New biomaterials for bone regeneration

Fabrizio Matassi[1]
Lorenzo Nistri[2]
Diana Chicon Paez[2]
Massimo Innocenti[2]

[1] University of Florence, Italy
[2] First Orthopaedic Clinic, University of Florence, Italy

Address for correspondence:
Fabrizio Matassi, M.D.
First Orthopaedic Clinic, University of Florence, C.T.O.
Largo P. Palagi 1 - 50139 Florence, Italy
Ph. +39 055 7948286
Fax +39 055 432145
E-mail: fabriziomatassi@yahoo.it.

Summary

Bone-grafting techniques either with autografts or allografts still represent a challenge for reconstructive surgery. Allografts and autografts are the current strategies for filling bone defects and subsequent repair but each have drawbacks. Synthetic bone-graft substitutes, developed in an effort to overcome the inherent limitations of autograft and allograft, represent an alternative strategy. Synthetic bone graft substitutes have the goal of mimicking the physical and mechanical nature of native tissue and to promote osteoconduction for bone regeneration. In addition these substitutes are capable to release drugs or growth factors in a temporally and spatially manner. Some biomaterials are employed to design biomimetic scaffold such as natural and synthetic polymers, ceramics, metallics and composites.
The purpose of this paper is to provide an overview of the main biomaterials used for bone reconstruction.

KEY WORDS: tissue engineering, scaffold, biomaterials, regenerative medicine.

Introduction

Bone is a tissue that has the ability to heal and regenerate itself. Occasionally a bone defect can be formed in orthopedics and traumatology and in this case the bone fails to heal and needs bone reconstruction.
Successful bone reconstruction requires osteoproduction, osteoinduction, osteoconduction, mechanical stimulation, and vascularisation. Besides drugs that acts in to the bone metabolism can play an important role for bone ingrowth (1).
Autogenous cancellous bone is the current gold standard treatment in bone loss for a number of reasons, including the osteogenic, osteoconductive and osteoinductive properties of autograft and the lack of disease transmission or immunogenicity when utilized. However, there are major drawbacks to the use of autologous bone, such as limited availability and variable quality, hematoma, infection, increased operative time and bleeding, chronic donor site pain, and additional cost (2,3).
Because of this limits the expanding need for bone reconstruction is paired by the growth of interest in the discipline of bone substitutes and tissue engineering.Tissue engineering in bone reconstruction includes the utilisation of growth factors, scaffolds and mesenchymal stem cells.
Some investigators have developped synthetic scaffolds with the goal of mimicking the physical and mechanical nature of native tissue and to promote osteoconduction for bone regeneration. This graft substitutes, or biomaterials, or matrices, are formed from a variety of materials that are designed to mimic the three-dimensional characteristic of autograft tissue while also providing the ability to sustain cells proliferation onto the construct (4).
The purpose of this paper is to provide an overview of the main biomaterials employed for bone reconstruction.

Scaffold generality

Two major categories of scaffold can be distinguished (5):
1. Human tissue derived scaffols. There are homologous cancellous bone and demineralized bone matrix.
Urist in the 1965 defined the organic matrix of bone as “a scaffold/delivery system for bone morphogenetic protein”. DBM may be generated by the acid extraction of processed allograft bone, giving rise to a demineralized matrix consisting of osteoconductive type 1 collagen and noncollagenous proteins, including osteoinductive bone morphogenetic proteins (BMPs) that stimulate the formation of bone at a defect site. These properties make DBM both osteoconductive and osteoinductive. DBM is available in several forms, including freeze-dried powder, granules, gel, putty, and strips (6,7).
The advantage of these materials is their good biocompatibility and the bioresorbable property. Disadvantages however are the natural source, processing, possible disease transmission and immunogenicity (8).
2. Medical devices scaffolds (biomaterials). These synthetic graft substitutes, or matrices, are formed from a variety of materials, including natural and synthetic polymers, ceramics, metallics and composites, that are designed to mimic the three-dimensional characteristics of autograft tissue while maintaining viable cell populations.
Ideally, biomaterials for tissue engineering should meet several design criteria:
1. the surface should permit cell adhesion, promote cell growth, and allow the retention of differentiated cell functions;
2. the scaffolds should be biocompatible, with lack of immunogenic response, neither the polymer nor its degradation by-products should provoke inflammation or toxicity in vivo;
3. the scaffold should be biodegradable and eventually eliminated (not for metallic biomaterials);
4. the porosity should be high enough to provide sufficient space for cell adhesion, extracellular matrix regeneration, and minimal diffusional constraints during culture, and the pore structure should allow even spatial cell distribution throughout the scaffold to facilitate homogeneous tissue formation. Ideally the structures of the scaffold must be highly porous, with open pored and fully interconnected. Microporosity with pores less than 10 μm is needed for capillary ingrowth and cell-matrix interactions. Macropo-

