Electric field-mediated processing of biomaterials: toward nanostructured biomimetic systems

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ABSTRACT

Significant opportunities exist for the processing of synthetic and biological polymers using electric fields ('electroprocessing'). We review casting of multi-component films and the spinning of fibers in electric fields, and indicate opportunities for the creation of smart polymer systems using these approaches. Applications include 2-D substrates for cell growth and diagnostics, scaffolds for tissue engineering and repair, and electromechanically active biosystems.

1. INTRODUCTION

A central dogma of materials science is that synthesis and processing dictate structure and composition, which in turn determine properties and, ultimately, performance. Electric fields can be exploited as a useful processing aid, allowing the creation of structures and morphologies that are difficult to otherwise achieve. We review here some examples of electric field-mediated polymer processing (electro-processing) and related issues studied in our laboratories, along with work in progress, with specific attention toward the fabrication of biomaterials with tailored properties. Our efforts are motivated by the quest to develop 'ideal' tissue engineering scaffolds that mimic the structure and biological functions of natural extracellular matrix (ECM), and to fabricate substrates having patterned surfaces for spatially resolved cell culturing, as well as genomics and proteomics applications.

2. ELECTROPROCESSING

2.1 Electro-casting of films

It is possible to generate 'pearl-chains' of biological cells in electric fields and, under appropriate conditions, to induce cell fusion.[1] This is the result of mutual dielectrophoresis wherein electrically neutral bodies can move in electric field gradients. These observations with cells inspired the first studies of the effect of electric fields on the morphology of solvent-cast polymer blends.[2] Most polymer pairs are incompatible in the solid state due to low entropies of mixing and thus phase separation occurs, often with the polymer in lower concentration forming droplets eventually embedded in a continuous major phase. Indeed, as was observed with biological cells, minor phases of polymer in a blend can be seen to 'pearl-chain' if casting is done in modest electric fields (ca. 3 - 12 kV/cm).[2-4] Examples include poly(ethylene oxide) (PEO) in poly(styrene) (PS) and poly(ethylene-co-vinylacetate) (PEVA) in PS. Unlike biological cells, however, solvent-swollen polymer droplets can undergo very large deformations in electric fields, and in fact rather long columns of polymer minor phase can be observed under certain conditions.[2-4] Chaining and column formation in these systems are complicated processes, arising from mutual dielectrophoresis as well as droplet deformation (elongation), sometime followed by break-up into apparent pearl chains. This is seen clearly in Figure 1, which also shows classical Rayleigh instabilities in a few of the columns.

Polymer droplet deformation occurs more readily as the interfacial tension between polymer phases is lowered, the latter being accomplished by, for example, addition of a block copolymer effectively acting as

a surfactant.[3] The polymer phases just described have sizes of a few microns to tens of microns, although with block copolymer systems much smaller phases can be manipulated during film casting using electric fields.[5] For example, ca. 20 nm 'micelles' of PEO-b-PS in PS can aggregate and form thin (ca. 20 nm) threads when cast from solution in the presence of a ca. 8 kV/cm field.

While not discussed explicitly here, we note that effects of electric fields on polymer films having long-range order (e.g., liquid crystalline polymers and block copolymers films) has also been studied in detail.[6]

Figure 1: Poly(ethylene-co-vinyl acetate)/poly(styrene) film cast from toluene between two evaporated electrodes on glass. PEVA is the minor phase. Applied field was 10 kV/cm. Arrow indicates field direction. From Ref. 4.

2.2. Electrospraying and Electrospinning

Electrostatic spraying (electrospraying) and electrostatic spinning (electrospinning) represent attractive approaches for polymer biomaterials processing with the opportunity for control over morphology, porosity, and composition using simple equipment. Electrostatic spraying is the basis upon which the technique of electrospray mass spectrometry is able to achieve molecular mass analysis of single polymer molecules.[7] In the case of electrospraying, charged droplets are generated in a strong electric field and delivered to a grounded target. It is also possible to generate small, solid particles of polymers in this manner with applications in medicine. For example, PEVA particles have been prepared containing proteins and the release of the latter has been studied.[8]

In electrospraying, charged droplets are generated at the tip of a metal needle (or pipette with a wire immersed in the liquid) and are subsequently delivered to a grounded target. The droplets are derived by charging a liquid typically to 5-20kV vs. a ground a short distance away, 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 the ground electrode of opposite polarity, forming a so-called Taylor cone at the needle tip. Droplets are formed when electrostatic forces between the charged liquid and the ground exceed the liquid's 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 and, ultimately, individual macromolecules.

Electrospinning[9,10] is fundamentally an extension of electrospraying, and is of particular interest because of 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. In electrospinning, polymer solutions or melts are deposited as fibrous mats rather than droplets, with

advantage taken of chain entanglements in melts or solutions at sufficiently high polymer concentrations to produce continuous fibers. The electrospinning phenomenon is mechanistically similar to electrospraying, a key difference being that chain entanglements yield a fiber rather than droplet ejection from the Taylor cone. Recent studies[10] have indicated that the fiber does not splay into thinner ones, but rather progressively thins itself prior to deposition. Thus, electrospinning is essentially the continuous deposition of a single fiber. The basic elements of a laboratory electrospinning system are simply a high voltage supply, collector (ground) electrode/mold, source electrode, and a solution or melt to be 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 (Figure 2). In actuality, the technique is even more versatile, as we find that electrospinning (or electrospraying) can also be done on a dielectric material interposed between the ground and the spinning/spraying solution. Thus, a wide variety of substrates can be coated by these electrostatic processing techniques.

