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Bio-inspired virus imprinted polymer for prevention of viral infections

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ABSTRACT

A novel virus-imprinted polymer for prevention of viral infection was prepared by anchoring molecularly imprinted polymer (MIP) on the surface of poly-dopamine (PDA)-coated silica particles. The imprinting reaction was carried out via self-polymerization of dopamine in the presence of a virus template. Plaque forming assay indicated that the MIP exhibited selective anti-viral infection properties for the template virus in complex media containing different interfering substances, and even other types of viruses. Remarkable dose-dependent and time-dependent inhibition of virus infection was observed due to the MIP's selective binding to the template virus. When the MIP was incubated with the virus and host cells together, rapid and selective adsorption of template viruses by the MIP prevented the viruses to infect the host cells in a period of 12 h. The MIP was biocompatible and non-toxic, and had excellent stability and reusability. Furthermore, the MIPs prepared using different viruses as templates showed similar anti-viral infection properties. The MIP synthesized using dopamine as monomer and crude virus as template provided an attractive possibility for clinical applications in the field of antiviral therapy.

Statement of Significance

This is the first report to prepare artificial antibody (molecularly imprinted polymer, MIP) that can selectively prevent virus infection using dopamine self-polymerization system. Only MIP anchoring on the surface of poly-dopamine coated silica particles and polymerized using ammonium persulfate as radical initiator showed dose-dependent and time-dependent inhibition of template virus infection in complex media containing interferences and even other viruses. Viruses bond to MIP lost infectious capability. When incubated with virus and host cells, MIP rebond viruses rapidly and selectively to prevent viruses infecting host cells for 12 h. The achieved MIPs were biocompatibility, non-toxicity with excellent stability and reusability, and can be used to different viruses. The bio-mimic MIPs provided an attractive prospect for clinical applications in antiviral therapy.
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1. Introduction

Virus-induced diseases have become a major health risk in the world. Antiviral drugs and vaccinations are two main routes of controlling viral diseases. At present, antiviral drugs targeting different stages of viral infection have been explored. Antibodies and immunoglobulins that can neutralize and block virus infection are among the few of the antiviral drugs being used [1]. On the other hand, vaccines have been very successful in preventing viral infections by activating the body's immune system to neutralize or kill viruses [2]. For example, smallpox has been eradicated through mass vaccinations. However, the use of vaccines can cause allergic reactions or other side effects. Finding an alternative way to produce non-allergic materials to neutralize virus will have great clinical applications [3].

Molecular imprinting represents a cost-effective approach to synthesizing artificial antibodies (also named molecularly imprinted polymers, MIPs). The artificial antibodies possess

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stereo-specific molecular recognition and binding capabilities for target molecules (templates). Compared with natural antibodies, MIPs are more chemically stable and inexpensive to produce on a large scale. Thus, MIPs have been widely used as substitutes for antibodies in separation and purification [4], sensing [5] and drug delivery [6–8]. The imprinting of viruses has attracted great attention because virus-selective MIPs have the potential applications in clinical diagnostics and even for in vivo therapy [9]. Until now, most virus-imprinted MIPs are developed as recognition materials for virus detection [10–18]. Whole virus [14–16,19] and an epitope (a short amino acid sequence of the virus protein) [20,21] have been used as templates to prepare virus-selective MIPs. The MIPs prepared using these methods could be used to detect the target virus or the virus coat proteins effectively. For example, MIPs prepared by the epitope imprinting [21] and the whole virus-imprinting [22] have enabled detection of the target virus using a quartz-crystal microbalance sensor.

Recently, a MIP prepared by miniemulsion polymerization was shown to be able to hinder virus infection. The specific adsorption of virus by the MIP was considered as the mechanism of the anti-viral effect. Despite the interesting anti-viral activity, the process of preparing the MIP was tedious, and the temperate virus must be immobilized first before the actual imprinting reaction could be performed. Besides, it was not clear if the virus-imprinted polymers had acceptable biocompatibility and toxicity [19]. Clinical applications of virus-imprinted MIPs are not conceivable due to the lack of biocompatible and non-toxic MIPs. In the previous investigations, only single type of virus was used in each study. As the shape, structure and viability of every virus can differ significantly, a generic imprinting method that can be applied to imprint different types of viruses will be highly valuable for clinical applications, e.g. as specific anti-viral reagents.

Dopamine (DA) is a hydrophilic monomer containing both amino and catechol groups, which can self-polymerize in aqueous conditions at room temperature. DA is suitable to be used to prepare water compatible MIPs when fragile proteins and enzymes are used as templates [23–29]. The amino and catechol groups of dopamine can interact with protein via non-covalent interactions such as hydrogen bond, ionic bond and π-π interactions to generate the binding selectivity of the MIPs. Theoretically, whole virus is an ideal template because it can provide multiple copies of proteins to bind to dopamine. However, until now there has no study reported where whole virus was used as template to prepare virus-imprinted polymer through the self-polymerization of dopamine.

