



### RESEARCH ARTICLE

#### TISSUE SCAFFOLDING: AN UPCOMING TISSUE FABRICATION TECHNIQUE

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#### Abstract

Tissue engineering (TE) is a multidisciplinary research that combines clinical medicine, biology, biochemistry, and materials science. TE involves the creation of scaffolds that can replicate the characteristics of the extracellular matrix (ECM) of tissues to ensure complete and whole regeneration. During the past decade, a wide range of materials have been chosen for bone tissue engineering and combined with these production techniques. Several production methods, including electro-spinning, freeze-drying, bio-printing, and de-cellularization, have been used to create scaffolds for tissue engineering applications. In this paper, we review the capabilities and operating methods of the most promising techniques for the future.

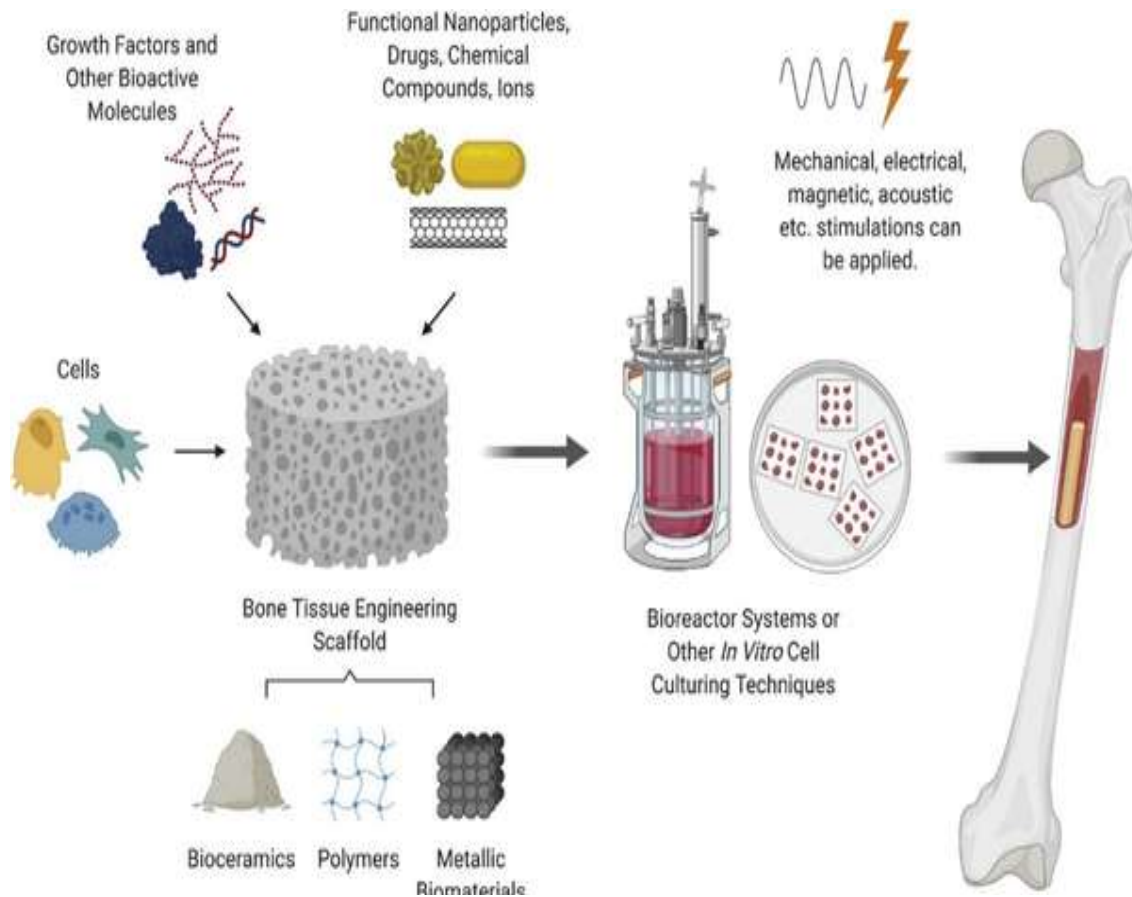
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#### Introduction:-

