Bone Scaffolds and their Assistance in Bone Repair

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Abstract

Bone fractures are a serious condition which can cause pain or infection if not treated. The natural healing process is only useful for smaller fractures, and the process can take anywhere from months to years to fix completely. Current techniques to speed up this process are bone grafts, which, despite their efficiency, have major downsides to all their versions, making a need for an alternative. Bone scaffolds, specifically porous scaffolds are a potential alternative which utilized a cell seeded scaffolding which is applied to a fracture. This results in mediated cell growth in the area, which speeds up healing and does not have the major downsides that the bone grafts have, making it a strong candidate.

Background

Bone fractures occur for a multitude of reasons. From age-related bone disorders to bone diseases and injury. Fractures and breaks are treated as different, however, for all intents and purposes, both are synonymous with the discontinuity of the bone tissue occurring from extreme or unusual pressure loads onto the bone or underlying health defects. A fracture compromises the structural integrity of the bone, and depending on the severity of the fracture, bone shards can jut out of the skin and cause extreme pain and lead to potential infection.

Under normal circumstances, bone fractures are healed through three phases: The Inflammatory Phase, The Reparative Phase, and The Remodeling Phase.[7] The Inflammatory Phase occurs after the fracture initially occurs. Blood from the exposed marrow resulting from the fracture begins to pool and clot, forming a pseudo scaffold which is used by leukocytes which flood into the area, removing pathogens and necrotic bone tissue. Afterwards, the Reparative Phase starts, which involves the movement of bone progenitor cells such as osteoblasts and angiogenic factors to allow for differentiation into bone cells and proliferation at the fracture area to initiate repairs. After the repairs have been done, the Remodeling Phase begins. This phase in particular can take months to years to complete depending on the type and severity of the fracture, as the newly repaired bone must be reacclimated to the load forces that occur during the patient' daily life. Although this mechanism works sometimes, severe fractures or the healing of some types of bones lead to misshapen bones or bones not being repaired at all. As such, a remedy is necessary to speed up the healing process, as well as raising the efficiency of fracture repairs.

Bone Grafts have been a staple technique to assist in repairing fractures. The process involves the sourcing of bone tissue from another part of the body, grinding the bone into powder and mixing the bone with stem cells and progenitor cells and packing the graft into pre-drilled canals in the fracture area.[2] Regrowth of the bone is mediated in the proper areas, decreasing the likelihood of improper regrowth, as well as speeding up the repair process. There are three types of grafts: autografts, which utilize bone from the patient for the graft but due to the amount needed depending on fracture severity, is not usable for severe fracture. Allografts are an alternative, which utilizes donor bone for the graft, but it necessitates the use of immunosuppressants as an immune response usually occurs, as well as having lower osteoinductivity. Synthetic grafts use synthetic materials to create a graft, which does undermine the disadvantages of the allograft and autograft, however the main issue lies in the lack of biodegradability of the graft, requiring manual removal to prevent infection. Although this has been the golden standard for fracture repair, with the current donor shortage as well as the downsides of each graft type, this necessitates an alternative that may be able to overcome these shortcomings.

Main text

Bone scaffolds

Bone scaffolds are scaffolds made through seeding progenitor cells and vascularization factors onto a material with macro-to-nano scale pores and is shaped to be applied onto the fracture area. These scaffolds allow for cell differentiation and proliferation around the fracture area in a controlled manner, allowing for native progenitor cells to be recruited, as well as the pores in the scaffold allowing for the growth of vascular networks which will provide nutrients to the growing area. The design for bone scaffolds modifiable, allowing for different materials and ways to induce vascularization to be used in the scaffold. Depending on the material, once the fracture is healed, the scaffold can even degrade safely within the body and is integrated into the new bone, which is contrary to the current technique. Bone scaffolds are produced in a multitude of ways, and depending on the technique, is more effective than bone grafts. Despite the advantages that scaffolds provide, the main disadvantage of the technique is the bone not being able to handle loads it was able to previously even when fully repaired, however this is a downside that most, if not all methods including the natural healing process have.

