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Scaffolds Used for Bone Tissue Regeneration: Review.

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ABSTRACT

The bone is a remarkable organ which performs many important functions in the body. Hence, any injury or defect to it is a critical issue. Although it possesses an intrinsic capacity for regeneration, the process is limited or impaired in several cases. Bone grafting techniques are used to overcome this insufficiency. Autografting, which was the most preferred technique, has drawbacks of donor site morbidity, lack of donor supply, and multiple surgery requirements. Other conventional techniques such as allografts and xenografts are also limited by donor supply and host responses. To cope with this, a biomaterial-based alloplast approach was adopted. Alloplasts serve as temporary three-dimensional scaffold or framework for the repair and regeneration of the damaged bone, which can later be removed by a surgery or naturally degraded by the body itself. There are several parameters which determine scaffold performance and success of regeneration. Over the years, various biomaterials have been experimented as scaffolds. In this review, some of such biomaterials, which include metals, polymers, ceramics, and their composites, are discussed and compared.

Keywords: Bone regeneration, fracture, scaffold, graft, osteoconduction, biomaterial.

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INTRODUCTION

Bone is a living, complex, hard tissue that constitutes the vertebral skeleton. It has an organic matrix phase composed primarily of collagen, with the inorganic phase embedded in it [1]. The calcium and phosphate containing inorganic crystals ultimately form hydroxyapatite. The bone morphology can be described as comprising of the cortical bone - the outer compact region, and the trabecular bone - the inner spongy region [1, 2]. The functions of the bone in the body include mechanically supporting soft tissues, protecting vulnerable organs, producing blood cells, serving as an anchor to muscles, and acting as a calcium reservoir [3]. It possesses high tensile and compressive strengths owing to the presence of the organic and mineral phases respectively [1, 3]. Yet it is susceptible to fracture and defects due to trauma injuries, infection, aging, bone diseases or others.

Bone can intrinsically regenerate itself as part of its response to injury, or during remodeling and skeletal development. The regeneration process is a well-controlled series of events, involving various cellular components and signaling pathways, aimed to restore the bone function [4]. The main cells responsible for the formation of bone are osteoblasts, which are derived from osteoprogenitor cells [5, 6]. They are present on bone surfaces, and aid in matrix formation and mineralization. They can further turn into bone lining cells if flattened or into osteocytes if they get trapped in the matrix. Osteoblasts are fully differentiated cells, and so formation of bone is entirely dependent on the presence of osteoprogenitor cells, which can migrate to target regions, proliferate and undergo differentiation into osteoblasts [7]. Osteoclasts also play a major role in bone remodeling [7, 8]. They are associated with the breaking down and resorption of bone tissue during its growth and healing.

Typical fracture healing process involves three main phases- reactive, reparative, and remodeling. The reactive phase constitutes inflammation (hematoma formation) of the fracture site, and formation of granulation tissue which decreases the strain at the site. The osteoclasts remove the dead tissue ends. In the reparative phase, the fracture gap is bridged by a callus composed of hyaline cartilage (formed from chondroblasts) and fibrous woven bone (formed from osteoblasts). The callus constituents are then replaced by lamellar bone. This occurs by endochondral ossification in cartilage and by bony substitution in the woven bone. Further, in the remodeling phase, the lamellar bone present in the form of trabecular bone is substituted with compact bone. This proceeds by the formation of a shallow pit (Howship's lacuna) by the osteoclasts followed by the filling of pit by osteoblasts. Thereby the callus is remodeled into a structure which closely resembles the strength and shape of bone [9, 10].

This regeneration process is however limited and does not provide adequate repair rate in cases like tibial fracture, maxillofacial injury, tumor resection etc. [8, 11]. The complications mainly arise due to infections at the defect site or due to non-union, mal-union or delayed union of the fracture gap [12]. In other cases such as avascular necrosis and osteoporosis, the regeneration process is impaired [8, 12].

To overcome this insufficiency and promote regeneration, bone grafting techniques were adopted. The grafting approaches are based on three basic mechanisms- osteoinduction, osteoconduction, and osteogenesis. Osteoinduction involves use of appropriate growth factors like bone morphogenetic proteins (BMP) that can stimulate the osteoprogenitor cells to differentiate into osteoblasts, subsequently aiding bone formation [13, 14]. Osteoconduction involves bone grafts acting as a scaffold which serve as a framework for the generation of the new bone [15]. The third mechanism, osteogenesis, promotes bone growth via osteoblasts originating from the graft [16]. All grafts show all or any one of the above three mechanisms.

