Protein Polymers

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Towards Bioactive Nanovehicles Based on Protein Polymers**

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Nanomedicine is smoothly paving the way to drug nanocarriers in healthcare and becoming a genuine alternative to traditional medicinal chemistry, improving drug tolerability and efficacy. The emergence of theranostic nanoparticles, which allow clinicians to simultaneously visualize the boundary of a disorder, treat it locally, and monitor its evolution, holds tremendous promise for targeted and personalized medicine. The few nanocarriers that have already gained clinical approval and reached the market were designed based on biologically inert scaffolds (phospholipids, (co)polymers, stealth proteins) and have led to improved pharmacological properties (circulation time, distribution, and degradation). However, in the next generation of nanocarriers, the passive backbone will be replaced by an active one capable of interacting with specific tissues, micro-environments, cells, and subcellular components, responding to local environmental stimuli, releasing the therapeutic payloads according to spatially and temporally controlled processes, and sending out real-time feedback signals to the clinician.

Toward this goal, nanovehicles made of self-assembled copolymers are extremely attractive. Targeting properties can be subsequently endowed to the self-assembled polymer nanoparticles by surface-grafting of biologically relevant receptor-specific ligands (e.g. saccharides, peptides, full antibodies or fragments, aptamers, low-molecular-weight organic molecules). To date, two ligand-targeted polymer nanoparticle systems (namely BIND-014 and CALAA-01) have already entered phase I clinical trials.[1] Strategies for surface functionalization after self-assembly (Figure 1 A), however, present significant pitfalls regarding clinical translation. Scalability and batch-to-batch reproducibility become tricky when multiple functionalities have to be incorporated independently onto self-assembled nanoparticles. Yet, it is not acceptable that the design of superior drug-delivery systems can be accompanied by a decreased degree of precision of the underlying biomaterial structure. To overcome this limitation, one approach consists of designing materials that encode both the self-assembly and the biological functionalities at the molecular level, in analogy to naturally occurring molecules that form biological self-assembled materials. Particularly interesting are macromolecules that combine the advantageous features of block copolymers (i.e. orthogonal block solubility, self-assembly propensity, elasticity) with those of peptides and protein domains (i.e. secondary structure, biological activity, target specificity, diversity, biocompatibility).

For this purpose, synthetic amphiphilic polymer–peptide and polymer–protein conjugates have been developed. Polymer–peptide chimeras can be obtained by covalently attaching biologically relevant peptide fragments to synthetic polymer chains (Figure 1 B). The latter thus display self-assembly properties arising from their amphiphilic character and those eventually afforded by the particular nature of the polymer block, while the peptide sequence of the conjugate confers surface bioactivity to the resulting self-assembled micellar or vesicular structures. Extraordinary complex synthetic architectures have been devised in this way.[2] However, if one considers the extensive diversity and bioactivity that can theoretically be reached from the association of the twenty natural amino acid building blocks, the self-assembled nanoparticles from polymer–peptide conjugates that have been described to date remain relatively primitive.[3] Most peptide blocks are actually poly(amino acids) rather than truly biomimetic peptides. Furthermore, the level of control in terms of macromolecular architecture (primary sequence, length) is intrinsically limited by conventional synthetic polymerization processes.

To obviate this limitation, material scientists have turned towards protein-engineering techniques to produce de novo protein-like polymers by recombinant methods. Mainly constituted from repeating peptide sequences, these macromolecules have been called "protein polymers", "recombinant polymers", and "recombinamers".[4] The principle is based on the exquisite design of an artificial gene encoding for the complete macromolecule, including structural polymeric sequences and specific peptide motifs that feature the desired biological activities (receptor-binding, stimuli-responsive, cross-linking, and enzyme-degradable motifs) placed at

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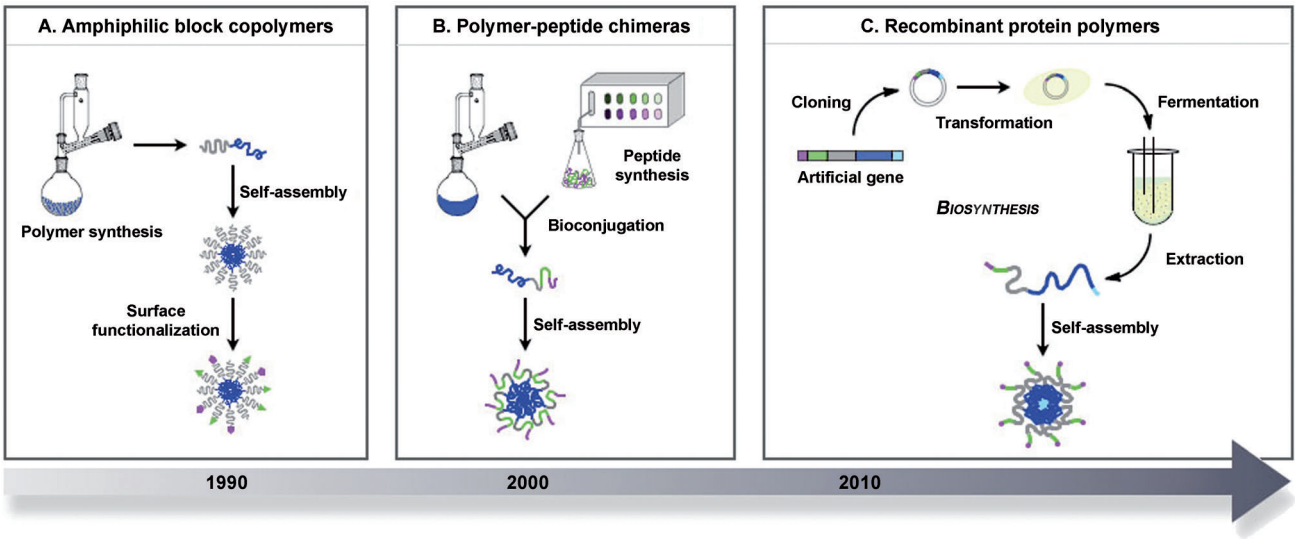

