Colloids and Surfaces B: Biointerfaces 158 (2017) 453–459

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Surface imprinted bacterial cellulose nanofibers for hemoglobin purification

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ARTICLE INFO

Article history:
Received 2 April 2017
Received in revised form 6 July 2017
Accepted 7 July 2017
Available online 10 July 2017

Keywords:
Hemoglobin purification
Surface imprinting
Metal ion coordination
Bacterial cellulose nanofibers

ABSTRACT

There is a significant need for the development of the novel adsorbents in the field of protein purification. In this study, thin hemoglobin imprinted film (MIP) was fabricated onto the bacterial cellulose nanofibers' (BCNFs) by surface imprinting method using metal ion coordination interactions with N-methacryloyl–(L)-histidinemethylester (MAH) and copper ions. The hemoglobin surface imprinted bacterial cellulose nanofibers (MIP-BCNFs) was applied to selective recognition of hemoglobin and purification from hemolysate. The characterization of the MIP-BCNFs was carried out by the Fourier Transformed Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), micro-Computerized Tomography (μCT), atomic force microscopy (AFM) and surface area measurements. The adsorption experiments of hemoglobin onto the MIP-BCNFs and NIP-BCNFs from aqueous hemoglobin solutions were investigated in a batch system. The results showed that MIP-BCNFs are promising materials for purification of hemoglobin with high adsorption capacity, significant selectivity and reusability.

1. Introduction

There exists significant demand for the production of hemoglobin (Hb) based oxygen carriers (HBOCs) which present promising alternative to the blood obtained via donation [1]. The HBOCs can be utilized in blood shortages and in various medical application areas such as surgery, ischemia and internal hemorrhaging, etc. The major step for the synthesis of HBOCs is the purification of Hb, which can be obtained by lysing red blood cells [2]. Therefore, the development of easy, cheap and effective technique for the purification of Hb from red blood cells with high selectivity is of great interest [3].

The molecularly imprinted polymers (MIPs) can be obtained through self-assembly of the functional monomers around a template molecule in the presence of cross-linkers via polymerization and then, the subsequent removal of template molecule leading specific recognition cavities [4]. However, protein imprinting is still a challenging task since it is hard to extract template protein from the matrix as well as the rebinding onto recognition sites because of the poor mass transfer properties between imprinted polymer and solution due to the large molecular size [5]. Additionally, organic solvents, which are used generally in the synthesis of MIP, are not suitable for proteins due to lack of solubility. Lastly, non-specific binding generally occurs because of the structural complexity of proteins [6]. To overcome these limitations, the surface imprinting is a promising approach having the specific recognition sites located at the surface or near to the surface of MIP thus enabling extraction and binding of template proteins [7].

The creation of complexes between the metal ions and imidazole groups of the histidine(s) exposed to the surface of the protein is one of the general approaches for the specific recognition of proteins and first developed by Mallik et al. [8]. Metal ion coordination with proteins is well suited to molecular recognition due to its specificity and stability by orienting proteins as a mediator to establish a stable complex with high specificity [9–11]. Additionally, metal ion coordination is a fast binding process and binding strength can be adjusted by choosing appropriate metal ion for the protein molecule.

Affinity supports for selective separation of proteins show significant importance in the application field of biotechnology such as separation, purification, biosensors and diagnostics [12–15]. Up to now, various kinds of affinity materials such as nanoparticles, nanospheres, nanotubes and nanofibers were synthesized and used for protein recognition [16–18]. Nanofibers have been gaining significant interest since they avoid intraparticle diffusion resistances with high surface area [19,20]. There have been many reports on protein purification using nanofibers' surface functionalization via

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http://dx.doi.org/10.1016/j.colsurfb.2017.07.023
0927-7765/

