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Review

Targeting dendritic cells through gold nanoparticles: A review on the cellular uptake and subsequent immunological properties

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ABSTRACT

Gold nanoparticles (NPs) have been proposed as a highly potential tool in immunotherapies due to its advantageous properties including customizable size and shapes, surface functionality and biocompatibility. Dendritic cells (DCs), the sentinels of immune response, have been of interest to be manipulated by using gold NPs for targeted delivery of immunotherapeutic agent. Researches done especially in human DCs showed a variation of gold NPs effects on cellular uptake and internalization, DC maturation and subsequent T cells priming as well as cytotoxicity. In this review, we describe the synthesis and physiochemical properties of gold NPs as well as the importance of gold NPs in immunotherapies through their actions on human DCs.

1. Introduction

Nanoparticles (NPs) defined as particles with a diameter of less than 100 nm (Horikoshi and Serpone, 2013) have been proposed to be highly useful in several medical applications from diagnostics to disease therapy. The minute size of NPs enables them to infiltrate tissues including lymphoid tissues and subsequently target immune cells, making them potentially useful in immunotherapies (Almeida et al., 2014). There are different types of NPs, composed of inorganic metals (*e.g.* gold, silver, carbon), metal oxides (*e.g.* titanium oxide, iron oxide), metalloids (*e.g.* crystalline silica, amorphous silica), organic biodegradable (*e.g.* lipid, polysaccharide, polymeric matrix) or inorganic biocompatible polymers (*e.g.* polystyrene) (Mohamud et al., 2014). Among these, gold NPs have been the choice for immunotherapies applications given its advantageous physicochemical properties.

Gold NPs are customizable in their size and shape which have functional significances in immunotherapies. The size and shape of gold NPs may influence the ability of gold NPs to reach the targeted cells (*e.g.* cancer cells) and antigen-presenting cells [*e.g.* dendritic cells (DCs) and macrophages], as well as their interaction capacity with these cells (*e.g.* internalization process). Studies have shown that smaller sized gold NPs (< 2 nm) were more readily internalized into cellular compartment (Fernandez et al., 2015; Oh et al., 2011) as they can pass cellular membrane passively through diffusion and other internalization mechanisms. As for shape, rod-shaped gold NPs were more efficiently taken up by DCs than those of sphere- or cubic-shaped gold NPs (Niikura et al., 2013) because of the larger surface volume of rod shape compared to other shapes (Vacha et al., 2011). These reflect the importance of physicochemical properties of gold NPs in modulating the targeted immunological effects.

In addition, the functionality of gold NPs can be fine-tuned after surface functionalization with numerous molecules including antibodies, antigenic peptides, nucleic acids, polymers and radioisotopes (Oh et al., 2011; Lee et al., 2016a; Paul et al., 2014; Safari et al., 2012). High surface energy as well as affinity of gold NPs toward these molecules enable gold NPs to be attached either by covalent or non-covalent mechanisms. Furthermore, gold NPs are chemically inert and relatively stable. For example, gold NPs conjugated with M2e, the ectodomain of M2 protein present on influenza A virus, could be lyophilized and stably resuspended in water demonstrating the potential of

Abbreviations: Ag, Antigen; APCs, Antigen presenting cells; AuNP, Gold nanoparticles; AVNs, Artificial virus nanoparticles; CLRs, C type lectin receptors; CTAB, Cetyltrimethylammonium bromide; CTL, cytotoxic T cells; CTLA-4, cytotoxic T lymphocyte associated protein 4; DCs, Dendritic cells; DC-SIGN, Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; DLS, Dynamic light scattering; EPR, Enhanced permeability and retention effect; ER, endoplasmic reticulum; HSCs, Haematopoietic stem cells; LPS, Lipopolysaccharides; LSPR, Localized surface plasmon resonance; MHC, Major histocompatibility complex; mPEG, Methylpolyethyleneglycol; NCs, Nanoclusters; NK, Natural killer; NPs, Nanoparticles; PEG, Polyethyleneglycol; PLGA, Poly (D L-lactide-co-glycolide; PVA, Polyvinyl alcohol; rhTNF, Recombinant human tumor necrosis factor; TCR, T cell receptor; TEM, Transmission electron microscopy; Th, T helper cells; TLRs, Toll-like receptors; Teff, Effector T cells; Tregs, Regulatory T cells; UV-vis, UV–vis; Zw, Zwitterion; ZwMUA, Zwitterion-mercaptoundecanoic

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Table 1
Methods of gold NPs synthesis.

