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CONTENTS

Calcium phosphate nanoparticles: a potential vaccine adjuvant

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ABSTRACT: Nanotechnology is a branch of science and engineering, which plays a crucial role in numerous areas like medical, agricultural, pharmaceutical sector. Various nanoparticles are considered to be efficient drug delivery carrier. The usage of nanoparticles in vaccine formulation facilitates improved antigen stability, immunogenicity as well as slow release and targeted delivery. Several organic and inorganic nanoparticles have been evaluated for application in nanomedicines for targeted drug delivery as vaccine adjuvant system. Various Polymeric nanoparticles such as Poly Lactic Acid (PLA), Poly Lactic-Co-Glycolic Acid (PLGA), Polycaprolactone (PCL), Chitosan, Gelatin etc. have been studied as drug delivery agent for the treatment of diseases. Among inorganic nanoparticles, Calcium phosphate nanoparticles have been considered as most suitable and safe vaccine adjuvants due to their high biodegradability and biocompatibility. These nanoparticles are cost-effective and have hydrophilic nature, better stability and potential efficacy to produce better immune response in comparison to other nanoparticle adjuvant system.

Key words: Calcium phosphate nanoparticles, Nanotechnology, vaccine adjuvants

Adjuvants can be defined as substance or material, which comprises of chemically complex molecules that enhances an immune response to an antigen (Schijns and Lavelle, 2011).They have been used to enhance and improve the efficacy of vaccines from last century. The adjuvants act by different ways such as by depot generation, improving antigen presentation or by immunomodulation (Reed *et al*., 2013). Several particulate and non-particulate adjuvants have been used to enhance the immunity response to an antigen. Particulate adjuvants such as mineral salts (alum), lipid particulate and microparticles are most commonly used in human and animal vaccines while non-particulate adjuvants (Saponins, mucosal adjuvants, Chitosan etc.) also play an important role in enhancing the immunogenicity. Advancement in the field of Nanotechnology offers the opportunity to design nanoparticles varying in size, shape, composition and surface properties, for application in the field of medicine (Couvreur and Vauthier, 2006; Moghimi *et al*., 2005). These nano-sized particles are able to enter living cells and play a crucial role in diagnosis of diseases as well as drug delivery for exploring the treatment and prevention of various diseases such as Alzheimer's (Chackerian, 2010), hypertension (Tissot *et al*., 2008)and nicotine addiction (Maurer *et al*., 2005). Several nanoparticles such as Gold nanoparticles, Carbon nanoparticles, Silver nanoparticles, mesoporous silica nanoparticles etc. are most commonly used as drug delivery agent as well as diagnostic agents (Chen *et al*., 2012; Wang *et al*., 2011; Yu *et al*., 2013).

Calcium phosphate nanoparticles as nano-adjuvant system

Nanotechnology plays a pivotal role in development of new generation and effective adjuvant system. The application of nanoparticles in vaccines enhanced targeted delivery, immunogenicity and stability. In past few years, nanoparticles of varying size and composition have been used as adjuvant to enhance immune potential of antigens and as drug delivery system for prevention and treatment of disease (Couvreur and Vauthier, 2006). Several different studies have reported the application of nano-materials as adjuvant for human use and many of them are still under clinical trials (Correia-Pinto *et al*., 2013).The nano-based adjuvant system is divided into organic and inorganic nanoparticles. The organic nanoparticles include polymeric nanoparticles such as Poly (d,l-lactide-co-glycolide) (PLG), poly(ethylene glycol) (PEG) (Vila *et al*., 2004) poly(g-glutamic acid) (g-PGA) (Akagi *et al*., 2011)and poly(d,l-lactic-coglycolic acid)(PLGA) (Lu *et al*., 2009)which have been evaluated as vaccine and drug delivery systems due to biodegradability and efficient biocompatibility. Apart from synthetic polymeric nanoparticles, natural polymers such as chitosan (Feng *et al*., 2013), inulin (Saade *et al*., 2013) and alginate (Li *et al*., 2013) were also used as adjuvant system for delivery of antigens. These natural polymers have high biocompatibility, high biodegradability (Chua *et al*., 2012; Akagi *et al*., 2011).Therefore, they were used for animal (New castle disease) and human (HBV) vaccines (Borges *et al*., 2008; Zhao *et al*., 2012).On the other hand inorganic nanoparticles have better stability and resistance from microbial attack. They can be prepared at low temperature and are relatively inexpensive than organic nanoparticles. Inorganic nanoparticles can be further divided into two categories i.e., metallic and non-metallic nanoparticles. Metallic nanoparticles like gold and silver nanoparticles have been used for delivery of antigens for various diseases like FMD (Chen *et al*., 2010),influenza (Tao *et al*., 2014)human immunodeficiency virus-acquired immunity deficiency syndrome (HIV-AIDS) (Xu *et al*., 2012)and generated an effective immune response but the toxicity caused by metallic nanoparticles is a debatable issue (Lin *et al*., 2017).Non-metallic nanoparticles such as Silica nanoparticles, Carbon nanoparticles and Calcium phosphate nanoparticles were developed for drug delivery and adjuvant system (He *et al*., 2002;Yu *et al*., 2013; He *et al*., 2000). Among these non-metallic inorganic nanoparticles, Calcium phosphate nanoparticles have immense potential to function as nano adjuvants for inducing more balanced T helper type (Th1 and Th2) immune responses for better cell mediated and humoral immunity (Lin *et al*., 2017).These nanoparticles are highly biocompatible, biodegradable and non-toxic for the cells (Joyappa *et al*., 2009).These nanoparticles were mainly used for delivery of DNA vaccines (He *et al*., 2002;He *et al*., 2000; Joyappa *et al*., 2009).Calcium phosphate nanoparticles have been extensively used in therapeutics and diagnostics (Desai and Uskokovic, 2013). Calcium phosphate is a normal constituent of body that is well tolerated and rapidly absorbed *in vivo*(Chu *et al*., 2017). Initially, it was used as an adjuvant of human vaccine (Diptheria-tetanus-pertussis) in France (Goto *et al*., 1997; Masson *et al*., 2017).Calcium phosphate nanoparticles are made up of Calcium, Phosphorous and Oxygen therefore these have high biocompatibility and biodegradability in comparison to other nanoparticles likes Zn and Cu (Lalk *et al*., 2013).