The term "tissue engineering" (TE) was first used in 1988 at the UCLA Symposia on Molecular and Cellular Biology [1]. A thorough definition of TE was provided there, stating that it is the application of life sciences and engineering to develop a fundamental understanding of the structural and functional relationships of both pathologic and natural mammalian tissues, as well as the development of bio-substitutes that can be used to repair, preserve, or enhance tissues that have been damaged or lost due to various disease conditions [2]. The goal of tissue engineering is to regenerate and repair sick or damaged tissue, as well as to create new bio-functional tissues [3]. The creation of bio-mimetic scaffolds is a field of growing attention as a means of overcoming such restrictions [4]. Compared to more conventional bone grafting techniques like auto-grafts or allo-grafts, bone tissue engineering (BTE) methodologies (**Figure 1**) appear promising for replacing missing or damaged bone tissue [5]. A new tissue with the appropriate form and characteristics is created throughout the process of cell regeneration, with the scaffold serving as a temporary aid. Gradually, the scaffold biodegrades, either during or after the healing process [6]. Because of the scaffold's biodegradability, there is no longer a need to remove the material afterwards, which minimizes the possibility of any negative consequences from residual foreign bodies. Therefore, in order to enable cell dispersion and the development of three-dimensional tissues, the scaffold that is used must satisfy certain chemical, mechanical, and physical criteria [7].

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**Figure 1:-** Bone tissue engineering strategies.

Tissue engineering (TE) is advancing several scaffold construction processes, including electro-spinning, freeze-drying, bio-printing, and decellularization [8]. A wide range of materials have been chosen for BTE and combined with these production techniques. The selection process often takes into account the functional and biological requirements of bone tissue, which is a composite material, made up of naturally occurring and artificially created elements arranged in a highly hierarchical manner. The concept of bioactive materials has recently replaced that of bio-inert materials, with an emphasis on naturally occurring biopolymers because of their easy chemical modification and innate capacity to interact with developing cells [9]. Biomaterials originating from extracellular matrix de-cellularization have been utilized in BTE scaffolding recently. De-cellularized bone extracellular matrix (ECM) improves cell survival and proliferation for tissue repair and regeneration by preserving the natural matrix structure, growth factors, and cytokines [10].

### Significance

In the multidisciplinary discipline of tissue engineering (TE), engineering concepts and the living sciences are combined to create bio-substitutes that enhance, preserve, or repair tissue or organ function. In order to obtain therapeutic applications, TE is a multidisciplinary research that combines clinical medicine, biology, biochemistry, and materials science [11]. In an effort to avoid the problems that arise with using traditional organ donation methods, TE, or regenerative medicine, has emerged as a potentially effective method of healing damaged tissues. Because of the growing need for organ transplants in clinical practise, TE has emerged as a substitute [12].

Several cell types—expanded or non-expanded—extracted from a patient or donor are incorporated into the scaffold during cell regeneration. The main source of non-expanded cells is bone marrow aspirate-derived platelet-rich plasma cells, whereas the source of extended cells is adult stem cells, such as those found in bone marrow, fatty tissue, teeth, blood cells, embryonic stem cells, induced pluripotent stem cells (iPS cells), peripheral blood-derived stem cells, and genetically engineered cells [12]. Since these cells may be produced in vocal or chemical substances in a variety of ways, a scaffold with certain characteristics is essential. Hydrogels, for instance, have been used to

promote the regeneration of spinal cord tissue because of their ability to conform to the mechanical properties of viscoelastic, spongy, soft neural tissue. Furthermore, the death of façade tissues is caused by a severe mismatch between the tissues and the implant [13].