Among the bone grafts, the current gold standard is the use of autologous grafts (autografts) where the graft is taken from another part of the body of the same individual [17-19]. Usually the iliac crest, tibial plateau, romus, tori, etc. are utilized for this purpose [20]. This is the most effective and preferred method, but has certain limitations such as donor site morbidity, lack of donor supply, and multiple surgery requirement [17, 21]. Grafts obtained from another individual (allograft) [22, 23] or other species (xenograft) [24] may also be used for guided bone regeneration. These are also limited by donor availability and triggers immunological responses in the host [25]. To compensate these drawbacks, artificial grafts (alloplasts) were introduced [26, 27]. These grafts are made of biomaterials, either natural or synthetic, and serve as a framework or scaffold for the repair and growth of damaged bone (osteoconductive mechanism). These scaffolds may also be incorporated with mesenchymal stem cells, osteoblasts, BMPs and chondrocytes to promote regeneration.

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TARGETED BIOMATERIAL PROPERTIES FOR AN IDEAL SCAFFOLD

There are various factors which dictate the kind of biomaterial to be used. Primarily, the material used has to be biocompatible, that is, it should not generate any systemic or local toxicity in the body [28]. Additionally, it should be non-carcinogenic, non-teratogenic, and non-immunogenic. Secondly, bioresorbability or biodegradability should be optimum [17, 29]. The material should get absorbed in the body without causing any adverse effects after successful regeneration. The rate of degradation should match with the rate of regeneration. The degradation products must be non-toxic and preferably should stimulate osteoblastic cells and enhance bone formation. Further, it should have sufficient porosity and pore distribution so as to promote the migration, adhesion, proliferation, and differentiation of the bone cells [30-32]. The porous architecture and interconnected pore networks are important characteristics which determine the amount of mass transport across the scaffold and the extent of cell seeding. It is also important for angiogenetic responses or vascularization of the newly forming bone. Since the graft acts as a scaffold, it should have adequate strength, stiffness, and fatigue resistance [30, 33]. Stiffness is a critical parameter. Any mismatch of its value with that of natural bone may lead to bone failure or atrophy. It should also provide the required mechanical and thermal stability as it has to undergo continuous stress cycles, especially if non-resorbable materials are used. Availability of the material, cost, and ease of processing are also important parameters [34, 35].

Various metals, ceramics, polymers, and composite materials have been experimented and analyzed as scaffolds which is shown in **Table 1**. Earlier only bioinert materials were utilized, however, now bioactive materials are widely used. Bioactivity is the ability by which it can interact with its surrounding normal tissues without causing any harmful effects [36-38]. Such a scaffold can effectively undergo bioactive fixation, unlike mechanical or bio fixation in inert scaffolds, by way of formation of an apatite layer on its surface. In the following paragraphs, some scaffold fabrication techniques and various types of biomaterials used for their fabrication are discussed.

Table 1: Merits and Demerits of various scaffold used for the regeneration of bone

Material	Merits	Demerits
Metals and alloys		
Porous tantalum	Highly porous, high strength, elastic modulus matches that of bone	Non-bioresorbable
Magnesium and its alloys	Bioresorbable, mechanical properties similar to bone	Mg ion toxicity, rapid corrosion rate
Titanium and its alloys	Highly biocompatible, corrosion-resistant	Non-bioresorbable
Ceramics		
CaP-based ceramics	Chemical composition similar to mineral phase of bone, high bone affinity, bioresorbable, osteoconductive	Extremely brittle, poor strength, slow bioactivity
Silica	Excellent bioactivity, highly biocompatible, osteoconductive, osteoinductive, high hardness, easily processable into different forms	Brittle, low tensile strength, low biodegradability
Alumina, zirconia	Possess load-bearing strength	Bioinert
Polymers		
Collagen	Highly biocompatible, can be molded into various geometries	Poor mechanical properties, may produce immunological responses
Chitosan	Minimal foreign body reaction, antibacterial	Low bioactivity, swelling, poor mechanical properties, may produce immunological responses
Silk fibroin	Controllable biodegradability, biocompatible, soluble in aqueous solvents	Low osteoconductivity
Synthetic polymers	Better strength, flexible, biodegradable	Acidic degradation products, hydrophobic

SCAFFOLD FABRICATION TECHNIQUES

Among various techniques available for scaffold preparation, the simplest method is *solvent casting*. It involves evaporation of solvent after either dipping a mold into a polymeric solution or adding a polymeric

solution into a mold. It is an inexpensive method which ensures control over pore size and crystallinity. The method is however limited by the toxicity that may arise due to residual solvents [39, 40].

