Electrospun materials for affinity-based engineering and drug delivery

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Abstract. Electrospinning is a process which can quickly and cheaply create materials of high surface to volume and aspect ratios from many materials, however in application toward drug delivery this can be a strong disadvantage as well. Diffusion of drug is proportional to the thickness of that device. In moving from macro to micro to nano-sized electrospun materials drug release rates change to profiles that are too fast to be therapeutically beneficial. In this work we use molecular interactions to further control the rate of release beyond that capable of diffusion alone. To do this we create materials with molecular pockets, which can “hold” therapeutic drugs through a reversible interaction such as a host/guest complexation. Through these complexes we show we are able to impact delivery of drug from electrospun materials, and also apply them in tissue engineering for the reversible presentation of biomolecules on a fiber surface

1. Introduction

Electrospinning and spraying are processes which have been known for more than 100 years, however have only recently be “rediscovered” and applied toward applications in medicine including drug delivery and tissue engineering. The process uses a high voltage course to inject charge on a polymer solution or melt. As this solution is accelerated toward a collector of opposite polarity, electrostatic and solution forces drive the formation of structures ranging from droplets (electrospraying) to micro and nanofibers (electrospinning) (Figure 1).

Electrospinning has been exponentially increasing in its usage for tissue engineering and drug delivery over the last decade [1]. Recent advances using polymer fibers with composite materials has even allowed for new directions to take these materials [2]. Some of the major advantages of electrospun materials are that they can be easily manufactured using a fairly inexpensive benchtop apparatus, and simple polymer solvents or melts. Many different polymers can be electrospun from natural materials to synthetic. Also the resulting fibers are easily within the size range of biological matrix molecules (100 nm – 1 µm).

However one of the major limitations that these materials, particularly in regards to drug delivery, is that small fiber diameter. Fick’s First Law of Diffusion \( J = D \frac{dc}{dx} \) which relates drug diffusive flux \( J \) to concentration \( dc/dx \), or drug amount per unit distance; and as such the smaller the distance the drug has to travel, the more rapidly it will diffuse across that distance \( x \) [3]. \( D \) is the empirically determined diffusivity. Acceleration of drug release corresponding to a decrease in device size is a phenomenon that had been well-known for the micro- and nano-particulate world but had to be re-learned for electrospun micro- and nano-fibers. Basically this means that drug which would take weeks to months to get out of a macro-sized polymer slab, would get out of an electrospun fiber of similar mass within minutes to days. Typically drug delivery applications require therapeutic rates
across biological time frames such as cell proliferation cycles (days) to tissue remodeling (weeks). So delivery that is completed within hours is not much better than a bolus injection of that drug.

Figure 5. Electrospinning Device Setup. Variables altered are the solution, the needle arrangement, and collector layout (plate, rotating drum, metal gap plate, biomedical device).

In our laboratory we have been exploring the use of molecular interactions to control the rate of drug release. These interactions are specific affinities between device polymer and drug, which if strong enough can slow down the rate of drug release beyond that capable of diffusion alone. Essentially drug-decomplexing from the material interactions becoming the rate-limiting step. This new research field is termed “affinity-based drug delivery” [4]. In this paper we explore whether through affinity-based drug delivery strategies we can overcome this major limitation of utilizing electrospun fibers for drug delivery.

Another recently popular application for electrospun materials is as tissue engineering or regenerative medicine scaffolds. Often these materials are electrospun from natural polymers such as collagen to improve biocompatibility; however, the most commonly used solvent for electrospinning collagen has previously been hexafluoroisopropanol (HFIP), which is extremely cytotoxic, even with the miniscule residual amounts found in well-washed electrospun collagen mats. Only recently have investigators begun to electrospin collagen from more biologically friendly solvents [5].

One way to gain the benefits of biological integration and cell attachment, but also keep the benefits of the high flexibility of electrospinning, is to decorate electrospun fibers with cell attachment peptides such as the ubiquitous Arginine-Glycine-Aspartic Acid (RGD). With this strategy one can encourage endothelial cell attachment and ingrowth of new blood vessels to form a vascular tissue bed [6]. Decorating an electrospun fiber with RGD is done either after the spinning is complete, or as polymer modification before the electrospinning process. The former method is limited in its chemical conjugation strategies, and the latter method limits electrospinning since the peptide is molecularly more fragile than most synthetic polymers, and as a charged molecule it changes spinning dynamics.
Further another limitation of RGD decorated fibers is that they are simultaneously both chemically unstable and biologically unchangeable. The instability comes from general peptide instability, which can either spontaneously hydrolyze or specifically be degraded by a number of nonspecific proteases. Once degraded, the precious cell attachment signal that the investigator has laboriously added to the electrospun fiber is now lost. The unchangability is of concern due to the highly dynamic environment of tissue engineering and regenerative medicine in terms of tissue remodeling. One cell signal might be needed at one time point while a different cell signal might be needed at another time point. As an example for bone tissue engineering, initially a signal to recruit endothelial cells and generate blood vessels is required. While at later time points no more new blood vessels are needed, but rather osteogenic cells are needed. A chemically attached signal such as RGD cannot change post-implantation.

