Chapter

Innovative Biomaterials for Tissue Engineering

Anna Dolcimascolo, Giovanna Calabrese, Sabrina Conoci and Rosalba Parenti

Abstract

In the field of regenerative medicine, biomaterials play a crucial role since they may serve as a support (scaffold) to promote cell growth and differentiation in order to promote the healing of tissue lesion. The aim of this chapter will be to analyze the properties of more recent biomaterials suitable for tissue engineering strategies, to end to define better and innovative materials for scaffold production. To this purpose, we will analyze the main materials (natural and synthetic) and their characteristics, such as biocompatibility, bioactivity, and biodegradation, and it will be discussed how their chemical-physical properties (surface morphology, porosity, stiffness, and mechanical strength) could affect the interaction with cells and living system. Moreover, the chapter will be focused on methods of extraction or production of biomaterial suitable for scaffolds.

Keywords: biomaterial, scaffold, polymers, biocompatibility, surface, porosity, synthesizing

1. Introduction

The promising field of tissue engineering (TE) purposes to restore damaged tissues by combining cells with biomimetic materials able to act as templates for tissue regeneration and to drive new tissue growth. The term "tissue engineering" was formally conceived at a National Science Foundation workshop in 1988 as "the application of principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function" [1].

According to the definition of Langer and Vacanti, tissue engineering is "*an interdisciplinary field of research that applies the principles of engineering and the life science toward the development of biological substitutes that restore, maintain or improve tissue function*" [2].

The tissue engineering is a highly multidisciplinary field that associates several areas including clinical medicine, mechanical engineering, materials science, genetics, and related disciplines to both engineering and the life sciences. This field is based principally on the use of biomimetic materials (3D scaffolds) that provide not only a suitable environment for the new developing tissue but also offers a structure for cell adhesion, proliferation, and extracellular matrix (ECM) deposition until

new tissue is totally restored [3]. Furthermore, the scaffolds are often combined with cells and signaling molecules or growth factors representing the key elements of tissue engineering.

2. Biomaterials for tissue engineering

The first definition of biomaterial was developed in the 1980s, during the Consensus Development Conference (Chester, UK, 1982) in which the biomaterials were defined as "*any substance, other than a drug, or a combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system, which treats, augments or replaces any tissue, organ or function of the body*" [4]. Since ancient times, men searched in nature animal or plant-derived materials able to heal wounds, maintain, or restore body functions. In fact, the ancient Egyptians and Romans used vegetable fibers to sew skin lesions, and they were able to model wooden limb prostheses. Later, the industrial revolution allowed the development of a series of synthetic biomaterials (first metallics and then polymerics) with characteristics more and more suitable for the development of medical devices.

More recently, natural and synthetic biomaterials have become one of the important elements for regenerative medicine and tissue engineering strategies.

Nowadays, several types of scaffolds have been produced with a multiplicity of manufacture systems but the main challenge for tissue engineering is represented from the choice of appropriate materials for the scaffold production. To this aim, different types of biomaterials have been currently used, such as, natural or synthetic polymers, ceramics, metals, composites, and hydrogels. Furthermore, it is important when planning or determining the suitability of a scaffold to evaluate that it fulfills the following key requirements: (i) biocompatibility, (ii) bioactivity, and (iii) biodegradability.

The main requirement of the scaffold for tissue engineering is its biocompatibility or capability to promote cellular adhesion, proliferation, and migration onto the surface and eventually through the scaffold *in vitro* and *in vivo*. Moreover, after implantation, it must integrate into the host tissue without eliciting immune response in order to avoid an important inflammatory reaction that might decline healing or induce rejection [5].

The bioactivity represents the ability of a biomaterial to interact with surrounding tissue ensuring cell adhesion, proliferation, and differentiation [6]. Generally, biomaterials with chemical composition comparable to the host tissue have a higher bioactivity and can promote cellular recognition evoking specific cellular response to support tissue growth. To this aim, it is possible to modify the surface of the biomaterial by adding extracellular matrix macromolecules, including collagen, fibronectin, and laminin, to produce a biomimetic environment equivalent to the native tissue able to modulate cellular behavior and response [7].

