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Nanomaterials as Potential Antimicrobial Agent against Drug-Resistant Pathogens

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Abstract: ***Infectious diseases are one of the major causes of mortality and morbidity throughout the world. Emerging pathogens pose major threat to human health. Antibiotics are oftenly used to kill infectious pathogens. Exhaustive and overuse of antibiotics, have led the emergence of drug-resistant pathogen. Genes responsible for the antimicrobial resistance are oftenly present in plasmid. These genes are disseminated from one bacterium to another through horizontal gene transfer. Recent decade has faced problems of inefficacy of existing antibiotics. This has posed development of new candidate antimicrobial agents, which are effective against microbial pathogens. Nanomaterials having immense applications can overcome the existing lacunae for usage of conventional antibiotics. Nanomaterials are newer to bacteria and due to their inorganic nature the bacteria may not acquire resistance against them. Due to stability of nanoparticles the nano-based drugs can overcome the problem of low shelf life.***

Keywords- Drug resistance, Antibiotics, Nanoparticles, Escherichia coli and Salmonellae

I. INTRODUCTION

The deterioration in the microbiological quality of water and food adversely impacts human health worldwide. Rapid population growth has placed new challenges on management of the frequency of food and water-borne disease outbreaks. The majority of water and food borne infectious diseases that occur include diarrhea, typhoid, and cholera among others. An estimated 94% of the diarrheal burden of disease is attributable to the environment, and associated with risk factors such as unsafe drinking water, lack of sanitation and hygiene [1]. The diarrheal diseases kill an estimated 1.8 million people each year. Approximately, 20 million episodes of travelers' diarrhea occur annually and out of which 80% of cases are due to bacterial agents. Diarrheagenic Escherichia coli, including enterotoxigenic E. coli (ETEC) and enter aggregative E. coli (EAggEC), are responsible for approximately 50% of cases [2]. Globally, the three regions can be alienated depending upon the risk of acquiring travelers' diarrhea for visitors. India is categorized under the high risk area, having 30-50% chances of acquiring diarrhea [3]. Enterotoxigenic E. coli (ETEC) is among the most common E. coli diarrheal pathotypes. In the developing countries about 0.7 million childhood deaths due to ETEC diarrhoea are reported every year. Enterohemorrhagic E. coli (EHEC), a potential path ovar of E. coli, causes bloody diarrhea and hemorrhagic colitis. They can cause hemolytic uremic syndrome (HUS), an important cause of childhood acute renal failure [4].

It has been documented that countries in south Asia, particularly south-east Asia, exhibit high burden of typhoid fever. India and neighboring countries have been identified as high risk sites for infections caused by Salmonella spp. [5].

Consequently, the detection, reporting and characterization of food and water-borne illnesses help in identifying the origin of disease, the causative agents and the sources involved. Conventional methods for detection of pathogenic bacteria involve several rounds of selective enrichment and biochemical identification. Stressed bacterial cells, generally undergo viable but non-culturable (VBNC) state, and cannot be detected by culture based methods [6]. These methods are time consuming, less sensitive and generally require at least three to five days for identification and confirmation. The immunoassay based diagnostics have disadvantages involved with the use of antibodies for pathogen recognition including the potential for nonspecific binding, the need for physiological pH and temperature during immunoassay procedures. Apart from these, sensitivity to chemicals in samples, inability to distinguish viable from nonviable organisms and limited shelf life inhibit the detection of pathogens in proficient manner. The indiscriminate use of antibiotics in clinical infections, easy availability of antibiotics without prescription and international travel are the factors that contribute to the emergence and spread of multi-drug resistance in bacteria. In India and other developing countries, pathogen diagnostics based on antimicrobial agent resistance and virulence gene profiles of E. coli pathotypes particularly STEC/ EHEC and ETEC of surface and potable water resources is not well established. Therefore, antibiotic resistance and plasmid profile of *E. coli* isolates recovered from surface and potable waters will be helpful in future therapeutic advancement of clinical management of STEC infections in humans.

The over-usage of antibiotics has led emergence of new multi-drug resistant (MDR) pathogenic bacteria. In strong contrast to clinics, there are no data on emergence of antibiotic resistance in ETEC and *Salmonellae* in potable water. Hence, predictions on risk of emergence of new MDR are extremely difficult. It is for these reasons; a better understanding on the prevalence and emergence of MDR ETEC and *Salmonellae* in drinking water is an urgent need. This leads to necessity for the development of potent new candidate materials to overcome this problem.

