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Single molecular weight discrete PEG compounds: emerging roles in molecular diagnostics, imaging and therapeutics

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Abstract

“The use of PEGylation has subsequently become commonplace in the development and modification of numerous biopharmaceuticals and has led to many advancements in molecular diagnostics, imaging and therapeutics.”

Keywords

branched; linear; PEG; PEG conjugation; PEG length; PEGylated antibodies; PEGylated peptides; PEGylated protein; PEGylation; polyethylene glycol

PEGylation is the process of covalently attaching polyethylene glycol (PEG) chains to various biomolecules, such as oligonucleotides, peptides, proteins and antibody fragments. The PEGylation process was first described in 1977 by Frank Davis and Abraham Abuchowski [1,2]. It is noteworthy that the first US FDA-approved PEGylated biopharmaceutical became commercially available in 1990 [3-10]. The use of PEGylation has subsequently become commonplace in the development and modification of numerous biopharmaceuticals and has led to many advancements in molecular diagnostics, imaging and therapeutics [5-10].

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PD Davis is the salaried founder, CEO and owner of Quanta BioDesign, Ltd, the company that invented the dPEG® process technology, which also develops, manufactures and markets products based on this core-process technology. Many of the studies referenced in this editorial article use products purchased from Quanta BioDesign, Ltd, or from companies who distribute the same. However, there are no financial interests in the specific projects related to the studies referenced herein, nor are there any financial gains by referencing the aforesaid. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Any given PEG chain is made up of a repeating backbone of ethylene oxide units [5-10,201]. This backbone can be flanked by terminal methoxy groups and functional groups that match the chemistry on the intended biomolecule. This basic construct of the PEG chain is essential for allowing the creation of a modified or conjugated biopharmaceutical product. The molecular construct of the ethylene glycol backbone of PEG is nonionic, hydrophilic, nontoxic, nonimmunogenic and nonantigenic. The goal of PEGylating various biomolecules is to increase their hydrodynamic volume, water solubility and systemic circulatory half-life, while decreasing their proteolytic enzyme degradation and immunogenicity.

The manufacturing process of these traditional polymeric PEG compounds has relied exclusively upon conventional polymerization technology, thus resulting in a polydispersed admixture of many different PEG molecules of varying sizes and molecular weights [5-10,201]. This heterogeneous admixture of polydispersed PEG molecules can vary from manufacturing batch to batch, and can possibly contain hundreds of different PEG species. Functional terminal chemistry groups can vary dramatically in their incorporated yield, thus creating an additionally complicated modification profile. Typically, commercially available PEGylated products contain polymers of molecular weight range averaging from 10, 20 to 40 kDa. Most common is the 40-kDa polymer, which is used as a branched, 2×20 -kDa compound off of a lysine core. Any attempt to commercially market such polydispersed PEG admixtures for consideration towards molecular diagnostics, imaging and therapeutic applications may be fraught with major practical barriers for successful FDA approval, as ideally the production process for any intended PEG product should be practical, easily reproducible and straightforward to characterize. The attempt to use such complex admixtures of polydispersed PEG for conjugation to a given biopharmaceutical can lead to significant losses in the intended biological activity of that biopharmaceutical. These activity losses are largely due to secondary factors, such as steric hindrance and other activities like binding/interaction site issues.

In an effort to overcome the shortcomings of polydispersed PEG admixture products, techniques for generating monodispersed PEG products have been developed. Typically, the generation of a monodispersed PEG product is accomplished by separating a polydispersed PEG admixture into multiple fractions by way of various modes of chromatography, thus attempting to create a PEG product with a narrower range of chain lengths and molecular weight distribution. The mode of chromatography most commonly used is size exclusion chromatography, and the modes less commonly used are chromatographic techniques of hydrophobic interaction chromatography and membrane affinity chromatography [11,12]. However, the resultant so-called monodispersed PEG products, even when optimized to a so-called 'high purity', are still bimodal at best, and generally represent complex admixtures of some 40–60 different species of PEG molecules that are covalently linked to the intended biomolecule [13]. Furthermore, the additional steps needed to attempt to create these 'high purity' monodispersed PEG products are costly, but do not necessarily add significant value to the ultimate efficacy or utility of the intended PEGylated biomolecular product.

