ROLE OF POLYMER CAPPED METAL NANOPARTICLES IN HEALTH

Dissertation submitted in partial fulfilment for the degree of

Master of Science in Biotechnology

Submitted By

Manjistha Sen Gupta

School of Biotechnology (Campus 11)

KIIT University

Bhubaneswar, Odisha, India

Under the Supervision of

Dr. Luna Goswami

Associate Professor

KIIT School of Biotechnology

KIIT deemed to be University

Bhubaneswar, Odisha, India

KALINGA INSTITUTE OF INDUSTRIAL TECHNOLOGY

Deemed to be University U/S 3 of the UGC Act, 1956

CERTIFICATE

This is to certify the dissertation entitled "***Role of Polymer capped metal nanoparticles in health***" Submitted by *Manjistha Sen Gupta* in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology, KIIT School of Biotechnology, KIIT University, Bhubaneswar bearing Roll No. *1661014* & Registration No. *16675658452* is a *bona fide* research work carried out by his/her under my guidance and supervision from 01.01.2018 to 18.05.2018.

(*Research Supervisor full Signature*)

Full name and Designation

KALINGA INSTITUTE OF INDUSTRIAL TECHNOLOGY

Deemed to be University U/S 3 of the UGC Act, 1956

CERTIFICATE

This is to certify that the dissertation entitled "Role of polymer capped metal nanoparticles in health" submitted by '*Manjistha Sen Gupta, Roll No. -16610114*, *Registration No.16675658452*' to the School of Biotechnology, KIIT University, Bhubaneswar-751024, for the degree of Master of Science in Biotechnology/ Applied Microbiology is his/her original work, based on the results of the experiments and investigations carried out independently by him/her during the period from 01.01.2018 to 18.05.08 of study under my guidance.

Further, it is also to certify that the above said work has not been previously submitted for the award of any degree, diploma, or fellowship in any Indian or foreign University.

Date:

Place:

Dr. Luna Goswami
Associate Professor
KIIT School of Biotechology
KIIT deemed to be University

DECLARATION

I hereby declare that the dissertation entitled "***Role of polymer capped metal nanoparticle in health***" submitted by me, for the degree of Master of Science to KIIT University is a record of *bona fide* work carried by me under the supervision and guidance of Dr. Luna Goswami, Associate professor, KIIT School of Biotechnology, KIIT deemed to be University, Bhubaneswar, Odisha, India.

Date:

Place:

Manjistha Sen Gupta

Table of Contents

LIST OF ABBREVIATIONS

AuNP - Gold nanoparticle

AgNP - Silver nanoparticle

$HAuCl_4$ - Chloro auric acid

NP - Nanoparticle

CMT - Carboxy methyl tamarind

UV- Vis spectra - Ultra Violet - Visible spectroscopy

SEM - Scanning electron microscopy

TEM - Transmission electron microscopy

LSCM - Laser scanning confocal microscopy

XRD - X-Ray Diffraction

DLS - Dynamic light scattering

FT-IR - Fourier Transform Infrared Spectroscopy

E. Coli - Escherichia Coli

µM - micromolar

µL - microlitre

OD - Optical density

PBS - Phosphate buffer saline

MRI - Magnetic Resonance Imaging

CFU - Colony forming unit

SPR - Surface Plasmon resonance

DCFDA - Dichlorofluorescein diacetate

MTT - Dimethylthiazol - diphenyl tetrazolim bromide

List of tables

List of figures

Fig 18 : TEM images of AuNPs at (a) low magnification power, (b) high magnification power

Fig 19 : Antimicrobial activity of AuNP at (a) Control, (b) 10 µM, (c) 20 µM,(d) 50 µM, (e) 100 µM, (f) 200 µM concentration of AuNP capped with CMT

Fig 20 : Proliferative effect of AuNP on Saos - 2 cell line (a) Control, (b) 10µM, (c) 20µM, (d) 50µM, (e) 100µM, (f) 200µM.

Fig 21 : Bar chart Showing dose Dependent Cytotoxicity effect of Gold Nanoparticle.

Fig 22 : Detection of ROS using Saos-2 cells, (a) Positive control -50 µM H_2O_2, (b) Negative control- Cell culture medium, (c), (d) and (e) Cells treated with gold nanoparticles.

Fig 23 : ALP activity in Saos 2 cell lines at different concentration of AuNP.

ACKNOWLEDGMENT

With the name of God, I would like to express my pleasure in bringing my project entitled “Role of Polymer Capped Metal Nanoparticles in Health”. I greatly accept this opportunity to convey my heartiest thanks and express my deep sense of gratitude to my project guide Dr. Luna Goswami, Associate Professor, KIIT School of Biotechnology, KIIT deemed to be University for her valuable guidance, kind co-operation and meticulous care through out the duration of the project work.

I would like to thank Dr. Mrutyunjay Suar, Director, KIIT School of Biotechnology for providing basic facilities for the work.

I would also like to convey my gratitude to Dr. Chandan Goswami (NISER) and his lab members for allowing to use some of their lab facilities.

I would like to extend my gratitude to my laboratory seniors Mr. Abhishek Singh, Mr. Satish Kumar, Mrs. Priyanka Choudhury, Ms. Hera Khan, Ms. Deepa Mandal, Ms. Sunita Panda, Ms. Ananya Nanda, Ms. Srijita for their personal interaction, valuable suggestion and positive criticism to improve my work.

Last but not the list I would like to thank my parents for their constant moral support.

ABSTRACT

Polymer coated metallic nanoparticles have found application in biomedical and therapeutic field as they have antimicrobial activity, can be used for targeted drug delivery, bio-imaging, etc. They are cost effective and less toxic than chemically synthesized nanoparticles. In this study polymer (Carboxymethyl tamarind, CMT) capped colloidal gold nanoparticles were synthesized which was characterized by UV- Vis spectroscopy, Dynamic light scattering, Transmission electron microscopy (TEM), XRD and FTIR. Antimicrobial activity of the synthesized gold nanoparticles was investigated against *E.coli*. Cytotoxic effect of this metal nanoparticle was investigated against Saos -2, osteoblast like cells. Different concentration of AuNP was used to detect ROS using Saos - 2 cell line. This nanoparticle shows antimicrobial property and no cytotoxicity towards mammalian cells. We propose that the CMT capped gold nanopartcle has a potential application in the biomedical field.

Key words :

Green synthesis, CMT polysaccharide, Gold nanoparticle, antimicrobial activity, cytotoxicity, reactive oxygen species (ROS).

INTRODUCTION

The unique properties of metal nanoparticles have been applied in almost every field of science and technology, making nanotechnology a promising field in modern life. Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic and molecular structures. The size of these tiny nanoparticles ranges from 1 to 100 nm and have significantly different characteristics from the particles which are not in nanoscale.

1. ***Background and context*** :

Due to high surface area and smaller size nanoparticles possess unique physical and chemical properties. Nanoparticles also show size dependent properties. Optical properties of nanoparticles are reported to be dependent on size which imparts different colors due to absorption in visible region. The antimicrobial activity of different metal nanoparticles is related with their size. For instance, the smaller the size of the silver nuclei, the higher will be its antibacterial activity [1].

Metal nanoparticles can be synthesized by two different methods - one is physical approach and another is the chemical approach. Physical method utilizes evaporation or condensation whereas chemical approach includes the formation of small metal clusters or aggregates under favored condition [1].

Over the past few decades researches are emphasizing on the “green synthesis” of nanoparticles using a suitable stabilizing and reducing agent. It is the simple and cost effective, eco-friendly method in which sugars, plant extract and microorganisms are used as reductants and capping agent. Polymers are used most frequently as stabilizing agent for chemical synthesis of nanoparticles. Now a days, biodegradable, bio polymers are being used for green synthesis.

Silver nanaoparticles are the prominent nanomaterials and have been widely used in a range of biomedical application including diagnosis, treatment, drug delivery, medical devices coating and for personal health care. The shape, size and composition of silver nanoparticles have significant effect in their function and can induce toxicity in living beings [2]. The toxic effect of silver nanoparticles can risk human health and hence an alternative to silver nanoparticle is needed.

In the present study, gold nanoparticles are synthesized using polysaccharide based biopolymer CMT (carboxymethyl tamarind polysaccharide). CMT gum is an easily available low cost reducing as well as stabilizing agent. It is easily soluble in water and can resist microbial degradation. The cytotoxic effect of AuNP and its potential to resist ROS formation was studied on osteoblast cells.

2. ***Scope and Objectives***:

In recent studies it has been confirmed that nanoparticles can detect the surface of the bio-macro molecules as an artificial receptor. The small size nanoparticles interact with the biomolecules by covalent or non-covalent linkage. Nanoparticles has its application in biological sensing, delivery and imaging of live cells and tissues. Bio-imaging technologies like optical imaging and magnetic resonance imaging has been benefited from the current development of luminescent and magnetic nanoparticles. An important goal for biomedical application, forensic analysis and environmental monitoring is to sense the biological agents disease and toxic material which can be easily done by using nanoparticles as a sensor [3].

Nanoparticles due to their advantageous characteristics can be used as potential drug delivery system. It can be used in treatment of different infectious disease which is hampered due to microbial resistance against all classes of microbial agents. The use of silver nanoparticles as antibacterial, antifungal, antiviral, antiprotozoal and anti-cancer drug have recently attracted the eye of the scientist everywhere in the globe [2,3].

Application of gold nanoparticles in this field is not pronounced. The objective of this study was to synthesize gold nanoparticles in a green synthetic method and to investigate its toxic effect on osteoblast cells.

3. ***Achievements*** :

Nanoparticles have the same dimension as biological molecules. In living organisms they may immediately adsorb onto the surface of large molecules and can enter into the tissues through body fluids. Some nanoparticles easily dissolve in blood and their effect on the living systems are same as the effect of chemical they are made up of.

4. ***Overview of dissertation***:

During in vitro studies a variety of mechanisms of AgNP toxicity have been reported, including ROS generation, DNA damage and cytokine induction [4]. This study includes the synthesis of gold nanoparticles. Here the cytotoxic effect of less toxic CMT- capped AuNP is reported along with its potential to generate ROS. The effect of gold nanoparticles was also observed during this experiment.

REVIEW OF LITERATURE

Nanoparticles are considered to the “next industrial revolution”. A fraction of nanomaterials are already near to commercialization. Application of the nanoparticles are strongly influenced by the size and aggregation state. Dedicated by their environment, size and physical dimension nanoparticles interact with light strongly. This is why nanoparticles exhibit vibrant colors. This unique optoelectronic properties are being utilized in high technology application. The surface plasmon resonance can be adjusted with varying shape and size of the nanoparticles leading to particles with modified optical properties for different application. The surface to volume aspect ratio, the most important feature, allow the nanoparticles to interact with other particles easily. Many optical properties of metallic colloids may led to many application utilizing their plasmon absorbance or their ability to enhance the Raman effect [5].

The current trend is to develop more complex nano-objects with improved morphology and better controlled chemical structures. From the known methods of producing nanoparticles with multifunctionality it has been identified that there are four main strategies to combine several function into one nanostructure. These strategies are categorized as core-shell strategy, surface multifunctionalization, multifunctionalization by asymmetric nanoparticles and multimeric assemblies of nanoparticles. The core shell strategy include polymer, silica and metal based structures, layer - by - layer assemblies, mesoporous and hollow particles while the surface modification comprises of one- step, multi-step or solid phase methodologies [4].

The use of different types and use of nanomaterials continues to evolve with the growth of nanotechnology for the in - vivo applications in fields like drug delivery, medical imaging, diagnostics and engineering technology. Combination of the optical and electronic properties of the nanoparticles leads to its application in optical data storage devices, biosensors and new catalyst. These properties also have advantages in single electron tunneling devices, non - linear optical devices, electron microscopy markers and the emerging era of plasmonics [6].

Metal nanoparticles can be prepared by both top - down and bottom - up approaches. A bulk-state metal is systematically broken down to obtain nanoparticles of desired size and dimension, in case of top - down procedure. In the bottom - up strategy, nanoparticles originates from individual molecules which include chemical and biological reduction. Chemical reduction is the classical method and one of the better methods for the preparation of particles with narrow size distribution. This process involves chemical reduction of metal salts by microemulsion, coprecipitation, carbon nanotube and polymer protection methods [7].

