 doi:10.20944/preprints202010.0580.v1

Exploring the modulation of immune response and oxidative stress of intracellular pathogens using nanoparticles encapsulating drugs

Ramendra Pati Pandey[a], Anjali Priyadarshini[a], Archana Gupta[a], Arpana Vibhuti[a], Elcio Leal[e], Utpal Sengupta[b],Vishwa Mohan Katoch[c], Pawan Sharma[d], Catrin E Moore[f],V. Samuel Raj[a]

[a]Centre for Drug Design Discovery and Development (C4D), SRM University, Delhi-NCR, Rajiv Gandhi Education City, Sonepat - 131 029, Haryana, India; **ramendra.pandey@gmail.com** (R.P.P.); **anjali.p@srmuniversity.ac.in** (A.P.); **archana.g@srmuniversity.ac.in** (A.G.); **arpana.v@srmuniversity.ac.in** (A.V.); **directorcd4@srmuniversity.ac.in** (V.S.R.); [b]Stanley Browne Research Laboratory, The Leprosy Mission, Nand Nagari, Sahadra, New Delhi, 110093; [b]**usengupta2002@yahoo.com** (U.G.); [c]Rajasthan University of Health Sciences (RUHS), Jaipur, President, JIPMER, Puducherry; **vishwamohankatoch18@gmail.com** (V.K.M); [d]ICGEB (International Centre For Genetic Engineering And Biotechnology), New Delhi 110067, India; **pawan37@gmail.com** (P.S.) [e]Institute of Biological Sciences, Federal University of Para, Para 66075-000, Brazil; **elcioleal@gmail.com** (É.L.)
[f]Nuffield Department of Medicine, University of Oxford, Big Data Institute, Li Ka Shing Centre for Health Information and Discovery Old Road Campus, Headington, Oxford, OX3 7LF, United Kingdom, **catrin.moore@ndm.ox.ac.uk** (C.M.)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

Abstract:

The immune system is a dynamic network of cells and cytokines are the major mediators of immune responses which combat pathogens. Based on the cytokine production, effector T cells differentiate into subsets known as Th1, Th2, Th17 or Treg (T regulatory). This system serves as a barrier to intracellular pathogens, bacterial infections and stimulates the production of reactive oxygen species (ROS), reactive nitrogen intermediates (RNI) and nitric oxide (NO), which diffuses across membranes and engulfs intracellular pathogens. Oxidative stress occurs when ROS, reactive nitrogen species (RNS) production and antioxidant defences become imbalanced. Oxidative stress generated by infected cells produces a substantial amount of free radicals which enables killing of intracellular pathogens. Intracellular pathogens are exposed to endogenous ROS as part of normal aerobic respiration, also aexogenous ROS and RNS are generated by the host immune system in response to infection. Nanoparticles which are designed for drug delivery are capable of trapping the desired drug in the particles which protects the drug from enzymatic degradation in a biological system. The small (subcellular) size of nanoparticles enables higher intracellular uptake of the drug which results in the reduction of the concentration of free drugs reducing their toxic effect. Research on the modulation of immune response and oxidative stress using nanoparticles used to encapsulate drugs has yet to be explored fully. In this review we illustrate the immune activation and generation of oxidative stress properties which are mediated by nanoparticle encapsulated drug delivery systems which can make the therapy more effective in case of diseases caused by intracellular pathogens.

Key words: Immune system, Oxidative stress, Nanoparticles, Intracellular Pathogens.

Introduction:

The immune system is constantly in a flux, it encompasses a dynamic network of cells, tissues and organs within a host that work in a coordinated manner to defend the body against attacks by "foreign" invaders while also protecting against disease by recognising both "self" and "non-self". Antigens, usually a toxin or foreign substance recognised by the host, are recognized by specialized cells which facilitate their initial destruction followed by elimination from the host. Any microorganism able to cause disease in a host organism can be termed a pathogen. When a pathogen (for example a bacterium**,** virus or protozoal parasite) infects the human body, after which an internal battle ensues between the host's innate and adaptive immune system and the pathogen's assorted virulence mechanisms, together with factors which are able to overcome the immune attack and establish disease. Detection of antigens by the host is complicated as pathogens evolve rapidly; are able to adapt quickly and escape the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

immune surveillance which allows the pathogens to infect their hosts and cause disease (Christensen & Thomsen, 2009). Pathogens can be extracellular and intracellular and the mechanism to counter their attack by immune system is varied. As intracellular pathogens reside within the host cell, their elimination and clearing is more complex, the cell-mediated immune response plays a vital role in the host defence against intracellular pathogens such as those causing tuberculosis and leishmaniasis (Urdahl, 2014) .

The immune response can be both innate and adaptive; the innate immune response is the first line or primary defence immediately stimulated upon infection. This first line of defence initiated by the host upon the entry of microorganisms to the body involves responses by phagocytic cells that fight pathogens in a nonspecific manner. Antigen presenting cells (APCs) such as macrophages and dendritic cells (DCs), which are spread extensively throughout the body, swallow and process potential microbial antigens *via* phagocytosis, antigen presentation and activation of T and B lymphocytes to generate an adaptive immune response (Heit *el al.*, 2008). These activated cells cooperate with activated macrophages within the host to abolish intra and extra-cellular pathogens (Storni *et al*, 2005). Antigens are collected by APCs after which they migrate to the draining lymph nodes with maturation signified by enhanced presentation of antigenic material to major histocompatibility complex (MHC) class I and and/or class II receptor molecules that are subsequently presented to the immune system for development of the acquired immune response. The presentation of antigen alone is not adequate to cause naive T cells to mature into effector T cells. Upon receiving signal, APCs can initiate key T cell responses to the antigen, such as cell survival, differentiation of naive T cells to develop into effector T cells and cytokine secretion (Rescigno *et al*, 1999; Lima *et al*, 2004; Reed *et al*, 2008). $CD4^+$ T cells identify antigens that have been processed by APCs, DCs, macrophages and B cells that express MHC class II molecules.

