Understanding Basics Of 3-D Printed Bone Tissue Engineering Scaffolds



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Introduction:

Congenital abnormalities, trauma (such as fractures and non-unions), bone diseases (such as osteoporosis, osteosarcoma, and osteonecrosis), or surgery (such as tumor removal, spinal fusion) can all result in bone defects, which can have clinical as well as socioeconomic implications (1). Bone can self-heal; however, because bone repair-related cells lack a growth and differentiation platform, it cannot regenerate in some circumstances, such as large defects. Nowadays, autologous bone grafting, allogenic bone grafting, and synthetic bone graft substitutes (i.e., bone tissue engineering scaffolds, BTES) are available as therapies for critical-sized bone deficiencies. Nevertheless, secondary damage, inadequate size matching, immunological response, and other dangers from auto- and allo-transplantation have restricted their clinical utility. BTES with excellent biocompatibility and robust osteogenesis ability have been extensively explored to address the aforementioned issues with clinical treatment processes, particularly the polymer-based composite bone scaffolds made by 3D printing in accordance with the clinical needs of patients (2).

In order to elicit particular biological responses from the local environment, scaffolds are used in a damaged tissue niche and bone tissue engineering (BTE) principles are utilized in order to promote the healing process. Within bone tissue scaffolds, a 3D porous matrix consisting of cells, biocompatible materials (as substrates) and essential biological and biochemical cues are present. Biomaterials are employed in BTE as transient substrates that offer the perfect milieu for cells to attach to, multiply, differentiate, and maybe produce new bone tissue where an injury has occurred. The 3D porous matrix also contains cells and crucial biological and biochemical cues in addition to substrates (3). Inflammation and other natural bodily reactions brought on by an injury trigger the recruitment of cells to a spot, which is the first step in the process of bone repair. Next, where there is a void, cells will proliferate, create their own extracellular matrix (ECM), differentiate, and finally deposit the building blocks of fresh, sound bone formation. The cells of most BTE strategies are Mesenchymal stromal cells

(MSCs), bone marrow-derived stromal cells (BMSCs), and adipose-derived stromal cells often (ADSCs). The most common base materials for scaffolding are scaffold substrates made of either polymer, ceramics, metals, or, in most situations, some mixture of these materials. The presence of biophysical and biochemical stimuli in the local microenvironment is largely responsible for the creation of new bone tissue. Porosity and substrate stiffness, two biophysical factors, have made it possible for BTE scaffolds to better regenerate tissue after damage and other losses of natural bone, as well as to choose the lineage fate of MSCs in vitro. Faster regeneration of healthy bone tissue is feasible by including biological cues (such as growth factors and bioactive substances like nitric oxide) into BTE scaffolding. Recent research has demonstrated that electrical stimulation (ES) in combination with biophysical signals, including scaffold conductivity, can better support the development of healthy bone tissue without the need for extra growth hormones. Additionally, by using stimulus-responsive (i.e., piezoelectric) materials as scaffolds for BTE applications, cells can transform the stimuli generated by the scaffold into biochemical signals that cause changes to other cellular signalling events, potentially producing scaffolds that closely resemble natural bone tissue (3).

BTE scaffolds have the following functions: i) supplying temporary mechanical support to the affected area and filling in the space left by bone defects; (ii) encouraging the adhesion and growth of circulating precursor cells and enabling ECM deposition onto the scaffold surface (osteoconduction); (iii) inducing vessels and bone in-growth into the porous scaffold; (iv) enhancing osteogenic differentiation via molecular signalling and the formation of new bone tissue; (v) inducing cellular activity to facilitate osteointegration (the fusion of bone with natural tissue); and (vi) providing medications or bioactive substances to quicken the healing process (4).

BTES are frequently produced using 3D printing. In contrast to subtractive manufacturing techniques, three-dimensional (3D) printing, also known as additive manufacturing (AM) and fast prototyping, is a method of connecting materials to create objects with customized shapes and porosity from 3D model data. Due to its versatility, ease of use, precise control over the fabrication process, and ability to produce products with customized shape and structure and unique architecture and properties that are highly sought after for biomedical applications, 3D printing technology has attracted significant attention in the biomedical field (5). Engineering and biological sciences are combined throughout the 3D printing process to create scaffolds filled with cells and bioactive growth agents that may eventually replace conventional tissue grafts. By combining the use of computer-aided design (CAD), computer-aided manufacturing (CAM), computer numerical control (CNC), laser technology, and computed tomography (CT), 3D printing technology can turn digital signals into tangible objects. The steps involved in creating the bone scaffold are as follows: first, the repair site's three-dimensional data is captured using a CT scan or magnetic resonance imaging. Next, the three-dimensional model is "sliced" using CAD software to capture the data for each layer and imported into the 3D printing system. In order to prepare the bone scaffold, the device stacks the components layer by layer in accordance with the layered data. One of the best scaffolds for clinical use is BTES made using computer-aided design (CAD) modelling and 3D printing technology because it has the highest accuracy, repeatability, and spatial control over the scaffold's microstructure (2).