On other essential property of scaffold for tissue engineering is the biodegradability. The biomimetic scaffolds are not permanent implants but they must be biodegradable to allow cells to produce their own extracellular matrix. Further, the by-products of this degradation must also be nontoxic and easily eliminable from the body without interfering with other tissues [8]. On the other hand, it is critically important to also know the in vivo degradation kinetics of a biomaterial to avoid an excessively rapid or slow elimination. In the first case, the scaffold could not satisfy its function of support for cells, while in the second one, it could cause necrosis or inflammation [9].

3. Scaffold design: the importance of structural and mechanical properties

The scaffold for tissue engineering must possess structural and mechanical characteristics appropriate to the anatomical site in which it must be implanted and, moreover, must be strong enough to allow its surgical manipulation during implantation. The structural features include macro- and micro-structural properties. The macro-structural properties refer to a temporary 3D architecture, of critical importance, which mimic the ECM and allows cell to maintain their native differentiated phenotypes; while, the micro-structural properties refer to scaffold porosity, pore shape, pore size, and interconnectivity. The mechanical properties include mechanical strength and stiffness.

3.1 Structural properties

Scaffold micro and macro architecture critically influences cell survive and surface adhesion, but also cellular proliferation, differentiation, vascularization, and specific gene expression [10].

If on the one hand, a scaffold may be strong enough to support the physiological load of the body and to allow surgical handling during implantation, on the other hand, it is important to obtain a porous structure to avoid cellular colonization. It is clear that a balance between mechanical strength and high porosity is a significant challenge in scaffold production.

3.1.1 Pore interconnection, porosity, and pore size

Pore interconnection, porosity, and pore size represent very important parameters for the scaffold production. All three features allow cellular penetration, vascularization, adequate diffusion of nutrients and oxygen to cells within the construct, and neo-formed extracellular matrix ensuring cell viability [5, 11].

In particular, pore size is a key element for the scaffold efficiency. In fact, the pores must be large enough to allow cells to penetrate and migrate within the scaffold structure, but also small enough to allow the binding of a critical number of cells at the same. Pores can be classified into micropores (0.1–2 nm), mesopores (2–50 nm), and macropores (>50 nm) according to their dimension. All the scaffolds used for tissue engineering may have a macroporous structure with a specific pore size as a function of the type of host tissue. In particular, a pore size of 20 micron is required for hepatocyte and fibroblast growth, while the dimension is around 20–150 micron for soft tissue healing. For bone tissue engineering, researchers propose a pore size range between 200 and 400 micron.

The most common techniques used to obtain a porous structure are gas foaming, salt leaching, phase separation, sintering, and freeze-drying.

3.2 Mechanical properties

The scaffold for tissue engineering must have adequate mechanical integrity, so that it can offer support from the time of implantation until the remodeling process is fully completed.

3.2.1 Mechanical strength and stiffness

The mechanical strength depends on the bonding forces that hold together the atoms in scaffold architecture. It is an important parameter to avoid the

solid structure deformation due to cellular loading on the scaffold or caused by scaffold handling.

Another important feature of the scaffold surface is the stiffness that is measured by Young's modulus. Cells respond to scaffold stiffness *via* different mechanisms such as activation of ion channels or protein unfolding, and by this way, stiffness affects cell proliferation and differentiation. Hadjipanayi *et al.* demonstrated that the increasing of free-floating collagen matrix stiffness led to a higher proliferation rate for human dermal fibroblasts [12].

4. Biomaterials for scaffold production

Biomaterials for tissue engineering have a considerable importance for the success of a tissue replacement or regeneration. In addition to interacting with the implant site, they have the ability to influence biological processes that are important for tissue regeneration.

Different kinds of biomaterials have been used for scaffold production such as ceramics and polymers, naturals and synthetics, metals, composites, and hydrogels.

4.1 Ceramics

For several decades, ceramic biomaterials have been used to reconstruct damaged body parts and for skeletal repair.

