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Polymer-antibody fragment conjugates for biomedical applications

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ABSTRACT

Many proteins benefit from improved solubility, immunocompatibility, pharmacokinetics or stability upon conjugation to polymers. For protein-conjugates used in delivery or imaging *in vivo*, this can mean increased efficacies due to longer circulatory half-lives or increased intracellular stability. *In vitro*, conjugation to polymers can increase stability, reduce aggregation, or mediate multimerization or phase separation of proteins to increase assay sensitivities or improve signal detection. The emergence of recombinant antibody technologies over the past two decades has allowed relatively simple isolation *in vitro* of (human) antibody fragments, such as Fabs and single chain variable fragments (scFvs), that retain the binding properties of their parent molecules and may exhibit additional properties such as reduced immunogenicity, improved tissue penetration or increased packing density on sensor surfaces due to their small sizes. In addition, protein engineering approaches that facilitate their chemical functionalization have seen antibody fragments linked to a broad spectrum of chemically and functionally diverse polymeric molecules. Of the varied strategies used in polymer–protein coupling, amine and cysteine conjugation are the most widely applied chemistries with antibody fragments. Simple conjugation to poly(ethyleneglycol) can increase half-life, decrease renal clearance, improve stability and reduce aggregation of antibody fragments without compromising their antigen binding. Meanwhile, engineering of antibody fragments can be used to control conjugation to polymers and coupling to responsive polymers can enable intracellular delivery or context responsive release of a drug payload from a polymer–antibody fragment complex. Recent years have seen polymer–antibody fragment conjugates increasingly encroach into areas traditionally associated with monoclonal antibody–polymers and we discuss the potential of such conjugates, *in vivo* and *in vitro*, in applications such as drug delivery, tissue engineering, diagnostics and bioseparation.

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Nomenclature

ABC	accelerated blood clearance
CuAAC	copper-catalyzed azide alkyne 1,3-dipolar cycloaddition
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide)
E. coli	*Escherichia coli*
EIA	enzyme immunoassay
EPR	enhanced permeability and retention
Fab	"fragment antigen binding" antibody fragment
GAD	glutamic acid decarboxylase
HEL	hen egg white lysozyme
LCST	lower critical solution temperature
NCL	native chemical ligation
NHS	N-hydroxy succinimide
PAA	polyacrylic acid
PAMAM	poly(amido amine)
PEG	poly(ethylene glycol)
PLGA	poly(lactic-co-glycolic acid)
PNiPAAm	poly(*N*-isopropylacrylamide)
PS	polystyrene
PSA	polysialic acid
QD	quantum dot
scFv	single-chain variable antibody fragment
SPAAC	strain-promoted azide-alkyne cycloaddition
SPR	surface plasmon resonance
VEGFR	vascular epithelial growth factor receptor

1. Introduction

Engineering of polymeric conjugates represents one of the most rapidly emerging areas of nanotechnology and nanomedicine. Many cytotoxic and tissue regenerative agents, including small molecule drugs, nucleic acids (DNA, plasmids, siRNA), peptides, hormones and proteins suffer from poor solubility, are rapidly cleared from the circulation or accumulate inappropriately in non-target cells or tissues *in vivo*. The past two decades have seen the development of a plethora of chemically and functionally diverse synthetic and biomimetic systems to protect these agents in circulation, target their delivery to the appropriate cells or tissues or improve their activity or stability *in vitro* for improved performance. Improved *in vivo* half-life, increased stability, targeted delivery and optimized solubility or immobilization are just some of the advantages that can be achieved by conjugation to these carrier polymers, leading to huge potential impact in fields such as drug delivery, tissue engineering, diagnostics and monitoring, and biotechnology. Antibody molecules are immune system-derived molecules that possess exquisite ligand binding specificity which can be harnessed by tethering to polymeric carriers for tissue targeting or diagnostic applications. Increasingly, however, the use of antibodies is being surpassed by antibody-derived fragments which combine the target specificity of whole antibody molecules with smaller size, ease of production, reduced costs and simplicity of engineering for improved conjugation and performance.

In this review, we consider recent advances in the design and creation of polymer–antibody conjugates, focussing in particular on engineered antibody fragments. We describe the major polymer partners of antibody fragments and detail the chemical and protein engineering approaches by which polymer–antibody fragment conjugates can be realized. Finally, we highlight some of the major biomedical applications of these conjugates to date and consider possible developments over the coming decade.

1.1. Polymers in biomedicine

Polymers have found widespread uses in fields such as biomaterials due to their biodegradability, relative biocompatibility, mechanical strength, conductivity and stimuli responsiveness properties [1]. They can be naturally occurring or synthetic in origin and typically exist in linear chain, cross-linked or surface-grafted forms [2]. The past decade has seen the emergence of large numbers of novel polymeric biomaterials, bio-functionalized polymers and other polymers of medical importance at the interface of polymer chemistry, materials science, engineering, biophysics and biology, leading to rapidly expanding biomedical and industrial applications [3].