2.General requirements for bone scaffolds:

The biological and mechanical performance of fabricated bone scaffolds is typically dictated by the interplay of characteristics classified into three broad groups:
(1) inherent material characteristics, (2) surface characteristics, and (3) scaffold or architectural characteristics

2.1 Inherent material characteristics:

A substance's bulk characteristics, such as its stoichiometric content, crystal microstructure, and elastic modulus heavily influence its bioactivity. The bioactivity of a material has been proven to be impacted by ions released in small amounts from the material, which alter the pH of the immediate microenvironment and the biochemical reaction of the surrounding cells and tissues. The material's elastic modulus determines how resistant it is to local deformation at the cellular level, which has an impact on cell fate. It has been demonstrated, for instance, that mesenchymal stem cells (MSCs) can be guided towards various lineages by altering the elastic modulus of the matrix: soft matrices (E = 0.1–1 kPa) are neurogenic, stiffer matrices (E = 8–17 kPa) are myogenic, and stiffer matrices (E = 25–40 kPa) that mimic the collagen of osteoids are osteogenic.

2.2 Surface characteristics:

The topographical characteristics like roughness, nano/micro-porosity, waviness, hydrophilicity, and surface charge have an impact on the in-vivo biological integration of tissue scaffolds. These characteristics impact protein and cell adhesion and entrapment, which in turn affects the material's overall bioactivity. Together with roughness, nano/micro-porosity influences ion release and surface topology, which in turn controls cell adhesion, proliferation, and differentiation.

2.3 Architectural characteristics:

It has been demonstrated that the interior structure of scaffolds influences their bioactivity. Permeability, pore interconnectivity, pore size, pore shape, and scaffold stiffness are examples of architectural qualities. Pore connectivity and permeability have an impact on cell migration, nutrition flow, and waste elimination, which has an impact on the ability of the scaffolds to support tissue regeneration (6). Moreover, porosity promotes cell migration within the scaffold and expands the surface area accessible for cells to adhere to the scaffold and communicate with the neighbouring tissue. The microstructure of the scaffold must be properly planned during the BTES design stage using factors that are advantageous to cells and tissues, such as the scaffold's porosity, pore size, and interconnected pore structure, among others (2). Pore size and shape affect the amount of surface area accessible for the release of ions and degradation products as well as the amount of space that can be used to grow tissues. An essential factor is the size of each individual pore in the scaffold. Large pores (a few hundred microns) help angiogenesis, ECM aggregation, and tissue development while small pores (usually 100–150 micro in size) encourage cell adhesion, intracellular signal transmission, cell proliferation, and migration (2). Tiny pores are undesirable because they offer little room for the growth of new tissue, which restricts the development of blood vessels and vascular networks within the tissue. Yet, retaining the internal architecture while expanding the pore size reduces the effective surface that cells can bind to (6).

The scaffold's interconnected pore structure, in addition to the scaffold's porosity and pore size, has a significant impact on how well the scaffold functions. The effectiveness of four distinct pore shapes (circular holes, polygonal holes, randomly oriented holes) was tested. According to the findings, round and polygonal shaped scaffolds were more effective at promoting early osteogenesis, proliferation, and adhesion. Although the scaffolds had directional and random orientations, both of which demonstrated robust cell adherence, there was no early evidence of any appreciable cell proliferation. Scaffolds with random orientations may encourage osteogenesis and cell proliferation in later observation species (2). Another important property that should be comparable to the replacement tissue is scaffold stiffness, which relates to the material's resistance to macroscopic deformation. It is critical to distinguish between the scaffold's stiffness, which controls the degree of macroscopic deformation, and the material's elastic modulus, which influences cell destiny at the microscopic level. The inherent elastic modulus of the material and the design of the scaffold both influence stiffness. Hence it is possible to customise the stiffness of a porous scaffold by changing its structural design. A stiffer scaffold can cause bone resorption due to stress shielding, whereas a softer scaffold compared to native tissue may deform greatly and fail under physiological pressures. As a result, it's crucial that the scaffold match the stiffness of the replacement tissue since the mechanical stimulation of nearby osteoblasts controls how the original bone is remodelled. Scaffolds' general properties, surface, and internal design should all be managed to match their rate of deterioration with the rate at which new bone forms in vivo. Extremely high porosity and weak chemical bonds between atoms of a material are not desired because they cause rapid degradation and, as a result, a loss of contact at the implant-tissue interface, which can result in implant failure. These materials are susceptible to extensive hydrolytic, oxidative, or enzymatic degradation. On the other side, slow degradation limits the area available for the formation of new tissue, which prevents tissue healing. Implant loosening results from fibrous encapsulation of the implanted material, which happens if breakdown is too slow or the substance is fundamentally non-degradable. To promote tissue regeneration and to match the scaffolds' rate of disintegration to the rate of bone production, the surface and architectural features of the scaffolds should be rationally designed (6).

