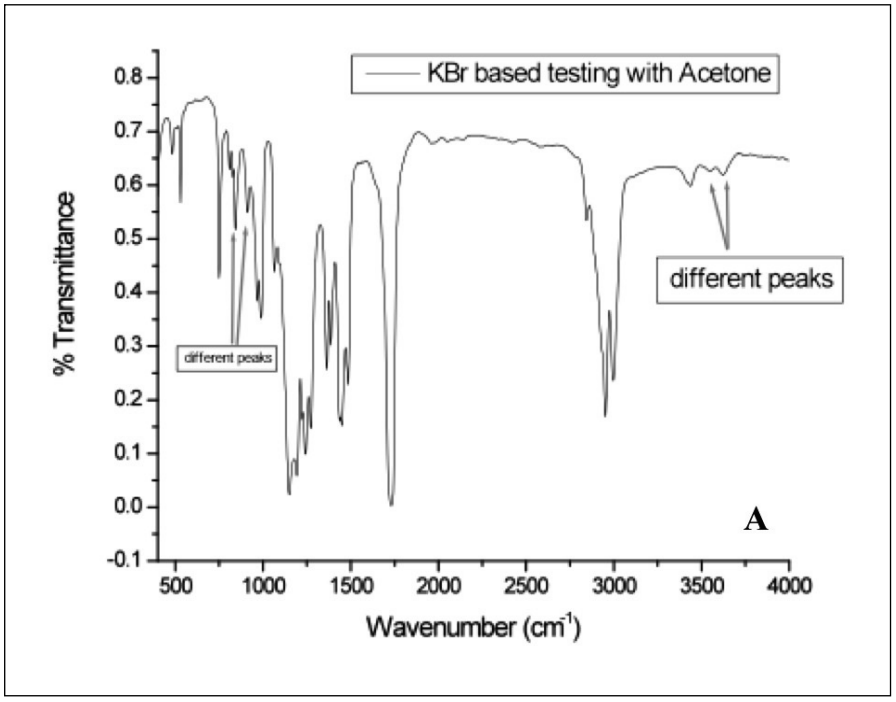
Figure 6. (A) SEM micrograph of Test 3 from Table-1. (B) SEM micrograph of Test 4 from Table 1. (C) SEM micrograph of Test 5 from Table-1. (D) SEM micrograph of Test 6 from Table-1.

4. Discussion

Two striking observations from this work are: a) the synthesis of 500 nm or smaller sized PMMA fibers under specific D, V and C conditions, and b) a close dependency of D and V on C in the outcome of the process. It is probable that the synthesis of nanofibers is due to whipping instability of the fluid jet under an applied electric bias of 15 kV (Li, 2004). The whipping instability is mainly caused by the electrostatic interactions between the external electrical field and the surface charges on the jet (Rutledge, 2001). It has also been occasionally observed that a single jet can "splay" into one or more jets, which others have shown yielded a web of PMMA nanofiber matrix (Li, 2004; Rutledge, 2001). Also related to the synthesis of 500 nm or smaller sized PMMA fibers are the effects of D, V and C on the polymer stretching process (region of "whipping" instability), as shown in Figure 2 during Stage (C). This includes elongation, evaporation, and deposition. During elongation, the polymer chains disentangle from macro to sub-micron sizes due to electrostatic attraction of individual molecules in the charged polysol (Ramakrishna, 2005). In the evaporation process, the solvent evaporates from the polymer, drying the fibers (Ramakrishna, 2005). Finally, the oppositely charged polymer fibers are attracted to the grounded collection substrate (evaporation also occurs after deposition) (Li, 2004). In this study, it appears that the effect of D, V, and C on the polymer stretching process occurred in Test 4, enabling the formation of nanoscale fibers. However in Tests 1-7, the effect of varying D, V, and C on the polymer stretching process allowed for the formation of PMMA beads and micro/macro sized fibers.

These observed effects of varying D, V, and C on the polymer stretching process may be explained as follows. First, varying D is known to directly influence both flight time of the jet and the **E** field strength (Ramakrishna, 2005). The flight time of the jet affects the dryness of the deposited fibers and the **E** field strength affects the resultant fiber diameter. If D is reduced, the flight time of the jet is reduced. The jet will travel a shorter distance, **E** field strength will increase, and jet acceleration will increase (Ramakrishna, 2005). This results in the deposition of polymer beads and increasingly wet deposits.