NPs exert their antimicrobial effects through membrane protein damage, superoxide radical production, reactive oxygen species formation, protein disruption of microbes, damge to proton efflux pump & disruption of electron transport chains and the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

generation of ions that interfere with the cell granules leading to the formation of condensed particles **(Figure 1).**

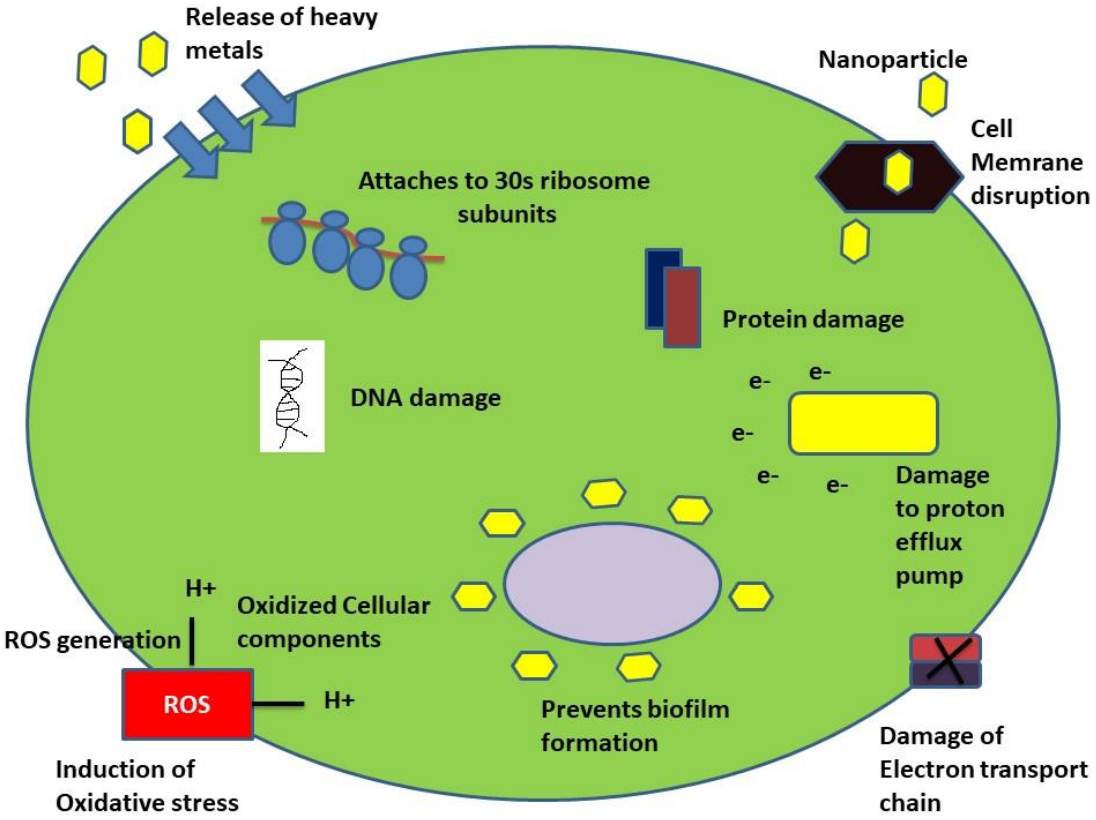

FIGURE 1. Antimicrobial activities exhibited by nanoparticles include damage of the proton pump, prevention of biofilm formation, oxidation of cellular components, disruption of the electron transport chain & cell membrane of microbes.

Immune evasion:

Many pathogens which have the ability to cause acute infections are often cleared effectively by the hosts immune system. However, some pathogens which invade the hosts cells become intracellular pathogens, and are able to establish persistent and sometimes lifelong infections. Several of these intracellular pathogens manage to evade the host immune system causing disease by replicating inside the host cells.

Some bacteria, such as *M. tuberculosis* are able to disrupt the phagosome-lysosome fusion using the PtpA tyrosine phosphatise which preventing the acidification of the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

phagosome (Bach *et al*., 2008). Other mechanisms use the phagosome to create a suitable microenvironment for proliferation: *Legionella pneumophila* safeguards itself from the hosts innate immunity by creating a vacuolar environment which is lacking MHC class II molecules (Clemens and Horwitz, 1992), whereas *C. burnetii* needs an acidic environment for growth and virulence which eliminates the other pathogens (Maurin *et al*., 1992). Some bacteria can enter a hardy, non-replicating state, termed dormancy for self protection (Rittershaus *et al*., 2013) *M. tuberculosis* undergoes a period of reduced cellular growth which maintains a basal metabolism called cellular quiescence (Betts *et al*., 2002) and true dormancy, a metabolically-arrested spore state promoting survival under adverse conditions, as exhibited by the *Clostridium* spp. (*Rittershaus et al*., 2013).

Endoparasites are parasites that live in the tissues and organs of their hosts, they encompass both parasites and helminths. Protozoa avoid contact with human immune cells by living in immune priviledged sites, *Plasmodium falciparum*, which causes malaria in humans, matures in liver (Patarroyo *et al*., 2011). The parasite can then travel in the peripheral blood to infect erythrocytes, they target these cells as they lack MHC I receptors, and are not recognised by cytotoxic immune cells (Gomes *et al*., 2016). *Trypanosoma brucei* is able to invade the central nervous system causing African sleeping disease, the parasite proliferates in blood and lymph and is not recognised by the immune system (Masocha *et al*., 2007). In addition to evading the immune systems, genetic polymorphisms within the parasites also play a role in immune evasion by a number of protozoans. Two examples are i) *P. falciparum* which has multiple stages during it's lifetime, their surface antigens are altered after every stage, and ii) *T. brucei* survives through remodeling it's subsurface protein, this protein is involved in signaling transitions during developmental stages of dormancy and disease progression (Batram *et al*., 2014). These polymorphic modifications reduce the ability of B cells to make highly specific antibodies against the parasitic antigens (Zambrano-Villa *et al*., 2002).

T. brucei uses its "vector host" to its advantage, the saliva of the Tsetse fly is transmitted along with the parasite, the saliva contains a Gloss2 peptide which suppresses human host release of cytokines TNF-α, IFN-γ, IL-6, and IL-10

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

(Stijlemans *et al*., 2016). Helminths are able to survive in humans for many years due to their ability to secrete immunomodulatory products, including proteases, protease inhibitors, venom allergen homologues, glycolytic enzymes and lectins (Hewitson *et al*., 2009). As central components of the "respiratory burst" in activated macrophages and neutrophils, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) cause an oxidative burst which plays an essential role in the host immune defenses against pathogens (Grant and Hung., 2013, Belkaid and Hand., 2014) which can be harnessed to tackle drug resistant infections. Apart from intracellular pathogens there are various extracellular ones which use different modes to evade the hosts immune defence. The Gram positive organism, *Staphylococcus aureus* (*S. aureus*) is one of the most important human bacterial pathogens, with worlwide distribution, is a commensual organism which also causes infection in almost any human tissue. Effective treatment of staphylococcal infections has been hampered by the emergence of antibiotic resistance (Spaans, 2015).