rosity with pore sizes of 150-900 μm allows for nutrient supply and waste removal of cells grown on the scaffold;
5. the material should be reproducibly processable into three-dimensional structure;
6. mechanical structure: bone responds to the absence and presence of physical load. In response to these loads, the body either resorbs or forms bone. Given this principle, it is important to design a matrix that possesses mechanical properties that are similar to the tissue in the immediate surrounding area of the defect. An overengineered matrix may result in bone resorption around the implant site, while an underengineered matrix may fail as a mechanical support to the skeleton.
A number of three-dimensional porous scaffolds fabricated from various kinds of biodegradable materials have been developed and used for tissue engineering of the bone tissue (9).

Biomaterials

Synthetic bone-graft substitutes, developed in an effort to overcome the inherent limitations of autograft and allograft, represents an alternative strategy. Biomaterials are temporary matrices for bone growth and provide a specific environment and architecture for tissue development. In addition scaffolds can be used as a vehicle for drug delivery such as antibiotics, chemotherapeutic agents or growth factors (10,11). Depending on the specific intended application of the matrix, whether for structural support, drug-delivery capability, or both, certain material categories may be more or less well suited to the final structure. Choices for matrix material include polymers, ceramics, and composites of the two.

1. Polymers
Polymers are *natural* or *synthetic.* Natural biodegradable polymer such as type-I collagen, fibrin, hyaluronic acid and chitosan, exhibit good biocompatibility and osteoconductive properties. However the use of these materials is limited due to their very low mechanical stability. Biodegradable synthetic polymers, such as polyanhydrides, polypropylene fumarate, polycaprolactones, polyphosphazenes, polylactide, polyglycolide, and associated copolymers (polylactide-co-glycolide), are widely used as scaffolds for tissue engineering (12-14).
Different polymers express different physical attributes, mechanical properties, degradation times, and modes of degradation that can be chosen on the basis of the intended application of the matrix.
Polymers like polylactide-coglycolide and polycaprolactone, for example, undergo bulk degradation and may be less well suited for drug-delivery purposes than are surface-eroding polymers, such as polyanhydrides, that would more predictably deliver loaded factors and therapeutic substances.

2. Ceramics
A ceramic is a material made from an inorganic, non-metallic material that can possess a crystalline structure. Ceramics typically have a high compressive strength and low ductility, meaning that they provide high resistance to deformation but that they tend to fail because of their brittle nature. Often the compressive modulus of such ceramics exceeds the value commonly seen in trabecular bone.
Calcium phosphates, calcium sulfates, and bioactive glass have been used as matrices for bone regeneration. These substances, especially the calcium phosphates, are ideal candidates for use as matrices because the inorganic component of bone is composed of the ceramic calcium hydroxyapatite. Calcium phosphate and bioactive glass are also considered biomimetic, in that they stimulate the formation, precipitation, and deposition of calcium phosphate from solution and can result in enhanced bone-matrix interface strength.
Ceramic such as calcium sulfates also have potential as drug and/or factor delivery vehicles as a result of the high binding affinities between ceramics and proteins (15-17).
The ceramics can form either solid preformed structures or injectable forms that harden in situ.

