NANOCYTES-Technology – Biomimetic nanoparticles for molecular recognition by molecular imprinting

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ABSTRACT

Living systems communicate by molecular recognition. This central principle of the living world is performed at the contact sites of different objects such as single macromolecules or highly complex supramolecular assemblies as which living cells may be described. Molecular recognition capabilities are evoked at artificial materials by the NANOCYTES-technology of the Fraunhofer IGB and biomimetic nanoparticles are synthesized that possess molecularly recognizing properties.

NANOCYTES carry molecularly defined binding sites at their surface. These binding sites are either composed from biologically derived macromolecules or fully synthetic receptors. Core-shell nanoparticles are particularly suited for this purpose, e.g. to immobilise a specific protein or a protein complex at their shell surface. Entirely synthetic molecularly recognising nanoparticles can also be prepared by chemical nanotechnology. A cooperative chemical reaction evokes the formation of specific molecular binding sites at the surface of copolymer nanoparticles. Such synthetic receptors may be employed e.g. as specific absorbers to remove micropollutants from the drinking water cycle or as functional unit of a biosensor. The talk will highlight the design and application of biomimetic nanoparticles based on the structural concepts described above.

Keywords: synthetic receptors, core-shell nanoparticles, biomimetics, specific absorbers.

1 NANOCYTES BY MOLECULAR IMPRINTING

Molecular imprinting of polymers (MIP) is a highly attractive route to synthesize artificial receptors which combine the specificity of biological binding sites with the superior chemical stability of synthetic materials [1], Selective molecular binding sites are induced in a growing cross-linked polymeric material by template interaction of a non-polymerising agent which interact non-covalently with specific parts in the polymer. The templates are washed from the generated polymer monoliths and the induced artificial binding sites can be applied for molecular recognition reactions.

2 SYNTHESIS AND APPLICATION OF MOLECULAR IMPRINTED NANOCYTES

Nanostructured MIP monoliths (nanoMIP) can be prepared by a modified miniemulsion polymerisation technique, where the monomer, the template, the cross-linker, and the initiator are reacted in the droplet cavities of an emulsion [2,3]. The reaction, although complex, runs in a single reaction chamber and in a single-step chemical process. Thus a variety of active agents ranging from low molecular weight drugs to peptides, proteins and biomacromolecules can be imprinted [4,5].

NanoMIP dispose of the high surface area of nanostructrued material and are thus highly attractive for use as specifically absorbing material. Moreover, nanoMIP synthesis overcomes some crucial limitations of the preparation of imprinted material such as chemical inhomogeneity of a molecular imprinted polymeric matrix.

The technique of miniemulsion polymerization results in particles with typical sizes between 50 nm and 300 nm. Besides classic miniemulsion polymerization (hydrophobic phase emulsified in hydrophilic phase – here water), a MIP technique also based on inverse miniemulsion polymerization is established. Thus, possible templates can be chosen from the full range of hydrophilic over amphiphilic to hydrophobic molecules [5]. Additives like inorganic nanocrystals or organic fluorophores can be easily added to polymerization process.

Nanostructured hybrid materials with multiple properties (fluorescence, magnetism) and specific binding sites is a promising functional material for modern bio-analytics and diagnostics as well as in down stream processing in chemical and pharmaceutical industry. Widespread use in applications ranging from medical technology to environmental technology can be envisaged with the approach of nanostructured molecularly recognizing core-shell particles – NANOCYTES.

2.1 Magnetic cores

The described technique of miniemulsion polymerization (in figure 1) results in particles with typical diameters between 50 nm and 300 nm. Besides classic miniemulsion polymerization (hydrophobic phase emulsified in hydrophilic phase), a MIP technique based on inverse miniemulsion polymerization was also established.