Oxidative Stress:

Superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$) are molecules commonly defined as ROS. They are generated as metabolic by-products by biological systems (Sato *et al*., 2013; Navarro-Yepes *et al*., 2014). A proper ROS production forms the basis of other processes such as protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity and differentiation (Rajendran *et al*., 2014). Redox regulation *via* ROS/RNS and the antioxidant defences represents a tightly controlled system that can have both deleterious and beneficial effects within the cellular environment. ROS can be generated both intrinsically or extrinsically within the cell. Molecular oxygen generates O_2 ·−, the primary ROS via one-electron reduction catalyzed by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Further reduction of oxygen may lead to H_2O_2 or OH· via dismutation and metal-catalyzed Fenton reaction, respectively (Vallyathan and Shi, 2000). Free radicals occur as essential byproducts of mitochondrial respiration and transitionmetal ion-catalyzed Fenton-type reactions (Vallyathan and Shi, 1997).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

Inflammatory phagocytes such as neutrophils and macrophages induce oxidative outburst as a defense mechanism towards environmental pollutants, tumor cells and microbes, this results in the generation of both non-specific and specific immune responses. A variety of NP including metal oxide particles induce ROS as one of the principal mechanisms of cytotoxicity (Risom *et al*., 2005). Nanoparticles have been reported to influence intracellular calcium concentrations, activating transcription factors, and modulating cytokine production via the generation of free radicals (Li *et al*., 2010) thus contributing towards oxidative stress.

Glutathione-S-transferase (GST) are a family of Phase II detoxification enzymes catalyzing the detoxification of electrophilic compounds, it protects cells from mutagens and carcinogens as a free radical scavenger along with glutathione (Hemachand, 2002). Glutathione removes H_2O_2 in the cytosol (Reed,1969). H_2O_2 is formed near the membrane during phagocytosis, and it can simply diffuse into the cytosol. In the cytosol, glutathione reacts with H_2O_2 through a chemical reaction catalyzed by glutathione peroxidase which results in glutathione disulfide (GSSG) (Paul, 1970). Glutathione reductase (GR) catalyzes the regeneration of glutathione from GSSG, utilizing NADPH generated by the hexose monophosphate shunt (HMPS).

Glutathione Peroxidase (GPx) isoenzymes use GSH as a donor of reducing equivalents to detoxify H_2O_2, in various organic compounds (Briviba, 1998). Mitochondria in normal mammalian cells are the principal endogenous sources of ROS, the main exogenous sources of ROS are drugs and other xenobiotics. Cells have an elegant defense system against ROS, consisting of antioxidant enzymes and low molecular weight substances capable of scavenging many different ROS. Glutathione Peroxidase (GPx) has a crucial role in the defence mechanisms against damage by catalyzing the reduction of H_2O_2 and a large variety of hydroperoxides into water and alcohols (Sies, 1995). The cellular pool of glutathione is replenished by two mechanisms: glutathione regeneration from GSSG mediated by GR and de novo glutathione synthesis. By regenerating glutathione from GSSG, GR facilitates cytosolic H_2O_2 detoxification, which protects phagocytes from oxidative damage and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

sustains oxidative burst-mediated bactericidal activities (Reed, 1969). In oxidative stress there is either an excessive production of ROS or a significant decrease or lack of antioxidant defense. The removal of H_2O_2 or other hydroperoxides by GPx requires GSH as cofactor. GSH is a tripeptide of glutamate, cysteine, and glycine, which is found ubiquitously in eukaryotic cells at a concentration between 1 and 10 mM. GSH has a potent electron-donating capacity. Its high redox potential reduces GSH which is both a potent antioxidant and a convenient cofactor for enzymatic reaction.

These molecules are all associated with the regulation of apoptosis (Riley *et al.*, 2006). NO is a component of the innate immune system, and is involved in both the pathogenesis and control of several types of viral, bacterial and parasitic infections (Bogdan, 2001). Furthermore, NO is able to modulate the immune response *via* the regulation of apoptosis and the upregulation of cytokine mRNA expression (Hanum *et al*., 2003). The regulation of NO production in tuberculosis appears to be very complex, due to the ability of various mycobacterial cell wall components to stimulate the release of NO (Underhill, 1999). Basu *et al* reported that *M. tuberculosis* secretory proteins control cell signaling, ROS and MTSA-10 are also important in the modulation of macrophage immune functions (Basu *et al*., 2006, 2009). Secretory proteins of *M. tuberculosis* induce a TH1 bias in T cell response (Pandey *et al.*, 2011). *Mycobacterium abscessus* is a non-tuberculous mycobacteria (NTM) which represents a pathogen causing a substantive but often underappreciated burden of disease worldwide. Although causing pulmonary diseases that resembles tuberculosis (TB), *M. abscessus* does not respond to standard anti-TB therapy due to its natural resistance to most antibiotics.

The integrated dynamics between oxidative stress and the immune cell function of an intracellular pathogen that can sense and acclimatize to the continuously changing host environment during infection. Both oxidative and reductive stresses induce redox cascades that have the potential to alter pathogens signal transduction, DNA and RNA synthesis, protein synthesis and drug resistance (Manke *et al*., 2013). Maintaining a suitable redox balance is necessary for a good clinical outcome,many prodrugs are only effective upon bio reductive activation. Proper homeostasis of oxido-reductive

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

systems are essential for pathogens survival, persistence and subsequent reactivation. As in humans, the exposure of bacteria to ROS causes damage to a variety of macromolecules, resulting in mutations and often in cell death thus contributing to the generation of a host defence. ROS function as signaling molecules that lead to a coordinated response in bacteria under redox-stress conditions. Metallic nanoparticles (NPs) offer a novel potential means of fighting bacteria becaue they exert their effects through membrane protein damage, superoxide radicals and the generation of ions which may cause oxidative stress.

Role of Nanoparticles in specific immune response against intracellular pathogens:

Microorganisms in infected tissues can become resistant to antimicrobials through a number of routes, these may include, amongst others, protection by biological structures around the infection foci. The bacteria will secrete glycocalyx through during adhesion to the host cells which can lead to increased protection and provide an increased resistance to antibacterial agents (Bakker, 2002), eradication of the intracellular pathogen will not occur (Allen, 1998). Research has concentrated in this area, loading antibiotics into the colloidal carriers, liposomes and nanoparticles, has shown an improved drug delivery to infected cells (Allen and Martin, 2004). Currently there are two types of delivery systems: liposomes and nanoparticles. Intracellular drug delivery systems need to be biocomatable with the host and and should minimize nonspecific cytotoxic effects to healthy tissues (Seale-Goldsmith, 2009) . Nanoparticles have been produced using a variety of materials, including poly(lactide-co-glycolide) (PLGA), poly-lactic acid (PLA), polymethacrylic acid (PMA), polyethylene glycol (PEG), natural polymers such as chitosan, gelatin, or alginate. Nanomaterials have physicochemical properties which are different from other substances. The possible routes by which nanomaterials migrate in the human body include the following:

1) Endocytosis: Nanomaterials enter the cells when they are surrounded by the cell membrane but don’t pass through it.