Stable nanoparticles are synthesized by biological reduction which is considered as a green approach for nanoparticle synthesis. The high volume, low cost chemical approach limits the use of nanoparticles in biomedical application due to contamination from toxic chemicals. “Green Synthesis” is a non toxic, eco-friendly approach for nanoparticle synthesis in order to allow them to be used in a wide range of industries. While preparing nanoparticles through green synthesis the major concerns is to select proper solvent system, to select an environmentally benign reducing agent and proper choice of stabilizing agent [8]. Different polymeric stabilizing agent like dendrimers, latex particles, microgels, mesoporous inorganic materials, hydrogels have been employed [7,10].

Although having disadvantages many bacteria, fungi and plants have shown their ability to synthesize metal nanoparticles [9]. The hydroxyl groups, hemiacetal group and other functionalities present in polysaccharides play important roles in both the reduction and the stabilization of metallic nanoparticles. Chitosan, a derived from of chitin, has improved solubility and therefore the exposed primary amine supports the immobilization of metal nanoparticles [11]. Silver nanoparticles can be obtained by the reduction of silver ions using starch as both reducing and capping agent. Dextran, a polysaccharide, is used as capping agent with high biocompatibility for the fabrication of nanoscale particles such as iron oxide. The weak chemical interaction between the polysaccharide and the nanoparticle ensure that the obtained nanoparticles could be easily separated from the reaction mixtures, making the production more energy efficient [12].

The biosynthetic method of using plant extract has received a greater attention with respect to the chemical and physical methods and even the use of microbes. Silver nanoparticles were biosynthesized from *Curcuma longa* tuber powder extracts using silver nitrate. In this simple and eco-friendly process the tuber extracts act as a reductants and stabilizer simultaneously [13].

Natural gums are also utilized for synthesizing nanoparticles. The plant based exudates gum such as gum Acacia and gum kondagogu can be utilized as stabilizing and reducing agent for silver nanoparticle synthesis. A microbial heteropolysaccharide, gum gallen, was used for the same purpose to synthesize gold nanoparticles. Gum ghatti, the biopolymer is used for the synthesis of nanoparticles due to its edible nature, low cost, intermediate viscosity, higher emulsification stability at low concentration. It is an polysaccharide derived from the bark of *Angeissus latifolia* whichact as a prebiotic by supplying the matrix required to sustain the microbial flora of human colon. Silver nanoparticles produced by using gum ghatti shows antimicrobial activity against gram positive and gram negative bacteria and hence can be used in various environmental and biomedical application [14]. Cashew gum, another natural product extracted from *Anacardium occidentate*, is a heteropolysaccharide and is used in synthesizing AgNPs. This cashew gum based silver nanoparticles shows cytotoxicity against VERO cells. The cell morphology did not change in comparison to control group where highest concentration of cashew gum was used [15].

AgNPs show antimicrobial activity against *Vibrio cholerae* and *Escherecia coli* which can be used as an new effective and inexpensive approach towards therapeutics [16]. Chitosan, a positively charged polysaccharide biopolymer display inhibiting activities towards human cell. CS-AgNPs do not cause any DNA damage of the host cell after treatment with a bactericidal dose of nanoparticles. For this reason it is vastly used in the field of food, medicine and pharmaceuticals [14].

The major problem in treatment of many diseases is to deliver a drug to its target site. Controlled drug delivery system (DSS) overcomes this limitation. Developments in nanotechnology has shown that nanoparticles smaller than 100 nm have a great potential to be used as a drug carrier [18]. Nanoparticles help in early diagnosis and localized treatment which give an broad range of application in biomedicine. For an example, nanomedicine are being used in breast cancer treatment. The basic prerequisites for design of new materials should comprise of the knowledge on

✓ Drug incorporation and release

✓ Formulation stability and shelf life

✓ Biocompatibility

✓ Biodistribution and targeting and

✓ Functionality

The adverse drug effect should be considered when carriers are used solely. Biodegradable nanoparticles with limited life span would be optimal in this case [19].

1. ***History of Nanoparticles*** :

Nanoparticles have their existence in nature for a long time and hence their use can be traced back to ancient time. More than 4500 years ago the control supplementation of ceramic matrix with natural asbestos nanofibers is more fascinating than the application of clay minerals as natural nanomaterials. Metallic luster decoration of glazed ceramics was found in Mesopotamia during 9^{th} century [20]. As a precious metal gold has been playing an important role in human history. "Soluble" gold appeared in China and Egypt around 4^{th} or 5^{th} century B.C. Gold nanoparticle was first used to make ruby red colored glass and in 4^{th} century it was applied to make Roman opaque glass cup. Lycurgus cup in the British Museum is one of the example of such cup which changes its color from green to red because of illumination [20,21].

Fig 1 : First application of gold nanoparticles in Lycurgus Cup (www.awesomestories.com)

People began to realize the use of gold in red colored glass during the period of 5th to 15th century. In 17th century a colorant in glasses, “Purple of Cassius” was popular. It is a colloid resulting from the hereto-coagulation of gold particles and tin oxide [21,22].

Scientist began to investigate in preparation colored gold materials from 19th century onwards. Silver nanoparticles was initially used to satin the glasses yellow. In 1960’s and 1970’s metallic nano powders were used in magnetic recording tape. The prefix “nano” is derived from a Latin word “nanos” which means dwarf. The concept of ‘nanometers’ was first proposed by a Nobel laureate Richard Zsigmondy. It takes a long time for the nanoparticles to be associated with the modern science. Modern nanotechnology is the innovation of physicist Richard Feynman.

In 1959, his lecture “There’s plenty of room at the bottom” introduce the concept of manipulating matter at the atomic level. A Japanese scientist Norio Taniguchi used nanotechnology for the first time to describe semiconductor process which occurred on the order of nanometer. In late 1960s nanoparticles for drug delivery system was first developed by Paul Elrich [37]. The golden era of nanotechnology began in 1980s with the discovery of fullerenes. In 1986 Eric Drexler used ideas from Feynman’s work and independently used the term “nanotechnology” in his book ‘Engines of creation: The Coming Era of Nanotechnology’. This book provide an idea about nanoscale “assembler” and his vision of nanotechnology is known as molecular naotechnology. In the beginning of 21st century nanoscience and nanotechnolgy were the major area of interest [23].

2. ***Classification of Nanoparticles*:**

To understand and appreciate the diversity of nanomaterials some categorization is are required. According to their origin nanomaterials are classified as natural nanomaterials and artificial nanomaterials. Minerals, such as natural colloids, clay, fog, volcanic ash, ocean spray are some of the examples of natural nanomaterials. Carbon nanotubes, quantum dots - nanoparticles prepared through a well defined mechanical and fabrication process, are well known artificial nanomaterials [47]. Nanoparticles differentiated as per their dimension, morphology, composition, uniformity and agglomeration are mentioned herein:

2.1. ***Dimensionality* :-**

Dimension wise nanoparticles are divided into three categories - One dimension(1D), two dimension(2D) and three dimension(3D).

1D - Thin films or surface coatings fall in this category. These are the materials with one dimension in nanometer scale.

2D - Two dimensional materials in the nanometer range include nanotubes, nanowires, dendrimers and nanofibers.

3D - These nanomaterials are nanoscale in all the three dimension. Fullerenes, nanoparticles, nanocrystals, colloids and precipitates.

Due to their intrinsic differences in physical and chemical properties, their methods of preparation and their biological targets it is difficult to correlate the reports on one particle with the other [38].

2.2. The Morphology of nanoparticles and nanocomposites :-

In case of hybrid nanoparticles flatness, aspect ratio and spatial arrangement are taken into account as morphological characteristics. A general classification is done on the basis of high and low aspect ratio. High aspect ratio include nanotubes and nanowires while small aspect ratio include spherical, oval, pillar, helical shaped morphologies [39].

2.3. *Chemical Composition :-*

2.4. Nanoparticles can be composed of single constituent or can be made up of several materials. Depending on their chemical composition nanoparticles are categorized as follows -

❖ Mixed nanoparticles can be either random or ordered. Randomly mixed are solution of solids whereas ordered nanoalloys correspond to the ordered arrangement of two different atom.

❖ Core- shell nanoparticles are shell consisting one type of atom surrounded by another type of atom.

❖ Layered nanoparticles consist of two atoms sharing a common interface.

On the basis of their chemistry and electromagnetic properties nanoparticles can exist as aerosols, suspension or colloids and in an agglomerate state [39].

3. ***Properties of Nanoparticles*** :

Nanoparticles possesses unexpected properties. Bulk materials have constant physical and chemical properties. But the properties of nanomaterials change as per their sizes. Large surface area, mechanical stability, optical activity and chemical reactivity, are the various physicochemical properties that make nanoparticles unique and dominating over the bulk materials [24].

3.1. ***Electronic and Optical Properties*** *:-*

The electronic and optical properties of nanoparticles are interrelated. Metal nanoparticles show size dependent optical properties and hence exhibit a strong UV- visible excitation band. The excitation band results from the resonant oscillation of the free electrons present in the conduction band of the metal. This spectroscopic feature, stimulated by the incident light, is called surface plasmon resonance (SPR). SPR spectrum is dependent upon the size, shape and interparticle spacing of the particles and also on the local environment. That is why silver nanoparticles appear as yellow and gold appear in rusty colors [24,48].

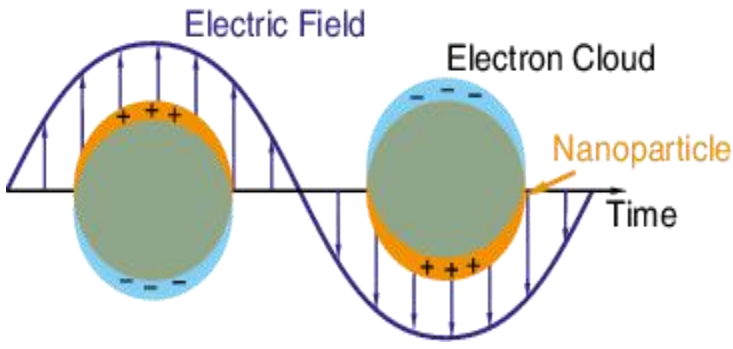

Fig 2 : Graphical representation of SPR on outer surface of nanoparticles (www.researchgate.net/publication/300850269_Nanoparticle_Biosensing_with_Interferometric_Reflectance_Imagingb)

*3.2.**Magnetic Properties** :-*

Nanoparticles exhibit magnetic property due to their uneven distribution of electrons. The magnetic properties of nanoparticles dominate effectively when their size is less than the critical limit of 10 - 20 nm. These properties are also dependent on the synthesis procedure of nanoparticles [24]. These magnetic properties of nanoparticles are widely used in magnetic data storage and magneto-optical systems. Some geometric and magnetic data such as size, shape, composition, crystal structure, coercivity, temperature dependent magnetization, blocking temperature are required before examining the magnetic properties of nanoparticles [50].

*3.3. **Mechanical Properties** :-*

Mechanical properties of the nanoparticles include surface coating, coagulation and lubrication along with the hardness, stress, strain and adhesion. The measurement of the hardness of nanoparticles was measured by Shorey et al, about ten years ago, In lubricated contact the nanoparticles can be deformed due to high pressure at the point of contact [24].The hardness and elastic modulus of nanoparticles deviates from that of the bulk materials and have been measured primarily with AFM [49].

*3.4.**Thermal Properties** :-*

Nanoprticles have higher thermal conductivities than that of fluid or solids. Small size of the nanoparticles provides a large surface area which provides superior properties to the nanofluids. This is because heat transfer takes place at a faster rate than the conventional one [24].

4. ***Types of Nanosystems*** :

The European Commission released a specific recommendation on the definition of the nanomaterial in 2011. According to this recommendation 'nanomaterials' are "a natural, identical or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1nm - 100nm". As per as health, threshold and environment is concerned the number size distribution threshold of 50% is replaced by threshold in the range of 1-50%. In 2015, ISO defined nanomaterials as "material with an external dimension in the nanoscale (size range from approximately 1-100 nm) or having internal structure or surface structure in the nanoscale" [ISO/TS 8004-2 : 2015]. Nanomaterials that are naturally occurring or are generated unintentionally as a byproduct of combustion process are heterogeneous in nature. These particles are often called as "ultrafine particles". Engineered nanomaterials are produced and designed to be used for a specific purpose.

Nanomaterials are classified into nano- crystalline and nanostructured materials. Nano crystalline materials can be easily manufactured and can replace bulk materials. These are raw materials and can be used in bone replacement, implant and drug encapsulation. Nanostructured materials are derived from raw materials and include carbon nanotube, fullerenes, dendrimers and quantum dots [44].

❖ ***Carbon nanotubes :***

Carbon nanotubes fall into the major class of carbon - based nanoparticles. These structurally resembles rolled up graphene sheet. The sp^2 hybridized carbon atom form an layered cylindrical structured by weak out of plane van der Waals bonds and strong in plane bonds. It can be divided into single well and multi- well known as multi- well carbon-nanotubes (MWCNT).