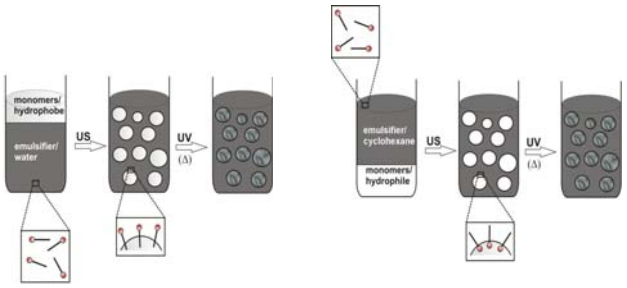

Figure 1: Scheme of a miniemulsion polymerization (left) and an inverse miniemulsion polymerization (right) process. Nanodroplets are formed from a reactive mix containing monomers and template by ultrasonication (US) and are emulsified in the continuous phase. Subsequently the polymerization is started by UV-initiation.

Core-shell nanoparticles equipped with molecular recognition properties of MIPs at the shell surface and a magnetizable core are promising tools in separation and purification processes.

Four concepts of magnetic nanoparticles are described in literature [6]. The most popular concept is co-precipitation: coated magnetite nanoparticles can be obtained from aqueous Fe(II) and Fe(III) salt solutions within a relatively narrow particle-size-range. Another concept describes the thermal decomposition of organometallic compounds in high-boiling organic solvents [7, 8]. Figure 2 shows magnetite nanoparticles produced via thermal decomposition of iron(III)acetyl acetonate.

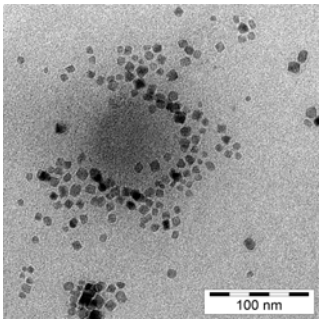

Figure 2: TEM-micrograph of oleic acid coated magnetite nanoparticles with a mean diameter of 8 nm via thermal decomposition of iron(III)acetyl acetonate.

The surface of the magnetite nanoparticles was modified for use with a hydrophilic shell or hydrophobic shell. Hydrophilic surface carboxy-groups were introduced via co-precipitation or vinyl-groups were introduced via a ligand exchange procedure.

2.2 Specific adsorbers for water treatment

In recent years, the excessive exploitation of natural resources and widespread use of chemicals has led to the accumulation of various (micro-)pollutants in our environment. Some of the existing, pending or potential emerging contaminants are synthetic organic compounds (SOCs), such as pesticides, pharmaceutical active compounds (PhACs) and endocrine disrupting compounds (EDRs) and have been identified so far in the aquatic cycle. The decomposition of such organic compounds using physicochemical methods, such as ozonolysis is either rather costly or the process itself produces toxic decomposition products. Thus, specific adsorbers to remove the contaminants from the water cycle open a promising way to tackle the problem.

Bisphenol A (BPA) is one of the highest volume chemicals in the world [9]. BPA concentrations in the range of up to 320 ng L^{-1} in river waters [10] and 700 ng L^{-1} in sewage effluents [11] have been reported. BPA is a xenoestrogen that can disrupt endocrine function and adversely affect the reproductive systems of wildlife and humans. Its weak estrogenic activity has been confirmed *in vitro* and *in vivo* [12]. By reason of the widespread applications of BPA and its associated disposition in the aquatic environment, BPA is an eminent target molecule for developing specific adsorbers for application in aqueous media.

MIPs and non-imprinted polymers NIPs as control polymers were prepared with methacrylic acid (MAA) or 4-vinylpyridine (4-VP) as functional monomer. The influence of the functional monomer on non-specific binding and binding capacity was examined. For all syntheses a cross-linker (ethylene glycol dimethacrylate EGDMA) amount of 80 mol% was used and the template-to-functional monomer-ratio was 1:4 (Figure 3). The polymerization was UV-initiated and performed in double-walled reactors which were cooled with water at 5°C.

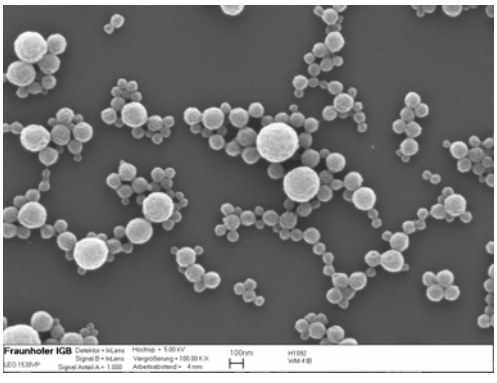

Figure 3: SEM micrograph of molecularly imprinted polymers. Synthesized with the polymer system poly(methacrylic acid-*co*-ethylen glycol dimethacrylate).

