American Journal of Botany and Bioengineering
https://biojournals.us/index.php/AJBB
ISSN: 2997-9331

Study of the Preparation of Bone Scaffold Via the Replication Technique

Mohaimen Ali Razzaq
Madenat al-Elem University College, Department of Medical Physics

Received: 2025 19, Jun
Accepted: 2025 28, Jul
Published: 2025 04, Aug

Annotation: Bone scaffolds play a vital role in orthopedic and dental applications by facilitating bone regeneration through biomimetic structural support. Despite extensive research, optimizing scaffold porosity, mechanical strength, and biocompatibility remains a challenge. This study investigates the preparation of bone scaffolds using replication techniques with polymer, ceramic, and metal-based templates, focusing on material selection, template design, and sintering or curing protocols. The process involves coating sacrificial templates with tailored slurries followed by thermal treatment to produce highly porous, interconnected structures. The results demonstrate that scaffold properties such as pore size (60–350 μm), porosity (up to 90%), and compressive strength can be effectively controlled, ensuring compatibility with physiological loads and cellular environments. These findings highlight the replication method's potential to produce cost-effective, customizable scaffolds suitable for clinical use in tissue engineering and orthopedic repair.

Keywords: Bone scaffold, replication technique, porosity, biocompatibility, tissue engineering, polymer template, ceramic slurry, sintering, orthopedic applications.

1. Introduction to Bone Scaffolds

Bone scaffolds exhibit significant clinical potential in various fields such as dentistry,

orthopedics, and tissue engineering. These controlled porosity structures play a crucial role in supporting tissue regrowth effectively while enhancing the process of implant osseointegration. Advanced replication techniques allow for the preparation of high-quality bone scaffolds from a range of materials including polymers, ceramics, and metals, catering to diverse clinical needs and applications. [1]

2. Overview of Replication Techniques

Replication techniques offer a promising route to engineering bone scaffolds from a variety of materials. The process consists of coating a sacrificial template, usually a porous foam to mimic tissue, with the scaffold material. Polymer, metal and ceramic powders were used, with polymer, conformal and replica techniques employed to manufacture bone scaffolds. Selective replication can be used to promote porosity in the scaffolds, while additives modify the properties of the foams and coatings. Characterisation includes the analysis of physical, mechanical and biological properties after sintering or curing. Applications of scaffolds fabricated by replication are typically found in orthopaedics, dentistry and tissue engineering [2].

3. Materials Used in Scaffold Preparation

Three-dimensional bone scaffolds are of considerable interest for achieving sizeable bone defect repair in vitro, as well as in several other fields. They are macroporous materials with interconnected pores, used to provide a matrix or framework to support the attachment and growth of new bone cells [1]. Porosity can induce osteointegration and facilitate vascularization and the exchange of nutrients with surrounding tissues in vivo. Scaffolds are usually made from biomaterials or their composites, which can be natural or synthetic polymers, ceramics, and metals.

3.1. Types of Biomaterials

Bone scaffolds are fabricated using various biomaterials, including metals, bioceramics, polymers, and composites. The selection depends on biological functionalities such as osteoconductivity, osteoinductivity, osseointegration, and osteogenesis, as well as on economic, processing, and design considerations. Additional components like pore formers, binders, dispersants, and surfactants can be introduced to modify the properties of the final samples. Metals and their alloys must be verified for biocompatibility. Several polymer-processing routes support both natural and synthetic polymers, including diblock copolymer templates, the TIPS-TCL-SL technique, and immersion capillary-force-induced binder infiltration. Ceramic materials—primarily apatites, calcium phosphates, and calcium carbonate—are commonly used, with processing routes like adhesive tape casting, direct ink writing, and conventional ceramic methods. For metals, the replication technique remains one of the few viable shaping options to achieve the necessary mechanical characteristics and porosity. Due to the specific nature of each replication method, it is essential first to understand the types of materials employed in preparing bone scaffolds [2].

3.2. Additives and Modifiers

Additives in biomaterials used for bone scaffold preparation via replication techniques extend beyond structural modifiers. Polymers serve multifunctional roles, enhancing adhesion and providing stabilization against catalytic or thermal influences during processing. Viscosity modifiers are incorporated to prevent sudden draining of the slurry during the infiltration process, ensuring uniform coating of the template. Additionally, foaming agents, including organic acetates or inorganic gases, facilitate the generation of porosity within the scaffold structure. Certain biomaterials, such as titanium, can act simultaneously as base materials and porogens, influencing both the intrinsic properties and pore architecture of the scaffold.

Table 2 provides examples of additives employed in replica-derived bone scaffold fabrication. The variation in porosity and pore size is chiefly dictated by the choice of template and the

slurry's composition. Replication methods enable control over the size and interconnectivity of pores within the final structure, though it is important to note that this technique primarily yields open-cell porous scaffolds, as the polymer foam is subsequently dissolved in the process. [3][4][5]

4. Design Considerations for Bone Scaffolds

Biomaterials have been employed in tissue engineering as scaffolds because they offer a physical template for cell colonization, spreading, and tissue organization and formation. Scaffolds provide temporary support and a desirable matrix that enables cells to form functional tissues. Bone scaffolds are three-dimensional matrices structure that provide a temporary support for bone formation, functioning similarly to native extracellular matrix.

Bone scaffolds must possess several essential requirements and unique properties to allow their use in various medical applications. Porosity is the most significant factor because it dictates the ingrowth of host bone and the formation of new bone tissue, crucial for scaffold integration. The porosity range for scaffolds is typically between 45% and 95% for interconnected pores, with optimal values for cell transplantation for bone and cartilage regeneration around 90%. The scaffold must deform under physiological load without breaching, with mechanical properties matching native bone, considering site-specific and load direction factors, especially for long bone injuries in load-bearing parts. Non-toxicity and non-immunogenicity ensure the scaffold does not trigger an immune response. Degradability allows the scaffold to be resorbed after the host tissue forms, with a resorption rate matching the curling rate of host tissue, while maintaining sufficient mechanical strength during this process. Surface chemistry influences the interaction of cells with the scaffold, and the scaffold must be sterilizable. [6][7][8]

4.1. Porosity and Pore Size

Bone scaffolds prepared using replication techniques constitute an active and topical research field in the biomaterials area. Fundamental biomaterials for bone scaffold preparation include calcium phosphate-based ceramics, calcium-silicate-based ceramics, calcium-phosphate-based ceramic–polymer composites, and metal–ceramic composites, sometimes modified with additives such as manganese oxides and reduced graphene oxides. For preparing such scaffolds, the polymer replication, ceramic replication, and metal replication techniques are studied. The replication technique is among simple manufacturing processes suitable for mass production because of facile production steps, equipment requirements, and rapid manufacturing. The porous space, pore size, interconnection, and bone scaffold design can be customized through the replication technique to meet clinical applications, such as bone-regeneration-support materials, dental implants, bone-tissue-engineering scaffolds, bone-healing materials, and cancellous bone substitutes.