In this paper, we present a facile method to produce virus-imprinted MIP based on the polymerization of dopamine. As poly-dopamine (PDA) provides a convenient route to modify various materials, we fabricated virus-imprinted MIP on PDA-coated silica particles. Different crude (unpurified) bacteria viruses (bacteriophages) were used as templates in our investigation. After confirming their binding selectivity, the virus-imprinted MIPs were tested to evaluate their efficiency in preventing the viral infection. The biocompatibility, toxicity and stability of the MIPs were also investigated.

2. Materials and methods

2.1. Materials

Dopamine hydrochloride was from Sigma-Aldrich. Agar, tryptone and yeast extract were from Oxoid. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum were from GIBCO BRL. Commercial MTT assay kit was from Corning Inc. Ammonium persulfate, tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and other chemicals were purchased from Sinopharm Chemical Reagent Company, China. Human serum was provided by one anonymous volunteer in our lab. Doubly distilled water was used throughout the experiments.

2.2. Preparation of crude virus samples

A bacteriophage (also known as phage) is a virus that infects and replicates within a bacterium. Bacteriophages f2, T4, P1and M13 were used in this study. Phage f2 and its host bacterium (*E. coli* 285) were obtained from the Institute of Hygiene and Environmental Medicine, Chinese Academy of Military Medical Sciences. Phage M13 (host bacterium *E. coli* TG1), T4 (host bacterium *E. coli* BL21), P1 (host bacterium *E. coli* W3110) and their host bacteria were provided by Professor He-ping Dai, Institute of Hydrobiology, Chinese Academy of Sciences. Stocks of phages were prepared by small-scale liquid culture. Unpurified phage precipitation (containing unpurified crude phage particles) was obtained using large scale lysis method according to standard protocols [30]. The phage precipitation was re-suspend in SM buffer (pH = 7.5) to a titer of about 1×10^{13} PFU/mL.

2.3. Synthesis of virus-imprinted MIPs

Virus-imprinted MIPs were prepared with or without using ammonium persulfate as radical initiator. For the method using ammonium persulfate, PDA-modified silica particles were prepared by mixing silica particles (750 mg), dopamine (100 mg), ammonium persulfate (60 mg) in 50 mL Tris-HCl (10 mmol/L, pH = 8.5) under mechanical stirring at room temperature for 24 h. After centrifugation, the PDA-modified silica particles were collected. Virus-imprinted polymers (MIPs) were then synthesized by putting the PDA-modified silica particles (750 mg), dopamine (100 mg), ammonium persulfate (60 mg) and the template virus (1×10^{10} PFU/mL unpurified phage precipitation) together into 50 mL Tris-HCl (10 mmol/L, pH = 8.5) under mechanical stirring at room temperature for 24 h. Then the MIP particles were collected and washed with 3% (v/v) acetic acid containing 1 mol/L NaCl for 6 times, followed by washing with distilled water for 6 times. Non-imprinted polymers (NIPs) were prepared under identical conditions except that the template virus was omitted. For the method without using ammonium persulfate as radical initiator, only the ammonium persulfate was omitted without changing any other conditions.

The morphology of the particles was studied with Tecnai G2 20 TWIN transmission electron microscope (TEM) at 200 kV. Attenuated total reflection (ATR) infrared spectra were recorded using a Perkin-Elmer FTIR instrument. Elemental analysis of carbon, hydrogen, and nitrogen was performed using a Vario Micro cube Elemental Analyzer from Elementar (Germany). For each elemental analysis, 2 mg dried sample was used.

2.4. Selective rebinding and inhibition of virus infection

Batch rebinding experiment and plaque forming assay were used to evaluate the binding characters and the anti-viral properties of MIPs respectively. The rebinding tests were performed under three different conditions: (1) MIPs (40 mg/mL) prepared using different phages as template were mixed with about 1×10^3 PFU/mL corresponding phages in SM buffer at 25 °C for 2 h. (2) The f2-imprinted MIP or NIP (40 mg/mL each) was mixed with different phages (about 1×10^3PFU/mL each) in SM buffer at 25 °C for 2 h. (3) Different amounts of f2-imprinted MIP and T4-imprinted MIP were mixed and added to about 1×10^3 PFU/mL alive phages in SM buffer at 25 °C for 2 h. After a given period of incubation, the titer of the infectious phages remaining in the supernatant (reported as plaque formation units, PFU/mL) was

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