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Fabricating electrospun cellulose nanofibre adsorbents for ion-exchange chromatography

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ABSTRACT

Protein separation is an integral step in biopharmaceutical manufacture with diffusion-limited packed bed chromatography remaining the default choice for industry. Rapid bind-elute separation using convective mass transfer media offers advantages in productivity by operating at high flowrates. Electrospun nanofibre adsorbents are a non-woven fibre matrix of high surface area and porosity previously investigated as a bioseparation medium. The effects of compression and bed layers, and subsequent heat treatment after electrospinning cellulose acetate nanofibres were investigated using diethylaminoethyl (DEAE) or carboxylate (COO) functionalisations. Transbed pressures were measured and compared by compression load, COO adsorbents were 30%, 70% and 90% higher than DEAE for compressions 1, 5 and 10 MPa, respectively, which was attributed to the swelling effect of hydrophilic COO groups. Dynamic binding capacities (DBCs) at 10% breakthrough were measured between 2000 and 12,000 CV/h (2 s and 0.3 s residence times) under normal binding conditions, and DBCs increased with reactant concentration from 4 to 12 mg BSA/mL for DEAE and from 10 to 21 mg lysozyme/mL for COO adsorbents. Comparing capacities of compression loads applied after electrospinning showed that the lowest load tested, 1 MPa, yielded the highest DBCs for DEAE and COO adsorbents at 20 mg BSA/mL and 27 mg lysozyme/mL, respectively. At 1 MPa, DBCs were the highest for the lowest flowrate tested but stabilised for flowrates above 2000 CV/h. For compression loads of 5 MPa and 10 MPa, adsorbents recorded lower DBCs than 1 MPa as a result of nanofibre packing and reduced surface area. Increasing the number of bed layers from 4 to 12 showed decreasing DBCs for both adsorbents. Tensile strengths were recorded to indicate the mechanical robustness of the adsorbent and be related to packing the nanofibre adsorbents in large scale configurations such as pleated cartridges. Compared with an uncompressed adsorbent, compressions of 1, 5 and 10 MPa showed increases of 30%, 110% and 110%, respectively, for both functionalisations. The data presented show that capacity and mechanical strength can be balanced through compression after electrospinning and is particular to different functionalisations. This trade-off is critical to the development of nanofibre adsorbents into different packing configurations for application and scale up in bioseparation.

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1. Introduction

The contribution of biotechnology products to the global prescription and over-the-counter pharmaceutical markets were estimated to be worth $118 billion in 2011 with increased focus in the therapy areas of oncology, anti-diabetes and vaccines [1]. Some individual products are reaching annual sales of over $1 billion [2]. As the market moves towards developing more complex biomolecules such as fusion proteins and antibody fragments, purification stages in downstream processing are becoming more expensive. The advancement of cell line engineering in upstream processing, including transfection methods and media development, in upstream processing have realised increased product titres over the past two decades [3]. However, downstream processing has yet to achieve a dramatic improvement in process efficiency partly due to limitations in widely used packed-bed resins

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including diffusive mass transfer, achievable flow rates and scale-up volumes. Protein bioseparation media using convective mass transfer such as porous membranes and monoliths have received increased attention because they avoid this diffusion limitation and have a higher capture efficiency and reduced buffer use to improve overall productivity [4]. In the last 30 years, rigid porous monoliths have also been introduced and developed. The single solid continuous matrix has no interstitial voids and can also vastly improve productivity by operating at much higher flowrates than packed-bed chromatography [5]. Current advantages in industry have been realised in the polishing stage of monoclonal antibody purification using flowthrough mode where a membrane column binds impurities and allows the target to pass through [6].

Nanofibre electrospinning involves passing a viscous polymer solution through a microneedle charged at a high voltage (>5 kV) to deposit a continuous fibre strand to a grounded collector and form a non-woven mat with a fibre diameter of less than 1 μm [7]. Electrospun nanofibres have been investigated for a multitude of applications including tissue engineering [8], catalysis and sensors [9,10], filtration [11] and composites [12]. Cellulose is a commonly used material in membrane chromatography and filtration for being chemically resistant, cheap and has good non-specific binding properties [4]. However, cellulose raises many challenges in electrospinning because it is difficult to dissolve and the solvent systems required can lead to non-uniform nanofibre deposition [13]. As such, electrospinning readily dissolvable cellulose derivatives such as cellulose acetate are preferred followed by regeneration to cellulose via hydroxide treatment. For uniform fibre deposition of cellulose acetate, controlling polymer solution (viscosity), flow rate and voltage as well as environmental conditions have been shown to be critical [14]. Annealing cellulose acetate nanofibres with heat is a common step to improve mechanical strength by creating “spot welds” at fibre strand overlap points. Fig. 1 shows scanning electron microscopy (SEM) images of the different morphologies for a cast porous membrane, packed-bed resin and an annealed electrospun regenerated cellulose nanofibre adsorbent. A nanofibre adsorbent balances a high surface area and porosity with the benefits of convective mass transfer.