Porosity influences permeability and the mass-transfer rate of nutrients and support of new tissue growth. The porosity can be adjusted from 30 to 85 vol % according to the range exhibited by cancellous bones using the replication technique. A porosity of 70 vol % tends to manifest support for osteoblast growth. However, porosity in excess of 80 vol % tends to result in poor mechanical strength. Interconnection constitutes an essential factor in bone-tissue engineering because the drug delivery and protein-transport mechanisms can occur easily. A porosity of 30 vol % can support bone regeneration and differentiation of stem cells into bone cells. Generally, the repair duration is 10–12 weeks. Pore sizes in the range 60–350 μm facilitate adequate space for bone cells and adequate supply of nutrients for new bone growth. [9][10][11][12]

4.2. Mechanical Properties

A scaffold’s mechanical properties defines its functionality. Bone is a living tissue used in a wide variety of load bearing orthopaedic applications. In bone scaffolds, the mechanical properties must be sufficient enough to withstand bulk stress and support bone growth. The mechanical behavior is not only dependent upon the constituent biomaterial, but also on the

structural design of the scaffold (i.e., pore size, pore shape). It has been reported that scaffolds with pores that are too large, or too many pores, have mechanical strengths below the range of cancellous bone. Recently, different porosity in polymer-bonded ceramic scaffolds is utilized for bone tissue engineering application. To fulfill the scaffold requirements, scaffolds with porosities of more than 50% are prepared and evaluated, thereby adhering to the principles of bioengineering. Several researchers have experimented on pore size, mechanical properties, cell migration, vascularization, and so on, to develop a structure close to the native bone. Zirconia-based scaffolds with porosities ranging from 45 to 55% appear to be appropriate for load bearing applications.

4.3. Biocompatibility

Biocompatibility testing is a vital step in the evaluation of bone scaffolds for tissue engineering applications. The biocompatibility levels of scaffolds can be recognized using several biological assay techniques. In vitro cytotoxicity testing is the most common biocompatibility test for assessing cellular interactions and responses. The simplest in vitro cytotoxicity evaluation is the direct contact test, capable of detecting both cytotoxic and non-cytotoxic responses. Indirect testing methods include agar diffusion and extract dilution assays. The preparation of human osteoblast culture used for biocompatibility testing involves aseptic harvesting from human trabecular iliac crest bone under ethical guidelines. Cells are expanded in specialized proliferation media composed of α-MEM with GlutaMAX, supplemented with fetal bovine serum, ascorbic acid-2-phosphate, HEPES buffer, penicillin-streptomycin, and fungizone.

Cell proliferation assays, such as XTT, together with non-invasive in vitro NBT/BCIP staining, are standard approaches for evaluating biocompatibility. In the XTT assay, metabolically active cells reduce XTT to form orange formazan, the concentration of which is proportional to cell viability and can be quantified by measuring absorbance at 450 nm. The NBT/BCIP staining reveals alkaline phosphatase activity, producing violet precipitates that indicate areas of cell proliferation. [13][14][15]

5. Replication Techniques in Detail

Replication techniques can be used to prepare scaffolds for bone tissue engineering. Materials applied range from polymers to ceramics and metals. Various composite materials, together with bioglass and additives, have also been used to cover a broad range of applications in orthopaedics, dentistry and tissue engineering. The fabrication of porous titanium scaffolds by replication of NaCl crystals is presented, with a focus on the influence of the process on the final structure.

A detailed introduction to bone scaffolds and their design, together with a discussion of the materials involved, is given to enable commentary on the processing techniques. A functional porous replica Ti metal scaffold with sufficient mechanical strength was successfully fabricated by coating titanium slurry on NaCl crystals followed by sintering. NaCl acted as a pore-forming agent and was completely leached out by water without introducing defects in the final sample. The level of porosity can be controlled by altering either the slurry content or the size of the NaCl crystals. The replication method presented is simple, efficient and suitable for bone scaffolds.

5.1. Polymer Replication

Polymer replication techniques use polymer replicas as templates around which scaffolds are formed. Their high temperature resistance enables subsequent heating processes that remove the polymer, yielding the scaffold's final form.

Firstly, polymer scaffold replicas are created via rapid prototyping or salt leaching. These replicas are then immersed in biomaterial slurries and dried to form a cohesive layer. Coated replicas undergo sintering or curing to produce a skin layer, and when using ceramic materials, a

subsequent sintering step solidifies the structure. Finally, rinsing eliminates the polymer template, completing the process. Biotemplating involves similar immersion and curing steps but omits the final sintering stage. [16][17][18][19]

5.2. Ceramic Replication

Ceramic replication is a widely used technique for preparing bone scaffolds with a porous structure and improved mechanical properties. The process is relatively simple and involves dipping a highly porous template into a ceramic slurry, enabling the ceramic to coat the surface of the template. It is critical to avoid over-thickening the ceramic layers, which can fill the pores of the template and lead to reduced porosity in the final ceramic scaffold. Following coating, the template structure is removed by heat treatment—calcination or sintering—depending on the components of the template. Calcination is typically preferred for polymer templates as it effectively removes the template to expose the ceramic while mitigating the formation of cracks during thermal decomposition. Sintering is used if the template contains ceramic elements, providing enhanced stability to the scaffold.

This approach has been successfully employed to prepare ceramics with porosities exceeding 90% and desirable pore sizes. A crucial factor during fabrication is establishing a strong bond between the ceramic coating and the porous template in the initial sintering step, which serves as a compensatory mechanism to enhance the mechanical strength of the porous ceramic scaffold. The precise control of coating thickness and sintering parameters is essential to balance porosity and mechanical integrity, thereby achieving scaffolds that fulfill the functional prerequisites for bone tissue engineering.