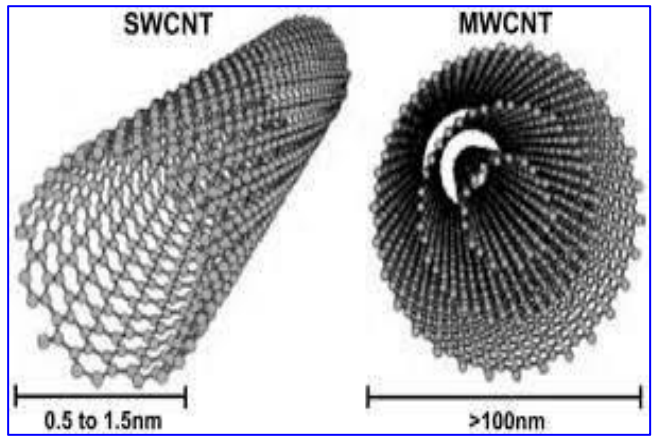

Fig 3 : Carbon nanotubes (http://lolesinmo.com/2018/armchair-single-wall-nanotubes.php)

The diameter of SCNTs ranges from 0.5 to 3 nm while that of MWCNTs ranges from 0.4nm to 100nm. Carbon nanotubes have unique electrical properties which can be used as conducting, semi- conducting and insulating materials. As they can penetrate cell cytoplasm and nucleus they can be used as nano- carriers in gene delivery and peptide delivery [25,26].

❖ ***Fullerens :***

Another carbon based nanomaterial fullerene consists of sp^2 hybridized allotropic carbon atoms arranged in hollow sphere, tube or ellipsoid form.

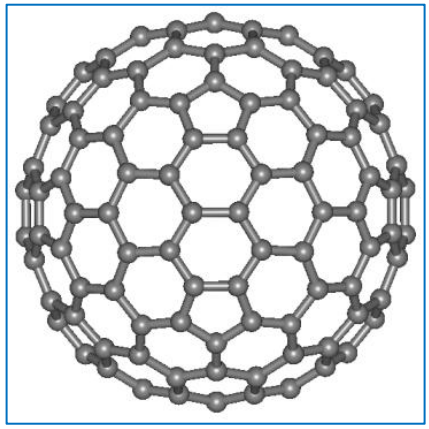

Fig 4: C_{60} Fullerene (http://www.nanotube.msu.edu/fullerene/fullerene-isomers.html)

By convention, fullerenes are closed convex cage made up of hexagonal or pentagonal carbon atoms. Spherical fullerens are known as buckyball or Buckminsterfullerens named after Richard Buckminster Fuller. C_{60}, the most common buckyball cluster is a truncated isohedron and made up of twenty hexagon and twelve pentagon. It show antiviral and antioxidant activity and can be used in drug delivery.

❖ ***Dendrimers*** *:*

A Greek word ‘dendron’ means trees. Dendrimers are nano - sized radially symmetrical molecules of diameter less than 10nm.

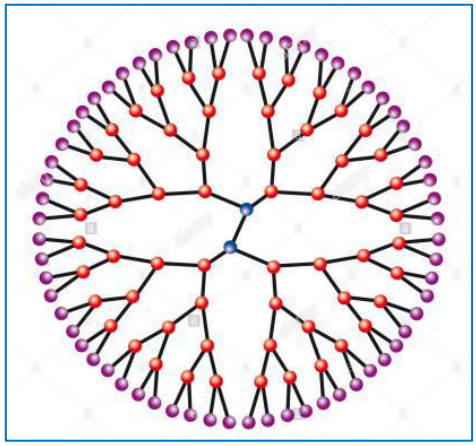

Fig 5 : Dendrimer (https://www.alamy.com/stock-photo-dendrimer-illustration-of-the-molecular-structure-dendrimers-are-artificially-126899070.html)

The well defined, homogeneous and monodisperes structure tree like arms or branches. The symmetrical structure is divided into a core, a shell and a surface layer. Dendrimers are mostly used in liver targeting. It is also used as targeted delivery of bioactives to macrophages [44].

❖ ***Quantum dots*** *:*

Quatum dots fall in the section of nanocrystalline materials size ranging from 2 to 10 nm. This semiconductor nanostructure traps the motion of the valence band holes and conduction band electrons or bound pairs of the conduction band electrons and valence band electrons in all the three spatial direction.

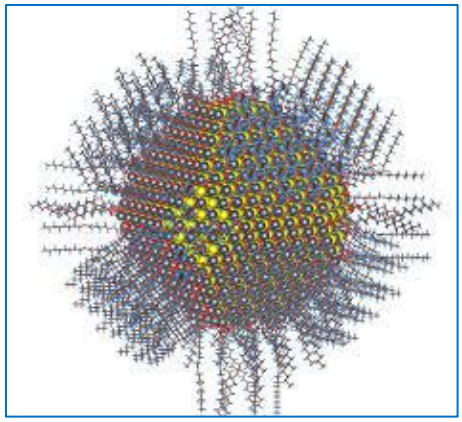

Fig 6 : Quantum dots
(www.futuretimeline.net/blog/2017/11/1.htm)

The core shell of the quantum dots consist of type II-IV, IV-VI and III-V semiconductors configurations of CdS, CdSe, ZnS, ZnSe, InAs etc. It show narrow emission and broad UV excitation with bright fluorescence and high photo stability. These characteristics can be applied to multiple color liver cells imaging, immunoassay and labeling breast cancer cells [25,27].

❖ ***Liposomes :***

Liposomes are sphere shaped small artificial vesicles made up from one or more cholesterol and natural non - toxic phospholipid bilayers. These phospholipids form a closed structure in aqueous solution in which the non polar groups surrounds the aqueous unit and the polar head group are oriented inwardly, exterior to the aqueous unit.

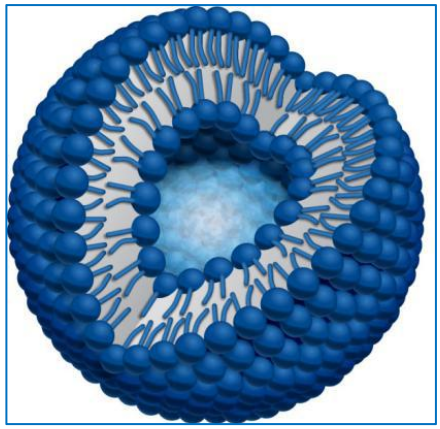

Fig 7 : Liposomes (http://www.probonobio.com/technology/sequessomes)

The size of the liposomes being 50 - 100 nm show versatile characteristics along with a good entrapment efficiency which offers its application in transfer of genes, proteins and peptide [25,28].

❖ ***Polymeric nanoparticles :***

Polymeric nanoparticles are biodegradable and biocompatible particles with a size range of 10 - 1000nm and used as an excellent carrier for control and sustained delivery of drugs [26]. Most polymers are easily process-able and a transparent, permeable or conductive material can be chosen as required for specific application. Numerous methods for preparation of nanoparticle - polymer composites have been developed. Polymer - metal nanoparticle composites have been synthesized by the condensation of metal vapors into liquid monomers followed by polymerization, by reduction of metal complexes in solution where the solvent also acts as a polymerizable ligand, by emulsion polymerization in the presence of nanoparticles and by electro - synthesis at liquid - liquid interface. Supercitical CO_2 solutions have also been used to load bulk polymer films with metal salts and to reduce their salts to metal nanoclusters by chemical post - treatment.

Capping agents play an important role in NP synthesis. The commercially available chemical surfactants are hazardous to environment and can lead to health problems. Biomolecules due to their non- toxic nature has recently gained interest in synthesis of NPs. This biomolecules do not involve any harsh synthetic procedure. But the binding affinity of AuNPs with amino acid surface is weaker compared other surfaces. Polysaccharides, on the other hand, are well known capping agent in nanoparticle synthesis. The less toxic, biodegradable, hydrophilic, stable and safe coating of metal nanoparticles enhances its use in medical field [44,51].

Over recent years, thiol- stabilized AuNPs have attracted increasing interest attributed to their potential in divergent field like nanoelectronic and optics similarly as DNA diagnostics. Alkanethiolate- stabilized AuNPs of small size distribution are simple to synthesized and of comparatively high stability. The shell of these organic-inorganic hybrid materials can be functionalized by site exchange reactions thus permitting the controlled change of properties of the particles and adjustment of the surface for further reactions. A polymer shell is fascinating in the control of the surface properties such as a rise in chemical or thermal stability and the functional groups present in the polymeric chain furnishes activity [35,53].

Marine polymers have grabbed the attention in producing biomimetic nanoparticles with tunable surface properties. Fucoidan, a naturally occurring sulfated polysaccharide, extracted from marine seaweeds is an excellent candidate for pharmaceutical application. It was used as an immuno - therapeutic function polymer [36].

Here an efficient green synthesis of gold nanoparticles is reported by in situ reduction and capping by a synthetic polysaccharide based biopolymer carboxymethyl tamarind (CMT). The synthesis of AuNPs involves the reduction of positively charged gold atoms and their stabilization by CMT.

5. *Strategies for Synthesis of Nanoparticles* :-

Scientists are doing research to develop nanoparticles of better properties and more functionality with low cost. Several physical and chemical method applied for this purpose are broadly divided into two categories - (I) top down approach and (II) bottom up approach. These approaches are further divided into different sub-classes depending on their reaction condition, protocol and operation method. In top down approach bulk materials are made to undergo size reduction. Bottom up method include the synthesis of nanosized materials by combining atom scale materials [40].

➢ *Topdown approach :*

This method is simply the solid - state processing of bulk materials. It is a destructive method in which larger molecules are decomposed to smaller units and then these smaller units are converted to nanoparticles. The processes like grinding or milling, crushing, chemical vapor deposition and physical vapor deposition are employed in top down synthesis. This route was employed to synthesize colloidal carbon spherical particles with controlled size. The biggest problem with top down approach is the imperfection of surface structure which leads to crystallographic damage of the processed structure.

➢ *Bottom up approach :*

This method is exactly reverse to the top down approach. That is it refers to the build- up from atom by atom, molecule by molecule and cluster by cluster. Sedimentation and reduction techniques are employed in this route to synthesize nanoparticles which includes sol- gel, spinning and biochemical techniques. This process has the ability to generate nanoparticles with uniform size, shape and distribution.

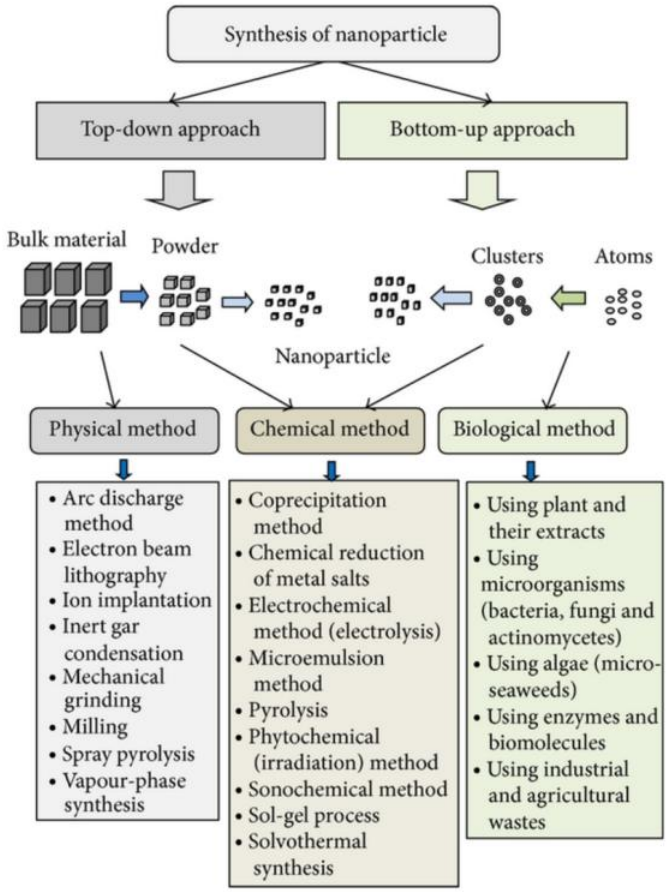

Fig 8 : Schematic diagram for the synthesis of nanoparticles (https://www.researchgate.net/figure/Different-approaches-and-methods-for-synthesizing-nanoparticles_fig1_287698425)

6. ***Methods of Synthesis of Nanoparticles*** :-

It is important to understand the various possible methods of synthesis to obtain design desired nanoparticles. High throughput nanoparticles are required to for their commercialization and can be prepared in two phases - liquid phase or solid phase. Two basic approaches to synthesize nanoparticles are further classified into (a) physical method, (b) chemical method and (c) bio-assisted methods [40].

