Tissue Regeneration using Nano Particles

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Introduction:

Tissue engineering is very fast growing scientific area in this era which is used to create, repair or replace cells, tissues and organs by using cell or combination of cells with biomaterials or biologically active molecules and it helps to produce materials which very much resembles to body's native tissues. From tissue engineering current therapies got revolutionised and life quality of several million patients got improved. Tissue engineering is the connecting discipline between engineering material science, medicine and biology (1). In typical tissue engineering cells are seeded on bio mimicked scaffold providing adhesive surfaces, then cells deposits on their own protein to make them more bio compatible, but unable to vascularise properly, lack of functional cells, low mechanical strength of engineered cells, host's immune incompatibility and nutrient limitation are the classical issues in the field of tissue and tissue engineering (2). Novel bio mimetic Scaffold and Modern technology been developed for more accuracy on positioning viability, complexity, interaction etc. using micro and Nano technology for production and analytical control through tools(3). Micro and Nano technology are providing simple substrate for adhesion, proliferation, active agents for their growth. Nano fabrication techniques, material sciences, surface, micro and nano patterning in tissue engineering helps in providing best micro environment where cells grows(4).

Tissue Engineering from Nano technology:-

There are several benefits of using micro and Nano fabrication techniques for tissue engineering. Nano technology can be used to create Nano fibres, Nano patterns and controlled release Nano particles with applications in tissue engineering, for mimicking native tissues since bio materials to be engineered is of Nanometre size like extracellular fluids, bone marrow, cardiac tissues etc. (5)

Types of Nano particles:-

Particles can be prepared with various types of materials such as Ceramics, MetalsNatural and Synthetic polymers. Their Nano compositions and characteristic advantages like high penetrability, high surface area with tunable surface properties these are one of widely preferred concepts in Tissue Engineering and RegenerativeMedicine field for imaging, mechanical strength enhancement as anti microbial and bio active agent, bio ink supplements etc. (6)

- **Metallic Nano particles:-**

Nano particles provides a link between bulk materials and molecular or atomic structures (7). Metallic Nano particles can be manufactured and modified through utilising different functional groups that provides conjugation of anti bodies, ligands and as delivery systems(8).

- **Gold Nanoparticles:-**

Gold Nano particles can be (Au NPs) can be described as a colloid of Nano meter sized particles of gold. Colloid gold solutions present different properties compared to the bulk gold for example, their optical property due to their unique interaction with light(9).

On gold surface it is possible to conjugate various ligands including poly peptide sequences, antibodies and proteins with various moieties such as phosphines, amines and thiols as they have strong affinity to gold (10).

Potential use of gold Nano particles in the context regenerative medicine is a safety measure, if the implanted tissue replacing aresected tissue or organ due tumour growth.

Eg: Use of Au NPs for disturbing the cancer cell division by selectively transporting the particles in to affected cells nuclei.

- **Silver Nano particles:-**

Silver Nano particles (Ag NPs) can also be described as a colloid of Nano meter sized particles of silver and are one the most widely used metallic Nano particles in bio medical field mainly for their anti microbial properties(11). As bacterial infection is a significant risk with engineered tissues. Use of Ag NPs as a safety measure is a potential solution. These particles can be produced by either physical or chemical processes. (12)

