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Appendix 2

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ELECTROSPRAYING AND ELECTROSPINNING OF POLYMERS FOR BIOMEDICAL APPLICATIONS. POLY(LACTIC-CO-GLYCOLIC ACID) AND POLY(ETHYLENE-CO-VINYLAACETATE)

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ABSTRACT

Significant opportunities exist for the processing of polymers (homopolymers and blends) using electric fields. Specific attention is given here to electrospinning, but we note that electroaerosol formation and field-modulated film casting represent additional processing options. Of particular interest is the ability to generate polymer fibers of sub-micron dimensions using electrospinning, down to about 0.05 microns (50 nm), a size range that has been traditionally difficult to access. In our work, poly(lactic-co-glycolic acid), PLA/PGA, poly(lactic acid) PLA, and poly(ethylene-co-vinylacetate) (PEVA) have been deposited from solutions in methylene chloride or chloroform by electrospaying or electrospinning to afford morphologically tailored materials for tissue engineering and related applications. Low solution concentrations tend to favor electrostatic spraying ('electro-aerosolization') while higher concentrations lead to spinning on fibrous mats. Preliminary observations of muscle cell growth on PLA electrospun mats are reported.

KEY WORDS: Biomaterials, applications-medical, fibers

1. INTRODUCTION

One of the challenges to the field of tissue engineering/biomaterials is the design of ideal scaffolds/synthetic matrices that mimic the structure (mechanical aspects) and biological functions of natural extracellular matrix (ECM). The main purpose of the scaffold is mechanical support to allow for tissue regeneration while at the same time guiding (cell-matrix and cell-cell interactions; morphology guides structure of engineered tissue) cell differentiation and function (1). Some of the ideal scaffold requirements include biocompatibility, not inducing an undesirable host response and completely biodegradable while remaining non-toxic during replacement by cellular ECM components. Another challenge for the scaffolding is to be reproducibly produced in a variety of shapes and compositions (chemically and morphologically) with minimal time and cost.

Electrostatic spraying (electrospaying) and electrostatic spinning (electrospinning) represent attractive approaches for polymer biomaterials processing with the opportunity for control over morphology, porosity, and composition using simple equipment. In electrostatic spraying, charged droplets are generated in a several kV dc field and delivered to a grounded target. Microspheres of steroids for controlled drug delivery may be produced in this manner (2). In electrospinning, polymer solutions or melts are deposited as fibrous mats rather than droplets, with advantage taken of chain entanglements in melts or at sufficiently high polymer concentrations in solution to produce continuous fibers. Of particular interest is the ability to generate polymer fibers of sub-micron dimensions, down to about 0.05 microns (50 nm), a size range that has been heretofore difficult to access yet one which is great interest for tissue engineering. To date, examples of electrospun polymers include acrylics, poly(hydroxybutyrate-co-valerate), poly(ethyleneoxide), and DNA (3).

As early as 1977, Martin and Cockshott (4) reported on the use of electrospinning for biomaterials applications with the production of fibrillar mats for wound dressings and vascular prosthetics. They noted that polymer concentration required for spinning depended upon the molecular weight of the polymer, with lower molecular weights requiring higher concentrations. Only recently has interest in electrospinning for polymer biomaterial processing been revived (5,6). We have initiated an effort to explore the scope of electrospinning and electrospinning in biomaterials processing, and report here on the fabrication of sheets and tubes of poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLA/PGA), and poly(ethylene-co-vinylacetate) (PEVA). PLA and PGA have enjoyed widespread use as a biodegradable scaffold in tissue engineering (1), and PEVA is a biocompatible (but non-biodegradable) polymer exploited as a matrix for the controlled delivery of both small molecules and macromolecules (7).