A. Pathogenic Microorganisms

1) *Enterotoxigenic Escherichia coli:* Enterotoxigenic *Escherichia coli* (ETEC), a potential pathovar of *E. coli* is regarded as a major cause of diarrhea worldwide in humans, mainly affecting children and travelers [7]. The contamination of drinking or recreational waters with ETEC has been associated with waterborne disease outbreaks. Diarrhea due to ETEC is caused by the consumption of contaminated water and food [8]. In case of improper sanitation and hygiene, the ETEC is a major cause of diarrhea. In the developing countries, surface waters are the potential reservoirs of ETEC and transmission can occur while bathing and/or using water for food preparation. Further, these forms of transmission lead to the infection of local populations and in international travelers visiting these areas, hence often referred to as Traveler's diarrhea. A few studies report the prevalence of ETEC in surface waters and in macrophytes [9].

The pathogenesis of ETEC secretory diarrhea involves the colonization of small intestine epithelial cells by means of fimentous adhesins known as colonization factors (CFs) followed by the production of at least one out of two enterotoxin types, the heat-labile toxin (LT) and/or the heat-stable toxin (ST). LTs produced by ETEC strains are a heterogeneous group of toxins. Two major LT families have been identified, LT-I and LT-II. LT-II is rarely found among human-derived ETEC strains. The enterotoxin gene *LT1* commonly present in strains associated with human illness has been observed abundantly in ETEC recovered from surface waters in gangetic riverine system.

The traditional methods for the detection of ETEC are mostly culture or microscopy-based are being replaced with techniques that rely on molecular recognition for the specific targeting of the pathogen. Serotyping has been used to identify and characterize ETEC strains. The ETEC has more than 78 O groups and 34 H groups identified so far making the determination of O serogroups associated with the lipopolysaccharides in the cell wall and H serogroups of the flagella somewhat difficult [7]. There are no serotype that dominate and the huge number of combinations of O and H groups makes serotyping less suitable for identification of ETEC.

Molecular technique such as Polymerase Chain Reaction (PCR) has become the gold standard and has been used for the detection of pathogens.

B. Salmonella

Salmonella is an important food and water-borne pathogen. Each year, approximately, 93.8 million human cases of gastroenteritis and 155, 000 deaths occur due to Salmonella infection around the world [10]. The typhoid caused by *Salmonella enterica* serotype Typhi remains an important public health problem in developing countries. Further, Salmonellosis causes substantial medical and economic burdens worldwide. It has been demonstrated that countries in south Asia and particularly south-east Asia exhibit high burden of typhoid fever [5].

Salmonella have a wide range of hosts and are commonly associated with food animal products [11]. Outbreaks of Salmonella infections have been reported due to consumption of eggs, cheese, ice cream premix, a variety of fresh sprouts, juice, fishes, and other fresh vegetables [12]. Eggs and poultry meat products are one of the most important sources of infection by Salmonella in humans. Salmonellae can enter the food chain at any stage and has ability to multiply to harmful levels. The type of food plays a major role in the severity of illness. Salmonellae present in the fatty foods can pass through the acidic environment of stomach and become invasive in the intestine. Low numbers of *Salmonella* in food recreational, surface and potable water may pose a public health risk.

Water and sediments in aquatic environments are the potential reservoirs of Salmonella as these provide dissolved organic carbon and protection from contaminants and predation. An epidemiological study suggests that the sources of Salmonella infections in children were more likely a result of environmental contamination than from food sources. The runoff from fields with animal husbandry, poultry waste, addition of untreated sewage from nearby civilization contributes Salmonella enterica serovar Typhimurium in natural water resources. In addition, agricultural produces from crops irrigated by contaminated water have also been found associated in large number of outbreaks [13].

Selective toxicity is an important feature of an ideal antimicrobial drug in which the drug is harmful only to the parasite without being harmful to the host. Targets of ideal antimicrobial agents are anatomic structures and or biosynthetic functions present uniquely in micro-organisms rather than the host cell. Development of resistance to antimicrobial agents in bacteria is mediated by several mechanisms, which mainly include changes in bacterial cell wall permeability, removal of antimicrobial agents through efflux pumps of membrane, drug action site modification, antimicrobial agent's inactivation, etc.