✧ ***Physical Methods :***

This method applies mechanical pressure, high energy radiation, thermal energy or electrical energy to generate nanoparticles by the process of melting evaporationor condensation.

High energy ball milling, inert gas condensation, pyrolysis, physical vapour deposition, melt mixing are the commonly used physical methods for synthesizing nanoparticles. This process operates in top down approach. The advantage of the physical process lies in the fact that this process is free from solvent contamination although become less economical due to large amount of waste product [40].

Table 1 : Synthesis of nanoparticles by physical methods [40]

Name of the process	Mechanism of the process	Example of NP
High Energy Ball Milling	The kinetic energy of the balls is transferred to the milled materials which results in the breaking of the chemical bond and hence rupturing the milled materials into smaller particles with newly formed surface	Fe, Co
Inert Gas Condensation	Evaporated materials are transported with inert gas and condensed on to the substrate attached with liquid nitrogen	Cu
Physical Vapour Deposition	Vacuum deposition technique consisting of three steps- vaporization, transportation, nucleation and growth	TiO_2
Laser Pyrolysis	Resultant condensable product are generated from the laser induced chemical reactions	SiC
Electrospraying	Charged droplets are produced due to high voltage and the particles are collected after evaporation of the solvent in the electrochemical device	Ti

✧ ***Chemical Methods*** **:**

Chemical methodology of synthesis of nanoparticles involves a number of stages taking place in the liquid phase.The formation of metal ions are first accomplished by the reduction of metal precursors by chemical reductants and then the formed metal atoms undergo nucleation followed by slow growth process which leads to the formation of nanoparticles. Sol- gel, microemulsion, hydrothermal syntheses are some of the most commonly used chemical techniques for synthesis of nanopaticles [40].

Compared to others, chemical liquid deposition (CLD) method is more versatile techniques in order to obtain purified metal NPs. In this method no byproducts of metal salt reduction are present and hence can be the obtained material can easily to handle and analyzed [30].

Table 1 : Synthesis of nanoparticles by physical methods [40]

Name of the process	Mechanism of the process	Example of NP
High Energy Ball Milling	The kinetic energy of the balls is transferred to the milled materials which results in the breaking of the chemical bond and hence rupturing the milled materials into smaller particles with newly formed surface	Fe, Co
Inert Gas Condensation	Evaporated materials are transported with inert gas and condensed on to the substrate attached with liquid nitrogen	Cu
Physical Vapour Deposition	Vacuum deposition technique consisting of three steps- vaporization, transportation, nucleation and growth	TiO_2
Laser Pyrolysis	Resultant condensable product are generated from the laser induced chemical reactions	SiC
Electrospraying	Charged droplets are produced due to high voltage and the particles are collected after evaporation of the solvent in the electrochemical device	Ti

✧ ***Biological Methods :***

This method provides cost - effective, environment friendly, less - toxic and an efficient protocol to synthesize fabricated nanoparticles. Many bacteria fungi and plants have shown the ability to synthesize metallic nanoparticles. Intra- cellular and extra- cellular synthesis, growth temperature, synthesis time - all play an important role in biological synthesis. “Natural” biogenic metal nanoparticles can be split into two categories - bioreduction and biosorption [52]. Extra- cellular method is more efficient and less time consuming for recovery of gold nanoparticles [53].

Plant tissues like leaf, stem, root and bark are used to the production of gold nanoparticles. Different ratio of gold salt and plant extract can be used to synthesize gold nanoparticles at different temperature and pH [53]. Biological method of synthesis can be broadly divide into the following categories.

Biological synthesis is also called as green approach towards the synthesis and stabilization of metal nanoparticles. Three main steps should be followed during the synthesis of nanoparticles using a green chemistry perspective -

✓ Choice of solvent medium used for the synthesis

✓ Choice of environmentally benign reducing agent

✓ Selection of a non - toxic material for stabilization [9]

Table 3 : Biological methods of synthesizing nanoparticles[40]

Name of the process	Name of the process	Example of NP
Microorganisms assisted biogenesis	Metal ions present in their environment is converted to elemental metal through enzymes generated by cellular activities	CdS
Bio - Templates assisted biogenesis	Nucleic acid, membranes, viruses and diatoms are used as template to synthesize NPs	Pt
Plant extract assisted biogenesis	Plant extract or plant biomass is used to synthesize NPs	Ag

The pH value of the plant extract has a great influence on the formation of the nanoparticles. The charge of the natural phytochemicals changes with the change in pH which affects their ability to bind with the metal cations or anions during the synthesis of nanoparticles. This also affect the shape, size and yield of nanoparticles.[31]

7. ***Characterization of Nanoparticles*** :

Characterization refers to the study of material's features such as size, composition and different properties. Characterization of nanoparticles is important to understand and control nanoparticles synthesis and applications. First the color change is observed with naked eyes. UV-visible spectroscopy is a useful technique for studying nanoparticles due to the characteristic surface plasmon resonance observed for different nanoparticles. Characterization is performed using a variety of different techniques such as dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS),transmission and scanning electron microscopy (TEM, SEM), atomic force microscopy (AFM),powder X-ray diffractometry (XRD), Fourier transform infra-red spectroscopy (FTIR) and UV–Vis spectroscopy.

These techniques are used for determination of different parameters such as particle size, shape, crystallinity, fractal dimensions, pore size and surface area. The orientation, intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials could be determined by these techniques.

For instance, the morphology and particle size could be determined by TEM, SEM and AFM. The advantage of AFM over traditional microscopes such as SEM and TEM is that AFM measures three-dimensional images so that particle height and volume can be calculated. Furthermore, dynamic light scattering is used for determination of particles size distribution. X-ray diffraction is used for the determination of crystallinity, while UV–Vis spectroscopy is used to confirm sample formation by showing the plasmon resonance [42,43].

❖ ***UV - Visible spectroscopy:***

A wide range of spectroscopic methods are available for the analysis and characterization of nanoparticles. UV - vis spectroscopy offers the characterization of nanoparticles like quantum dots and organic based nanoparticles. The relationship between absorption spectra and particle size distribution for quantum sized nanocrystals can be studied by using UV - vis spectroscopy. In this spectroscopic technique light of wavelength 200- 700 nm is used which is related to the electronic transition of the molecules present in the sample [42].

❖ ***Powder X - Ray diffractometry :***

X - Ray diffraction or Powder X - Ray diffractometry (XRD) is a powerful nondestructive technique which provides information about the structure, phase, crystal orientation and crystal defects of a crystalline material. Monochromatic beam of X - Rays are scattered at a specific angles from each set of lattice plane in a sample and X - Ray diffraction peaks are produced by the constructive interference. This XRD pattern is the fingerprint of periodic atomic arrangements in the given material [41].

❖ ***Scanning Electron Microscopy (SEM) :***

The high energy electron beam falls onto the surface of the sample and the secondary electron reflected back from the surface of the specimen are detected by the detector. The energy of the electron is converted to provide the image of the surface of the specimen. SEM analysis gives a 3D structure of the sample [42].

❖ ***Transmission Electron Microscopy (TEM) :***

In this microscopic techniques electron from the high energy electron beam falls onto the sample and the energy transmitted is detected by the detector. This energy is converted to produce the image [42].

❖ ***Dynamic Light Scattering (DLS) :***

Surface charge and particle size are the two most commonly known factors responsible for biological effects of the nanoparticles which can be detected by DLS. This instrument uses Brownian motion of the particles to characterize the colloidal nanoparticles. When light falls on the moving particles it causes a Doppler shift and the wavelength of the incident light is changed and it is directly proportional to the size of the particle. Zeta potential measures the potential at the particle - fluid interface and provides information about the surface charge [43].

❖ ***Laser Scanning Confocal Microscopy (LSCM) :***

3D structure images are produced by LSCM. It has the ability to image structure at the discrete level within an intact biological specimen.

❖ ***Atomic Force Microscopy (AFM) :***

AFM is a very high resolution type of scanning probe microscopy (SPM) used to determine the roughness of the sample at high resolution which distinguish sample based on its mechanical properties [44].

❖ ***Fourier Transform Infrared Microscopy (FT - IR) :***

Fourier transform infrared spectra (FT- IR), a non destructive technique for characterization, is the most acceptable for surface characterization of nanoparticles. The surface reactive sites, responsible for surface reactivity, along with the chemical composition of nanoparticles surface can be identified using FT- IR [45].

8. *Application of nanoparticles* :-

The properties of materials alter as their size reaches the nanoscale length. Nanoparticles have unpredictable optical, electronic and catalytic properties as they are capable to lock up their electrons and generate quantum effect due to their small size. Nanoparticles are found naturally in water, soils and sediments. Both the natural and synthetic nanoparticles have application in various fields that combines chemistry, physics, engineering, biology and medicine.

It is surprising that a large scale use of nanoparticles has entered our everyday life decades ago. A prominent example of this is the use of carbon black in the car tires instead of rubber [23].

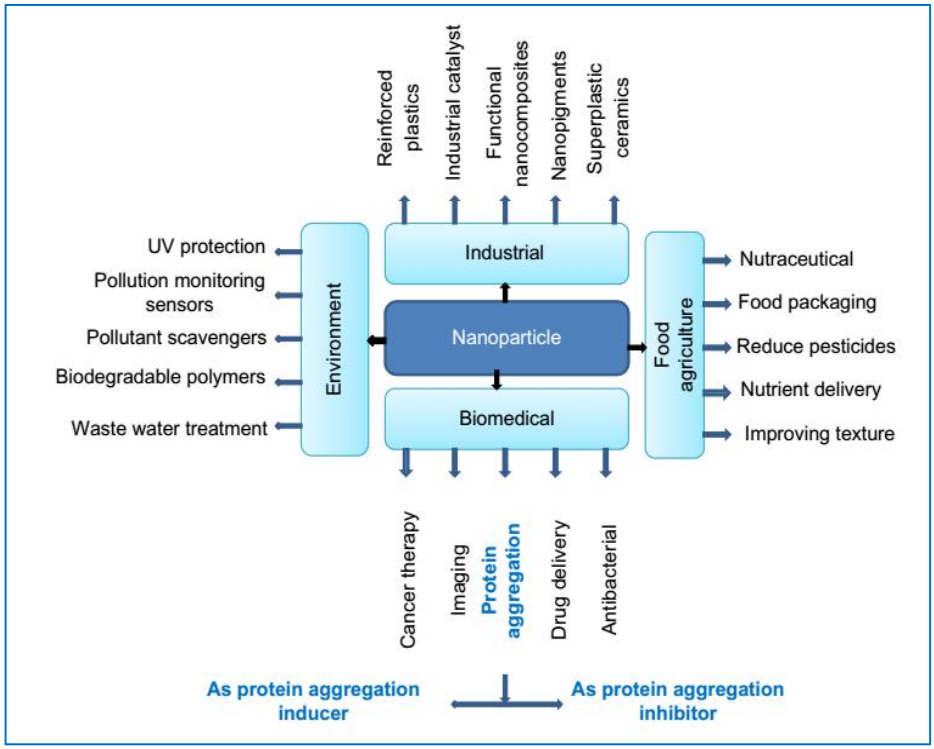

Fig 9 : Different application of nanoparticles (https://www.researchgate.net/figure/Application-of-nanoparticles-in-various-fields-such-as-in-the-biomedical-environmental_fig1_260647467)

The main challenge of Alzheimer's disease, the blood brain barrier can be conquered by the application of nanoparticles as drug carriers. The anatomical changes due to abnormal cellular activity can be detected by MRI or CT scans which uses nanoparticles for imaging. Nanoparticles undergo aggregation in contact with biological fluids and media. Gosens demonstrated the aggregation of nanoparticles by using pulmonary inflammation animal model in male rats. The application of nanotechnology provide a hope for the development of novel cancer therapeutic diagnosis. The small size enhances the retention time in the body fluids and hence nanoparticles can be used as drug delivery system when conjugated with ligands, proteins, drugs and enzymes. Researchers have developed nanosponges that absorb toxins and remove them from the blood stream. They are also

investigating the use of quantum dots to treat antibiotic resistance application.

Nanoparticles based technologies are favorable as it gives improved sustainability, efficiency and higher speed compared to other existing technologies. This is possible because very less amount of materials are used in nanoparticle based technologies which are already in its 'more' reactive state.