5.3. Metal Replication

Metal replication techniques prepare highly porous metal structures by introducing a metal slurry into polymeric saturated templates. The organic polymeric network provides the overall shape and pore size of the final product. These materials possess exceptional dimensional stability and are readily fabricated, yet they exhibit significant defect concentration and relatively low strength, making them suitable only for non-load-bearing biomedical applications.

Structures yielding load-bearing scaffolds are predominantly ceramic. Several approaches have been employed to impart necessary mechanical strength to ceramics, including optimizing composites, laminates, porosity, and processing conditions. Adjusting the porosity of materials remains the most efficient method for controlling mechanical properties. In this context, metallic porous materials are especially interesting due to their esthetic appearance and mechanical properties closely resembling those of bones.

6. Process of Scaffold Fabrication

Bone scaffolds are three-dimensional biomaterials designed to assist and guide the regeneration of natural bone tissue. Through cell adhesion, migration, growth, and differentiation, they promote the formation of new bone tissue. Bone scaffolds should provide mechanical support in the defect, repair the environment for attachment, proliferation, and differentiation of stem cells, and are typically lost after the growth of new bone tissue. Tissue engineering commonly uses scaffolds to simulate the extracellular matrix, promoting cell adhesion and differentiation in vivo or in vitro and guiding tissue growth. Preparation methods include multiple, phase separation, electrospinning, template, freeze-drying, and impregnation techniques.

The replication technique involves immersing a polymeric sponge or a close-packed template of spheres with high porosity into a highly viscous slurry of the scaffold material, followed by drying, pyrolyzing to remove the polymeric template, and sintering to obtain the ceramic scaffold. The technique can produce scaffolds with high porosity and high pore interconnection, replicating a hierarchical structure similar to natural bone. Mainly, polymer sponges or templates composed of ceramic or metal spheres packed in a body-centered imploding mode are used.

Although suitable for various functional biomaterials, the process is mainly applied to ceramic biomaterials (such as calcium phosphate, bioactive glass-ceramics, and bioactive glass) and titanium metal-alloy bone scaffolds.

6.1. Template Preparation

Materials such as replica foam or wood serve as porous substrata, while ceramic slurries or metal formulations form the coating phases. The surfaces of polymer templates undergo treatment to enhance the wetting characteristics necessary for subsequent coverage with ceramic slips or concentrated metal slurries.

Polymer templates supporting the ceramic or metal slurry coating are eradicated through thermal processes; any residual carbonaceous material from incomplete combustion is eliminated during ceramic sintering cycles or metal curing phases. Once the polymer has been removed, the ceramic or metallic coating attains sufficient self-supporting strength, enabling it to preserve the microcellular structure that replicates the original configuration of the polymer template.

6.2. Coating Techniques

Coating techniques represent a critical step in preparing scaffolds via the replication method. Both polymer and metal scaffolds can be manufactured by replication of polymer foam to produce a porous skeleton. However, current research on polymer foam replication primarily aims to fabricate ceramic and metallic materials. The main deficiency of the replication technique lies in the inability to independently manufacture polymer foam with a porous structure. Given the excellent properties and diverse applications of polymer foam, the question arises of whether polymer scaffolds can be obtained by coating polymer foam with ceramic or metallic materials. By selecting appropriate metal and metal compound powders and formulating suitable slurries, porous structures with distinct properties can be replicated. This approach facilitates investigation of properties that are otherwise difficult to examine in porous polymer materials.

The replication method for polymer scaffold preparation can be divided into three categories according to the coating material: ceramics, metals, and ceramics–metals. Taking ceramic coating as an example, the process involves coating the polymer foam template with ceramic slurry and then sintering or curing it to obtain the ceramic scaffold. This process generally includes pre-treatment of the template, preparation of the slurry, coating of the template, and sintering or curing of the coated template. However, it is challenging to prepare scaffolds with high porosity by the replication of polymer foams with porous ceramics or metals. [20][17][21]

6.3. Sintering and Curing

Sintering of ceramic/polymer-coated templates plays a crucial role in fabricating ceramic or metal bone scaffolds. The primary purpose of sintering is to remove a sacrificial template used during the scaffold preparation process while simultaneously densifying the ceramic matrix within those templates. Achieving complete removal of the sacrificial template and a dense ceramic structure is influenced by the sintering temperature and temperature ramp rate. Materials possessing lower melting/sintering points facilitate this process. Typical ceramic sintering temperatures range from 1150 to 1500 °C, but preparing strontium-doped bioactive glass scaffolds at temperatures below 900 °C is also feasible if the material crystallizes at higher temperatures. Geometric shapes like rods and rectangular prisms can be produced according to patient needs by adjusting mold shapes.*

The curing process is generally associated with polymer replication methods, particularly in fabricating porous polydimethylsiloxane (PDMS) scaffolds. In this context, a specific ratio (15:1) of pre-polymer to curing agent is mixed and degassed to eliminate bubbles. This mixture is then infiltrated into a sugar template via a vacuum chamber to ensure thorough penetration. Curing proceeds for 24 hours at 65 °C, resulting in a solid and porous polymer scaffold after

subsequent template removal. [22][23][24]

7. Characterization of Bone Scaffolds

Physical, chemical, and biological analyses are conducted on replicated polymer-, ceramic-, and metal-based bone scaffolds. Physical analyses evaluate parameters such as apparent density, porosity, and compressive strength. Chemical analyses investigate compounds, functional groups, crystal phases, or corrosion resistance. Biological analyses test biocompatibility and cell adhesion.

In physical analyses, apparent density and porosity relate to the contents of naphthalene or sponge, while compressive strength depends on the type of slurry and sintering temperature used. Chemical analyses of compound and functional group determine whether materials with the expected formula are synthesized. Crystal phase assessment reveals whether nacre or coralline properties remain after sintering. The corrosion resistance of metal scaffolds is also assessed. Finally, cytotoxicity and cell adhesion analyses indicate the potential for biomedical applications in bone tissue engineering.

7.1. Physical Characterization

Performance assessment of scaffolds requires the understanding of relationships between physical properties such as porosity, morphology, and pore size. These scaffold parameters provide information for their evaluation and are required for conforming to specific requirements for developing bone scaffolds that meet the suitable standards of the host bone. Additives and modifying agents have been used during the preparation of bone scaffolds with replication techniques to enhance the physicochemical properties of scaffolds. Moreover, the use of different types of templates is considered as an important factor in the replication technique to achieve controllable porosity and pore size and in turn influence the mechanical properties of scaffolds.

