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Well-defined biohybrids using reversible-deactivation radical polymerization procedures

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ABSTRACT

The use of reversible deactivation radical polymerization (RDRP) methods has significantly expanded the field of bioconjugate synthesis. RDRP procedures have allowed the preparation of a broad range of functional materials that could not be realized using prior art poly(ethylene glycol) functionalization. The review of procedures for synthesis of biomaterials is presented with a special focus on the use of RDRP to prepare biohybrids with proteins, DNA and RNA.

1. Introduction

Biomaterials include a full spectrum of natural, synthetic or hybrid materials used for specific, targeted biological applications [1–3]. The development of novel procedures for synthesis of functional hybrid materials within the field of biomaterials has accelerated the treatment of many diseases and provided a benefit to both researchers and patients [4–8]. Applications for biomaterials range from sutures (permanent, bioresorbable or tailored to enhance wound healing) [9], transplanted cell-loaded scaffolds, tissue and cell sheet engineering [10], drug delivery systems, as well as new diagnostic and therapeutic agents [11].

The design of highly functional biomaterials requires knowledge of both chemical reactivity and compatibility of reaction conditions with biomolecules in order to optimize procedures for linkage and retention of biological activity. This requires developing biocompatible reaction conditions and techniques for structure–function assays. Additional considerations include overcoming scale up challenges, due to the requirements of stringent purity and minimal batch-to-batch variation as desired by both regulatory agencies and basic research [12,13]. Facilitating this synergy between chemistry and biology allows for the creation of new biomaterials with custom designed properties.

A classic biomaterial that exemplifies the combination of chemical and biological reactivity is PEGylated protein [14,15]. These biohybrids are prepared by careful selection of reactive groups inherently present on a protein surface with complementary functionality incorporated into the chain-end of poly(ethylene glycol) (PEG) to yield a conjugate with enhanced biological activity. Site selective modification of a protein surface, critical for maximized bioactivity, can now be accomplished using either non-natural amino acids or careful control of reaction conditions to select for specific amino acid residues to react specifically with selected polymer chain-end functionality [16–19].

A new generation of polymer modified proteins is being created with enhanced conjugate properties compared to the first generation PEGylated proteins. These new polymers include molecular bottle-brush architecture PEG structures (i.e. a polymeric chain with oligomeric PEG extending from the backbone) or polymers that can enhance or tailor the bioactivity of the modified biologicals [20,21]. Conjugation with zwitterionic polymers greatly alters the solvation properties and can extend the activity of a protein for longer periods at extreme pH or at high protease and inhibitor concentrations [22]. These next generation biomaterials have built on traditional biohybrids and advanced the fields of organic and material chemistry.

There are many ways to subdivide the field of biomaterials, however this review will focus on the synthesis of biohybrids comprising DNA, RNA or proteins conjugated to synthetic polymers derived from RDRP [23,24]. The covalent modification of biomolecules requires the presence of a suitable pair of reactive complementary functionalities at

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specific sites on the biomolecule and the synthetic material. Biomolecules have a palette of functional handles that are found endogenously or introduced using chemical derivation or genetic engineering. Each class of biomolecules has their own endogenous reactive groups. However, the chemical reactivity of polynucleotides such as DNA and RNA is limited due to the labile nature of the phosphate backbone and lack of synthetic handles on the native monomers. Functional groups can be introduced into DNA and RNA during solid phase synthesis at the 5′ and 3′ ends of the nucleic acids using reactive phosphoramidites. Once cleaved from the solid support, the DNA/RNA can be further derivatized and modified [25–29]. The most commonly incorporated functional groups for subsequent conjugation with polymers are amines [30], thiols [31] and alkynes [32].

A critical requirement for the preparation of bioconjugates is the ability to synthesize well-defined polymers with control over the composition, molecular weight and molecular weight distribution [33,34]. The most commonly used methods to prepare the next generation of functional polymers for biomolecule modification are reversible-deactivation radical polymerization (RDRP) procedures [35,36]. The three most commonly used RDRP methods are nitroxide mediated polymerization (NMP) [37], reversible addition–fragmentation chain transfer polymerization (RAFT) [38,39] and atom transfer radical polymerization (ATRP) [36,40–44].

ATRP and RAFT are most commonly used for the preparation of bioconjugates due to their tolerance of functional groups, range of reaction conditions and polymerization media, and provide the capability to be conducted under biologically relevant conditions [36,45,46]. Although NMP has been successfully used to prepare reactive polymers to graft-to polypeptides [47] the high temperatures (~80 °C) required for polymerization exceed biologically relevant temperatures and therefore NMP has not been explored for grafting-from reactions.