2) Penetrates the cell membrane using hydrophobic particles.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

3) Nanoparticles <5 nm are transported across the cell membrane channel.

The nanoparticle structures allow a better retention of the drug inside the polymeric network and can slowly be degraded by esterase action.

There are two types of nanoparticles:

a. Nanospheres (these have a solid framework)

b. Nanocapsules (these have a liquid central cavity surrounded by a wall).

Nanoparticles are classically prepared by the emulsion polymerization of alkylcyanoacrylic monomers in the presence of a specific drug. This reaction is induced by a nucleophilic agent. In some cases, the antimicrobial agent may itself induce the anionic polymerization of the monomer. The linkage of the drug to polymer by covalent bond may result in its non availability which is not desirable as a therapeutic drug. Therefore, the antimicrobial activity of the nanoparticle drug formulation must always be compared *in vitro* with the free drug as the control to understand the bioavailability. The release of the drug from the nanoparticles is dependent on the degradation rate of the nanoparticle. The release can be maximised using enzymes for its degradation. The drug may be used to form the nanoparticle through encapsulation within a polymer matrix, or it can through attachment to the surface of a solid nanoparticle which acts as a carrier. The incorporation of surface targeting components helps to localize to the affected tissue which may improve the therapeutic efficacy, along with the presence of molecules to enhance the cellular penetration such as cell-penetrating peptides (CPPs). CPPs are short, cationic peptides, typically derived from the HIV TAT proteins (Fonseca, 2009), which readily translocate through cell membranes. The addition of CPPs, such as TAT, to the surface of a nanoparticle can increase the efficiency of intracellular delivery of the nanoparticles (Juliano,2009; Schmidt,2010). The CPP probably promotes direct translocation through the membrane via electrostatic interactions or the it binds to specific receptors on the membrane and induces rapid receptor-mediated endocytosis (Schmidt, 2010) which promotes cellular penetration and as a result helps combat infection by intracellular pathogens. Cellular internalization of NP activates immune cells including macrophages and neutrophils, contributing to reactive oxygen species and reactive nitrogen species (ROS/RNS). Based on these findings, it is postulated

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

that the oxidative stress and inflammatory responses may induce the occurrence of immunotoxicity directly or indirectly. Cytokines are soluble signaling molecules used for intracellular communication. Upon such recognition, activation of $CD4^+$ T cells and T helper (Th) cells, differentiation can take place into Th1 and Th2 type subsets (Seder and Hill, 2000). These two subsets secrete a variety of cytokines. A typical Th1 type cytokine is IFN-γ which induces phagocyte-mediated defense against infection, particularly intracellular microbes. In contrast, Th2 type cytokines such as IL-4 and IL-10, down regulate Th1 type immune responses and activate B cells to stimulate antibody synthesis (Lima *et al*, 2004).

Nanoparticles against pathogen:

Nanomedicine is an application of nanomaterials that provides solutions to current unmet medical needs. Recent advances includes the development of innovative nanoparticles to target reactive oxygen species (ROS) (Figure 2). Oxidative stress has been known to contribute a great deal in altering the bacterial membrane permeability resulting in damage of cell membranes. There are various examples of metallic NPs regarding this aspect such as nano silver ions activate oxygen and produce reactive oxygen ions and hydroxyl radicals, which can hinder bacterial proliferation or destroy bacterial cells.

Metallic nanoparticles (NPs) offer a novel potential means of fighting bacterial infections and combating drug resistant infections. Nanoparticle activities can also target multiple biomolecules and have the potential to reduce or eliminate the evolution of multiple drug resistant organisms (MDROs) (Slavin *et al.*, 2017).Different NPs employ different mechanisms to destroy bacteria. Metallic NPs use several modes of action to destroy bacteria: they penetrate the bacterial cell wall and form pores on the surface of the membrane which, in turn, cause free radical formation which can destroy the cell membrane (Prabhu and Poulose, 2012).

A recent report describes an enhanced activity of ciprofloxacin and gentamicin, among others, with both synergistic and additive effects, when applied in combination with AgNPs (Panáček, 2016). The enhancement effect was higher in antibiotic-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

resistant strains than in antibiotic-susceptible strains, because AgNPs affect the cell membrane and cell wall integrity favoring antibiotic action, leading to a "restored" susceptibility for some antibiotic resistant strains (Panáček, 2016). It has been reported that RBx 11,760 loaded nanoparticles have potential to be used as nanomedicine against drug resistant *S. aureus* infections (Barman, *et al.*, 2018).

Bio-polymeric nanoparticulate drug delivery system:

Nanoparticles offer the ability to interact with complex biological functions at the scale of biomolecules, implying enormous opportunities for novel applications in medicine, including diagnostics, medical imaging, targeted delivery and immunotherapy (Zhang, 2015). Nanoparticles are defined as a material with dimensions of 0.1-100 nm that behaves as a whole unit in terms of its transport, properties and unique characteristics.

Polymeric nanoparticles are defined as nanoscale drug delivery platforms assembled by biodegradable polymers, dendrimers, conjugates or micelles (Poilil Surendran *et al*., 2017). The nanoparticles can easily be manipulated through the particle size and surface characteristics. Such modifications increase drug therapeutic efficacy and reduce the side effects. Polymeric biodegradable nanoparticles have been explored for vaccine formulations, in which antigen is encapsulated in polymers such as PLGA, polylactide or Chitosan (Pandey *et al.*, 2011). For example, it has been reported that smaller nanoparticles (~25 nm) travel through the lymphatic more readily than the larger particles (~100 nm) and accumulate in lymph node resident dendritic cells (Reddy *et al.*, 2007). Polysaccharide-derived nanoparticles and nanostructured surfaces help to improve biocompatibility of cell toxic material, together with new immobilization approaches, which are currently in development for novel bionanoparticle-derived pharmaceutical formulations. Panda *et al.*, (2009) revealed that microparticles elicit better antibody response than nanoparticles, and improve the immunogenicity of polymer particle entrapped antigen. Admixture of micro-particulate antigen and alum elicit memory antibody response. Biopolymeric nanoparticles like Albumin (Goosen, 1985), Alginate (Wee and Gombotz, 1994), Collagen (Gilbert *et al*., 1990), Gelatin (Shinde and Erhan, 1992; Saffran *et al*., 1991),

