



# Tuneable nanoparticle-nanofiber composite substrate for improved cellular adhesion



Ariana M. Nicolini<sup>a</sup>, Tyler D. Toth<sup>b</sup>, Jeong-Yeol Yoon<sup>a,b,c,\*</sup>

<sup>a</sup> Biomedical Engineering Graduate Interdisciplinary Program, The University of Arizona, Tucson, AZ 85721, USA

<sup>b</sup> Department of Biomedical Engineering, The University of Arizona, Tucson, AZ 85721, USA

<sup>c</sup> Department of Agricultural and Biosystems Engineering, The University of Arizona, Tucson, AZ 85721, USA

## ARTICLE INFO

### Article history:

Received 21 December 2015

Received in revised form 17 April 2016

Accepted 27 May 2016

Available online 28 May 2016

### Keywords:

Reverse potential electrospinning

Surface tension

Surfactant

Functionalized nanoparticles

RGD ligand

## ABSTRACT

This work presents a novel technique using a reverse potential electrospinning mode for fabricating nanoparticle-embedded composites that can be tailored to represent various fiber diameters, surface morphologies, and functional groups necessary for improved cellular adhesion. Polycaprolactone (PCL) nanofibers were electrospun in both traditional positive (PP) and reverse potential (RP) electrical fields. The fibers were incorporated with 300 nm polystyrene (PS) fluorescent particles, which contained carboxyl, amine groups, and surfactants. In the unconventional RP, the charged colloidal particles and surfactants were shown to have an exaggerated effect on Taylor cone morphology and fiber diameter caused by the changes in charge density and surface tension of the bulk solution. The RP mode was shown to lead to a decrease in fiber diameter from  $1200 \pm 100$  nm (diameter  $\pm$  SE) for the nanofibers made with PCL alone to  $440 \pm 80$  nm with the incorporation of colloidal particles, compared to the PP mode ranging from  $530 \pm 90$  nm to  $350 \pm 50$  nm, respectively. The nanoparticle-nanofiber composite substrates were cultured with human umbilical vein endothelial cells (HUVECs) and evaluated for cellular viability and adhesion for up to 5 days. Adhesion to the nanofibrous substrates was improved by  $180 \pm 10\%$  with the addition of carboxylated particles and by  $480 \pm 60\%$  with the functionalization of an RGD ligand compared to the PCL nanofibers. The novel approach of electrospinning in the RP mode with the addition of colloids in order to alter charge density and surface tension could be utilized towards many applications, one being implantable biomaterials and tissue engineered scaffolds as demonstrated in this work.

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## 1. Introduction

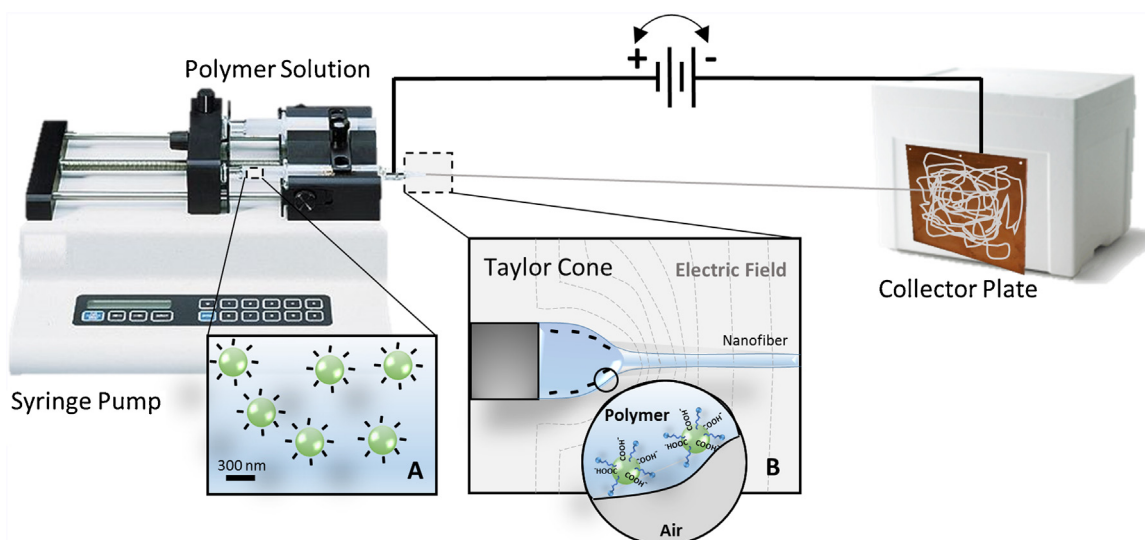
Electrospun fibers of various materials and properties have been utilized in research such as tissue engineering, drug delivery systems, textiles, and biosensor applications [1]. According to Persano et al. [2,3], in the first decade of the 21st century, the number of publications investigating properties and potential applications of electrospinning has increased exponentially. The volume of patents has also expanded at an astounding rate, and over 50% of these patents were related to biomedical scaffolds for use in implants. Electrospinning techniques are likely popular due to the formation of nano- and micrometer scaled structures that can be easily tailored to form smooth/rough, porous/non-porous, composite, 2D or 3D fibers, tasks unachievable through other filament-forming processes. Despite the volume of research to this end, there remain

areas of electrospinning that require additional research and development, which will be discussed later in this paper.

