Colloids and Surfaces B: Biointerfaces 143 (2016) 1–6

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Feasibility of using DNA-immobilized nanocellulose-based immunoadsorbent for systemic lupus erythematosus plasmapheresis

Changgang Xu*, Daniel O. Carlsson, Albert Mihranyan*

Nanotechnology and Functional Materials, Department of Engineering Sciences, Uppsala University, Box 534, 75121 Uppsala, Sweden

ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form 3 March 2016
Accepted 4 March 2016
Available online 7 March 2016

Keywords:
Nanocellulose
Immunoadsorption
DNA immobilization
Systemic lupus erythematosus
Autoimmune diseases

ABSTRACT

The goal of this project was to study the feasibility of using a DNA-immobilized nanocellulose-based immunoadsorbent for possible application in medical apheresis such as systemic lupus erythematosus (SLE) treatment. Calf thymus DNA was bound to high surface area nanocellulose membrane at varying concentrations using UV-irradiation. The DNA-immobilized samples were characterized with scanning electron microscopy, atomic force microscopy, and phosphorus elemental analysis. The anti-ds-DNA IgG binding was tested in vitro using ELISA. The produced sample showed high affinity *in vitro* to bind anti-ds-DNA-antibodies from mice, as much as 80% of added IgG was bound by the membrane. Furthermore, the binding efficiency was quantitatively dependent on the amount of immobilized DNA onto nanocellulose membrane. The described nanocellulose membranes are interesting immunoadsorbents for continued clinical studies.

1. Introduction

Autoimmune diseases are diseases wherein antibodies, which are normally produced to protect the organism against external pathogens, target one's own tissues and organs. The traditional treatment of autoimmune diseases involves immunosuppression with steroids or cytotoxic drugs such as cyclophosphamide, azathioprine, or mycophenolate mofeil. However, considering that women are more prone to autoimmune diseases (in some cases up to 90% of the patients being women of childbearing age) prescription of immunosuppressant and cytotoxic drugs during pregnancy is contradicted. Furthermore, immunosuppressant drugs cannot be administered in patients with active infection, e.g. tuberculosis.

In cases when administration of immunosuppressant drugs is contradicted and when acute intervention in severe cases is necessary, immunoadsorption can be used [1]. Further, immunoadsorption plasmapheresis is also used during the so-called "synchronization" treatments, when extracorporeal immunoadsorption is combined with high-dose administration of immunosuppressant (e.g. cyclophosphamide) to achieve long lasting therapeutic effect [1]. During immunoadsorption apheresis, blood (or plasma) is circulated over special adsorbent materials and then recycled back to the patient. The adsorbent is typically decorated with various ligands featuring high affinity for harmful antibodies. This method has been shown useful for treating many severe autoimmune diseases, including systemic lupus erythematosus (SLE), myasthenia gravis, Guillain-Barré syndrome, Miller-Fisher syndrome, Sjögren's syndrome, rheumatoid arthrirtis, multiple sclerosis, lupoid sclerosis, idiopathic thrombocytopenic purpura, and haemophilia [2]. Another important application of immunoadsorption includes transplantation medicine since autoimmune reactions are the main cause of organ rejection. In this study, we focus on immunoadsorbets for treatment of SLE as a prototype autoimmune disease.

SLE is a potentially fatal disease, characterized by evolution of autoimmune antibodies and autoreactive T cells against one's own double stranded (ds) DNA-protein complexes (anti-ds-DNA, anti-histone, antichromatin antibodies), ribonucleoproteins (anti-Sm, RNP, Ro/SS-A and La/SS-B antibodies) and nucleosomes [3]. SLE can affect almost any organ system, with the kidneys being the most commonly involved target. The prevalence of SLE varies from 15 to 50 cases per 100,000 persons, and each sixth patient in whom SLE is diagnosed at 20 years of age is likely of dying by 35 [4]. Female patients of childbearing age constitute 90% of the total number of patients with SLE [5].

