News and Views

Subject: Chemoenzymatic catalysis

Title: Polymer-decorated Bacteria for cascade catalysis

Standfirst

Polymer/whole-cell hybrid catalysts were created by synthesizing catalytically active polymers from the surface of *Escherichia coli* cells that recombinantly expressed enzymes. The surface-engineered bacteria allowed for orthogonal tandem catalysis, involving photo- or chemocatalytic steps by the polymers on the cells and biocatalytic steps by the enzymes within the cells.

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Main text

The use of biocatalysis and, in particular, whole-cell biocatalysis has become a significant method for synthesizing high-value organic compounds, including active pharmaceutical ingredients, biofuels, and polymers.[1,2] In these catalytic systems, workhorse organisms from molecular biology, such as bacteria and yeast cells, are genetically modified to conduct the desired chemical transformations. This may be achieved by expressing recombinant enzymes within the cells or by displaying such catalysts on their surface. Cells constantly interact with their surroundings through their cell membrane or cell wall. Moreover, the cell surface plays a great role in cell stability and how a cell interacts with neighboring cells or other surfaces. Thus, it is no surprise that it has become important to engineer and modify cell membranes and surfaces to create advanced whole-cell biocatalysts. One way to tune and tailor the properties of a cell is to engineer its surface, which can, most obviously, be done by genetic engineering. Another possibility, however, is to coat the cells with a thin layer of synthetic polymers.[3,4] Synthetic polymers are often much more stable than the compounds found in natural cell membranes and cell walls. Moreover, they can easily be equipped with functional moieties not found in nature, allowing the creation of multifunctional and stimuli-responsive polymer-cell hybrid systems. Several strategies can be pursued to encapsulate single cells in a thin layer of polymer, including the formation of a hydrogel around the cell,[5] chemical conjugation of preformed polymers to cell surfaces,[6] or the extrusion of cells and polymer vesicles together through track-edged membranes.[7] Moreover, polymer chains can be synthesized directly from the cell surface.[4,8-10] This grafting-from approach has the advantage of providing better surface coverage and more precise control over polymer properties. Controlled radical polymerizations such as reversible addition-fragmentation chain transfer (RAFT) polymerization[8] and atom transfer radical polymerization (ATRP)[9,10] have proven to be particularly applicable to cell surface engineering. They result in polymers whose properties, such as molecular weight and functionality, can be controlled by the composition of the monomer mixture and the reaction conditions. Moreover, these reactions

tolerate the presence of a variety of functional groups and can, therefore, be carried out in the presence of whole cells and in complex biological media. Although many monomers of radical polymerizations, such as acrylates, methacrylates, or acrylamides, are cytotoxic, several studies have shown that radical polymerizations can be carried out directly on and even within living cells if the reaction conditions are carefully chosen and if robust organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* are used.[4] Previous studies of cell surface engineering *via* cell surface-initiated polymerization have focused on proof-of-concept, on increasing the stability of the cells, and on demonstrating that synthetic polymers on the cell surface alter the aggregation properties of the cells, e.g., by promoting cell clustering upon addition of molecules that bind to the polymers.[4,8-10] In addition, the polymers have been used as attachment points for enzymes that are not native to the cell, thereby endowing the cells with novel functionalities, such as the ability to degrade cell-toxic surfactants or to digest carbon sources on which they would not otherwise grow.[10] However, the full catalytic potential of polymer-modified cells has yet to be explored.

Now, writing in Nature Catalysis, Wu and coworkers show that the grafting of polymers from *E. coli* can transform bacterial cells into powerful hybrid catalytic systems, in which polymeric catalysts on the surface of the cells work synergistically with enzymes expressed inside the bacteria.[Ref11] The authors used cell-surface initiated ATRP to graft polymers that contain photocatalytic anthraquinones or ruthenium complexes onto bacteria, which recombinantly express a variety of enzymes. This approach establishes a versatile catalytic platform for cascade reactions, combining the catalytic activity of the polymers with the biocatalytic activity of the enzymes. For example, anthraquinone-polymer-modified *E. coli* catalyzed a three-step photoenzymatic reaction converting benzyl acetate to *R*-benzoin (Figure 1a). In this cascade, *Candida antarctica* lipase B (CalB), expressed in one set of cells, hydrolyzed benzyl acetate to produce benzyl alcohol. The polymer-bound photocatalyst on the cells then oxidized the benzyl alcohol to benzaldehyde under UV irradiation. Separately, polymer-decorated *E. coli* cells overexpressing benzaldehyde lyase (BAL) converted the benzaldehyde to *R*-benzoin. Another example is the chemoenzymatic conversion of α-methylbenzylamine to *(R)-α-methylbenzyl alcohol*, where amine transaminase (ATA) from *Silicibacter pomeroyi* transformed the substrate into acetophenone within *E. coli* cells. The acetophenone was then reduced to the chiral alcohol by a polymer-bound Ru(II) catalyst on the cell surface (Figure 1b). The proximity of the polymers and enzymes significantly enhanced reaction efficiency. The polymers also protected the cells and enzymes from stresses such as toluene, low pH, and UV exposure. The system demonstrated excellent recyclability, allowing easy recovery by filtration or centrifugation, with no loss of activity over multiple uses.