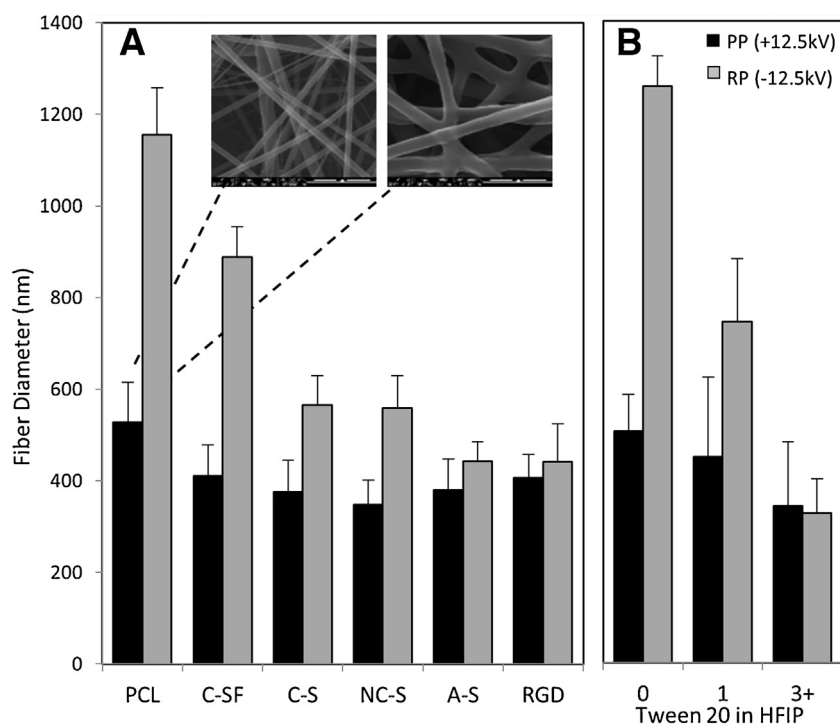
Briefly, electrospinning is the process of creating fibers through the extraction of a solution – polymer:solvent – through a capillary tube while subjected to an electric field force. This field force drives the solution through an air interface where the solvent dissipates, thus resulting in ultrafine, polymeric fibers on a collection substrate. This event can only occur at a critical point in which the repulsive electrostatic forces at the surface of the polymer (*i.e.* air-liquid interface) exceed the surface tension forces [4–6].

There are many methods that can be utilized to manipulate fiber diameter, surface chemistry, and topography during the electrospinning process. Such variables include applied voltage, tip-to-ground distance, inner needle diameter, extrusion rate, viscosity, volumetric charge density, and polymer:solvent ratio or type [6–8]. The addition of surfactants to polymer solutions has been a popular topic of research aimed at minimizing both beading during the spinning process as well as fiber diameter. By increasing the ratio of surfactant to polymer solution, the resulting fiber diameter

\* Corresponding author at: Department of Agricultural and Biosystems Engineering, The University of Arizona, 1177 E. 4th St., Tucson, AZ 85721-0038, USA.  
E-mail address: [jyoon@email.arizona.edu](mailto:jyoon@email.arizona.edu) (J.-Y. Yoon).



**Fig. 1.** Schematic of the horizontal electrospinning setup in which positive and negative potentials can be applied (arrows) to the needle tip and collector plate. Particles in the polymer solution maintain a certain distance based on surface charge (A). Similar to surfactants, particles adsorb to the air-liquid interface, thus altering the solution surface tension and Taylor cone morphology (B).



**Fig. 2.** (A) Average fiber diameters of positively and negatively electrospun 10% w/v PCL-HFIP solution containing: 0.1% w/v 300 nm polystyrene carboxylated particles with (C-S) and without (C-SF) anionic surfactant, non-carboxylated particles with surfactant (NC-S), aminated particles with cationic surfactant (A-S), and GRGDSPK coupled carboxylated particles containing albumin (RGD). (B) Average fiber diameters with the addition of Tween 20 (T20) to HFIP solvent with incubation time of 0, 1, and >3 days. All surfactant concentrations = 0.01% v/v. Error bars represent standard error ( $n=5$ ).

can be estimated with respect to the forces on the Taylor cone and surface tension of the solution [9–11].

It is known that in certain circumstances micro- and nanometer solid particles can function similarly to surfactants and micelles [12]. Adsorption of particles and/or micelles to an air-liquid interface can affect surface tension [13,14]. This interaction occurs at a slower rate for colloids compared to that of individual surfactant molecules. However, for particles, adsorption is considered irreversible, whereas for surfactants, desorption occurs readily [15,16].

To our knowledge, there has been no research to date that describes the use of particles for controlling surface tension and charge density during the electrospinning process. In addition to affecting surface tension and thus fiber morphologies, particles containing charged functional groups (carboxylated particles carrying negative charge and aminated particles carrying positive charge) could be integral in altering the electrostatic mobility of a nanofiber through the electric field during the electrospinning process. Reversing the potential of the electric field, *i.e.* from a positive applied voltage to the needle tip and grounded collection plate,

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