Method	Stabilizing agent	Gold NPs synthesized	Year	Ref.
Turkevich	Trisodium citrate	< 40 nm Sphere	1951	(Turkevich et al., 1951)
Frens	Trisodium citrate	15–150 nm Sphere	1973	(Frens, 1973)
Brust-Schiffrin	CTAB	1–3 nm Sphere	1994	(Brust et al., 1994)
Seed-growth	Ascorbic acid	12 nm Sphere, Rod	2001	(Jana et al., 2001)
Kimling	CTAB	9–120 nm NA	2006	(Kimling et al., 2006)

CTAB: cetyltrimethylammonium bromide.

gold NPs as a stable vaccine delivery agent (Tao et al., 2014).

Another important properties of gold NPs is their unique optical properties pertaining to the surface plasmon resonance and light-scattering that render gold NPs as a promising candidate in cancer detection and treatment (Arosio et al., 2011; Wang et al., 2011). Moreover, gold NPs are efficiently interacting with targeted cells compared to other NPs in terms of immunological responses and cytotoxicity (Singh et al., 2010; Yen et al., 2009). Therefore, these unique properties make gold NPs a suitable immunotherapeutic agent as compared to other NPs.

The purpose of this review is to highlight the effects of gold NPs in manipulating biological functions of human DCs to regulate T cells, specifically on their cellular uptake of gold NPs by DCs, the subsequent maturation of DCs and activation of T cells as well as potential cytotoxicity of gold NPs on DCs.

1.1. Synthesis of gold nanoparticles

The customizable size and shape of gold NPs, which are the advantageous properties of gold NPs, are produced through well-explored synthesis methods (Table 1). Gold (III) salts are normally employed for chemical synthesis of gold NPs. The most popular route in synthesizing gold NPs is through citrate reduction of hydrogen tetrachloroaurate (III) ($HAuCl_4$) in boiling water. This chemical synthesis is first proposed by Turkevich and coworkers back in 1951 (Turkevich et al., 1951) and further modified by Frens in 1973 (Frens, 1973). Turkevich's group introduced the use of trisodium citrate which plays dual roles: as a reducing agent to promote the reduction of Au^{3+} to Au^0, and as a stabilizing agent later as it adsorbs onto the surface of gold colloids formed and electrostatically stabilizes them in aqueous environment (Turkevich and Colloidal Gold Part, 1985). Later, this citrate route was improved to regulate the particle size by controlling the ratio of trisodium citrate to gold salt, resulting in gold NPs with diameters ranging from 15 to 150 nm (Frens, 1973).

The Turkevich-Frens method has been used for about three decades until it was further improved by Kimling and colleagues (Kimling et al., 2006). According to their findings, the general relation between gold-to-reductant ratios in determining the final size of gold NPs is independent of the absolute concentration of gold precursor. In addition, they initiated the reaction with UV exposure and ascorbate, respectively, resulting in a more defined spherical gold NPs (Kimling et al., 2006).

