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3-D nanocomposite scaffolds: Tissue engineering for bone reconstruction

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Abstract

A challenge to the orthopedic surgeons in veterinary and human fields is the treatment of delayed union, malunion and nonunion. Apart from renovation of arrangement and constant fixation, in many cases, adjunctive measures such as bone-grafting or the use of bone-graft substitutes, are of dominant significance. In general bone-graft materials comprise one or more components: an osteoconductive matrix, which acts as a scaffold to new bone growth; osteoinductive proteins, backing the mitogenesis of indistinguishable cells; and osteogenic cells, which can form bone in the proper environment. Our review focuses on the currently existing bone graft and graft substitutes for the novel therapeutic approaches in the clinical situation of orthopedic surgery. This review is based on an extensive literature search of different composite scaffolds developed as bone regenerative therapies. The settlement and drawbacks of different composite scaffold developed techniques, the properties of generally used ceramics and polymers, and the properties of presently investigated synthetic composite grafts. To follow, an exhaustive review of *in vivo* models is used to test composite scaffolds in segmental bone defects (SBDs) to serve as a guide to design suitable translational studies and to recognize the challenges that require to be overcome in scaffold design for successful transformation. This includes the formative of the anatomical position within the animals, selecting the accurate study period, and an overview of scaffold presentation evaluation.

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Introduction

The idea of repairing an injured body have existed since the start of humanity, with initial history establishing it as a mythology and magical. Innovative thoughts of the ordinary world, disease, trauma, and the overview of logical approaches allowed synthetic prosthetic resources to repair the missing purposes of body parts and tissues. By the unfolding of the twentieth century, the conception of substituting one tissue with a new was industrialized. This substitution has put the basis for developing body parts and tissues manufacturing that officially started in 1987 (1). The discipline of manipulative and constructing innovative tissues or materials for injury preservations has been extensively considered and continuously growing. The bone has the maximum renewal abilities that offer a typical model of a perfect standard of a tissue engineering model (2).

Presently, unique nanotechnology methodologies are involved in tissue engineering. The human hard tissue (the bone) signifies unique of the furthermost essential structures

of the human build. These hard structures' performance is essential for providing the wanted sustenance, defense, and effort. These exclusive topographies of the hard tissues (the bones) are fine operated in body parts and tissues manufacturing in a continuous examination for a perfect hard tissue (the bone) spare structure. A chief problem for bone operation often presents secondary bone tumor, trauma, or malformation (3-5).

Bone damage is mainly a consequence of age, deteriorating diseases, or accidents. Numerous renovation procedures have been recommended over the previous decades. Conversely, practically wholly of them were unsuccessful in building continuing structure renovation (6,7). Bone replacement includes implanting an innovative bone or an appropriate spare structure among the places of a broken hard structure (the bone) or a deserted hard structure (the bone) to help the therapeutic procedure. Relocation of bone is a profligate developing field, which substantially affects patients that hurt from bone tissue damage and contamination (8). For more than a century, the progression of hard structure (the bone) implanting has been applied by orthopedic specialists because of the ongoing requirement for hard structure (the bone) spare. In therapeutic techniques, implanting is usually used to substitute injured tissue. Currently, the replacements to treat these damages are insufficient because they depend on autografts, allografts, and biomimetic or diversity of artificial resources and approaches (9). Autografts are osteoconductive, osteoinductive, with osteogenic appearances (10,11). Although autografts are considered typical for bone transfer, they also have limitations due to possible donor illness, establishing other therapeutic difficulties, and low tissue availability (11-13). The expectancy of an implant substitute is highly reliant on the environment of the break or imperfection of the hard structure (the bone). This limits the implant usage, whether modest invalid filler or greater hole filler that doings similar a scaffold substantial to simplify the construction of innovative bone. In together cases, the substantial implant performances like basic sustenance and strong point supplier (11).

