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Advancement in Scaffolds for Bone Tissue Engineering: A Review

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Abstract: *In last decade, Tissue Engineering has moved a way ahead and has proposed solutions by replacing the permanently or severely damaged tissues of our body. The field has expanded to tissue regeneration of cartilage, bone, blood vessels, skin, etc. The domain of tissue engineering is very wide and is the combination of bioengineering, biology & biochemistry. This review is focus on recent research advancement in bone tissue engineering. Bone grafting techniques are used to replace the severely damaged due to any accident, trauma or any disease. These are either allograft, autologous or synthetic bone properties similar to bone. Bone Tissue Engineering is part of a synthetic technique and overcome the limitations faced in other two mentioned techniques. Bone Tissue engineering is rapidly developing field and has become important due to its remarkable therapeutic properties. Mesenchymal stem cells are used as starting cells in tissue regeneration. These cells get differentiated into bone cells and start multiplying to form bone. One inevitable requirement of these growing human cells is a strong support which helps in the proper growth. This support is known as scaffold, in tissue engineering. For proper regeneration of cells scaffold materials plays vital importance in the field of bone tissue engineering. This review attempts is illustrate the biology of natural bone, various desirable properties of scaffold, biomaterials used for fabrication of scaffold and various fabrication techniques with examples of bone regenerate.*

Keywords*: Bone regenerate, Grafting, Mesenchymal stem cell, Scaffold, Tissue Engineering.*

I. Introduction

Bone and cartilage all together provide incomparable support to our body and protect the fragile organs. It is important to ensure that their proper structure is retained for proper functioning of organs. Unfortunately some inadvertent accidents cause permanent damage to these tissues and thus disturb the entire body balance. Here comes the role of regeneration of tissues. Regeneration of vital tissues of our body parts like bone, cartilage, ligaments, etc. has opened wide gates in the field of therapeutics of damaged tissues. The field of engineering which deals with the development of these tissues is Tissue Engineering and encompasses several disciplines in it. It is blend of Biochemical Engineering, Cell biology, Biomaterials science and Cell Imaging. The involvement of multitude of disciplines in this field has helped it in making one of the most lucrative branches for research and innovation directed towards the development of human health.

The review focuses on various aspects of bone tissue engineering, wherein an attempts to understand on the paradigm shift observed in the field of bone tissue engineering. There have been tremendous advancements in the choice of biodegradable materials and techniques of 3-D scaffold designing for better regeneration of bone tissues. These techniques have helped in overcoming the problems observed in autograft and allograft grafting techniques[1-2].

The two major bone graft techniques are autograft and allograft. In the first, bone is harvested from the patient's body while in the latter one cadaver is the source of bone. However, both of these techniques come along with serious concerns and limitations[3]. In autograft, donor site morbidity that is damage of remaining tissue at the site of harvest is major limitation. Besides this, limited availability and unpredictable resorption characteristics of the bone are also matters of concern. In allograft, the immune-rejection from the host body and increase chances of disease transmission comprises the major limitations. In the above discussion bone tissue engineering seems to be a best option.

First of all mesenchymal stem cells (MSCs) are obtained from patient's body and are cultured outside the body. They are further seeded into the diligently tailored scaffolds and are surgically implanted into the patient's body[4]. The choice of materials is such that it ensures proper development of the tissue in vivo and simultaneous degradation of scaffold into non-toxic components which can easily be excreted out through body metabolism or utilized in body. Throughout the development of tissue it is required by the scaffolds to maintain the mechanical integrity and provide the mechanical stresses required for the differentiation of cells. Intensive research in this field has provided with several options for scaffold materials including bioresorbable natural polymers, synthetic polymers, composites, bioactive ceramics, hydrogels and their combinations. Each and every material has its own merits and demerits but neither of them has been capable to mimic the natural extracellular matrix (ECM). Other factors such as cell sources, regulating molecules, mechanical simulation,

bioreactor designing, in vitro evaluation, in vivo evaluation and clinical considerations also play significant role in tissue engineering but are beyond the scope of this paper.

1.1 Bone Biology

To mimic the structure of bone, it is essential to know about the peculiarities and structural features of the natural bone. Bone tissues present in our body are essentially abundant in compact or cortical bone which comprises mostly 80% of the skeletal system. The remaining proportion is of cancellous or spongy bone. The proportion varies at various junctions[5-7].

Cortical bones are mostly solid in nature and have mostly around 10% of porosity which is in contrast to the cancellous bones where the porosity may range from 50-90%. Consequently, cortical bones mechanical strength supersedes the mechanical strength of cancellous bones with significant amount. The fact can be corroborated by the compressive strength test and modulus experiments [8].

Here, it becomes important to discuss about the bone cells and their vital functions. Bone cells are of three types namely, osteoblasts, osteocytes and osteoclasts and every bone cell has been allocated to perform different functions[9].

I. **Osteoblasts:** Osteoblasts are cuboidal and polarized cells. They are present on the surface of the bone and help in the formation of tight layer of cells. It is done by the synthesis of ECM and its further mineralization.

II. **Osteocytes:** Osteocytes, stellated in shape are the cells formed on the entrapment of osteoblasts. Their major function lies in the calcification of the osteoid matrix and blood calcium homeostasis. Osteocytes also work as sensory organs for the bones for the transmission of signals.

III. **Osteoclasts:** Osteoclasts are multinucleated polarized cells which play major role in the resorption of bone.

The extracellular matrix of bone which has been archetype in development of the scaffolds comprise of two components: mineral part constitutes hydroxyapatite and forms the larger portion of ECM whereas the other component, organic part comprising glycoprotein's, proteoglycans and sialoproteins and forms the minor part of the matrix [10].Thus, it is observed that the best suited synthetic model of the scaffold would be the one which is analogous to the natural bone, i.e. composite of organic and mineral components. The details structure of the bone at hierarchical level is shown in Fig.1[11].

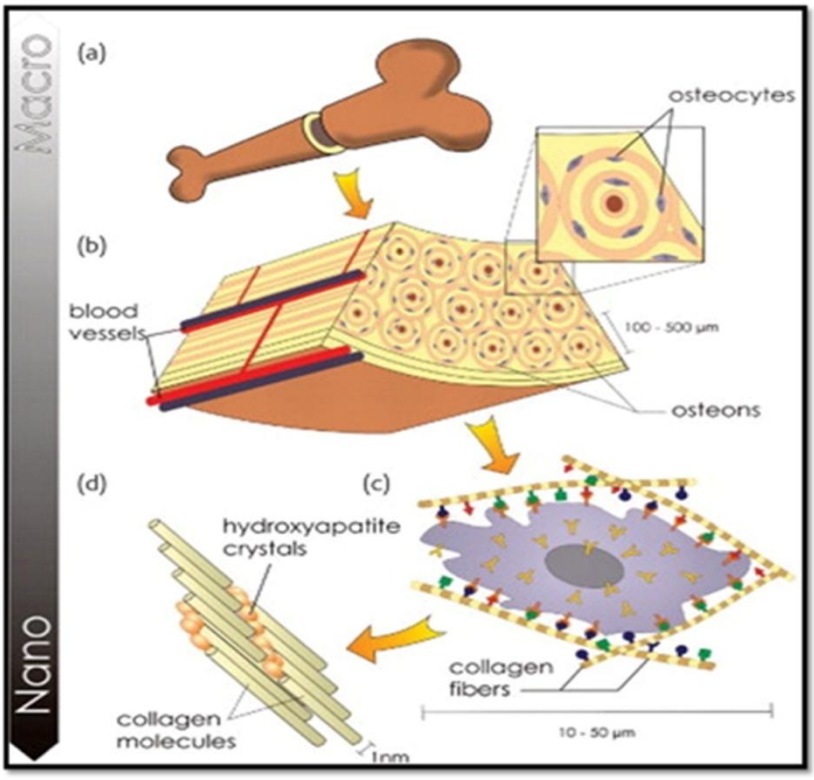

Fig.1. Hierarchical organization of bone over different length scales. Bone has a strong calcified outer compact layer (a), which comprises many cylindrical Haversian systems, or osteons (b). The resident cells are coated in a forest of cell membrane receptors that respond to specific binding sites (c) and the well-defined nanoarchitecture of the surrounding extracellular matrix

