Journal of Controlled Release 190 (2014) 485–499

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Review

Insight into nanoparticle cellular uptake and intracellular targeting ☆

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ARTICLE INFO

Article history:
Received 13 May 2014
Accepted 21 June 2014
Available online 28 June 2014

Keywords:
Nanomedicine
Nanoparticle cellular uptake
Non-targeted and targeted nanoparticles
Intracellular distribution
Subcellular targeting

ABSTRACT

Collaborative efforts from the fields of biology, materials science, and engineering are leading to exciting progress in the development of nanomedicines. Since the targets of many therapeutic agents are localized in subcellular compartments, modulation of nanoparticle–cell interactions for efficient cellular uptake through the plasma membrane and the development of nanomedicines for precise delivery to subcellular compartments remain formidable challenges. Cellular internalization routes determine the post-internalization fate and intracellular localization of nanoparticles. This review highlights the cellular uptake routes most relevant to the field of non-targeted nanomedicine and presents an account of ligand-targeted nanoparticles for receptor-mediated cellular internalization as a strategy for modulating the cellular uptake of nanoparticles. Ligand-targeted nanoparticles have been the main impetus behind the progress of nanomedicines towards the clinic. This strategy has already resulted in remarkable progress towards effective oral delivery of nanomedicines that can overcome the intestinal epithelial barrier. A detailed overview of the recent developments in subcellular targeting as a novel platform for next-generation organelle-specific nanomedicines is also provided. Each section of the review includes prospects, potential, and concrete expectations from the field of targeted nanomedicines and strategies to meet those expectations.

1. Introduction

Multidisciplinary and integrative research efforts in the field of nanomedicine have led to the development of a variety of nanoparticle-based carrier systems suitable for site-specific delivery of diagnostic and therapeutic agents [1]. The original foundation for the recent dramatic progress in the use of nanomaterials for biomedical applications is considered to be the famous 1960 lecture of R. Feyman, "There is plenty of room at the bottom" [2]. However, the work of Paul Ehrlich, who coined the visionary term "magic bullets" to describe cell-specific diagnostics and cell-targeted therapies, is also of seminal importance [3,4]. The field of nanomedicine has established its capability to overcome the low solubility, non-specific cytotoxicity, poor bioavailability, and suboptimal pharmacokinetics and pharmacodynamics associated with the cytotoxic agents employed in cancer chemotherapy. With some nanomedicines already making their way into the clinic, liposomes, polymeric nanoparticles, dendrimers, and gold nanoparticles have demonstrated remarkable potential as carrier systems [5–8]. On one hand, the entire field of nanomedicines has been greatly expanded by the development of a wide range of nanomaterials with a high degree of control over their physical (e.g., size, surface charge, shape, mechanical strength) and chemical attributes. At the same time, a better understanding of the physiopathological nature of different diseases and insight into the interaction of nanomaterials with biological systems at various levels (i.e., systemic, organ, tissue, and cell) are of paramount importance for further progress towards bench-to-bedside translation. The recent strides forward in nanomedicines stem from some key multidisciplinary efforts. The non-fouling nature of hydrophilic materials such as polyethylene glycol (PEG) and polycarboxybetaine (PCB) [9,10] against biological materials, and recognition of the enhanced permeability and retention (EPR) effect are two such examples [11]. The development of hydrophilic polymer functionalization at the surface of nanoparticles imparts a stealth character against the immune system and enhances their systemic circulation [12]. The groundbreaking discovery of the EPR effect [13,14], which stems from the abnormal and leaky microvasculature common to tumors, has laid the foundation for the first generation of passively targeted nanomedicines that preferentially accumulate in tumor tissue [15,16]. While the EPR effect has also been observed during inflammation caused by other diseases, in that context this review is mainly focusing on the EPR effect in tumor tissues. The combination of long systemic circulation made possible by hydrophilic polymers and the EPR effect results in the accumulation of nanoparticle-based carrier systems in the tumor tissue followed by the release of therapeutic agent, either in proximity to diseased tissue or inside the cells after internalization. The EPR effect results from many complex biological processes including differences in

☆ This review is part of the 30th Anniversary Issue of the Journal of Controlled Release.