The selection of implant spare advertised satisfies these conditions, and single or extra of the strategic rulers of bone therapeutic (osteoconduction, osteoinduction, and osteogenesis) is not everything. At the very least possible, an implanting substantial design must be osteoconductive in its environment to be used as modest void fillers, simplifying the construction of innovative bone cells. Combining growth factors, such as Bone Morphogenic Proteins (BMPs) helps cells grow, an osteoinductive environment might be consulted to an implanting structure to stimulate an even quicker therapeutic level. The persistent appearance of new revolutionized or enhanced implanting materials preserves the area of the hard tissue (the bone), manufacturing an exciting opportunity for future researches in demand to achieve these blank voids in fabricating an implanting structure that achieves the chiefs of an adequate bone temporary structure. Prosthetics from metals and bone cement fillers, polymers, and ceramics are supplementary treatment choices (14).

Moreover, bone defect repairs or alters fragmented bone tissue. The whole expectable methodologies to repair and substitute bone may be hurting, captivating extended time, and maybe rejected by the body (15,16). In the latest decades, body parts manufacturing ascended as an altering technique to renovation and improved injured body parts to evade the requirement for perpetual graft (17-19).

Tissue engineering can be separated into varied approaches. The top method for the manufacture of resilient tissue (for example, bone and cartilage) replacements is by the mixture of alive cells, naturally active molecules, and impermanent Three-dimension (3-D) spongy scaffoldings (20). Substitute methods have been strongly discovered and studied constructed on body parts and challenging (the bone) and soft tissue manufacturing methods trying to increase beyond the native restrictions of the presently existing resolutions to hard tissue (the bone) deficiencies. Using this methodology, tissue engineering develops hard tissue (the bone) from scattering cells that can grow into osteoblasts on significantly porous biomaterials (5,15). Improper on Williams (21), body parts, and hard (the bone) and soft tissue manufacturing is defined using a multidisciplinary area that usages the ideals of manufacturing and life disciplines to enhance biological substitutes that preserve, regenerate, or advance the purpose of tissue. These replacements are usually proprietary as scaffoldings.

In the last few decades, body parts and hard (the bone) and soft tissue manufacturing have become an encouraging substitute for treating or standby loss of tissues and organs resulting from contamination or pain (22,23). The utmost studied methodology comprises the usage of synthetic extracellular ground (the scaffolding) typically premeditated to be momentary and consequently prepared from bioresorbable or biodegradable polymers. Tissue engineering has lately advanced up the attentiveness in manufacturing spongy constructions to support tissue redevelopment. The significant typical in body parts and hard (the bone) and soft tissue manufacturing is culturing of cells separated from a patient, prolonged, and even stimulated to isolate *in vitro*. In *in vitro*, the cells are cultured onto a platform that promotes developing *in vitro*, ultimately in lively seeding locations, subsequently which is set into the receiver insufficiency, which will act as an inductor for tissue regeneration (24). Tissue engineering provides a forthcoming technique to reconstruct tissues, organs, and artificial implant products below laboratory environments in overcoming the difficulties of grafting refusal, diseases related to xenografts conduction, and allografts with a deficiency in the donation of an organ (25-27).

Bone tissue engineering is a multidisciplinary study field in which novel methodologies are developing to treat human patients' misery from bone injury or disease. Like tissue engineering, artificial bone is shaped by planting cells that

can develop osteoblasts on 3D porous scaffolds for cultivation *in vitro* or *in vivo* to stimulate bone matrix construction (5,15,27). The natural, synthetic bone is expected to substitute the autogenous bone implant with that equivalent vital machine. Hard (bone) tissue manufacturing can resolve many difficulties, such as bacterial contamination, donor deficiency, high cost, and slow vascularization (8,27,28). Bone rebuilding is the typical objective for bone tissue engineering. It might be suitable in therapeutic or fixative expansive variation of bone deficiencies (5,25,27). As clarified above, tissue engineering of bone wants three substantial essentials: cellular apparatuses, extracellular matrix (ECM), and growth factors (14,29). Many diverse methodologies possibly will be used in the construction of hard (bone) tissue manufacturing. One methodology is planting autologous osteogenic cells *in vitro* alongside a decomposable scaffolding establishing a scaffold–cell hybrid, which can be named a tissue manufacturing concept. Mesenchymal stem cells, chondrocytes, and osteoblasts from stiff and lenient structures of the patient could be prolonged in planted and culture onto a scaffolding that would in an insufficient method die, allowing entirely typical bone tissue substitution (30-32). A present statement on the world marketplace of orthopedic grafts and materials manufacturing showed that the whole drug orthopedic graft and device market to produce at a CAGR of almost 8.8% over the next decade to reach around $91.42 billion by 2025 (33).