II. Osteoblast Proteins

2.1. Extracellular matrix proteins

The bone is made of osteoblast embedded in an extracellular matrix which contains collagenic proteins and non-collagenic proteins (NCP) in the ratio of 90:10.Collagenic protein contains two type of collagen viz type I collagen 97% and type V collagen 3%, along with non-collagenic proteins (NCP) viz osteocalcin 20%, osteonectin 20%, bone sialoproteins 12%, proteoglycans 10%, osteopontin, fibronectin, growth factors, bone morphogenetic proteins, etc. Several proteins (fibronectin or vitronectin) are synthesized by osteoblast which also helps in the adhesion while the roles of other plasma proteins (α2HS glycoprotein, transferrin, albumin, immunoglobulin, etc.) are not cleared but they are linked with mineralized bone matrix [12-13].
Bone proteins exhibit chemotactic or cell adhesive properties which may be due to the presence of RGD sequence (Arg–Gly–Asp). Which are due to fixation of cell membrane receptor like integrin [14].
Human osteoblasts adhere more preferentially with fibronectin and vitronectin as compared to other collagen

2.2. Cytoskeleton proteins

Tissue cultured cells exhibit adhesion properties to surface of substrate or junction, which may be attributed to the presence of specific receptor protein like integrin present in external faces of focal contacts where internal faces have proteins like talin, paxillin, vinculin, tensin etc. These proteins are also involved in signal transduction at cellular level shown in the Fig.2 [15].
Actin cytoskeleton architecture is essential for the maintenance of osteoblast shape and adhesion.

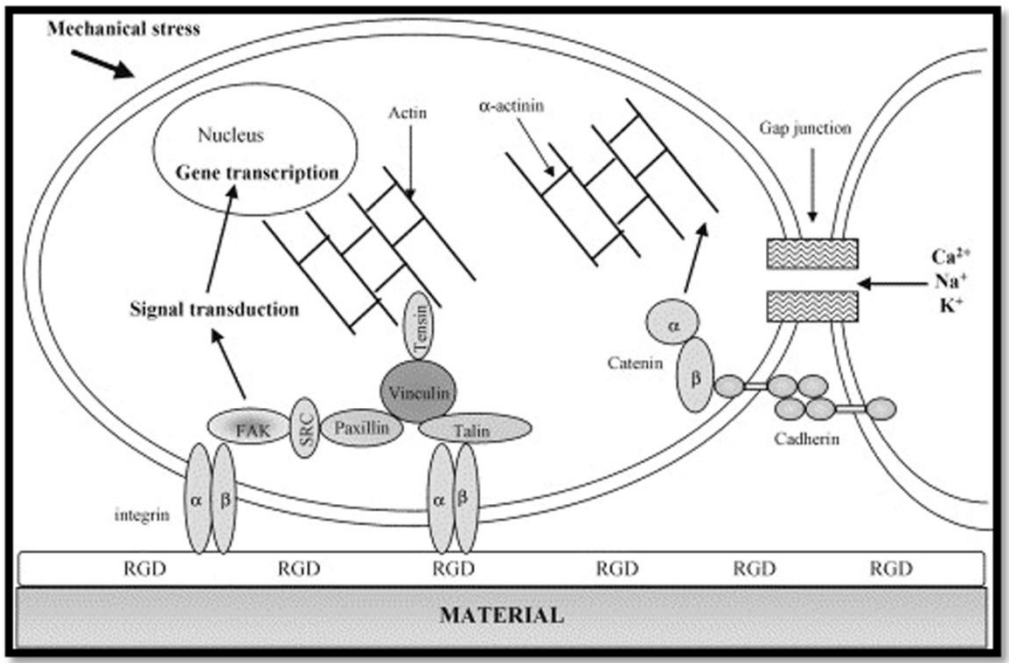

Fig. 2. Representation of the cell proteins involved in cell adhesion on biomaterial.

2.3. Adhesion molecules

Adhesion molecules in general are of four types, these are selectins, immunoglobulin super family, cadherins and integrins. Among them cadherins and integrins plays an important role in the osteoblast functions.
2.3.1. Integrins: The integrin class of protein with 22 heterodimers having two types of sub-units α and β, 16 α sub-units and 8 β sub-units which have exhibited ligand-binding ability. These integrins are transmembrane heterodimers consisting of α and β sub-units. Each sub-unit comprise of large extracellular domain. The integrin serve as interface between the extracellular and intracellular compartments.
2.3.2. Cadherin: Cadherins are observed to be transmembrane glycoprotein's performing with intracellular partners catenins which interact with intracellular proteins. Union with α, β or γ-catenin is required for the adhesive function of cadherins.
2.3.3. Gap junctions: They are involved in cell-cell communications. These proteins help in intercellular communication through direct exchange of ions by the help of gap junctions. Gap junctions are made of proteins called connexins, which provides anchorage to nearby cells and allow direct exchange of ions or small molecules between the cells [16].

III. Scaffolding approaches in tissue engineering

Development of tissue requires the matrix. The 3D scaffolds developed serve as the matrix for the regeneration of the tissues. They act as a store house for nutrients, water, cytokines and growth factors and regulate the cell proliferation. Their role extends as the presence of matrix governs the vascularisation of the neo-tissue which can further involve in the regenerative activity over the release of differential factors present in the structure[17].

Four main scaffolding approaches used in tissue engineering applications which are briefly discussed below.

3.1. Pre-synthesized scaffolds for cell seeding

Pre-synthesized porous scaffolds used for cell seeding are the usual technique in tissue engineering field. This has led to a substantial research in the field of biomaterials and fabrication technologies. As a result, today's tissue engineering is supported by bulk of biomaterials (both natural and synthetic) and highly developed fabrication techniques. Natural biomaterials are conducive to the growing cells and are highly biocompatible. Synthetic biomaterials provide control over physical and mechanical properties and are used for the scaffold preparation in case of both hard and soft tissues. Various fabrication technologies that have developed can be broadly categorised into three forms.

1. Freeze drying, phase separation, gas foaming, solvent casting and particulate leaching are some examples of process in biomaterials using porogen.
2. Rapid prototyping technologies or solid free-form, for instance, selective laser sintering, 3D printing, etc.
3. Techniques that use woven and non-woven fibres for fabrication of scaffolds. Electro spinning is an example of such a technique. These are discussed below in detail.

The approach has got several advantages. It has got bulk of options for choosing the best suited biomaterial. Secondly, the controlled structure of the scaffolds with controlled properties may be established. Scaffolds showing the properties of physiochemical can be governed to quite an extent. The approach has some disadvantages as well. The process of cell-seeding after the fabrication of scaffolds is highly inefficient as well as time consuming. Nonuniform distribution of cells inside the scaffold and thus heterogeneous properties are observed in the tissue. The complete process of forming pre made porous scaffold is shown in Fig.3[18].