Figure 1. Strategies towards bioactive self-assembled nanovehicles based on polymers: from synthetic block copolymers to biosynthetic protein polymers.

strategic locations into the protein polymer backbone. (Figure 1 C) The tailor-made gene is then cloned into an expression plasmid, transformed, and expressed in a suitable heterologous host. Among the significant advantages of the method are: 1) the potential huge liberty and creativity in material design arising from the infinite combinations of amino acid building blocks; 2) the monodispersity and exquisite control over sequence (primary structure) and length in contrast to chemically synthesized polymer materials; 3) the possibility to study structure–activity relationships ensuing from the latter that are extremely difficult to establish with polydisperse polymers; 4) the molecular length attainable as compared to that by traditional solid- or solution-phase peptide synthesis; 5) the scalability to large batches and/or continuous production with perfect reproducibility.

With the specific aim of developing drug nanovehicles, most recombinant polymers described so far have been based on the pentapeptide repeat [-VPGXG-] derived from the hydrophobic domain of elastin. Elastin-like polypeptides (ELPs) indeed present a lower critical solution temperature (T_t) that has a dual benefit: Firstly, the T_t constitutes a means to control the self-assembly process, a critical issue with peptide and protein materials. Secondly, the T_t greatly facilitates isolation and purification of the protein product from the protein lysate soup. Significant advances have been made since Conticello and co-workers described the self-assembly of the first diblock ELP, which featured consecutive hydrophilic and lipophilic blocks, into nanometer-sized micelles.[5] Comprehensive studies on the design of ELP block copolymers have identified critical parameters (hydrophilic-to-hydrophobic block ratio, copolymer size, distribution of polar and apolar regions along the polymer chain, cross-linking) for the preparation of stable monodisperse core–shell nanoparticles.[6] Temperature responsiveness has been identified as the key to triggering the site-specific targeting and accumulation of ELPs in vivo. Accumulation of ELPs in tumors can be prompted by local thermal cycling; local aggregation of ELP is induced by the heating phase above the T_t and its dissolution and massive extravasation occurs upon return to physiological temperature.[7] Several attempts to increase material complexity and to display bioactive peptide motifs on the surface of ELP nanoparticles have already translated into successful stories. Last year, Chilkoti and co-workers described a recombinant diblock ELP preceded by the $\alpha_v\beta_3$ integrin-targeting linear GRGDS sequence which changed from a low-avidity state as a unimer into a multivalent high-avidity ligand above its T_t.[8] Raucher's group has explored several membrane-translocating sequences fused at the N-terminal end of ELPs to promote intracellular delivery of different therapeutic peptides.[9] A fusion product was thus produced in *E. coli* featuring an elastin-based recombinamer terminated by a penetratin motif and dragging a peptide inhibiting c-Myc oncogene transcriptional function. In vitro cellular uptake and anti-proliferative effects were potentiated by external local hyperthermia prompting ELP phase transition. In applications of ELPs as drug carriers, high-molecular-weight hydrophilic ELPs have also been conjugated at their C-terminal end to hydrophobic doxorubicin derivatives, inducing self-assembly into a drug-rich core surrounded by a soluble protein corona. In vivo, the drug-nanoparticle formulation had a fourfold higher MTD (maximum tolerated dose) than doxorubicin and induced nearly complete tumor regression after a single systemic dose.[10] In addition to ELPs, recombinant polymers based on other structural motifs, such as silk and resilin, have just appeared for drug-delivery applications, providing new properties to the carriers.[11]

En route to highly functional drug nanovehicles, we foresee that protein polymer materials, with a greater potential to mimic the functional complexity of native proteins, are good substitutes for the traditional, synthetic polymers that were highly regarded in the last century. Their biocompatibility and biodegradability into natural metabolites (amino acids) are obvious advantages in biomedical applications. One possible drawback of such protein structures to be considered, however, is that oligomeric degrada-

tion products might have unwanted biological activity. As recombinant protein polymers do not require specific complex post-translational modifications, their large-scale production can be performed in *E. coli* at a lower cost than most recombinant proteins currently used in the clinics (antibodies) which require mammalian cell cultures. The perfect batch-to-batch reproducibility of the material structure obtained by such a process is also a key parameter for future clinical and industrial development.

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