1. Preformed Matrices

Preformed matrices can be designed of different shape such as blocks, granules, powders with pore structure, diameter and interconnettivity nearly to the bone.
This type of matrices are indicated in defects in which the shape can be largely predicted prior to surgery such as in osteotomy (Figure 1 A-B), tumor resection or spinal fusions. Restoration of an entere long-bone segment that has been damaged as a result of injury or tumor removal requires preformed matrices for structural integrity (18).

2. Injectable Matrices

Injectable matrices are gaining favor in the orthopaedics field as materials and techniques. This type of matrices are indicated for use in trabecular defects in which the damaged skeletal tissue in not load-bearing, in contained defects when the major structural osseous tissue is still intact and in small tumor-removal sites in appendicular skeleton (Figure 2).
The primary advantage of cements over preformed matrices is the ability to custom-fill defects and increased compressive strength. The material for injectable matrices must be in a liquid or gelatinous state and will harden in an aqueous environment at 37°C (body temperature). This property allows the polymer to be injected into a bone-defect cavity or space through a narrow-bore syringe, minimizing the surgical site to a small cutaneous incision. Theoretically, radiopaque markers can render the material visible under fluoroscopy to determine appropriate deposition into the defect site.
Investigators have evaluated the use of calcium phosphate cement products for augmentation of the repair of fractures of the distal radial metaphysis, tibial plateau, calcaneus, hip and spine (16, 19-21).

3. Metallics
There is a novel class of biomaterials employed in clinical use: the metallic scaffolds such as porous titanium or tantalum.
Porous metals are an expanding family of porous structures or scaffolds that have the innate characteristic of a 3-dimensional interconnected pore structure comparable to trabecular bone.
Futhermore titanium and tantalum are biocompatible, highly corrosion resistant, durable and not biodegradable with an elastic module very similar to that of the trabecular bone and can be prepared in many different shapes and textures without affecting its biocompatibility.
However the bioinert character of its protective naturally forming surface oxide does not readily form a strong interface with surrounding tissue. Furthermore, the relatively high stiffness of titanium, as compared to surrounding bone, can lead to problems of stress-shielding and subsequent implant loosening.
This type of scaffolds are employed to coat the surface of the prosthetic implants to promote bone ingrowth and secondary implants stability (22,23).

4. Composites
The composites consist of a combination of materials of different properties and might therefore use the advantages of the individual materials to optimize another material class. The combination of bioactive ceramics such as calcium phosphates with polymers improves the mechanical properties of scaffolds. Furthermore, the addition of polymers to the ceramics reduces their overall brit-