C. Multi Drug Resistance

The easy availability, indiscriminate use of antibiotics in clinical infections and international travel are the factors that contribute to the emergence and spread of multi-drug resistance in bacteria. In addition, the dissemination of antibiotic resistance genes among human and non-human pathogens is the paradigm for horizontal gene transfer on a global scale. It is likely that close contact of the human population with surface and potable water can enrich the environmental gene pool of pathogens and lead to emergence of new pathogenic variants. In India and other developing countries, pathogen diagnostics based on antimicrobial agent resistance and virulence gene profiles of E. coli pathotypes particularly ETEC and EHEC of surface and potable water resources is not well established. Salmonellae are one of the most common causes of water-borne illness in humans. Enteric fever in humans is most commonly caused by Salmonellae. Salmonellae, usually acquired by the consumption of contaminated water and food have been a major human pathogen since decades. In India, the typhoid occurs with an incidence ranging from 102 to 2,219 per 100,000 populations [14]. With the frequent use of antibiotics to kill Salmonellae in previous decades, the pathogen has evolved resistance mechanism to combat against them. As a result, the multidrug-resistant (MDR) Salmonellae strains have been prevalent in environment and spread worldwide, resulting in high rates of morbidity and mortality. The extensive use of antibiotics have generated and disseminated drug-resistant S. Typhi in the environment and potable water drinking system. The emergence of MDR S. Typhi strains to existing antibiotics such as ampicillin, chloramphenicol and co-trimoxazole has complicated the treatment of typhoid fever. This leads to necessity for the development of potential new alternative materials in order to combat this problem.

D. Nanoparticles and Their Properties

Nanoparticles (NPs) are small sized (1-100 nm) compounds that are able to function as whole units. The dimension of matter important in nanoscience and nanotechnology is typically on the 0.2- to 100-nm scale (nanoscale). The properties of materials change as their size approaches the nanoscale. Further, the percentage of atoms at the surface of a material becomes more significant. Bulk materials possess relatively constant physical properties regardless of their size, but at the nanoscale this is often not the case. As the material becomes smaller the percentage of atoms at the surface increases relative to the total number of atoms of the material bulk. This can lead to unexpected properties of nanoparticles which are partly due to the surface of the material dominating over the bulk properties. At this scale, the surface-to-volume ratios of materials become large and their electronic energy states become discrete, leading to unique electronic, optical, magnetic, and mechanical properties of the nanomaterials. In general, as the size of inorganic and organic materials decreases towards the nanoscale, their optical and electronic properties largely varies from the bulk material at the atomic/molecular levels and is size and shape dependent. The various size dependent properties that can be observed are quantum confinement in semi-conductor particles, surface plasmon resonance in noble metal particles and super paramagnetism in magnetic materials. Thus, the crystallographic surface structure and the large surface to volume ratio make the nanoparticles exhibit remarkable properties. Moreover, the increased catalytic activity due to morphologies with highly active facets and the tailoring of its synthesis as per the requirement makes the nanoparticles an attractive tool to solve various technological problems. In the field of medicine, nanoparticles are being explored extensively because of their size dependant chemical and physical properties. The size of nanoparticles is similar to that of most biological molecules and structures. This makes them an interesting candidate for application in both in vivo and in vitro biomedical research. The result of their integration in the field of medicine has led to their application mainly in targeted drug delivery, imaging, sensing, and artificial implants. Another interesting avenue for their exploration in medicine is their use as antimicrobials to target highly pathogenic and drug resistant microbes. But, for the application of nanoparticles in biology, biocompatibility is a highly desired trait. Biocompatibility is the materials ability to perform medically without exertion of undesired local or systemic effects.

E. Nanoparticles as Potential Antimicrobials

Nanoparticles (NPs) are small sized (1-100 nm) compounds that are able to function as whole units. These compounds are becoming widespread for their use in consumer products and medical applications; with potential for utilization as therapeutic compounds, transfection vectors, anti-microbial agents and fluorescent labels [15]. Due to their outstanding properties, they are highly suitable as

antimicrobial agents against drug-resistant pathogens (Figure 1). Silver NPs are the most commercialized and prominent group of nano-compounds, attributed to their diverse applications in the health sector physical as well as biological properties. Silver, in a colloidal form, is used for the treatment of bacterial infections in open wounds, and preparation of ointments, bandages and wound dressings [16]. Additionally, nanosilver has been used as a contraceptive, and marketed as a water disinfectant. Silver NPs are now being exploited for the treatment of various diseases such as retinal and acquired immunodeficiency syndrome as a result of human immunodeficiency virus [17]. Concerns on environmental exposure to AgNPs have initiated toxicity studies. Silver NP-hydrogel induced DNA damage and the production of reactive oxygen species (ROS) in cultured HeLa cells [18]. A study using human lymphocytes revealed that AgNPs caused DNA damage and cell death [19]. Additionally, AgNPs induced oxidative stress and caused impairment of nuclear DNA in Swiss albino mice. Recently, the use of AgNPs as anti-cancer agents has proved promising.