As early as 1977, Martin and Cockshott[11] 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.[12-14] Our initial work has focused on PEVA (Figure 3) and biodegradable poly(lactic acid) and poly(glycolic acid) and copolymers. However, we have recently been successful in the electrospinning of 100 nm fibers of collagen,[15] which may be an ideal scaffold for tissue engineering.
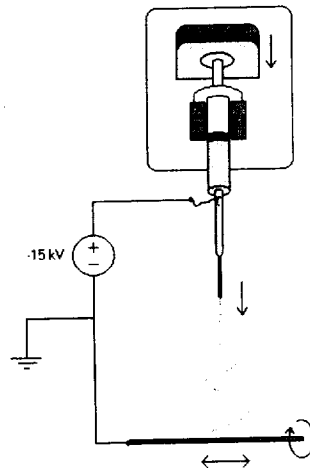

Figure 2: A schematic of the electrospinning setup as discussed earlier. The syringe needle is charged to 10 -20 kV and directed towards a target that may be translated and rotated during spinning.
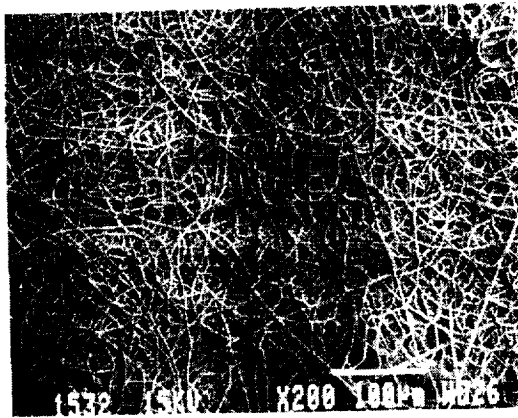
Figure 3: Poly(ethylene-co-vinyl acetate) electrospun from chloroform (15wt% solution)

2.3. Electrostatic cell seeding and spraying

It is also possible to use electric fields to deliver living cells to biomaterial surfaces, and while this is different from field-assisted polymer processing, we discuss it briefly here since this represents a promising set of approaches for conferring biological function and biocompatibility to polymeric materials.

Since the inception of endothelial cell transplantation and the subsequent barrage of published research in the late 1980's, advancements have been few and very limited in addressing the recognized needs for clinical application of endothelial cell transplantation. The obstacles to clinical application include: 1) improving the efficiency of transplanted endothelial cell attachment and 2) minimizing cellular losses upon implantation. Recently, a novel electrostatic endothelial cell transplantation technique has been proposed and evaluated which addresses many of the concerns that presently prevent the clinical application of endothelial cell transplantation.[16,17] The novel aspect of this technique is that it is capable of enhancing endothelial cell adhesion (surface density as well morphological maturation) by inducing a temporary positive surface charge, or a "temporary glue", on the negatively charged e-PTFE graft luminal surface (electric field mediated process). Upon completion of the electrostatic transplantation procedure (removal of electrical potential from the apparatus), the graft luminal surface reverts to its original highly negative charged surface. This is a critical aspect because any non-endothelialized graft surfaces or any exposed graft surfaces that result from endothelial cell losses upon restoration of blood flow remain non-thrombogenic due to the presence of the natural negative surface charge of the graft material. Preliminary data are very promising for this technology also indicating enhanced/accelerated EC adhesion/maturation to metallic stents.

3. OPPORTUNITIES AND PROSPECTS

Interesting possibilities exist to further develop electroprocessing for the creation of biomimetic systems. For example, electro-casting of blends using patterned electrodes may be a simple approach to obtaining interesting 2-D structures for cell culturing and biological assays (e.g., biochip technology). PEVA is an attractive minor phase as it is biocompatible and is a useful matrix for controlled drug delivery.[18] Nano-sized domains are accessible using block copolymers. As another example, electromechanically responsive gels are promising materials as artificial muscles,[19] and it may be possible to prepare materials with very fast response times via electrospinning. Electromechanical response in ionic gels can be the result of field-induced ion gradients in the material leading to osmotic pressure gradients. Ion transport kinetics dominates the response, and facile transport is expected with the small fibers. Indeed, gel swelling and shrinking kinetics have been shown to be proportional to the square of the diameter of a gel fiber. Electrically conducting polymers (e.g., polyaniline) exhibit electromechanical responses during doping and de-doping, here the result of ion diffusion into and out of the films with concomitant dimensional changes.[20-22] Once again, ion diffusion is rate-limiting, and thus electrospun electroactive polymer fibers may offer fast response kinetics. We note that conducting polymers have been spun and fiber diameters of < 100 nm can be routinely obtained.[23] Of particular interest is the growth of cells and tissue within electroactive, nanostructured scaffolds where the material serves to modulate cell and tissue growth through electrical and/or mechanical stimuli. Electrostatic cell seeding may be a useful approach to

homogeneously seed these scaffolds. We believe that there is much promise for the exploitation of one or more of these ideas to create novel and useful biomimetic, nanostructured materials.

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