Orthopedic grafts improve with a progress ratio of 7% to 10% over the last decade, and this development is expectable to continue in the years to come up (34). The universal dental grafts and prosthetics market is expectable to rise at a CAGR of 7.2% through the prediction period to impact USD 12.32 Billion by 2021 (35).

The central portion of this market was thoracolumbar fixation followed by interbody devices and cervical fixation, which comprise the total spinal union market (36). The universal foot and ankle devices marketplace raised at CAGR to about 7.9% above the following decade to around $7.82 billion by 2025 (35).

The achievement of body parts and soft and hard tissues manufacturing scaffolding will perform in conclusion out if it will tolerate affection of cell, growing, and lastly cell division into the suitable structure. For this reason, the bioresorbable scaffolding must be biocompatible and having a permeable related connection to create informal vascularization and fast-developing of a newly shaped structure (36-38). Subsequently, several necessities were documented as vital for the engineering of scaffoldings. In body parts and soft and hard tissues manufacturing, the scaffolding must have: (I) linking holes of a balance suitable to sustenance combination and vascularization of structures over permitting cell movement, the transmission of O_2 and CO_2, metabolites, nutrients, and indication molecules together inside the scaffolding and among the scaffolding and the indigenous atmosphere, (II) materials that controlled the biodegradability or bioresorbability in direction for the host tissue to end with substitute the scaffold over permitting to be disruption down by biotic processes at a ratio harmonious to the ratio of tissue growth through supportive mechanical consistency at a charitable time which differ from weeks to numerous months, (III) appropriate surface chemistry to sustenance cell linking, division and developing, (IV) acceptable mechanical possessions, (V) not motivate a negative response, and (VI) modest range of formulae and dimensions (26-28,37). Having these necessities in attention, numerous constituents have been recognized or shaped and made up into scaffoldings (38).

A numeral of polymers is typically used in bone scaffolds such as hydroxyapatite, collagen, polyglycolic acid (PGA), polycaprolactone (PCL), and polylactic acid (PLA). One-time synthetic scaffolding might tolerate other surface alterations to develop their connections with cells (39-41).

Bone graft substitutes

Lately, the general traits, properties, and performance abilities essential for appropriate bone graft replacements have been reviewed (42,43). Autograft, allograft, and xenograft are other common bone graft materials. Autograft is when the bone is obtained from the patient's body (44). It might also be vascularized or non-vascularized. Autograft is not possible for patients with compound hard tissue (the bone) injury that needed a significant quantity of hard tissue (the bone) implant constituents. Allograft is defined as tissues transplanted from one person to another. It is usually used for spinal fusion surgery (45). Allograft bone transplants used as scaffolds could have possibilities of infection diffusion, such as HIV, and Hepatitis B and C. In addition, it could have a high infection rate of ten to twelve percentage, and an additional eighty percentage of diseased allografts have related to experimental letdown (25). A xenograft is a bone scaffold graft that can be transplanted among (2) diverse classes, such as bovine, porcine, or coralline bone grafted into humans (3,46). Many studies have proven that coralline xenograft has the same performance as autografts if used as a filler in deficiencies secondary to trauma or tumors and cysts (47-51). Xenografts are also subjected to immunogenicity and could take the propensity to putrefy in room temperature or temperature lower its decomposed point. Numerous revisions lately have established xenograft mixed with Osteoinductive factors, for example, bone morphogenetic proteins (BMPs), to increase the in situ hard tissue (the bone) production (52-54).