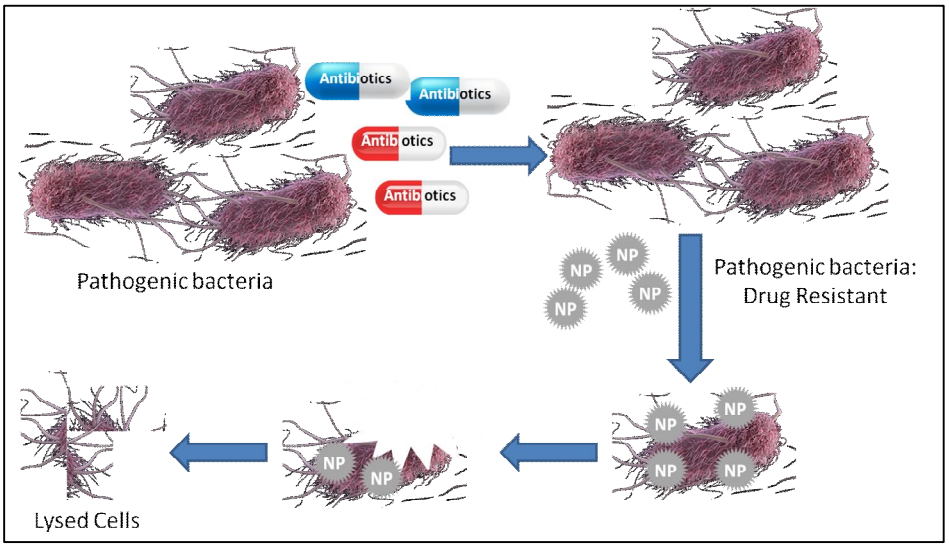

Fig. 1 Bactericidal effect of nanomaterials on drug-resistant bacteria

F. Silver Nanoparticles

Silver nanoparticles are used as effective antimicrobial agents. They have bactericidal potential against MDR organisms. Panacek and coworkers (2006) synthesized silver nanoparticles by developing one-step protocol and evaluated their antimicrobial activity against Gram-positive and Gram-negative bacteria, including MDR strains such as MRSA [20]. Colloidal silver nanoparticles were found to possess significant bactericidal potential against MRSA and Gram-positive and Gram-negative bacteria. Gram-negative bacteria include members of the genera Acinetobacter, Escherichia, Pseudomonas, Salmonella and Vibrio. Gram-positive bacteria include Bacillus, Clostridium, Enterococcus, Listeria, Staphylococcus and Streptococcus. Antibiotic-resistant bacteria include methicillin-and vancomycin-resistant Staphylococcus aureus (MRSA and VRSA) and Enterococcus faecium, by preventing biofilm formation, which act as efficient barriers against antimicrobial agents and the host immune system to protect the bacterial colony. In a study of antibacterial activity of the silver nanoparticles against MRSA and non- MRSA, minimal inhibitory concentration (MIC) and minimal bactericidal concentration were evaluated in LB broth using nanoparticles of 100 nm. They observed the dose-dependent bactericidal activity of silver nanoparticles against MRSA and non-MRSA and found that both MRSA and non-MRSA were inhibited at different concentrations when the inoculum was 10^5-CFU/ml). Silver compounds have been used to treat burns, wounds and infections. Various salts of silver and their derivatives are used as antimicrobial agents. Previous studies have reported that nanosized silver particles exhibit antimicrobial properties [21, 22]. Nanoparticles of silver have been studied as a medium for antibiotic delivery, and to synthesize composites for use as disinfecting filters and coating materials. Several mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on bacteria. It is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur-containing proteins on the bacterial cell membrane, silver nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions (particularly Ag^+) released from silver nanoparticles can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or can react with sulfur-containing proteins, leading to the inhibition of enzyme functions.

The general understanding is that Ag nanoparticle of typically less than 20 nm diameters get attached to sulfur-containing proteins of bacterial cell membranes leading to greater permeability of the membrane, which causes the death of the bacteria. The dose dependent effect of silver nanoparticles (in the size range of 10-15 nm) on the Gram-negative and Gram positive microorganisms has been studied. At micromolar levels of Ag+ ions have been reported to uncouple respiratory electron transport from oxidative phosphorylation, inhibit respiratory chain enzymes, or interfere with the membrane permeability to protons and phosphate. In addition, higher concentrations of Ag^{+} ions have been shown to interact with cytoplasmic components and nucleic acids [23].