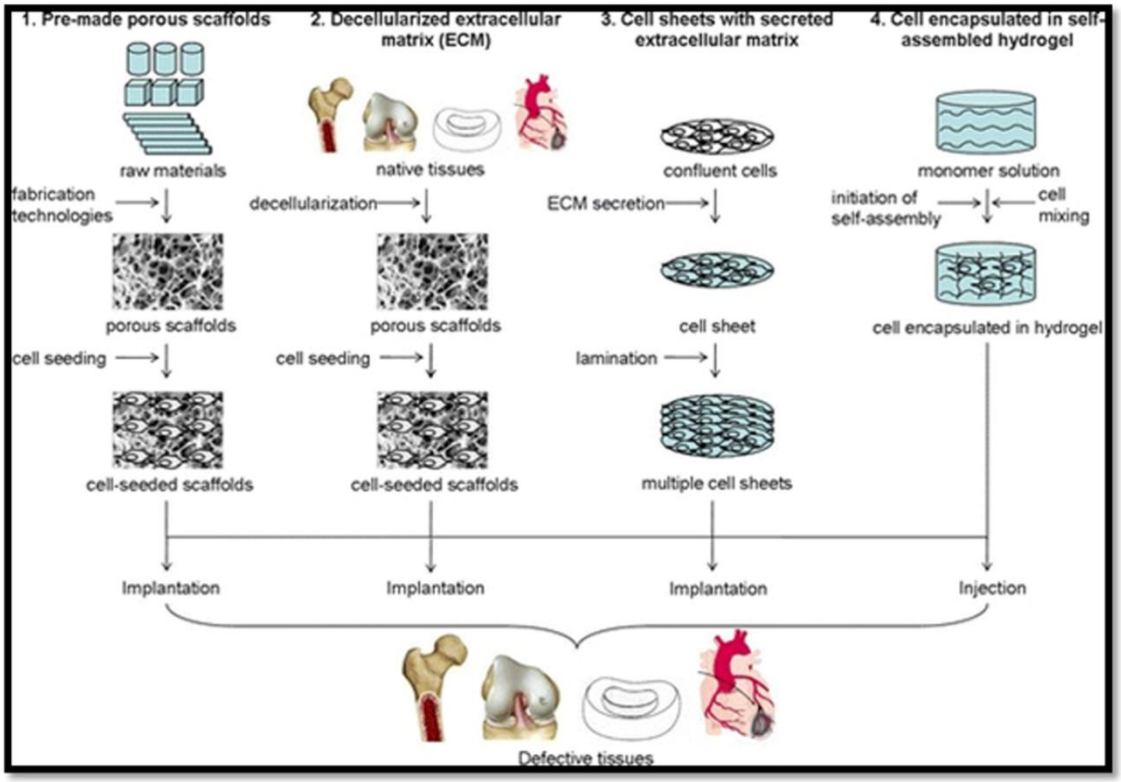

Fig 3: Schematic diagram showing different scaffold approaches.

3.2Decellularized extra cellular matrix (ECM) for cell seedings

Decellularized ECM is highly simulating scaffolds that have been used in heart valves, vessels, nerves, tendons and ligaments. Generally in this method the cellular antigens by the allogenic or xenogenic tissues are eliminated and the ECM components are preserved[19]. Decellularization is attained through physical,

chemical and enzymatic methods. Cell membrane is lysed through physical methods like freeze-thaw cycles or ionic solution. This is initially solubilised and then removed with the help of handful of detergents. The above procedure is carried out keeping in mind the minimum disturbance to biochemical composition and mechanical properties of ECM. This decellularized ECM serves as replacement for damaged analogous tissues. It's close to mechanical and biological properties acts as its greatest advantage. However, cell seeding in ECM might be in homogeneous[20].Process of forming Decellularized ECM is shown in Fig.3.

3.3 Self-secreted ECM by cell sheets

In this method the cells are usually cultured on thermo-responsive polymer coated cultural dish. On confluence the cells secrete their possess ECM. Such approaches are repeated to obtain multilayer thick matrix. This approach has its major application in regeneration of epithelium, endothelium and cell-dense tissue. This approach is being attributed between high cell density and close association. Development of corneal epithelium, vessel endothelium and tracheal epithelium are main applications of this method. Besides that cells sheet engineering help in rapid neovascularisation of tissues. Nevertheless, the development of thick matrix is a tedious job to perform and it can't be used for the development of ECM rich tissues like bones, cartilages and intervertebral discs. Fig.3 showing the process of forming cell sheets with self-secreted ECM.

3.4. Cell encapsulation in hydrogel matrix

Cell encapsulation is a method of entrapment of living cells inside a homogenous solid mass otherwise semi-permeable membrane. Immunoisolation through allogenic or xenogenic cell transplantation is significantly used by this method. Hydrogels are prepared by natural, synthetic and semi synthetic water soluble polymers. Naturally occurring materials includes agarose, chitosan and natural polysaccharides derived through algae while poly (ethylene glycol) and poly vinyl alcohol are in the list of synthetic polymers. Biomaterials irrespective of whatever kind need to be biocompatible to ensure proper growth of tissues.

Semi-permeable membrane has a major role to play in proper working of immunoisolation. This membrane in general permeable to nutrients and metabolite like oxygen, glucose, lactic acid, etc, on the other hand it is impermeable to large molecules such as antibodies and antigens. Biomaterials used for encapsulation have remarkable property to self-assemble from liquid monomers to solid polymer meshwork. Factors controlling the initiation are pH, temperature, ionic strength, light and others. This feature gives the remarkable chance of combining the cell-seeding and scaffold fabrication into one step process and increases the chances of uniform cell distribution in the matrix with admirable cell viability.

IV. Scaffold characteristics

The scaffolds to be used for bone tissue regeneration satisfy various macro and micro structural properties which cannot be neglected if the proper growth of the tissue is desired[21].
The following properties are important for scaffolds in bone tissue engineering:

4.1 Biocompatibility

The scaffold chosen should concord with the host's tissue and should not elicit any immunological responses. This property of the Tissue Engineering gives it upper hand over the allograft and autograft techniques where the rejection of the tissue due to immunological responses is frequently encountered.

Various types of novel scaffolds are getting fabricated comprising organic and inorganic materials. The factor of biocompatibility draws concern with the advent of multitude of these composites. Synthetic polymers like PLA, PLGA, PDLA, PLLA hydrolyses to give lactic acid and glycolic acid which without causing any harm are excreted out of the body. Other synthetic polymers like Poly (e-caprolactone), Polyanhydride, Poly(phosphazenes), Poly(propylene fumarates) also lie in the league of biocompatibility[22]. Natural polymers have an upper hand over synthetic polymers for biocompatibility. In case of composites most of the composites which are prepared using Hydroxyapatite (HA), bioactive glass and other bio ceramics are biocompatible as well. However, use of Carbon nanotubes (CNTs) which has shown remarkable results for improving the mechanical strength and bioactivity has not been assured to be biocompatible in nature[23].

4.2 Porosity

The unavoidable need of high porosity in scaffold was shown by the scientist using rat ectopic model[24]. There used of solid as well as porous hydroxyapatite particles for the delivery of BMP-2[25].It was observed that, there was no regeneration of bone in solid scaffold where the osteogenesis was evident in porous scaffold. Higher porosity and interconnectivity of pores ensures larger surface area to volume ratio and contribute in vascularisation of recently developed tissues. Though porosity doesn't play any significant role in cell attachment but with the increased space transport of oxygen and other nutrients is enhanced. These pores are responsible for the removal of waste metabolites which consequently improve high mass transfer rate and

improve the proliferation of tissues. However, the role of porosity in vitro and in vivo differs to some extent and can be further studied here [26].

Mechanical integrity relies on the porosity as well. Highly porous structures are more fragile as compared to the solid scaffolds or less porous scaffolds. Therefore, it is essential to maintain equilibrium connecting porosity and mechanical integrity of the scaffolds.

4.3 Pore Size

Pore size and porosity play synergic effect in development of the tissue. To prevent the occlusion transfer of oxygen and nutrients it's better to have larger pore size this helps to Increase surface area to volume ratio. Proper vascularisation and development of ECM by growing tissues is corroborated by the large pore size.

It was reported that bone reconstruction can be achieved employing the scaffold with the large pore of 1.2-2.0 mm. The development of such large pores in the scaffold won't be feasible for the proper growth of the tissues as it will lower down the mechanical strength of the scaffolds to quite an extent[27].

The significance of high porosity and proper pore size in scaffold fabrication is inevitable. In case of synthetic polymers Poly(lactide-co-glycolide) was generally used for culturing human osteoprogenitor cells by the scaffold having mean pore size of 200μm[28].The polymer was also used for fabricating tooth implants having mean 65% porosity and pore size of 100μm[29]. Composite of hydroxyapatite/chitosan-gelatine was used for the in vitro study in rat calvarial osteoblasts. The scaffold used had pore size lies between 300 and 500 μm and the porosity increased with decreasing concentration of gelatine-chitosan and increase of chitosan-hydroxyapatite/gelatin proportion[30].

4.4 Surface Properties

Chemical and topographical properties of scaffolds help in proper adhesion of osteogenic cells to the scaffolds and their proper proliferation [31-33].