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 28 October 2020 doi:10.20944/preprints202010.0580.v1

Hyaluronic acid (Illum, 1994), Polysaccharides (Artursson, 1984), Chitosan (Verma, 2011; Artursson, 1984), have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs, their ability to target cells / tissues, and in their ability to deliver drugs in optimal dose at targeted sites (Langer, 2000). The synergistic effect of NPs when placed in combination rather than using a single NP and for combinations to target a broad spectrum of both Gram-positive and Gram-negative bacteria, could be useful to healthcare practitioners and biomedical engineers in the development of medical devices that target a wide range of bacterial pathogens (Bankier *et al.*, 2019).

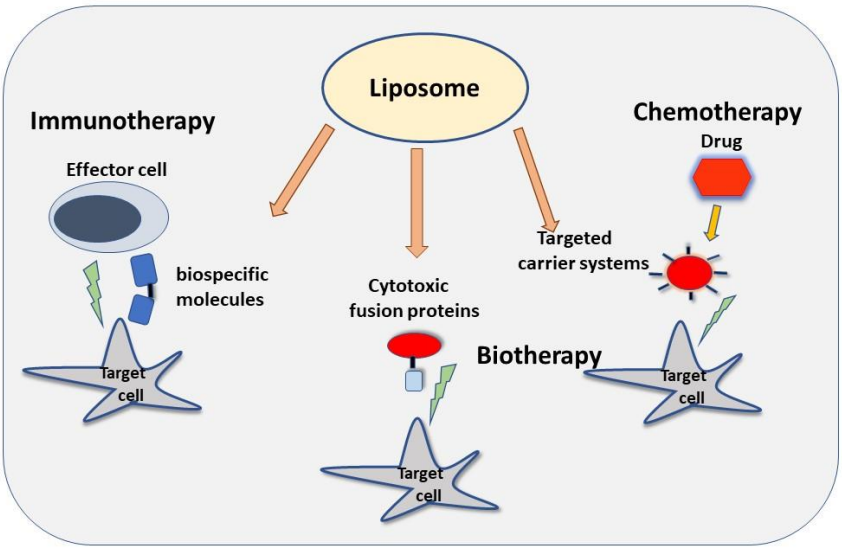

FIGURE 2. Schematic representation of nanoparticles as a carrier system and its applications.

Conclusion:

Infectious diseases caused by pathogenic bacteria are one of the most common causes of death worldwide and are a constant health risk in all countries (Marova *et al.*, 2011). Today, the burden of infectious disease on health, economy and other social aspects is so complex, that the worldwide cost cannot be estimated (El-banna *et al.*, 2012). Multi-drug resistant (MDR) pathogens constitute one of the most severe worldwide public health problem. Apart from those, the biodegradable polymers can

be utilized as drug delivery system because of several advantages such as biodegradability, biocompatibility, enhanced circulation and reduced toxicity. The main objective in using the nanoparticles as a delivery system is the control led release of encapsulated controlled drugs in order to accomplish the site specific action at the therapeutically optimal level. In addition, they can also influence the entrapment efficiency, release of the drugs from the nanoparticles and stability of nanoparticles. The area of modulation of immune response and oxidative stress using nanoparticles encapsulating drugs is yet unexplored. In this review we are trying to discuss the possible hypothesis whether the immune activating and generation of oxidative stress properties of nanoparticles encapsulated drugs delivery system can be co-related for progressing toward more effective therapy and immune response in case of infections caused by intracellular pathogens. Using antibacterial encapsulated nanoparticulate systems we may lead to a delay or inhibition of the resistance development. The shining ray of hope amidst the plethora of antimicrobial resistance is that the microbes would require multiple gene mutations in the same bacterial cell to become resistant to NPs. It will be interesting to explore further; an interplay of immune response, ROS and RNS created by intracellular pathogens, which warrant detailed evaluation on the signaling pathways to ascertain the extent of interdependence.

References:

1. Alexandra-Cristina Brussel, Oana Gherasim, Alexandru Mihai Grumezescu et al. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. Nanomaterials. 2018; 8: 681.
2. Allen TM. Liposomal drug formulations. Rationale for development and what we can expect for the future. Drugs. 1998;56(5):747–756.
3. Allen TM, Martin FJ. Advantages of liposomal delivery systems for anthracyclines. Semin Oncol. 2004;31(6 Suppl 13):5–15.
4. Bakker Woudenberg IA. Long-circulating sterically stabilized liposomes as carriers of agents for treatment of infection or for imaging infectious foci. Int J Antimicrob Agents. 2002;19(4):299–311.
5. Ansari, MA, Khan HM, Alzohairy MA, Jalal M, Ali SG, Pal R, Musarrat J. Green synthesis of Al2O3 nanoparticles and their bactericidal potential against clinical isolates of multi-drug resistant pseudomonas aeruginosa. World J. Microbiol. Biotechnol. 2015; 31: 153–164.
6. Artursson P, Edman P, and Sjoholm I. Biodegradable Microspheres. I. Duration of Action of Dextranase Entrapped in Polyacrylstarch Microparticles in Vivo. J. Pharmacol. Exp. Ther. 1984; 231: 705-712.