Chemical modifications of chromatographic media using hydroxyl groups on the support for application in bioseparation have been researched [15]. Electrospun nanofibre adsorbents in bioseparation have been reported for cellulose [16] and other polymers including polysulfone [17] and polyacrylonitrile [18]. Diethylaminoethyl (DEAE) cellulose electrospun nanofibres have been fabricated by Williamson ether synthesis using 2-(diethylamino) ethyl chloride hydrochloride (DAECH) to show improved separation productivity compared with porous membranes [19,20]. Alcohol groups on cellulose have been controllably oxidised to carboxylate (COO) groups using (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) as a catalyst and sodium hypochlorite as the oxidant [21,22]. The main application of TEMPO-mediated oxidation is in the preparation of nanocellulose from wood pulp where the ionic repulsion of COO groups helps force cellulose fibres apart during processing, reducing the mechanical energy required [23]. The use of TEMPO-mediated oxidised electrospun cellulose nanofibre has been used before to bind metal ions [22] and viruses [24]. The physical and chemical methods applied in fabricating electrospun cellulose nanofibre adsorbents affect bioseparation performance and controlling parameters is important to fabricating a reproducible material. Compressing nanofibre sheets combined with annealing via heat treatment is used to further improve mechanical properties than heat treatment alone. A robust nanofibre adsorbent is essential in scaled up packed bed configurations such as pleated cartridges, as seen in membrane chromatography. However, chemical modifications applied to nanofibres may adversely affect morphology and structure.

2. Materials and methods

2.1. Fabricating cellulose nanofibre adsorbents

To shorten the electrospinning time and produce nanofibre mat of consistent bed height, four microneedles (100 mm length; 0.5 mm i.d.) were used and the collector was moved side-to-side in line with the needle array. The operating voltage was 30 kV, the humidity set to 70% and temperature to 25 °C. A 20 wt.% cellulose acetate (Mr = 29,000, 40% acetyl groups, Sigma-Aldrich, Dorset, UK) solution was prepared in acetone:DMF:ethanol (Sigma–Aldrich) at a ratio of 2:2:1 as previously described [14,19]. The solution was spun at 2.5 mL/h for 10 h. The collector was a rotating drum (200 mm dia.; 300 mm length) set at 60 rpm on a translation stage set at 300 mm *x*-axis displacement (150 mm either side of the needle array centre) at a rate of five loops per minute. A sheet 600 mm × 180 mm was produced equating to approximately 30 g/m^2, which was comparable to the nanofibre mat used in our previous study but a reduction in spinning time from 36 h to 10 h [19]. Squares (80 mm × 80 mm) were cut, layered and placed in between two 100 mm × 100 mm square aluminium blocks to act as the die. Compression was performed for 2 min in a manual hydraulic press (Specac, Kent, UK) under different loads of 1000,

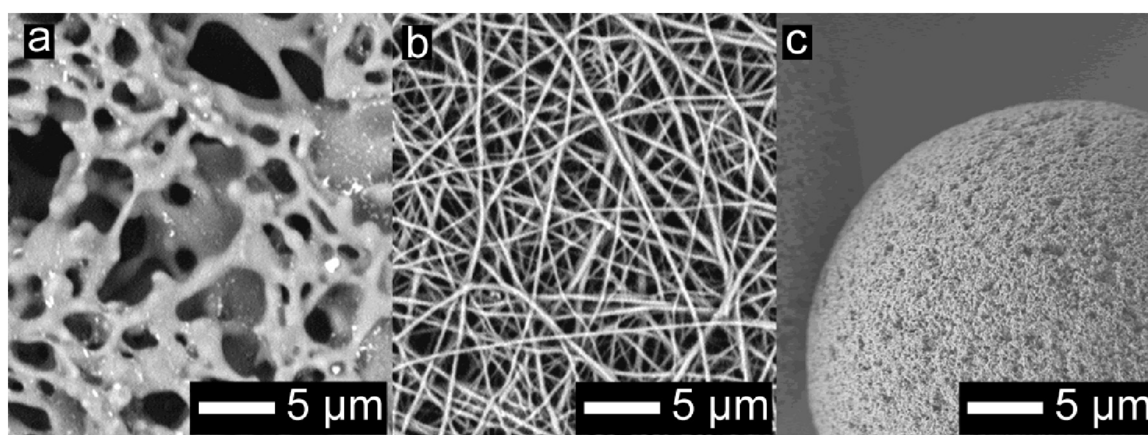

Fig. 1. Scanning electron microscopy images comparing protein purification media. (a) Sartobind S cellulose membrane (Sartorius Stedim, Epsom, UK). (b) Compressed and heat treated regenerated cellulose nanofibre adsorbent. (c) Fractogel EMD TMAE HiCap packed-bed resin (EMD Millipore, Darmstadt, Germany) with 40–90 μm bead diameters and approximately 0.1 μm pore diameter.

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