Types of bio-scaffolds

Bone fractures are very common and are increased especially in the median age. In tissue engineering the use of scaffolds promises a lot in the future. Scaffolds in regenerative medicine are structures that are designed to promote cell growth and proliferation after transplantation into a patient and can be categorized in fibrous, porous and hydrogel.[10] Fibrous scaffolds can mimic the extracellular matrix (ECM) composed of nanoscale structural and adhesive protein fibers such as collagen, elastin, and laminin.[12-13] Porous scaffolds are useful for growing cells into functional tissues, because of their large surface area they allow cell adhesion.[14] This type of scaffolds has been used effectively for bone regeneration by creating a 3D hybrid scaffold with controlled structure and mechanical properties. The third type of scaffolds are hydrogels. This type can be synthetic or natural having tissue-like properties. They can be used for encapsulating cells and can provide the cells within the gel with proper structural growth cues.[15] To add on, bone scaffolds can be made from different materials such as polymers, bioceramics, and metals. With this range of materials to make these scaffolds, it alleviates the potential risk of a shortage of a certain material, once again contrary to current bone grafts. To focus further on the advantages of the technique, a particular scaffold, namely the Porous scaffold design will be described and the methods of its creation will be evaluated to see how effective it is on repairing bone fractures.

Porous scaffold fabrication techniques

There are various methods for the fabrication of porous scaffolds that can be used for bone substitutes. As shown in figure 1, the combination of freeze-drying and leaching techniques can generate porous structures. The pore size can be specific for the demands of the project by controlling the space of the leaching template, the temperature and the viscosity of the polymer solution during the freeze-drying technique.[8] Another way to produce porous scaffolds is the CO2 foaming and melt processing where the molecular weight of the polymer is changed and this can affect the pore structure.[9] Other methods include rapid prototyping, freeze drying, salt leaching and laser sintering.[20] Scaffolds with large and small pores and interconnectivity can be obtained by melt blending the two polymers. With the electrospinning method the scientist can deliver fibers with nanometer dimensions ensuring a suitable surface for cell

adhesion. The most promising and appropriate technique is the direct melt electro writing for producing homogeneous porous biomaterials with large pore size. These techniques can provide a suitable substrate that can be well penetrated by cells by controlling the deposition of filaments on the collector, resulting in custom pore geometries for specific pore sizes.[21]

