Journal of Controlled Release xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Stimuli-responsive nanomaterials for therapeutic protein delivery

Yue Lu, Wujin Sun, Zhen Gu

Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University, Raleigh, NC 27695, USA
Center for Nanotechnology in Drug Delivery and Division of Molecular Pharmaceutics, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Received 13 June 2014
Accepted 11 August 2014
Available online xxxx

Keywords:
Stimuli-response
Nanomaterials
Protein/peptide therapeutics
Drug delivery
Nanomedicine

ABSTRACT

Protein therapeutics have emerged as a significant role in treatment of a broad spectrum of diseases, including cancer, metabolic disorders and autoimmune diseases. The efficacy of protein therapeutics, however, is limited by their instability, immunogenicity and short half-life. In order to overcome these barriers, tremendous efforts have recently been made in developing controlled protein delivery systems. Stimuli-triggered release is an appealing and promising approach for protein delivery and has made protein delivery with both spatiotemporal- and dosage-controlled manners possible. This review surveys recent advances in controlled protein delivery of proteins or peptides using stimuli-responsive nanomaterials. Strategies utilizing both physiological and external stimuli are introduced and discussed.

1. Introduction

Proteins, the "engines of life", play the most dynamic and diverse roles among all the macromolecules in the human body, including catalyzing biochemistry reactions, controlling cell fates, forming cellular structures, providing tissue scaffolds, and transporting molecules [1]. The history of protein therapeutics usage can be traced back to 1922, when insulin was first purified from bovine pancreas and served as a life-saving daily injection for type 1 diabetes treatment [2]. However, protein therapeutics remained rarely used until the emergence of the first FDA approved recombinant protein therapeutic human insulin 32 years ago [3]. Ever since then, the development of protein therapeutics has experienced an explosive growth and protein drugs now play a pivotal role for treating a broad range of diseases, covering cancer, metabolic disorders and autoimmune diseases. To date, more than 130 proteins or peptides have been approved for clinical use by the FDA [1]. Compared with small-molecule drugs, protein therapeutics possess several advantages attributed to their highly specific and complex set of functions and superior biocompatibility [1]. Protein therapeutics can also bypass the requirement of permanent or random changes to the genetic makeup of the cell, and is therefore a safer alternative compared with gene therapy [4].

Although the last few decades have witnessed significant progresses in the development of protein therapeutics, several challenges still remain to be addressed. Direct delivery of protein therapeutics suffers from their *in vitro* and *in vivo* instability, immunogenicity and a relatively short half-life within the body [5]. Also, most proteins are negatively charged at neutral pH, resulting in poor membrane permeability for intracellular delivery [6–8]. Therefore, vast efforts have been put into the design of versatile protein delivery systems for enhancing stability of cargoes, achieving "on demand" precise release and enhancing therapeutic efficacy [9]. In light of this, delivery approaches based on stimuli-responsive smart materials have drawn extensive attention these years [10]. Stimuli-responsive design is capable of conformational and chemical changes in response to environmental stimuli, and these changes are subsequently accompanied by variations in their physical properties [11]. Such action can not only facilitate release of drug with desirable pharmacokinetics, but also guarantee that drug can be spatiotemporally released at a targeting site. As summarized using a "magic cube" in Fig. 1, based on the distinct functions of target proteins, specific nanomaterials and formulations were engineered and tailed with integration of stimuli triggers. As the central component of a design, stimuli can be typically classified into two groups, including physiological stimuli such as pH, redox potential, enzymatic activities and glucose concentration and external stimuli such as temperature, light, electric field, magnetic field and mechanical force [12]. Other three "faces" of the "magic cube" could involve a variety of diseases, specific targeting sites and bio-inspired designs. We will also incorporate these elements during our discussion.

The emphasis of this review is to introduce and classify recent progress in the development of protein/peptide delivery systems *via*

E-mail addresses: zgu@email.unc.edu, zgu3@ncsu.edu (Z. Gu).