Chemical properties of scaffolds help in adhering of the material to the cells and interaction of the growing tissue with the proteins and other bioactive agents present on the scaffold. Topographical properties come into picture with osteoconduction (supporting growth of bone and simultaneously encouraging the growth of surrounding bone), osteoinduction (osteoprogenitor cells to differentiate into osteoblasts and then begin with new bone formation) and osteointegration (integration into surrounding bone)[34-35]. The tailoring of the surface properties can be done by integration of biologically active agents and growth factors.

Fabrication of chitosan/hydroxyapatite scaffold has shown increase in osteoconduction of cells which lacked in pure chitosan scaffold. The dearth of chemical integration between cells and scaffold in various polymers has greatly subsided by the use of various inorganic materials like bioactive glass, calcium phosphates, HA, etc. The reason for this increased bioactivity is the change in the surface morphology of these scaffolds. Bioceramic coupled polymer scaffolds have shown the formation of hydroxycarbonate apatite on their surface when come in continuous contact with biological fluids[36-37].It has also improved the adhesion of cells to the scaffold resulting in increased proliferation required for tissue regeneration.

4.5 Mechanical Properties

The development of bone in vivo is accompanied by regular stresses. Thus, it is mandatory for the scaffolds to bear these mechanical stresses and ensures the mechanical integrity and protection to the developing tissues. The property of the scaffold should be such that with the increase in mechanical stresses the strength of the growing cells increases [38-39]. In order to perform these requirements the use of bioactive nano-composites are extensively researched. The incorporation of these mineral composites helps in better proliferation of cells and at the same time provides enhanced mechanical strength of the matrix.

According to Woff's law, mechanical loading of the bone (well within limits) is proportional to the growth of bone. Interstitial fluid flow in our body is source of continuous mechanical stress and has shown significant effects on the growth of bone. For instance, increase in vascularisation (kind of fluid flow) has shown increase in osteogenic cells and their activity during bone healing. Contrary to it the decrease in mechanical stress has shown dramatic results of bone loss. Significant bone loss has been observed with bed rest of 30-36 weeks and in case of astronauts or experimental animals residing in microgravity for long time[40].

4.6 Biodegradability

The material for building framework of tissues must be biodegradable in nature. Degradation rate must be such that it is in accord with the generation of the tissues. The best suited material will be the one which provides all the desired above mentioned properties and gets completely degraded at the time of complete proliferation of cells. Degradation rate be able to tailor by varying the monomer composition and scaffold fabrication technique and the best model can be opted for the tissue in concern[41].

The rate of degradation in case of polymers can be governed through the monomer constituents. The discussion on variance in degradation is explained under the section of 'Synthetic Polymers'.

V. Porosity and Pore Size measurement

Porosity and pore size are very vital factors in the scaffold preparation, thus significance of their measurement can be understood. Different types of methods are used for measuring porosity and pore size.

5.a. Gravimetry

Using the gravimetric analysis porosity is measured in the following way:

$\Pi = 1 - (\rho_{scaffold}/\rho_{material})$

Here, Π denotes the porosity of scaffold, $\rho_{scaffold}$ denotes density of the scaffold and $\rho_{material}$ denotes density of the material used for fabricating scaffold.

5.b.Porosimetry

Porosimetry involves the use of any non wettable liquid. In most of the cases Mercury is used as a standard non-wettable liquid to measure porosity and pore diameter of the scaffold. The technique involves the use of instrument, porosimeter for the intrusion of mercury into the scaffold by applying high pressure. The pressure applied against the surface tension of liquid for the intrusion of mercury helps in determining the scaffold size.

$P = 2\sigma\cos\Theta/r$

Where P is external pressure applied, σ denotes surface tension of the mercury, Θ denotes the contact angle of the mercury with the scaffold material & r is the radius of the pore. Porosity handy to intrusion is recognized as open porosity and can be calculated as[42]:

$\pi = V_{intrusion}/V_{scaffold}$

Where $V_{intrusion}$ denotes the whole volume of mercury which intruded the pores and $V_{scaffold}$ is the volume of scaffold.

5.c. Liquid Displacement Method

In this intuitive method scaffolds immersed into the cylinder in ethanol with volume (V_1). Cylinder is kept below vacuum for the purpose to force the ethanol by the pores of scaffold. Ethanol soaked scaffold remains immersed in the cylinder and the whole volume of ethanol & scaffold is volume (V_2). Scaffold skeleton volume is (V_2-V_1). If the saturated scaffold is separate from the cylinder after that ethanol volume left will be (V_3). Ethanol volume there in the scaffold can be calculated, which will be (V_1-V_3). Total volume of scaffold can be calculated by (V_2-V_1) + (V_1-V_3) =(V_2-V_3) and porosity (Π) is given by equation [43-44]:

$\Pi = (V_1-V_3)/(V_2-V_3)$

5.d. Scaning Electron Microscopy

Pore size, porosity, surface morphology can be determined by the cross-section of scaffold using scanning electron microscopy (SEM). Cross-section of the scaffold is analysed in two-dimensional (2D). With the help of image analysis software pore to polymer area is calculated and it is further extended to 3D estimate of porosity.

VI. Biomaterials for scaffold preparation

The extensive research in the field of finding novel and better option for the scaffold material has provided today's age with development of several materials which are or may be used in future. The prerequisite requirements of these materials have already been discussed in the above paragraphs. They need to be biologically active and integrate easily. Natural polymers, bioactive ceramics, bioactive glass, semi-synthetic polymers, synthetic polymers or composite of them are main substitute materials for the preparation of scaffolds. For the preparation of scaffold natural Polymers like polysaccharides (starch, alginate, chitosan, and hyaluronic acid derivatives), collagen, fibrin gels and various lingo cellulose bio fibres are used. However, the problem of using natural polymers is their increased immunogenicity and high chances of pathogenic contamination. However, in this review we will be focusing our attention more towards the use of synthetic polymers in the application of scaffold designing.

6.1 Synthetic Polymers

Broad use of synthetic polymers has been driven by its various advantages. The mechanical and physical properties exhibited by these polymers are reproducible, material impurities are in control, the freedom of tailoring the degradation profile has helped in ensuring the mechanical integrity for growing tissues. These are better as correlate to natural polymers in the regard that possibility of toxicity and immunogenicity is reduced.

6.1.1 Bulk Eroding Polymer

Synthetic polymers used for constructing 3 D scaffolds are poly-α-hydroxy esters as well as poly (lactic acid) (PLA), poly (glycolic acid) (PGA) & poly (lactic-co-glycolide) PLGA. PLA also exist in other three forms L-PLA (PLLA), D-PLA (PDLA) and racemic mixture of D, L-PLA (PDLLA).

The extensive use of the polymers for scaffold designing and their FDA approval [45] for using these polymers for human health system are strong evidence of their numerous benefits. Lactic acid and glycolic acid is degraded product of the polymer, & body has got well developed mechanism for the elimination of these products.

The degradation is regulated by hydrolytic degradation through de-esterification. The degradation rate is able to govern by varying the monomers composition. For an instance PGA being more hydrophilic degrades faster than PLA which is more hydrophilic, so in the polymer PLGA the high concentration of PGA will contribute to faster degradation whereas high concentration of PLA will lead to slow degradation. The amorphous and crystallinity also contribute to the different degradation rate. More is the polymer amorphous in nature faster it will degrade in the solvent (mostly aqueous).The ease in processing, control over the degradation rates and reproducibility of physical and mechanical properties have increased the propensity towards these polymers. Polypropylene fumrate (PPF) is also one of the polymers which fall in this category. The best property about PPF is that its mechanical strength matches somewhat to cortical bone[46] and like other polymers of this category its degradation rate can be controlled.

However the degradation of these polymers is due to bulk erosion which may cause abrupt release of the degradation products being acidic in nature. The sudden fall in pH may cause strong inflammatory reactions. To counter this problem, bioactive glasses, hydroxyapatites, basic salts and calcium phosphates are used which serve dual purpose [47-49]. They regulate the pH and improve the mechanical strength of the scaffold as well. These composites being more hydrophilic in nature help in fresh spread of serum proteins serving helpful in the proliferation of the tissues.