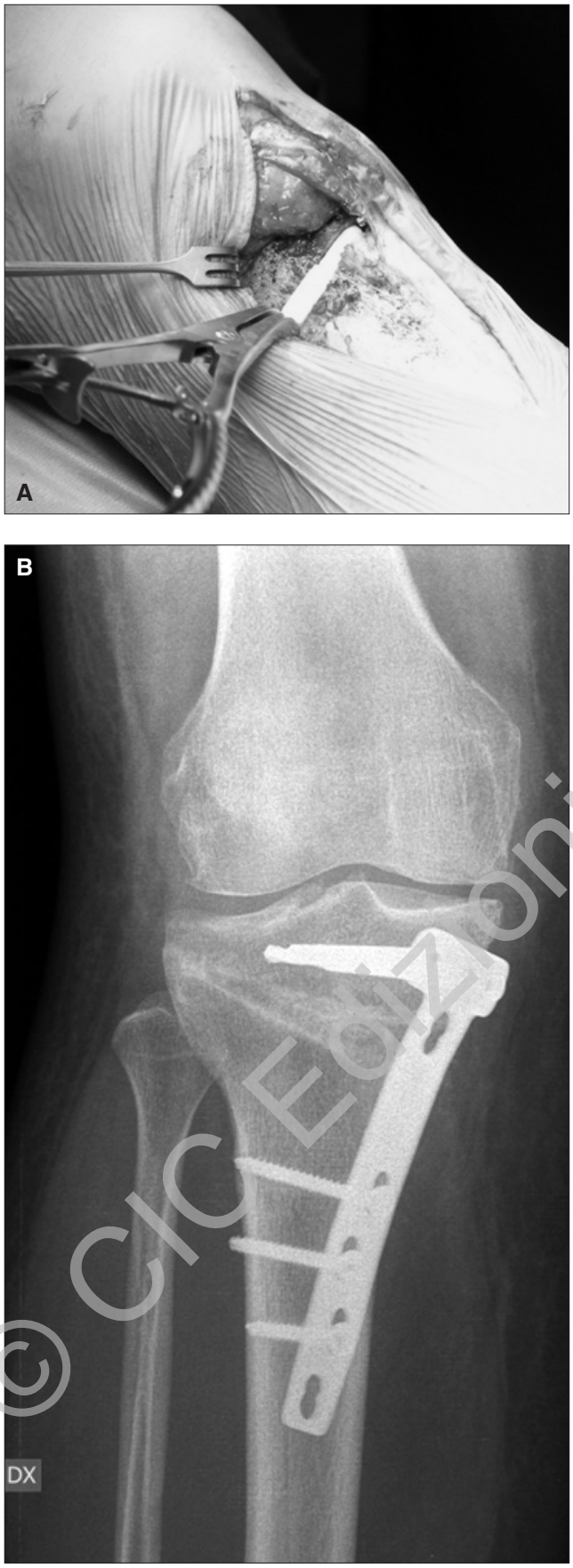

Figure 1 A-B - Wedge preformed matrices employed to fill the bone defect in osteotomy.

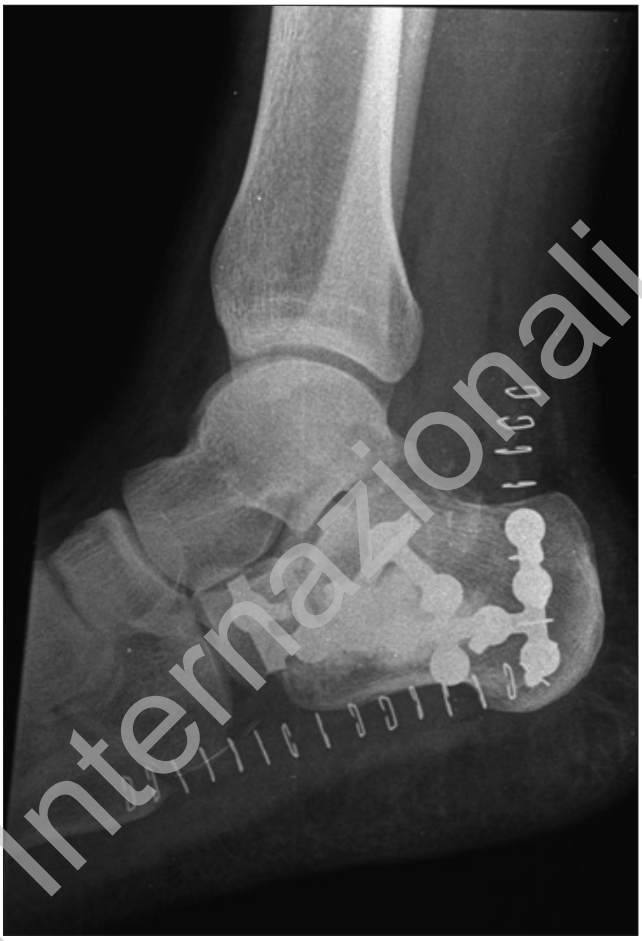

Figure 2 - Injectable calcium phosphate cement in calcaneus fracture.

tleness, while the addition of ceramic to a polymer increases both its bioactivity and its capacity to take up and deliver factors and therapeutic substances.
For example, the combination of natural polymers with calcium sulphate ceramics might enhance their mechanical stability.
Recently some investigators have combined titanium surfaces with hydroxyapatite to reduce the bioinert character of the metal and to enhance bone osteointegration.

