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Molecularly imprinted submicronspheres for applications in a novel model biosensor-film

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ABSTRACT

Two different molecularly imprinted polymers (MIP1 and MIP2) in the form of submicron-particles were obtained by radical polymerization, using acetonitrile as a solvent, methacrylic acid (MAA) as a functional monomer, a cross-linking agent (trimethylolpropane trimethacrylate (TRIM) for MIP1 and pentaerythritol triacrylate (PETRA) for MIP2) and phenylalanine aminoacid (Phe) as a template molecule. The extraction of Phe template from polymer submicron-particles by washing steps allowed the formation of free recognition sites for the selective rebinding of template molecule. Rebinding ability was evaluated in acetonitrile and in phosphate buffered saline by chromatographic methods and compared to that of corresponding control polymers. As MIP1 showed a superior specificity towards Phe rebinding as compared to MIP2, it was selected as a component of a poly(L-lactic acid) (PLLA) based model sensor-scaffold. Impedance measurements, carried out on PLLA films loaded with as-produced, extracted or rebound MIP1 submicron-particles demonstrated the possibility to build a scaffold capable of sensing the amount of rebound template. In the future, devices incorporating MIP submicron-particles able to rebind extracellular matrix or transmembrane proteins will be respectively used as structures promoting cell adhesion and proliferation and sensor-films monitoring cell colonization.

1. Introduction

Molecular imprinting is a technique that allows the synthesis of innovative polymers containing highly specific recognition sites for molecules of interest in a wide range of applications [1–3]. Due to their ease of preparation and their mechanical and chemical robustness, molecularly imprinted polymers (MIPs) have been successfully and extensively employed in several applications, such as stationary phase in chromatography [4], chiral separation materials [5], solid phase extraction adsorbents [6], drug delivery devices [7], artificial antibodies in immunoassays [8], recognition components in chemical sensors [9] or biosensors [10] and enzyme mimics in catalysis [11]. Furthermore MIPs have been used as antibodies, since the selectivity and affinity properties of MIPs are comparable with the ones displayed by natural recognition systems [12].

Molecularly imprinted polymers are generally prepared by two different approaches: (i) phase inversion and (ii) polymerization. In the molecular imprinting technique by phase inversion [13], the cavities for molecular recognition have been introduced into the polymer though the dissolution of the preformed polymer in a solution containing the print molecule to be recognized (template). The molecular imprinting by polymerization have generally been based on three steps: (1) the template molecule interacts with the functional monomer(s) in a predetermined orientation; (2) the monomer–template complex is copolymerized with a cross-linking agent, leading to the formation of a rigid polymeric network with the print molecule in a sterically fixed arrangement; (3) the template is removed, so the resulting polymer molded around the template presents free recognition sites able to specifically rebind the same template.

The imprints of the template, having complementary shape, size and functional group orientation with respect to the print molecule, have been found to be involved in the specific recognition of the template rather than of another chemically similar molecule. The interactions between template and monomers have been found to be reversible covalent bonding [14–16], non-covalent [8,17,18], or metal ion coordination [19–22]. Typically, molecular imprinting technique has involved the formation of ionic interactions and hydrogen bonds [23] between the template and the monomer. Many unsuccessful attempts have been performed to obtain an efficient recognition system in a polar medium such as water, with the aim to mimic the typical in vivo binding environment [24].

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Conventional methods for the preparation of molecular imprinted polymers have allowed the obtainment of MIPs with different physical configurations, such as monoliths [25–27], membranes [28–31] and monolayers [32,33]. Submicron or microspheres have ensured a better accessibility of template molecules to the specific binding sites. Polymer beads have been realized by time-consuming grinding/sieving steps, or by complicated multistep swelling in microemulsion systems [34–37]. On the other hand, the method developed by Ye et al. [38] has allowed the production of regular shaped microspheres without the use of any interfering components as surfactants or stabilizers [39–41]. This technique has been based on precipitation polymerization of functional monomer and cross-linking agent, in the presence of template molecule under condition of high dilution. According to this method, during the cross-linking process the growing polymer chains exceed the limit of solubility, leading to phase separation. Dilution of the reaction system results in a dispersion of macrogel particles in the solvent, and further increasing of dilution finally leads to microscale beads.

The molecular imprinting of macromolecules, such as proteins, has been found to be difficult due to the competing action of the solvent against the analyte in the interaction within the binding site, and to sterical and thermodynamic reasons, specifically large non-rigid templates may yield less well-defined recognition sites for the successive rebinding [42].

The results achieved nowadays with molecular imprinting technology offer very interesting perspectives for its applications in the tissue engineering of intelligent matrices for cell adhesion and proliferation (scaffolds) [43,44]. Molecular imprinting of transmembrane and extracellular matrix (ECM) proteins, such as integrins, collagen and fibronectin, could allow the realization of scaffolds promoting the ligand-receptor type bonds between cells and imprinted proteins.