2. Protein bioconjugates — general considerations for synthesis

The most commonly modified biomaterials are proteins, due to their unique ability to act as signaling agents, structural components, and enzymes in vivo. Proteins have a diverse range of functional groups that can be modified compared to most biopolymers. The dichotomy of an amino acid backbone is that while it allows for a number of ligation chemistries, the procedures for the synthesis of well-defined conjugates are elusive since most amino acid residues are represented multiple times in the backbone. Therefore the following major challenges must be resolved when designing protein based bioconjugates: 1) selection of the specific amino acid residue over other residues on a different region of the same protein; 2) a statistical distribution of products, that is typically observed due to the multiple reactive groups available; and 3) excessive modification of proteins as this can be accompanied by a loss of the protein's activity [48,49]. The most readily modified amino acid is lysine whose amine side chain is used to form amide linked hybrids. However, due to their hydrophilic nature, lysine residues are commonly found on all surfaces of a protein and therefore site-specific modification is not usually accomplished [50].

Cysteine is an important structural amino acid that typically forms disulfide bridges extracellularly which can link protein fragments or lock in a protein's tertiary structure. Modification of cysteine residues with thiol-terminated polymers, to form disulfides or with maleimides to form thioethers, has the advantage that only one or two sites on a protein are modified by conjugation with polymers [51]. However, even these lower levels of modification can alter the protein structure, thereby losing bioactivity in the hybrid [52]. Reagents bearing dithiomaleimides have two sites available for reaction with thiols and therefore retention of disulfide bridges can be attained [53,54]. Recently, tyrosine selective oxidation reactions using cerium(IV) salts [55], 4-phenyl-3H-1,2,4-triazoline-3,5(4H)-diones [56] and diazonium [57] have been developed. Thus selective modification of tyrosine is a useful bioconjugation strategy. However, the proteins being modified need to be oxidatively stable.

Selective modification of a protein N-terminus is a powerful strategy for preparation of a mono-functional bio-hybrid. Exact control of the reaction media's pH is needed in order to selectively modify the N-terminus. One must protonate all available lysine residues while leaving the N-terminus in its non-protonated form. An aldehyde or ketone modified polymer is then introduced into the reaction media to form a Schiff base which can be reduced with sodium cyanoborohydride to form a stable secondary amine bond [58,59]. This method is used to prepare a next generation multiple sclerosis drug, Plegridy™, which has superior pharmacokinetics and bioactive properties compared to prior generation materials prepared using non-specific lysine modification [60].

An emerging strategy to selectively introduce reactive functional groups into proteins is the genetic encoding of non-canonical amino acids into a protein [61,62]. There are two primary strategies that are employed in genetic code expansion for incorporation of non-canonical amino acid and both utilize amino acid derivatives that bear a new functional group instead of the native side chain. The first method, called residue-specific mutagenesis, uses amino acyl-tRNA synthases to load a tRNA with a cognate codon so that the new non-canonical amino acid side chain can be incorporated into the protein sequence during translation [61]. This method can insert a non-natural amino acid at every site that is encoded by the native residue. The second strategy, site-specific genetic code expansion, involves a modified amber codon (UAG) tRNA synthetase to recognize a non-canonical amino acid. In this strategy, the non-canonical amino acid is only incorporated when the amber codon is present in the mRNA thereby providing exact control over the position number of non-canonical amino acid's residues [62]. Both of these genetic code expansion methods are used to incorporate azides, alkynes, ketones and aldehydes, among other functionalities, into proteins. The site-specific non-canonical amino acid strategy has the most promise as an applicable procedure for the preparation of discrete bioconjugates with minimal perturbation of protein function.

3. Protein bioconjugates — “grafting from” vs. “grafting to”

The formation of covalent polymer biomolecule hybrids can be accomplished by grafting a polymer “from” or “to” a protein (Scheme 1). The “grafting to” (GT) method uses a pre-formed polymer with a reactive chain-end that is directly conjugated to a suitably reactive protein [14,63,64]. In the “grafting-from” (GF) method, a polymerization initiator is attached to a biomolecule and a polymer is grown in situ [33,34,63,65].

While the GT allows for the straightforward formation of a functionalized protein and subsequent conjugation of the preformed polymer to proteins, the procedure has several limitations. Typically GT suffers from substantial batch-to-batch variability and production of a broad distribution of products. This variability is due to the presence of a multiplicity of reactive groups on a protein surface as well as steric and reactivity constraints that arise when grafting two large macromolecules together. Consequently, in the purification step there is the need to separate high molecular weight polymer from the biohybrid products and thus purification of these materials may be challenging.

The advantages of the GF approach are high yields, compared to GT, and simple purification procedures, due to the large molecular weight differences between the product and the starting materials (i.e. monomers and catalyst). The current limitation is the characterization of the grafted polymer's molecular weight and composition in the case of copolymers. New advances in both GF and GT procedures are overcoming these challenges and both remain viable methods for the preparation of biohybrids.