The graft replacements must be accessible to a specialist on diminutive notice, stimulates the hard tissue (the bone) ingrowth, absorb expected performance with bone growth, and do not promote lenient structure development at the bone-culture boundary (55). Many scaffolds were used as bone graft substitutes mainly derived from a natural bone in powder form positioned near a break or union. The advantages of consuming ordinary resources such as bone

powders that are not toxic may have a specific protein binding site to contribute to bone healing (39).

Most bone banks offer demineralized bone matrix (DBM) fabricated mainly using collagen type I after extraction from human cadaver bones (56,57). DBM is offered in a powder that can be mixed with liquid to the right consistency to facilitate the application, small chips, and blocks or strips (58-60). The DBM is aseptic and sterile and could be more critical in recent years due to the necessity of inactivating viruses such as the human immune-deficiency virus (HIV). DBM consistency was argued to have considerable influences on the final Osteoinductive capability.

In other words, the implant should be absorbable, biocompatible component, unique mechanical and bodily possessions for presentation, malleable to unbalanced wound location, regular pore size between two hundred to four hundred microns, maximum bone development via Osteoinduction and/or Osteoconduction, nope harmful properties to neighboring tissue, good bone apposition and sterilizable without loss of properties.

Bone replacement

Williams and Lewis (61) were the first to define biomaterials as non-sustainable resources that can be used in a therapeutic device that is planned to interrelate with the biotic atmosphere. There are three general criteria reported by Katti (62), who stated that materials for bone replacements must be: neither inflammatory nor toxic, possess mechanical properties to correspond with natural hard structures (the bones) at the grafting position and cost-effective.

The biomaterials also must not impose any pressure or interfere with the surrounding host's systems, and they must not be affected by the host systems themselves (30,63,64). The surface and mechanical compatibility are two other essential aspects of an implant produced for orthopedic use (14,65-67). Implant surface morphology, chemical reaction, and toxicity of that surface would be the most critical factors contributing to surface compatibility.

In vivo interaction among the host hard structures (the bones) and the surface of the graft is dynamic. Within few seconds of the implantation time (the initial stage), water, ions, and other biomolecules will be in uninterrupted interaction with the graft surface (65).

Surface roughness (friction) would be significant for the integration and stability elements carrying orthopedic braces. Surface permeability is an additional vital issue in bone substitutes (65,66).

Approach of bone tissue engineering

More than forty years ago, hard structure (the bone) manufacturing developed as a new novel part of a study that used the ideologies of engineering with biology to develop viable replacements that renovate and preserve the task of hard human structures (the bones). The technique includes seeding Osteoprogenitor cells or stem cells on porous biodegradable 3D scaffolds fabricated using biomaterials (68). This could help promote a new bone tissue formation at the affected area when implanted *in vivo*.

Materials for hard structure (the bone) manufacturing

There are numerous kinds of materials that have been successfully used in scaffolds development:

i-Ceramics

Are resistant to corrosions, highly strong, and have sufficient biocompatibility. However, they have low mechanical reliability, which makes them difficult to fabricate. Additionally, ceramics are fragile and exhibit low fracture strength, such as Cockle (*Anadara granosa*) shells (69,70).

ii-Normal polymers

Such as collagen, alginate, chitosan, fibrin, hyaluronic acid-based materials, and agarose frequently have more organized structures and have good surface compatibility that makes them easily link to cell receptors. However, natural polymers have some degree of immunogenicity stimulating immune response post-implantation (71).

iii-Synthetic Polymers

Have more advantages over natural polymers. Their manufacturing could be controlled to produce polymers with high chemical and physical properties. Moreover, they could be produced in high quantities, cost-effective with good mechanical properties, and relatively low degradation time. These synthetic polymers have been used for cartilage, bone, and skin replacements (72).

iv-Hydrogels

Are mixed types of natural and synthetic polymers with a high affinity toward the water. These hydrogels are typical of standard polymers such as chitosan, fibrin, collagen, agarose, alginate, gelatin, and hyaluronic acid (HA) (73). Hydrogels could be ideal polymers that can mix with the ceramics to fabricate the scaffolds quickly.