In polymer replication, the polymer is coated on a polymer template. The solvent for the polymer is removed during sintering and bone scaffolds are obtained in the hollow form. The skeleton of the polymer template enables the formation of a porous structure in polymer-scaffolds. Ceramic and metal scaffolds are manufactured by coating the ceramic or metal slurries on polymer templates. In these cases, different powder additives are used during slurry preparation to reduce internal stresses and shrinkage during the sintering process. Inorganic and metallic additives are used to enhance the mechanical properties of scaffolds. [25][26][27]

7.2. Chemical Characterization

TE bones have been prepared using replication techniques employing NaCl or paraffin spheres as repellents for MT porosity. A technique similar to slurry dipping has been utilized to prepare pieces coated with a slurry of conventional bone cement and bioactive glass. The regular replication routes that have been proposed different support materials; these purposely prepared frameworks determine the MT geometry.

Bovine bone spheres have been used as a template to prepare bioactive glass composite scaffolds. Saos-2 osteosarcoma cell line seeding has demonstrated the potential role of silicone addition in increased cell adhesion and proliferation. The presence of mustard stalk fibre in the scaffolds has resulted in improved cell viability. Porosity conferred by gold foams has been used to manufacture new scaffolds based on polyhydroxyalkanoates (PHA) coating, the major class of biopolyesters synthesized by bacteria. Biocompatibility of the coated foams (supporting titanium) has been tested by seeding a set of cell lines.

7.3. Biological Characterization

Bone scaffolds designed for implant applications should be free from toxic residues; therefore, it is crucial to remove residuals from the preparation process and assess the scaffold material's cytotoxicity. The commonly used cytotoxicity assay for screening a broad range of scaffold

materials for tissue engineering is the MTT assay. The initial step in the biological characterization of the prepared bone scaffolds is to assess their cytotoxicity against a suitable cell line, such as osteosarcoma (MG63) cells. The MTT assay has been employed to evaluate the cytotoxicity of these scaffolds.

MG-63 cells are cultured and harvested after 10–13 d. The MTT assay requires approximately 5 × 10^4 cells; for other cell lines with different sizes and growth rates, careful consideration is necessary when maintaining the same numbers of cells per well. In vitro cell culture studies are conducted to detect cytotoxic effects of residuals. The MTT assay is based on the cleavage of the tetrazolium salt MTT by mitochondrial dehydrogenase in viable cells, producing a blue formazan product—insoluble in aqueous solutions—that can be extracted with dimethyl sulfoxide (DMSO). The absorbance of this product at 570 nm, measured using an enzyme-linked immunosorbent assay (ELISA) reader, correlates with the number of living cells in culture. [28][29][30]

8. Applications of Bone Scaffolds

Bone scaffold replication method is widely employed in tissue engineering [1]. Bone scaffolds are frameworks for tissue-engineered bone and play many important roles in orthopedics, oral implants, and periodontal surgery [31]. Bone scaffolds should have high porosity and interconnected pores, good cytocompatibility, suitable mechanical strength, relatively slow degradation rate, and ease of surgical operation [32].

The polymer replication technique, ceramic replication technique, metal replication technique, and more are often used to prepare bone scaffolds. Scaffolds fabricated by the replication technique are highly porous with interconnected pores, strong, and biocompatible. The preparation process starts with the preparation of a sacrificial template, fully immersed in slurry, dried, and then sintered or cured. The characteristics of the replicas are similar to those of the starting templates.

8.1. Orthopedic Applications

Bone scaffolds are considered a new option in tissue engineering and are also used in various clinical applications such as bone augmentation in dental surgery. The preparation of bone scaffolds using replication techniques has been heavily studied. Bone constitutes the main supporting framework structure of the body. Damage to the bone from trauma, disease, or injury causes the support structure of the body to become weak. In orthopedic surgeries bone, bone scaffold or related materials are used to support the main skeleton structure. In bone scaffolds, the biomechanics, structures, and porosities are very important for proper functioning and are made by replicating polymer structures using different materials. The polymer replicated ceramic, polymer replicated metal, and polymer-replicated composite materials techniques for bone scaffolds are heavily studied. Bone scaffolds are prepared with different materials using the replication technique, including ceramic, metal, and composites. [33][34][9]

8.2. Dental Applications

Bone scaffolds used in dental surgery must be designed with great care, given their role in supporting the regeneration of the facial skeleton and the complex compromise required between compression strength, porosity, and infiltration ability. The replication technique enables the successive addition of different material phases, including functionally graded materials, allowing sophisticated designs. Làzàr et al. employed the foam replication process to prepare biphasic ceramic composites suitable for dental surgery, utilizing a hydroxyapatite (HAP)–β-tricalcium phosphate (β-TCP) starting material with MgO and SiO2 additions.

The application of various amounts of MgO and SiO2 as sintering aids resulted in different porosity and compression strength values; moreover, the use of a biphasic HAP–β-TCP matrix influenced the overall scaffold behavior. A direct correlation was found between the amount of

MgO added and the content of the β-TCP phase formed during sintering. The presence of the second phase had a greater influence on mechanical strength than the porosity of the samples. Finally, samples showing promising results were coated with α-quartz (α-SiO2), which greatly improved stability during in vitro tests. [35][36][37][38]

8.3. Tissue Engineering

Tissue engineering is an emerging discipline with direct clinical applications. It offers a possible solution to challenges such as donor-site shortage, local tissue necrosis, donor-tissue morbidity, and postoperative scarring and deformity. One strategy in tissue engineering focuses on the repair of damaged bone tissues using scaffolds loaded with cells and growth factors, followed by transplantation to defect sites. Critical requirements for these bone substitutes include biocompatibility, proper degradation characteristics, appropriate porosity and pore sizes, mechanical strength, and structural support during new bone growth.

