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SCIENCE DIRECT
Biosensors and Bioelectronics 21 (2006) 2329–2338
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

Supported imprinted nanospheres for the selective recognition of cholesterol

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Received 24 September 2005; received in revised form 30 November 2005; accepted 19 December 2005
Available online 29 March 2006

Abstract

The preparation of innovative polymeric systems using molecular imprinting technology for application in extracorporeal blood purification is described. Membranes based on a methylmethacrylate-*co*-acrylic acid copolymer, produced through the phase inversion method, were modified introducing into their structure specific binding sites for cholesterol molecule by adding molecularly imprinted nanoparticles in the membrane matrix. Membranes prepared are intended to selectively remove cholesterol from the blood by using interactions at a molecular level, between the membrane/nanoparticles devices and the template, created during the preparation of polymers. Three polymeric systems in form of nanoparticles were prepared differing in the polymerisation solvent (a mixture of acetonitrile and ethanol (1:1) or pure ethanol), and the molar ratio between the functional monomer and the cross-linker (2.3:1 and 1:1). Two out of three of the prepared polymers showed a very good template rebinding capacity both in phosphate buffer solution (pH 6.9) and in ethanol. In particular the nanoparticles rebound 115.4 mg cholesterol/g polymer in buffer solution, and 57 mg cholesterol/g polymer in ethanol.

The deposition of the nanoparticles on the surface of the phase inversion membranes produced devices with interesting rebinding performances towards cholesterol in buffer solution: a specific recognition of 14.09 mg cholesterol/g system (membrane and nanoparticles) was detected, indicating maintained binding capacity of supported particles as well.

Keywords: Molecular imprinting; Selective membranes; Imprinted nanoparticles; Cholesterol

1. Introduction

Atherosclerosis is one of the major causes of morbidity and mortality in industrialized societies. While there has been significant advance in the treatment of atherosclerosis there is still a great need for more effective treatment interventions. The factors associated with atherosclerosis include: high levels of cholesterol, triglycerides, low density lipoproteins (LDL) and low levels of high density lipoproteins (HDL).

A significant reduction in blood levels of cholesterol and LDL by diet and lipid reducing drugs was found to result in regression of atherosclerosis (West of Scotland Coronary Prevention Study Group, 1998; Scandanavian Simvastatin Survival Study (4S) Group, 1994). However, oral lipid lowering drugs, such as statins, are risky and may cause liver damage; their efficacy is relatively limited, even when they are taken in association with a strict diet. A striking example is found in patients who have the homozygous form of familial hypercholesterolemia (FH) with extremely high levels of LDL-cholesterol even after an appropriate drug treatment. In these patients cholesterolemia can be reduced by specific removal of blood LDL; in practice the patient has to be treated once every 2–4 weeks by selective LDL-apheresis for 2–4 h.

Lowering of LDL by extracorporeal treatment of blood is significantly more effective in reducing blood cholesterol and LDL levels and therefore coronary heart disease risk in severe types of FH patients (Borberg et al., 1983). Cholesterol and LDL

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doi:10.1016/j.bios.2005.12.027

Nomenclature

AA	acrylic acid
AIBN	azobis(isobutyronitrile)
BPO	benzoyl peroxide
CHOL	cholesterol
CP	control polymer
MAA	methacrylic acid
MIP	molecularly imprinted polymer
PMAA	polymethacrylic acid cross-linked with trimethylolpropane trimethacrylate
P(MMAcoAA)	copolymer of methylmethacrylate and methacrylic acid cross-linked with trimethylolpropane trimethacrylate
THO	theophylline
TRIM	trimethylolpropane trimethacrylate

can be removed by affinity adsorption, utilizing as the adsorbent antibodies to LDL or other specific chemical adsorbents, such as heparin (Lupien et al., 1980) or dextran sulfate (Denizli, 2002). LDL removal can also be achieved by heparin precipitation (Seidel et al., 1991) and by double filtration plasmapheresis (Yokoyama et al., 1988).

An interesting way to reduce the level of cholesterol or other lipids that are etiological in atherosclerosis is to use an extracorporeal treatment device based on selective adsorbents prepared by molecular imprinting technique.