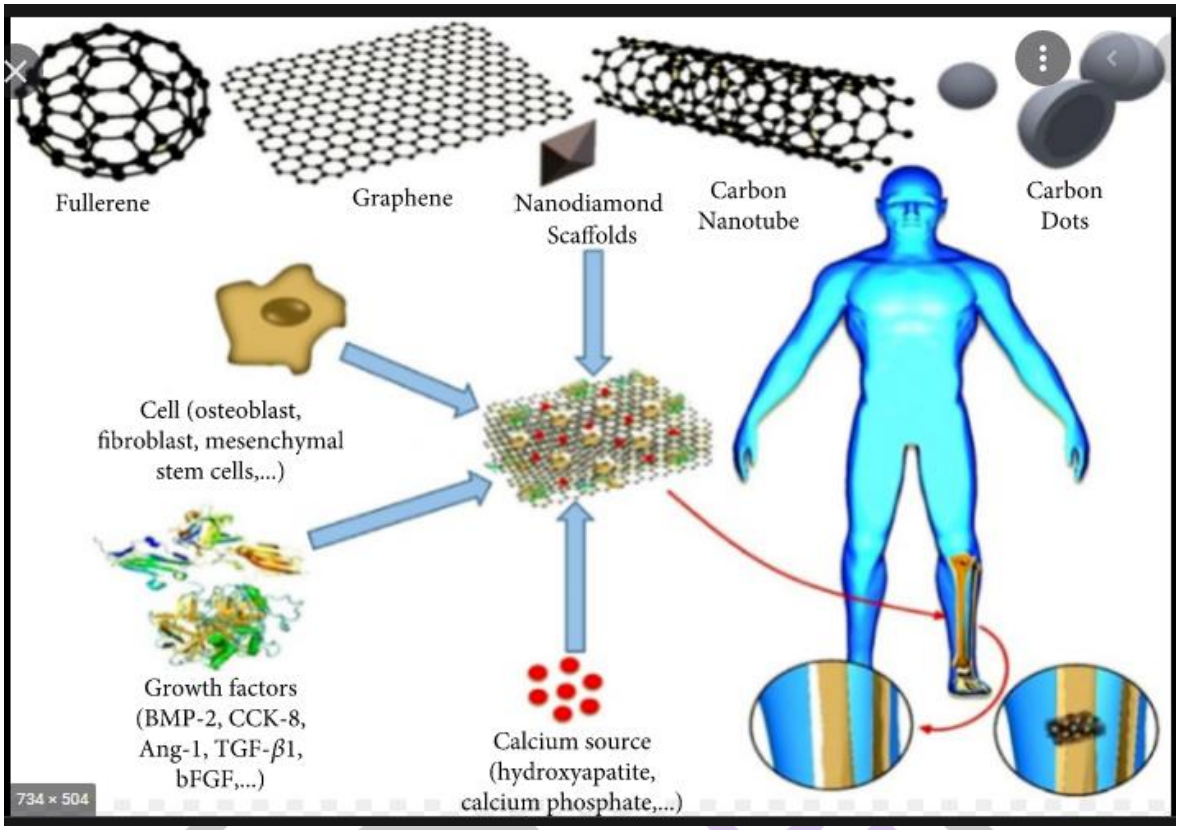
The physical methods used to synthesize Ag NPs are evaporation, condensation process, laser ablation of metallic bulk material, gamma irradiation or ultrasonic irradiation. Chemical methods are mostly based on the utilization of sodium borohydride as reducing agents, in order to reduce silver salt solution. (13) After the reduction of silver ions (Ag^+) in to metallic silver (Ag^0), Nano particles will be formed through nucleation followed by the growth.

Depending on the method used (chemical or physical) and the choice of reducing agent the size of the Nano particles can range from few Nano meters to more than 500nm diameters. To stabilize and control the size of the particles, all most all methods comprise the use of surfactants. (14) Silver ions have been used for a long time for their anti microbial properties towards a wide range of micro organisms. It has shown that silver ions are able to block the microbial respiratory chain system and precipitate bacterial cellular protein.

- **Ceramic Nano particles:-**

Ceramic Nano particles are basically comprised of inorganic compounds, besides metals, metal oxides and metal sulfides they can be used in production of nanoscale materials of various shape, size and porosity. (15) In general,

Ceramic Nano particles can be classified according to their tissue response as being inert, bio activeor bio resordable ceramics and magnetic Nano particles. (16)



3D Nanostructures for tissue engineering

- **Bio resordable Nano Ceramics:-**

Bio resordableNano ceramics have calcium phosphate(CaP) based composition which include variety of materials such as hydroxyapatite(HAP), calcium aluminate, tricalcium phosphate, calcium phosphate, dicalcium phosphate dehydrate, calcium carbonate (CaCO3), calcium sulfate hemihydrate. HAP is major component of natural bone and under neutral or alkaline conditions. It is most stable form of phosphate salts. These materials have been applied in orthopaedics such as bone substitutes. (17) Various ions can be incorporated to the HAP lattice to modulate the characteristic features of the Scaffold for desired TERM application such as degradation properties and cellular responses. Cadmium, Silicon, Yttrium, Silver, Zinc, Copper, Magnesium and trace elements have been used fir modifying the HAP properties. (18)

Multidoping may also improves the properties of HAP and substitutions are gaining huge attention now a days. Ba+2and HO3+ ions as contrast agents for computed tomography (CT) have been doped in to nHAP via micro wave assisted synthesis and under various operating voltages, significant enhancement in the contrast efficiency was observed. (19)

- **Bio inert Nano ceramics:-**

Bio inert nano ceramics including titaniumdioxide (TiO2), zinc oxide (ZnO) are utilised for different medical applications as they show positive interactions with body tissues. TiO2 Nano particles can be synthesised with different manufacturing processes including hypothermal, solvothermal, solgel process and emulsion precipitation methods. (20) By using this method it is possible to manufacture uniformly distributed, bio ceramics in targeted size range.