### Scaffold Features for Tissue Engineering

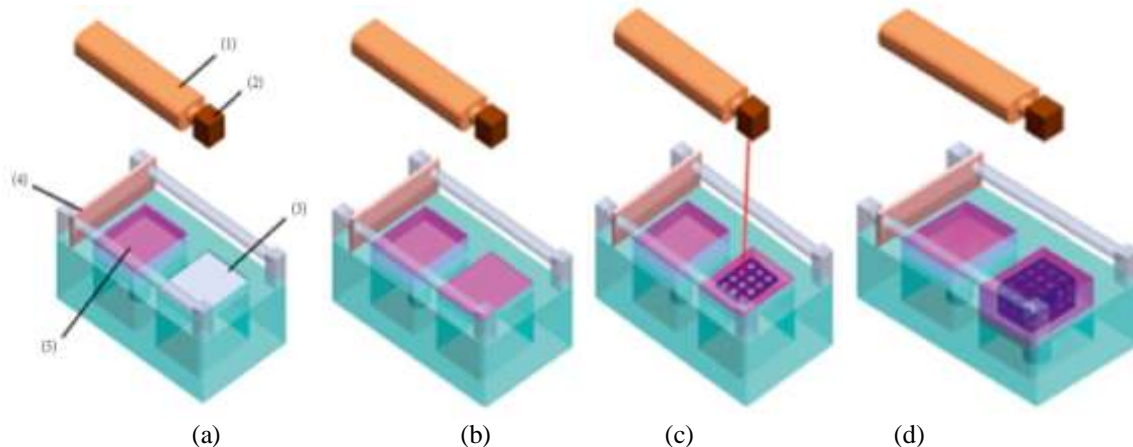
Despite the fact that several researches have documented various discoveries in TE, the medicinal application of these results has led to a notable rise in the commercialization of these recently identified functions. Therefore, in order to increase the level of acceptance for clinical applications of these technologies, it is important to include certain biological, clinical, and mechanical components that are not just theoretical but may also be useful in real-world applications. The minimal criteria for a suitable scaffold should be able to heal damaged body tissues; moreover, the materials used should naturally disintegrate during or after the healing process, allowing for cell development, vascularization, proliferation, and host integration. A scaffold, however, has unique properties relating to its chemical composition, structure, and biological aspect [12].

### Biological Characteristics of Scaffolds

The nontoxic and biocompatible characteristics of scaffolds constitute some of their biological features. For the cells grown on scaffolds to generate a new matrix, they need to be allowed to divide and distinguish without interruption. Considering the fact it can replicate the characteristics of the extracellular matrix (ECM) of tissues to ensure complete and whole regeneration, a scaffold is considered excellent for use in tissue engineering applications. As previously stated, the supporting cell's ability to perform its duty is dependent on a number of factors, including the scaffolding structure, the underlying material, the surface characteristics, and the cell line used [2]. Since a scaffold's biological characteristics influence how it interacts with tissues and organs, they provide a serious modifying challenge. Bioactive scaffolds have been used to promote proper cellular interaction, migration or differentiation, tissue information, and incorporation into the host, as well as to prevent undesirable processes like scarring, because biological material has a limited capacity to cross-talk with the environment [13].

### Structural Characteristics

Biological tissue is a very complicated three-dimensional structure with complicated mechanical properties related to mass transport properties. In order to overcome this structural complexity and function, tissue engineering (TE) must use biological scaffolds that supply the cells, proteins, and genes needed for tissue rebuilding. There is no doubt that biological materials and structures are unable to replicate complex tissue environments, which include a multitude of cell types interacting with different cytokines to generate extracellular matrices within cells with hierarchical properties demonstrating mechanical function with high nonlinearity and two-phase behavior [2]. One of the main obstacles in the creation of vascularized engineering scaffolds is the absence of vascular insufficiency, which results in the ineffective integration of osseo, indicating that material selection influences the scaffold's final physical characteristics [14]. In order to encourage cell development, it is frequently desirable for the scaffold's porosity to increase its mechanical characteristics. A scaffold with the right pore size also encourages high mass transfer of oxygen across the scaffold and enhances cell motility and water absorption [15].



**Figure 2:-** 3D printer assembly and fabrication of the scaffold: (a) servo motor driving: (1) CO2 laser, (2) laser scanner, (3) working platform, (4) scraper, and (5) feeder; (b) paving material; (c) start of 3D printing; (d) end of 3D printing.