❖ ***Nanoparticle application in material :***

The unique properties of nanoparticles are related to their size and it is put to use in a nanocomposite materials. Despite the difficulties of agglomeration the use of nanomaterials grew markedly in the 21st century. Nanocomposites and nanomaterials are mainly used as the building blocks of dielectric and magnetic materials.

✓ **Food packaging :**

Nanoparticles bring advanced functional properties to packaging. Silver NPs, nanotitanium dioxide, nano zinc oxide nanoclay are introduced as functional additive to food packaging. Nanoparticles are used to improve the gas barrier properties, humidity resistance of packaging. Carbon nanotubes can be used as antibacterial agents. It is also used for smart packaging [54].

✓ **Batteries and supercapacitors :**

A key factor in battery efficiency is power density. Lithium - ion batteries store high amount of energy, but slower to charge and are related to safety issues. Engineered nanocomposite materials have very high internal surface area which offer sorting of electrical charge and make them valuable for use in batteries and supercapacitors. Colloidal nanoparticles can be used as electrode to enhance the charge transport when combined with carbon - based conducting materials [30].

✓ **Light control :**

Nanocomposite materials are used to coat diodes in order to convert blue light into white light. The quantum effect of semiconductor particles is used to obtain a desired light. The nanocomposite polymers optimize the plant growth by effectively converting sunlight to blue and red wavelength used for photosynthesis. This can be achieved with sub-micron particles of inorganic phosphor materials incorporated into the polymer [2].

✓ **Polymers :**

Carbon and silica oxide nanoparticles are used as filler in rubber to improve the mechanical properties of tires whereas nanoclays have been used to improve their strength and impact resistance. Biopolymers, derived from alginate, cellulose or starch is used in conjugation with natural nanoclay. These polymers are biodegradable and do not leave behind harmful residues [2].

❖ ***Biomedical application of nanoparticles :***

Low toxicity, high stability, small dimension and the possibility to interact with various substances make nanoparticles important for application in the biomedical field. Treatments and diagnostics based on the use of nanoparticles have important benefits [2].

✓**Sensors :**

Nanoparticles can tolerate high sensitivity related to biosensors due to their large surface to volume ratio. Target and traducer, the two main parts of biosensors, can be improved by using nanoparticles. In this case the surface chemistry should be optimized for specificity. The high signal to noise ratio provided by the small size of biosensors make the system useful in sensing application. Inter-particle plasmon phenomenon is used in colorimetric sensing. The conductivity and catalytic properties can be used for electro - analytical sensing [2,44].

✓**Drug delivery system :**

Nanoparticles are used for the entrapment of the drug to enhance their delivery to target cells and to reduce the toxic effect of the free drug to the adjacent non - target organs. Both physical properties and the surface chemistry of nanoparticles are utilized to design Drug delivery system. Safe delivery of the biomolecules like DNA, RNA, proteins, peptide across the cell membrane provide access to gene therapy and protein based therapeutic approaches. For an example, nanoparticle covered folic acid can enter into cancer cells via endocytosis [18].

✓ **Bioimaging :**

In vivo imaging is important for medical diagnosis to develop targeted therapies. Hybrid nanomaterials which combine multiple imaging functionalities can be used for obtaining dynamic images at organ or even cellular level. Progress has been achieved by combining several techniques such as single photon emission computed tomography (SPECT), two photon excited fluorescence, X - ray computed tomography (CT), magnetic resonance imaging (MRI) and up conversion luminescence (UCL). Quantum dots are in use for optical imaging. High resolution cellular imaging can be achieved by using two different contrast agents [2].

✓**Therapy :**

Therapy including nnanoparticles is not restricted to drug delivery. Nanoparticles can be used in the treatments complementary to chemotherapy. It can be also used in tissue engineering. Nanoparticles can restore, maintain or improve tissue function. Carbon nanotube is more efficient to improve regulation of cell adhesion vascularisation compared to nanofibers on glass [2,44].

9. ***Advantage and Disadvantages of nanotechnology*** :

✧ ***Advantages*** : -

1. Stronger, lighter, cheaper and precise unique materials can be created.

2. Industrial computers can be made a million time smaller and faster.

3. Medical fields

4. Improve food storage and packaging.

✧***Disadvantage*** : -

1. Nanoparticles are transparent to cell dermis and hence can cause irritation.

2. There are no hard and fast disposal policies for nanoparticles.

3. Highly pure nanoparticles are difficult to produce as they interact with impurity as well.

4. Fine metal particles with high surface area can cause explosion in contact with oxygen.

OBJECTIVE

- Synthesis and characterization of biopolymer capped gold nanoparticles.
- Investigation of AuNP for their antibacterial efficacy on *Escherichia coli.*
- To study the cytotoxic effect of biopolymer capped AuNP.
- Investigation of effect of AuNP on osteoblast.

MATERIALS AND METHODS

Materials : -

Escherichia coli strain O, CMT (Carboxy Methyl Tamarind) was purchased from Hindustan Gum & Chemical Pvt.Ltd. Ahmadabad, India. Auric Chloride ($HAuCl_4$) was purchased from Sigma Aldrich, USA, Saos - 2 cell lines. Milli-Q water is used for preparing all the solution.

Methods : -

Auric Chloride ($HAuCl_4$) reagent & CMT gum was used for synthesis.

Synthesis of gold nanoparticle :

In this present study we have synthesized gold nanoparticles (AuNPs) using aqueous solution of carboxy methyl tamarind (CMT) polysaccharide as a reductant and capping agent. The optimization of AgNP synthesis was achieved by varying the concentration of CMT polysaccharide solution and auric chloride solution alternatively. The CMT- capped gold nanoparticles were synthesized in the microwave at 450W.

Table 4 : $HAuCl_4$ solution containing different concentration of gum solution

Gum Concentration (%)	Gum Volume (mL)	$HAuCl_4$ concentration (mM)	$HAuCl_4$ volume (μL)
0.1	20	1	400
0.2	20	1	400
0.3	20	1	400
0.4	20	1	400
0.5	20	1	400

Table 5 : Gum solution containing different $AuCl_4$ concentration

Gum Concentration (%)	Gum Volume (mL)	$HAuCl_4$ concentration (mM)	$HAuCl_4$ volume (mL)
0.1	20	1	10
0.1	20	2	10
0.1	20	5	10

Characterization of Nanoparticle :

The UV-visible spectroscopy(Cary 60 Agilant technology, USA) is widely used as a useful technique for studying the nanoparticles owing to the characteristic surface plasmon resonance observed for different metal nanoparticles including AuNPs. The influence of variation in concentrations of both CMT and auric chloride was studied. The DLS analysis was carried out to assess the size and dispersity pattern of silver nano- particles. The DLS (Zetasizer nano S, Malvern instruments) result reveals particle sizes which are the sizes of the shell, while the real size of AuNP core is smaller. Further, DLS measurements can indicate the hydrodynamic volume representing the size of overall solvent associated nanoparticle and thus can provide qualitative information about the nanoparticles.

CFU Assay :

To determine the antimicrobial efficacy of the AuNP, the overnight grown *E.coli* cultures (5ml)were grown overnight. 1mM concentration of the AuNPs (50µM, 100µM,150µM, 200µM, 300µM) and LB medium with E.coli culture incubated for 3 hrs. All samples were plated and incubated for 3hrs and number of colony forming units (CFUs) was assayed and values were averaged from it.

MTT assay :

The cytotoxic effect CMT- capped AuNPs against Saos-2 cells were evaluated by MTT Assay. Approximately 10,000 cells in passage 4 were seeded in 96-well plates (Tarsons Product Pvt. Ltd.) and were incubated for 24 hours at 37°C in a 5% CO2 incubator. Cells seeded without AuNPs were used as control. To determine the cell viability, MTT dye (100µl from 0.1 mg/ml stock) was added to each well and incubated for 4 hours at 37°C, 5% CO2 in dark. In this assay, metabolic active cells reduce the MTT salt into water insoluble purple MTT formazan crystal by mitochondrial dehydrogenase. The formazan crystals formed as a result of cellular reduction of MTT were dissolved in buffer solution (4g NP-40 detergent in 50 ml 0.02 M HCl and 50 ml isopropanol) and incubated for 1h at 37°C and the absorbance was measured at 570 nm in an ELISA reader (Biotek, Germany).

Measurement of Reactive Oxygen Species (ROS) :

ROS generation was determined by fluorometric assay using intracellular oxidation of DCFH-DA (Sigma,USA). Approximately 10,000 mouse macrophage Raw214.7 cells were seeded on cover slips placed inside the 12 well tissue culture plate (Tarsons Pvt.ltd.) and incubated for 12 hr at 37°C and 5% CO_2. After 12 hour cells were treated with After that media was removed and cells were washed three times with 1X PBS. Then cells were incubated with 10µm DCFH-DA at 37°C for 30 min. Cover slips were mounted on fluorescent mounting media over glass slide and observed under fluorescent microscope. Cells containing H_2O_2 media was taken as positive control and cells containing only cell culture medium was taken as negative control.

ALP assay :

Alkaline phosphatase activity on hydrogel surface: Approximately 8 X 103 Saos-2 cells were seeded in nanoparticle coated and uncoated well with and without osteogenic media (McCoy's 5A medium containing 10 mM β-Glycerophosphate, 100mM L-ascorbic acid and 10nM dexamethasone) for 7 days. After that cells were washed and lysed in cell lysis buffer (0.1 vol% Triton X-100). The Alkaline phosphatase activity in the cell lysate was evaluated by p-nitrophenyl phosphate measurements (pNPP tablet, Sigma Aldrich). For that, 10 µl solution from cell lysate solution was added in 190 µl of pNPP substrate solution and incubated in dark for 30 minutes. Subsequently, the reaction was stopped by adding 3M NaOH solution to the reaction mixture. The absorbance was measured at 405 nm using an ELISA microplate reader (Biotek, Germany).

RESULT

Synthesis of gold nanoparticles :-

Gold nanoparticles exhibit a distinct optical feature commonly referred to as localized surface plasmon resonance (LSPR). That is the oscillation of electrons in the conduction bands are in resonance with a specific wavelength of incident light. This LSPR is dependent on shape and size of gold nanoparticles and it results in a strong absorption band. In order to monitor the synthesis of gold nanoparticles the absorption spectra of the AuNPs was studied using a UV-Visible spectroscopy. The influence of different parameters was studied.

Time based synthesis:

The gum based gold nanoparticles were synthesized in microwave. A 2mL solution was aliquot at every minutes and scanned in UV-Vis spectrophotometry. The color intensity of the solution increased with time with an absorption peak at 535nm. Milli-Q water was taken as a blank in this case.

Fig 10 : Picture showing AuNP colour changing pattern. 0.1% gum solution with 1mM concentration of $HAuCl_4$

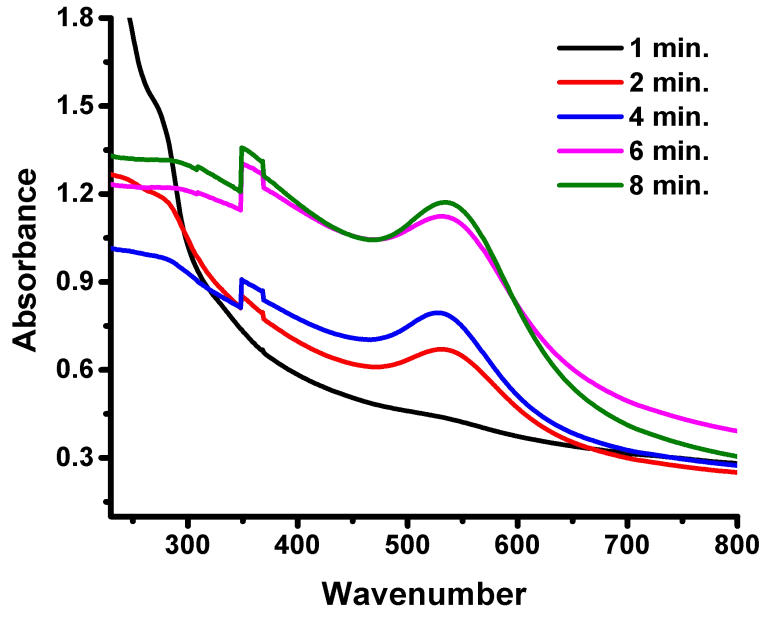

Fig 11 : The UV-Visible absorption spectra of gold nanoparticles from 1 minute to 8 minutes

Synthesis of AuNPs in different gum concentration:

Different concentration of CMT solution was prepared and absorption peak was observed. There was a very small change in the absorption peaks which indicate almost same size of nanoparticles.