Immunoadsorbents for treating SLE were among the pioneering materials for medical apheresis. In particular, immobilized Protein A columns against SLE were first suggested for immunoadsorption by Terman and co-authors [6]. DNA-colloidon-charcoal membranes were also shown efficient in binding human anti-DNA antibodies [7]. DNA-immobilized adsorbents to adsorb anti-DNA

* Corresponding authors.
E-mail addresses: changgang.xu@angstrom.uu.se (C. Xu), albert.mihranyan@angstrom.uu.se (A. Mihranyan).

http://dx.doi.org/10.1016/j.colsurfb.2016.03.014
0927-7765/

antibodies have been studied for SLE treatment in clinics [6–10]. An immunoadsorbent column consisting of ss-DNA-cellulose incorporated in agar gel demonstrated a 65% reduction in DNA binding of serum [10]. Following these studies, the first clinical application of DNA-immobilized immunoadsorbents for treatment of a female patient with SLE was reported, demonstrating decreased titre of ss-DNA auto-antibodies, resolution of immune complexes in biopsied kidneys, and overall improvement of clinical status [11]. Additional clinical studies on numerous patients conducted over the past decades by the Nankai University group in China confirm the usefulness of the apheresis approach using DNA-immobilized adsorbents for treatment of SLE, especially in acute cases [12–16].

As an alternative to relatively expensive and sensitive biologically active proteins and nucleic acid ligands, more robust albeit less specific ligands were developed during the 1990s. In particular, non-specific ligands featuring both hydrophobic and charged ligands were tried. It was shown that anionically charged cellulose based immunoadsorbents (decorated with dextrane sulfate, polyacrylate, or sulfanilic acid) show higher anti-ds-DNA/ss-DNA binding capacity than cationic (decorated with lysine) or hydrophobic (decorated with tryptophane or phenylalanine) immunoadsorbents [17,18]. It should be noted that in general DNA immobilized immunoadsorbents show an order of magnitude larger anti ds-DNA antibody binding capacity for SLE treatment than non-specific immunoabsorbents e.g. decorated with dextran sulfate, tryptophan or phenylalanine [19]. Although immunoadsorbent columns packed with beads are prevalent [19–21], membranes can also be used for immunoadsorption. In particular, DNA immobilized immunoabsorbent membranes on polyethylene terephthalate (PET) [22] and polyvinylidene difluoride (PVDF) [23] have been realized in the past.

Cellulose is a common immunoadsorbent material [16]. It offers many of the desirable properties including chemical inertness, mechanical strength, insolubility in water, hydrophilicity, ease of derivatization, possibility of sterilization by autoclavation, and biocompatibility. Immobilization of nucleic acids onto cellulose can be achieved both chemically, e.g. covalent binding after surface activation by periodate oxidation, and physically, e.g. by using UV-light [24,25]. Non-covalent binding of nucleic acids onto cellulose with UV light is a particularly attractive and facile method of immobilization, previously explored to develop affinity chromatography columns and adsorbents for removal of DNA-binding endocrine disruptors [26–30]. Several reports studied the feasibility of using cellulose beads for selective removal of immune complexes, including anti-DNA antibodies and cytokines [22,31–36]. Thus, while the specific binding capacity of an immunoadsorbent is important, the choice of the matrix material is also based on several other factors such as absence of toxicity, previous clinical record, ease of manufacturing, robustness, ease of derivatisation, and overall cost-efficiency. For this reason, direct UV-light catalyzed immobilization of DNA on cellulose without chemical cross-linkers is highly appealing for development of immunoadsorbent materials.

Although cellulosic materials have been studied extensively for medical apheresis and affinity chromatography applications, it has been pointed that "*cellulose fiber has no effective porosity or extended surface area*" which limited the capacities for ligand and target molecules [24]. Nanocellulose offers extensive surface area as compared to ordinary cellulose, i.e. almost two orders of magnitude larger [37] and therefore may potentially provide enhanced binding capacity for antibodies. Nanocellulose membranes have been studied for various biomedical applications including virus removal, cell culture and hemodialysis [38–46]. Further, bacterial cellulose has been studied for affinity separation of human serum albumin [47] and peptide-modified cellulose nanofibers as IgG-binders in vitro. [48] The goal of this project was to study the feasibility of using a DNA-immobilized nanocellulose-based immunoadsorbent for possible application in medical apheresis such as SLE treatment.