Biodegradable and bioresorbable

Biodegradable is to decompose naturally with nontoxic remnants. Pharmaceutical and surgical fields use biodegradable polymers implants in medical applications due to their bio-absorbability and degradability (74). These polymers could be degraded inside the host's body in a certain implantation period, leaving nontoxic metabolites that could be eliminated. Degradation occurs in two ways: 1) hydrolysis degradation that is arbitrated through the water and 2) enzymatic dilapidation, which is merely arbitrated by enzymes.

Nowadays, many commercial materials have been used as bio-absorbable orthopedic implants like Poly glycolide (PGA) and Polylactic acid (PLA) which used for three-

dimensional polymer scaffold for cell relocation; Poly ($_{L}$-lactide) ($_{L}$-PLA) that used for break fixation, suture anchor, ACL rebuilding, rotator cuff restoration and meniscus restoration and Poly ($_{D, L}$-lactide) which used for Break fixation, ACL restoration, suture anchor only (75,76). The advantage of using decomposable polymers above metallic tools is to decrease stress. Hence, the polymer will be degraded eventually, eliminating the requirement for an additional operation to take away metal transplants.

Scaffolds

A scaffold provides the essential 3D structure that accelerates the new tissue regeneration. A tissue culture study on a 3D system (scaffold) provides more accurate findings such as physiological responses than 2D cell culture systems (77). Biocompatibility of materials used to fabricate the 3D scaffolds can be tested and the structural design. In addition, the 3D system cell culture is beneficial to examine the scaffold architecture and its permeability, which is a significant factor of any scaffold efficiency (78-80). The latter influences the cell culturing factor and determines the capability of the scaffolding to exchange nutrients and oxygen that are vital to sustenance the growth of the cells within the scaffolds. A scaffolding with higher permeability is favorable and well known to improve *in vivo* bone tissue formation scaffolds (81-87).

The SG cell cultures system is complicated because of the trouble of cell culturing and preservation. In the SG system, cells can be cultured through the scaffoldings at the beginning and/or on the surface of the scaffolding, only then permitted to transfer inside through seeding procedure. In either scenario, the cells growing inside the scaffold need nutrients and waste disposal (88,89).

Thus, bone tissue engineering is a suitable matrix for osteoblasts proliferation that could be helpful to the restoration method of bone integrity. The osteoblasts can be cultured on the surface of the spongy net of Osteoconductive and decomposable scaffoldings, then transplantation in the bone deficiency (90,91). Instead, the cell-seeded scaffold could be seeded *in vitro* before relocation (92). The latter can produce extracellular matrix (collagen) deposits produced by cells that proliferate through the early phase of *in vitro* culture. This process ensures early mineralization of the seeded scaffold in prolonged cultures (93).

The human osteoblast cell line is an excellent choice for cell culture to determine the compatibility and the characteristics of the 3D scaffolds. Additionally, the human osteoblast cell line was proven superior qualities for bone 3D scaffolds besides its similarities to the bone tissues (77,86,87,94-98).

Composite scaffolds

Composites scaffolds are fabricated using two or more materials mentioned previously to produce an improved scaffold putting together the advantages from all composing materials (5). Sometimes, reinforcement (fibers) is needed to get the best of the composite scaffolds to improve the compressive modulus and the strong point of the compound scaffold (99,100).

Recently, fiber enforcement has been achieved using spider silk and silkworm as strengthening material because of their high resistance, and they can be absorbed by the body (101).

Scaffold requirements

Materials for scaffolds application must fulfill a specific criterion to be considered an ideal fit for tissue engineering or implant. However, most of the requirements are unique to tissue to restore size and location (102). Scaffolds must generally have a high flexible modulus to fit in the intended space and allow adequate growth (27,103). In addition, scaffolds must have the significant mechanical strength to stand the load weight for a specific period. The chemical and physical structure of scaffolds is a very critical factor to allow degradation and breakdown. Moreover, scaffolds must have enough porosity with a macro-pore structure of 300-500 µm to improve nutrient exchange besides waste removal (84).