G. Copper Oxide Nanoparticle

Copper oxide (CuO) is a semiconducting compound with a monoclinic structure. It is the simplest member of the family of copper compounds and exhibits a range of potentially useful physical properties such as high temperature, superconductivity, electron correlation effects and spin dynamics. Therefore, it finds a wide application. There is limited information available about the antimicrobial activity of nano CuO. As CuO is cheaper than silver, easily mixes with polymers and relatively stable in terms of both chemical and physical properties, it finds a wide application [24]. It is suggested that highly ionic nanoparticulate metal oxides, such as CuO, may find potential application as antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal morphologies. CuO nanoparticles were effective in killing a range of bacterial pathogens involved in hospital-acquired infections. But a high concentration of nano CuO is required to achieve a bactericidal effect [25]. It has been suggested that the reduced amount of negatively charged peptidoglycans make Gram-negative bacteria such as Pseudomonas aeruginosa and Proteus spp. less susceptible to such positively charged antimicrobials. However, in the time–kill experiments the Gram-negative strains showed a greater susceptibility to nano CuO combined nano Ag. Studies have been conducted to assess the potential of nano CuO embedded in a range of polymer materials. A lower contact-killing ability was observed in comparison with release killing ability against MRSA strains. This suggests that a release of ions into the local environment is required for optimal antimicrobial activity Copper nanoparticles have a high antimicrobial activity against B. subtilis. This may be attributed to greater abundance of amines and carboxyl groups on cell surface of B. subtilis and greater affinity of copper towards these groups. Copper ions released may also interact with DNA molecules and intercalate with nucleic acid strands. Copper ions inside bacterial cells also disrupt biochemical processes [26]. The exact mechanism behind bactericidal effect of copper nanoparticles is not clear.

F. Zinc Oxide Nanoparticle

Zinc oxide (ZnO) has some similar properties to TiO2 (i.e. its nanoparticles scatter light so it can be used for transparent UV filters, in creams or coatings). Like TiO_2, it is used for solar photocatalytic remediation but, compared to TiO_2, it has a weaker photocatalytic effect. Zinc oxide also suffers from the same limitation of absorbing only a fraction of the solar spectrum so research is underway to increase its photoresponse.

A peculiarity of ZnO is that it has a tendency to grow in self-organised nanostructures. By controlling crystal growth conditions, a variety of crystal shapes are possible. Researchers have been able to grow nanoscale wires, rods, rings, etc. The bactericidal mechanism of ZnO NPs is complex and still under investigation. However, it is believed to involve the release of Zn2C ions leading to the generation of hydrogen peroxide [27]. Huang et al showed that ZnO NPs attached to the cell walls of both gram-positive and gram-negative bacteria, resulting in membrane disorganization, elevated membrane permeability, and cell damage [28]. Biocidal effects and cellular internalization of ZnO NPs on *E. coli* were also reported. ZnO NPs were synthesized in di (ethylene glycol) (DEG) medium through the hydrolysis of ionic Zn^{2+} salts. *E. coli* cells were damaged, showing membrane disorganization of gram-negative triple layer units after the contact with DEG and ZnO. This behavior caused an increase of membrane permeability, leading to accumulation of ZnO NPs in the bacterial membrane as well as cellular internalization of NPs [29]. In another study, bactericidal effects of ZnO NPs (bare and thioglycerol (TG)-capped ZnO NPs) were also confirmed to result from membrane lipid peroxidation caused by ROS which is generated during the interaction of ZnO NPs with the culture medium. Reactivity of nanoparticles toward bacteria depends on their size and shape. In general, toxicity is inversely proportional to particle size. Just as smaller-sized AgNPs release more AgC ions against *E. coli*. Interestingly, truncated triangular AgNPs showed higher antibacterial activities compared to those of similar-sized spherical or rod-shaped AgNPs [30]. Recent results suggest that the surface charge of nanoparticles affects the toxicity of AgNPs. Negatively charged nanoparticles do not adsorb to negatively charged cell membranes due to electrostatic repulsion, thus their cellular internalization is greatly inhibited.