Inorganic bone scaffolds are commonly fabricated from bioactive ceramics like calcium phosphate, alumina, and zirconia. Various methods—freeze-drying, solvent casting, polymeric sponge, vapor deposition, fibre bonding, and electrochemical deposition—have been employed in their production. Among these, the replication technique using polymeric sponges stands out for its ability to create highly porous and interconnected ceramic and metallic foams, attributes that render them suitable for tissue engineering scaffolds and load-bearing implant coatings. [39][40][9]

9. Challenges in Scaffold Preparation

Preparation of Bone Scaffolds Using Replication Techniques The ceramic replication technique involves coating a 3D open-porous template with appropriate material slurry to obtain a replica scaffold. This method is commonly used to prepare low-cost, highly porous scaffolds with excellent mechanical properties. By adjusting the size, shape, and manufacturing technology of the open-porous template, ceramic replication allows the preparation of porous scaffolds in virtually any form. The technique is primarily used for the preparation of ceramic and metal scaffolds. Various bone scaffolds prepared using the replication technique are summarized, with the addition of functional additives mentioned. Bone is highly vascularized tissue that self-repairs; however, certain injuries, such as large bone defects or bone cancer, inhibit the healing process. Traditional unnatural reconstruction methods do not lead to the formation of new tissue. Bone scaffolds enable the regeneration process by supporting the growth of new blood vessels and connective tissue. In comparison, the regeneration process is similar for other tissues, such as cartilage and skin, and a supporting framework also promotes faster healing in these cases. The use of a suitable template of the required shape for the replication method is advantageous for the preparation of scaffolds for craniofacial and other part replacement. [41][42][43]

9.1. Scaling Up Production

A brief outline of the main materials and methods used for preparing bone scaffolds by replication techniques highlights key design aspects, materials parameters, and the characterization and applications of resulting bone scaffolds. Bone scaffolds prepared by replication techniques form an important group of biomaterials with applications in tissue engineering and medicine, especially in orthopaedics and bone tissue damage treatments. The main replication methods of bone scaffolds with different biomaterials in the form of powders or fibres, including polymers, ceramics and their composites, and metals, together with the main additives for viscosity control, wettability modification, phase-stabilization or fibre-binding ability, are reviewed.

Many growth factors, drugs and ions in addition to allowing bone-tissue regeneration with the scaffolds prepared by replication methods can also provide an additional added value. Certain design parameters such as pore size, pore morphology and porosity are essential for cell/tissue ingrowth and biofluid circulation of the material. Mechanical strength and elasticity modulus are

two key factors for practical applications of scaffold materials. A brief outline of the main manufacturing methods used for the preparation of bone scaffolds by replication techniques summarizes key design aspects, materials parameters, and the characterization and applications of the resulting bone scaffolds. [5][8][44][45] [5][8][44]

9.2. Maintaining Consistency

Porosity is a very important attribute in bone scaffolds. The porosity of bone scaffolds prepared by replication techniques shows a significant variation due to variations in the shape, size, and volume fraction of porogen and biomaterial slurries. Achieving the desired pore characteristics in sintered scaffolds can thus be difficult because of undissolved parts of the pore-forming agent, breakage of porogen particles during slurry coating and drying stages, segregation of porogen and slurry particles, and delamination during the manufacturing process. Studies have explored the use of various porogenic particles, including sodium chloride, sodium bicarbonate, carboxymethyl cellulose, and deflocculated silica, in the preparation of calcium phosphate scaffolds through replication methods. High compressive strength has also been obtained in scaffolds prepared in-house by coating polyurethane foams with silica-based slurry.

The consistency of porosity and pore shape is affected by the shape and volume fraction of the template. Irregular-shaped templates pose an elevated challenge in controlling density and porosity. Silicone or wax-based foam can be manufactured at the industrial stage and used as templates. To achieve consistent production in sodium chloride crystals, the manufacturing method must be as simple and controlled as possible. Controlling porosity and density may be more straightforward in ceramic scaffolds prepared with template replication but using silica sol coating. Sintering temperature, the number of coating cycles, and slurry properties can be adjusted to maximize mechanical properties. It has been reported in studies that the strength and toughness of scaffolds increase as a non-linear function of densification in the replication technique, scCO2-foaming method, and vendidoze method.

9.3. Regulatory Considerations

Establishing premarket review, investigational device and exemption programs, adequately addressing questions of safety and effectiveness, applies equally to scaffolds prepared by replication methods. Considerable effort has been directed at establishing cohorts of products prepared by these manufacturers to obtain regulatory approval and thereby demonstrate substantial equivalence with reference products that are on the market. Regulatory clearance to market the products and the subsequent commercial success signals the bidirectional benefit to patients and manufacturers.

While the investigation of bone-scaffold preparation using replication techniques does not specifically address regulatory considerations, any application of the replication technique for this purpose must recognize such issues. The use of bone scaffolds prepared by replication techniques for orthopaedic and dental applications falls under the Medical Device Directive and therefore has to fulfil stringent clinical performance and safety requirements. It is thus necessary to address the regulatory aspects of scaffolds prepared by these replication techniques to be able to take advantage of a legitimate commercial opportunity. [46][47][48]

10. Future Directions in Bone Scaffold Research

Research in bone scaffold preparation using replication techniques continues to advance in several key areas. The development of composite scaffolds aims to integrate the benefits of different material classes by combining ceramics with polymers or metals. Such composites show promise in matching the graded properties of native bone tissue, mimicking the transition from hard, dense cortical bone to soft, pliable cancellous bone. The exploration of new materials for replication extends beyond traditional systems, with efforts to enhance biomimetic properties, processing efficiency, clinical compatibility, and cost-effectiveness. Additionally, the optimization of process parameters remains a priority to achieve better mechanical and

biological performance of the scaffolds. These multidisciplinary investigations benefit from emerging two-dimensional materials, such as graphene, which find increasing applications in tissue engineering.

The scaling-up of the production of replication-derived scaffolds presents challenges in terms of repeatability and consistency. Furthermore, considerations of end-users' needs and regulatory issues are critical in guiding the clinical application of these technologies.

10.1. Innovative Materials

The preparation of bone scaffolds using replication techniques is a key enabling technology in tissue engineering. Various innovative materials and their combinations have been developed, including boron nitride-reduced graphene oxide (B-rGO) nanocomposites in a polyvinyl alcohol–chitosan (PVA–chitosan) matrix. These nanocomposites have demonstrated non-cytotoxic behavior and antioxidant activity that are beneficial for osteoblast formation. Bone scaffolds prepared by replication techniques are especially well suited for applications with high porosity requirements because templates with defined porosity can be easily used. The high strength of these materials makes them useful for bone repair after trauma or surgery.