Cell culture systems

Living organisms have a complex niche. Therefore, *in vitro* systems, such as cell cultures, lead to advantageous study replacements that can be simply reproduced and compared under specific conditions. The *in vitro* valuation of scaffoldings was approved as a foundation for defining the functional applicability of the developed scaffolds within any biological system. The *in vitro* experimentation offers a similar yet simplified version of the biological system to place the foundation and determine the scaffolds' suitability and implacability when used for *in vivo* studies (104,105).

Cell culture with 3D solid geometry (SG), regularly used to evaluate material's cytotoxicity or any other effects on proliferation and differentiation. However, cells behavior in a cell culture environment (SG) was found to be different compared to a 2D flat or plane geometry (PG) (106-109). Nonetheless, the SG is indispensable to observe the cell's behavior in applied conditions.

***In vitro* cell culture**

Various *in vitro* experimentation has shown that the affection of osteoblast cells in the primary few hours post-inoculation differed considerably depending on the protein surface is covered or not (110,111). Proteins such as fibronectin and vitronectin are well identified to promote the linkage of osteoblasts. These extracellular proteins perform as a transition component among synthetic surfaces and osteoblasts, promoting osteoblast adhesion, maturation, and matrix mineralization (112). However, some other studies have advocated the contrary to the latter belief that the rise of linkage interactions among the cells and extracellular background might not be continuously advantageous (113,114). Few adhesive ligands (fibronectin, vitronectin) is

insufficient, and the cells cannot get tight grips to migrate. In contrast, more than few ligands are favorable, and the cells adhere and stick up for optimal cell migration (115,116).

The most common culturing procedure for tissue engineering purposes is standing culturing, which is frequently categorized as non-homogenous cell spreading. It detains prevalent cells on the scaffold's external faces, which results in an inhomogeneous spreading of the *in vitro* produced extracellular ground (29,117-124). However, this disadvantage could be avoided by using other culturing structures that mainly involve growing cavities with stirrers and sensors to deliver suitable nutrients and gases and remove the waste products. These systems are named bioreactors that provide adequate nutrient supply and waste elimination as well as providing a physiochemical environment conducive for tissue formation, for instance, spinner flask (125), rotating wall vessel bioreactor (126), concentric cylinder bioreactor (127), and perfusion bioreactor (128). These bioreactor systems have advanced the construction, purpose, molecular possessions of manufactured cartilage (129) and bone (130). They could provide an atmosphere that simulates the *objective structure is in vivo* physiological niche, supporting cell migration and development and separating the cells into the required lines.

Cell culturing of scaffold constructs *in vitro*

Origin of bone marrow stem cells

One of the utmost significant considerations in body parts manufacturing approaches and most extensively studied is the *in vitro* cultivation of cells on the scaffolding previously grafting to evaluate cells capacity to control the multiplying and cell differentiation (131-136). Cells resulting from the patient's well tissues (autogenic cells) would be the top primary select to evade immune rejection of foreign tissue.

Friedenstein and colleagues (137) were first to apply *in vitro* cell culture and transplanted it in research laboratory animals to describe the cells that produce the physical stroma of bone marrow. These cells are cultivated at low concentrations; the stromal cells from bone marrow will stick quickly and can modestly be detached from non-adherent hematopoietic cells via repeated washings (138-140).

Friedenstein *et al.* (137) have reported that cultivate adherent cells existing in the marrow stroma can distinguish into cartilage and bone tissues if placed into a suitable environment *in vivo* (110-116,124,140-142). These revisions could have directed to the theory that stroma comprises mesenchymal stem cells capable of distinguishing to several cell lineages comprising osteoblasts and chondrocytes if positioned in suitable *in vitro* and/or *in vivo* atmospheres.

