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ANTIMICROBIAL ACTIVITY OF NANO – BIOCOMPOSITES FOR TISSUE ENGINEERING APPLICATIONS

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ABSTRACT

Nanotechnology mainly includes designing of new materials which contains unique properties by varying the composition at the atomic level, which can be utilized for several applications like biomedical and biotechnological, including drug delivery, enzyme immobilization, DNA transfection, imaging, sensing, gene delivery system and artificial implants. Biomaterials can mimic surface properties of natural tissues. The loss or failure of an organ or tissue is one of the most frequent, devastate and economical problem with regard to human health. A new field, tissue engineering, applies the principles of biology and nanotechnology for the development of functional substitute for damaged tissues.

KEYWORDS:- **Nanotechnology, Implants, Biometerials**

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INTRODUCTION

Nanotechnology is a primary research area which includes development of materials, devices and systems exhibiting physical, chemical, and biological properties that are different from those found on a larger plate

Nano comes from the Greek word for “dwarf” (Freitas, 2002). The size and surface of the particle are crucial factors in targeting, and the attachment of cell-specific ligands can lead to increased selectivity (Davis, 1997). Nanoparticles and dendrimers were first prepared in 1970’s. Colloidal gold particles in nanometer sizes were first prepared by Michael Faraday more than 150 years ago (Larsson, 1979). In 1990’s, scientists began to design experiments to specifically couple biology with nanofabricated devices and tools (Zhang, 2003). Nanoparticles are sized between 1 and 100 nanometers. These may or may not exhibit size-related properties that differ significantly from those observed in fine particles or bulk materials (Buzea *et al.,* 2007). Nanoparticles are taken up by cells more efficiently than larger micromolecules and also can be used as effective transport and delivery systems (Suri *et al*., 2007). Nanotechnology involves the use of materials such as fibers, grains and particles that has dimensions of less than 100nm (Sato and Webster, 2004). Biological systems are composed of nanoscale building blocks, and most investigations in molecular biology are already pursued on a nanoscale (Walker and Mouton, 2006). Nanotechnology involves the tailoring of materials at the atomic level to attain unique properties, which can be suitably manipulated for the desired applications like biomedical and biotechnological, including drug delivery, enzyme immobilization, DNA transfection (Martin and Kohli, 2004), imaging, sensing, gene delivery system and artificial implants (Shrivastava *et al*., 2007). Nanotechnology is the most prominent and rapidly emerging field. The vision of nanotechnology was introduced in 1959 by late Nobel Physicist Richard P Faynman: “*There is plenty of room at the bottom. Many of the cells are very tiny, but they are active: they manufacture substance; they walk around; they wiggle: and they do all kinds of marvelous things all on a very small scale. Also, they store information. Consider the possibility that we too can make things very small which does what we want, when we want and that we can manufacture an object that manoeuvres at that level”* (Walker and Mouton, 2006). Innovations in nanoscale processes includes, nanofiber enhanced clothing that protects the wearer or resists stains, nanoparticle based cosmetics and sunscreens that improve product efficacy (Wissing and Muller, 2003). According to Piegorsch and Schuler nano-composites that improve automobile impact strength, body lightness and durability. Nanotechnology has applications in every branch of medicine, which are cancer (nanooncology), neurological disorders (nanoneurology), cardiovascular disorders (nanocardiology), diseases of bones and joints (nanoorthopedics), diseases of the eye (nanoophthalmology), and infectious diseases (Jain, 2008).

Biomaterials

In 1880, Gluck, used ivory prosthesis as implants in the body. In 1902, gold was used in capsule form interposing between the articular heads of the implant. The first metal prosthesis made of Vitallium alloy (Willes and Bursch, 1939). This prosthesis was used until 1960. Biomaterial can be defined as “ any substances (other than a drug) or combination of the substances synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ or function of the body” (Recum and Laberge, 1995). Biomaterials in the form of implants (structures, bone plates, joint replacements, ligaments, vascular grafts, heart valves, dental implants etc.) and medical devices (pacemakers, biosensors, artificial hearts, blood tubes etc.) are widely used to replace or degenerated tissues or organs to improve function and to correct abnormalities. The various materials used in biomedical applications are grouped into bioinert, bioactive, biostable and biodegradable (Ramakrishna *et al*., 2001). Biomaterials can mimic surface properties of natural tissues (Rho *et al*., 1998).. A new field, tissue engineering, applies the principles of biology and engineering to the development of functional substitute for damaged tissue (Langer and Vacanti, 1993). Bone tissue engineering is an increasingly important research area whose goal is to supress the limitations of conventional treatments based on organ transplantation and biomaterial implantation (Kusmanto *et al*., 2008).

