

Fabrication of polymer-based bone scaffolds—Conventional vs. advanced methods

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Abstract: This review comprehensively summarizes various preparatory methods of polymeric bone scaffolds using conventional and modern advanced methods. Compilations of the various fabrication techniques, specific composition, and the corresponding properties obtained under clearly identified conditions are presented in the commercial formulations of bone scaffolds in current orthopedic use. The gaps and unresolved questions in the existing database, efforts that should be made to address these issues, and research directions are also covered. Polymers are unique synthetic materials primarily used for bone and scaffold applications. Bone scaffolds based on acrylic polymers have been widely used in orthopedic surgery for years. Polymethyl methacrylate (PMMA) is especially known for its widespread applications in bone repair and dental fields. In addition, the PMMA polymers are suitable for carrying antibiotics and for their sustainable release at the site of infection.

Keywords: bone scaffold; polymer; polymethyl methacrylate; tissue engineering; orthopedic surgery

1. Introduction

Bones are dense connective tissues with a solid calcified outer layer (cortical bone) that comprises more than three-fourths of the bone's mass. Cortical bone has a relatively low porosity, ranging from 5% to 10%. The soft inner spaces of bone (usually described as cancellous or trabecular bone) form the remaining one-fourth of the bone mass. Cancellous bone has a high porosity, ranging from 60% to 90%, and contains the bone marrow, which consists of blood stem cells, adipose cells, osteoblasts, and osteocytes. From these, the osteoblasts are essential for the deposition and mineralization of the extracellular matrix of new bone, while osteocytes are the supporters of bone matrix calcification. In addition, specific growth factors and proteins, mainly residing in the extracellular matrix of bone, regulate cellular activity and stimulate the intracellular environment.

Bone possesses a high compressive strength of 170 MPa but a low tensile strength of 104–121 MPa and a very low shear stress strength of 51.6 MPa [1,2]. This means that bone can be fractured given that torsion force is exerted. In other words, bone is more sensitive to pulling or torsion than pushing. Though bones are naturally brittle (80% of CaP), a significant degree of elasticity is shown due to collagen. However, the likelihood of human bones failing due to mechanical problems, injuries, diseases, infections, and tumors increases with age. This means that wrong movement of the body or diseases like osteoporosis [3–5], scoliosis [6–8], and osteomyelitis [9–11] can cause the bones to fracture or deteriorate.

Naturally, bones can regenerate in case of minor injury and continuously remodel

throughout adulthood [12–14]. These bone injuries heal without forming scar tissue while regenerating the bone with its pre-existing properties. In addition, the regenerated bone is indistinguishable from the uninjured bone. However, significant injuries involving the load-bearing bone require orthopedic surgery to place a bone graft over the defective bone site to encourage new bone growth while preventing other tissues from interfering with osteogenesis. During osteogenesis, the osteoblasts (cells with single nuclei that form the bone) originating from the bone graft enhance the growth of new bone. This is conducted through osteoinduction and osteoconduction processes. Osteoinduction is the recruitment of immature cells and stimulation of these cells to develop into bones [15–17]. Osteoconduction is when bone grows on a surface- a phenomenon seen in the case of bone implants [18]. Polymers are unique synthetic materials [19–43] that are extensively used in various industrial applications [44–50]. Likewise, it is primarily used for bone and scaffold applications. This paper provides various fabrication techniques and their potential use in orthopedic surgery.

2. Tissue engineering

Tissue engineering combines cells, materials, engineering methods, and suitable biochemical and physic-chemical factors to improve or replace biological functions [52,53]. The main goal of tissue engineering is to regenerate and replace the structural and functional of the injured bone beyond its natural healing capacity [53]. For this to happen, external regenerative materials such as scaffolds, cell growth factors, or a combination of either are required [54]. Tissue engineering uses undifferentiated cells seeded within the scaffold, which defines the geometry of the replacement tissues and provides environmental indications to promote the development of new tissues, as demonstrated in **Figure 1** [55]. However, the interaction between the cell and the material used to develop the scaffold plays a vital role in tissue engineering. This is because the developed scaffold must mimic the properties of the injured bone structurally and bio-functionally. Therefore, it is essential to understand the requirements and properties of the injured bone before designing the scaffold [56–58].