Phase separation technique is based on a thermally induced separation of different phases (polymer-lean and polymer-rich phases) in the polymer solution. The former phase forms the pores and the latter forms the matrix. A non-solvent based phase separation also exists, but it produces heterogeneous pore structure. Thermally induced phase separation (TIPS) is further divided as solid-liquid and liquid-liquid PS. In solid-liquid PS, solvent crystallization occurs at low temperatures and these crystals are removed to form pores. In liquid-liquid PS, the solution forms a bicontinuous structure as it has an upper critical temperature [41-43].

Electrospinning technique is widely used for fabrication of fibers having dimensions in the nano or microscale. It utilizes a syringe pump, a high voltage source, and a collector. The polymer solution or melt is filled in a capillary tube and is held at its tip by surface tension. When a very high voltage is applied to the capillary, charge repulsion is induced in the melt which opposes the surface tension. At sufficiently high field intensities, charge repulsion overcomes surface tension and a jet is formed. As the jet moves towards the collector, the solvent evaporates to form fibers. Fiber size can be controlled by regulating factors such as solution properties, electric field strength, tip-collector distance etc. Scaffolds prepared by this method are highly preferred as they promote cellular growth efficiently due to the ultrafine oriented fibers [44, 45].

Freeze drying, used for fabricating porous scaffolds, is based on the sublimation principle. It involves freezing the solution to a low temperature, followed by primary and secondary drying processes in which ice is removed by direct sublimation and unfrozen water is removed by desorption respectively. The process is carried out in partial vacuum. It however produce mall pore sizes and requires long processing times [40, 46].

The most advanced form of scaffold synthesis is *rapid prototyping.* It is a computer controlled layer manufacturing technique in which the scaffold design in a Computer Aided Design (CAD) software is expressed in several slices and printed into a 3D form layer-by-layer. The 3D object construction can be carried out by 3D printing, selective laser sintering (SLS), fused deposition modeling, or stereolithography [47-49].

Several other techniques such as particulate leaching, porogen leaching, melt molding, self-assembly, gas foaming, fiber mesh, fiber bonding, etc. are also widely used [40, 50].

COMMON BIOMATERIALS USED AS BONE SCAFFOLDS

Metals and alloys

Metals and metal alloys were the first materials to be utilized in orthopedics. They are used as scaffolds, bone implants or substitutes. In the case of implant or bone replacement materials like stainless steel, Co based alloys, Ti alloys etc., inertness and stability were of great importance. However, for their use as scaffolds, they lack surface recognition abilities. Unless appropriate surface modifications or incorporation of growth factors and cells are done, they can neither effectively communicate with cells nor promote regeneration [51]. Another limitation is their degradation via corrosion. Although they are generally considered biocompatible, corrosion may lead to release of toxic metallic ions that may trigger allergic or inflammatory reactions [52]. Again, surface coatings or modifications are necessary to prevent this. Further, processing metals and alloys into the required porous architectures is also a challenge. It is highly dependent on the material properties [51, 53].

Porous tantalum was found to be a viable scaffold material due to its favorable mechanical and physical properties. Its high porosity, interconnected pore architecture, a Young's modulus similar to that of bone, and strength high enough to support physiological loads enhance its bone in-growth and volume filling properties [54]. However, a second surgery is required for the removal of the scaffold post regeneration.

Magnesium and its alloys gained importance due to their bioresorbability [53]. This avoids the need for a second surgery. They also have mechanical properties similar to bone, play a role in cell adhesion, exhibit no toxicity, and are osteoconductive. Magnesium alloys based on rare-earth elements like neodymium, cerium etc. and Mg-Ca alloy have been experimented both *in vitro* and *in vivo* as scaffolds [53, 55]. Limitations, however, arise from the toxicity of Mg ions. Even though excess of Mg is effectively removed by the body,

degradation rate of pure Mg in physiological conditions is very fast. Surface coatings or the use of corrosion-resistant alloys are necessary to control the corrosion rate [52].