7. Basu SK, Kumar D, Singh DK, Ganguly N, Siddiqui Z, Rao KV, Sharma P. Mycobacterium tuberculosis secreted antigen (MTSA-10) modulates macrophage function by redox regulation of phosphatases.FEBS J. 2006 Dec;273(24):5517-34.
8. Basu SK, Kumar D, Ganguly N, Rao KV, Sharma P. *Mycobacterium tuberculosis* secreted antigen (MTSA-10) inhibits macrophage response to lipopolysaccharide by redox regulation of phosphatases. Indian J Exp Biol. 2009; Jun;47(6):505-19.
9. Basu SK, Kumar D, Singh DK. Ganguly N, Siddiqui Z, Kanury V. S. Rao and Sharma P. Mycobacterium tuberculosis secreted antigen (MTSA-10) modulates macrophage function by redox regulation of phosphatases. FEBS Journal. 2006;273:5517–5534.
10. Bogdan C. Nitric oxide and the immune response. Nat. Immunol. 2001; 2(10): 907-16.
11. Briviba K; Kissner, R.; Koppenol, W. H.; Sies, H. Kinetic study of the reaction of glutathione peroxidase with peroxynitrite. Chem. Res. Toxicol. 1998;11: 1398- 1401.
12. Baxt LA, Garza-Mayers AC, Goldberg MB. Bacterial subversion of host innate immune pathways. Science. 2013;340:697-701.
13. Bach H, Papavinasasundaram KG, Wong D, Hmama Z, Av-Gay Y. Mycobacterium Tuberculosis virulence is mediated by Ptp A dephosphorylation of human vacuolar protein sorting 33B. Cell Host Microbe. 2008;3(5):316-322.
14. Batram C, Jones NG, Janzen CJ, Markert SM, Engstler M. Expression site attenuation mechanistically links variation and development in Trypanosoma brucei. 2014;20;3:e02324.
15. Baptista PD, McCusker MP, Carvalho A, et al. Nano-Strategies to Fight Multidrug Resistant Bacteria—"A Battle of the Titans". Microbiol. 2018.
16. Belkaid Y and Hand T W. Role of the microbiota in immunity and inflammation.Cell. 2014; 157(1):121–141.
17. Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K. Evaluation of a nutrient starvation model of Mycobacterium tuberculosis persistence by gene and protein expressing profiling. Mol Microbiol. 2002;43:717-731.
18. Briviba. K.; Kissner, R.; Koppenol, W. H.; Sies, H. 1998. Kinetic study of the reaction of glutathione peroxidase with peroxynitrite. Chem. Res. Toxicol.,11: 1398 1401.
19. Cheloni G, Marti E, Slaveykova V I. Interactive effects of copper oxide nanoparticles and light to green alga Chlamydomonas reinhardtii. Aquat. Toxicol. 2016, 170, 120–128.
20. Christensen JE, Thomsen AR. Co-ordinating innate and adaptive immunity to viral infection: mobility is the key. APMIS. 2009;117: 338-355.
21. Clemens DL, Horwitz MA. Membrane sorting during phagocytosis: selective exclusion of major histocompatibility complex molecules but not complement receptor CR3during conventional and coiling pnagocytosis. J Exp Med. 1992; 175:1317-1326.

22. Dakal TK, Kumar A, Majumdar RS, Yadav V. Mechanistic Basis of Antimicrobial Actions of Silver Nanoparticles. Microbiol. 2016
23. Duran N, Nakazato G, Seabra AB. Antimicrobial activity of biogenic silver nanoparticles, and silver chloride nanoparticles: an overview and comments. Applied Microbiology and Biotechnology. 2016;100:6555-6570.
24. El-banna A A E , El-razek A M , El-mahdy A R . 2012. Isolation, identification and screening of carotenoid-producing strains of *Rhodotorula glutinis.* Food Nutr Sci.2012; 3 627-633.
25. Fialkow L, Wang Y, Downey GP. Reactive oxygen and nitrogen species as signalling molecules regulating neutrophil function. Free Radic Biol Med. 2007;42(2):153-64.
26. Fonseca SB, Pereira MP, Kelley SO. Recent advances in the use of cell-penetrating peptides for medical and biological applications. Adv Drug Deliv Rev. 2009;61:953–964.
27. Ghasemi F, Jalal R. Journal of Global Antimicrobial Resistance Antimicrobial action of zinc oxide nanoparticles in combination with ciprofloxacin and ceftazidime against multidrug-resistant *Acinetobacter baumannii*. J Glob Antimicrob Resist.2016; 6:118–122.
28. Gilbert DL, Kim SW. Macromolecular release from collagen monolithic devices. J. Biomed. Mater. Res. 1990;24: 1221-1239.
29. Goosen MFA, O Shea G.M. Gharapetian HM, Chou S, Sun A. Optimization of microencapsulation parameters: semipermeable microcapsules as a bioartificial pancreas. Biotech Bioeng. 1985;27: 146-150.
30. Grant SS and Hung DT. Persistent bacterial infections, antibiotic tolerance, and the oxidative stress response. Virulence. 2013;4(4):273–283.
31. Guo C, Xia Y, Niu P, et al. Silica nanoparticles induce oxidative stress, inflammation, and endothelial dysfunction in vitro via activation of the MAPK/Nrf2 pathway and nuclear factor-κB signaling. Int J Nanomedicine. 2015;10:1463–1477.
32. Gomes PS, Bhardwaj J, Rivera-Correa J, Freire-De-Lima CG, Morrot A. Immune escape strategies of malaria parasites. Front Microbiol. 2016;7:1617.
33. Hemachand, T.; Gopalakrishnan, B.; Salunke, D.M.; Totey, S.M. and Shaha, C. 2002. Sperm plasma membrane associated glutathione S- transferases as gamete recognition molecules. J Cell Sci., 115: 2053-2065.
34. Hanum PS, Hayano M, Kojima S. Cytokine and chemokine responses in a cerebral malaria-susceptible or -resistant strain of mice to Plasmodium berghei ANKA infection: early chemokine expression in the brain. Int Immunol. 2003; 15 (5):633-40.
35. Heit B, Robbins SM, Downey CM, Guan Z et al. NPTEN functions to 'prioritize' chemotactic cues and prevent 'distraction' in migrating neutrophils. Nature Immunolgy. 2008; 9(7).
36. Hewitson JP, Grainger JR, Maizels RM. Helminth immunoregulation: the role of paracite secreted proteins in modulating host immunity. Mol Biochem Parasitol. 2009; 167(1):1-11.

 doi:10.20944/preprints202010.0580.v1

37. Ismail N, Ismail F, Omar SV et al. Drug resistant tuberculosis in Africa: Current status, gaps and opportunities. Afr J Lab Med. 2018; 7(2): 781.

38. Illum L, Farraj NF, Fisher AN, Giu I, Miglietta M. and Benedetti LM . Hyaluronic acid ester microspheres as nasal delivery systems for insulin. J. Control. Rel. 1994; 29:133–141.

39. Juliano RL, Alam R, Dixit V, Kang HM. Cell-targeting and cell-penetrating peptides for delivery of therapeutic and imaging agents. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2009;1(3):324–335.

40. Li JJ, Muralikrishnan S, Ng CT, Yung LY, Bay BH. Nanoparticle-induced pulmonary toxicity. Experimental Biology and Medicine. 2010; 235:(9).1025–1033.

41. Knaapen A M, Borm P J A, Albrecht C, and Schins R P F. Inhaled particles and lung cancer, part A: mechanisms. International Journal of Cancer. 2004; 109(6): 799–809.

