

# Application of nanoparticles in the control and management of pathogenic microorganisms

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**ABSTRACT:** In the past few decades, there have been growing interests in the use of nanoparticles (NPs) in combating infectious diseases. This is due to their unique size, shape and significantly improved physical, chemical, biological and antimicrobial properties at the nanoscale. Different types of NPs like gallium, copper, zinc, titanium, magnesium, gold and silver have been developed. However, silver nanoparticles have proved to be most effective as they exhibit potent antimicrobial efficacy against pathogenic bacteria, fungi, virus and parasites. Diagnosis and treatment of diseases can be done using NPs because they have been found to be very sensitive and specific in detection of pathogens. More so, some are inexpensive and easily reproducible. The progress in development of nanopesticides, nanovaccine as well as nanodiagnostic tools are opening up new avenues in the management of infectious diseases. However, there are challenges particularly the toxicity, which is a big issue of public health concerns. This review outlines the conceivable opportunities regarding the application of NPs for the control and management of pathogenic microorganisms.

**Keywords:** Nanoparticles, nano pesticides, parasites, toxicity.

## INTRODUCTION

Nanotechnology is rapidly sweeping through all vital fields of modern sciences and promises a pool of research opportunities in the field of medical sciences, life sciences, biotechnology and many others (Ismail and Vejayakumaran, 2012). According to Patel et al., 2014, nanoparticles (NPs) sizes range from 1 to 100 nanometres. It is also considered as enabling technology since the property and activity of NPs changes with change in size and shape (Ahmed, 2017). At the nanoscale,

physical, chemical and biological properties differ from the properties of individual atoms and molecules of bulk matter (Ismail and Vejayakumaran, 2012; Berekaa, 2016). Therefore, it provides opportunity to develop new medical equipment and drugs that can be used for the control and management of pathogenic microorganisms. Since most of the natural processes also take place in the nanometre scale regime, a confluence of nanotechnology and biology can address several biomedical problems and can

revolutionize the field of health and medicine (Zhang et al., 2006). Nanotherapy aims at maximising the physical and chemical characteristics of NPs for the treatment of pathogenic diseases (Sundar and Kumar, 2012).

Mohanraj and Chen (2006) outlined the key advantages of NPs in the field of biomedical science to include the following;

1. Surface characteristics and size of NPs can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent removal of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
3. Controlled release and particle degradation characteristics can be readily altered by the choice of matrix constituents.
4. Targeting specific site can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, parenteral, intra-ocular, nasal, and subcutaneous.

## NPs AND INFECTIOUS DISEASES

The ability of pathogenic microorganisms to resist antimicrobial agents has emerged seriously in recent times as a major health problem. Nanoparticles have been shown to possess size dependent interaction with pathogenic microorganisms and antimicrobial property (Dastjerdi and Montazer, 2010). According to Berekaa (2016), NPs can serve as a medium for antibiotic delivery, disinfection of filters and coating materials and can inhibit the growth of viruses. However, the bactericidal and bacteriostatic properties of these NPs depend on their stability and the retention time for bacterium-nanoparticle interaction. The use of antimicrobial enzymes covalently attached to NPs as an antibiotic-free approach to treat microbial infections because of enhanced stability of protein-nanoparticle conjugates and the possibility of targeted delivery is of great interest.

### Antimicrobial activity of NPs use in the control of pathogens

According to Banik and Pérez-de-luque (2017), NPs consist of metal and metal oxides, carbon based NPs have antibacterial activity. The antibacterial activity of NPs depends on the dosage and are more effective against Gram negative than Gram positive bacteria. Naturally occurring bacteria are independent of acquisition of resistant activity for NPs. Sundar and Kumar (2012)

reported that the antimicrobial mechanisms of action of NPs is a matter of active research, the NPs exert antimicrobial activity via photocatalytic production of reactive oxygen species that damage cellular and viral components, compromising the bacterial cell wall/membrane, interruption of energy transduction, inhibition of enzyme activity and DNA synthesis. The following NPs that are used in the control of pathogens;

### Silver Nanoparticles

Silver NPs is the most widely studied NPs for its antibacterial activities basically, it is also used in treatment of burn wound and dental work in form of silver nitrate or silver sulfadiazine. Patel et al. (2014) revealed that Silver NPs can inhibit *Pseudomonas aeruginosa* and *Escherichia coli* bacteria by blocking the respiratory chain and cell cycle division step. Silver NPs interact simultaneously with sulphur containing proteins of bacterial membranes and phosphorous containing compounds like DNA to inhibit replication. The silver NPs penetrate the bacterial cell wall, modulate the cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues.