### Advantages and Disadvantages of Scaffold Fabrication Techniques

Technique	Advantages	Disadvantages	References
Freeze-drying	<ol style="list-style-type: none"> <li>1. Possess higher porosities</li> <li>2. Adjustable scaffolds structure and pore size</li> <li>3. Greater interconnectivity of the porous structure with the extracellular matrix</li> </ol>	<ol style="list-style-type: none"> <li>1. Process is high energy and time consuming</li> <li>2. Use of cytotoxic solvents</li> <li>3. Leads to shrinkage of the tissue</li> </ol>	[16]
Gas foaming	<ol style="list-style-type: none"> <li>1. Due to the use of organic solvents, they are non-flammable</li> <li>2. Carbon dioxide is used as porogen gas</li> </ol>	<ol style="list-style-type: none"> <li>1. Process cannot be used as a hydrophilic material</li> <li>2. Carbon dioxide used is of low solubility</li> </ol>	[17]
Electrospinning	<ol style="list-style-type: none"> <li>1. Can be used in large scale productions</li> <li>2. Abilities to generate homogeneous mixtures with nanoscalefibres</li> <li>3. Develop polymers of high tensile strengths</li> </ol>	<ol style="list-style-type: none"> <li>1. Process is limited in producing 3D scaffolds due to poor control over pore structural size and shape</li> <li>2. Due to the use of the wide range of biomaterials, the solvents used sometimes might be cytotoxic</li> </ol>	[18,19,20]
Stereolithography (SLA)	<ol style="list-style-type: none"> <li>1. Enables to overcome the challenges related to wastage in subtractive abrication methods</li> <li>2. High resolution</li> <li>3. Uniformity in pores interconnectivity</li> </ol>	Has limitations in the process of photopolymerization	[21,22]
Bioprinting	<ol style="list-style-type: none"> <li>1. Low costs</li> <li>2. Higher accuracy and greater shape complexity</li> </ol>	Depends on existence of cells	

### Chemical Composition of Scaffolds/Materials used for Scaffolds

In general, polymers, bioceramics, and hybrid materials—whether natural or man-made—make up the majority of scaffolds. Biocompatibility, composition, and breakdown products of such matrices are of concern, depending on where the components used to fabricate the scaffold came from. Some materials have been observed to not support cell development within scaffolds, despite a wide range of materials having been investigated for use as scaffolds [2].

There are two kinds of polymers: synthetic and natural. Natural polymers with favourable biological compatibility, minimal immunogenicity, and osteoconductivity include collagen, hyaluronic acid, fibrin, and chitosan. Nevertheless, they have limited mechanical stability and high rates of free degradation [12]. Polyphosphazene, polypropylene fumarate (PPF), polyanhydride, polycaprolactone (PCL), polyether ether ketone (PEEK), polylactic acid (PLA), and poly (glycolic acid) (PGA) are examples of synthetic polymers with regulated rates of breakdown. Their better cell attachment (negatively-charged chemical groups) and capacity to transport soluble chemicals are other advantages. They can also be manufactured into various geometries. Moreover, artificial polymers have a longer shelf life and may be made in big quantities for a reasonable price [12].

According to certain *in vitro* research, the substance itself, as opposed to natural tissue matrices, could destroy the outcomes of *ex vivo* tissue creation. Degradation products' side effects, unanticipated degradation times, and material immunogenicity can all have a significant impact on poor regeneration in an *in vivo* setting. Based on these factors, the most promising matrices in TE are those that are most similar to the natural extracellular matrix. The goal of the newly created methods for extracorporeal tissue engineering is to avoid using non-biodegradable scaffolds that reabsorb at a pace that differs from the regeneration of skeletal tissue. So, by doing away with scaffolds, new techniques have been devised to solve these issues.