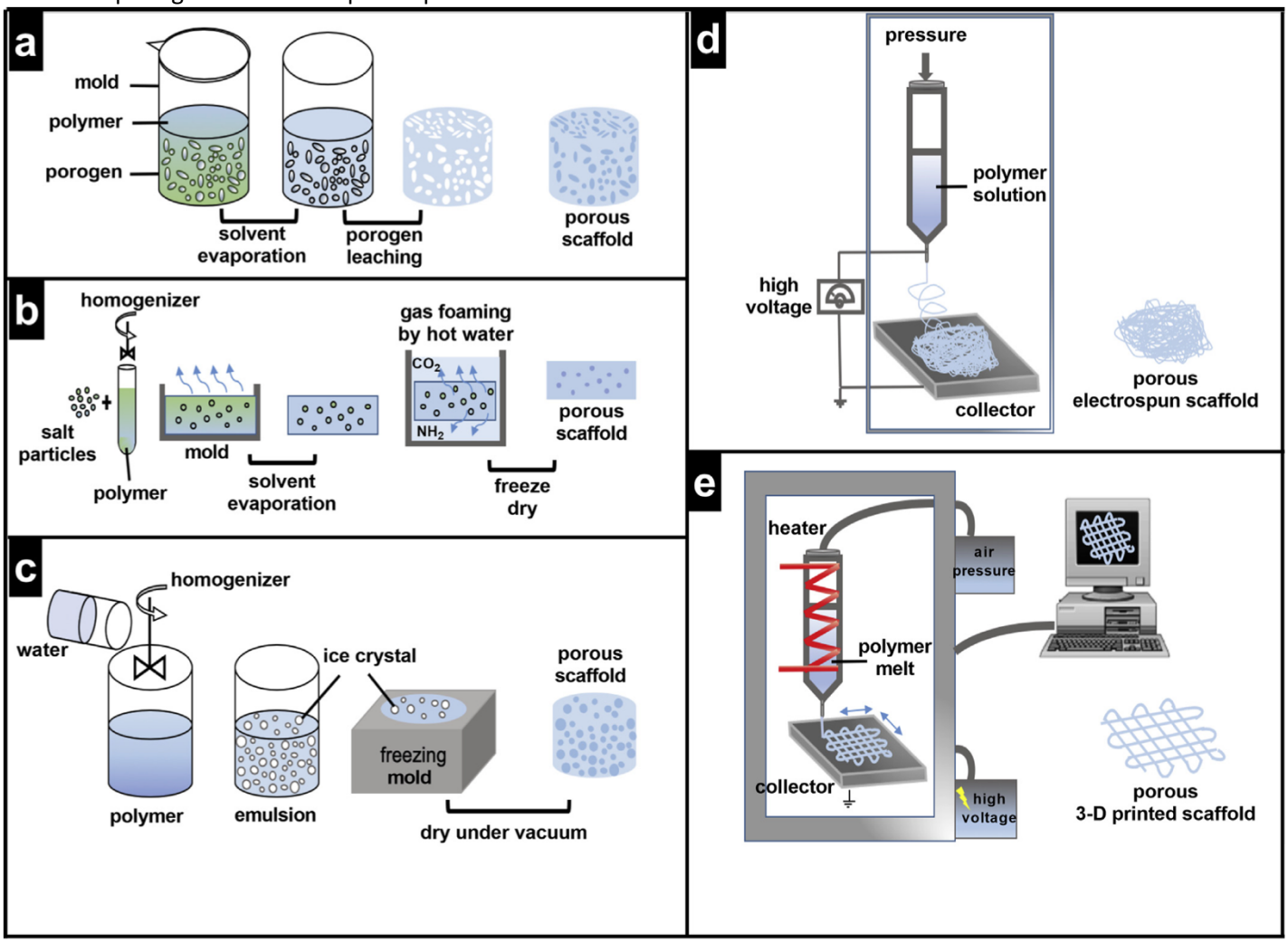

Figure 1: Various porous scaffold fabrication techniques. (a) Porogen leaching, (b) Gas foaming, (c) Freeze-drying, (d) Solution electrospinning, (e) Melt electro writing and 3-D printing. (Abbasi et al. 2020)

Criteria for scaffold success

A lot of different scaffolds have been used in tissue engineering for regeneration of different tissues and organs. There are some considerations that are important to determine if the scaffold is suitable for use. One of the first criterion for any type of scaffold for tissue engineering is biocompatibility. Scaffolds have to be able to integrate into the host tissue without any immune response such as severe inflammatory response which can lead to rejection of the scaffold by the body.[11] The goal of tissue engineering is to allow the body's own cells to replace an implanted tissue engineered scaffold over time. The scaffold's biodegradability is another important requirement for effective regeneration, allowing cells to produce their own ECM.[16] By-products of this degradation must be nontoxic and can be removed from the body without affecting other organs. In order for degradation to occur with tissue formation, an

inflammatory response is required in combination with controlled injection of cells such as macrophages.[17,18] To have the expected results, the scaffold must have specific mechanical properties that are similar with them anatomical site into which will be implanted and must be strong enough to allow surgical manipulation during implantation.[19] Last, the architecture of the scaffolds for tissue engineering is very critical. Scaffolds should be highly porous with high surface area to volume ratio to allow for cell growth and proper distribution, as well as microporosity to allow for capillary growth for nutrient and waste movement within the scaffold. The surface should have proper chemical and topographical properties to allow for influencing of cell adhesion, differentiation, proliferation, and ECM establishment.

Scaffold loaded with rhBMP-2 (growth factor)

Bone defects of critical size impose great demands on efficient bone regeneration materials. Mimicking the hierarchical porous structure and specific biological signals of native bone is considered an effective strategy to promote bone regeneration. In research made by Tang et al. (2016), they made successfully an effective bioinspired scaffold loaded with growth factor for complete regeneration of large bone defects. The trimodal macro/micro/nano-porous scaffold was loaded with a human bone morphogenetic protein-2 (rhBMP-2) included highly interconnected macropores and osteogenesis-related micropores. To investigate the cooperation and success of the trimodal scaffold, the researchers created 2 bimodal scaffolds with macro/nano-porous and macro/micro-porous structures. In figure 2A they depict 3D images of the rat bone marrow stromal cells (rBMSCs) penetrating into the 3 different scaffolds. With green are the live cells and with red the dead ones (apoptosis). As shown in figure 2B, nearly confluent rBMSCs were tightly anchored and spread on the macropore walls both around and inside the scaffold. These results are indicators for excellent cytocompatibility providing a wanted environment for cell attachment and ingrowth. For detailed observation of cell morphology, the researchers conducted SEM and they observed the spread rBMSCs with outstretched filopodia extensions (Fig.2C). As shown in figure 2C (right column) the researchers observed a leaf-like hydroxy-carbonate apatite crystal deposition which is similar to the morphology of apatite in human bone in the surface of TMS and BMS-M. This was not observed the same in the BMS-N scaffolds. These findings implied that the bioactivity of the scaffolds and the cell attachment might benefit and make faster the osteogenic process in vivo. The RhBMP-2 loaded trimodal scaffold prompt cell attachment, ingrowth and osteogenesis in vitro. Additionally, in vivo results on ectopic bone formation and critical size defects in orthotopic rabbit radius showed that TMS/BMP-2 compared to bimodal macro/nano and macro/micro-porous scaffolds loaded with rhBMP-2, showed better bone regeneration capabilities.