**Antibacterial activity of Silver NPs:** In studies by Mohanraj and Chen (2006), silver NPs is an alternative antibacterial agent to antibiotics and have the ability to overcome the bacterial resistance against antibiotics. Therefore, it is necessary to develop silver NPs as antibacterial agents. Among the several promising nanomaterials, silver NPs seem to be most promising antibacterial agents due to their large surface-to-volume ratios and crystallographic surface structure. In related studies Dastjerdi and Montazer (2010) reported the antimicrobial activity of silver NPs against *Escherichia coli*, in which *E. coli* cells treated with silver NPs showed the accumulation of Silver NPs in the cell wall and the formation of "pits" in the bacterial cell walls, eventually leading to cell death. In the same *E. coli*, smaller particles with a larger surface-to-volume ratio showed a more efficient antibacterial activity than larger particles. Furthermore, the antibacterial activity of Silver NPs is not only size-dependent but also shape-dependent. Silver NPs were synthesized by four different types of saccharides with an average size of 25 nm, showing high antimicrobial and bactericidal activity against Gram-positive and Gram-negative bacteria, including highly multi-resistant strains such as methicillin-resistant *Staphylococcus aureus* (Liu et al., 2016).

**Antifungal activity of Silver NPs:** Fungal infections are more frequent in patients who are immunosuppressed and overcoming fungi-mediated diseases is a tedious process, because currently there are limited numbers of available antifungal drugs. Therefore, there is an inevitable and urgent need to discover antifungal agents, which should

be biocompatible, non-toxic and environmentally friendly. As reported by Ouda (2014) that silver NPs play an important role as anti-fungal agents against various diseases caused by fungi. Silver NPs showed potent anti-fungal activity against clinical isolates of *Trichophyton mentagrophytes* and *Candida* species with concentrations of 1 to 7  $\mu\text{g/mL}$ . Biologically-synthesized silver NPs showed enhanced antifungal activity with fluconazole against *Phoma glomerata*, *Phoma herbarum*, *Fusarium semitectum*, *Trichoderma* species, and *Candida albicans*. Silver NPs stabilized by sodium dodecyl sulfate showed enhanced antifungal activity against *Candida albicans* compared to conventional antifungal agents (Liu et al., 2016). Biologically synthesized Silver NPs exhibited antifungal activity against several phytopathogenic fungi (Rai and Kratosova, 2015).

**Antiviral activity of Silver NPs:** Viral mediated diseases are common and becoming more deadly in the world; therefore, developing anti-viral agents is essential (Qasim et al., 2014). The mechanisms of the antiviral activity of silver NPs are an important aspect in antiviral therapy. Silver NPs mechanisms of action on human immunodeficiency virus type 1 (HIV-1) was investigated by Banik and Sharma (2011). They found out that silver NPs kills HIV-1 virus via preferential binding to the envelop glycoprotein GP120 (gp120) glycoprotein knobs. In another studies by Liu et al. (2016) revealed that silver NPs have unique interactions with viruses based on certain size ranges and shapes. The antiviral activity of silver NPs incorporated into polysulfone ultrafiltration membranes was evaluated against MS2 bacteriophage, which shows that significant antiviral activity was a result of increased membrane hydrophilicity.

### **Gallium Nanoparticles**

Ahmed (2017) investigated the anti-bacterial properties of quaternary gallium NPs with various concentrations. The concentration of 1 mol% gallium NPs could provide a sufficient antibacterial efficiency and had a good potential promising therapeutic agent for pathogenic bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*. In a similar studies by Alghuthaymi et al. (2015), reported ionic radius of  $\text{Ga}^{3+}$  is very similar to  $\text{Fe}^{3+}$ , biological systems cannot distinguish these two ions. Therefore,  $\text{Ga}^{3+}$  can get to the cell as a substitute of  $\text{Fe}^{3+}$ . However,  $\text{Ga}^{3+}$  is not able to play a key role as  $\text{Fe}^{3+}$  in cell metabolism. This phenomenon leads to the lysis of the bacterial cell.