Types of biomaterials	Examples	Advantages	Disadvantages	Application	Fabrication technique
Ceramics	Hydroxyapatite, $\beta$ -TCP, $\alpha$ -TCP, calcium silicate, calcium sulphate	Non-toxic, Biocompatible Anti-inflammatory	Slow degradation rate, low mechanical strength, brittle	Bone and dental tissues applications	Inkjet printing, salt leaching, SLS
Metals and alloys	Iron magnesium Alloys, stainless Steel, titanium	Non-toxic, light-Weight, biocompatible	Poor osteo-integration With the nearby bone, Non-biodegradable	Bone and dental application	SLS, stereo Lithography, Vacuum foaming
Natural polymers	CS, alginate, dextran, collagen, gelatin, cellulose, keratin	Biodegradable, non-toxic, biocompatible, anti-allergenic	Rapid degradation, complex structure, less mechanical strength, water-soluble	Drug delivery, Bone and tissue Application, gene therapy	Electro-spinning, Inject printing, Solvent casting
Synthetic polymers	Polyesters, polycarbonates, polyhydroxy acids, polylactones, polyanhydride	Biodegradable, good Mechanical strength, Biocompatible, non-Toxic, low melting point	Toxic, hydrophobicity, high production cost, slow degradation	Bone, tissues, Cartilage and Dental application	Freeze drying, Electro spinning, Gas foaming, SLS
Composites	Different polymers, Ceramics and metals are blended. Calcium silicate, Calcium sulphate	Good mechanical Strength, non-toxic, Biocompatible, light weight	Slow degradation, Less cell-cell interaction, compact In nature.	Bone, tissues, Cartilage and Dental application	Freeze-drying, Stereo lithography

### Scaffolding Fabrication Methods

The ideal manufacturing method would be able to create scaffolds that are repeatable and have a regulated hierarchical porous structure. This is because the shape of the pores in the scaffold has a significant impact on the biological and mechanical responses of the bone tissue [23]. These days, the most popular fabrication techniques for the best scaffold creation are those that make it easier to incorporate cells and growth factors [24]. Modern processing methods undoubtedly help create highly customizable scaffold geometries for implants made specifically for each patient, but they are also necessary for specific therapeutic requirements.

The functionality and operating methods of most promising techniques for the future are discussed as follows-

#### Freeze Drying

The process of freeze drying, also known as lyophilization, involves drying polymeric solutions. The procedure can be divided into three parts: (i) preparation of the solution; (ii) casting or moulding of the solution; and (iii) low-pressure freezing and drying. In the course of the third stage, sublimation and desorption are used to extract the ice and the unfrozen water, respectively. It is possible to use freeze drying the ability to create scaffolds with roughly 90% porosity and pores with diameters between 20 and 200  $\mu\text{m}$ . Pore size regulation according to temperature, polymer content, and freeze rate [25]. Within A high, strong vacuum is needed to create a scaffold with high porosity and connectivity.