Replication techniques in polymer, ceramic, and metal processing provide an alternative route for the production of bone scaffolds based on polymer foaming principles. A crucial role in replication techniques is played by the material that forms the scaffold and the modifiers that direct the scaffold properties, such as the level of porosity, the porosity grade, or specific chemical elements in the composition that favor the interaction between the bone and the reconstructed tissue. Material selection is also related to the application area of the scaffold. The preparation of materials according to the properties of the final scaffold follows a methodical approach: the choice of synthesis procedure depends on the presence of materials that direct self-assembly, the reactivity of the material with other groups or functional groups for self-assembling and the microenvironment, and balances hydrophobicity and hydrophilicity. Through the careful selection of additives that direct the properties or strengthen the material, a much-improved preparation of bone scaffold is possible.

10.2. Advanced Fabrication Techniques

Scaffolds provide a porous and biocompatible structure for supporting the growth and differentiation of new cells. A porous scaffold network permits the transmission of nutrients and waste as well as gas exchange. A high amount of porosity ensures higher water uptake and mimics the functions of the extracellular matrix in cell activity. Bone scaffolds are utilized in bone repair and regeneration; they must therefore possess a bone-like three-dimensional (3D) microstructure and sufficient mechanical strength. Orthopedic and dentistry implants are designed in such a way that they should be light-weight, fatigue-resistant, non-toxic, strong, biologically inert, stable, and biocompatible. Besides stoichiometric considerations regarding the synthesis of scaffold materials, the design-related parameters such as porosity, pore size, pore shape, interconnectivity, and mechanical strength also greatly influence the biological and mechanical response of a biomaterial in-vivo. The processing of bone scaffold–bone substitutes involves a number of methods which vary for different materials (metals and alloys, ceramics, bioglasses, and bioplastics). For the preparation of organic and inorganic porous scaffolds, different techniques such as phase separation, gas foaming, freeze drying, solvent casting, particulate leaching, thermally induced phase separation, fiber meshes, additive manufacturing, electrospinning, replication, and other approaches are employed.

Replication techniques are utilized to deposit titanium and titanium dioxide layers on sponges. Scaffolds are prepared using replicate sponge method for hard tissues by coating mineral-loaded slurries onto a porous polymer template that is subsequently burned out and sintered during heat treatment. The slurries can be loaded with biologically active minerals to promote osseointegration as well as the presence of the mineral omegas in body tissue. Salt or sugar

particles can be added to the primed template to increase the porosity filling capabilities of the polymer support. The replication technique is widely used for the preparation of ceramic (hydroxyapatite, calcium silicate, titania, tricalcium phosphate) and metallic (titanium, magnesium, tantalum) porous bone scaffolds. Porous green body scaffolds are molded by emulating the architecture of the porous template, which is then sintered or strengthened.

10.3. Personalized Medicine Approaches

The objective of personalized medicine is to tailor drugs and devices to patients considering their lifestyle and genetic makeup. Personalized medicine for bone scaffolds involves designing and manufacturing scaffolds tailored to patientsâ€™ specific age group and physical activity, mainly by adjusting physical properties, such as porosity and mechanical strength. Imaging modalities, such as computed tomography (CT) and magnetic resonance imaging (MRI), expertly reconstruct three-dimensional bone models tailored to each patient using computer-aided design (CAD) software. The scaffold trabecular structure may be designed to mimic the actual internal framework of the bone, thus improving mechanical strength and allowing for tissue growth. Porous scaffold metrological parameters, such as porosity and scaffold thickness (trabecular bone thickness), can also be designed to correspond to the patientâ€™s medical history and daily activity.

Bone scaffolds that meet the medical standards and patient-specific needs can be fabricated with almost any shape using the 3D printing technique. The area of 3D printing is rapidly growing and promises the fabrication of scaffolds tailored for each patient. Tomographic studies, computer-aided design (CAD) of the scaffold geometry, and the conversion to stereolithographic (STL) files enable the reconstruction and fabrication of scaffolds that either correspond to the dimension of the defect or that actively promote tissue growth in any ledge bone.

11. Case Studies on Bone Scaffold Applications

Replication techniques have been extensively employed to prepare bone scaffolds for orthopedic and dental applications as well as in tissue engineering [32]. Notable clinical examples include hydroxyapatite and bioactive glass scaffolds that successfully function in maxillo-facial and calvarial defects [1]. Other replication-based scaffolds, such as those incorporating ceramic additives, hold promise for similar clinical uses.

11.1. Successful Clinical Trials

Several bone substitutes have been introduced for clinical use; however, their application remains limited due to inadequate tissue formation or mechanical strength.

This combination results in a material that is easily manipulated and facilitates cellular infiltration and neovascularization. Histological evaluations confirm that the scaffold material is gradually resorbed and replaced by new woven bone, indicating successful integration. Subsequent clinical application in 11 cases demonstrates favorable guidelines for the use of the bFGF-loaded BCP scaffold in bone regenerative procedures.

[49] focus on the development of artificial bones capable of conforming to bone defect shapes. Utilizing a three-dimensional (3D) printer, b-tricalcium phosphate (β-TCP) scaffolds are fabricated to meet specific structural and geometric requirements. The scaffolds are further coated with recombinant collagen peptide (RCP) to enhance bone regeneration potential. To optimize cellular contribution to the regenerative process, the culture duration of bone marrow cells prior to loading onto the scaffold is investigated. Osteogenic markers peak at 7 days of culture, with later-stage differentiation observed at 14 days. Transplantation of cells cultured for 7 days onto the scaffolds yields positive outcomes in both subcutaneous and cranial defect models, supporting the protocol for clinical application.

11.2. Comparative Studies

Replication techniques use ceramic, metallic, polymeric, or composite templates, which are filled

with the desired biomaterial and subsequently treated to solidify the structure. Extraction of the original template material creates an interconnective open-porosity network that transfers to the resulting bone scaffold. Several methods exploit different template materials, including metal impregnation, polymer burning, polymer nodule agglomeration, ceramic burning, metal sponge impregnation, and polymer sponge replication [32]. Foam replication typically involves impregnation of a porous, open-cell structure (such as polyurethane foam) with a ceramic slurry, followed by drying, polymer burnout, and sintering. The polymer foam acts as a temporary support, facilitating the formation of a replica with an open and interconnected porous network and wall structure similar to human trabecular bone.

Foam replication is also employed with metallic samples, where the ceramic slurry consists of a mixture of metal powders and acids. After thermal treatment, metal ions or powder support the porous structure. Polymeric scaffolds can be prepared by using PU sponges as porous templates coated with ceramic slurries containing bicomponent polymers, which are then thermally treated at lower temperatures to cure the polymers, a method described as cane sugar replication.