42. Kenneth A, Fields, Wolf K, Betts J Helen. Chlamydia trachomatis in ocular and urogenital tissues. Current opinion in microbiology. 2009;12(1):81-7.

43. Klemens SP, Cynamon MH, Swenson CE, Ginsberg RS. Liposome encapsulated gentamicin therapy of Mycobacterium avium complex infection in beige mice. Antimicrob. Agents Chemother. 1990;34:967-970.

44. Kumar S, Singh BR. An overview of mechanisms and emergence of antimicrobials drug resistance. Adv Anim Vet Sci. 2013;1(2S):7–14.

45. Kuo AJ. Shu JC, Liu TP, Lu JJ, Lee MH, Wu T. S et al. Vancomycin-resistant Enterococcus faecium at a university hospital in Taiwan, 2002–2015: fluctuation of genetic populations and emergence of a new structure type of the Tn1546-like element. J. Microbiol. Immunol. Infect. 2018;51:821–828.

46. Kot AM, Błażejak S, Kurcz A, Gientka I, Kieliszek M. Rhodotorula glutinis—potential source of lipids, carotenoids, and enzymes for use in industries. Appl Microbiol Biotechnol. 2016; 100 (14): 6103-6117.

47. Langer R. Biomaterials in drug delivery and tissue engineering: one laboratory's experience. Acc. Chem Res.2000; 33: 94-101.

48. Lima KM, Dos Santos SA, Rodrigues Jr JM, Silva CL. Vaccine adjuvant: it makes the difference. Vaccine. 2004;22: 2374-2379.

49. Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. Biomed Res Int. 2013;942916.

50. Marova I, Certik M, Breierova E. Production of enriched biomass by carotenogenic yeasts-application of whole-cell yeast biomass to production of

pigments and other lipid compounds. In: Dr. Darko Matovic, editor. Biomass-detection, production and usage. InTech;.2011; ISBN: 978-953-307-492-4.

51. McLean KJ, Jacobs-Lorena M. Plasmodium falciparum Maf1 confers survival upon amino acid starvation. ASM mBIO. 2017;8(2):e02317-16.

52. Maurin M, Benoliel AM, Bongrand P, Raoult D. Phagolysosomes of Coxiella burnetii-infected cell lines maintain an acidic pH during persistant infection. Infet Immun. 1992;60:5013-5016.

53. Masocha W, Rottenberg ME, Kristensson K. Migration of African trypanosomes across the blood-brain barrier. 2007;92:110-4.

54. Miller BH, Fratti RA, Poschet JF, Timmins GS, Master SS, Burgos M, Marletta MA. and Deretic V. Mycobacteria inhibit nitric oxide synthaserecruitment to phagosomes during macrophage infection. Infect Immun. 2004;72(5):2872-8.

55. Mundargi RC, Babu VR, Rangaswamy V, Patel P and Aminabhavi TM . Nano/micro technologies for delivering macromolecular therapeutics using poly(D,L-lactide-co-glycolide) and its derivatives. J. Control Release.2008; 125:193-209.

56. Navarro-Yepes J, Zavala-Flores L, Anandhan A, Wang F, Skotak M, Chandra N. Antioxidant gene therapy against neuronal cell death. Pharmacology & Therapeutics. 2014;142:206–230.

57. Nathan, C.F. and Ehrtm S. 2004 Tuberculosis. Nitric Oxide in Tuberculosis. Lippincott, Williams & Wilkins.

58. Nayak B, Panda A K, Ray, P. et al. Formulation, characterization and evaluation of rotavirus encapsulated PLA and PLGA particles for oral vaccination. J Microencapsul. 2009;26:154-165.

59. Panacek A, Smekalova M, Vecerova R, Bogdanova K, Roderova M, Kolar M, et al. Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Enterobacteriaceae. Colloids Surf B Biointerfaces.2016;142:392–399.

60. Patarroyo ME, Bermudez A, Patarroyo MA. Structural and Immunological principles leading to chemically synthesized multiantigenic, multistage, minimal subunit based vaccine development. Chem Rev. 2011;111(5):3459-3507.

61. Pandey R, and Khuller GK. Nanotechnology based drug delivery system(s) for the management of tuberculosis. Indian J. Exp. Biol. 2006;44:357-366.

62. Paul, B. B.; Strauss, R. R.; Jacobs, A. A.; and Sbarra, A. J. 1970. Function of H2O2, Myeloperoxidase, and Hexose Monophosphate Shunt Enzymes in Phagocytizing Cells from Different Species. Infect Immun., 1(4): 338–344.

 doi:10.20944/preprints202010.0580.v1

63. Pelgrift RY, Friedman AJ. Nanotechnology as a therapeutic tool to combat microbial resistance. Advanced drug delivery reviews. 2013.

64. Poilil Surendran S, George Thomas R, Moon MJ, Jeong YY. Nanoparticles for the treatment of liver fibrosis.Int J Nanomedicine. 2017 Sep 20;12:6997-7006.

65. Prabhu S & Poulose E K . Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett. 2012;2:32–42.

66. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of applied microbiology. 2012;112(5):841-852.

67. Rajaonarivony M, Vauthier C, Couarraze G, Puisieux F and Couvreur P. Development of a new drug carrier made from alginate. J. Pharm. Sci. 1993;82(9):912-7.

68. Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas E N, Lakshminarasaiah. Antioxidants and human diseases. Clinica Chimica Acta. 2014;436:332–347.

69. Rodrigues V, Cordeiro-da-Silva A, Laforge M, et al. Impairment of T cell function in parasitic infections. PLoS Negl Trop Dis. 2014;8(2):e2567.

70. Ritterhaus ES, Baek SH, Sassetti CM. The normalcy of dormancy: common themes in microbial quiescence. Cell Host Microbe. 2013;13(6):643-651.

71. Reddy ST, Van der Vlies AJ, Simeoni E, Angeli V, Randolph GJ, O'Neil, CP Lee LK, Swartz MA and Hubbell JA. Exploiting lymphatic transport and complement activation in nanoparticle vaccines. Nat. Biotechnol.2007;25: 1159-1164.

72. Reed PW. Glutathione and the hexose monophosphate shunt in phagocytising and hydrogen peroxide-treated rat leukocytes. J. Biol. Chem. 1969;244: 2459–2464.

73. Reed SG, Bertholet S, Coler RN, Friede M. New horizons in adjuvants for vaccine development. Trends in immunology. 2008;30(1):23-32.

74. Rescigno M, Granucci, F. and Ricciardi-Castagnoli, P.. Dendritic cells at the end of the millennium. Immunology and Cell Biology. 1999;77(5):404-10.