http://dx.doi.org/10.1016/j.jconrel.2014.08.015
0168-3659/

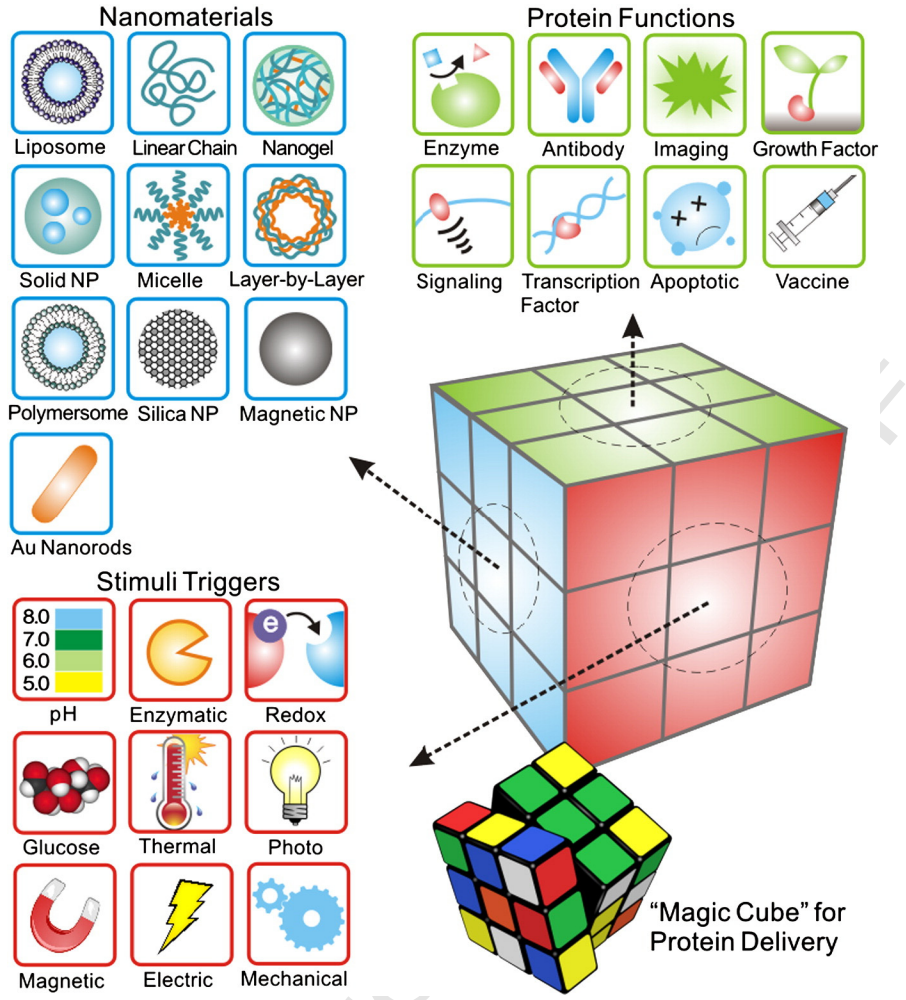

Fig. 1. Schematic of "Magic Cube" for protein delivery: combination of a variety of triggering mechanisms and carrier formulations for delivery of a broad spectrum of functional proteins.

nano-scale formulations integrated with stimuli-responsive moieties. We will survey representative examples of each stimulus type. Advantages and limitations of different strategies, as well as the future opportunities and challenges will also be discussed.

2. Physiological stimuli-triggered delivery

2.1. pH-sensitive nanosystems

Physiological pH gradients have been widely utilized in the design of stimuli-responsive nanosystems for controlled drug delivery to target locations, including specific organs, intracellular compartments or micro-environments associated with certain pathological situations, such as cancer and inflammation [9]. These delivery systems are typically based on nanostructures that are capable of physical and chemical changes on receiving a pH signal, such as swelling, charge conversion, membrane fusion and disruption and bond cleavage [13]. There are two general strategies to make such pH-responsive nanomaterials. One strategy is to utilize the protonation of copolymers with ionizable groups [14,15]. The other strategy is to incorporate acid-cleavable bonds [16–20]. Adopting these two fundamental mechanisms, researchers have developed numerous pH-responsive nanomaterials to achieve controlled delivery of protein/peptide therapeutics at both cellular and organ level [21]. At the cellular level, pH-responsive nanomaterials have been designed to escape acidic endo-lysosomal compartments and lead to cytoplasmic drug release [22,23]. At the organ level, pH-responsive oral delivery systems for controlled delivery of proteins and peptides have been developed for differential drug uptake along the gastrointestinal tract [24,25]. Herein, we will introduce recently developed approaches for intracellular delivery and oral delivery. The relevant systems covered in this manuscript are summarized in Table 1.

2.1.1. pH-responsive nanosystems for intracellular protein/peptide delivery

After endocytosis, rapid endosomal acidification occurs due to a vacuolar proton ATPase-mediated proton influx [26]. As a result, the pH levels of early endosomes, sorting endosomes, and multivesicular bodies drop rapidly to pH < 6.0 [27]. The process of endosomal acidification can be harmful to the cargo molecules, especially macromolecules such as DNA, small interfering RNA (siRNA) and proteins. However, endosomal acidification can also be used as a trigger for endosomal escape and cargo release. As the most studied stimuli-responsive mechanism, pH-triggered intracellular drug release has been extensively investigated and applied in the development of intracellular protein delivery system. pH-responsive protein/peptide delivery systems utilizing various formulations including micelles, liposomes, polymersomes, protein nanocapsules and inorganic nanoparticles such as mesoporous silica nanoparticles (MSNs) have been developed.

Utilizing the above mentioned pH reduction, Kataoka and co-workers developed an intracellular protein delivery strategy based on charge-conversional polyionic complex (PIC) micelles [28,29]. The core–shell structured nanomicelles were prepared *via* electrostatic

Please cite this article as: Y. Lu, et al., Stimuli-responsive nanomaterials for therapeutic protein delivery, J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.08.015