Zinc oxide is non-toxic and becomes one of the most important functional materials due to its unique optical, electronic properties such as near-UV emission, optical transparency, electric conductivity, piezoelectricity. ZnO have superior durability, greater selectivity and heat resistance than organic and inorganic materials. Due to noble properties such as high refractive index, high

thermal conductivity, binding energy, antibacterial and UV-protection of ZnO it could be used in various materials and products, including medicine, cosmetics, varistors, solar cells, rubber and concrete, foods. ZnO has high biocompatibility and fast electron transfer kinetics, such features advocate the use of this material as a biomimic membrane to immobilize and modify the biomolecules. Among a variety of semiconducting materials, zinc oxide is rich in nanostructures and it has the capability to produce into a variety of morphologies such as nanowires, nanorods, nanocombs, nanoflowers and nanosheet.

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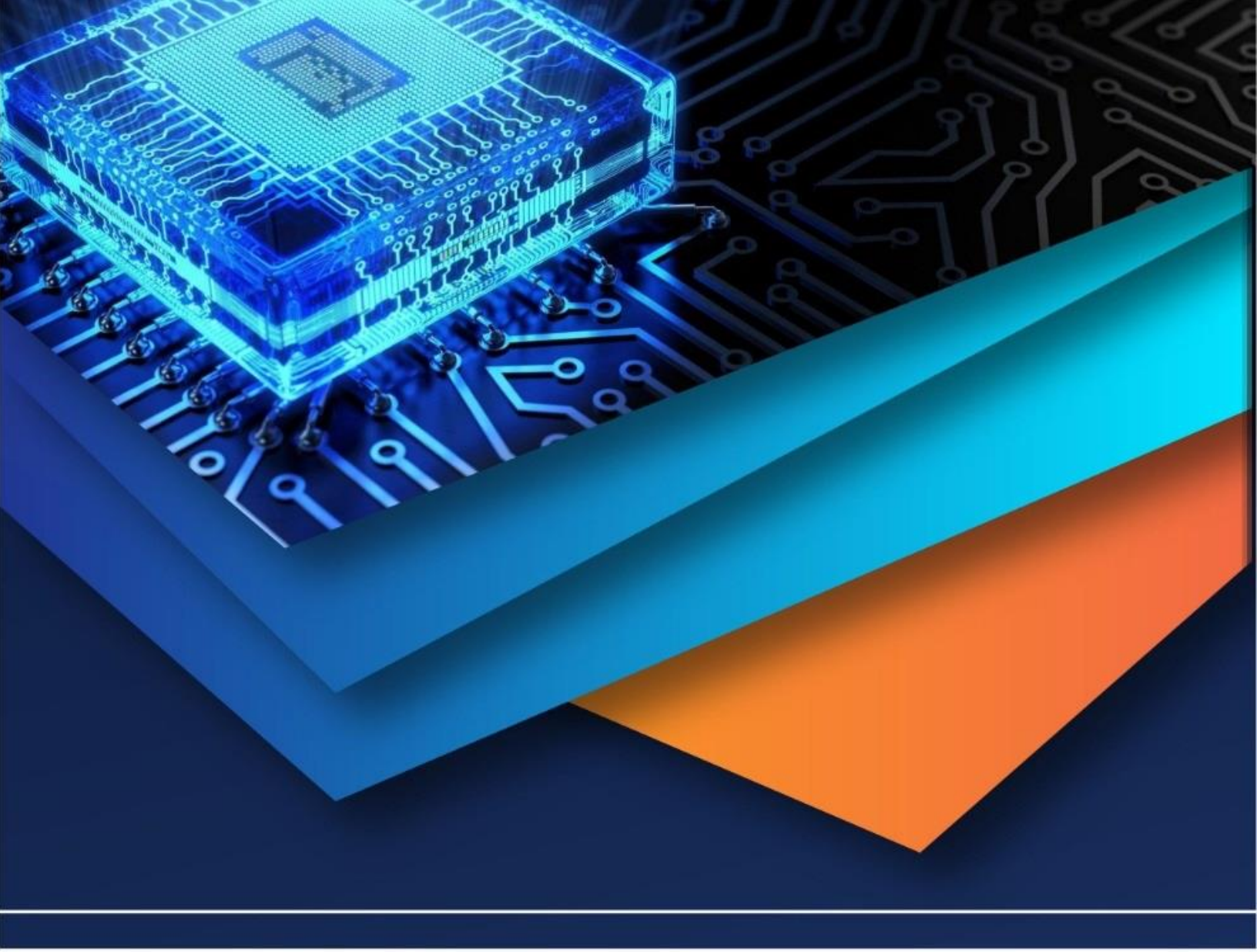

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