Bone marrow contains both hematopoietic cells and adherent stromal cells of non-hematopoietic derivation. These cells composed with the extracellular matrix could offer scaffolding support, the so-called bone micro-environment. The bone marrow micro-environment comprises reticular endothelial cells, macrophages, adipocytes, fibroblasts, and osteogenic precursor cells.

Human osteoblast cells line

The human osteoblast cell line provides an excellent choice as a cell culture system to determine the characteristics of the developed scaffolds. These cell lines are proving to be superior candidates for bone scaffold cell-material studies due to their enhanced tissue reaction and high phenotypic similarities to the target tissues (94-96). Harvest of Mesenchymal stem cells in bone marrow-derived cells, which have more superior capacity for chondrogenesis, or it can be adipose-derived cells, which are 500x more than those found in the bone marrow. They are easily assessable, non-invasive repeatable harvesting method, relatively little donor site morbidity, cultured more efficiently, grow more rapidly, proliferation and differentiation potential are less affected by age, and have better immunosuppressive properties.

The MG63 human osteosarcoma-derived cell is an example of the cell line that characterizes the human osteoblast-like cells. Though originating from a tumor cell line, the MG63 cells are often used to produce phenotypic osteoblasts. It is well-known in the region of bone tissue cultivate revisions because of their capability to grow a hard tissue (the bone) precise alkaline phosphatase (ALP) reaction and osteocalcin in reaction to osteogenic agents and differentiating to bone developing cell with characters and forms that mark them an outstanding selection for *in vitro* studies (143,144). The MG63 cell line also has an accelerated proliferation response in high calcium monolayer culture system (145).

ALP is an enzyme and an initial indicator of HOB variation, and it is related to calcification. The concentration of this enzyme is increased through the first few days of the bone defect and immediately before the mineralization phase of the matrix. The ALP provides the local enrichment of inorganic phosphate, which is a part of the mineralization phase of bone reconstruction (146-148). Minerals, such as calcium, show an essential part in the bone tissue construction-resorption process. It also contributes to the bone formation-repair process as an indicator of osteoblast maturation (149).

The first basic *in vitro* assessment of a developed scaffold regularly involves determining cytotoxic properties of the scaffolding materials on the cells. The test for biocompatibility is usually carried out via cell viability evaluation using a simple MTT (3-dimethylthiazo-2,5-diphenyl tetrazolium bromide) colorimetric analysis. This test is used to quantify the cytotoxic effects of scaffold materials on cells and as a pointer for the multiplying rate of the cells (150).

Additionally, ALP assay in culture medium is used to determine cell proliferation and functional properties. The ALP concentration is an index of osteoblast activity toward

the scaffold materials and the rate of cell differentiation within that scaffold (151).

***In vivo* studies**

The United States only has over one million surgeries include bone and cartilage replacement, every year. Therefore, using autologous cancellous bone implanting is advantageous as it offers the crucial fundamentals for bone construction, principally living osteogenic cells, bone inductive proteins that motivate cell propagation and differentiation, and a scaffolding that maintenances the ingrowth of freshly shaped bone (152,153).

Vascularized autogenous cancellous bone can be collected from the fibula, scapula, pelvic bones, and ribs. Nonetheless, these bases of cancellous bone are restricted and have high morbidity in their respective donor sites. In addition, the allograft is also limited because of its immunological refusal, the spread of infections, untimely resorption, and above all, giver lack (154-157).

Lately, the practices of using biocompatible and decomposable scaffoldings cultured with living cells have allowed the formation of purposeful tissue (158). Previous work had proven that osteoblasts polyglycolic/polylactic acid concepts can be made up and when transplantation into an animal model, a novel bone construction was grown with the last morphology comparable to that of the polymer scaffolding (159).

Calcium phosphate and hydroxyapatite ceramic are the most hopeful bone replacements due to their chemical structure and mechanical possessions, which are like the bone. This was one of the several synthetic materials available as a bone replacement (28,32,160). In addition, these materials have suitable pore form, pore dimension, and pore interconnection passageway, as well as structural density (24).