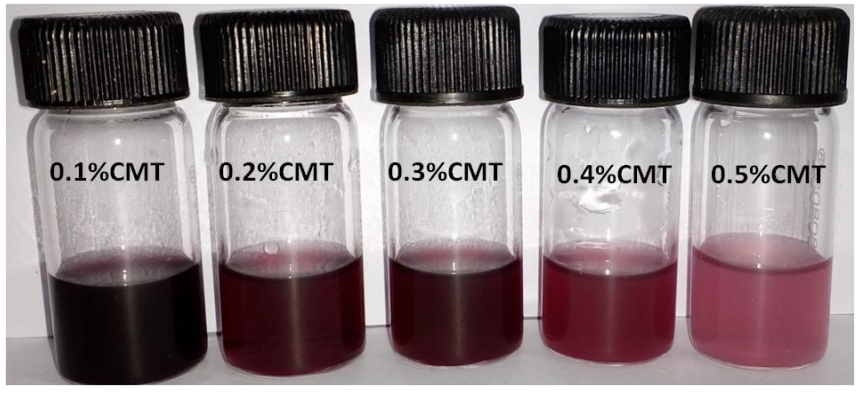

Fig 12 : AuNP colour changing pattern according to concentration. 0.1% - 0.5% gum solution with 1mM concentration of $HAuCl_4$.

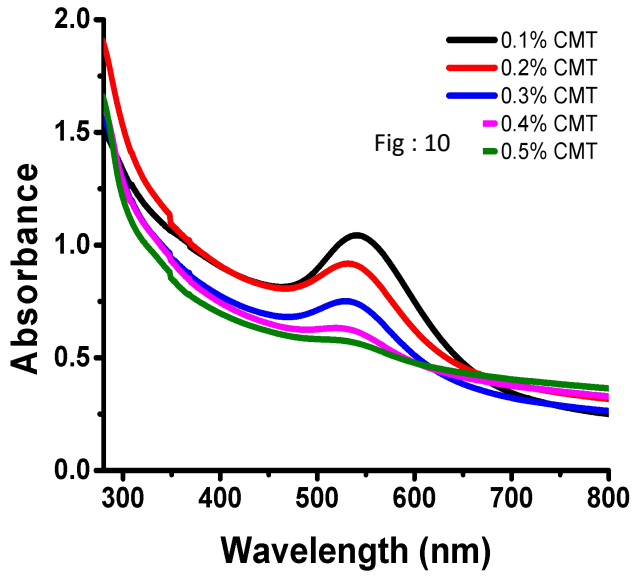

Fig 13: UV-Visible absorption spectra of gold nanoparticles synthesized by dissolving 1mM $HAuCl_4$ solution with different concentration (0.1%, 0.2%, 0.3%, 0.4%, 0.5%) solution of CMT polysaccharide.

Table 6 : Comparison of absorption peaks at different gum concentration

Different concentration of gum (%)	Absorption Peaks (nm)
0.1	544
0.2	536
0.3	531
0.4	529
0.5	527

Synthesis of gold nanoparticles at different $HAuCl_4$ concentration:

This experiment indicates that with change in concentration of auric chloride solution the size of the nanoparticles can be regulated.

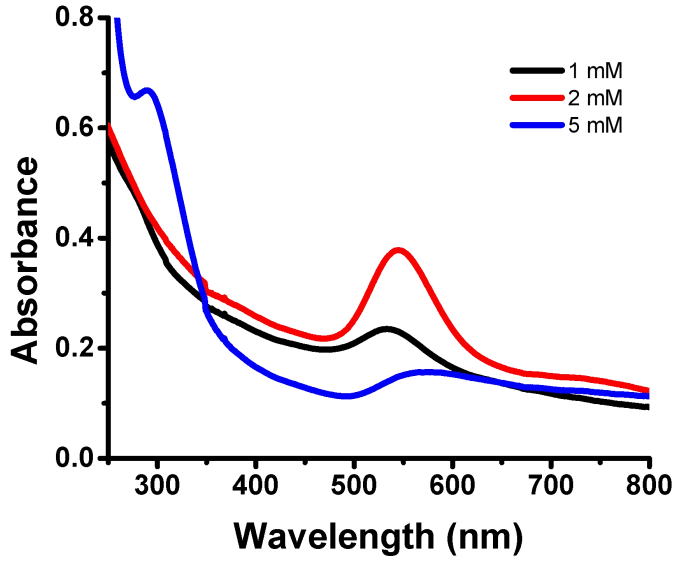

Fig 14 : The UV-Visible absorption spectra of gold nanoparticles synthesized by dissolving different concentration (1mM, 2mM, 5mM) of $HAuCl_4$ solution with 0.1% solution of CMT polysaccharide.

Table 7 : Comparison of absorption peaks at different HAuCl4 concentration

Concentration of $HAuCl_4$ (mM)	Absorption peaks (nm)
1	533
2	544
5	547

The UV-visible absorbance spectrum for synthesized CMT-capped AuNPs having surface plasmon resonance (SPR) peak centred at around 535 - 540 nm. The occurrence of peak at this wavelength (λmax value) reflects the size of AuNPs.The variation of concentration of CMT has not affected the AuNPs, however the variation of auric chloride with respect to a fixed concentration CMT polysaccharide resulted in the gradual colour change to dark red. This is due to the better seeding and higher yield of AuNPs (Fig : 10,12) which is typically facilitated in presence of CMT polysaccharide.

Fourier Transform Infrared Microscopy (FT - IR) :

FT-IR is used widely to study the surface phenomenon of the nanoparticles. The decrease in absorption peak height indicates that the nanoparticles are capped by CMT - polysaccharide.

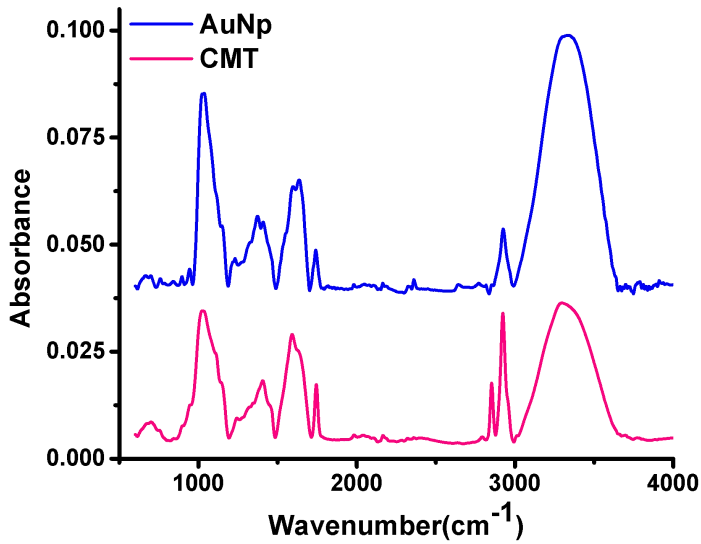

Fig 15 : FTIR spectra showing the formation of AuNP crystals.

X-Ray Diffraction (XRD) study of gold nanoparticles :

This study detects the existence of crystal defect, texture, size and other variable related to samples basic structure. Here the analysis showed the formation of AuNPs.

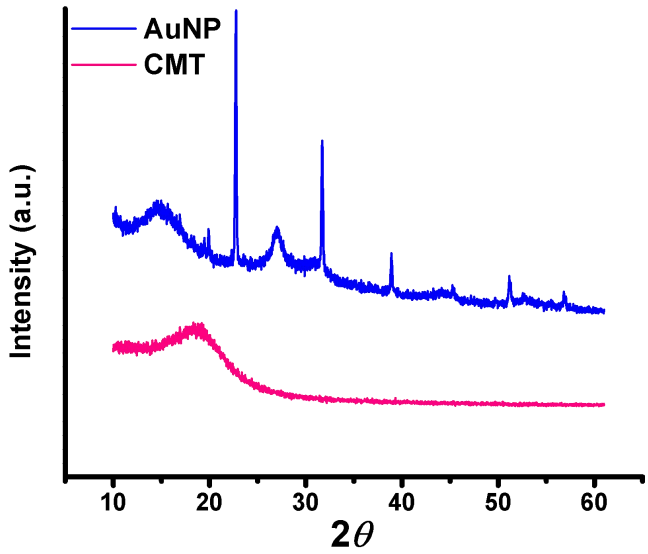

Fig 16 : XRD analysis showing the formation of AuNPs

***Detection of size and charge distribution of gold nanoparticles by DLS*:-**

Depending on the light source and detector specific properties of molecules can be studied by DLS. Surface charge and particle size, the two important characteristics of nanoparticles was measured by DLS. Homogeneous sample of 1mL in 1mM KCl solution was used to obtain the specific data. The size of the AuNP was found to be different for different concentration of $HAuCl_4$. The average size measured from DLS was found to be 112 nm. in terms of percent intensity distribution and 10nm by volume distribution.

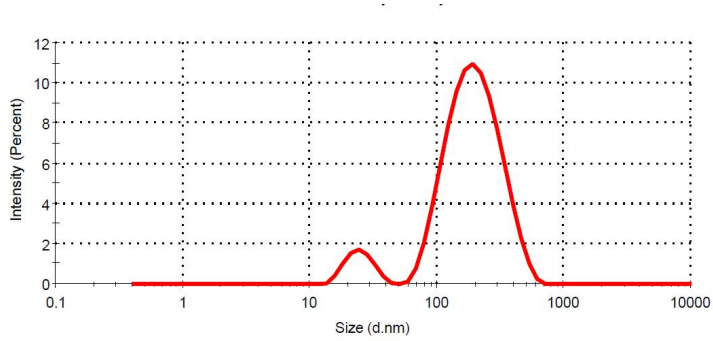

Fig 17 : DLS image showing the size of nanoparticles synthesized by dissolving 1mM of $HAuCL_4$ in 0.1% CMT polysaccharide.

Table 8 : Size of nanoparticles containing different concentration of $HAuCl_4$

Concentration of $HAuCl_4$ (mM)	Size of nanoparticles (nm)	Zeta Potential
1	112.4	-19.5
2	402.8	-16.8
5	241.6	-11.2

Transmission Electron Microscopy :

The size distribution of the nanoparticles is presented by TEM analysis. Transmission electron microscopy (TEM) can be used directly to image nanoparticles at scales approaching to single atom. The following TEM images represent the size of AuNPs at low and high magnification power. The 20 nm size of the nanoparticles at low magnified power indicates the agglomeration of nanoparticles whereas a single nanoparticle (5 nm) was found in the highly magnified image. It concludes that the AuNPs were not aggregated during synthesis.

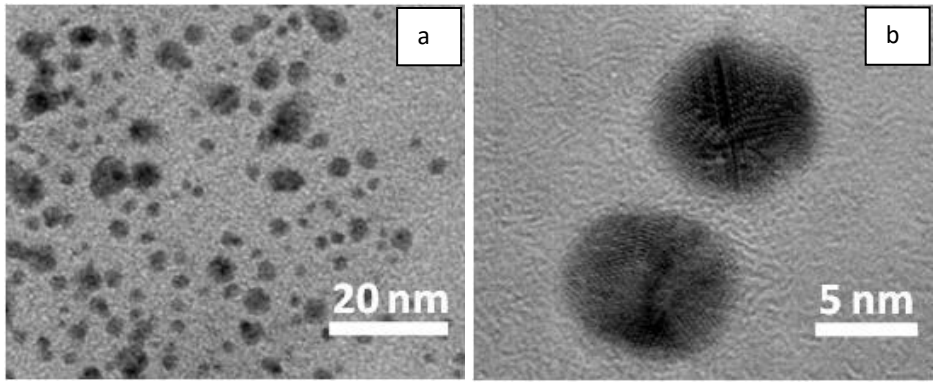

Fig 18 : TEM images of AuNPs at (a) low magnification power, (b) high magnification power

Antimicrobial activity of gold nanoparticles :

The antibacterial activity of AuNPs was investigated against one species of bacteria like *E.coli* by colony forming unit assay (CFU). The number of CFU were analyzed by harvesting bacteria for 3hrs of incubation time points by plating the serial dilutions on LB agar plate & surviving colonies were calculated after 12hrs of incubation. AuNPs significantly killed *E. coli* in a dose dependent manner.