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http://dx.doi.org/10.1016/j.jconrel.2014.06.038
0168-3659/

cancer genetics as reviewed in ref. [11], consequently the therapeutic outcomes based on exploiting the EPR effect can be inconsistent due in part to the heterogeneity of tumor tissue. Recently, exploitation of the specific affinity of receptors to certain ligand molecules has led to the second generation of nanomedicines, which are preferentially targeted to particular organs, tissues, or cells. The ligands, with specific affinity towards a particular receptor or molecule differentially expressed at the target site, are displayed on the surface of nanocarriers, resulting in the preferential accumulation and uptake at the site of action [1,17]. Although some concerns have been raised about poor systemic circulation, enhanced clearance by the mononuclear phagocyte system, and limited tissue penetration, the new paradigm of ligand-conjugated actively targeted nanocarriers has been shown to improve the cellular uptake and efficacy of their payload when compared to their passively targeted counterparts [18,19]. The enhanced cellular uptake of nanoparticles at the disease site is of paramount importance, because targets for many theranostic agents against several disorders (including cancer) are localized in the subcellular compartments [20]. This fact not only highlights the importance of a better understanding of cellular uptake mechanisms but has also fueled recent research into the development of nanocarriers capable of subcellular- and organelle-level targeting, referred to as the third generation of nanomedicines [21]. After giving an account of the endocytic pathways relevant to non-targeted and ligand-conjugated targeted nanoparticles, we provide a comprehensive review of recent developments and outline future strategies in designing nanomedicines capable of efficient intracellular trafficking and subcellular targeting.

2. Endocytic routes and non-ligand targeted nanomedicines

Precise release of drugs in specific organs, tissues, and cells [22] has been the primary focus of nanoparticle-based therapeutic strategies. However, drug-loaded nanoparticles must overcome a number of transport barriers to reach their target [23]. Particularly for intracellular targeting, efficient translocation of nanoparticles across the plasma membrane barrier is a prerequisite. The plasma membrane is highly complex and provides an independent environment necessary to develop the normal function of different types of cells. This membrane also plays a critical role in cellular adhesion, communication, and division, and endocytosis is crucial to the regulation of these functions. Endocytosis involves the generation of new intracellular membrane-enclosed vesicles from the plasma membrane with a concomitant internalization of lipids, proteins, and extracellular fluid (Fig. 1). The opposite phenomenon, exocytosis, is the fusion of inner vesicles with the plasma membrane to transport molecules either to plasma membrane or to extracellular space [24]. Endocytic and exocytic trafficking are highly dynamic and well regulated, and it has been estimated that cells can internalize up to five times their volume and membrane area in one hour [25]. Phagocytosis and pinocytosis are the two main endocytic pathways employed by cells. Phagocytosis is mainly used by dendritic cells, neutrophils, and macrophages [26]. Pinocytosis occurs in all types of cells and can be further subdivided into clathrin-mediated endocytosis, caveolae-mediated endocytosis, clathrin/caveolae-independent endocytosis, and macropinocytosis. Because efficient uptake of nanoparticles is central to effective intracellular drug delivery, we believe that a deeper understanding of the biological pathways for cellular internalization of nutrients and solutes can facilitate the development of nanoparticles with precise intracellular targeting and enhanced therapeutic outcomes.

2.1. Phagocytosis

Phagocytosis is an endocytic process exhibited by several types of cells, including epithelial cells, fibroblasts, immune cells, specific phagocytic cells (monocyte, macrophages, and neutrophils), cells that generate inflammatory mediators (basophils, eosinophils, and mast cells), and natural killer cells [26]. In mammalian organisms, phagocytosis is used to engulf disabled particles, senescent cells, and infectious