Table 1
Nano-Biocomposites for Bone tissue engineering applications

Nano-Biocomposites	Literature collection
Bovine collagen–hydroxyapatite composite: Bone tissue engineering.	Rodrigues *et al*., 2003
Nanocrystalline hydroxyapatite and calcium sulphate as biodegradable composite: local delivery of antibiotics in bone infections	Rauschmann *et al.,* 2005
Biomimetic nanocomposites of Gelatin-hydroxyapatite: Tissue engineering scaffolds.	Kim *et al*., 2005
Chitosan-metal composite: Antimicrobial agent.	Wang *et al*., 2005
Nano-carbonated hydroxyapatite/collagen/PLGA composite: Tissue regeneration.	Liao *et al.,* 2005
Poly(L-lactide) and surface grafted Hydroxyapatite nanocomposite: Mechanical properties and biocompatibility.	Hong *et al.,* 2005
Hydroxyapatite in 3-D chitosan–gelatin composite: Human mesenchymal stem cell construct.	Zhao *et al*., 2006
Gelatine-Hydroxyapatite nanocomposites: Orthopaedic applications.	Vidyarthi *et al*., 2007
Poly (amidoamine) Dendrimers with Amino and Poly(ethylene glycol) Groups: Antibacterial activity.	Calabretta *et al.,* 2007
β-chitin–hydroxyapatite composite membranes: Tissue engineering applications.	Madhumathi *et al*., 2009
Silver-Hydroxyapatite nanocomposite: Antibacterial and Osteogenic properties.	Chen *et al*., 2007; Diaz *et al*., 2009
Ag–TiO_2/HAP/Al_2O_3 bioceramic composite: Bactericidal activity.	Ma *et al*., 2009
Nano-hydroxyapatite reinforced poly(vinyl alcohol) gel composites: Articular cartilage.	Pan and Xiong, 2009
Poly (lactide-co-glycolide) and hydroxyapatite surface-grafted with Poly(L-lactide) composite: *Invivo* mineralization and Osteogenesis	Zhang *et al.,* 2009
Nano-hydroxyapatite/ chitosan/carboxymethyl cellulose: Bone tissue engineering	Liuyun *et al*., 2009

Ceramics

Ceramic nanocomposites were first developed by K. Niihara and Basu *et al*., in 2004. Ceramics are inert, where implantation is performed without cement anchorage to the tissue which leads to implants loosens very quickly. Loosening leads to clinical failure including fracture of the implant or the bone adjacent to the implant. To improve this, biologically active or bioactive materials were developed, such as bioglass and hydroxyapatite by Hench (1971) and Jarcho (1970). Organic polymers coated with the bone like apatite layer are useful as substitutes, not only for hard tissues but also for soft tissues (Kokubo, 1991). Ceramics are defined as calcium phosphate materials with a composition and structure that are similar to the inorganic components of bone (Jaffe and Scott, 1966). 'Nanotechnology' called the materials technology of 21st century has also entered the field of ceramics (Rajalakshmi *et al*., 2003). Some of the ceramics are hydroxyapatite (HA), alumina, zirconia, bioglass (Niinomi, 2002). Ceramic materials in biomedical applications have been universally accepted specifically in terms of their strength, biocompatibility, hydrophilicity, and wear resistance in articulating joints (Heness and Nissan, 2007). Ceramic and metallic materials have been widely accepted for the developments of implants, their non-resorbability and necessity of second surgical operation (like for bone repair), which induce extra pain for the patients, limit their wide applications (Lau *et al*., 2010).