With the advancement of Nano technology, TiO2 Nano particles, Nano tubes or Nano probes labelled with the fluorescent dye or magnetic resonance contrast agents have been successfully prepared for all imaging through fluorescent analysis or magnetic resonance imaging (MRI) (21)

- **Magnetic Nano particles:-**

Magnetic Nano particles are iron oxide Nano particles which are widely studied in bio medical field because of their lower toxicity. These consists of various applications like imaging cancer cells, pursuing stem cells in vivo and monitoring of engineered tissues, Moreover Supra Magnetic Nano crystal Clusters (SMNC) can be utilised for cell imaging. They are prepared by coating with polyetherimide, citric acid or silica. These SMNCs possessed very high sensitivity towards magnetic resonance and had no adverse effect on cell viability. (22)

Applications:-

- **Stem cell tissue engineering:-**

Techniques used are:

- **Electro spinning:**

Helps to improve adhesion and expansion of hematopoietic stem/progenitor cell at animated nanofiber mesh and in Bone marrow these acts as efficient captor and carrier for hematopoietic stem cells. (23)

- **Soft lithography**:

It is used in regulating the distributor, alignment, proliferation and morphology of Human Mesenchymal Stem cells (24), initiation of differentiation of embryoid bodies of greater uniformity in cell culture in vitro (25), ease to study the growth and differentiation of human embryonic stem cells under defined conditions and homogenous aggregation of human embryonic cells.(26)

- **Photolithography:**

Used to maintain the cells to be in the grooves not ridges and maintaining
Uniform shape and it also have effects the rate of lipid production and thus differentiation of cells to adipocytes. (27)

- **Neural cells tissue engineering:**

Techniques used are

- **Electro spinning**:

Helps in cell differentiation, orientation and behaviour like embryoid bodies will differentiate in to mature neural cells including neurons, oligodendrocytes and astrocytes when they will be cultured on polycaprolactone, poly nano fibres neural stem cells differentiation is more.

- **Replica moulding:**

Helps in maintaining cell shape and behaviour. Eg: Bovine aortic endothelial cells can be cultured with higher cell alignment frequency and smaller circular index when they are cultured on Poly (glycerol sebacate) on sucrose coated micro fabricated silicon. (28)

- **Micro contact printing:**

Helps to form synaptic connections on defined protocol with poly styrene andpolydimethylsiloxane also rat hippocampal neurons when culture with silicon oxide showed resting potential andafter one day of culture they becomes capable to reach action potential. (29)

- **Cartilage cell tissue Engineering:**

Techniques used are

- **Photolithography:**

Used to maintain cell behaviour Eg: Chondrocytes isolated from avian sterna were cultured on micro patterned agarose gal which acts as bio mimicked scaffolds and helps in maintaining chondrogenic phenotype. (30)

- **Replica moulding:**

Helps to maintain controlled micro environment and is integrated with inverted microscope to monitor real time for cell size change in articular chondrocyte. (31)

- **Bone cell tissue engineering:**

Techniques used are

- **Soft lithography:**

Used to maintain cell orientation and behaviour
Eg: Mesenchymal osteoprogenitor M cells are cultured on collagen and thus appropriate surface topography enhances bone formation.

- **Photo lithography:**
- It is providing better groove topography for primary human osteoblasts and helps in cellular adhesion and osteospecific function and in determining cellular response also used on patterned cells on photo cross linkable chitosan by using lysozyme. (32)

- **Micro contact printing :**

Helps in osseo integration of Rat mesenchymal stem cell derived osteoblasts cultured on poly (3-hydroxy butyrate-co-3-hydroxy valerate) which can guide selective osteoblast adhesion and alignment.

Vascular cells tissue engineering:

Techniques used are

- **Soft lithography:**

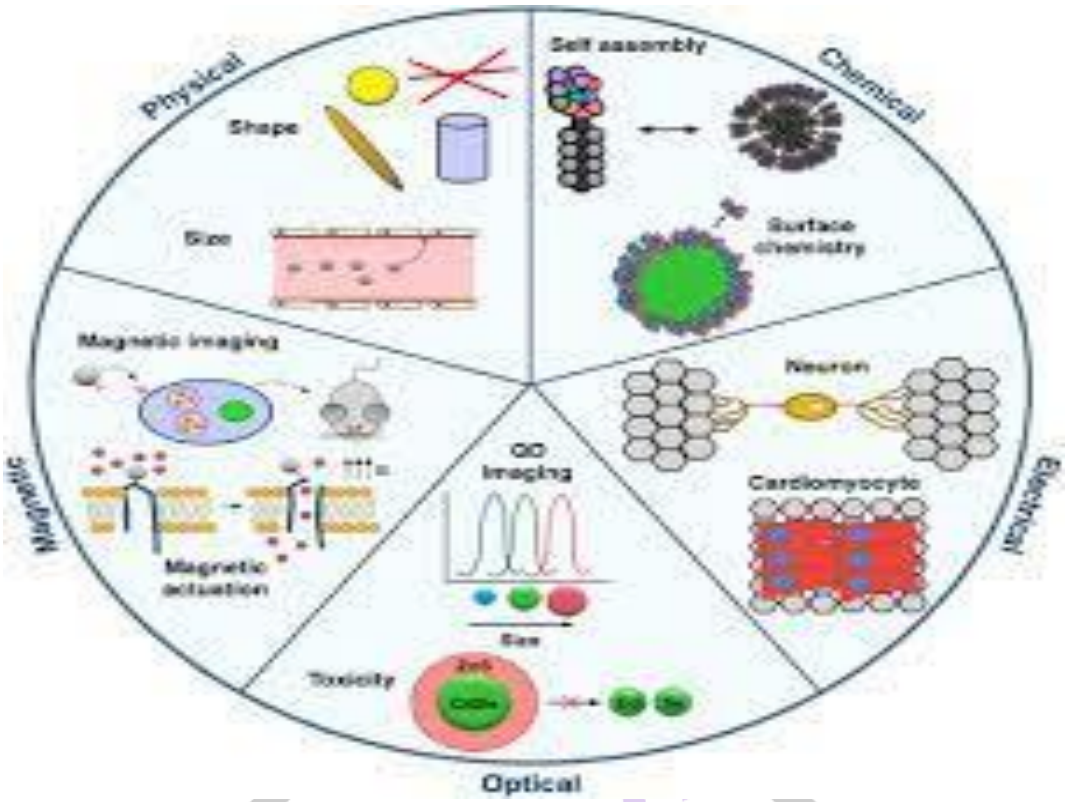
Helps to induce global gene expression and alteration in cell signalling in mesenchymal stem cells culture with polydimethylsiloxane and also helps to increase retention of endothelial cells with poly -urethane which results in reducing thrombogenecity during its implantation.

- **Microfluidic patterning:**

Helps to form contractile cardiac organoids from cardio myocytes with the help of hyaluronic acid and helps in cell-ligand attachment and spatial distribution for culturing human umbilical vein endothelial cells with poly (ethylene glycol). (33)

- **Electro spinning :**

Helps in attachment and migration of cells along the axis in human coronary artery smooth muscle cell culture with poly (L-lactid-co-caprolactone)



Applications of Nanotechnology for Regenerative medicine

- **Hepatic cells tissue engineering:**

Techniques used are

- **Electro spinning:**

Promotes the formation of integrated spheroid Nano fibreconstruct in rat primary hepatocytes culture with poly(e-capro lactone-co-ethyl ethylene phosphate) . (34)

- **Soft lithography:**

Along with some defined design help to provide sufficient oxygen and nutrient mass transfer to maintain viability in hepatoma cells culture and primary rat hepatocytes culture with poly dimethyl siloxane and poly carbonate. (35)

- **Photo lithography:**

Helps to maintain cell-cell 3D structure in hepatocytes culture with poly (ethylene glycol) and also be able to maintain phenotypic functions for many weeks in primary rat hepatocytes and primary human hepatocytes culture with dimethyl siloxane. (36)

Conclusion:

Tissue engineering evolved from the field of bio materials development and refers to the practice of combining scaffolds, cells and biologically active molecules in to functional tissues.

The goal of tissue engineering is to assemble functional constructs that restore, maintain or improve damaged tissues or whole organs.

Bone tissue engineering requires the complex formation of cell types such as osteoblasts, osteoclasts and osteocytes with in a cellular material component. High strength carbon Nano tubes are fully compatible with bone cells .(37)In future bone tissue surgery can be carried out by reinforcing artificial bone implants by using carbon Nano tubes.

Regenerating cardiac tissue with carbon Nano tubes improves the prognosis of heart pathologies such as cardio vascular defects and heart failure.

Carbon Nano tubes are being used to develop devices for functional regenerative purposes. (38)

In comparison to bone tissue repair, the regeneration of neural tissue has proved more challenging and the ability to regrow nerves for paraplegic patients has not yet been reached. Nano technology may provides a promising new strength for treatment. Nano tubes are especially suited for neural tissue engineering as their structure mimics the natural tubular forms of microtubules and axons.

Hence target drug delivery treatment with Nanotechnology is most effective, acceptable when compared with conventional type of drug delivery systems

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