Binding studies were performed in different aqueous solvent mixtures to evaluate the effect of the incubation media on binding capacity and non-specific binding. Figure 4 shows the effect of the aqueous solvent mixtures and the effect of the functional monomer on binding capacity and non-specific binding. It can clearly be seen that the amount of adsorbed BPA increased with increasing the polarity of the incubation media and with increasing the hydrophobicity of the polymer.

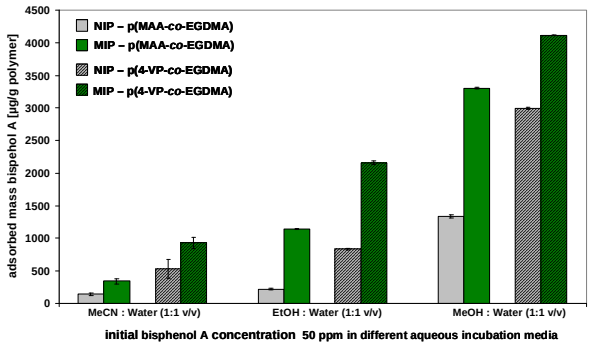

Figure 4: Results of the binding experiments with p(MAA-*co*-EGDMA) and p(4-VP-*co*-EGDMA) MIPs and NIPs in a variety of incubation media.

The highest amount of BPA adsorbed at the MIPs which were synthesized with 4-VP as functional monomer and in a methanol-water solution as incubation media. However, it is obvious that with the total amount of bound BPA increasing the amount of non-specific bound BPA is increasing too. The best ratio of non-specific binding to total binding capacity was found for the polymer with MAA as functional monomer and the incubation media ethanol-water. These results show the strong impact and correlation of hydrophobicity of the polymer, hydrophobicity of the target molecule and the polarity of the incubation media. It seems that the main driving forces for the adsorption are hydrophobic interactions between target molecules and polymer. Further experiments have the ambition to minimize the non-specific adsorption by increasing the hydrophilicity of the polymer by building a permeable, hydrophilic shell around the polymer.

2.3 Rational design of synthetic receptors for biomacromolecules

MIPs for applications in purification processes of biomacromolecules such as proteins are highly attractive goals. MIPs have been reported to show specificity and selectivity similar or superior if compared to natural receptors such as antibodies. Acdditionally, the synthetic MIPs may be able to withstand more drastic conditions like elevated temperature or extreme pH if compared to regular proteins. On the other side, imprinting of water-soluble biomolecules represents a great challenge. The imprinting in aqueous media is quite demanding as water lowers the effectiveness of hydrogen bonds between template and monomers significantly.

Aiming for the rational design of synthetic receptors for biomolecules the polypeptide insulin was chosen. Insulin is one of the most important biotechnological synthesized proteins worldwide due to its crucial role in the treatment of diabetes mellitus.

Insulin is a water-soluble molecule and thus inverse miniemulsion polymerisation is applied for the synthesis of polymers which shall become functionalised by evoking artificial binding sites for insulin sequences at their surface. The structure of insulin was analyzed and a short and sterically easily accessible sequence from the macromolecule was chosen as preferential template sequence for the creation of MIPs for specific recognition of insulin. The peptide Thr-Pro-Lys-Thr (TPKT) from the C-terminus of the b-chain of the insulin was selected.