In view of corrosion resistance and biocompatibility, *titanium and its alloys* were found to be superior. They are characterized by the formation of a passive titanium oxide layer on its surface which can get rebuilt if damaged [56, 57]. Titanium scaffolds have been used in surgery for decades. It is commonly used in spinal fusion, especially for anterior lumbar interbody fusion [58]. The bioactivity of the Ti mesh cage used for this purpose can be improved by conjugating hydroxyapatite. Ti scaffolds and meshes are mainly used in load-bearing defect sites [59]. They have the drawback of being non-bioresorbable.

Ceramics

Ceramics are inorganic materials comprising largely of covalent and ionic bonds. This type of bonding results in their high compressive strength, hardness, and low strength-weight ratio. Ceramic materials have been employed in bone applications including bone replacements and scaffolds for the same reason. An added advantage is that processing ceramics with porous architecture is relatively easy. Commonly used bioceramics are calcium phosphate based ceramics, silica, alumina, and zirconia. However, their extreme brittle nature is a challenge [60].

As mentioned earlier, *calcium phosphate* (CaP) is a major component of bone. Hence, many researches have concentrated on scaffolds based on CaPs and its analogues [61]. The widely focused forms of CaP are hydroxyapatite (HA), beta-tricalcium phosphate (β-TCP), and biphasic calcium phosphate (BCP, a mixture of HA and β-TCP). Hydroxyapatite has the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ and is usually obtained from corals or bovine bones [62]. Its excellent biocompatibility, osteoconductivity, and bioactivity make it a promising candidate for bone scaffolding [30]. Several studies have demonstrated high affinity of bone towards scaffolds or implants with high percentage of HA, TCP or BCP [30, 61-64]. Hence, scaffolds are prepared with its pure form or its composites with other ceramics, polymers, or metals [65-67]. Their weak mechanical properties require it to be used in combination with other biomaterials.

The importance of *silica* or silicon dioxide in bone regeneration and healing was discovered only recently [68, 69]. It is one of the most abundant minerals found in the Earth's crust, existing both in crystalline and amorphous forms. It is also found in bones, teeth, eyes, glands, skin, and organs in trace amounts [70]. Silica is a crucial component which provides strength and support to these structures. It was found to work with calcium and promote bone strength, bone formation and repair [68]. It is infact more prevalent than calcium, because it is a major constituent of collagen that makes up the organic matrix of the bone (80% of total bone mass). It ensures assimilation of calcium and other important minerals, and prevents their leaching [69, 70]. Silica gained importance as a bone scaffold material due to these reasons. It exhibits high bioactivity and excellent biocompatibility. It has low tensile strength and high brittle nature, but its hardness makes it perfect for use as a component in composites [71, 72]. The strength was found to be enhanced when size of the particles were reduced to the nanometer range [73]. These nanosilica particles embedded in bones is what gives it the stiffness, strength, and resistance to wear and tear. Nanosilica is also more biodegradable than silica. It also stimulates proliferation and differentiation of pre-osteoblasts on it, and suppresses osteoclasts, thereby being osteoinductive in nature [38]. Silica based bioactive glasses are also implemented in bone regeneration. Bioactive glasses (BAG) are a family of melt-derived or sol-gel-derived glasses which can bond with the bone [74]. The most important and commercialized BAG is the 45S5 Bioglass. Several *in vitro* and *in vivo* studies have proved their ability to stimulate bone formation [75-78].

Alumina and *zirconia* have excellent biocompatibility and mechanical properties, but are bioinert materials [79-82]. Hence they require appropriate surface modifications and coatings to promote bioactivity. Comparatively, only a few studies are available which uses them as scaffolds in their pure form, as they are mostly utilized as permanent implants. Bioactive alumina has been found to be important in load-bearing scaffolds [81].

Natural polymers

Polymers became the center of interest for bone applications due to its biodegradability. Almost all polymers which were obtained from natural sources could be degraded *in vitro* and *in vivo* by hydrolytic or

enzymatic degradation mechanisms. Natural polymers like collagen, chitosan, and silk are commonly used as scaffolds.

Collagen, the most abundant protein found in mammals, has been intensively investigated for bone applications. Collagen, being a natural constituent of bone, is highly biocompatible. There are 28 types of collagen with types I, II, III and IV making up 90% of the body collagen. Although widely used in scaffolds, it suffers from a lack of well-defined commercial source, cost, poor mechanical properties, and difficulty to control the processing [34, 83].