2. OVERVIEW OF ELECTROSTATIC SPRAYING AND SPINNING

In electrostatic spraying, or more simply electrospinning, charged droplets are generated at the tip of a metal needle (or pipette with a wire immersed in the liquid) with a several kV dc field, and are subsequently delivered to a grounded target. The droplets are derived by charging a liquid typically to 5-20kV, which leads to charge injection into the liquid from the electrode. The sign of the injected charge depends upon the polarity of the electrode; a negative electrode produces a negatively charged liquid. The charged liquid is attracted to an electrode of opposite polarity some distance away, forming a so-called Taylor cone at the needle tip. Droplets are formed when electrostatic repulsions within the liquid exceed its surface tension. If the liquid is relatively volatile, evaporation leads to shrinkage of the droplets and an increase in excess charge density, leading to break-up into smaller droplets. This can happen many times prior to reaching the target, thereby affording very small droplets.

In electrospinning, polymer solutions or melts are deposited as fibrous mats rather than droplets, with advantage taken of chain entanglements in melts or at sufficiently high polymer concentrations in solution to produce continuous fibers. Electrospinning is mechanistically similar to electrospinning, a key difference being that chain entanglements yield a fiber from the Taylor cone. Moreover, rather than break-up into small droplets, entanglements lead to splaying of fibers into thinner ones, and herein is a particularly attractive aspect of electrospinning. The basic elements of a laboratory electrospinning or electrospinning system are simply a high voltage supply, collector (ground) electrode/mold, source electrode, and a solution or melt to be sprayed or spun. The sample is confined in any material formed into a nozzle with various tip bore diameters (such as a disposable pipette tip), with a very thin source electrode immersed in it. The collector can be a flat plate or wire mesh, or in more sophisticated modifications can be a rotating metal drum or plate on which the polymer is wound.

3. EXPERIMENTAL

PLA/PGA (RESOMER RG503, Boehringer Ingelheim), 100L PLA (Alkermes Medisorb[®]), and PEVA (Elvax 40, Dupont) were employed in this study. PEVA pellets were soaked in ethanol for several days to remove antioxidants. PLA/PGA was electrospun from 0.19g/ml solutions in methylene chloride, 100L PLA was spun from 0.19 and 0.14 g/ml solutions in chloroform, and PEVA was sprayed from 0.06 g/ml and spun from 0.21 g/ml solutions in chloroform or methylene chloride. Chloroform is preferred to mitigate clogging of the pipette tip at high polymer concentrations due to solvent evaporation. The electrospinning/spinning set-up consisted of a glass pipette (held parallel to ground or angled at 45° downward), 0.32 mm diameter silver-coated copper wire (positive lead), various targets/molds with results presented from 303SS flat plates and 4 mm diameter mandrels, and a Spellman CZE1000R high voltage supply. A schematic of the set-up is shown in Figure 1. Voltages in the range of 10-20 kV were employed. Scanning electron micrographs (SEMs) were recorded using a JSM-820 Scanning Microscope (JEOL, Ltd.). Smooth muscle cell seeding and proliferation was examined using SEMs as well.

Figure 2 shows an SEM of 50:50 PGA/PLA electro-processed at 16 kV from 0.19 g/ml in methylene chloride. It shows a 'beads-on-a-string' morphology which is more an aerosol than fibrous. This structure seems to have been more electrospayed than spun. Figures 3a and 3b show SEMs of PEVA electro-processed (12 kV) from 0.06 g/ml in methylene chloride and 0.21 g/ml in chloroform, respectively. The

former shows a porous microstructure that appears to have been principally formed by electrospraying and partial coalescence of droplets. The material from the more concentrated solution is clearly fibrous and is the result of electrospinning, which is favored from more concentrated solutions due to greater entanglement density. Like solvent-cast PEVA films, both electro-processed materials are rubbery, although unlike PEVA cast films both are white due to light scattering by the microporous structures. Figure 4 shows an SEM of 100L PLA electro-processed onto a rotating flat plate at 16 kV, at a flow rate of 20 ml/hr, for 10 minutes. The original polymer solution was 0.14 g/ml (wt%) in chloroform. The resulting structure is more aligned but still gives uniform fibers of 10 μ m diameter. Figure 5 shows an SEM of 100L electro-processed at 16 kV from 0.19 g/ml in chloroform onto which muscle cells have been seeded and allowed to proliferate. It shows relatively uniform 10 μ m diameter fibers randomly oriented (developed on a static mandrel). The resulting structure was sterilized for one hour in 100% ethanol, rinsed with sterile water, then soaked in SMC media for an hour before seeding with smooth muscle cells. The smooth muscle cells were allowed to proliferate for 30 days in static conditions, then fixed in formalin for SEM examination.