Additionally to the selection of a suitable template, the choice of applicable monomers is of particular importance for the creation of MIPs. For rational design of MIPs for insulin three water-soluble monomers were chosen: acrylamide (AAm), *N*-(1,1-dimethyl-3-oxobutyl)-acrylamide (OxobutylAAm) and N-isopropylacrylamide (NIPAAm). To learn about the possible attractive interaction between the monomers and the template, molecular modelling calculations were applied. The stronger the interactions between the functional monomer and the template are, the higher the probability to create MIPs which successful recognise and bind the template. In this simulatios, each of the chosen monomers were brought in contact with the amino acid threonine which is contained twice in the selected peptide TPKT. Applying a semi-empirical quantum mechanics method [13], the interaction potentials were calculated and exhibited the most attractive interaction between threonine and acrylamide (Figure 5). First experimental approaches by imprinting MIPs with TPKT and applying them for adsorption confirmed the result of the simulation.

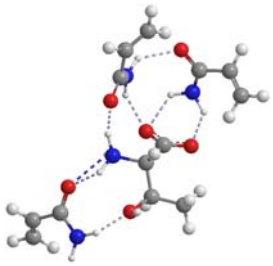

Figure 5: Interaction between threonine and three molecules of acrylamide. The dashed lines exhibit the H-bonds between the template and the monomers.

2.4 Process development – Scale-up aspects of NANOCYTES synthesis

While the previous results were solely conducted at laboratory scale the subsequent section covers the conversion of the NANOCYTES synthesis to pilot scale.

Originating from a laboratory scale of 20 mL the target scales for process development are 1 L and 5 L which represents a cubic scale-up factor of 50 and 250, respectively.

The overall process can be divided into three process steps: emulsification, polymerization and down-stream processing. As dosing is facilitated at higher reactant volumes, certain unit operations cannot simply be converted to a larger scale. The emulsification by ultrasound, applied at the 20 mL laboratory scale, suffers from the limitation of an inhomogeneous droplet distribution at larger scales. The reason is a difference in the residence time of the fluid elements in the cavitational zone of the batch device. The increase of the sonication time would render the necessity for more efficient cooling of the miniemulsion. High-pressure homogenization is an attractive alternative method to produce emulsions. Therefore a 3^2 factorial analysis was carried out using a MICROFLUIDICS M-110P high-pressure homogenizator. The parameters homogenization pressure, number of passes, and homogenization cell type were analyzed by means of dynamic light scattering (DLS). The response values were the miniemulsion droplet size and distribution as well as polymerized particle size and distribution. Figure 6 illustrates the response surface of the polymerized MAA-co-EGDMA particles.

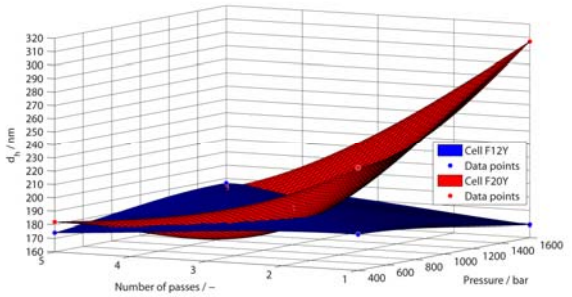

Figure 6: Particle size response surface in dependency of the number of passes and homogenization pressure.

The F12Y cell type produces over the whole range of parameters high quality miniemulsions with narrow size distributions (PDI 0.08 – 0.16). In contrast, the F20Y cell type shows an increase at the first passage and high pressure. Multiple passages can compensate this phenomenon. The acquired data show that with respect to the F12Y cell type at least one or two passages at moderate pressure are necessary to produce high quality miniemulsions. Subsequent to the emulsification process the miniemulsion is cooled to room temperature and directly advanced to the polymerization reactor.

The reaction unit consists of two temperate glass reactors of 1 L and 5 L in volume, agitators and a thermostat. The thermally initiated polymerization is controlled and monitored by a central software application programmed in LabVIEW facilitating a complete automation of this process step. Good thermal transfer and sufficient mixing results in a complete conversion within 3 h. After the cool-down routine down-steam processing by conventional centrifugation completes the overall process.

3 CONCLUSIONS

Biomimetic nanoparticles can be synthesized with a core-shell structure. The core may assure an interaction with an external field, e.g. an electromagnetic field, and the shell can be functionalized to be molecularly recognizing by imprinting. Thus a variety of applications can be tackled with the multifunctional nanomaterial.

4 ACKNOWLEDGEMENTS

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