Chitosan is a derivate of chitin, comprising of randomly distributed N-deacetylated and acetylated units. Chitin is a biopolymer found in the cell walls of fungi, and exoskeletons of crustaceans and arthropods. It is utilized for its non-toxicity, anti-bacterial nature, and biodegradability. Since it shows low bioactivity, it is mostly utilized in combination with other biomaterials. Swelling is also a prominent limitation [35, 84].

Silk fibroin became popular as a biomaterial due to its unique mechanical properties, biocompatibility, and controllable biodegradability [85]. Raw silk is usually isolated from silkworms or spiders. Silk fibroin is obtained by subjecting raw silk to a degumming process to remove the sericin glue protein coating [86, 87]. Its removal is necessary as it was found to induce certain immunological responses in the host. Its remarkable mechanical properties arise from the formation of secondary beta-sheet structures. These crystals add to its stability in different environments, with degradation depending on proteolytic activity. The degradation rates can be controlled by varying the degree of beta-sheet crystallization. This in turn depends on the beta-sheet conformations based on the chemical or physical treatment method used [71]. Silk fibroin is soluble in aqueous solvents, unlike other polymers which are only soluble in organic solvents. This highly reduces the cytotoxicity induced while processing the scaffold [86, 88, 89]. As a bone scaffold, silk does not exhibit osteoconductivity up to the optimum level. Hence, it is commonly used in combination with other biomaterials to improve its properties [86, 88, 90].

Scaffolds with gelatin and alginate have also been reported [91-95]. All natural polymers possess the drawback of poor mechanical properties when compared to metals and ceramics. There is also a possibility of rejection by the body due to immunological responses, when these are obtained from other species [96].

Synthetic polymers

Due to the weak mechanical properties of natural polymers, a need for synthetic polymers possessing superior properties arose. They had to maintain the biocompatibility and bioresorption properties of the natural polymers while providing sufficient strength and elasticity. Biodegradable polymers of naturally occurring hydroxyl acids have been utilized for this purpose and are extensively used [96]. These polyesters have been shown to be biocompatible and osteoconductive in various researches. They also undergo hydrolytic degradation via a de-esterification process. Although all esters undergo this degradation reaction, only those with aliphatic chains between ester bonds can comply with the resorption time frame required for regeneration [29]. A further advantage is that the degradation products are non-toxic and are removed naturally by the human body. Their degradation rates can be varied by controlling their molecular weights [97, 98]. Their synthesis is also comparatively simple, since they are soluble in common organic solvents. Hence, various thermal and solvent-based synthesis routes have been employed for their processing [39].

Poly(glycolic acid), formed by the polycondensation of glycolic acid or ring-opening polymerization of glycolide units, was one of the first synthetic polymers to be experimented as bone scaffolds. PGA exhibits high crystallinity, therefore showing high strength and faster degradation rates [99, 100]. *Poly(L-lactic acid)* (PLLA), a linear aliphatic polyester formed by the polymerization of L-lactic acid, has also gained much attention due to its renewable resource, biocompatibility, and favourable thermal and mechanical properties [98]. The amorphous copolymer of PLA and PGA – *Poly(lactide-co-glycolide)* (PLGA) is also popular as a bone scaffold. It however shows weak cell ingrowth and interaction due to its hydrophobicity [101-103]. Another widely experimented polymer is *poly(ε-caprolactone)* [37, 97, 104]. It is a semicrystalline polyester that shows excellent biocompatibility, high processability, and biodegradability. Its slow degradation rate and hydrophobicity poses a drawback. Other polymers such as polyurethane, poly(hydroxyalkanoates), etc. have also been experimented [105-108]. In general, synthetic polymers show weak cell interactions. The toxicity that may be produced due to their acidic degradation products is a challenge [109, 110].

Hybrid materials

None of the biomaterials individually achieve all the targeted properties for a scaffold. Therefore, researches focused on combining two or more of these biomaterials to improve their properties. This composite or hybrid approach opened up wide possibilities for a more efficient and faster regeneration process. The composite involves a matrix phase and a filler phase which acts as the reinforcement. Common combinations include metal-ceramics, metal-polymers, ceramic-polymers, polymer-polymers and ceramic-ceramics. Metal based composites are not used much now due to their non-biodegradability.