4. CONCLUSIONS

In summary, we have shown that synthetic polymers can be 'electro-processed' to produce a variety of biomaterials (composition and morphology) with an emphasis on tissue engineering scaffolds. As discussed, these matrices/scaffolds can be reproducibly produced in a short period of time (< 10 minutes). Electrospun PLA/PGA copolymer appears to be an excellent scaffold for muscle cell growth. Our next goals are to further characterize the materials produced in terms of mechanical properties (i.e. Young's Modulus) and porosity, and to tailor scaffolds, via processing parameters, toward specific cell and tissue types.

5. REFERENCES

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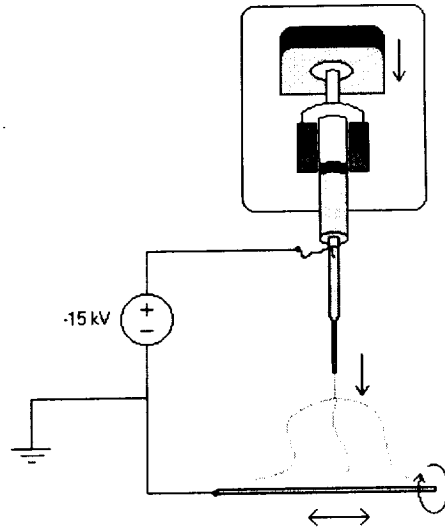


Figure 1. A schematic of the electrospinning setup. A syringe-pipette combination is charged to 10 - 20 kV and directed towards a target that may be translated and spun. Note the splaying fibers between the target and the pipette tip.

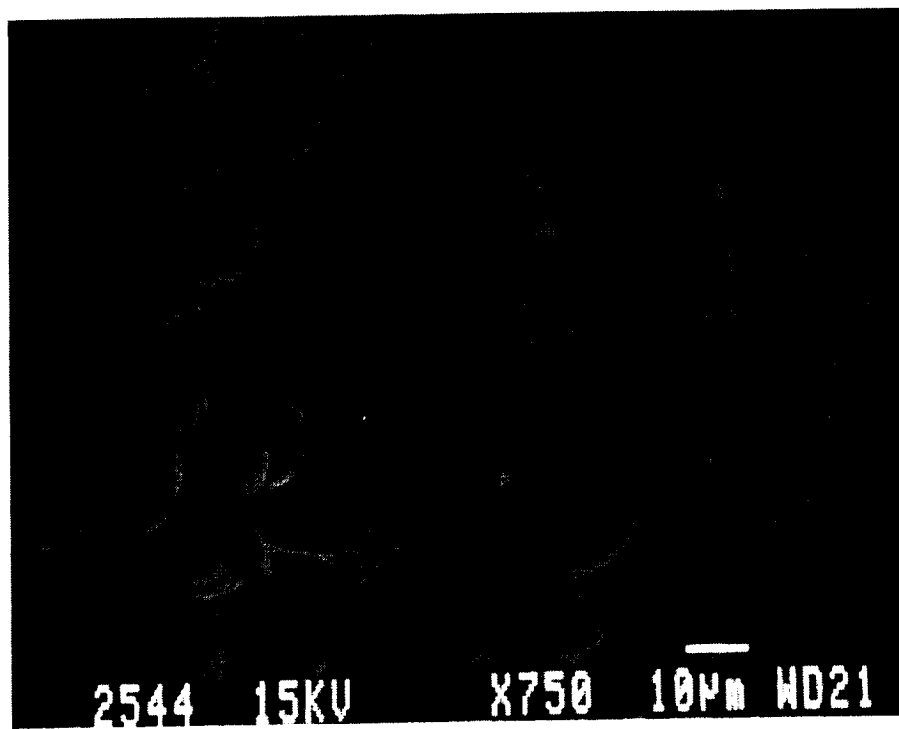


Figure 2. Electroprocessed PGA/PLA illustrating a 'beads-on-a-string' morphology which is more an aerosol than fibrous mesh.