6.1.2 Surface Eroding Polymers

PLA, polyortho esters and polyhydrazene are few of the surface eroding polymers. The erosion of these polymers depends upon the exposed total surface area of the polymer contrary to the bulk eroding polymers. Extensive use of these polymers has been in drug delivery applications. They are advantageous if used for building scaffolds as they maintain the mechanical integrity of the scaffold, the acidic products are not produced in burst and there is increase in porosity of the polymer with layer by layer erosion[50]. However, their uses for building scaffolds have not been significant and are under research.

6.2 Bioceramics

Bioceramics plays an essential role in the field of bone tissue engineering improving the properties of scaffold and making it more bioactive. The term 'bioactive' signifies the chemical bond among the bone and scaffold. Presence of biological fluids a coating of hydroxyl carbonated apatite is formed on the surface of bioceramic. The layer of HCA makes them bioactive and acts as an interface between scaffold and developing bone. In natural bone HCA attributes more than 50% of the bone weight where it solidifies and is known as bone mineral.

6.2.1 Bioactive Glass

After the success of 45S5 Bioglass® many other composition for developing better bioactive glass have been developed but have not been fortunate enough to gain the attention which prior got. The HCA coat produced on the surface of bioglass to keep scaffold intact with developing bone tissue and reduces the chances of scaffold loosening manifold.

The advantages of the bioglass extend in other directions as well. The dissolution products of the 45S5 Bioglass® regulate the gene expressions and stimulate osteogenic activity of developing bones[51]. Si incorporated in place of Ca has shown to improve the osteogenesis through gene activation. It has been observed that 45S5 Bioglass® increases the secretion of VEGF in vitro growth factor & subsequently inducing quicker vascularisation of bones by in vivo method[52]. Experiments in recent past has shown that 45S5 Bioglass® when doped with AgO_2 exhibit anti-bacterial properties preventing the chances of bacterial contamination [53]. Supporting enzymatic activity, faster osteoblast adhesions are few of the other advantages of Bioglass. The rate of the bioglass desorption can be tailored by varying their structural and chemical properties at the molecular level. However, the major drawbacks are their low fracture toughness & low mechanical strength because it has not got much application in the load bearing conditions.

6.2.2 Calcium Phosphates

HA, one of the most dominant constituent of bone is a kind of calcium phosphate. Thus calcium phosphates show excellent biocompatibility because of their structural and chemical resemblance to bone mineral (solidified form HA). They support the strong attachment of cells to the scaffold, proliferation and differention of Mesenchymal cells into bone cells. However, their limited use in the bone application is because of their low biodegradation rate & low mechanical strength of crystalline HA[54].

6.3.0 Composites

Composite scaffolds are prepared by the fabrication of two or more constituents. The composites comprise of the advantageous properties of both the materials involved [55-57].For an instance, the composites of bioactive glass and polymers. On one side, bioactive glass improves the bioactivity and mechanical integrity of scaffolds while on the other polymer induces strength in the scaffold. Higher the inclusion of bioactive glass over the surface area of the polymer, higher the bioactivity is observed. It has been observed that the addition of Bioceramics improves the hydrophilicity of the scaffold[58-59]. Consequently composites provide better opportunities for tailoring their degradation rate. The variance in the degradation can be seen in both bulk as well as surface eroding polymers[60].

VII. Scaffold Fabrication Mechanism

Different type of fabrication methods used for scaffold preparation. Few of the widely used techniques will be discussed here. The best technique is the one which produce the scaffold with all the desired qualities mentioned below.

7.1 Particulate Leaching and Solvent Casting

Particulate Leaching & Solvent casting is the easy and general technique taken into use for 3D scaffold fabrication.Fig.4 shows the detailed process of Solvent casting-particulate leaching scaffold fabrication technique[61] . In this technique polymer solution is pour into mould of the desired shape which contains any water soluble salt (e.g. Sodium Chloride, Sodium Citrate)[62-63]. It is further followed by the removal of solvent through evaporation or lyophilisation and salt particles leach inside the polymer particles[64]. The mould is dipped into water bath for sufficient time to remove the salt leached inside the matrix. Porous structure formed by the removal of salt. Pore size optimized on the basis of salt/polymer ratio or on the basis of size of salt particles. Simple fabrication technique, control over pore size and porosity are few of its advantages[65-66]. Though, difficulty confronted in the removal of the soluble leached salts from the scaffold limit the thickness of scaffold to 0.5 to 2 mm.

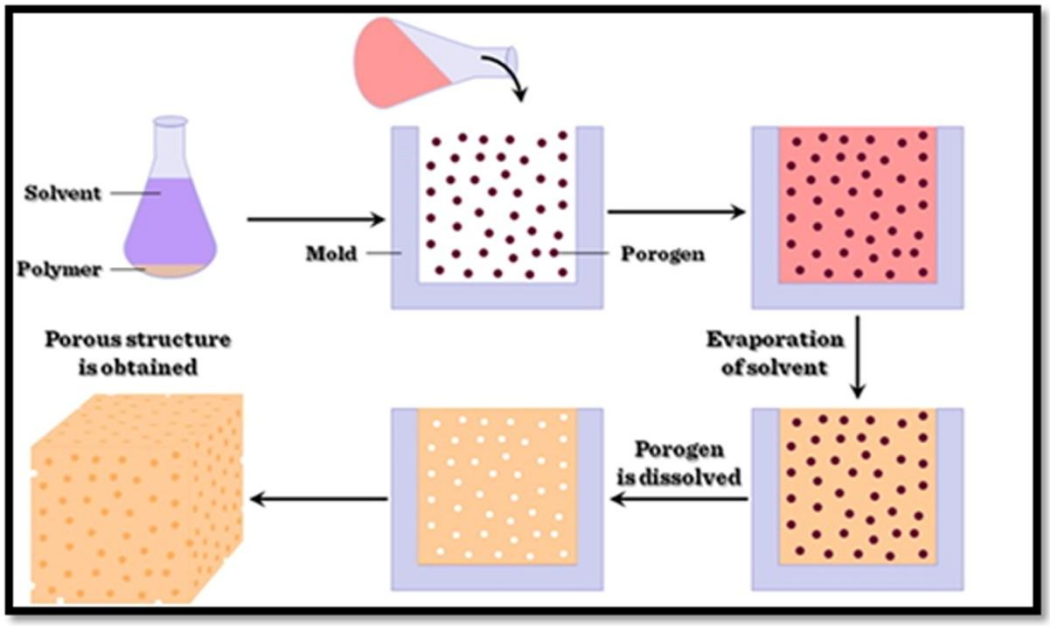

Fig 4. Schematic diagram of Solvent casting-particulate leaching scaffold fabrication technique.

Porous polyurethane (PU) scaffolds formulated by this method. Pellets of PU were dissolved in the dimethyl formamide at room temperature having volume proportion of 50:50.Solution was pour into polypropylene .The solution was kept into polypropylene dish with required diameters. To this solution salt

particles were added after that mixed properly. Solution was vacuum dried or freeze dried to remove the solvent. Salt leached out by keeping the container in distilled water. Further freezing at -20^{0}C provided with porous scaffolds [67].

7.2 Electro spinning Technique

Electro spinning is also widely used method for preparation of non-woven scaffolds. The scaffold prepared consists of fibres with their diameter ranging from micrometre to several nanometres[68-69]. Presence of smaller diameter fibres gives larger surface area to volume ratio. It uses electric voltage for the scaffold preparation[41, 69]. The polymer solution is loaded into the capillary further subject to high electric voltage. When electric field supplied overthrown the surface tension of the polymeric solution than the jet of solution is thrown out. After that polymer solution evaporates in the air and the fibres of the polymer are collected on the collector kept at ground voltage[70]. The operation parameters or the properties of the solution can be changed by varying the porosity. The choice of getting an aligned scaffold can be achieved by connecting a rotating drum collector at the other end. Fig.5[71].,showing the Electro spinning fabrication technique.