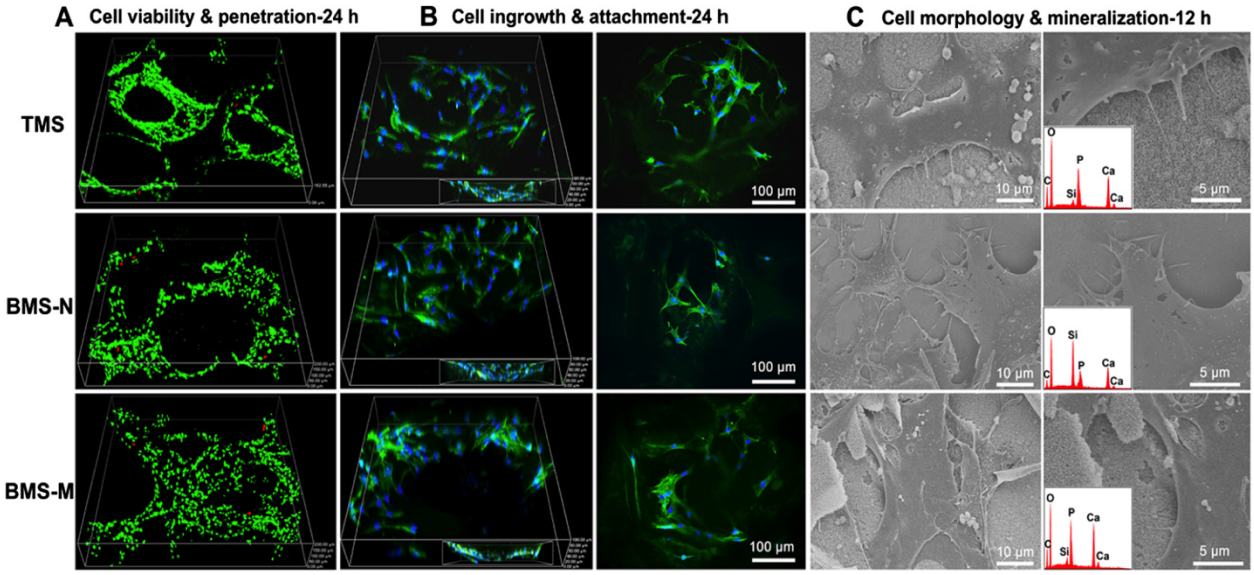

Figure 2. In vitro cytocompatibility and bioactivity of scaffolds (rBMSCs as a cell model). (A) Cell viability and penetration by live/dead assay (green: live cells, red: dead cells); (B) Cell ingrowth and adhesion observation by cytoskeletal staining (left column:3D visualization, right column: 2D visualization, green: cytoskeleton, blue: cell nuclei). (C) rBMSCs morphological observation by cytocompatibility and provided a desirable environment for cell attachment and ingrowth. More elongated cell morphology and hydroxyapatite depositions were observed in presence of micropores (on TMS and BMS-M) (Tang et al. 2016)

Conclusions

Critical and large bone fractures resulting to severe trauma or infection have raised challenges around the world. Because of the limiting self-regenerating capacity of the human body, the restoration of critical size bone defect cannot be fully successful. There are current techniques and bone substitutes, but they tend to fail and show unwanted therapeutic effects due to poor osteoconductivity or low osteinductivity. A team of researchers (Tang et al.2015) constructed effectively a trimodal scaffold for full bone regeneration. Viscosity controlled casting process as well as homogeneous particle reinforcing strategy was the solution for the improvement of the structural stability and mechanical power of the scaffold. Common problems in growth factor delivery like protein denaturation and low loading were solved by the customized nano-cavity entrapment concept. For the first time the trimodal porous scaffold loaded with growth factor was investigated in vitro and in vivo. The bioinspired trimodal macro/micro/nano-porous scaffold was effective because of its mechanical properties and properties in terms of osteoconductivity, osteoinductivity and biodegradability. This is merely an example of one of the applications of this bone scaffold technology. Currently, not enough clinical research has been done for bone scaffolds to test their efficacy on humans but based on models on smaller animals such as mice, this technology has great potential to be the next step in remedying bone fractures while having less risk than the alternative. With the high osteoconductive property of some models of scaffolds, as well as the biodegradability of scaffolds allows for optimal cell proliferation and bone regrowth of the fracture area while making sure the scaffolds can incorporate themselves onto the host bone tissue. These scaffolds are not limited by quantity, does not induce an immune response, and incorporate into host tissue. The only downside is the shared downside with other bone fracture repair techniques, that being low load

resistance post-healing. Regardless, the bone scaffold seems to be the future of bone fracture repair, and more research should be done to allow for ease of access to bone repair techniques, especially with the aging population in many nations in the world.

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