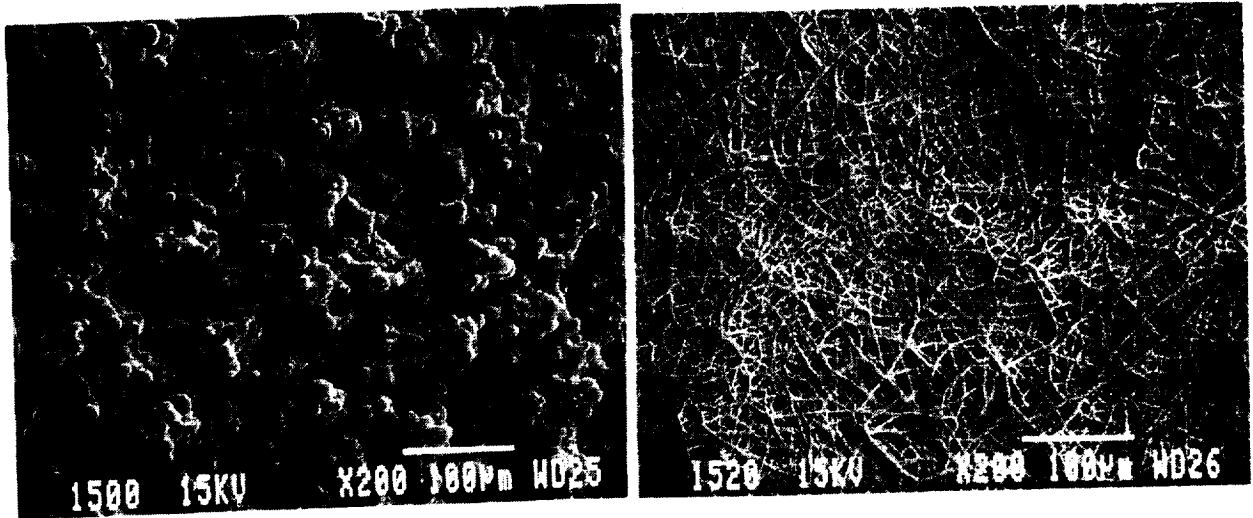


Figure 3. (a) PEVA deposited from 9wt% solution in chloroform; (b) PEVA deposited from 15 wt% solution in chloroform.

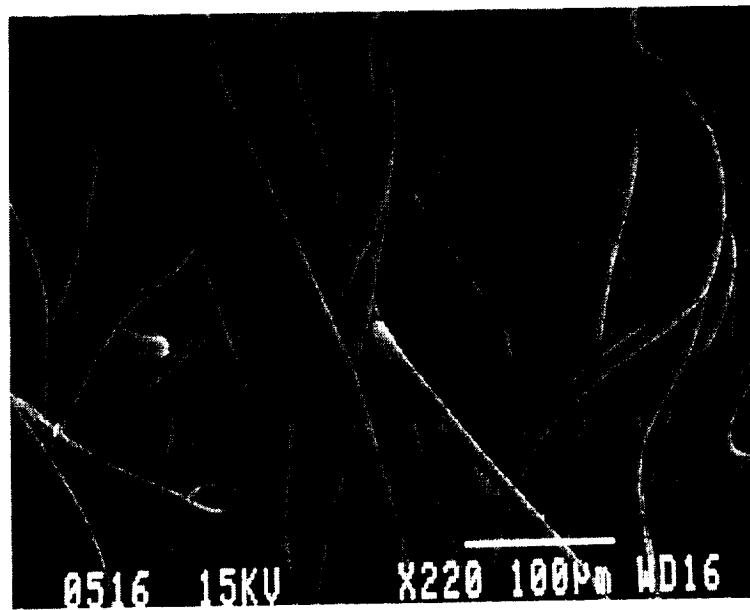


Figure 4. 100L PLA electrospun onto a rotating flat plate illustrating the aligned fibrous mesh that can be produced.