2. Materials and methods

Calf thymus DNA solution (5 × 1 mL, 10 mg/mL; Invitrogen^{TM}) was purchased from Life technologies (USA). The nanocellulose was of algae origin, viz. *Cladophora* cellulose (G3094-10), as obtained from FMC Biopolymer, USA. The 37% hydrochloric acid was purchased from Merck KGaA, Darmstadt, Germany. Albumin from bovine serum (BSA, lyophilized powder) was obtained from Sigma-Aldrich. Mouse anti-dsDNA IgG ELISA Kit (Cat. No. 5120) and mouse anti-dsDNA IgG (Cat. No. DNA12-M) were purchased from Alpha Diagnostic International Inc., Texas, USA. General purpose filter paper (Munktell), herein below denoted as *ordinary filter paper*, was purchased from VWR International.

2.1. Preparation of the nanocellulose membrane

The nanocellulose was washed 5 times with ethanol and rinsed 3 times with distilled water, and finally freeze-dried before use. The cellulose was then mixed with 75 mL distilled water and sonicated for 20 min with 30 s on/off pulse at 70% amplitude using a high shear ultrasonicator (Sonics Vibra Cell, Sonics & Materials, Inc. Newton, CT USA). The nanocellulose dispersion was then filtered for 2.5 h by vacuum suction, and the formed nanocellulose membrane was then heat-pressed (Rheinstern HP-3805, Mainz, Germany) for 20 min at 100 °C.

2.2. Immobilization of DNA onto nanocellulose by UV irradiation

The calf thymus DNA solution (10 mg/mL) was diluted to 2 mg/mL of DNA solution with distilled water. Aliquot of 5 mL of the diluted DNA solution was then sonicated for 2 min with 5 s on/off pulse at 40% amplitude using a high shear ultrasonicator (VCX 130 Sonics Vibra Cell, Sonics & Materials, Inc. Newton, CT USA). The sonicated DNA solution was then diluted with distilled water to produce aliquots of the following concentrations, i.e. 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL and 0.125 mg/mL. About 35 μL from each dilution was then applied onto both sides of the nanocellulose membranes and ordinary filter paper (each circular membrane weighing around 3.5 mg, and 8 mm in diameter) and dried for 5 h at room temperature. The samples were then irradiated with UV-light for 3 h at 254 nm (TUV PL-S 11W, Philips, Koninklijke Philips N.V.). The UV treated samples were then repeatedly washed with distilled water (10 mL × 5) for 1 h to remove the traces of non-immobilized DNA. The amount of immobilized DNA on the cellulose samples was quantified by the following procedure: the samples were treated with 1 mL of 1 M HCl solution in an Eppendorf tube (DNA Lobind), while shaken at 50 °C for 1 h on a mechanical shaker (500 rpm). The total amount of immobilized DNA was determined from UV-absorption at 266 nm (UV-1800 model, Shimadzu Co., Ltd., Kyoto, Japan) in the HCl solution as previously described by Yamada et al. [30] To control the extent of ligand leakage, the DNA-immobilized samples were also treated with distilled water at 23, 37 and 50 °C, and the amount of leaked DNA was measured spectrophotometrically at 260 nm in distilled water.

2.3. SEM and AFM

The samples were studied with a scanning electron microscope (Merlin FEG-SEM, Zeiss, Germany) after they were sputtered with Au/Pd to avoid charging. The atomic force microscopy images were acquired on the DNA-immobilized samples in air with Dimension Icon (Bruker, Germany) instrument, equipped with ScanAsyst-Air probes. The sample was mounted on a magnetic holder using a