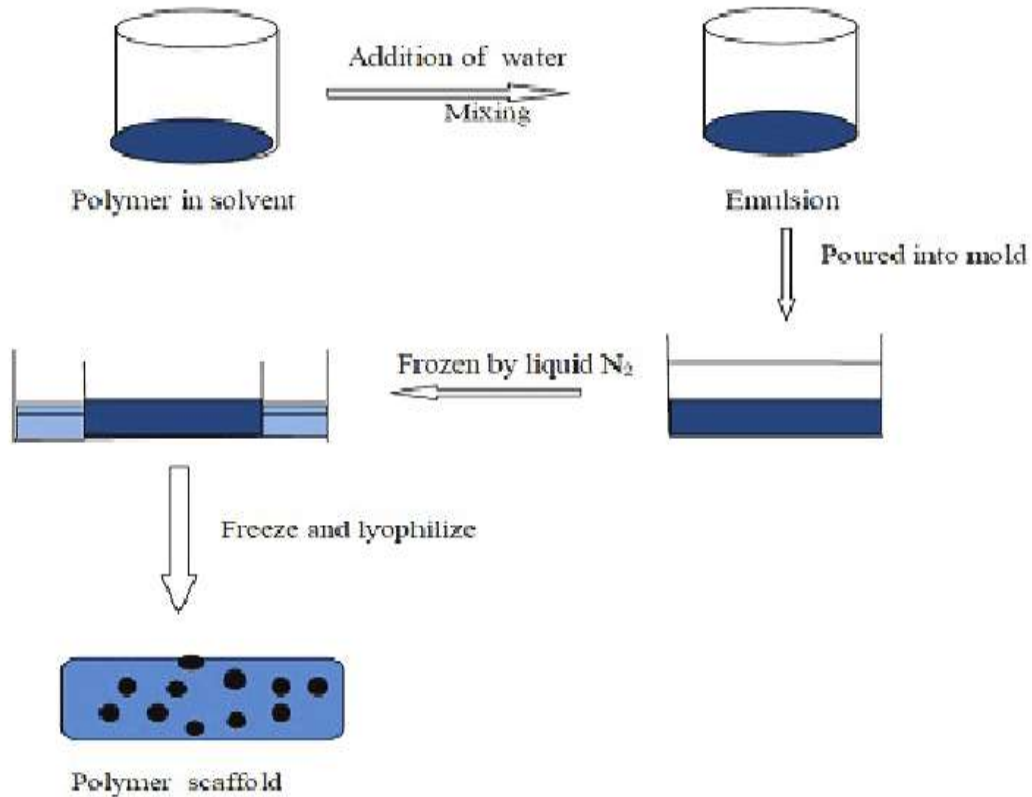


Figure 3:- Freeze Drying.

**Gas Foaming**

The gas foaming technique has developed as a means of handling organic cytotoxic liquids and high temperatures. This method makes advantage of comparatively inert gas foaming agents, such nitrogen or carbon dioxide to apply pressure to a biologically degradable polymer model using once they are saturated or gas-filled, add water or fluoro-form bubbles. This method typically yields formations similar to a sponge having pores between 30 and 700 μm in size and porosity up to 85% [26]. The technique's downside is that the resultant product may occasionally have a solid polymeric skin or a closed pore structure.

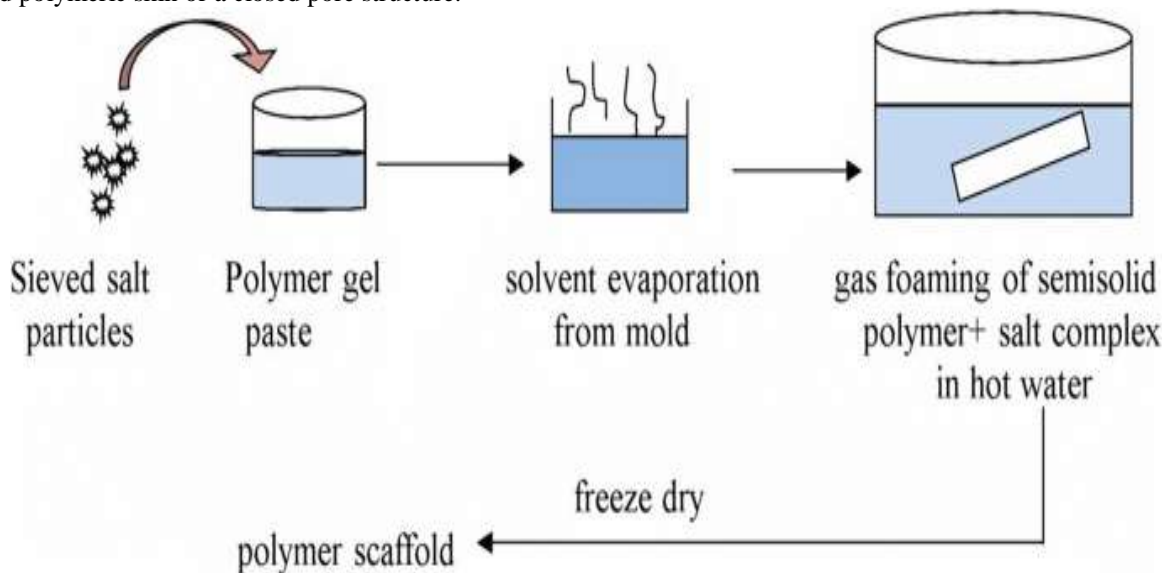


Figure 4:- Gas Foaming.

### Electro-spinning

One method for employing electricity to create fibres from a solution is called electro-spinning. This method is essential for creating nano-fibrous scaffolds. Electro-spinning is an extremely intricate method whereby Liquid charging at a high voltage results in the interaction between the electrostatic repulsion and surface tension that causes the spinneret's droplets to expand and erupt. A typical electro-spinning system is comprised of four primary parts: a syringe pump, steel needle, and high-voltage spinner energy source as well as a grounded collector.