To circumvent the issues that are related to polydispersed PEG admixture products and chromatographically separated so-called monodispersed PEG products, a new strategy for manufacturing single molecular weight, discrete PEG compounds has been developed [201,101]. These single molecular weight, discrete PEG compounds have been coined as 'discrete PEG' (dPEG®, Quanta Biodesign Ltd, OH, USA). In this regard, each specific dPEG® molecular species represents a discrete, single compound with a defined number of ethylene oxide units, thus giving it a specified chain length, molecular weight and purity. These dPEG® products are prepared by a patented, stepwise, organic chemistry methodology that does not involve conventional polymerization technology or

chromatographic separation techniques [201,101]. The dPEG® production process is extremely precise yet versatile, allowing for the generation of linear chain dPEG® constructs of four to 48 ethylene oxide units (i.e., from less than 0.2 kDa and up to 2 kDa), as well as branched chain dPEG® constructs consisting of three to nine linear chains (i.e., up to 8 kDa). In addition, all of these dPEG® constructs can be designed in such a fashion that they may contain any one of a variety of different architectures and functional groups for creating a wide variety of resultant conjugated biopharmaceutical products. The linear dPEG® constructs can be made in a wide range of specific dPEG® sizes, and are available as labeled compounds (i.e., with biotin and dyes), as crosslinkers (i.e., with all of the commonly used homo- or hetero-bifunctional chemistries) and as methoxy-terminated dPEG® constructs with concomitant functional chemistries. The use of branched dPEG® constructs can allow for a large apparent hydrodynamic radius with a much smaller increase in the molecular weight of any resultant biopharmaceutical than with the use of polydispersed linear PEGs, an effect that is thought to be due to the rigid nature of the branched dPEG® constructs. These branched dPEG® constructs, while more complex, remain as single unique molecular weight and purity products due to the sophistication of the process controls. Such branched dPEG® constructs can also have terminal charged groups that can be used as a tool to control renal clearance.

To date, many dPEG® constructs have been utilized in basic science investigations and clinical investigations related to molecular diagnostics, imaging and therapeutics. A summary of a few representative and the most important dPEG® strategies is shown in Table 1 [14-33,102-106]. The obvious advantages of utilizing dPEG® constructs for the discrete PEGylation of biopharmaceuticals is inherent within one's ability to absolutely control the molecular weight, chain configuration (i.e., linear or branched) and length for optimizing the pharmacokinetic properties of the biopharmaceutical, as well as for designing specific chemistries and properties for the biopharmaceutical to optimize specific performance characteristics related to the intended end-target site of action [201,101]. With the ever-growing number of newly engineered oligonucleotide, peptide, protein and antibody fragments that are available and of potential clinical relevance, the application of dPEG® constructs in optimizing the activity and function of these biomolecules opens the door to further significant advancements in molecular diagnostics, imaging and therapeutics.

In conclusion, the distinction between using a polymeric PEG admixture and using a single molecular weight discrete PEG compound cannot be overemphasized. As such, each specific dPEG® molecular species represents a discrete, single compound with a defined and specified chain length, molecular weight and purity. The discrete PEGylation of various oligonucleotide, peptide, protein and antibody fragments can allow for precise tailoring and optimization of the specific activity and function of these various biomolecules, thus bringing solutions to established applications in clinical medicine, as well as creating new and vital niches into previously under-represented areas of need within the fields of molecular diagnostics, imaging and therapeutics.

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Website

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Table 1

Representative and the most important dPEG® strategies.