### **Silica Nanoparticles**

Silica NPs provide biocompatible solid support for enzyme immobilization. The immobilized enzyme molecules on the

NPs surface have shown excellent enzymatic activity (Alghuthaymi et al., 2015). Ultrafine silica NPs, functionalized with amino groups, have been shown to bind and protect plasmid DNA from enzymatic digestion and make effective cell transfection *in vitro*. Dense silica NPs serve as an uptake-enhancing component by physical concentration at the cell surface; enhanced transfection due to the particles is seen with almost every transfection reagent tested with little toxicity. Zhang et al. (2016) have shown that mesoporous silica NPs are promising materials for biomedical applications, such as delivering drugs or biological molecules (siRNA or DNA), to the target cells or tissues.

### **Titanium Nanoparticles**

In the work of Mujeebur (2014), reported that titanium dioxide NPs avidly binds lipopolysaccharide with bridging calcium cations and the complex induces marked proinflammatory signalling in primary human mononuclear phagocytes. Photoactivated  $\text{TiO}_2$  NPs have been shown to inhibit the growth of bacteria in culture following UV irradiation, by generation of reactive oxygen species; the inhibition was highly effective although devoid of specificity (Alghuthaymi et al., 2015).

### **Zinc oxide Nanoparticles**

NPs of ZnO enriched with poly vinyl ferrocenium have significantly higher antibacterial effects on *Staphylococcus aureus* than the other metal oxide (Brigger et al., 2002). According to Berekaa, (2016), the potential application of ZnO NPs as a bacteriostatic agent in visible light may have future applications in the development of derivative agents for controlling the spread and infection of a variety of bacterial strains. ZnO NPs causes apoptosis (programmed cell death) in bacteria.

### **Copper Nanoparticles**

Banik and Pérez-de-luque (2017) described that CuO NPs in suspension generated by thermal plasma technology showed activity against a range of bacterial pathogens, including methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*, with minimum bactericidal concentrations ranging from 100 to 5000  $\mu\text{g/mL}$ . CuO NPs incorporated into polymers suggest release of ions, which is required for optimum inhibition of bacteria. The antibacterial activity investigation of zero valent copper NPs (size-12 nm) have shown that interaction of copper NPs with *E. coli* resulted in the formation of cavities/pits in the bacterial cell wall through scanning electron microscopic analysis (Ouda, 2014).

### **Lipid Nanoparticles**

The *in vivo* applicability of liposomal delivery system depends on its routes of administration, oral, intravenous, subcutaneous, dermal, transdermal, intraperitoneal, intramuscular or inhalation through the bronchial track. All these pathways have specific characteristics and limitations. The liposomes, when administered orally, can survive stomach digestion, but are lysed by the lipolytic enzymes in the intestine (Zhang et al., 2006). It has been shown that they can protect the entrapped materials such as drugs from such degradation and this route is used for liposomes in oral vaccination. When injected intravenously, liposomes are rapidly cleared from the blood and absorbed mainly by the phagocyte cells of the reticuloendothelial system. Liposomes injected through subcutaneous, transdermal or intramuscular routes may remain in the in circulation longer. Thus, it may act as a depot of drugs and facilitate the slow release of the entrapped materials from the vesicles. Encouraging results have been found of liposomal drugs in the treatment of a wide spectrum of diseases in experimental animals and in human (Sundar and Kumar, 2012).

Liposome-encapsulated antimonials were found to be 700 times more active than unencapsulated drug, thus confirming the potential of liposomal systems in the treatment of leishmaniasis (Ahmed, 2017).