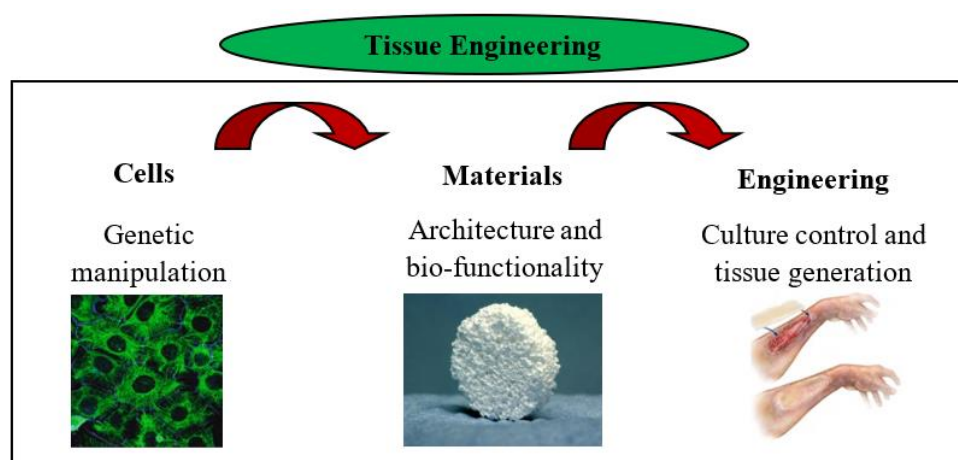


Figure 1. Schematic illustration of tissue engineering process.

3. Bone scaffolds and their requirements

Scaffolds are materials developed to perform in the body as devices able to support and possibly induce a complex pattern of events whose final goals are tissue repair and tissue function recovery [59–61]. Generally, the application of scaffolds can be summarized as:

- Allow cell attachment and migration.
- Deliver and retain cells and biochemical factors.
- Enable diffusion of vital cell nutrients and expressed products.
- Exert specific mechanical and biological influences to modify the behavior of the cell phase.

However, to achieve the goal of tissue engineering and bone regeneration, scaffolds must meet some specific requirements [62,63]. The scaffold should be biocompatible to integrate well within the tissue host without provoking any immune reaction and biodegradable into carbon dioxide and water forms. In addition, scaffolds should possess an open pore and be fully interconnected with highly porous structures [64,65]. These are the fundamental characteristics for providing space for cells to migrate and vascularize the tissue. In other words, the pore size of the scaffold is used to regulate cell survival, growth, and differentiation. Hence, the minimum pore size required is considered to be 100 μm due to the cell size, migration conditions, and transport [66,67]. However, pore sizes bigger than 300 μm are recommended to improve the new bone formation and to develop a net of capillaries. More on the effects of pore size on tissue regeneration is summarised in **Table 1**. Furthermore, the larger the surface area to volume ratio available, the more cell interactions will occur [68]. Scaffolds should also have the mechanical strength to retain their structure after implantation, mainly for the load-bearing tissues, as depicted in **Table 2**. Moreover, the scaffolds should be osteoinductive to recruit and stimulate the differentiation pathway of the stem and develop osteoblast cells to the defective bone [69].

Table 1. Effect of pore size on tissue regeneration [2].

Tissue formation/cell growth	Required pore size (μm)
Neovascularization	5
Fibroblas ingrowth	5–15
Regeneration of adult mammalian skin	20–115
Regeneration of bone	100–350
Osteoid ingrowth	40–100
Hepatocytes ingrowth	20
Fibrovascular tissue	500

In summary, a scaffold is required to match bone properties closely. However, achieving all these properties in one material is a complex challenge due to the lack of strength associated with porosity. Therefore, to make it possible, the scaffold materials must be optimized from the atomic level through the macroscale to the nanoscale structure with respect to the cellular response [70].

Table 2. Mechanical properties of human tissues.

	Tensile strength (MPa)	Compressive strength (MPa)	Young's modulus (GPa)	Fracture toughness (MPa.m ^{1/2})
Cancellous bone	-	4–12	0.02–0.5	-
Cortical bone	60–160	130–180	3–30	2–12
Cartilage	3.7–10.5	-	0.7–15.3 (MPa)	-
Ligament	13–46	-	0.065–0.541	-
Tendon	24–112	-	0.143–2.31	-

4. Bone scaffold preparation techniques—Conventional methods

Conventional, non-designed manufacturing techniques are used to fabricate an interconnected porous structured scaffold [71]. Some methods include solvent casting/particulate leaching, freeze-drying, phase inversion, and electrospinning. However, they lack precision when controlling the pore size, geometry interconnectivity, and spatial distribution of pores [72,73].