Secondly, varying V is known to affect the drawing acceleration of the polysol from the needle tip and subsequently, as described above, the size of the deposited fiber (Li, 2004). At a higher V, the polysol is pulled faster from the needle tip, leading to a bigger stretching effect with a consequent reduction in fiber size (Ramakrishna, 2005). A higher V will also encourage faster evaporation, yielding drier fibers upon deposition, although higher with a low C will increase the tendency for beads to form and be deposited. A lower V will decrease jet acceleration, increasing the



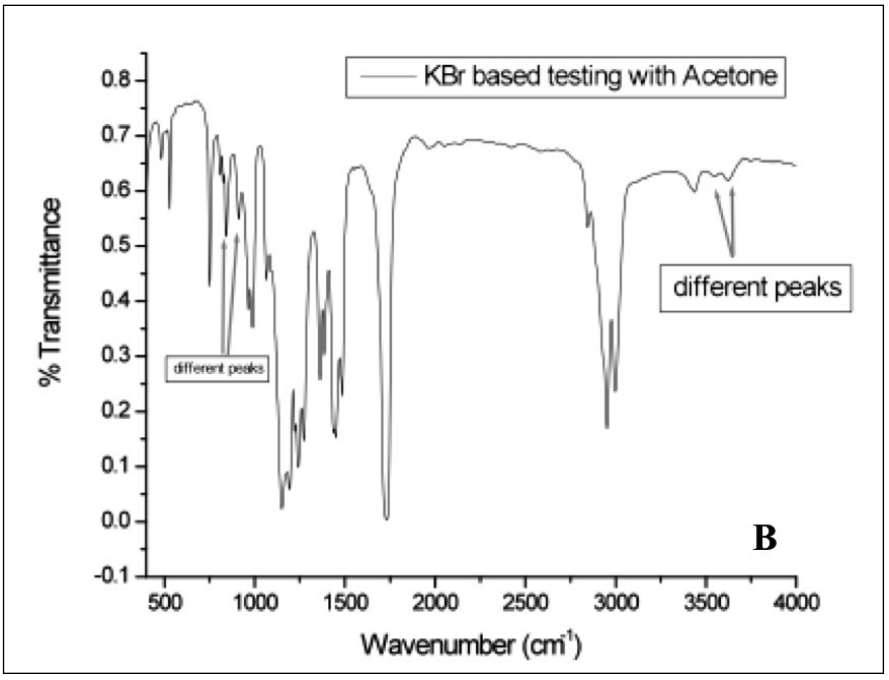


Figure 7. (A) FTIR spectrum for PMMA/DMF polymer solution sample. (B) FTIR spectrum for deposited PMMA fiber sample (both from Test 4 from Table-1).

jet flight time and may produce finer fibers (Ramakrishna, 2005).

Finally, the viscosity of a polysol depends directly on C, therefore varying C of the polysol directly changes the viscosity of the polysol and the effect viscosity has on fiber dimension (Li, 2004). When the viscosity of the polysol is too low, polymer beads form instead of fibers. However, when the viscosity is too high, polymer fibers form beads within their structures (Ramakrishna, 2004). Also, the retention of the PMMA polymer chemistry, determined by the FT-IR analysis, speaks to the potential of using PMMA fiber as a biocompatible scaffold-building material for biologically functionalized nanoparticle delivery and biocompatible prosthetic implants.

5. Conclusions and Future Work

In summary, the success of this research is apparent in two outcomes: fabrication and demonstration of an electrospinning system, and use of this system to deposit PMMA polymer in various form factors such as nanofibers, micro fibers and micro beads. The role of key process parameters and their interparametric dependency in the feasibility phase were also studied. The parameters included the distance between the polymer injection tip and the collection substrate, the voltage supplied, and the concentration of PMMA in the PMMA/DMF solution. This research on deposition of nanofibers was motivated by their potential use as carrier scaffolds for nanostructures such as drug molecules, HA nanoparticles, quantum dots, extracellular matrix mimicking scaffolds for tissue engineering advanced biocompatible implants and prosthetics. Future research will involve the analytical study of the process as well as applications of the established system to deposit chemically functionalized nanofibers on a 3D patterned scaffold mold used for printing, and ultimately for bio-printing applications.

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Mentor Comments: Professor Ajay Malshe and industry mentor Dr. Wenping Jiang served as joint advisors for Harsha Malshe's research, in part because the work involved initiatives of the company NanoMech, where Dr. Jiang serves as Chief Engineer, and in part because Dr. Malshe is Harsha's father (and every effort was made to avoid conflict of interest). Together they write:

Nano-bio materials and manufacturing is one of the fastest growing subjects to address the needs of growing and aging populations of the US and the world. Reduction of the cost of health care and providing better health care through innovations in materials and processes is at the heart of the progress. Harsha's project is at the intersection of these needs where NanoMech is interested in establishing an efficient and low cost manufacturing fiber coating process for producing basic nano building blocks, like nanofibers, and using them for drug and other chemical delivery.

More than a year ago, Harsha approached Dr. Wenping at NanoMech due to his interest in the biomedical field for a materials related project. The discussion between him and the co-advisors derived the direction to undertake this research. He contributed partly in conceiving this original project. His key contributions are in hands-on design, development and fabrication of electrospinning apparatus, and planning and execution of experiments interdependently working with advisors. This is a mainstream project for the company and he reported directly to the co-advisors. He also spent significant time in a detailed literature review, developing a fundamental understanding in physics and chemistry of process and materials, respectively. He performed analysis of the measurements and developed understanding of the process mechanisms in discussion with co-advisors. Last but not least, this is his first manuscript, and his enthusiasm, dedication, and writing impressed us. The work that he has presented is of good scientific importance and has laid the path for future advanced research, which we anticipate he will continue. Harsha's work is part of research and development for future nano materials with advanced health care applications.