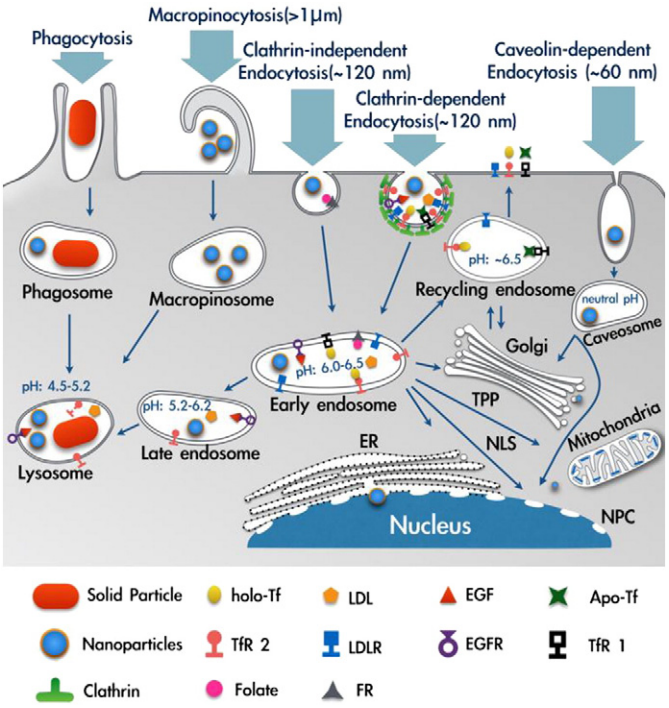

Fig. 1. Illustration of internalization pathways discussed in this article (phagocytosis, macropinocytosis, clathrin-dependent endocytosis, clathrin-independent endocytosis, and caveolae-dependent endocytosis). The fate of internalized cargo and localization to subcellular compartments are also depicted. ER: endoplasmic reticulum, NLS: nuclear localization signal, NPC: nuclear pore complex, TPP: triphenylphosphonium cation. Adapted and reproduced with permission from [90,92].

microorganisms (bacteria and viruses) as part of both the innate and adaptive immune response [27]. One of the main characteristics of this unique form of endocytosis is the large size of the endocytosed vesicles (>250 nm) known as phagosomes [28]. Phagocytosis can be triggered either through the interaction of cell-surface receptors with particular ligands presented by the foreign agent or through the interaction of specific cell-surface receptors with soluble factors that recognize the foreign agent and facilitate phagocytosis (opsonization). The soluble factors involved in opsonization include proteins of the complement system, antibodies, acetylcholine, laminin, fibronectin, C-reactive protein, and type-I collagen [29]. The most important receptors that participate in phagocytosis are the Fc receptor family for IgG (FcγRI, FcγRIIA, and FcγRIIA), the complement receptors (CR1, CR3, and CR4), and α5β1 integrin [30]. A great deal of scientific effort has been focused on controlling nanoparticle internalization via phagocytosis. The cellular internalization of nanoparticles via phagocytosis in macrophages involves attractive forces (i.e., van der Waals, electrostatic, ionic, hydrophobic/hydrophilic) between the cells and nanoparticle surfaces. In addition, the phagocytosis of nanoparticles can also be triggered by the receptor-mediated recognition of opsonins adsorbed on the surface of nanoparticles. Mitragotri and coworkers [31,32] discovered that the particle geometry can help in modulating their cellular internalization via phagocytosis. Different local particle shapes at the point of cell attachment generate different angles between the membrane and particle. This contact angle has a significant effect on the ability of macrophages to internalize particles via actin-driven movement of the macrophage membrane. Mitragotri et al. examined six different shapes of nanoparticles: spheres (radius 1.0–12.5 μm), oblate ellipsoids (major axis 4 μm, aspect ratio 4), prolate ellipsoids (major axis 2–6 μm, aspect ratio 1.3–3), elliptical disks (major axis 3–14 μm, aspect ratio 2–4, thickness 400–1000 nm), rectangular disks (major axis 4–8 μm, aspect ratio 1.5–4.5), and UFOs (sphere radius 1.5 μm, ring radius 4 μm). The authors demonstrated that elongated particles with higher aspect ratios are less prone to phagocytosis. Geng et al. [33] reported a similar finding.