When the electric field strength is greater than the droplet's surface tension, a liquid jet is created. This jet is then continuously extended and whipped by electrostatic repulsion until it lands on the grounded collector. During this process, the solvent evaporates and the jet solidifies to form a nonwoven fibrous membrane [26][27].

Although electrospinning is a quick and easy way to create nanofibrous scaffolds, it is still difficult to create scaffolds with complex geometries like uniform pore distribution, which limits its use in biomedicine [28].

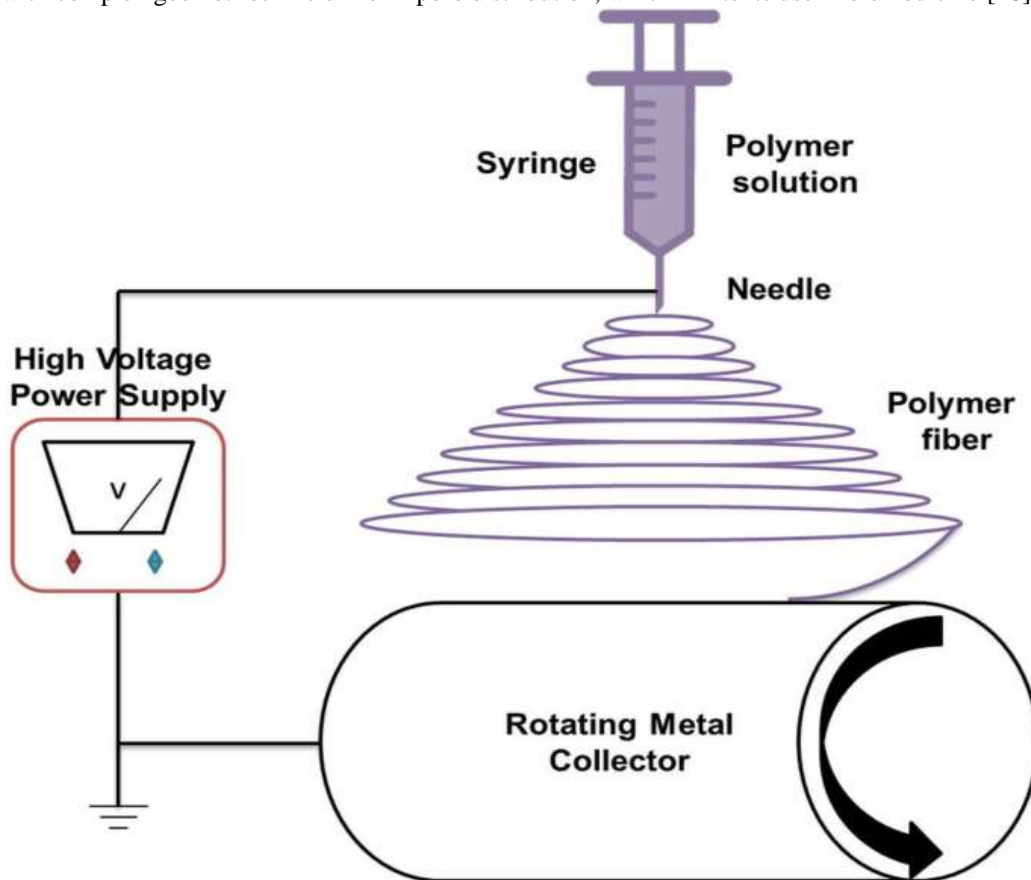


Figure 5:- Electrospinning.

### Rapid Prototyping

A group of manufacturing techniques called solid free-form fabrication (SFF) or rapid prototyping (RP) technologies can create direct forms from computer-aided design (CAD) models of an object without the requirement for specialised tools or experience.