Hydroxyapatite

The term apatite was the first applied to minerals by Werner, in 1788. It now denotes a family of crystals with formula ($Ca_{10}(PO_4)_6OH_2$). Bone mineral was found to be quite complex and included various types of hydrated calcium phosphates (Jaffe and Scott, 1996). Hydroxyapatite (HA) beads possess several properties which make them potentially useful for studying microbial adherence, growth and interaction (Sudo *et al*., 1975). Hydroxyapatite has been used extensively for the purification and fractionation of an array of biochemical substances, including enzymes, nucleic acids, hormones, and viruses (Berry and Siragusa, 1997). Hydroxyapatite ceramic has been applied widely in clinical for filling of bone defects due to its biocompatibility and bioactivity. It can form bone bonding with living tissue through osteoconduction mechanism (Wang *et al*., 2002). It is osteoconductive, non-toxic, non-inflammatory and non-immunogenic agent. HA has been proposed asa substitute for defective bones or teeth (Murugan and Ramakrishna, 2004). Hydroxyapatite is used widely for bone implant and bone cement applications due to its compositional and biological similarities to native tissues. It also can be used for coatings on metallic pins and to fill large bone voids resulting from disease or trauma (Degirmenbasi *et al.,* 2006). Natural bone tissue is composed primarily of carbonated hydroxyapatite crystals, 40-60 nm in length, 10-20 nm in width and 1-3 nm in depth (He *et al*., 2007). Natural HA and synthetic HA can differ in their chemical composition and behavior. The Ca/P molar ratio in bone is lower than 1.67, compared to a molar ratio of Ca/P in synthetic HA. This ratio can be an important factor in cell adhesion, proliferation and in bone remodeling and formation (Supova, 2009). Hydroxyapatite can also be used as delivery vehicles in various medical applications. These include the delivery of growth factors, antibiotics, anticancer drugs, enzymes and antigens for slow release vaccination (Motskin *et al*., 2009). Nano-hydroxyapatite may be a better candidate for an apatite substitute of bone in biomedical applications than microsized hydroxyapatite. Size control is always difficult when synthesizing well defined nano-HA particles (Shi *et al*., 2009).

Metals

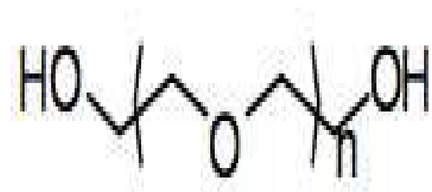
Metallic nanoparticles, such as silver, copper and zinc, have antibacterial capabilities. In order to facilitate the nanoparicle handling and to reduce health risks, nanoparticles in inorganic matrix are being studied as antibacterial agents. These materials present high antibacterial activity, low toxicity, chemical stability, long lasting action period thermal resistance versus organic antibacterial agents (Cubillo *et al*., 2006). One of the earliest nanomedicine applications was the use of nanocrystalline silver which is as an antimicrobial agent for the treatment of wounds (Taylor *et al*., 2005). Nanoparticles contain nitric oxide gas, which is known to kill bacteria (Gattorno *et al*., 2002). Heavy metal ions have diverse effects on bacterial cell function (Gajjar *et al*., 2009). Living organisms requires copper at low concentrations as cofactors for metalloproteins and enzymes, however at high concentrations; Cu (II) induces an inhibition of growth in bacteria (Sani *et al*., 2001), and has a toxic effect on most microorganisms. This effect may involve substitution of essential ions and blocking of functional groups of proteins, inactivation of enzymes, production of hydroperoxide free radicals by membrane bound copper, and alterations of membrane integrity (Faundez *et al*., 2004). Copper is created in volcanic areas, high in concentrations of hot sulfuric solutions. In addition, it has antibacterial properties that help to ward off microorganisms. Copper compounds have been widely used as algicide, fungicide, molluscide and acaricide agents in agriculture (Borkow and Gabbay, 2005).

Copper plated surfaces have been shown to have a significant antibacterial activity against a wide range of microorganisms, including *Salmonella enteric* and *Campylobacter jejuni* (Ibrahim *et al*., 2008). Copper, as a native copper, is one of the few metals to occur naturally as un-compounded mineral. Copper ions are soluble in water, where they function at low concentration as bacteriaostatic substances, fungicides, and wood preservatives. Copper ions can penetrate the cell walls of microbes and can disrupt reproduction and other cell functions (Feder, 2008). Copper oxide nanoparticles incorporated into polymers suggest release of ions may be required for optimum killing (Ren *et al.,* 2009).

Polyethylene Glycol

Polyethylene glycol (PEG) is widely used as a covalent modifier of biological macromolecules and particulates as well as a carrier for low molecular weight drugs (Zalipsky, 1995). PEG is a polyether compound with many applications from industrial manufacturing to medicine. It has also been used known historically as polyethylene oxide (PEO) or polyoxyethylene (POE) depending on its molecular weight (Kahovec, 2002). PEG coated nanoparticles have been found to be of great potential in therapeutic application for controlled release of drugs and site- specific drug delivery (Gerf *et al*., 1994; Quellec *et al*., 1998). It has been extensively used for preparation of biologically relevant conjugates, including stabilization of proteins and surface modification (Lee and Yuk, 2007).