75. Riley NA, Ward C, Sawatzky DA, Sheldrake TA, Dransfield I, Haslett C. and Rossi AG. Granulocyte Apoptosis and Macrophage Clearance of Apoptotic Cells as Targets for Pharmacological Intervention in Inflammatory Diseases. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry. 2006; 5(1): 3-12.

76. Rojas M, Barrera LF, Puzo G, Garcia LF. Differential induction of apoptosis by virulent M.tb. in resistant and susceptible murine macrophages: role of nitric oxide and mycobacterial products. J Immunol.1997;159:1352–1361.

77. Saffran M, Field JB, Pena J, Jones RH, Okuda Y. Oral insulin in diabetic dogs. J Endocrinol.1991;131(2):267-78.

78. Salcedo-Sora JE, Cammano-Gutierrez E, Ward SA, Biagini GA. The proliferating cell hypothesis:a metabolic framework for Plasmodium growth and development. Trends Parasitol. 2014;30(4):170-175.

79. Schmidt N, Mishra A, Lai GH, Wong GCL. Arginine-rich cell-penetrating peptides. FEBS Lett. 2010;584:1806–1813.

80. Seale-Goldsmith MM, Leary JF. Nanobiosystems. Nanomed Nanobiotechnol. 2009;1:553–567.

81. Sies, H.; Sharov, V.S.; Klotz, L.O. and Briviba, K. 1995. Glutathione peroxidase protects against peroxynitrite-mediate oxidation. J. Biol. Chem. 323: 40-46

82. Sato H, Shibata H, Shimizu T, Shibata S, Toriumi H, Ebine T. Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. Neuroscience. 2013;248:345–358.

83. Slavin Y. N., Asnis J., Hafeli U. O., Bach H. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J. Nanobiotechnology. 2017;15, 65. 10.1186/s12951-017-0308-z.

84. Seder RA and Hill AVS. Vaccines against intracellular infections requiring cellular immunity. Nature. 2000;406:793-798.

85. Shinde BG. and Erhan S. Flexibilized gelatin film-based artificial skin model: Release kinetics of incorporated bioactive molecules. Biomed. Mater. Eng. 1992;2(3):127-131.

86. Spaans SK, Weusthuis RA, van der Oost J et al. NADPH generating systems in bacteria and archae. Front Microbiol. 2015;6.

87. Storni T, Kundig TM, Senti G, Johansen P. Immunity in response to particulate antigen delivery systems. Advanced Drug Delivery Reviews. 2005;57:333-355.

88. StijlemanS B, Calijon G, Van Den Abbeele J, et al. Immune evasion strategies of Trypanosoma brucei within the mammaloan host: progression to pathogenecity. Front Immunol. 2016;7:233.

89. Tielens AG. Energy generation in parasitic helminths. Parasitol Today. 1994;10(9):346-52.

90. Ting SW, Lee CH, Liu JW. Risk factors and outcomes for the acquisition of

carbapenem-resistant Gram-negative bacillus bacteremia: a retrospective propensity-matched case control study. J. Microbiol. Immunol. Infect. 2018;51, 621–628.

91. Tsao LH, Hsin CY, Liu HY, Chuang HC, Chen LY, Lee YJ. Risk factors for healthcare-associated infection caused by carbapenem-resistant Pseudomonas aeruginosa . J. Microbiol. Immunol. Infect. 2018; 51:359–366.

92. Underhill DM, Ozinsky A, Smith KD. and Aderem A. Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. Proc. Natl. Acad. Sci. USA.1999; 96(25): 14459-63.

93. Urdahl KB. Understanding and overcoming the barriers to T cell-mediated immunity against tuberculosis. Semin Immunol. 2014 Dec;26(6):578-87.
94. Thannickal VJ and Fanburg BL. Reactive oxygen species in cell signaling, American Journal of Physiology. 2000;279,(6), L1005–L1028.
95. Vallyathan V and Shi X. The role of oxygen free radicals in occupational and environmental lung diseases. Environmental Health Perspectives. 1997;105 (1):165–177.
96. Verma AK, Sachin K, Saxena A, Bohodar HB. Release kinetics from bio polymeric nanoparticles encapsulating protein synthesis inhibitor-cycloheximide, for possible therapeutic applications. Curr. Pharm. Biotechnol. 2005; 6 (2): 121-130.
97. Verma A K, Pandey R P, Chanchal A, Siddiqui I, Sharma P. Encapsulation of Antigenic Secretory proteins of Mycobacterium tuberculosis in Biopolymeric Nanoparticles for possible aerosol delivery system. J. Bionanosciences. 2011;5, 88-95.
98. Vitco NP, Spahich NA, Richarson AR. Glycolytic dependency of high level nitric oxide resistance and virulence in Staphylococcos aureus. ASM. 2015;10.11145-15.
99. Wang, N. and Wu, X.S. Preparation and characterization of agarose hydrogel nanoparticles for protein and peptide drug delivery. Pharm. Dev. Technol. 1997; 2(2):135-142.
100. Wee, S.F. and Gombotz,W.R. Controlled Release of Recombinant Human Tumor Necrosis Factor Receptor From Alginate Beads. Proc. Intern. Symp. Control. Bioact. Mater. 1994; 21:730-731.
101. Whitin JC, Takahashi K and Cohen HJ. Activation of neutrophil superoxide production by concanavalin A can occur at low levels of intracellular ionized calcium. Blood. 1987; 62: 762.

102. Witkowski B, Lelievre J, Barragan MJ, Laurent V, et al. Increased to artemisinin in Plasmodium falciparum is mediated by a quiescence mechanism. Antimicrob Agents Chemother. 2010;54(5):1872-7.
103. Yan J, Bloom M, Bae Sung C, Luijten E, Granick S. Linking synchronization to self-assembly using magnetic Janus colloids. Nature. 2012; 491:578–581.
104. Yang W, Shen C, Ji Q, An H, Wang J, Liu Q, Zhang Z. Food storage material silver nanoparticles interfere with DNA replication fidelity and bind with DNA. Nanotechnology. 2009;20:085102.

 doi:10.20944/preprints202010.0580.v1

105. Zhang X. Cell Biochem Biophys. Gold Nanoparticles: Recent Advances in the Biomedical Applications.2015 Jul;72(3):771-5.
106. Zambrano-Villa S, Rosales-Borjas D, Carrero JC, Ortiz-Ortiz L. How protozoan paracites evade the immune response. Trends Parasitol, 2002; 18(6):272-278.