In this work we also wish to show how the use of these same affinity groups can begin to address some of these problems found in electrospun materials for tissue engineering, namely a) how to introduce a biofunctional molecule easily and stably, b) how to replace that molecule if needed after natural degradation; or c) how to replace that molecule if its signal needs to be replaced by that of a different biomolecule.

2. Methods
Poly(ethylene vinyl alcohol) (pEVOH) is modified with pendant β-cyclodextrins (CD), due to CD’s well known capacity to form inclusions with small molecular weight drugs. This is done in a two step process. pEVOH is first modified with the bi-functional crosslinker N-(p-Maleimidophenyl) isocyanate (PMPI). The resulting polymer is then electrospun using conventional techniques [7].

In parallel a CD-thiol is made by converting 6-Tosyl-β-CD by heating for 2 days 100°C in thiourea and methanol. Trichloroethylene is added to precipitate the product which is then washed and recrystalization from water. CD-thiol is reduced in basic conditions prior to use, and then added to pEVOH-PMPI to form pEVOH-CD. Conjugation is verified by NMR analysis, and conjugation efficiency by FTIR. Feed ratios were calculated to produce polymers with either 2.5 or 10% CD substitutions on polymer side chains. pEVOH which underwent the same processing steps but no CD conjugation was used as a non-affinity diffusion-only polymer control.

Fibers were hydrodynamically loaded with a model drug, Rhodamine B, in an aqueous solution, extensively washed, and then evaluated for drug release in an infinite sink of phosphate buffered saline agitated at 37°C, removing release aliquots every 12 h.

![Diagram](image)

**Figure 2.** Scheme for reversible and non-reversible attachment of peptides. Gold surfaces were conjugated either with peptide directly (a & b), or indirection through association with conjugated cyclodextrin (b & c).
For the tissue engineering studies, CD-thiol was directly conjugated to a gold surface, complexed with either the cell attachment peptide RGD, or the non-attaching control peptide RGE. To cause the association between the peptide and CD coated surface, by using a bifunctional crosslinker with N-hydroxysuccinimide at one end reacting to adamantine methylamine; and maleimide at the other end reacting to an extra cysteine residue; with a poly(ethylene glycol) spacer in between. The adamantine forms a strong but reversible complex with CD. In parallel RGD and RGE were directly attached to different gold substrates through use of the thiol on the extra cysteine residue. Green fluorescent protein (GFP) labeled fibroblast cells were added to the surfaces and evaluated by reflectance microscopy (Figure 2).

The pEVOH-CD was also evaluated for reversible modification with association with adhesion peptides. In this case an interpenetrating network was made between the pEVOH-CD polymer manufactured above, and tissue culture polyethylene, loaded with the various peptide constructs, and then incubated with fibroblast cells. Lastly the pEVOH-CD was electrospun, loaded with peptide constructs, incubated with GFP cells, and the resulting mat examined by fluorescence microscopy both top-down, as well as in cross-section after serial-sectioning by cryomicrotome.

3. Results

The drug delivery experiments show that addition of affinity through use of cyclodextrin substantially delays release of drug out of electrospun nanofibers, with the majority of the drug released after three days from the non-affinity pEVOH control, however at that same time point only ¼ of the drug was released from the affinity-based pEVOH-CD polymer (Figure 3).

In the tissue engineering studies reflectance microscopy shows that GFP labeled cells do not adhere to the control gold surface conjugated only with CD. However once an adamantane modified cell attachment peptide RGD is added there is rapid cell attachment with many focal adhesions and cell motility. When this peptide is exchanged with an adamantane (Ad) modified RGE cell attachment is rapidly diminished with few attached cells (Figure 4).
Fibroblasts on pEVOH-CD surfaces show similar behavior. Cells do not attach to the pEVOH-CD face of the interpenetrating network modified surface. However, upon incubation with an Ad-modified RGD, cells begin to attach and spread similar to tissue culture styrene controls (Figure 5).

Lastly, electrospun mats were seeded with GFP labeled fibroblasts. From top-down microscopy, individual cells can be seen which are approximately 10 times bigger than individual fibers estimated at 1µm in diameter. In cross-section, many individual cells can be seen adhering to the outermost fibers (Figure 6).

**Figure 3.** Cells on pEVOH-CD surfaces reversibly decorated with RGD. Left) TCPS control shows good cell attachment. Middle) PEVOH-CD face of interpenetrating network shows no cell attachment. Right) PEVOH-CD with Ad-RGD shows cell attachment and spreading.

**Figure 4.** GFP labeled cells on electrospun pEVOH-CD fibers with associated RGD peptides. Left) Cross-section showing cells adhered and spread (yellow arrows). Right) Top-down with individual (dark) fibers visible (red arrows).
4. Discussion
In this work we have shown that through affinity-based molecular interactions with cyclodextrin we can control the rate of drug release out of electrospun materials, reducing the disadvantage caused by their small diameter, and bringing release rates into therapeutically relevant timescales. Further we found that we could use the molecular interactions to cause reversible associate of affinity-modified cell-attachment peptides RGD, promoting cell adhesion on simple gold surfaces, affinity-polymer modified tissue culture surfaces, and electrospun affinity-based materials.

References
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