A gain benefit from the tissue engineering applications could also be the realization of scaffolds with the intrinsic capacity to monitor and quantify the cell adhesion in real time. The realization of molecularly imprinted scaffolds with recognition properties towards molecules produced by cell metabolism and capable of emitting a variable signal during the rebinding process of the molecules above, surely represents an attractive prospective. Although biosensors delegated to the recognition of specific molecules have generally consisted of appropriate biomolecules, such as antibodies or receptors [45], MIPs have been also proposed for this purpose offering advantages with respect to classic devices. MIPs advantages are their lower production costs, ease of preparation and high stability even in aggressive chemical environment or in the presence of high temperature or pressure [46]. The other key component of a biosensor is a transducing element which converts the signal emitted after the recognition of the target molecule into a measurable effect: the transduction can be carried out by a variety of transducing elements, including gravimetric [47–50], optical [51,52] and elettrochemical [53] sensors. Gravimetric sensors are typically based on acoustic wave devices, such as the Quartz Crystal Microbalance (QCM) or the Surface Acoustic Wave (SAW) sensor, that can resolve mass changes with sub-submicrongram precision. The QCM has been commonly applied to detect nandrolone [54], okadaic acid [55], caffeine [56], paracetamol [57], and to monitor the environmental contaminants [58,59]. Optical sensors have been recently developed for the detection of sialic acid based on Surface Plasmon Resonance (SPR) device coated with a MIP [60]. Moreover the researchers have fabricated submicronscale optical sensors coated with MIPs, able to significantly quench the photoluminescence emission when the template recognition occurs [61]. In addition to gravimetric and optical transduction, electrochemical methods have been extensively applied as sensor devices. Electrochemical sensors can be divided on the basis of their working principle in voltammetric (the most common), capacitive, conductometric, ion selective field effect transistor (ISFET), amperometric and potentiometric [62,63].

This work was aimed at the development of molecularly imprinted submicron-particles capable to support and enhance cell adhesion due to the presence of specific recognition sites for the selectively rebinding of molecules typically present in biological environment and favouring cell adhesion. Two different MIPs imprinted with phenylalanine (Phe) were realized. In this research, the precipitation polymerization was employed to produce Phe molecularly imprinted submicron-particles in acetonitrile, using methacrylic acid (MAA), trimethylolpropane trimethacrylate (TRIM) or pentaerythritol triacrylate (PETRA), and finally azobis(isobutyronitrile) (AIBN), respectively as functional monomer, cross-linking agents and radical initiator. The morphological properties of molecularly imprinted submicronspheres were investigated by scanning electron microscopy. Moreover, their capacity to selectively recognize phenylalanine was compared with that of non-imprinted control polymers. In particular, once removed the print molecule, dynamic rebinding experiments were performed and the results were analysed by high performance liquid chromatography (HPLC). In order to simulate a scaffold supporting MIPs submicronspheres and able to act as a sensor for the process of cell adhesion, poly(L-lactic acid) (PLLA) films [64] were loaded with MIPs submicron-particles, respectively obtained after the polymerization, extraction and rebinding processes. Impedance measurements were carried out on these systems to confirm the sensing ability of PLLA films loaded with MIPs. Further step will be the realization of a sensing structure containing MIP submicron-particles imprinted with transmembrane proteins, that favour cell adhesion and viability, that in this way can be monitored.

Table 1
Preparation of molecularly imprinted polymers.

Polymer[a]	Phe (mmol)	MAA (mmol)	TRIM (mmol)	PETRA (mmol)
MIP1	1.18	4.72	4.72	
CP1		4.72	4.72	
MIP2	1.18	4.72		4.72
CP2		4.72		4.72

[a] MIP: molecularly imprinted polymer; CP: control polymer.

2. Experimental details

2.1. Materials

Methacrylic acid (MAA > 99%), trimethylolpropane trimethacrylate (TRIM), and pentaerythritol triacrylate (PETRA) from Aldrich were used as supplied. Azobis(isobutyronitrile) (AIBN; purity grade > 98%) from Fluka and phenylalanine (Phe; purity grade > 99%) from Aldrich were of the highest commercially available purity grade. Anhydrous acetonitrile, trifluoro acetic acid, acetic acid and chloroform with HPLC purity grade were all purchased from Carlo Erba. Poly(L-lactic acid) polymer (PLLA, M_w 110,000) was supplied from Aldrich.

2.2. Preparation of molecularly imprinted submicron-particles

Molecularly imprinted polymers in the form of submicron-particles were prepared by precipitation polymerization. The polymerization process was performed in acetonitrile in the presence of traces of trifluoro acetic acid and acetic acid. Molecularly imprinted polymers, coded as MIP1 and MIP2, and synthesized with the compositions shown in Table 1, differed exclusively for the employed cross-linking agents (TRIM or PETRA, respectively).