Even though the chosen reactions are only model reactions to prove the concept but without providing significant synthetic value, the engineering of the cell surface with catalytically active polymers in tandem with enzymes inside the cells represents an important step in creating whole-cell catalytic systems. The work demonstrates the synergisms that can be created by combining two very different types of catalysts in a polymer-cell hybrid system. Polymer-modified cells with dual catalytic capability could not only find application as reusable catalysts of cascade reactions for the synthesis of pharmaceuticals, other fine chemicals or biofuels, but could also be very useful in applications further removed from catalysis, such as biosensing, the creation of functional and responsive engineered living materials, or the augmentation of synthetic biology systems with non-natural chemical conversion pathways. In addition, we envision that such hybrid systems will allow the study and subsequent engineering of molecular communication between living cells and polymer-based synthetic cells to create powerful hybrid systems in which synthetic cells influence and guide living cells and *vice versa*.

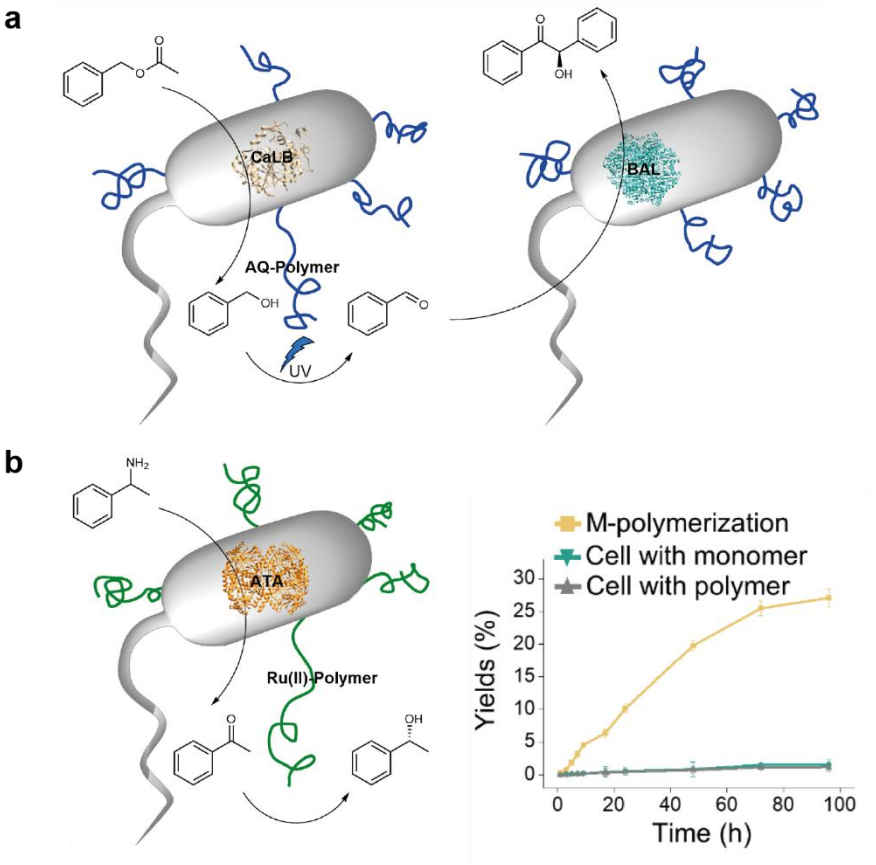

Fig. 1. **Polymer-decorated *E. coli* cells for tandem catalysis.** **a**, Photoenzymatic catalysis: Anthraquinone-containing polymers (AQ-Polymer, blue lines) were grafted by ATRP from the surface of cells that expressed *Candida antarctica* lipase B (CalB) and benzaldehyde lyase (BAL). The cells catalyzed the three-step cascade reaction of benzyl acetate to *R*-benzoin. **b**, Chemoenzymatic catalysis: Polymers containing Ru(II) complexes were grafted by ATRP from the surface of cells that expressed an amine transaminase (ATA). Cells with a medium amount of polymer catalyst (M-polymerization) led to a faster reaction and higher yield in the two-step cascade reaction of α-methyl benzylamine into the chiral (*R*)-α-methyl benzyl alcohol than the cells mixed with the Ru(II)-containing monomer (Cell with monomer) or the (Ru(II)-containing polymer (Cell with polymer). Data and graph reproduced with permission from Ref[11].

Competing interests

The authors declare no competing interests.

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