### **Gold Nanoparticles**

Alghuthaymi et al. (2015) suggested that the unique chemical and physical properties of gold NPs allow them to be used for transporting and unloading in pharmaceuticals. Cells can take up gold NPs without much adverse cytotoxic effects. Gold NPs are nowadays widely used in immunohistochemistry to identify protein-protein interactions. These have also been utilized as nontoxic drug carriers for selective drug delivery to target site. Gold NPs inhibits the growth of pathogenic bacteria by preventing cell division. Sundar and Kumar (2012) have shown that gold NPs covered with quaternary ammonium groups, as antimicrobial agents interact with plasmid DNA through electrostatic interactions, resulting in effective protection of DNA from enzymatic digestion. Gold NPs functionalized with covalently attached oligonucleotides activate genes related with innate immune system.

### **Iron Nanoparticles**

Rajasundari and Ilamurugu (2011) carried out several studies on the effect of NPs on pathogenic bacteria. In the investigation, they found out that the bactericidal effect of Zero-valent iron NPs (nano-Fe<sub>0</sub>) has a unique property of nano-Fe<sub>0</sub>, which was not observed in other types of iron-based compounds. Unlike the gold NPs, the iron oxide

NPs has an inhibitory effect on *E. coli* in a concentration dependant manner. Hamde (2013) used the phase contrast microscope to study the effect of both iron and gold NPs on bacteria cell division. It was observed that both iron and gold NPs prevent bacteria cell division.

### **Carbon Nanotubes**

The functionalized carbon nanotubes have numerous advantages when used as a nanovector for therapeutic molecules. Within NPs family, carbon nanotubes have emerged as a new alternative and efficient tool for transporting and translocating therapeutic molecules (Baetke et al., 2015). Carbon nanotubes can be functionalized with bioactive peptides, nucleic acids, proteins and drugs which can be used to deliver their cargos to cells and organs. One of the unique properties of carbon nanotubes is the network formation of carbon atoms in the nanometer scale, allowing the creation of nano-channels via cellular membranes (Sundar and Kumar, 2012). They are potential drug carriers in targeted delivery which is utilized for the diagnosis and treatment of infectious diseases and central nervous system disorders.

### **NANOVACCINE**

The nano-engineering of vaccines allows the creation of better adjuvants and vaccine delivery systems (Jain et al., 2007). The efficacy of a nanovaccine is measured by its ability to interact with and stimulate the immune system. Advancement of NPs has made possible its use as novel adjuvants and colloidal vaccine carrier to immunize the infected animals (Qasim et al., 2014). New generation NPs adjuvants are designed to minimize their side effects, prolong the immune response, and concurrently stimulate humoral, cellular, and mucosal immune responses. Calcium phosphate NPs can be used as potent adjuvant than alum. It induces high titers of immunoglobulin antibody which facilitates a high percentage of protection against Herpes simplex virus-2 infection, without inflammation at the site of administration. The size, physical and chemical property, surface charge of NPs makes it more suitable for the enhancement of mononuclear phagocytic system, stimulation of antigen presenting cells for the activation of immune system. Nanovaccine had several benefits over viruses and bacteria for vaccine delivery because of their size in micrometer and nanometer range. Oral delivery of NPs for the development of vaccine makes it stable in gastrointestinal tract, protective for encapsulated substances and able to modulate physicochemical properties. *In vivo* studies on mice by Alghuthaymi et al. (2015) had shown protection from influenza A virus in viral pneumonitis after single intranasal immunization and it does not require cold chain for storage.

## POTENTIAL NPs IN PLANT MICROBIAL DISEASE MANAGEMENT

Different types of organic and inorganic salts have been used for controlling disease for many years. Organic acids and salts were used to control citrus blue mold. The use of NPs in plant disease management is a novel approach that may prove very effective in future (Mujeebur, 2014). The NPs has potential prospects in plant disease control and management in different ways. The simplest and obvious way is in the direct application of NPs in the soil on seeds or foliage to protect plants from pathogen invasion. In this way, the NPs may suppress the pathogens in a way comparable to chemical pesticides. When NPs are to be applied directly in soil, their effects on non-target organisms especially the mineral fixing or solubilising microorganisms will be of great significance. Carbon tubes and NPs can also be used as a carrier of fine chemicals such as pheromones, systemic acquired resistance (SAR) inducing chemicals, polyamine synthesis inhibitors or even concentrated active ingredients of pesticides for their controlled release (Alghuthaymi et al., 2015). NPs use in plant disease control and management, there effects can be grouped into two, that is direct effect of NPs on pathogens and use of NPs in formulating the pesticides that is nanopesticides. In view of ultra-small size of NPs and their very high degree of reactivity/sensitivity, the NPs may also prove very effective in the diagnosis of plant pathogens/diseases and pesticide residue analysis (Rai and Kratosova, 2015).