Figure (i)



(Lee and Yuk, 2007)

PEG solutions have significant antibacterial activity against various pathogenic bacteria. PEG 400 appears to cause drastic changes in cell morphology within minutes after addition to the cells. It may have a potential value in medicine as a topical antibacterial agent. PEG 400 (as well as other polyethylene glycols) has several pharmaceutical applications not due to its antibacterial properties (Chirife *et al*., 1983 (a); Chirife *et al*., 1983 (b)).

Antibacterial activity

Antimicrobial materials have multiple applications in medicine, industry and commercial products. Metal nanoparticles are known to possess antibacterial properties (Weir *et al*., 2008). Microbial pollution and contamination by microorganisms have produced various troubles in industry and other vital fields, such as degradation and infection. Antimicrobial agents are divided into two groups, namely, inorganic and organic group. Inorganic antibacterial materials, especially doping silver ions. While antimicrobial agents have instantaneous antibacterial property, they cannot be used at high temperature (Huifang *et al*., 2009). Acting mechanism of silver has been known in some extent, silver inhibits phosphate uptake and exchange in *E.coli* and causes efflux of accumulated phosphate as well as of mannitol, succinate, glutamine and proline (Schreurs and Rosenberg, 1982). Silver has strong inhibitory and bacterial effects as well as broad spectrum of antimicrobial activities for bacteria, fungi and virus (Lok *et al.,* 2006). The antibacterial activity and acting mechanism of silver nanoparticles on *E.coli* was investigated by analyzing the growth, permeability and morphology of the bacterial cells (Li *et al*., 2010). Bacterial infection is a rising complication following the wide utilization of an implant, there is considerable attention on the issue of implant surface properties on bacterial attachment The effect of silver doped hydroxyapatite coatings on initial antibacterial adhesion and osteoblast cell proliferation and differentiation was investigated (Chen *et al*., 2007). To prevent the increasing frequency of per-operative infections, bioceramics was loaded with antibacterial agents, which will release with respect to their chemical characteristics. A novel hydroxyapatite was elaborated with specific internal porosities for using as a bone bioactive antibiotic carrier material (Chai *et al*., 2007). It was estimated that 2 million new dental implants are performed every year (Machtei *et al.,* 2008). The infection rates associated with prosthetic joints range between 1% and 9%, depending on the type of implant (Zimmerli *et al.,* 2004). The infection associated implant failures occur less frequently than aseptic ones. Moreover, infection rates after surgery revision are 40% higher than after primary replacement (Trampuz and Zimmerli, 2005).

The antimicrobial properties of silver and copper nanoparticles were investigated using *E.coli.* The average sizes of silver and copper nanoparticles were 3 nm and 9nm. The bactericidal effect of silver and copper nanoparticles were compared based on the diameter of inhibition zone in disk diffusion tests and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nanoparticles dispersed in batch cultures (Ruparelia *et al.,* 2007). In the case of dental implants, complications derived from an implant associated infection do not usually require patient hospitalization, but lead to a detriment in the patient quality of life and satisfaction because it is one of the reasons for early failures by lack of osseointegration and the major cause of late failures (Chen and Darby, 2003). One common and accepted strategy to treat and prevent infections associated with orthopedic implants is to deliver antibiotics in a controlled manner at the site of implantation in order to administer high local doses without exceeding the systematic toxicity of these drugs (Gerhart *et al.,* 1993). Most silver containing antimicrobial biomaterials consists of either elemental silver incorporated into organic (polymers) or inorganic (bioglass or HA) matrices. The *invitro* antimicrobial activity of silver containing polymers and bioglasses has been extensively studied the bactericide action. The bactericidal activity results show that this nanocomposite is strongly active against some of the most common Gram positive and Gram negative bacterial strains, so it can be considered as an antimicrobial biomaterial that can be be used in implant and reconstructive surgery applications (Diaz *et al*., 2009). Nanotechnology carries a significant potential for misuse and abuse on a scale and scope never seen before.They also have potential to take out significant outcomes, such as improved health, better usage of natural resources, and reduced environmental pollution.Nanotechnology has the potential to create many new materials and devices with wide range of applications, such as in medicine,electronics and energy production. on global economics, as well as speculation about various doomsday scenarios..Medical applications of nanotechnology are far more likely to involve improved delivery methods. Preliminary and complementary animal studies should be carried out to identify the risks associated with nanoparticle use, with particular attention should be paid for the elimination processes.

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