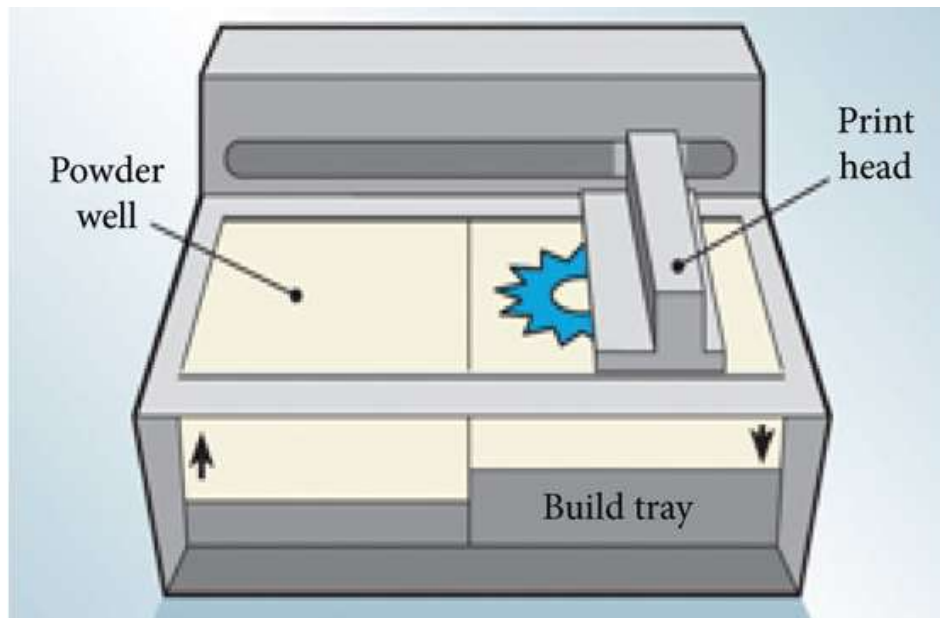
Some of the issues with conventional production methods can be addressed by the construction of designs with precise spatial control over polymer structure thanks to the RP scaffold fabrication technology [29]. The primary one advantage of these methods is that they facilitate the manufacture of With specialised scaffolds made for each patient that are appropriate for organs and tissues. The basic RP techniques include 3D printing (3DP), fused deposition modeling (FDM), selective laser sintering (SLS), and stereo-lithography [26][27].

### Three-Dimensional Printing (3DP)

The 3DP technology involves adding powdered material in stages and selectively fusing the powder by "inkjet," allowing for the creation of tools and functioning prototype features straight from computer models.

The 3D printing procedure can be used either directly or indirectly to manufacture a mould or the real part [30]. A novel fabrication technique called 3DP allows for fine control of scaffold structure down to the micron level.

Even so, its performance depends on the capacity to adhere precisely to the natural tissue's structure and the scaffold's mechanical attributes, the 3DP technique's scaffolds have a limited ability to emulate of the tissue's nanoscale extracellular matrix properties. What they want to do is take over. [31]



**Figure 6:-** Three-Dimensional Printing (3DP).

### Future Perspectives

When biomaterials and computer technologies are combined and applied to tissue engineering, new avenues for overcoming the limitations of current technology are opened up. Make scaffolding. The numerous uses of computational Software-controlled devices and software have transformed the regulated production of intricate artificial scaffolds made of tissue. The range of 3D tissue printing technology has expanded to include organ printing in the past because of the progress in printing technology. 3D printing of organs is currently developing to address the primary obstacles in tissue engineering, emulating the intricacy of biological tissues, and giving rise to vascularization. One day, endoscopic procedures may be used to deposit *in vitro* cultivated cells and tissues at the patient's injury site in order to print new tissues directly there. The fast expansion of this exciting field of study is anticipated to revolutionise traditional tissue engineering methodologies and significantly advance tissue engineering's medicinal potential.

### Conclusion:-

Tissue scaffolding is a vast area with a wide range of applications. Because it depends on so many different variables, the process of fabricating scaffolds is extremely intricate, meticulous, and delicate. Surveys of the literature support the conclusion that RP techniques are an advanced fabrication method for the creation of scaffolds for tissue engineering applications using computer-aided software and instruments. Tissue scaffolding relies heavily on the ability of the 3D scaffold to foster excellent cellular contact and an inherent cellular microenvironment. However, a number of obstacles, including porosity, pore size, cost, mechanical qualities, appropriate biomaterial selection, and interconnectivity within the pores, have been addressed with recent advancements in this field of study. Improving tissue regeneration, gene therapy, medication administration, bone repair, and other tissue-related engineering processes can result from tackling these types of issues during the creation of 3D scaffolds.

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