4.1. Solvent casting and particulate leaching

Solvent casting on its own is an attractive method in the polymer field due to its ability to obtain films with high quality [74,75]. Aside from being a relatively simple technique, the film's thickness, uniformity, and distribution can easily be controlled [76]. However, solvent-casting techniques can fabricate a scaffold in combination with particulate leaching. In this technique, the first stage involves dissolving the polymer into chloroform and casting it in a petri dish filled with porogen (sodium chloride, ammonium bicarbonate, or glucose). The composite is then placed in a dust-free environment to evaporate the solvent and washed with distilled water to remove the porogen. Here, the properties of the developed scaffold, such as porosity and pore size, can be controlled by the amount of salt added and salt crystals, respectively [77]. However, homogenous distribution of the salt into the polymer is difficult to attain because the density of the polymer and salt is different. Other factors, such as casting temperature and drying conditions, can also affect the properties of the scaffold. To overcome this limitation, researchers have suggested adding a centrifugation stage to improve the pore uniformity and interconnectivity of the scaffold, as shown in **Figure 2** [78]. In these studies, the polymer solution mixed with salt was centrifuged and dried (air-dried and vacuum-dried), followed by salt leaching in distilled water, resulting in the fabrication of an interconnected porous scaffold with porosity >90% [79].

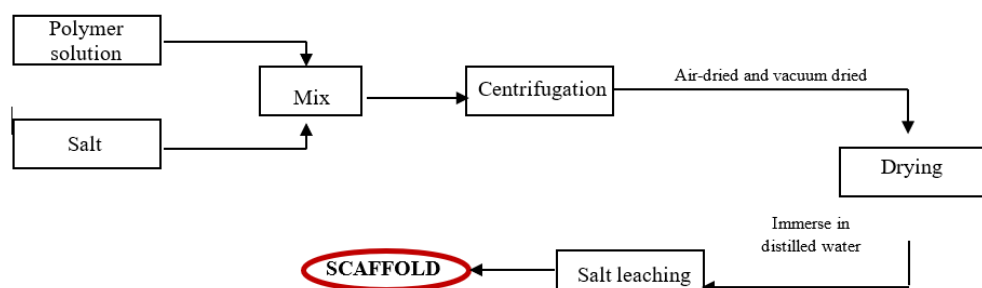


Figure 2. Schematic diagram for fabrication of scaffold using solvent casting and particulate leaching in addition to centrifugation stage.

4.2. Freeze-drying

In this technique, the polymer solution is mixed homogeneously with an acid (acetic acid) before the sublimation of ice using freeze drying, causing the formation of ice crystals, as shown in **Figure 3** [80–82]. The freezing temperature and rate can control the pore size properties of the developed scaffold. For instance, a scaffold freeze-dried pore size at $-20\text{ }^{\circ}\text{C}$ and $-196\text{ }^{\circ}\text{C}$ was $200\text{--}250\text{ }\mu\text{m}$ and $80\text{--}100\text{ }\mu\text{m}$, respectively [45,46,83–88]. However, in another research, the addition of an annealing stage after freeze-drying (low temperature) has been shown to increase the pore size from $96\text{--}150\text{ }\mu\text{m}$ to $85\text{--}325\text{ }\mu\text{m}$ ($\sim 40\%$ increase). This is due to the rise in temperature of the frozen suspension, which increased the ice crystal growth rate [89].

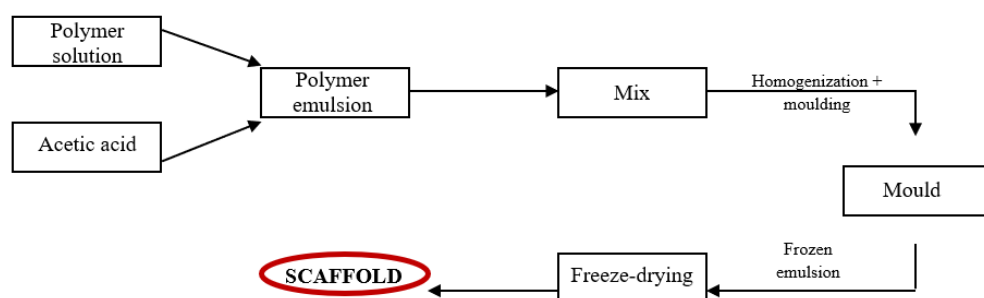


Figure 3. Schematic diagram for fabrication of scaffold using freeze-drying.