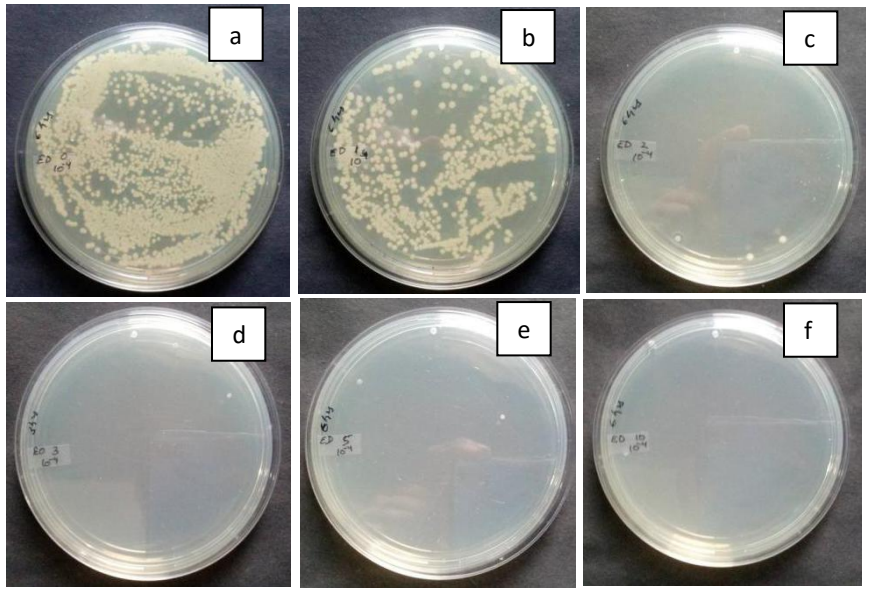

Fig 19 : Antimicrobial activity of AuNP at (a) Control, (b) 10 µM, (c) 20 µM,(d) 50 µM, (e) 100 µM, (f) 200 µM concentration of AuNP capped with CMT

Effect of AuNPs on osteoblast :

❖ ***Cytotoxicity effect on preosteoblast cells*** :-

The cytotoxicity effect of AuNPs was investigated against Saos - 2 cells using MTT assay. Cells seeded without nanoparticles were used as control. The enzyme present in the cells reduces MTT dye and produced formazan crystals. The absorbance of these purple color formazan crystals was measured at 570nm in an ELISA reader. The results indicate that with increase in AuNP concentration cell viability decreases.

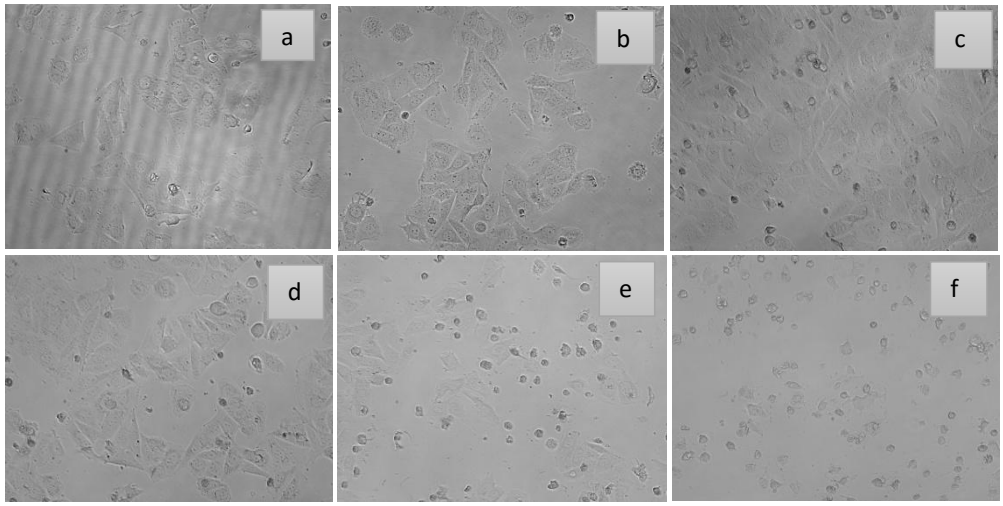

Fig 20 : Proliferative effect of AuNP on Saos - 2 cell line (a) Control, (b) 10µM, (c) 20µM, (d) 50µM, (e) 100µM, (f) 200µM.

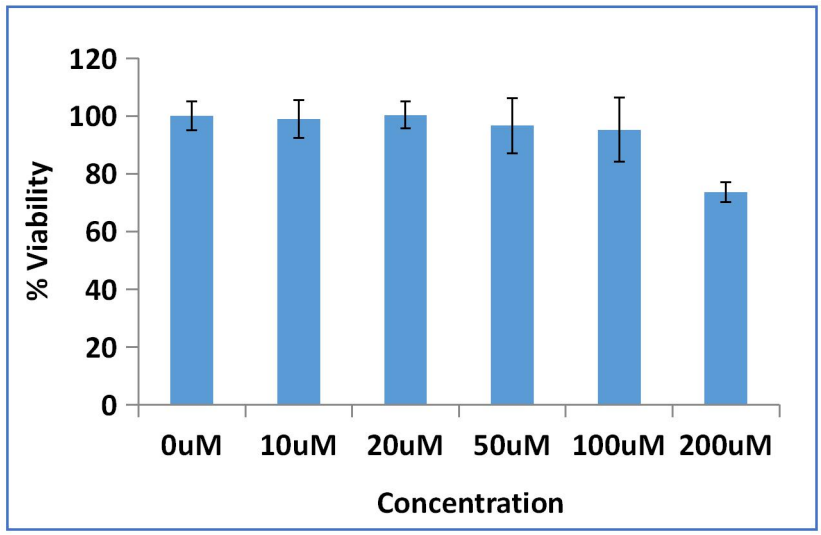

Fig 21 : Bar chart Showing dose Dependent Cytotoxicity effect of Gold Nanoparticle.

Detection of ROS using osteoblast like cells :

DCFDA was used to detect the formation of ROS in osteoblast like cells. Following the above mentioned protocol the cells were incubated for 12 hours and treated with 1X PBS. Cover slips were mounted on fluorescent mounting media and observed under fluorescent microscope. of The Saos-2 cells treated with AuNPs show no ROS formation.

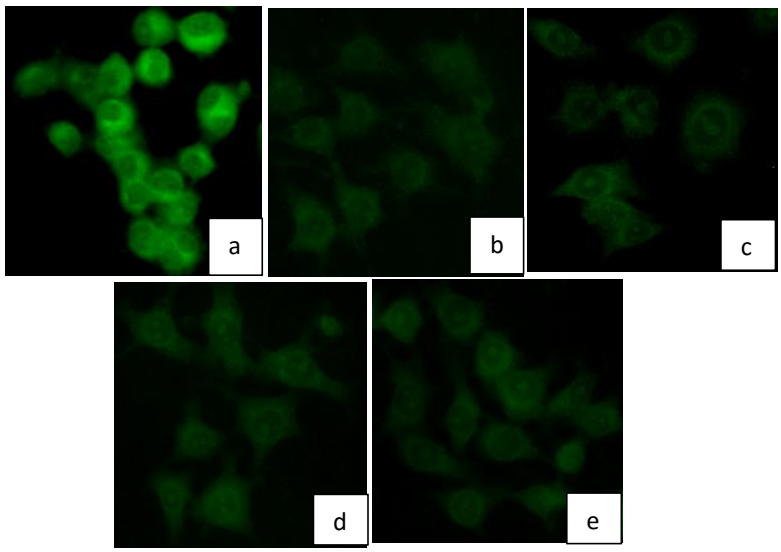

Fig 22 : Detection of ROS using Saos-2 cells, (a) Positive control -50 µM H_2O_2, (b) Negative control- Cell culture medium, (c), (d) and (e) Cells treated with gold nanoparticles.

❖ ***ALP Assay***:-

The alkaline phosphatase activity in the Saos - 2 cell lysate were evaluated by p-nitrophenyl phosphate (pNPP) measurement. The alkaline phosphatase present in the cells reacts with p-nitrophenyl phosphate and produce p-nitrophenol which give yellow color in alkaline medium. The absorbance of these mixture was measured at 405nm using an ELISA microplate reader. The cells showed a dose dependent effect of ALP activity where with an increasing dose of AuNP the ALP activity of the cells increases.

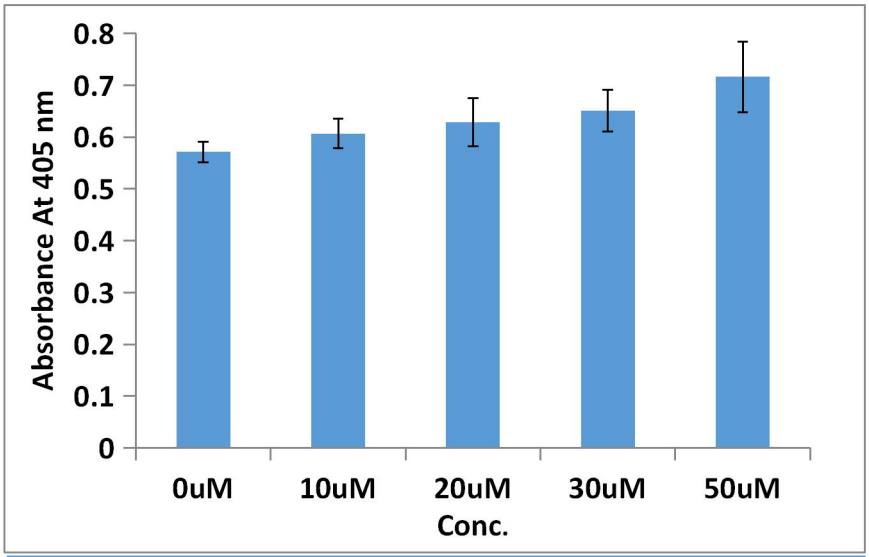

Fig 23 : ALP activity in Saos 2 cell lines at different concentration of AuNP.

DISCUSSION

The present experiment reports the green synthesis of metal nanoparticles and its effect on osteoblast cells. The method utilizes CMT polysaccharide as both reducing and capping agent. This natural polymer is not only very low cost and abundantly available but also has excellent surface active properties which are beneficial for metal nanoparticles. Being a natural polymer this gum is non toxic, renewable and also biodegradable [5]. Gold nanoparticles were synthesized by heating the gum based $HAuCl_4$ solution in a microwave at 450W for 60 minutes. Milli - Q water was used to reduce the cost of this technique. Sterilized condition was maintained using autoclave before using nanoparticles on cell line or for any other microbiological application. $HAuCl_4$ decomposes and reduces to metallic gold at high temperature. At this temperature the hydroxyl and carboxyl group of the gum become accessible to the gold nanoparticles and hence nanoparticles get incorporated into the polymer.

Fig 10 shows the color changing pattern of gold nanoparticles with time. The increasing color intensity of the solution indicates the synthesis of stable nanoparticles in gum solution. The color of the solution changes from pink to violet signifies the formation of Au^0 from Au^{3+} [10]. It was evident from the DLS study of the NPs (Table 8) that with different concentration of the gum solution the of the size of AuNPs remains almost same whereas with changing concentration of $HAuCl_4$ the size of the nanoparticles can be adjusted. The poly-dispersity index (PDI) of 0.0426 indicates the monodispersed pattern of nanoparticles. Zeta potential value of ±10 - 20mV suggest AuNPs are relatively stable. All the solution were analyzed in UV -Visible spectrophotometry in a wavelength ranging from 200 - 800nm.

The broad peak observed at different wavelength is proportional to the concentration of the NPs. This band was identified as the surface plasmon resonance band and assign to the free electron at the conduction band of the NPs.

The major problem related with nanoparticles use in health is its toxic effect. Therefore before application the cytotoxicity of the NPs should be investigated. The result of MTT assay explains the cytotoxicity effect of AuNP. The cell viability decreased at 200μM concentration of AuNP.

Another important factor related to health is generation of reactive oxygen species (ROS). The cell raptures with generation of ROS. It was evident from the experiment that the nanoparticles withstand the formation of reactive oxygen species (ROS). As the synthesized AuNPs are cytotoxic at 200μM concentration the potential of these AuNPs to form reactive oxygen species were investigated up to 100μM concentration and no ROS formation was found.

The ALP asaay on preosteoblastic cells indicates that nanoparticles can induce early osteogenesis events in preosteoblast cells. The ALP activity of the cells increases with an increasing concentration of AuNP.