Future direction: from bone tissue engineering to regenerative medicine

The biomaterials promotes bone formation by providing an osteoconductive matrix for host osteogenic cells to create bone under the influence of host osteoinductive factors.
Some of these biomaterials can be mixed with mesenchimal stem cells and growth factors to enhance the osteogenesis and osteoinduction property of the bone substitute. This procedure is the most used, not only because it is inexpensive and it does not require extra instrumentation, but also for regulatory reasons because it can be preformed as a "minimal invasive procedure". The procedure can be performed in the surgical room, and the cells are almost immediately reintroduced at the bone defect site.
Some authors have reported that a limited number of mesenchimal stem cells (MSCs) are implanted without expansion. Because of the limited number of MSCs in the bone marrow, several investigators have felt the need to increase the number of MSCs by ex vivo expansion (24).

1. Tissue engineering

In the tissue engineering the MSCs are isolated (typically from the patient), expanded ex vivo, before implantation, and seeded onto a synthetic scaffold, allowed to produce extracellular matrix on the scaffold in controlled culture conditions, and finally implanted into the osseous defect or void in the patient.

A primary obstacle in translating this technology from the bench to the bedside is that this technique involves an additional surgery and the patient must wait for the bone graft to develop in vitro. Transplanted scaffolds seeded with MSCs have been shown to enhance osteogenic capacity and integrate with native tissue faster than acellular scaffolds in many preclinical trials.

Despite the enormous potential of this approach for bone tissue engineering, there are still a number of barriers to address. The first and most significant barrier is that a number of studies have shown that MSCs which have been extensively cultured ex vivo lose their phenotypic behavior such as osteodifferentiation and bone forming capacity once implanted *in vivo*. Furthermore the low proliferative capacity of MSCs makes difficult to obtain sufficient cell density in a large scaffold. In addition to the increased risk due to a second surgery, there is a need to establish rigorous sterilization techniques for the cell-seeded scaffold which has been in culture ex vivo for up to several weeks (24-26).

2. Regenerative medicine

The new approach to bone regeneration involves implantation of a new generation of acellular scaffold immediately after injury or bone removal.

The performance of an acellular scaffold may be substantially enhanced through the incorporation of bioactive molecules which are released in a controlled manner as the scaffold degrades and native tissue replaces it. This type of scaffold are molecular designed for in situ regeneration and repair with minimally invasive surgery.

The governing principles of this approach are the same as the first approach, however, to ensure rapid healing, it is even more critical to design a scaffold as a bioreactor that mimics native bone tissue by driving local MSC migration into the scaffold, supporting and promoting osteodifferentiation and deposition of extracellular matrix (27).

Some clear advantages of this approach are that “smart” scaffolds are much easier to sterilize, they have a shelf-life, and they have the lowest potential for infection or immunogenicity of all the bone repair strategies.

To have smart biomaterials is important:

1 - design of a micro and nanoscale dimensional hierarchy representative of bone;

2 - the incorporation and controlled viable release of bioactive molecules and drugs;

3 - control of bioerosion to match native tissue synthesis rate (11, 27,28).