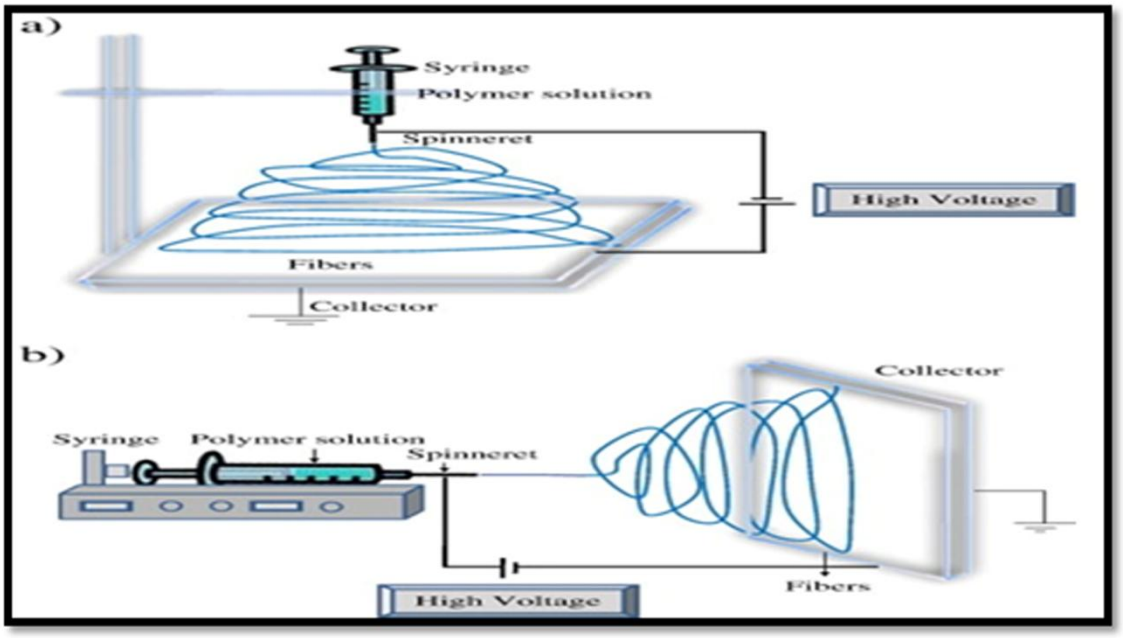

Fig.5. Schematic diagram of Electrospinning fabrication technique

Scaffold fabrication of Poly (e-caprolactone) has been used by electro spinning. PCL was dissolved in chloroform. Polymer solution flow through capillary was maintained at 0.1 ml/min. A fluid jet was ejected on applying high voltage of 13kV. As the fluid accelerated towards the ground collector the solvent got evaporated and polymeric fibre settled on the collector. Interwoven mesh with fibre diameter ranging from 20nm to 5 μm was obtained with average diameter of 400 nm having standard deviation of 200 nm[43, 72].Fig.6 showing the SEM image of scaffold prepared by Electro spinning technique[73].

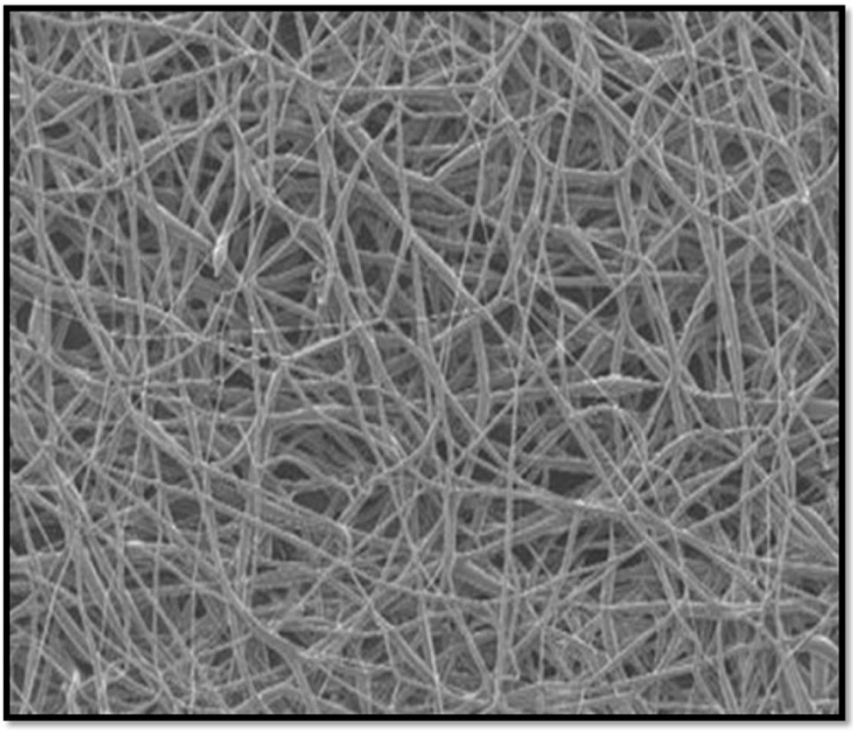

Fig 6. SEM image of scaffold prepared by Electrospinning technique

7.3 Gas-Foaming Process

This process is being used to fabricate the scaffold with high porosity without using any organic solvent [74-76]. The polymers used in this process have high amorphous character. The polymer disk is exposed to high pressure of CO_2 for several days in an isolated chamber such that polymer is saturated with the gas. The pressure in the chamber is restored back to the atmospheric pressure. This leaves the polymer with the spores formed from the purging of the gas forming sponge like structure[77-78]. In place of carbon dioxide nitrogen gas can also be used. The scaffolds prepared have only 10-30% of pore interconnectivity. The process gives the best results when used in conjugation to particulate leaching technique [79-80].Fig.7 showing the gas-foaming process[81].

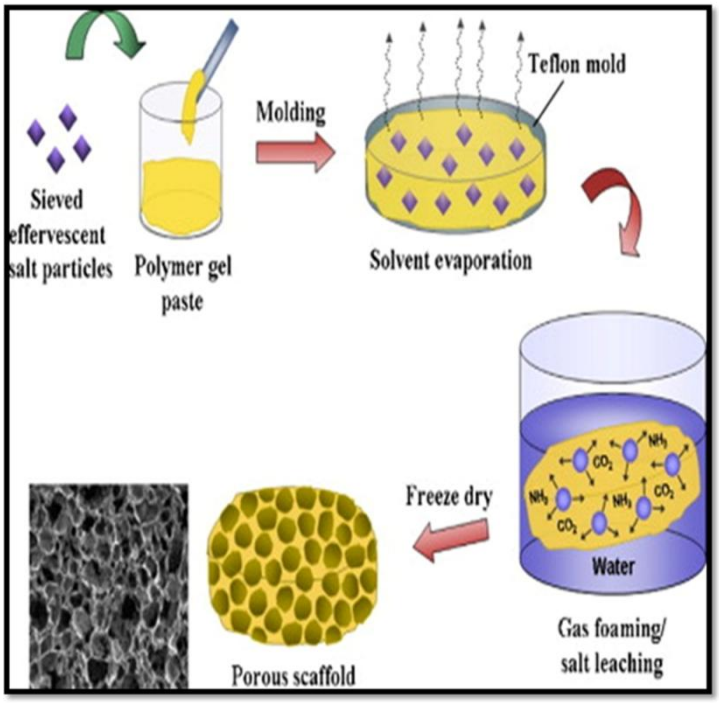

Fig.7.Schematic diagram of Gas-Foaming Scaffold fabrication Technique

PCL scaffold was also fabricated using gas-foaming process. PCL was dissolved in chloroform and was precipitated to gel paste by adding excess of ethanol (non-solvent) to the solution. The gel-paste was homogenously mixed with Ammonia carbonate particulates. The mixture of gel-paste and salt particles was kept in Teflon mould along with ethanol[82-84]. Semi-solid mass being left over when ethanol evaporated at room temperature. In gas-foaming and particulate leaching process left mass was used. Completion of effervescence provided with the scaffold which was further freeze dried for 2 days[85-86].Fig. 8 showing SEM image of scaffold prepared by Gas-Foaming Process[76].