CONCLUSION

This study relies on the role of polymer capped metal nanoparticles in health. Gold nanoparticles were synthesized from auric chloride solution using CMT as a stabilizing agent. In this green method of synthesis the gum act as both reducing and capping agent. At a given concentration of gum the efficiency of nanoparticle synthesis increases with time and a very slight change in the size of the NPs were observed when different concentration of gum was used. As the size of the NPs can be regulated by changing the $HAuCl_4$ concentration, it can be applied in a large scale production. The abundant availability of the low cost, plant derived bio-compatible biopolymer this method facilitate the use of these less toxic AuNPs in biomedical field. The monodispersed gold nanoparticles showed antimicrobial activity against *E.Coli* cells which can be used as a replacement of antibiotics. CMT - capped AuNPs induce osteogenic activity into the bone tissue upto 100μM concentration.

REFERENCES

1. Kholoud M.M. Abou El-Nour, Ala'a Eftaiha, Abdulrhman Al-Warthan, Reda A.A. Ammar (2010); Synthesis and application of silver nanoparticles; Arabian Journal of Chemistry, 3: 135 - 140.

2. Liangpeng Ge, Qingtao Li,Meng Wang, Jun Ouyang,Xiaojian Li, Malcolm MQ Xing(2014); Nanosilver particles in medical apllications : synthesis, performance and toxicity; International Journal of Nanomedicine; 9 : 2399 - 2407.

3. Mrinmoy De, Partha S. Ghosh, Vincent M. Rotello (2008); Application of nanoparticles in biology; Advanced Materials; 20 : 4225 - 4241.

4. Gabriel De Crozals, Romaric Bonnet, Carole Chaix (2016); Nanoparticles with multiple properties for biomedical application : a strategic guide; Nano Today; 11: 435 - 463.

5. Daizy Philip (2007); Synthesis and spectroscopic characterization of gold nanoparticles; Spectrochimica Acta : part A; 71 : 80 -85.

6. Dr. Ignác Capek (2017); Nobel metal nanoparticles; Nanostructure science and technology; pg : 125 - 210.

7. Y. Murali Mohan, K. Mohana Raju, K. Sambasivudu, Satyendra Singh, B. Sreedhar (2007); Preparation of Acacia- stabilized silver nanoparticles : a green approach; Journal of applied polymer science; 106 : 3375 - 3381.

8. Poovathinthodiyil Raveendran, Jie Fu, Scott L Wallen (2010); Completely green synthesis and stabilization of metal nanaoparticles; Journal of the American Chemical Society; 125 : 13940 - 13941.

9. Nikolaos Pantidos, Louise E Horsfall (2014); Biological synthesis of metallic nanoparticles by bacteria, fungi and plants; Nanomedicine and Nanotechnology; Vol 5 : 5 - 1000233.

10. Ghasem R Bardajee, Zari Hoosyar, Habib Rezanezhad (2012); A novel and green biomaterial based silver nanocomposite hydrogel : synthesis, characterization and antibacterial effect; Journal of inorganic Biochemistry; 117 : 367 - 373.

11. Y. Park, Y. N. Hong, A. Weyers, Y. S. Kim, R. J. Linhardt (2011); Polysaccharides and phytochemicals : a natural reservoir for the green synthesis of gold and silver nanoparticles; IET Nanotechnology; 5 : 69 - 78.

12. Haohong Duan, Dingsheng Wang, Yadong Li (2014); Green chemistry for nanoparticle synthesis; Chemical Society Reviews; 16 : 5778 - 92.

13. Kamyar Shameli, Mansor Bin Ahmad, Ali Zamanian, Parvan Sangpour, Parvaneh Shabanzadeh, Yadollah Abdollahi, Mohsen Zargar (2012); Green biosynthesis of silver nanoparticles using *Curcuma longa* tuber powder; International journal of nanomedicine; 7 : 5603 - 5610.

14. Aruna Jyothi Kora, Sashidhar Rao Beedu, Arunachalam Jayaraman (2012); Size-controlled green synthesis of silver nanoparticles mediated by gum ghatti (*Anogeissus latifolia*) and its biological activity; Organic and Medicinal Chemisrty Letter; 2 : 17.

15. Patrick V. Quelemes, Felipe B. Araruna, Bruna E. F. de Faria, Selma A. S. Kuckelhaus, Durcilene A. da Silva, Ronaldo Z. Mendonça, Carla Eiras, Maria José dos S. Soares, José Roberto S. A. Leite (2013); Development and Antibacterial Activity of Cashew Gum-Based Silver Nanoparticles; International journal of molecular sciences; 14 : 4969 - 4981.

16. Wesam Salem, Deborah R. Leitner, Franz G. Zingl, Gebhart Schratter, Ruth Prassl, Walter Goessler, Joachim Reidl, Stefan Schild (2015); Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxic *Escherichia coli;* International journal of medical microbiology; 305 : 85 - 95.

17. Prajna Jena, Soumitra Mohanty, Rojee Mallick, Biju Jacob, Avinash Sonawane (2012); Toxicity and antibacterial assesment of chitosan- capped silver nanoparticles on human pathogens and macrophage cells; International journal of nanomedicine; 7 : 805 - 818.

18. Agnieszka Z. Wilczewska, Katarzyna Niemirowicz, Karolina H. Markiewicz, Halina Car (2012); Nanoparticles as drug delivery systems; Pharmacological reports; 64 : 1020 - 1037.

19. De Jong WH, Borm PJ (2008); Drug delivery and nanoparticles : application and hazards; International journal of nanomedicine; 3(2) : 133 - 49.

20. Florian J. Heiligtag, Markus Niederberger (2013); The fascinating world of nanoparticle research; Materials today; 262 - 271.

21. Marie - Christine Daniel, Didier Astruc (2004); Gold nanoparticles : assembly, supramolecular chemistry, quantum - size - related properties, and applications towards biology, catalysis, and nanotechnology; Chemical society reviews; 104 : 293 - 346.

22. Ying Zhang, Thabitha P. Shareena Dasari, Hua Deng, Hongtao Yu (2015); Antimicrobial activity of gold nanoparticles and ionic gold; Journal of enviornmental science and health : Part C : 286- 327.

23. JE Hulla, SC Sahu, AW Hayes (2015); Nanotechnology : history and future; Human and experimental technology; 34 (12) : 1318 - 1321.

24. Ibrahim Khan, Khalid Saeed, Idrees Khan (2017); Nanoparticles : properties, applications and toxicities; Arabian journal of chemistry.

25. S. Bhatia (2016); Natural polymer drug delivery system; 33 - 93.

26. Ali Eatemadi, Hadis Daraee, Hamzeh Karimkhanloo, Mohammad Kouhi, Nosratollah Zargham, Abolfazl Akbarzadeh, Mozhgan Abasi, Younes Hanifehpour, Sang Woo Joo (2014); Crabon nanotubes : properties, synthesis, purification, and medical applications; 9 : 393.

27. D. Vasudevan, Rohit Ranganathan Gaddam, Adrian Trinchi, Ivan Cole (2015); Cor shell quantum dots: properties and applications; Journal of alloys and compounds; 636 : 395 - 404.

28. Abolfazl Akbarzadeh, Rogaie Rezaei-Sadabad, Soodabeh Davaran, Sang Woo Joo, Nosratollah Zarghami, Younes Hanifehpour, Mohammad Samiei, Mohammad Kouhi, Kazem Nejati-Koshki (2013); Nanoscale research letters; 8 : 102.

29. Croy SR, Kwon GS. (2006); Polymeric micelles for drug delivery; Current pharmaceutical design; 12 (36) : 4669 - 84.

30. M. F. Meléndrez, G. Cárdenas, J. Arbiol (2010); Synthesis and characterization of gallium colloidal nanoparticles; Journal of colloid and interficial science; 346 : 279 - 287.

31. Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV, Taliansky ME, Kalinina NO (2014); ”Green” nanotechnologies : synthesis of metal nanoparticles using plants; Acta naturae; 6 (1) : 35 - 44.

32. Masihuz Zaman, Ejaz Ahmed, gulam Rabbani, Rizwan hasan Khan (2014); Nanoparticles in relation to peptide and protein aggregation; International journal of nanomedicine; 9 : 899 - 912.

33. Silvia Vieira, Stephaine Vial, Rui. L. Reis, J. Miguel Oliveira (2017); Nanoparticles for bone tissue engineering; Biotechnology Progress; 33(3) : 590 - 611.

34. Mahendra Rai, Kateryan Kon, Avinash Ingle, Nelson Duran, Stefania Galdiero, Massimiliano Galdiero (2014); Broad spectrum bioactivities of silver nanoparticles : the emerging trends and future prospects; Applied Microbiology and Biotechnology; 98 : 1951 - 1961.

35. Stefan Nuβ, Henrik BÖttcher, Hellmuth Wurm, Manfred L. Hallensleben (2001); Gold nanoparticles with covalently attached polymer chains; Agnew. Chem. Int. Ed. Engl.; 40 (21) : 4016 - 4018.

36. Panchanathan Manivasagan, Subramaniyan Bharathiraja, Nhat Quang Bui, Bian Jang, Yun - Ok Oh, In Gweon Lim, Junghwan Oh (2016); Doxorubicin-loaded fucoidan capped gold nanoparticles for drug delivery and photoacoustic imaging; International Journal of Biological Macromolecules; 91 : 578 - 588.

37. Jörg Kreuter (2007); Nanoparticles - a historical perspective; International Journal of Pharmaceutics; 331 : 1-10.

38. Seshan K (ed) 2002; Handbook of Thin - film deposition process and techniques : Principle, Methods, Equipment and Application.

39. Maria Benelmekki (2012); An introduction to nanoparticles and nanotechnology; IOP Concise Physics : Designing hybrid nanoparticles; 1- 14.

40. Chetna Dhand, Neeraj Dwivedi, Xian Jun Loh, Alice Ng Jie Ying, Navin Kumar Verma, Roger W. Beuerman, Rajamani Lakshminarayanan, Seeram Ramakrishna (2015); Methods and strategies for the synthesis of diverse nanoparticles annd their applications : a comprehensive overview; RSC advances; 5 : 105003 - 105037.

41. Anderi A. Bunaciu, Elena Gabriela UdriŞTioiu, Hassan Y. Aboul- Enein (2015); X - Ray diffraction : Instrumentation and Application; Critical reviews in analytical chemistry.

42. Anna Dzimitrowz, Piotr Jamróz, George C. diCenzo, Iwona Sergiel, Tomasz Kozlecki, Pawel Pohl (2016); Preperation and characterization of gold nanoparticles prepared with aqueous extracts of *Lamiaceae* plants and the effect of follow- up treatment with atmospheric pressure glow microdischarge; Arabian Journal of Chemistry.

43. Sourav Bhattacharjee (2016); DLS and Zeta potential - what they are and what they are not?; Journal of controlled release; 235 : 337-351.

44. Nanoparticles types, classification, characterization, fabrication methods and drug delivery application; Springer; 33- 93.

45. Marie- Isabelle Baraton, Lhadi Merhari (2007); Dual contribution of FTIR spectroscopy to nanoparticles characterization : surface chemistry and electrical properties; Nanomaterials synthesis, interfacing, and integrating in devices, circuits and systems II; 6786.

46. MatthewCharles Stensberg, Qingshan Wei, Eric Scott McLamore, David Marshall Porterfield, David Marshall Porterfield, MarĪa Soledad Sepũlveda; 879- 898.

47. A. Alagarasi (2011) Introduction to nannomaterials;.

48. Susie Eustis, Mostafa A. El-Sayed (2006); Why gold nanoparticles are more precious than pretty gold: Noble metal surface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes; Chemical Society Reviews; 35 : 2009 -217.

49. Dan Guo, Guoxin Xie, Jianbin Luo (2014); Mechanical properties of nanoparticles : basics and applications; Journal of Physics D : Applied Physics; 47.

50. Bashar Issa, Ihab M. Obaidat, Borhan A. Albiss, Yousef Haik (2013); Magnetic nanoparticles : surface effects and properties retaed to biomedicine applications; International Journal of Molecular Sciences; 14(11) : 21266-21305.

51. Deepali Sharma, Suvardhan Kanchi, Krishna Bisetty (2015); Biogenic synthesis of nanoparticles : a review; Arabian Journal of Chemistry.

52. Nikolaos Pantidos, Louise E Horsfall (2014); Biological synthesis of metal nanoparticles by bacteria, fungi and palnts; Nanomedicine and Nanotechnology; 5(5) : 1000233.

53. Rainer Jordan, Nuki West, Abraham Ulman, Yen- Ming Chou, Oskar Nuiken (2001); Nanocomposites by surface -initiated living cationic polymerization of 2-Oxazolines on functionalized gold nanoparticles; American Chemical society; 34 (6) : 1606 -1611

54. Halen Jarvie, Stephen King, Peter Dobson (2017); Nanopaerticle; Britinnica Online encyclopedia.