12. Conclusion

Bone scaffolds provide temporary structural support for new bone tissues during the natural bone healing process. Replication techniques, capable of controlling porosity and interconnectivity, enable the production of scaffolds from various materials with excellent biocompatibility and bioactivity. Polymers are commonly employed as templates, whereas biomaterials such as ceramics, polymers, and metals serve as the scaffold matrix. The intrinsic tissue and bone regenerative properties of these scaffolds facilitate osseointegration and support patient health. At the same time, they meet the required mechanical performance for specific biomedical applications.

The preparation of bone scaffolds involves biological–material selection, design of the synthetic bone scaffold, and the replication fabrication process. Biological materials including polymers, ceramics, and metals must be biocompatible, bioactive or bioinert, and biodegradable. Synthetic bone scaffolds replicate the extracellular matrix for cells and new bone tissues, necessitating high porosity and interconnectivity for nutrient flow and waste removal, as well as adequate mechanical properties. The replication technique comprises the production of a polymer template, coating the template with a biomaterial slurry, and subsequent sintering or curing to form the final bone scaffold. Incorporating additives into the slurry can adjust viscosity and improve porosity, bioactivity, and mechanical strength. Culminating in a high-performance bone scaffold, the preparation process employs replication techniques based on the optimal combination of biological materials, scaffold design, and fabrication parameters.

References:

1. T. D. Szűcs, "Production of hard tissue scaffolds using three-dimensional printing method," 2009. [PDF]
2. J. Montero, A. Becerro, B. Pardal-Peláez, N. Quispe-López et al., "Main 3D Manufacturing Techniques for Customized Bone Substitutes. A Systematic Review," 2021. ncbi.nlm.nih.gov
3. G. Jia, H. Huang, J. Niu, C. Chen, J. Weng, and F. Yu, "Exploring the interconnectivity of biomimetic hierarchical porous Mg scaffolds for bone tissue engineering: Effects of pore size distribution on mechanical …," Journal of Magnesium, vol. 2021, Elsevier. sciencedirect.com
4. M. Guidoum, N. Verdú, J. L. Todolí, L. P. Maiorano, "Development of open-pore polymer and ceramic foams/Guefoams with tunable fluid-dynamic properties: Application in highly efficient water bacteria removal," Materials & Design, vol. 2023, Elsevier. sciencedirect.com

5. F. Mukasheva, L. Adilova, A. Dyussenbinov, "Optimizing scaffold pore size for tissue engineering: Insights across various tissue types," Frontiers in ..., 2024. frontiersin.org
6. M. Khajehmohammadi and R. Azizi Tafti, "Effect of porosity on mechanical and biological properties of bioprinted scaffolds," *Journal of Biomedical Engineering*, vol. 2023, Wiley Online Library. [HTML]
7. H. Mohammadi and M. Sepantafar, "How does scaffold porosity conduct bone tissue regeneration?" *Advanced Materials*, vol. 2021. [HTML]
8. A. T. Prakoso, H. Basri, D. Adanta, I. Yani, and M. I. Ammarullah, "The effect of tortuosity on permeability of porous scaffold," Biomedicines, 2023. mdpi.com
9. E. Fiume, S. Ciavattini, E. Verné, and F. Baino, "Foam replica method in the manufacturing of bioactive glass scaffolds: out-of-date technology or still underexploited potential?," Materials, 2021. mdpi.com
10. L. Tabard, V. Garnier, E. Prud'Homme, E. J. Courtial, "Robocasting of highly porous ceramics scaffolds with hierarchized porosity," Additive Manufacturing, vol. 2021, Elsevier. sciencedirect.com
11. A. Kumar and A. Jacob, "Techniques in scaffold fabrication process for tissue engineering applications: A review," J. Appl. Biol. Biotechnol, 2022. academia.edu
12. T. M. Koushik and C. M. Miller, "Bone tissue engineering scaffolds: function of multi-material hierarchically structured scaffolds," *Advanced Healthcare Materials*, vol. 2023, Wiley Online Library. wiley.com
13. B. Huzum, B. Puha, R. M. Necoara, "Biocompatibility assessment of biomaterials used in orthopedic devices: An overview," *Experimental and Therapeutic Medicine*, vol. 21, no. 1, 2021. spandidos-publications.com
14. M. Kus-Liśkiewicz, P. Fickers, and I. Ben Tahar, "Biocompatibility and cytotoxicity of gold nanoparticles: recent advances in methodologies and regulations," *International Journal of …*, 2021. mdpi.com
15. L. Khalef, R. Lydia, K. Filicia, et al., "Cell viability and cytotoxicity assays: Biochemical elements and cellular compartments," Cell Biochemistry and..., 2024. researchgate.net
16. E. Rosellini and M. G. Cascone, "Microfluidic fabrication of natural polymer-based scaffolds for tissue engineering applications: a review," Biomimetics, 2023. mdpi.com
17. I. M. Adel, M. F. ElMeligy, and N. A. Elkasabgy, "Conventional and recent trends of scaffolds fabrication: a superior mode for tissue engineering," Pharmaceutics, 2022. mdpi.com
18. A. K. Badekila, S. Kini, and A. K. Jaiswal, "Fabrication techniques of biomimetic scaffolds in three-dimensional cell culture: A review," Journal of cellular physiology, 2021. [HTML]
19. S. B. Khan, S. Irfan, Z. Zhang, and W. Yuan, "Redefining medical applications with safe and sustainable 3D printing," ACS Applied Bio Materials, 2025. [HTML]
20. T. L. do Amaral Montanheiro and V. M. Schatkoski, "Recent progress on polymer scaffolds production: Methods, main results, advantages and disadvantages," *Express Polymer Letters*, vol. 16, no. 9, pp. 823-836, 2022. academia.edu
21. T. Liu, Y. Wang, and T. Kuang, "Oriented porous polymer scaffolds in tissue engineering: a comprehensive review of preparation strategies and applications," Macromolecular Materials and …, 2024. wiley.com

22. K. Pandey, D. Poddar, S. J. Kim, and H. M. Yoo, "DEVELOPMENT OF INJECTABLE SCAFFOLDS ENCAPSULATING BIOACTIVE GLASS FIBERS: OPTIMIZATION AND FORMATION OF POROUS POLYMER …," Ceramics International, 2025. [HTML]