Ceramic biomaterials are inorganic compounds of natural or synthetic origin, which may contain metallic and nonmetallic elements. These biomaterials are generally made of polycrystalline solids, rarely of monocrystals and sometimes have an amorphous structure. Generally, their mechanical properties, including hard surface, high mechanical stiffness, low elasticity, low thermal expansion, chemical-physic refractoriness, depend on the way they are produced or extracted, but their properties can also depend on the composition and particle size of the starting powders.

Ceramic scaffolds are commonly used for bone regeneration practices because they are highly biocompatible, rarely evoke an immune response, and hardly cause the formation of fibrous tissue around the scaffold; instead they are osteoinductive, considering their high ability to recruit cells from the biological environment and promote osteogenic differentiation. Although the ceramics present these advantages, their use in tissue engineering applications is limited due to their fragility and slow degradation [1, 13, 14].

On the basis of their main features, they can be distinguished into three categories: (a) bio-inert ceramics: completely inert to biological environment; (b) resorbable materials: subjects to *in vivo* degradation for phagocytosis or dissolution of the material in biological fluids; and (c) bioactive ceramics: able to form chemical bound with the cell surface [15].

The most common ceramic biomaterials used for tissue regeneration are: (1) CaP, including hydroxyapatite (HA) ($Ca_{10}[PO_4]_6[OH]_2$), beta-tricalcium phosphate (BTF) ($Ca_3[PO_4]_2$), biphasic calcium phosphate (mixture of hydroxyapatite and beta-tricalcium phosphate), (2) bioglass, (3) alumina (Al_2O_3), and (4) zirconia oxide (ZrO_2).

CaP biomaterials are often selected for bone graft since they mimic bone tissue composition. One of the first used ceramic biomaterials for skeletal repair was BTF in 1920 by Albee and Morrinson [16].

HA may be natural or synthetic. Natural HA derives from particular species of coral or bovine bone and can contain traces of other elements such as Mg, Na,

CO_3, and F. Synthetic HA is prepared by sintering in dense or macroporous form as granules or blocks [17]. Ray and Ward, first, showed the high biocompatibility and biomimicry of synthetic HA in their study in which they used this material for bone tissue engineering application in the long bones and iliac wings of dogs [18]. Later, numerous other studies on HA have been carried out. Calabrese *et al.* in their studies tested a composite bi-layer type-1 collagen-HA/Mg scaffold for osteochondral regeneration, both *in vitro* and *in vivo*. They showed that the combination of this scaffold with mesenchymal stem cells (MSC) derived from adipose tissue (hADSCs) in the presence of specific differentiation conditions induce osteochondro differentiation both *in vitro* and *in vivo* [19–23].

Bioglass is composed by 45 wt% SiO_2, 24.5 wt% CaO, 24.5 wt% Na_2O, and 6.0 wt% P_2O_5. The first one (45S5 Bioglass) has been developed by Hench, which used it for biomedical applications *in vitro* and *in vivo* [24].

Bioglass materials can be synthesized through different methods such as polymer foam replication, thermal bonding of particles or fibers, and sol-gel processing. Similarly to HA, it is suitable for bone graft due to the high ratio of calcium to phosphorus promoting the formation of apatite crystals on its surface after grafting. Bioglass materials offer high osteoinductivity, control of rate of degradation, and excellent bioactivity even if they can present poor mechanical properties such as low strength and toughness [25, 26].

Alumina (Al_2O_3) is a ceramic biomaterial with a crystalline structure. Generally, a low porosity and reduced grain size increase its mechanical strength. Like other ceramic materials, alumina is fragile but it has good tribological properties such as resistance to wear.

Zirconia is characterized by a polymorphic structure and offer has a hard surface, a low thermal conductivity, and a high coefficient of thermal expansion. Its excellent biocompatibility and high breaking load make it a good candidate for prosthesis and bone grafting.

4.2 Polymers

Various biological polymers such as collagen, alginate, proteoglycans, chitin, and chitosan have been used to produce scaffolds. They are biocompatible and bioactive promoting cellular adhesion and growth on their surface. However, they often show poor mechanical properties and fast biodegradability, which limit their use.

Collagen and its derivatives are good candidates for osteochondral regeneration but also tendon and ligament reconstruction since the extracellular matrix of these tissues is mostly made of type-1 collagen fibers [27–29].