4.3. Phase inversion

Phase inversion is an effective technique for developing porous scaffolds by combining mass transfer and liquid phase separation, as depicted in **Figure 4** [61,90–93]. Initially, the mixed solution of polymer dissolved in a solvent and ethanol (non-solvent) would be cast or molded. Then, the dried casted gel will be induced to phase separation by immersing it into the non-solvent used [94]. After the extraction of the remaining solvent, the developed scaffold will be dried in a controllable environment. However, the drying stages can be avoided using supercritical fluid [95], such as carbon dioxide [96], which is biocompatible (non-toxic, non-corrosive, and non-flammable) and affordable [97]. The polymer solution is poured into a container and placed inside a heated high-pressure vessel. The supercritical fluid is pumped into the vessel with a high-pressure pistol. The fabrication of the scaffold was completed once the phase separation took place. A porous structure can be developed without any remaining solvent in this process. Nonetheless, the overall properties of the developed scaffold depend on the solubility and diffusivity of the supercritical fluid in the polymer. The phase separation technique has produced micro-patterned nanofibrous sheets ($50\text{--}500\text{ nm}$) with properties comparable to those obtained by electrospinning. The pore interconnectivity can increase when the phase inversion technique is combined with the particulate porogen leaching method, which can further be used for bone tissue regeneration applications [98].

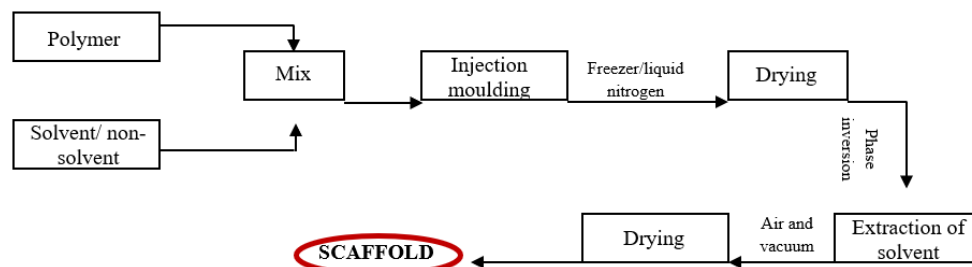


Figure 4. Schematic diagram for fabrication of scaffold using thermally induced phase inversion.

4.4. Electrospinning

Electrospinning, though not a new technique, has recently become significant in developing nanostructures in the form of fibers that can be used for scaffolds [99–105]. The basic tools required to fabricate this scaffold via the electrospinning technique include three components: a syringe, high voltage, and a collector plate, as shown in **Figure 5**. The polymer or composite solution prepared is poured into the syringe at a slow flow rate. Then, the tip of the needle is connected to the positive electrode of the high voltage, whereas the negative electrode is connected to the collecting plate. Finally, the polymer/composite is ejected from the syringe to the collecting plate as non-woven fibrous structures. These non-woven fibrous structures have unique characterization with a high surface area to volume ratio, flexible surface functionality, and mechanical properties superior to large fibers [105,106]. In addition, the pore size can be manipulated using either the properties of the polymer or composite solution prepared, the voltage applied, the processing temperature, or the distance between the collecting plate and the syringe [107–109]. However, the maximum pore size obtained through this technique is 10 μ m, which is relevant to applications related to hindering cell infiltration [110]. The porosity can be increased by either removing one of the composite components used or using phase separation technology during electrospinning [111].

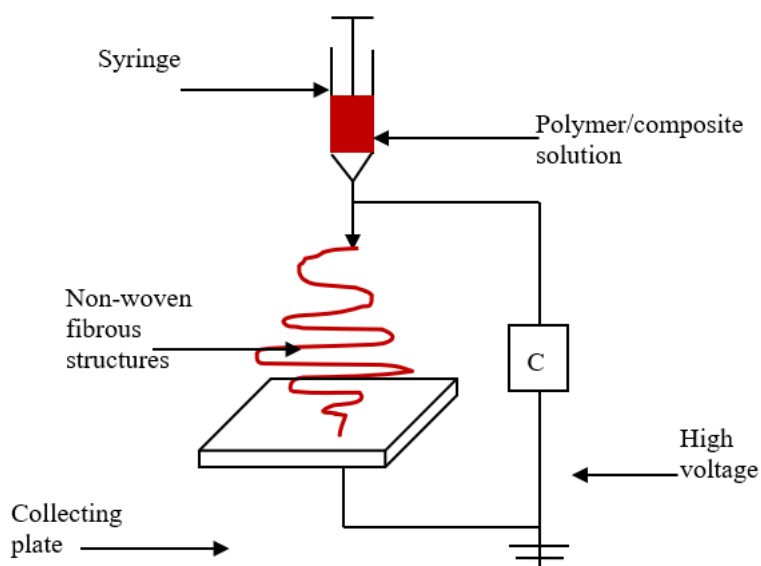


Figure 5. Schematic diagram for fabrication of scaffold using electrospinning.