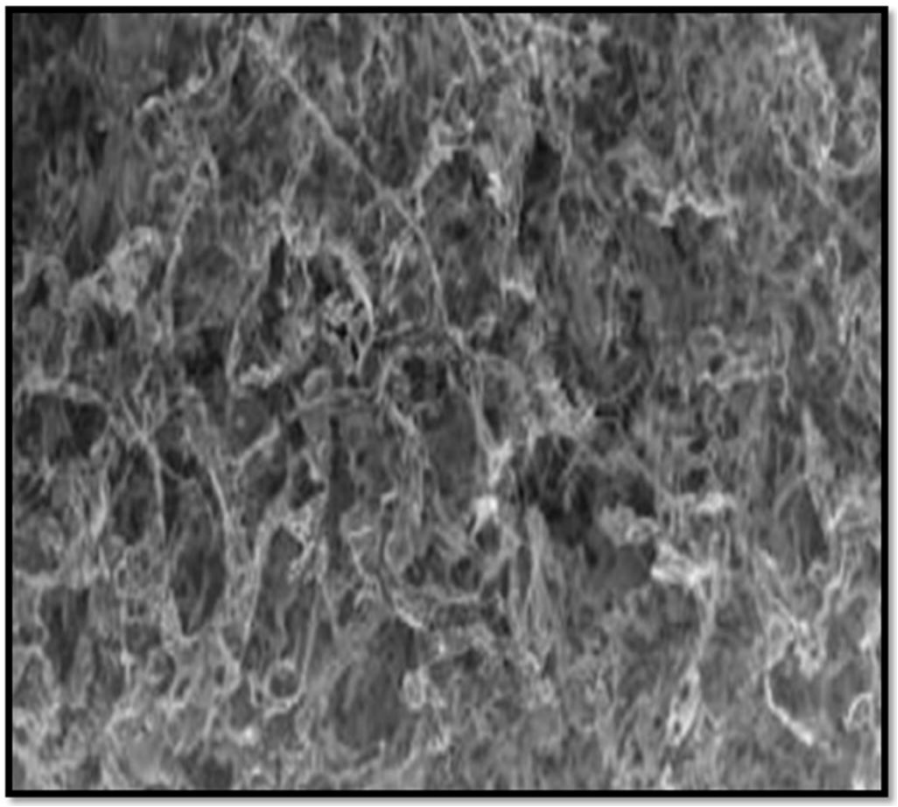

Fig. 8.SEM image of scaffold prepared by Gas-Foaming Process.

7.4 Emulsion Freeze Drying

Emulsion freeze drying method involves the polymer which is dissolved into its solvent (mostly organic solvent). To this solution, water is added and the resulting solution is mixed properly to form emulsions. The emulsion is poured quickly into the mould of desired shape before the separation of the two phases and quickly frozen[87]. The solvent and the water are removed from the emulsion through freeze drying leaving behind the porous scaffolds[88-89]. More than 90% of porosity can be expected from this technique with the pore size ranging from 20 to 200 micrometre[90]. However, the pore structures formed are closed[91].Chitosan/ Poly(l-lactide) composite scaffold was prepared by this process. Chitosan (CS) solution was prepared by dissolving in 2% (v/v) aqueous acetic acid solution to the concentration of 20g/l and PLLA concentration in chloroform was maintained at 40g/l. Tween-80 was added to CS solution (3mg/l). Varying ratios of PLLA were added drop by drop to CS solution supported with vigorous stirring[29, 92]. Centrifugation at 10,000 rpm for 3 minutes resulted in emulsion formation. This emulsion was poured into polysterene moulds which were subsequently frozen by liquid nitrogen. The solvent was evaporated in a lyophilizer. The product was washed with excess of ethanol to remove excess of Tween-80 and was re-lyophilized to obtain 3D scaffold. With the varying ratio of PLLA from 25% to 75% porosity ranged from 85% to 90% with the pore size ranging from 20-200μm. Fig.9 SEM image of scaffold prepared by emulsion-freeze drying process.

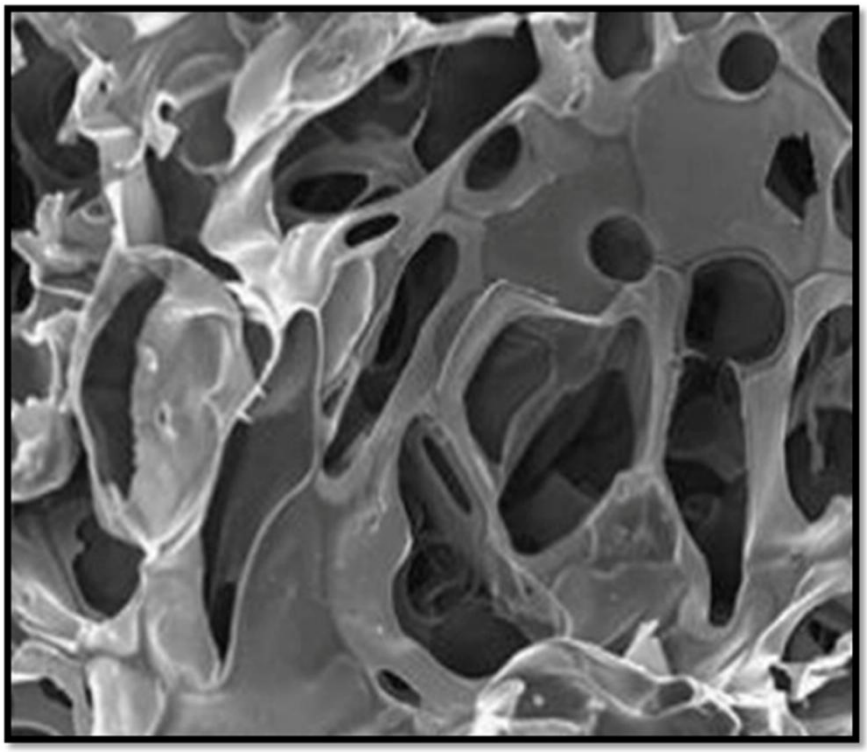

Fig.9.SEM image of scaffold prepared by emulsion-freeze drying process

7.5 Rapid-Prototyping Technique

Rapid-Prototyping technique is an advanced technique which takes into use the modern technology for 3D scaffold fabrication [78, 93-94]. Computer Aided Design (CAD) software used to design the scaffold[95]. Generally scaffolds designed through fused deposition modelling & ink-jet printing polymer. Then the Polymer is melted and comes out through the nozzle. The nozzle moves in the specific direction depending upon the algorithm. Consequently the scaffold is shaped layer by layer. This technology gives the independence of incorporation of biological agents in the scaffold[78, 96]. Though, provided with several benefits, the porosity of the scaffold is low and significant improvement is required in its mechanical properties as well[93].Fig.10 showing the rapid-prototype techniques[97-98].