Application	dPEG® species utilized	Biomolecule modified by dPEG®	Effect of dPEG® on biomolecule application	Ref.
Molecular diagnostics	12	PCR products detected by surface plasmon resonance	Helps to eliminate nonspecific binding and gives high signal/noise resolution	[14-16]
Molecular diagnostics	6	Synthetic DNA strands used for DNA-based attachment strategies	Acts as hydrophilic and longer spacer to attach synthetic DNA strands to cell surfaces	[17]
Molecular diagnostics	4, 8, 12	Chemically modified nucleic acids used as DNA probes	Optimizes antigen accessibility, antigenicity and binding in molecular diagnostics and immunoassays applications	[18,102]
Molecular diagnostics	16, 20, 24, 36	5'-phosphate-modified single chain nucleotides	Helps determine DNA sequence electronically at single molecule level with single base resolution	[19]
Imaging	4	Radiolabeled cyclic RGD peptides	Improves solubility, specific targeting, tumor uptake and pharmacokinetic properties	[20,21]
Imaging	12, 24, 27, 48	Copper-64-labeled CC49 diabodies	Results in increased tumor uptake secondary to decreased renal clearance and increased residence time in blood pool	[22,23]
Imaging	8	Magnetic iron oxide nanoparticles	Helps maintain saturation magnetization and relaxivity of magnetic iron oxide nanoparticles for MRI	[24]
Imaging	8	Phosphoramidate prostate-specific membrane antigen inhibitor	Optimizes <i>in vitro</i> imaging and surface accessibility	[25]
Imaging	4, 12, 24, 67	MMPs	Optimizes real-time <i>in vivo</i> video imaging of extracellular MMP expression	[26]
Therapeutics	9, 12	Long-acting insulin derivatives	Allows for pulmonary delivery of long-acting insulin derivatives	[103]
Therapeutics	5, 9	GLP-1 agonists	Allows for pulmonary delivery of insulinotropic peptides	[104]
Therapeutics	12	α -MSH	Developing a novel melanocortin receptor binding conjugate	[105]
Therapeutics	4	Antibody–maytansinoid conjugates (anti-EGF receptor, anti-EpCAM and anti-CanAg antibodies)	Bypass multidrug resistance pathway both in cultured human cell lines and xenograft tumors in immunodeficient mice, resulting in an increased therapeutic index of antibody–maytansinoid conjugates	[27]
Therapeutics	Branched dPEG® construct (NHS-dPEG®4-Tris [m-dPEG®12] ³)	Organophosphorus hydrolase	Results in improved pharmacokinetic and immunogenicity properties for <i>in vivo</i> detoxification of neurotoxic organophosphorus agents	[28]
Therapeutics	8	Cyclic RGD labeled gold nanoparticles	Improves targeting properties and reduces aggregation, toxicity and immunogenicity	[29]
Therapeutics	12, 24, 36	A β -Fc fusion proteins	Produces two-handed fusion molecules having more flexible and extendable nonprotein hinge regions for highly increased target binding affinity	[30]
Therapeutics	6	Gold nanoparticles bearing tumor-associated glycopeptide antigens	Provides hydrophilicity and reduces toxicity and immunogenicity for use as potential cancer vaccines	[31]
Therapeutics	4, 12, 24	PIE12-trimer (D-peptide inhibitor) and membrane anchor	Optimizes peptide geometry and localization to site of action, accommodates a variety of cargoes and chemistries and results in improved potency and retention for treatment and prevention of HIV-1	[32]
Therapeutics	2, 4, 5, 8, 12	Oligonucleotides	Allows systemic delivery of oligonucleotides for therapeutic purposes	[106]

Application	dPEG® species utilized	Biomolecule modified by dPEG®	Effect of dPEG® on biomolecule application	Ref.
Therapeutics	4	Anticonvulsant analogues of galanin and neuropeptide Y	Decreases hydrophobicity, increases <i>in vitro</i> serum stabilities, increases pronounced analgesia, while lacking apparent antiseizure activities	[33]

dPEG®: Discrete polyethylene glycol; RGD: Arginine–glycine–aspartate.