5. Bone scaffold preparation techniques—Advanced methods

5.1. Computer-aided tissue engineering (CATE)

Advanced techniques, also known as designed manufacturing techniques in cooperation with computer-aided tissue engineering (CATE), have been known for a while [112–116]. This technique integrates advanced imaging technologies such as computer tomography (CT), magnetic resonance imaging (MRI), computer-aided design (CAD) technology, and rapid prototyping (RP) with tissue engineering applications, as shown in **Figure 6** [117–119]. Generally, CATE consists of two major processes: 1) non-invasive imaging data acquisition, where an image or scan of 3D tissue structural view is produced using CT or MRI, and 2) 3D reconstruction, where the physical model of the image is fabricated using CAD followed by RP and finally used for tissue implementation [120,121].

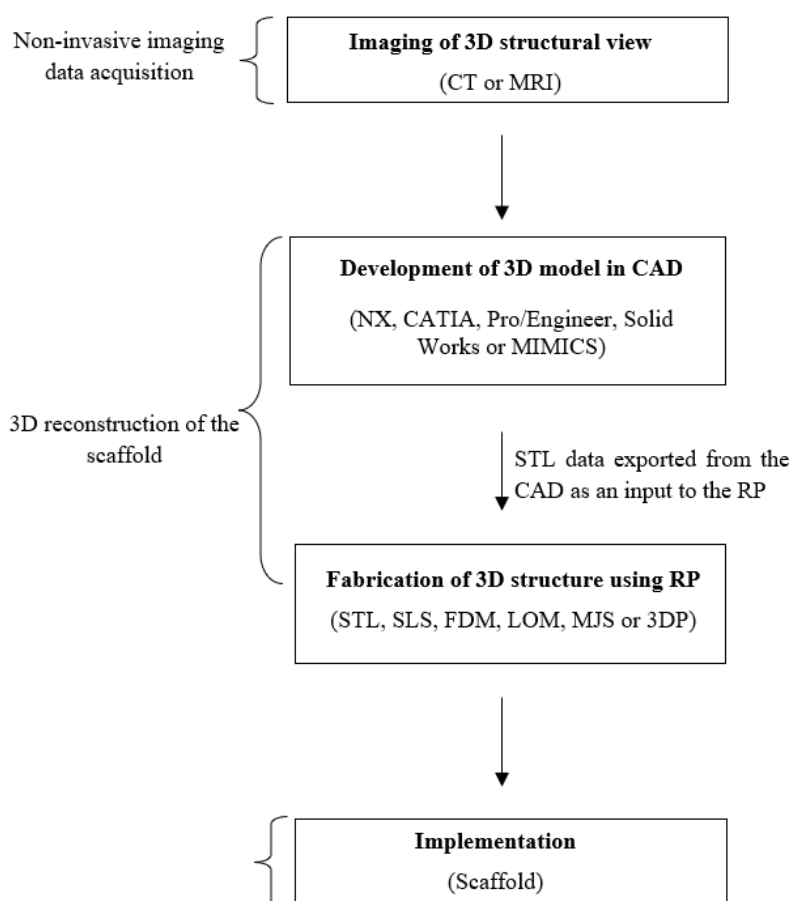


Figure 6. Schematic diagram for fabrication of scaffold using CATE.

5.2. Computed tomography (CT)

Computed tomography (CT) is one of the techniques employed to construct a 2D or 3D image of any tissue inside the body using special X-ray equipment with computer programs [122–126]. CT scanning has been used for many studies, including bone mass and morphology, growth and development analysis, mechanical loading and unloading, and evaluation of fracture healing [127]. The most crucial step in this technique is image acquisition, involving the preparation and positioning of the sample,

the selection of scanning medium, the determination of the X-ray energy required, the voxel size and image resolution needed, and the recognition of the area of interest for the study [128]. Then, the image obtained is filtered to reduce signal noises while maintaining its resolution. Furthermore, the mineralized and non-mineralized structures in the image are separated using a segmentation process for analysis [129].