### Effect of NPs on plant pathogenic fungi

Plant pathogens such as bacteria, fungi, viruses and nematodes are important limiting factors in the production of food material. Numerous methods are used to control pathogens but none of them offer perfect control of the disease causing agent. Hence, a great scope exists for the exploitation of NPs for the management of plant pathogens. The NPs have also been found suppressive to fungi. Ouda (2014) described that NPs of 15 micronutrients,  $\text{CuSO}_4$  and  $\text{Na}_2\text{B}_4\text{O}_7$  were found most effective in controlling rust disease of field peas. Microelements such as manganese and zinc also suppressed the damping off and charcoal rot diseases in sunflower. The silver NPs had been tested for fungicidal activity against different yeasts and molds such as *Candida albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata* and *Aspergillus brasiliensis*. The hybrid materials showed strong antifungal effects against the tested microbes. Fungicidal effect of zinc oxide NPs (ZnO NPs) against two post-harvest pathogenic fungi, *Botrytis cinerea* and *Penicillium expansum*, have been reported (Patel et al., 2014). Traditional microbiological plating, Scanning Electron Microscopy (SEM) and spectroscopy were used to study antifungal activities of ZnO NPs and to

characterize the changes in morphology and cellular compositions of fungal hyphae. The administer ZnO NPs ( $70\pm 15$  nm) at the concentrations greater than  $3 \text{ mmolL}^{-1}$  significantly inhibited the growth of *B. cinerea* and *P. expansum*, with the later fungus was being more sensitive to the treatments. The NP treatments caused deformation in the hyphae of *B. cinerea* and prevented the growth of conidiophores and conidia in *P. expansum* which eventually led to the death of fungal hyphae.

### Nanopesticides

There are various pests that are of adverse effect to the growth and development of crop. The conventional pesticides are not very effective in controlling these pests. Nanopesticides are quite promising and have great potentials to control plants pest in the soil (Mujeebur, 2014). Nanopesticides contain very small particles of pesticide's active compounds or other small engineered structures with useful pesticidal properties. This can increase the dispersion and wettability of agricultural formulations (reduction in organic solvent runoff) and unwanted pesticide movement. NPs exhibit useful properties such as stiffness, permeability, crystallinity, thermal stability, solubility and biodegradability needed to formulate the nanopesticides. The nanopesticide formulations have large specific surface area and this increased affinity to the target. Nanoemulsions, nanoencapsulates, nanocontainers and nanocages are some of the nanopesticide delivery methods that may prove very effective in plant protection programmes (Ouda, 2014).

## NANODIAGNOSTICS FOR MANAGEMENT OF PATHOGENIC MICROORGANISMS

The rapid and sensitive detection of pathogenic microbes at the point of care is extremely important. Limitations of most of the conventional diagnostic methods are lack of ultra-sensitivity and delay in obtaining laboratory results. A bioconjugated nanoparticle-based bioassay for in situ pathogen quantification can detect a single bacterium within 20 minutes (Polgoma et al., 2011). Several NPs have been used in the management and diagnosis of infectious diseases, the most frequently used are gold NPs, quantum dots, biomarkers, magnetic and viral NPs.

### Gold NPs for diagnostics

Polgoma et al. (2011) reported that small pieces of DNA can be attached to gold particles not larger than 13 nm in diameter. The gold NPs assemble onto a sensor surface only in the presence of a complementary target. If a patterned sensor surface of multiple DNA strands is used,

the technique can detect millions of different DNA sequences simultaneously; this was validated by the studies of Brigger et al. (2012) on NPs as diagnostic tools.