The principle of composites involve biomimicking the properties and structure of bone. Collagen/TCP [111-113] composite is considered to be an ideal material in this respect. CaP based composites are extensively experimented as they significantly improve the biocompatibility. Various combinations such as Collagen/HA [114, 115], HA/TCP [67], Collagen/HA/PLA [116], Collagen/BCP [117], nanoHA/PLLA [118], PLGA/HA [119], HA/PCL [120, 121], PCL/TCP [122], PVA/Collagen/HA [107], Chitosan/nanoHA [123, 124], Chitosan/TCP [125], Alumina/HA [126], Zirconia/HA [79, 80], Silk/HA [88], PCL/BCP [127] etc. have been reported. Although several other composites such as Collagen/glycosaminoglycan [128], PLGA/Collagen [103, 129], Chitosan/PLGA [102], and others [86, 130, 131] have been evaluated as scaffolds, CaP based composites remain superior. However, a recent study showed that the TCP or HA components in those composites did not provide bioactivity in simulated body fluid (SBF), but silica did [132-135]. The reproducibility of this result *in vivo* has already been verified [136]. Experiments revealed that the apatite forming ability and hence bioactivity came from Si-OH, Zr-OH, and Ti-OH groups [73, 135]. Hence there was a shift of interest from HA based materials to silica based materials.

As mentioned earlier, silica exhibits excellent biocompatibility, high hardness, osteoconductivity, and osteoinductivity.[137, 138] It also has the advantage that it is easily processable into different forms such as particles, fibres, whiskers, and more which can be incorporated in a matrix, forming the composite [60]. The simplest has been a silica/HA composite [139-142]. Several *in vitro* and *in vivo* studies have been published establishing its bioactivity, biodegradability, and biocompatibility. A bone grafting substitute based on nanocrystalline HA/nanoporous silica has even been commercialized (NanoBone) [143]. In an *in vivo* analysis, it showed excellent angiogenic response.

Collagen/silica composites also possess attractive angiogenic and osteogenic properties [72, 95]. The scaffolds were fabricated by different techniques such as freeze-drying, sol-gel, immersion, gelling etc. using silica precursors that mimic the biosilicification process. The silica-collagen interaction was found to be dependent on the source of silica. Heinemann et al. [144] analyzed a silica/collagen scaffold *in vitro*. 85-90% of the seeded human bone marrow stromal cells were found to adhere on it post 1 day culturing. Significant proliferation was also observed. An *in vivo* study employed this composite to repair calvarial defects in a rat model [145]. Larger regenerated bone area compared to pure collagen scaffold was observed. The composite also showed higher bioactivity due to formation of a large number of apatite crystals on its surface on exposure to SBF. Faster degradation rates leading to rapid bone regeneration was also observed.

BAG based composites have also been widely experimented [146-149]. Blaker et al. developed a highly porous PDLLA/Bioglass scaffold by thermally induced solid-liquid phase separation method followed by solvent sublimation [147]. Analytical modeling results revealed that the porous architecture of the polymer was not affected by the presence of Bioglass particles. Fabbri et al. developed a highly porous PCL/45S5 Bioglass scaffolds with varying glass content and analyzed their cytotoxicity and osteoblast proliferation *in vitro* [148]. The best result was observed in scaffolds with high glass content. They were found to be non-cytotoxic, but their limited wettability posed a limitation to cell adhesion and proliferation. In another study, poly(-hydroxybutyrate) (P3HB) microsphere/45S5 Bioglass scaffolds were evaluated [149]. The P3HB microsphere aqueous suspension coated the glass surface uniformly. The bioactivity of coated and uncoated samples was quantitatively similar, but the cell adhesion and proliferation was much more facilitated in the composite scaffold due to its higher surface roughness.

K.Madhumati and coworkers prepared a chitin/nanosilica composite and analyzed its bioactivity *in vitro* [73]. Bioactivity was tested in SBF and the biocompatibility with MG 63 cell line. Crystalline HA was formed on/in the scaffolds 7 and 14 days post biomineralization. The scaffold was also found to possess high biocompatibility. A ternary composite, chitosan/alginate/nanosilica, was prepared by another team by freeze

drying technique [150]. Its swelling, biodegradation, cytotoxicity, biomineralization, and protein adsorption properties were analyzed *in vitro.* The presence of nanosilica improved bioactivity and controlled its swelling. No cytotoxicity was observed towards osteolineage cells.