Collagen scaffolds are highly bioactive ensuring excellent cellular adhesion to their surface. However, since they have low resistance to mechanical stress often are coupled with other materials, which improve their mechanical properties.

Several studies have been focused on the use of collagen scaffolds for tissue engineering strategies. Aravamudhan *et al.,* for example, reported the fabrication and characterization of cellulose and collagen-based micro-nanostructured scaffolds exhibiting mechanical features similar to those of trabecular bone that promoted good adhesion of human osteoblasts to their surface. Moreover, they underwent a progressive calcium deposition process compared to control polyester micro-nanostructured scaffolds [30].

In another study, Schneider *et al.* developed a collagen I/III hydrogel scaffold and used it to seed hMSC isolated from bone marrow of femoral head spongiosa and from umbilical cord. When stimulated with osteogenic induction medium, both cell types showed comparable osteogenic gene expression, migration, and

scaffold colonization [31]. Collagen scaffolds may also be used to deliver osteogenic differentiation factors as demonstrated by Lu H. *et al,* who immobilized BMP4 in a collagen-PLGA hybrid scaffold to promote osteogenesis [32].

Polysaccharides such as chitin, chitosan, and alginate are suitable for both hard and soft tissue regeneration. In particular, chitosan scaffolds can be manufactured by freeze-drying techniques, which allow obtaining a porous scaffold with high pore interconnectivity. Chitosan ensures good cellular adhesion and thank to its positive charges can interact with glycosaminoglycans and proteoglycans present in living tissues. Costa-Pinto *et al.* cultured human bone marrow MSC on melt-based porous chitosan scaffolds using an osteogenic differentiation medium. They found an increase of cell viability and ALP activity after 21 days. They also investigated the capacity of the cell seeded scaffold to repair a cranial defect in mouse, and 8 weeks after implantation bone formation in the scaffold was analyzed using Bone μCT [33]. Chitosan may also be used as an injectable biomaterial as demonstrated by Bi *et al.,* who produced a composite scaffold of tricalcium phosphate (TCP), chitosan, and platelet rich plasma (PRP). MSC seeded on injectable biomaterial was used in vivo to test its capacity to repair bone fracture in goat femora [34].

Synthetic polymers are high molecular weight compounds composed of a series of monomeric units. On the basis of their structure, they can be linear, branched, or cross-linked. Considering their thermo-mechanical properties, they are thermoplastic or thermosetting. Polymeric materials can be produced in the form of fibers, films, bars, and viscous liquids, and they offer the important advantage to modulate their mechanical properties and biodegradation by varying synthesis process and reactants used. However, they could have low biocompatibility and mechanical strength and show *in vivo* toxicity due to the release of ions and other residual particles of polymerization.

Among the different synthetic polymers, the most suitable for scaffold production is the bio-erodible. These kinds of polymers undergo surface degradation with production of nontoxic low molecular weight compounds.

Numerous synthetic polymers have already been used such as: polystyrene, thermoplastic aromatic polymer with a linear structure; poly-l-lactic acid (PLA), hydrophobic polymer with slow degradation rate due to microorganisms; polyglycolic acid (PGA), hydrophilic polymer with good mechanical properties and fast degradation; poly-dl-lactic-co-glycolic acid (PLGA), biocompatible copolymers with fast degradation rate; and polycaprolactone (PCL), highly hydrophobic polymer with good permeability.

In particular, PGA and PLA and their copolymers are natural polyesters normally present in the organism and therefore well tolerated. They have been used for suture threads, orthopedic screws, and prostheses manufacture since 1970, and more recently, they have been evaluated for scaffold production and tissue engineering strategies. About this, Eğri *et al.* combined PLA and PGA to obtain a PLA-PEG-PLA scaffolds able to release VEGF and BMP-2 in bone tissue lesion. In relation to its chemical composition, the scaffold allows fast release of VEGF in about 1 week and slower constant release of BMP-2 [35].