References

1. Giannoudis PV, Einhorn TA, Marsh D. Fracture healing: the diamond concept. Injury 2007;38(Suppl 4):S3-S6.
2. Finkemeier CG. Current concept review: Bone-grafting and bone-graft substitutes. J Bone Joint Surg Am 2002;84:454-64.
3. Keating JF, McQueen MM. Substitutes for autologous bone graft in orthopaedic trauma. J Bone Joint Surg Br 2001;83:3-8.
4. Kelly CM, Wilkins RM, Gitelis S, Hartjen C, Watson JT, Kim PT. The use of a surgical grade calcium sulfate as a bone graft substitute: results of a multicenter trial. Clin Orthop Relat Res. 2001;382:42-50.
5. Brach del Prever EM, Donati DM, Fiorentino S, Macrì E. Regulatory aspects of regenerative medicine products and informed consent GIOT 2010; 36 p. 223, 242.
6. Urist MR, Silverman BF, Buring K, et al. The bone induction principle. Clin Orthop 1967;53:243-83.
7. Drosos GI, Kazakos KI, Kouzoumpasis P, Verrettas DA. Safety and efficacy of commercially available demineralised bone matrix preparations: A criticla review of clinical studies. Injury 2007; 38 S4:S13-S21.
8. Horowitz MC, Friedlaender GE, Qian HY. The immune response: the efferent arm. Clin Orthop Relat Res 1996;326:25-34.
9. Schieker M., Seitz H., Drosse I., Seitz S., Mutschler W., Biomaterials as scaffold for bone tissue engineering. European journal of trauma, 2006N.2 p. 114-124.
10. Schmidmaier G, Schwabe P, Strobel C, Wildemann B. Carrier systems and application of growth factors in orthopaedics. Injury, Int. J. Care Injured (2008) 39 S2, S37-S43.
11. Martin I, Riboldi SA, Jakob M, Wendt D. SnapShot: Bioreactors systems in tissue engineering (TE) & regenerative medicine (RM). Biomaterials. 2010 Apr;31(11):3114-5.
12. Vögelin E, Jones NF, Huang JI, Brekke JH, Lieberman JR. Healing of a criticalsized defect in the rat femur with use of a vascularized periosteal flap, a biodegradable matrix, and bone morphogenetic protein. J Bone Joint Surg Am. 2005;87:1323-31.
13. Burkoth AK, Burdick J, Anseth KS. Surface and bulk modifications to Photocrosslinked polyanhydrides to control degradation behavior. J Biomed Mater Res. 2000;51:352-9.
14. Lee KW, Wang S, Lu L, Jabbari E, Currier BL, Yaszemski MJ. Fabrication and characterization of poly(propylene fumarate) scaffolds with controlled pore structures using 3-dimensional printing and injection molding. Tissue Eng. 2006;12:2801-11.
15. Livingston T, Ducheyne P, Garino J. In vivo evaluation of a bioactive scaffold for bone tissue engineering. J Biomed Mater Res. 2002;62:1-13.
16. Larsson S. Calcium phosphates: what is the evidence? J. Orthop Trauma 2010 N. 3 Suppl.: S41-45.
17. Beuerlein MJS, McKee MD. Calcium sulfates: what is the evidence? J. Orthop Trauma 2010 N. 3 Suppl.: S46-51.
18. Khan Y, Yaszemski MJ, Mikos AG, Laurencin CT. Tissue engineering of bone: Material and matrix considerations. J Bone Joint Surg Am 2008;90 (Suppl.1):36-42.
19. Ambard AJ, Mueninghoff L. Calcium phosphate cement: review of mechanical and biological properties. J Prosthodont. 2006;15:321-8.
20. Low KL, Tan SH, Zein SH, Roether JA, Mourino V, Boccaccini AR, Calcium phosphate-based composites as injectable bone substitute materials, J Biomed Mater es B Apll Biomater. 2010 Jul.;94(1):237-86.
21. Ho E, Lowman A, Marcolongo M. Synthesis and characterization of an injectable hydrogel with tunable mechanical properties for soft tissue repair. Biomacromolecules. 2006;7:3223-8
22. Bobyn JD, Stackpool G, Toh K-K. Bone ingrowth characteristics and interface mechanics of a new porous tantalium biomaterial. J Bone Joint Surg. 1999;81-B:907-914.
23. Hahn H., Palich W. Preliminary evaluation of porous metal surfaced titanium for orthopedic implants. J Biomed Mater Res. 1970 Dec;4(4): 571-7.
24. Lucarelli E, Donati D, Cenacchi A, Fornasari PM. Bone reconstruction of large defects using bone marrow derived autologous stem cells. Transfus Apher Sci. 2004 Apr;30(2):169-74.
25. Mauney JR, Volloch V, Kaplan DL. Role of adult mesenchymal tem cells in bone tissue engineering applications: current status and future prospects. Tissue Eng. 2005;11:787-802.
26. Mistry AS, Mikos AG. Tissue engineering strategies for bone regeneration. Adv Biochem Eng Biotechnol. 2005;94:1-22.
27. Cartmell S. Controlled release scaffolds for bone tissue engineering. J Pharm Sci. 2009;98:430-441.
28. Mieszawska AJ, Kaplan DL. Smart biomaterials - regulating cell behavior through signaling molecules BMC Biol. 2010 May 19;8:59.