This technology allows us to introduce, into a polymeric material, recognition sites for specific molecular species (templates) through the polymerisation of a monomer and a cross-linking agent in the presence of a template (Mosbach, 1994), or through the dissolving of the preformed polymer in a solution containing the molecule to be recognised (Wang et al., 1996). In both cases, the spatial arrangement is maintained by the polymer even after the template has been removed, and confers a selective "memory" towards this molecule. The molecular imprinting technology is a valid alternative to molecular recognition systems present in biological systems, such as those activated by antibodies (Wulff, 1995). The macromolecular matrices prepared with this procedure, in fact, can be stable even in critical chemical and physical conditions, have a life of several years without any apparent reduction in performance and can be used repeatedly without any alteration to the "memory".

In the preparation of an imprinted polymer by radical polymerisation a template is introduced in a porogenic solvent to interact with complementary functional groups of appropriate monomers which are then fixed around the template molecule in the presence of a radical initiator and an excess of cross-linker. The polymer obtained maintains the specific orientation of functional groups even after elution of the template and allows rebinding of the original template with high selectivity.

The interactions of the template with the functional monomers involved in the imprinting and rebinding may be effected by reversible covalent (Wulff and Sarhan, 1972) or non-covalent bonding (Andersson et al., 1996).

Molecular imprinted polymers are being increasingly investigated as selective absorbents for cholesterol (Asanuma et al., 2000; Hishiya et al., 1999; Sreenivasan, 1998; Pérez et al., 2001, 2000; Kugimiya et al., 2001; Davidson et al., 2003). Whitcombe et al. (1995) prepared cholesterol selective adsorbents by a covalent molecular imprinting strategy using an easily cleavable carbonate ester linkage between a phenol monomer and cholesterol during polymerisation. This resulted in the formation of a non-covalent recognition site, bearing a phenolic residue, capable of interacting with the template through hydrogen bonding. The binding capacity of cholesterol for these materials was evaluated only in *n*-hexane to be 114 μmol/g. Sellergren et al. (1998) synthesized polymerisable derivatives of cholesterol and bile acids to be used as amphiphilic monomers in the imprinting of highly cross-linked methacrylates with cholesterol. The polymerisations were processed under conditions favouring apolar intermolecular interactions. The rebinding capacity of molecularly imprinted polymer for cholesterol was evaluated in intestinal mimicking fluids and was 17 mg/g as against 13 mg/g exhibited by the non-imprinted polymer. Through an approach similar to the one used by Sellergren, Hwang and Lee (2002) used cholesteryl (4-vinyl) phenyl carbonate for covalent imprinting and 4-vinyl pyridine for non-covalent imprinting of cholesterol. From chromatographic analysis, covalent imprinting resulted in more selective adsorption of template. Asanuma et al. (1998) synthesized receptors for cholesterol by cross-linking β-cyclodextrin with hexamethylene diisocyanate (HDMI) or toluene 2,4-diisocyanate (TDI) in dimethyl sulfoxide in the presence of cholesterol as template. The rebinding capacity was evaluated in a water/THF mixture (5/6, v/v). The choice of this mixture was justified by the too small solubility of cholesterol in water while the addition of THF provides homogeneous solutions. The molecularly imprinted polymer synthetised in presence of TDI as a cross-linker showed a better rebinding capacity than the one prepared with HDMI; probably TDI is more adequate to regulate the positions of β-CyD residues. Zhong et al. (2001) prepared polymers comprising acryloyl derivates of cyclodextrins which were imprinted using cholesteryl acrylate and *N*,*N*′-diacrylol piperazine as cross-linker. Gore et al. (2004) prepared two polymeric systems imprinted by cholesterol: the former was characterised by cholesterol conjugated monomers and hydrophilic cross-linkers; the latter was made up by cross-linkers containing covalently linked cholesterol. The rebinding tests of cholesterol were carried out from aqueous media. The polymers prepared from monocholesteryl itaconate as monomer and ethylene glycol dimethacrylate as cross-linker showed the best rebinding capacity: 32.5 mg/g for MIP and 23.3 mg/g for CP.

Usually molecularly imprinted polymers have been prepared in the form of a macroporous monolith, then ground and sieved to the required particle dimensions. Recent improvements in the morphology of MIP particles have been achieved using a precipitation polymerisation procedure that allows to obtain micro- or nanospheres with regular size and shape and particularly able to rebind effectively template molecule due to the high surface/volume ratio (Ye and Mosbach, 2001).