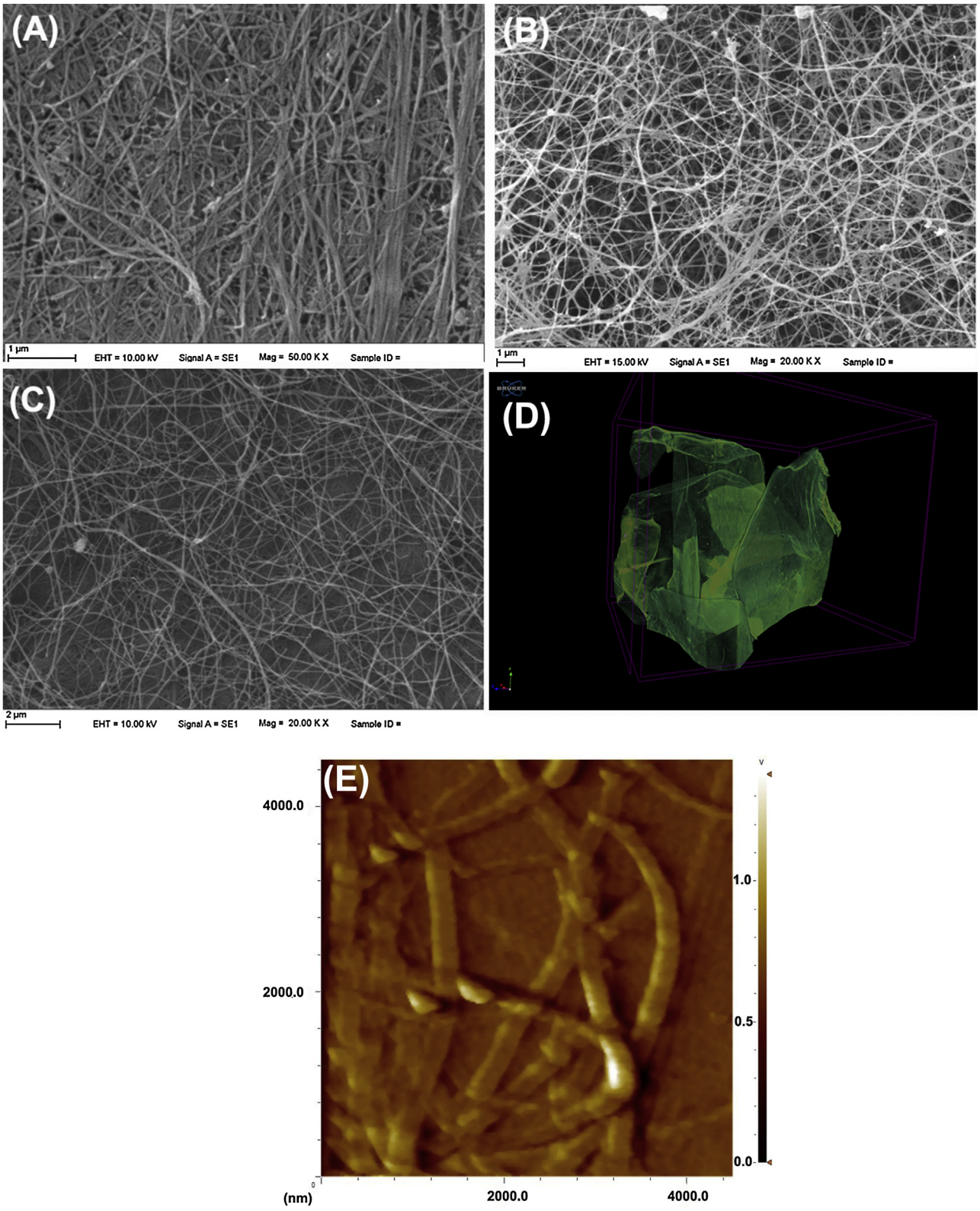

Fig. 1. (A–C) SEM photographs of BCNFs, MIP1 BCNFs and MIP2 BCNFs, and (D–E) three-dimensional μCT and AFM images of BCNFs, respectively.

chemical treatment. However, the affinity nanofibers possess low selectivity towards the template protein. Thus, the combination of the nanofibers with surface imprinting technique is becoming more interesting since the fabrication of the recognition sites onto the nanofibers' surface provide more accessible binding sites resulting selective removal of template protein molecules with high adsorption capacity and fast binding kinetics [21]. Moreover, bacterial cellulose nanofibers (BCNFs) with excellent surface area, high hydrophilicity, high purity and significant chemical and mechanical stability find many application areas such as bio-separation, paper, filler, packaging material and biosensors [22–24].