Although the aforementioned methods can produce gold NPs with variable sizes and shapes, they failed to produce NPs with uniform shapes and sizes necessary for significant effects on immunomodulation (Arosio et al., 2014; Fytianos et al., 2015; Brandenberger et al., 2010). Consequently, 1,3-acetonedicarboxylic acid, formed by the oxidation of citrate, is later modeled to be responsible for the gold NPs size in which the balance of degradation of 1,3-acetonedicarboxylic acid reaction and the nucleation reaction determine the particle sizes (Kumar et al., 2007). Other parameters such as pH, reaction temperature, solvent and reducing agents other than citrate are also modified to precisely control the shapes and sizes of gold NPs synthesized (Zhao et al., 2013). For example, sodium borohydride was used as the reducing agent under ambient conditions to produce long-term stable gold NPs with a diameter of 3 nm (Deraedt et al., 2014).

To confer functional properties to gold NPs as a future targeted delivery agent, a two-phase reduction method was initially introduced by Brust and Schiffrin's group by utilizing the alkanethiols that covered the surface of gold NPs, enabling gold NPs to be handled as a simple chemical compound (Brust et al., 1994). Besides producing smaller gold NPs with a diameter of 1–3 nm, the Brust-Schiffrin method is useful in maximizing the functionality of gold NPs by incorporating other molecules such as polymers, fluorescent dyes and drugs to the particle surface (Arosio et al., 2011; Fytianos et al., 2015; Rodriguez-Lorenzo et al., 2014).

Although the previous Turkevich-Frens method produces larger gold NPs (15–150 nm), the two-phase reduction method initiated by Brust and Schiffrin's group cannot be modified to obtain anisotropic gold NPs. In addition, other synthesis techniques focused on narrowing the size distribution (Jana et al., 2001) and making smaller particles (Duff et al., 1993a; Duff et al., 1993b) than the citrate route have since been developed. Gold NPs synthesized by the citrate route are less stable (Moller and Fritzsche, 2006) due to the presence of free ionic species such as Na^+ which imposes Debye Screening effects to screen through the electrostatic repulsion. In addition, because of the high density of gold, particles with a size of ~10 nm sediment form suspension even if they are colloidally stable (Moller and Fritzsche, 2006). To achieve a higher degree of colloidal stability, different capping agents with silane (Buining et al., 1997), amine (Newman and Blanchard, 2006), or thiol (Frenkel et al., 2005) functional groups have been employed to provide steric stabilization of the gold NPs.

Through years of research, many gold NPs techniques have been explored and studied to produce well-defined gold NPs (Table 1). The most current widely applied method called the seed growth method, pioneered by Richard Zsigmondy, synthesized gold NPs in two separate nucleation and successive growth steps (Jana et al., 2001). In this method, small-sized gold NPs called seeds (Duff et al., 1993a; Duff et al., 1993b) produced in the first step were enlarged with the addition of growth solution as well as the reducing and stabilizing agents in the second step. Although gold NPs produced are large in size, they are monodispersed and the shapes produced are more defined and varied as this method involves the most primary nucleation process (Fig. 1).

In addition, different types of gold NPs synthesis would produce gold NPs with different characteristics (size, shape, surface

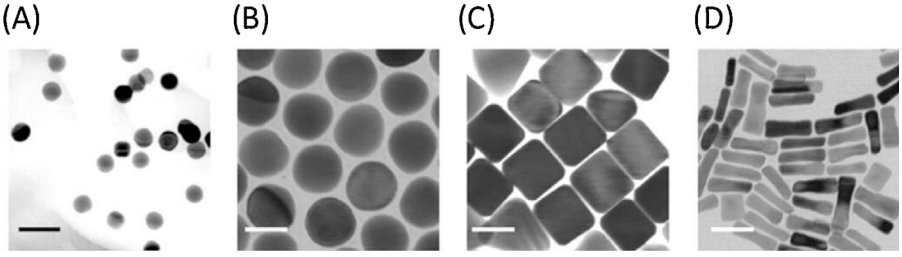

Fig. 1. TEM images of various sizes and shapes of gold NPs synthesized using seed growth method. (A) 20 nm-sphere; (B) 40 nm-sphere; (C) Cube; (D) Rod gold NPs. Scale bar represents 40 nm. Reprinted with permission from (Niikura et al., 2013), copyright 2013 American Chemical Society.