Critical sized defects (CSDs)

Bone defects due to disease or trauma can be a lifelong dilemma, hard to cope with inpatients, and it might too be tough to treat despite the advances of surgical procedures. Therefore, fragmental extended bone defect models with adjacent resemblance to experimental cases have been used for bone rebuilding to evaluate the effectiveness of growth factors and transporter substantial (161-163). A critical-sized defect is often used and definite as the most diminutive size of bone deficiency that cannot rebuild naturally if left untreated for a certain period (more than six months), Diagram 2 (164). It is well-identified that the size of the CSD in long bone is double its diameter.

There are many bone models for CSD commonly in the long bones such as femurs, radius, ulna, fibula, and tibia of dog, sheep, and rabbit was chosen based on the long bones criteria, which permit to create a segmental defect that allow convenient radiographic and histological evaluations, Diagram 3. Herold *et al.* (165) was the pioneer to use CSD on the rabbit radial bone. Then, it has been practiced as a standard and applicable animal model (8,77,85-87,166-177).

Conclusion

Investing in the future of bionanotechnology to serve the engineering of load-bearing *in vitro* and *in vivo* bone substitutes is the pathway to successful, relevant modules. An advanced combination of micro or nano calcium carbonate triturate mixed with natural and\or synthetic polymers is the ultimate applicable structure. The latter assurance and the achievement of the industrialized bone scaffolds propose an accurate biological system. Such mixture is vital in succeeding the bone scaffoldings' spongy construction that determines their subsequent appearances: perfect morphology, optimum physiochemical possessions, excellent mechanical strength, and Young's Modulus. Finally, it is expected to have a suitable cell attachment, cell scattering, and cell growing level in *the in vivo* system to accomplish the top necessities to be deliberated as a bone substitute.

Conflict of interests

The authors declare that they have no competing interests.

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السقالات النانوية المركبة الثلاثية الأبعاد: هندسة الأنسجة لإعادة بناء العظام

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الخلاصة

إن التحدي الذي يواجه جراحي العظام في المجالات البيطرية والبشرية هو علاج الالتئام المتأخر والمشوه وعدم الالتئام. وبصرف النظر عن طرق التثبيت المستحدثة، في كثير من الحالات تعتبر البدائل الاختزالية على سبيل المثال ترقيع العظام أو استخدام البدائل العظمية هي ذات أهمية كبيرة. بصورة عامة تتألف مواد ترقيع العظام من واحد أو أكثر من المكونات: مصفوفة العظام الموصلة والذي يعمل كسقالة لنمو العظام الجديدة; البروتينات العظمية، إعادة انقسام الخلايا التي لا يمكن تمايزها; والخلايا العظمية، والتي هي قادرة على تشكيل العظام في البيئة المناسبة. ركزت مراجعتنا على الترقيع العظمي الحالي وبدائل الترقيع للنهج العلاجية الجديدة في الحالة السريرية لجراحة العظام. ويستند هذا الاستعراض على بحث المراجع الواسعة النطاق من تطوير السقالات المركبة المختلفة والتي وضعت لتكون بمثابة علاجات تجديد العظام. وضعت تقنيات التطوير وعيوب السقالة المركبة المختلفة، وخصائص السيراميك والبوليمرات المستخدمة عموما، وخصائص الطعوم المركبة الاصطناعية قيد الدراسة والمتابعة حاليا. وللمتابعة، تم إجراء مراجعة شاملة في نماذج الجسم الحي المستخدمة لاختبار السقالات المركبة في عيوب العظام القطاعية لتكون بمثابة دليل لتصميم دراسات مناسبة ولتوضيح التحديات التي تتطلب التغلب عليها في تصميم السقالات للزراعة الناجحة. وهذا يشمل تحديد الموقع التشريحي داخل الحيوانات، واختيار فترة الدراسة الدقيقة، وأخيرا لمحة عامة عن تقييم مكونات السقالة.

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