4.3 Metals

Metals are particularly suitable for tissue engineering strategies for their good mechanical properties such as high elastic module, yield strength, and high ductility allowing them to bear a load without being deformed. If mechanical resistance makes them excellent candidates for scaffold production, however, the reduced cell adhesion to their surface could be a considerable limit to their use. Moreover, metal implants can release toxic metallic ions and/or particles, and biological fluids

can show corrosive action on their surface that can alter their function. Among the different metals used for scaffold production, there are stainless steel, cobalt, and titanium alloys.

Stainless steels are iron-based alloys with a low content of carbon and a high content of chromium. The presence of carbon ensures good mechanical properties but determines carbides formation that makes the scaffold subject to corrosion in a biological environment.

Cobalt-based alloys are of two types: cobalt/chromium/molybdenum alloy obtained with casting/melting methods and cobalt/nickel/chromium/molybdenum alloy worked by forging. Generally, the high level of chromium and molybdenum typical of these alloys increase granule size and improve mechanical properties.

Titanium alloys can be *alpha, beta or alpha/beta* biphasic. *Alpha* alloys contain alpha stabilizers such as aluminum and gallium and are characterized by good strength, hardness, resistance sliding, and weld ability; *Beta* alloys contain beta stabilizers such as vanadium, niobium, and tantalus molybdenum and show good ductility. *Alpha/beta* biphasic alloys show a mix of *alpha/beta* stabilizers, and they are quite ductile even if little resistant to high temperatures, and the most suitable one for biomedical application is Ti 6Al 4 V.

Wohlfahrt *et al.* tested the osteoinductivity and osteointegration capabilities of Ti and TiO2 scaffolds in rabbit tibia peri-implant osseous defects. After 4 weeks, the implant was removed and the new bone formation was observed. Moreover, a gene expression analysis was performed considering different osteogenesis differentiation markers such as osteocalcin and collagen-I [36].

In another study, Zuchuat *et al.* developed Cr-Co-Mo membranes and placed them in rabbit tibiae to analyze the volume of new bone formation. After the explant, histological analysis showed a huge number of osteoblasts and osteocytes on the scaffold [37].

4.4 Composites

Composite scaffolds are developed combining different biomaterials such as natural or synthetic polymers (PGA, PLA, gelatin, chitin, and chitosan), ceramics (hydroxyapatite and beta-tricalcium phosphate or bioglasses), and metals. They have technological, industrial, and applicative importance since they combine biocompatibility, biodegradation, and appreciable mechanical strength. Moreover, these kinds of scaffolds could be applied for both hard and soft tissue regeneration and greatly mimic tissue architecture being composed of cells and extracellular matrix.

Several studies displayed the efficacy of composite scaffolds (polymers/ceramics and synthetic/natural polymers) for tissue engineering strategies [38, 39].

Other researchers demonstrated that another interesting solution may be the combination of metallic implants with polymer coating or metal/ceramic scaffolds [40, 41].

4.5 Hydrogels

Hydrogels are hydrophilic polymers rich of polar moieties such as carboxyl, amide, amino, and hydroxyl groups, held together by chemical bounds or physical intra-molecular and inter-molecular attractions. Their main feature is the ability to absorb enormous amounts of water or biological fluids and swell without dissolving.

According to their origin, hydrogel can be classified into natural (made of polypeptides and polysaccharides), synthetic (obtained by traditional polymerization),

	Advantages	Disadvantages	Clinical uses
Ceramics	-Hard surface -High mechanical stiffnes -Chemical-physic refractoriness -High biocompatibility -osteoinductivity	- Brittleness - Slow degradation - Processing difficulties	-Hip prosthesis -Dental prosthesis -Bone and cartilage
Natural polymers	-Biocompatibility -Bioactivity	- Poor mechanical properties - Fast biodegradation	-Bone and cartilage - Tendon and ligament n
Synthetic polymers	- Possibility of modulating porosity and mechanical properties during the synthesis process.	- Low biocompatibility: possible release of ions and other residual particles of polymerization - Low mechanical strength	-Sutures -Catheters -Cardiovascular prostheses -Bone cements
Metals	-Good mechanical properties: high elastic module, yield strength and high ductility	- Reduced cell adhesion to their surface - Possible corrosion mediated by biological fluid	-Dentistry and orthopedic prostheses
Composites	-Biocompatibility -Good mechanical properties	- Processing difficulties	-Hard and soft tissue
Hydrogel	- Biocompatibility - Controlled biodegradation in vivo -Possibility to modulate their parameters [cross-linking density, porosity, pore size and inteconnectivity]		- Hard and soft tissue