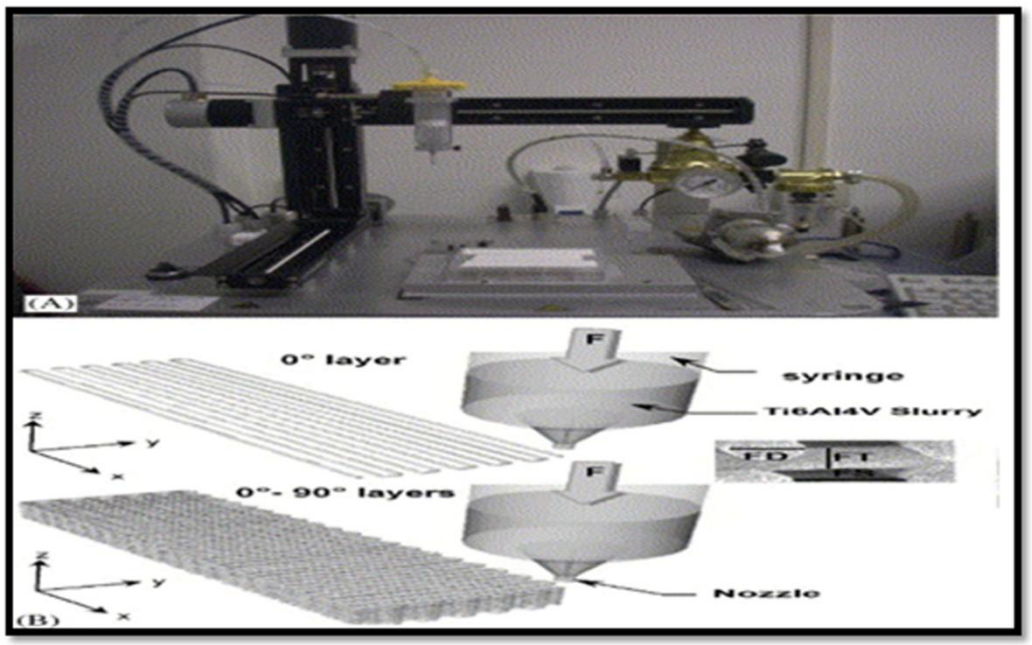

Fig.10.Schematic diagram of Rapid-Prototyping Technique

Rapid prototyping technique involves in different type of technique like 3D printing, fused deposition modelling (FDM), selective laser sintering etc. Scientist used FDM to fabricate PCL scaffolds having honeycomb structure with pore size ranging from 160-770μm[99]. And Some Scientist used 3D printing along with particulate leaching for preparation of scaffolds of Poly lactic-co-glycolic acid mixed through salt particles.Fig.11 showing the SEM image of scaffold prepared by rapid prototype technique[100].

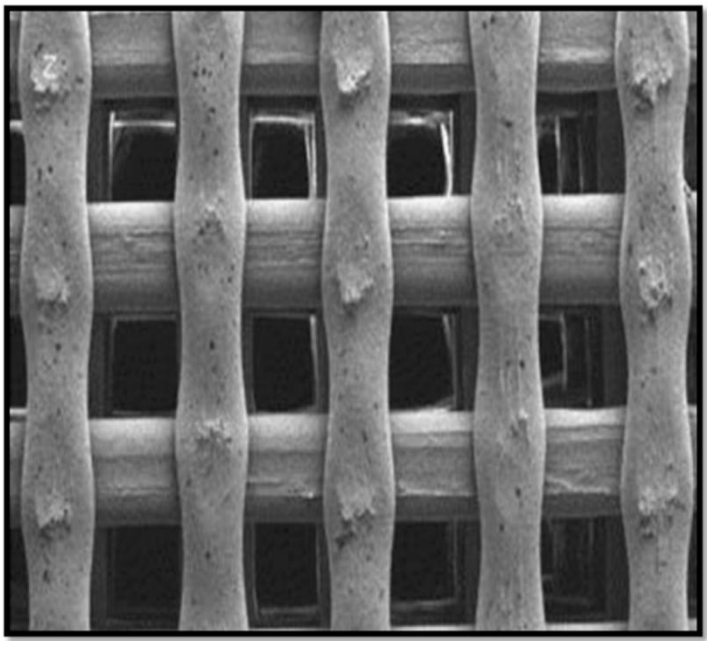

Fig.11.showing the SEM image of scaffold prepared by rapid prototype technique

7.6 Thermally induced Phase Separation (TIPS)

In this method first of all polymer is completely dissolved in a particular solvent, having low melting point such that it gets easily sublimed. For the phase separation Small amount of water is added into the solution. Polymer-rich phase and polymer-poor phase is formed. Solution is frozen using liquid nitrogen. Subsequently, the solvent is sublimed using freeze-drying[101]. This leaves the porous scaffold. The change in polymer concentration, solvent selected, degradation rate, temperature varies the pore morphology & mechanical [102]. The scaffold prepared has high porosity (approx. 93%) with the controlled macro and microstructure of the scaffolds[90].

The nano-fibrous structure of PLLA was prepared by TIPS. Liquid-liquid phase separation method was taken into use by to develop a 3D scaffold with fibre diameter varying from 50 to 500 nm. The resulting fibre structure was result of five sequential steps starting from solvent contraction, polymer dissolution, phase separation gelation & freezing to the final step freeze-drying under vaccum [103-104].Fig.12 showing SEM image of scaffold prepared by Thermally induced phase separation [105].

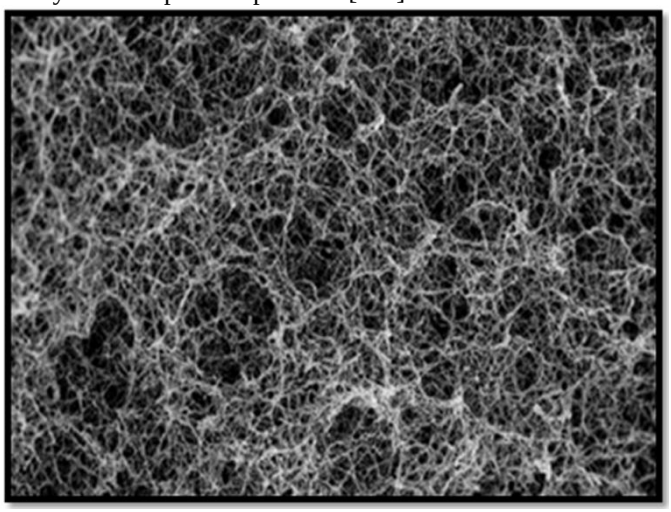

Fig.12. Showing SEM image of scaffold prepared by thermally induced phase separation.

VIII. Issues in Scaffold Designs

Although several studies (In-vitro) has been done extensively to advocate the use of several scaffolds for bone tissue engineering.

For the reliable human application there are various issues addressed.

[1] Carrying and release of growth factors/ protein in suitable concentrations by the scaffolds[106].
[2] Most of the studies in biomaterial has been performed using mainly animal cells[107].
[3] Clinical application of novel scaffolds and its handling and translation to patients required rigorous validations [108-110].
[4] Several issues of regulatory approval and clearness of validation of quality, safety and efficacy [111-113].
[5] Variations in sizes and doses of scaffolds use[114].

IX. Future Of Bone Tissue Engineering

Although there is tremendous advancement in the field of bone tissue engineering but still there is need to develop batter scaffolds having more strength along with timed bioresorption. Fabrication of composite scaffolds addresses the issue to some extent. However, polymeric materials degrade faster than ceramic material causing uneven degradation which can cause osteolysis. Thus, we need to have composites where polymeric and ceramic parts have comparable degradation rate. Amorphous calcium phosphates can serve as an alternative mitigating this problem. It degrades faster as compared to its crystalline counterparts and also helps in faster apatite deposition. Polymeric scaffolds with slower degradation rate can be tailored.

Advancement can be made in coupling actions like angiogenesis and osteogenesis while regeneration of tissues. For serving the purpose, sequential and substantial delivery of the suitable and specific biomolecules is required.

Optimization of scaffold pore size contributes in maintaining the rate of delivery of biomolecules. Development of scaffolds with optimum porosity will prevent the burst release of biomolecules. Thus, their delivery will depend on the resorption of the scaffold in an anticipated, time-dependent manner.

X. Conclusion

The past decade extensive research helped in innovations of several materials, processing techniques and better evaluation methods. Development of composites has open new frontiers of research. Controlled porosity, better interconnectivity between the pores, improved mechanical strength and mechanical integrity are the results of the modern technology. Though, there has been significant development in the field of tissue engineering but we far lag behind from mimicking the natural bone. The aim of the paper has been to address various developments acknowledged in bone tissue engineering field. For tissue regeneration fabrication of proper bioactive scaffolds are required. Biology of the bone has been discussed to understand its proper structure and giving better understanding of what we want to attain. The plethora of biomaterials is available for the fabrication of scaffold but the best results have been obtained by blending two or more materials. There is enough scope for the discovery of biomaterials as none of the discovered biomaterials or designed composites have been able to fulfil all the desired needs of the scaffold. Simultaneous improvement is required in the scaffold fabrication technique. The introduction of radio-typing fabrication technique has produced by far the best results for 3D scaffold fabrication. However, Electro spinning and TIPS techniques are the most common techniques which are used for fabricating scaffold and the improvement in these techniques will produce better results on a much larger scale. The introduction of mathematical modelling to tissue engineering has proved its importance by showing various effects on the parameters in the tissue regeneration and saving labour and time. The resonance in all these factors will definitely provide the result which is desired.

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