There are main advantages to using CT: 1) allows direct 3-D measurement of any morphology; 2) compared to the 2D image, a larger volume is studied; 3) it is faster than histologic analysis; and 4) the evaluation is non-destructive; hence can be used for other studies [130]. On the other hand, CT uses X-rays in the form of ionizing radiation, which can be harmful. However, compared to the naturally occurring radiation everyone is exposed to daily, 1-time low dose of CT radiation exposure is equivalent to 6 months of natural radiation. That being said, there are still three ways to reduce the overall exposure to radiation doses: 1) reduce the number of CT scans prescribed, 2) reduce the CT dose used in a person, and 3) whenever practical, replace CT use with MRI such as for imaging liver. As a result, the most recent CT machines, known as multi-slice CT or multi-detector CT scanners, take the image in a spiral manner rather than individual parts of the body, making it faster, producing better 3D images with fewer CT scans and detecting minor irregularities.

5.3. Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) is the other technique besides CT used to scan part of the body using a magnetic field and radio waves [131–133]. Due to its non-invasive and non-radiant nature and high resolution, MRI is preferred for in vivo assessments [134–136]. Unlike CT, MRI produces a magnetic field that temporarily releases hydrogen atoms into the body. That being said, the movement limiting its applications can easily affect MRI scanners. Furthermore, the images might suffer geometric distortions caused by variations in the magnetic field strength [137,138].

5.4. Computer-aided design (CAD)

Computer-aided design (CAD) is widely used to design an approach that provides a powerful tool to model 3D scaffold geometries [139–143]. CAD designs the model using constructive solid geometry (CSG) or boundary representation (B-Rep). CSG models are designed using Boolean operation whereas B-Rep uses software like NX (Siemens PLM Software), CATIA (Dassault Systemes), Pro/Engineer (PTC), SolidWorks (Dassault Systemes) and MIMICS (Materialize GmbH) to design the model [144]. Developing a CAD scaffold is well-suited when combined with rapid prototyping techniques to fabricate the physical scaffolds [145].

5.5. Rapid prototyping (RP)/Solid freeform fabrication (SFF)

Rapid prototyping (RP), also known as the solid freeform fabrication (SFF) technique, is a controllable 3D structure designed layer by layer [146–149]. Designing a scaffold using this technique allows excellent reproducibility and the possibility of designing a structure that mimics the natural bone structure to be replaced [150]. Some of the RP techniques employed include stereolithography (STL), selective laser sintering (SLS), fused deposited modeling (FDM), laminated object manufacturing

(LOM), multiphase jet solidification (MJS), and three-dimensional printing (3DP) [59,151,152].

6. Summary and future direction

This review comprehensively summarized various fabrication techniques of bone scaffold preparation techniques and their potential use in orthopedic surgery. Polymethylmethacrylate remains one of the most enduring materials in orthopedic surgery. It has a central role in the success of total joint replacement and is also used in newer techniques such as percutaneous vertebroplasty and kyphoplasty. The use of bone scaffolds is nowadays an important aid in the orthopedic field, both in situations in which it is necessary to fix a fracture in patients with severe osteoporosis (“augmentation”) and in cases where it must ensure greater stability in the system of the prosthetic hip, knee, and shoulder. In cases of prosthetic infection, joint antibiotic-loaded spacers are used, and PMMA has gained favor as a vehicle for the delivery of antibiotics. Antibiotic-loaded acrylic cement in joint replacement provides short- to medium-term protection against prosthetic infection. It aims to overlap with and replace the prophylaxis provided by peri-operative intravenous antibiotics. Recently, new materials such as bioglass and porous cement have been developed, which seem to provide good results in clinical trials. These materials exploit their potential biological value, allowing the bone to integrate within the acrylic cement structure and favoring the mechanical and biological stability of the bone cement system. For future development of these materials (PMMA, antibiotic-loaded cement, glass, and porous cement), the aim is to improve osseointegration to promote better mechanical stability and better biological integration on the interface of bone cement.

Bone scaffold research focuses on better mechanical quality and biocompatibility. Biomaterials, such as calcium phosphates and hydroxyapatite, more efficiently induce bone growth. Advances in the biocompatibility of PMMA bone scaffolds might be achieved by introducing osteogenic agents, such as bone morphogenic proteins or transforming growth factors, to scaffold surfaces that contact the surrounding bone. PMMA for vertebroplasty has greater stiffness than vertebral cancellous bone, causing higher incidences of fracture of neighboring vertebral bodies.

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