Synthesis of calcium phosphate nanoparticles

The Calcium phosphate nanoparticles are generally synthesized by following two approaches i.e,. Top down approach, which involves breakage of large sized microparticles into nano-sized particles by ultrasonication method (Bisht *et al*., 2005) and Top up approach that involves Co-precipitation method for synthesis of Calcium phosphate nanoparticles(He *et al*., 2002; Joyappa *et al*., 2009).Calcium phosphate nanoparticles synthesized by Co-precipitation method were used as adjuvant for vaccine development and provoked an effective immune response by Depot effect (Jones *et al*., 2014) and NLRP-3 inflammasome activation (Ronchi *et al*., 2016) as well as protective immunity against diseases like New castle disease, Human enterovirus-71, Friend rotavirus (Koppad *et al*., 2011; Saeed *et al*., 2015; Knuschke *et al*., 2016). These nanoparticles are more stable *in vivo* in comparison to polymeric nanoparticles (as polyacrylic acid nanoparticles) (Colby *et al*., 2013).In addition, the sterility and stability of Calcium phosphate nanoparticles *In vitro* are easier to control due to inorganic nature of Calcium phosphate nanoparticles. These nanoparticles are very cost-effective in comparison to other nanoparticles (Castro *et al*., 2016).

Mechanism of action

Calcium phosphate nanoparticles produce immune response by Depot effect (Jones *et al*., 2014), NLRP-3 inflammasome activation (Ronchi *et al*., 2016) and produce a balanced cell mediated and humoral immune response by activation of Th1 and Th2 responses. The small size of nanoparticles facilitates the uptake by macrophages. Therefore, Calcium phosphate nanoparticles have been used as adjuvant for vaccines of animals (FMD, New castle disease, Aeromonas vaccines) and humans (Human enterovirus-71, Herpes simplex virus type2 and Toxoplasma gondii) (Doel, 2003; Zhang *et al*., 2013;Saeed *et al*., 2015).

Advantages of Calcium phosphate nanoparticles

Calcium phosphate nanoparticles have a promising potential as targeted drug delivery agents. These nanoparticles are easy to deliver, cost-effective and readily absorbed, which allows their extensive use for entrapment of biomolecules. Various surface modifiers such as Cellobiose, Alginate, Gelatin etc. can be used for surface modification which leads to increased loading and releasing capacity of the drug in nanoparticles. The nano size of these nanoparticles helps in immobilizing and targeting biomolecules at specific site as they have high penetrability acting directly at cellular level. Several properties such as particle size, surface modification, surface charge etc. influence the potential of Calcium phosphate nanoparticles as drug delivery agent as well as efficient nano delivery adjuvant system for antigens.

CONCLUSION AND FUTURE PERSPECTIVES

Calcium phosphate nanoparticles have been proven suitable and safe for their use as a nano delivery adjuvant system in human vaccines. These nanoparticles are highly biocompatible and biodegradable and considered to be non-toxic at cellular level. Calcium phosphate nanoparticles have been reported to generate an effective cellular as well as humoral immune response and also can play a crucial role in site specific drug delivery system for many diseases at cellular level.

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