### Quantum Dots for diagnosis

Quantum dots are inorganic fluorophores offering numerous advantages as compared to the traditionally used fluorescent marker (Hamde, 2013). They are highly sensitive (brighter imaging signals), broad excitation spectra, stable fluorescence with simple excitation and do not need lasers. Their infrared colours enable whole blood assays and have a wide range of applications for molecular diagnostics and genotyping. Multiplexed diagnostics and integration of diagnostics with therapeutics is also a possibility, with the most important potential application for cancer diagnosis (Maksym et al., 2006).

### Biomarkers for diagnosis

Currently available molecular diagnostic techniques have been used to detect biomarkers of various diseases. NPs have refined the detection of biomarkers. Some biomarkers form the basis of innovative molecular diagnostic tests. The physicochemical characteristics and high surface areas of NPs make them potential candidates for developing biomarker-harvesting platforms (Amur et al., 2008).

### Magnetic NPs for diagnosis

As revealed by gel electrophoresis, this process facilitates nonspecific protein adsorption suppression, which is a requisite for NPs to be applied to carriers for bioscreening (Jain et al., 2007). Nanoparticles are used as labelling molecules for bioscreening. Superparamagnetism is a form of magnetism that appears in ferromagnetic or ferrimagnetic (types of permanent magnetism) nanoparticles. Superparamagnetic nanoparticles are useful for cell-tracking cells and for calcium sensing. Superparamagnetic nanoparticles measuring 2 to 3 nm have been used to reveal small and otherwise undetectable lymph node metastases. Ultrasmall enhances for imaging cerebral ischaemic lesions. A dextran-coated iron oxide nanoparticle enhances MRI visualisation of intracranial tumours for more than 24 hours (Kewal, 2007).

### Viral Nanobiosensors for diagnosis

Virus particles are biological NPs. Adenovirus and Herpes simplex virus can be used to trigger the assembly of

magnetic nanobeads as nanosensors for clinically relevant viruses. This assay can detect spectral differences between viruses, viral strains and viruses with gene deletions in biological media. The technique provides rapid diagnostics ( $\leq 60$  seconds) for detection and identification of viruses generating reproducible spectra without viral manipulation (Maksym et al., 2006). Rajasundari and Ilimurugu (2011) showed that it is possible to detect as few as 5 viral particles in a 10-mL serum sample. This system is more sensitive than ELISA-based methods and is an improvement over PCR-based detection because it is inexpensive, easily reproducible and faster.

### Nanoparticle as Biosensor

Synthesis of nanosensors which find applications in the public health sector in preventing food poisoning and in improving the existing clinical practices by allowing the more rapid quantification and detection of bacteria and viruses. A nanoparticle based bioassay is developed which can rapidly detect *E. coli* O157:H7 in food that cause one of the most dangerous and infectious food-borne diseases (Berekaa, 2016).

## SAFETY ISSUES OF NPs IN THE CONTROL AND MANAGEMENT OF PATHOGENS

There are many public health concerns about the *in vivo* use of NPs but not with the *in vitro* diagnostics, which forms the major part of laboratory diagnostics. There are environmental concerns about the release of NPs during manufacturing of NPs and the environmental hazards this may cause (Ismail and Vejayakumaran, 2012).

Despite the numerous potentials of NPs, there are still many unanswered questions about the fate of NPs introduced into the living body. Due to the huge diversity of materials used and the wide range in size of NPs, these effects will vary significantly. Quantum dots made with fluorescent labels of cadmium selenide or zinc sulphide increases stability which may release potentially toxic cadmium and zinc ions into cells (Zhang et al., 2016). The concerns centre on NPs smaller than 20 nm in diameter, which can penetrate the cells (Jain et al., 2007). A number of studies have been done, but at this stage, no conclusions can be drawn about the safety of NPs to be used *in vivo*.

## CONCLUSION

It's evident that many pathogenic microorganisms have developed resistance to conventional antimicrobial agents. NPs are very promising as antimicrobial agents. Pathogens are unlikely to develop resistance against NPs as compared to antibiotics, as NPs attacks a broad range

of targets in the microbes. However, extensive researches are needed to understand the physiological interactions of NPs, including toxicological effects of NPs *in vivo* and *in vitro*. Finally, the application of NPs may open up new avenues for biomedical research pertaining to the control and management of pathogenic microorganisms.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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