In another study, the properties of a PCL/silica scaffold synthesized via sol gel method were evaluated [151]. SBF assessment of bioactivity revealed successful formation of low crystalline apatite on its surface after 1 week of soaking at 36.5ºC. The PCL phase enhanced its biodegradability. Lee et al. evaluated a nanofibrous PCL/silica xerogel scaffold *in vivo* [152]. It showed improved mechanical properties, excellent biocompatibility, and bioresorbability. The hydrophobicity and low stiffness limitations involved with pure PCL scaffolds were overcome in this hybrid material.

A very promising candidate for bone repair is the silk-silica composite. It effectively combines the mechanical properties of silk and bioactivity of silica. Mieszawska et al. prepared a silk-silica scaffold by combining silica particles in a silk fibroin matrix [71]. *In vitro* analysis was done with human mesenchymal stem cells (hMSCs) subjected to osteogenic differentiation and excellent results were obtained. The incorporation of silica positively influenced gene expression resulting in upregulation of bone sialoproteins (BSP) and certain osteogenic markers (collagen type I). It also showed early bone formation. This was evident from the formation of collagen fibres and apatite nodules on the scaffold. The study also demonstrated that use of nanosilica can facilitate the biodegradability of silica via particle dissolution. This enables the fabrication of scaffolds with precisely controlled degradation/remodeling rates. Another study evaluated mesoporous bioactive glass/silk scaffolds *in vitro* and *in vivo* [153]. The scaffolds were fabricated in two different ways – a silk coated mesoporous BAG scaffold and a BAG particle integrated silk scaffold. Both were found to be highly suitable materials for bone repair. A better physio-chemistry and bone forming ability was shown by mesoporous BAG/silk scaffolds as compared to BAG/silk scaffolds.

A general comparison study on various scaffolds, which is synthesized for the study on regeneration of bones, using various fabrication techniques is shown in **Table 2**.

Table 2: Comparison study on various scaffold system

Material	Method	*In vitro/ in vivo*	Remarks
Chitosan/alginate/nanosilica	Freeze-drying	*In vitro*	- 20-100µm sized pores - Swelling ability controllable - Higher protein adsorption
Gelatin/silica	TIPS	*In vitro* (MC3T3-E1 cell line)	- Nanofibrous structure - 84-86% porosity - High biocompatibility for 30 wt% silica - E= 21.4±8.2 MPa for 30 wt% silica
Graphene/nano-58S BAG	SLS	*In vitro* (MG63 cells)	- Compressive strength and fracture toughness higher for graphene conc. of 0.5 wt% (48.65±3.19 MPa and 37.92±3.84 MPa resp.) - Ca/P ratio=1.69 (high bioactivity)
HA/nanosilica	Suspension	*In vitro* (rat calvarial osteoblasts)	As compared to sintered HA, it showed: - More stability - Higher ALP activity - Better mechanical and biological properties - Same cell proliferation rates
PLLA/silica xerogel	Electrospinning	*In vitro* (MC3T3-E1 cell line) *In vivo* (rat calvarial defect model)	- 40 wt% silica hydrogel showed better bioactivity compared to others tested (20 wt%, 60 wt%) - E= 50±16 MPa and tensile strength= 4.9±0.6 MPa for PLLA/40%silica xerogel
Poly(ɣ-glutamic acid)/silica	Sol-gel	*In vitro* (osteosarcoma SaOs-2 cell line)	- HCA layer formation after 72h SBF immersion - Non-cytotoxic

Silica xerogel/chitosan	Sol-gel	*In vitro*	- Mesoporous, drug eluting - Controlled drug release - E= 67.0±8.5 MPa for 50% chitosan
Silica xerogel-collagen	Sol-gel	*In vitro* (MC3T3 cells) *In vivo* (rat calvarial defect)	- Higher ALP activity at 30 wt% silica content - Higher rate of degradation - Higher regeneration area *in vivo* - Effective expression of osteoblastic phenotype
Silk/silica	Sol-gel	*In vitro* (hMSCs)	- 85% confluence by day 5 - Good cell viability - Upregulated BSP and Col1

CONCLUSION

As is clear above, no individual biomaterial possesses all the required properties for an effective scaffold. The composite or hybrid approach is considered to be the best in terms of scaffold fabrication. Recently, silica-based biomaterials have been proven to be superior to CaP-based materials, which were considered to be the gold standard. Silica hybrids exhibit excellent osteoconductivity and osteoinductivity in addition to being biocompatible and biodegradable. Among the hybrids, the most recent development is the silk-silica composites. They are high potential candidates for bone scaffolds and have been shown to promote faster and better bone regeneration.

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