Above all, the most crucial requirements of scaffolds are biocompatibility and biodegradability. The main standard for all tissue-engineered scaffolds is biocompatibility. The ability of bone tissue cells to adhere, migrate, and proliferate should not be inhibited by BTES. It shouldn't cause a strong immune reaction to stop serious inflammation, nor should it exhibit any notable cytotoxicity during or after transplantation. Polymer materials that are printable and biocompatible are the ideal material for 3D printing applications. Since they are very complex and adaptable and offer a preferred bionic environment for live cells (2).

Another unique quality that scaffolds can provide is controlled biodegradability. Biodegradation of implanted materials will inevitably affect the mechanical support of growing bone tissue. They offer the biological and mechanical foundation necessary for cell development and differentiation. It eventually gives way to tissue that has been grown back and is intended to mimic the mechanical characteristics of bone. The rate of scaffold deterioration should ideally coincide with the deposition of mineralized tissue. The new tissue gradually provides more mechanical support than the degradable scaffold, making up for the

mechanical support that was gradually lost. When new bone is formed, the load is likely to be greater than the capacity of the scaffold, which could result in fracture if the scaffold degrades too quickly. In contrast, if the scaffold does not break down quickly, it may cause an inflammatory reaction to any foreign materials present, halting the regeneration of new tissue. Many investigations on the relationship between degradation and osteoblasts in vitro have been conducted in attempt to create scaffolds that can both increase the activity of osteoblasts and display suitable degradation rates. The biodegradability of polymers is an attractive property that can be controlled by the molecular design of polymers (2).

3. Composition of 3D BTES:

For the creation of printing inks for 3D printing, bioceramic powders, natural and synthetic hydrogels, non-hydrogel-based polymers, and their composites have been used as raw materials. Poly (L-lactic acid), poly (vinyl alcohol), poly-hydroxybutyrate, polyurethane elastomers, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), poly (D,L-lactic acid), poly (lactic-co-lglycolic acid, and polycaprolactone are examples of biodegradable and biocompatible polyesters that can be processed into wires, pellets, or even powders to enable 3D printing of polymer scaffolds. At room temperature or below, hydrogel precursors made of aqueous solutions of natural polymers like chitosan, collagen, gelatin, gelatin methacryloyl, hyaluronic acid, sodium alginate, and polyethelyene glycol diacrylate could be 3D printed into scaffolds before being further stabilised by UV, ion, or temperature-assisted crosslinking. Bioceramic precursors, such as micro- or nanosized calcium phosphates (Ca-P), hydroxyapatite (HA), tricalcium phosphate (TCP), carbonated calcium deficient hydroxyapatite (CDHA), bioactive glasses, etc., can be combined with binders or photopolymers to create printing inks for 3D printed "green bodies" that are then sintered to remove the organic phase and leave only hard bioceramic scaffolds. To create 3D scaffolds without post-sintering, bioceramic micro/nanoparticles can be further combined with biodegradable synthetic/natural polymers. In addition to giving scaffolds osteoconductivity, polymer composite printing inks also improve the degradability of inorganic particle fillers and change the physical, mechanical, and biological characteristics of scaffolds (5). Composites are new hybrid biomaterials made of two or more constituents that can be arranged spatially to offer specific features in a single material system. During the construction of bone scaffolds, careful consideration of the constituents and optimization of their volume fraction and orientation may result in a design that is more reminiscent of nature. To achieve improved function, a variety of composites, including polymer-ceramic, polymer-metal, ceramic-metal, and polymer-polymer, have been developed. Typically, polymer and ceramic are coupled to strike a balance between the mechanical instability caused by too little stiffness in the polymer and the fracture generated by too much brittleness in the ceramic. However, other properties of some polymers, such as polycaprolactone (PCL), such as hydrophobicity, poor cell adhesion site, and limited biological interactions, can also be changed by using a ceramic, such as nano-sized hydroxyapatite (HA). Composites can be created using polymers and biodegradable metals, such as poly-L-lactic acid (PLLA) and magnesium (Mg), to give the necessary biodegradability rate while also having a better strength and structural integrity. Moreover, ceramic and biodegradable metal

4. Applications:

BTES have applications for the treatment of: a) osteomyelitis, where they release high levels of antibiotic at a local administration site; b) cancer bone metastasis, where anticancer drug can be specifically released in diseased tissue; c) osteosarcoma, where antitumoral efficacy of drug is enhanced; d) osteoarthritis, where they increase retention time of the drug, maintaining cartilage structure and composition; e) osteonecrosis where they decrease inflammation and facilitate osteogenic differentiation and maturation and f) delayed-non unions where they promote fracture healing and decreases the risk of secondary osteomyelitis by Osteoinductive agents and antibiotics (7).

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