Influence of chemical treatement of electrospun naonofibers on protein adsorption and delivery

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Statement of Purpose/Introduction: The response of host organism at cellular levels to biomaterials is closely associated with the materials' surface properties [1]. Conventional tissue engineered scaffolds do not possess bioactive abilities to accelerate/enhance extra cellular matrix (ECM) secretion and regeneration of tissue. Efforts have thus been focused on surface engineering of polymeric three dimensional (3-D) scaffolds [2]. Currently employed methods such as plasma or high energy radiation are limited to superficial surface modification of 3-D scaffolds and can be limiting for use on nanofibrous scaffolds due to the degradation of nanofibers on exposure to plasma / high energy radiation. Therefore, in this study we report the chemical treatment / functionalization of three dimensional nanofibrous scaffolds of synthetic polymers such as polylactide (PLA) and poly(lactide-co-glycolide) (PLGA).

Methods: The experiments have been classified into three sets (1) synthesis and chemical treatment (surface modification) of electrospun nanofibers (2) chemical and physical characterizations of modified matrices, and (3) protein adsorption/release. The above sets of experiments were performed for PLGA (85:15 - *M*w 45000-70000) and PLA (*Mw* 90,000-1,60,000), however, for the want of space, results pertaining to PLGA alone have been reported. PLGA was electrospun into nanofibers and the surface morphology, and diameter of matrices before and after chemical treatment were characterized by scanning electron microscopy (SEM). These matrices were subjected to hydrolysis and aminolysis for introducing –COOH and -NH_2 functionalities respectively on the nanofiber surfaces. The PLGA nanofiber matrices were exposed to NaOH (0.01N-10.0N) for hydrolysis, and N-Aminoethyl-1,3-propanediamine (AEPDA) (0.01M-5.0M) and ethylenediamine (ED) (0.01M-5.0M) for aminolysis. The exposure time for hydrolysis/aminolysis was varied from 1 - 60 minutes to enable the optimization of concentration of chemical agent used and time of exposure. The hydrophilicity of the modified nanofibrous matrices was characterized using a contact angle measuring goniometer. Atomic force microscopy was used to quantify the surface roughness after treatment. The extent of –-COOH and -NH_2 functionalities were quantified using fourier transform infrared spectroscopy (FTIR), and toluidine / methyl orange absorbance assay respectively. Further, thermal analysis (T_g/T_m) was conducted to study the influence of chemical treatment on thermal/mechanical properties of the electrospun nanofibers. For tissue engineering applications, protein adsorption and release studies were conducted.

Result/Discussion: Electrospun PLGA (22-20% w/v) nanofibers having diameter ranging from 400-1000 nm were used for the aminolysis and hydrolysis experiments. The concentrations of NaOH for hydrolysis and ED and AEPDA for aminolysis were optimized to 0.01N NaOH, 0.01M ED and 0.01M AEPDA at 1 min exposure (**Figure:1**). Since the chemical treatment processes produce functionality by virtue of surface erosion, one of the goals was to maximize the availability of number of functionalities while

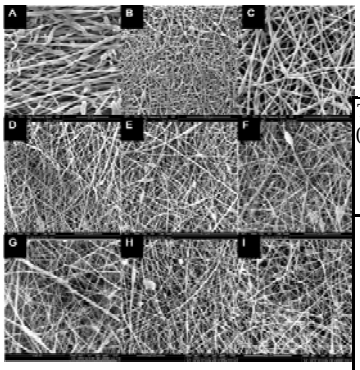

Figure1: PLGA matrix treated with A-C 0.01N NaOH treatment for 10,5&1 minute, D-F 0.01M ED treatment for 10,5&1 minute, G-I 0.01M AEPDA treatment for 10,5&1 minute.

Table 1: No. of carboxyl group micromole/cm^2 of PLGA matrix produced by 0.01N NaOH treatment for various time periods

Time (min)	Concentration of NaOH	Carboxyl group in micro mole / cm^2.
1	**0.01N**	**0.022865**
2	**0.01N**	**0.021300**
3	**0.01N**	**0.016939**
4	**0.01N**	**0.025046**
5	**0.01N**	**0.028344**
6	**0.01N**	**0.030469**
7	**0.01N**	**0.028120**
8	**0.01N**	**0.038240**
9	**0.01N**	**0.0344943**
10	**0.01N**	**0.0272823**

minimizing degradation. The highest number of –COOH and -NH_2 functionalities achieved were ~0.022865 (**Table 1**) and 0.024625 μM/cm^2 (data not reported) respectively while maintaining/minimally compromising fiber morphology. The contact angle measurement of modified matrices demonstrated a significant change in hydrophobicity (68^o for untreated to 52^o for chemically treated). Since the chemical treatment led to surface erosion of nanofibers, the surface roughness of chemically treated nanofibers was characterized using AFM. The results of the AFM study revealed increase in surface roughness after chemical treatment as compared to the untreated nanofibers. The effect of erosion on thermal properties of nanofibers was analyzed using a DSC and the results demonstrated that 0.01 M concentration of NaOH, ED, or AEPDA for duration of exposure of 1 min did not produce any significant change in the values of T_g. A major consequence of chemical treatment was enhanced adsorption of protein that was probably due to increased surface area. Bovine serum albumin (BSA) and lysozyme (data not shown) were used as model proteins for the immobilization and release study. BSA was immobilized on chemically treated (NaOH) nanofibers using a zero length crosslinker {1-ethyl-3(3 dimethylaminopropyl) carbodiimide} [3]. The release profile of BSA demonstrated improved sustained release from 48 hr to 120 hrs. that was proportional to the extent of chemical treatment / protein immobilization

Conclusion: These studies demonstrated that chemical treatment can enable functionalization / surface modification of 3D nanofibrous scaffold which in turn can be beneficial for protein / growth factor delivery.

References:

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