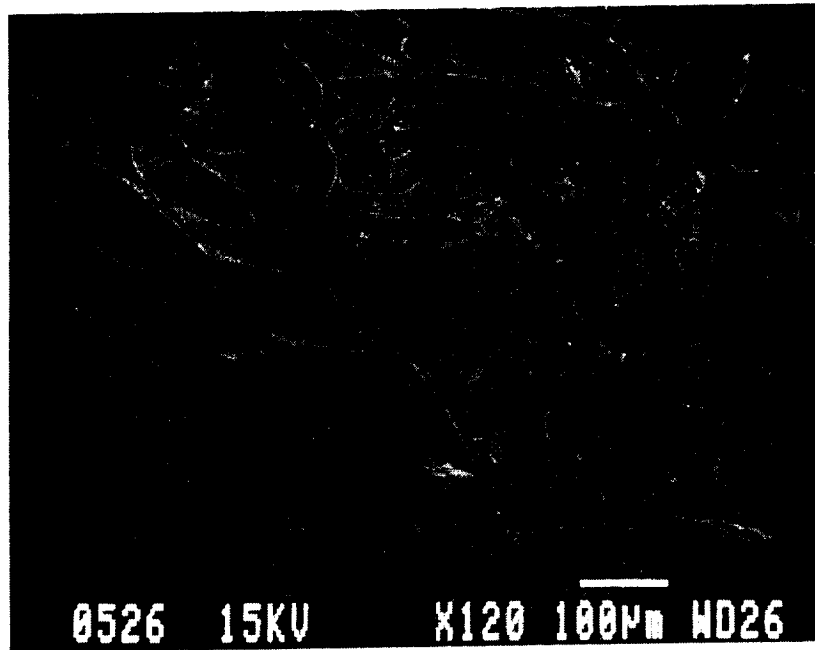


Figure 5. Illustration of 10 micrometer diameter PLA fibers seeded with Human Aortic SMC's for 30 days under static culture.

Biographical Sketches of the Authors

Joel Stitzel is a graduate student in the Department of Biomedical Engineering at VCU, working on the development of scaffolds for tissue engineering using electrospinning. Joel received his B.S. in Engineering Science and Mechanics from Virginia Polytechnic Institute and State University in 1997. Gary Bowlin is an Assistant Professor in the Department of Biomedical Engineering at VCU whose interests are vascular and muscle tissue engineering. Gary received his B.S. in chemical engineering from Youngstown State University in 1988, M.S. (1990) in chemical engineering from the University of New Hampshire, and a Ph.D. (1996) in biomedical engineering from the University of Akron. In 1995, Gary was a national award winner in the BFGoodrich Collegiate Inventors Program dealing with his work on vascular tissue engineering and holds several patents in this area. Kevin Mansfield just completed his sophomore year at VCU where he is majoring in Biomedical Engineering. Gary Wnek is Professor and Chair of the Department of Chemical Engineering at VCU, where his interests are in applications of polymers in medicine, biotechnology, and energy conversion. He received a B.S. in chemical engineering from Worcester Polytechnic Institute in 1977 and a Ph.D. in polymer science and engineering at the University of Massachusetts, Amherst, in 1980. David Simpson is an Assistant Professor in the Department of Anatomy at VCU where his research focuses on how mechanical activity regulates cardiac physiology and engineering of cardiac and skeletal muscle. David received his B.S. in human nutrition from Ohio University in 1986 and his Ph.D. (1991) from Northwestern University in cell, molecular, and structural biology.