23. A. Bahatibieke, S. Wei, H. Feng, J. Zhao, M. Ma, and J. Li, "Injectable and in situ foaming shape-adaptive porous Bio-based polyurethane scaffold used for cartilage regeneration," Bioactive Materials, vol. 2024, Elsevier. sciencedirect.com

24. T. A. Abdelrahman, A. Motawea, M. S. El-Dahhan, et al., "Erythropoietin-isosorbide dinitrate-loaded nanocomplex cryogel scaffold: a multifunctional smart dressing for diabetic wound healing," Life Sciences, 2025. [HTML]

25. H. Luo, Y. V. Kaneti, Y. Ai, Y. Wu, F. Wei, J. Fu, et al., "Nanoarchitectured porous conducting polymers: from controlled synthesis to advanced applications," *Advanced Materials*, vol. 2021, Wiley Online Library. uq.edu.au

26. H. Y. Zhao, M. Y. Yu, J. Liu, X. Li et al., "Efficient preconstruction of three-dimensional graphene networks for thermally conductive polymer composites," Nano-micro letters, 2022. springer.com

27. A. Shiohara and B. Prieto-Simon, "Porous polymeric membranes: Fabrication techniques and biomedical applications," *Journal of Materials*, vol. 2021. [HTML]

28. E. Jablonska, J. Kubásek, D. Vojtěch, T. Ruml et al., "Test conditions can significantly affect the results of in vitro cytotoxicity testing of degradable metallic biomaterials," Scientific Reports, 2021. nature.com

29. M. A. Siddiquee, M. U. Parray, M. R. Kamli, et al., "Biogenic synthesis, in-vitro cytotoxicity, esterase activity and interaction studies of copper oxide nanoparticles with lysozyme," *Journal of Materials Research*, vol. 2021, Elsevier. sciencedirect.com

30. H. Çobanoğlu, M. Belivermiş, E. Sıkdokur, Ö. Kılıç, "Genotoxic and cytotoxic effects of polyethylene microplastics on human peripheral blood lymphocytes," Chemosphere, vol. 2021, Elsevier. [HTML]

31. J. Wu, Y. Zhang, Y. Lyu, and L. Cheng, "On the Various Numerical Techniques for the Optimization of Bone Scaffold," 2023. ncbi.nlm.nih.gov

32. E. Fiume, S. Ciavattini, E. Verné, and F. Baino, "Foam Replica Method in the Manufacturing of Bioactive Glass Scaffolds: Out-of-Date Technology or Still Underexploited Potential?," 2021. ncbi.nlm.nih.gov

33. A. I. Ansari and N. A. Sheikh, "A review of bone regeneration mechanisms and bone scaffold fabrication techniques (conventional and non-conventional)," Journal of The Institution of Engineers (India), vol. 2022, pp. 1-12, Springer, 2022. [HTML]

34. M. N. Collins, G. Ren, K. Young, S. Pina, et al., "Scaffold fabrication technologies and structure/function properties in bone tissue engineering," *Advanced Functional Materials*, vol. 31, no. 1, 2021. wiley.com

35. R. A. Shakir and R. Géber, "Structure and properties of ZrO2-Al2O3-MgO porous ceramic for biomedical applications," Results in Engineering, 2023. sciencedirect.com

36. J. I. Kim, J. Kim, S. M. Lee, H. Jeong et al., "Low-temperature hot-press sintering of AlN ceramics with MgO–CaO–Al2O3–SiO2 glass additives for ceramic heater applications," Ceramics International, 2022. [HTML]

37. Y. Wang, S. Zhu, X. Zhou, and T. Zhang, "Effect of sintering process on micro-structure and properties of mullite porous ceramics containing magnesium oxide," *Journal of Physics*, 2022. iop.org

38. G. Yumuşak, "Enhancing Compressive Strength in Tialite Ceramics Through Additives and Sintering Optimization," Ceramics International, 2025. [HTML]

39. A. Sutygina, U. Betke, and M. Scheffler, "Manufacturing of Open-Cell aluminium foams: comparing the sponge replication technique and its combination with the freezing method," Materials, 2022. mdpi.com

40. R. Yeetsorn, S. Tungkamani, and Y. Maiket, "Fabrication of a ceramic Foam Catalyst using polymer foam scrap via the Replica technique for dry reforming," ACS omega, 2022. acs.org

41. Y. Man, G. Ding, L. Xudong, K. Xue, and D. Qu, "A review on porous ceramics with hierarchical pore structure by 3D printing-based combined route," *Journal of Asian Ceramic Society*, vol. 9, no. 1, pp. 1-12, 2021. tandfonline.com

42. V. S. Seesala, R. Rajasekaran, A. Dutta, and P. V. Vaidya, "Dense-porous multilayer ceramics by green shaping and salt leaching," Open Ceramics, vol. 2021, Elsevier. sciencedirect.com

43. K. Nazari, P. Tran, P. Tan, A. Ghazlan, and T. D. Ngo, "Open Ceramics," Additive Manufacturing. researchgate.net

44. A. C. Moreira, C. P. Fernandes, M. V. de Oliveira, et al., "The effect of pores and connections geometries on bone ingrowth into titanium scaffolds: an assessment based on 3D microCT images," *Biomedical Materials*, vol. 2021. researchgate.net

45. Y. Zhang, N. Sun, M. Zhu, Q. Qiu, P. Zhao, and C. Zheng, "The contribution of pore size and porosity of 3D printed porous titanium scaffolds to osteogenesis," Biomaterials, vol. XX, pp. XX-XX, 2022. [HTML]

46. K. M. Jurczak and T. A. B. van Der Boon, "Recent regulatory developments in EU Medical Device Regulation and their impact on biomaterials translation," *Journal of Medicine*, 2025. wiley.com

47. P. Ghelich, M. Kazemzadeh-Narbat, and others, "(Bio) manufactured solutions for treatment of bone defects with an emphasis on US-FDA regulatory science perspective," *Advanced Materials*, vol. 2022, Wiley Online Library. wiley.com

48. K. Ala-Mononen, "BIOMATERIALS IN THE MANUFACTURING OF ADVANCED THERAPY MEDICINAL PRODUCTS USED IN BONE REPLACEMENT," 2022. tuni.fi

49. R. Umeyama, T. Yamawaki, D. Liu, S. Kanazawa et al., "Optimization of culture duration of bone marrow cells before transplantation with a β-tricalcium phosphate/recombinant collagen peptide hybrid scaffold," 2020. ncbi.nlm.nih.gov