Table 1.
Advantages, disadvantages, and main clinical uses of different kinds of biomaterials.

and semi-synthetic. Moreover, they can present an amorphous or semi-crystalline structure that can be cationic, anionic, neutral, or ampholytic. Depending on their stability in a biological system, they can be considered durable if they do not undergo chemical-physical modification or biodegradable if they degrade into oligomers, which are subsequently eliminated from the body. In the last decades, smart hydrogels have been developed featured by the possibility to modify their structure and mechanical properties according to environmental stimuli such as pH or temperature. Already 50 years ago, these materials have been appreciated for their chemical-physical characteristics by Wichterle and Lim, who developed a poly(2-hydroxyethyl methacrylate)-based hydrogel for contact lens [42]. Since they present a soft and rubbery consistency very similar to that of ECM of different tissues, they have been recently studied for tissue engineering strategies. In particular, hydrogels used for scaffold production may respond to important requirements such as biocompatibility and controlled *in vivo* biodegradation. It is very important to modulate parameters such as hydrogel cross-linking density, porosity, pore size, and interconnectivity to obtain a suitable structural for cellular colonization and proliferation. Hydrogels can be modified at the surface by peptides or growth factor, which can promote cell attachment and differentiation process. Generally, natural hydrogels are less toxic

and more tolerated than synthetic ones, and Pasqui *et al*, for example, developed a natural cellulose-hydroxyapatite hybrid hydrogel for bone tissue engineering. For the chemical synthesis procedure, the freeze-dried hydrogel was immersed in a solution containing HA microcrystals, and then an *in vitro* study demonstrated that MG63 osteoblast-like human cell seeded into hydrogel samples adhered and proliferated rapidly. Moreover, an increase of ALP activity was identified at 3, 7, and 14 days [43]. Synthetic hydrogels could have limitations in the biocompatibility, but they offer the possibility to modulate their mechanical features and rate of degradation in biological environment. Kinard *et al.* developed a biodegradable oligo[poly(ethylene glycol)fumarate] hydrogel to deliver demineralized bone matrix (DBM) in a rat bone defect. They found that the *in vivo* degradation rate of the hydrogel depend on the DBM content, higher was the rate of DBM faster was the degradation. Moreover, high content of DBM could affect the mechanical properties of the hydrogel even if it increases its osteoinductivity in vitro and in vivo [44] (**Table 1**).

5. Processing techniques for scaffold production

After the choice of the biomaterial to use for scaffold production, it is quite important to select an adequate processing technique that allows to maintain high levels of control of the macro- and micro-structural properties of the same. The processing methodology must satisfy key requirements such as: process accuracy and repeatability. The scaffolds obtained will present regular shaped pores with consistent pore size and interconnectivity and should not show any physical-chemical variations when produced by the same method. Moreover, the processing conditions must not alter the mechanical properties of the biomaterial, and any toxic solvent used during the process must be totally removed not to limit scaffold clinical use [3, 11]. Among the most spread processing techniques, probably the most known are those that foresee the employment of a porogenous organic or inorganic agent such as sodium chloride, sodium tartrate, sodium citrate, citric acid, or saccharose. However, the use of porogens limits the scaffolds to thin membranes with a thickness of 2 mm to facilitate complete porogen removal [45].

Mikos *et al.* described solvent casting/particulate leaching for the first time, and it is chosen for the fabrication of porous scaffold used for bone tissue engineering. In this case, the porous agent is dispersed in appropriate solvent and then the dispersion is processed by casting or by freeze-drying. This technique allows obtaining thin membranes with 30–300 micrometer pore size and 20–50% porosity even if the pores have a shifting shape and the interconnectivity is quite low. However, the method presents some disadvantages like time consuming (it is necessary to wait for days or weeks for solvent evaporation) and the use of toxic organic solvents [46].

In melt molding/particulate leaching, an unrefined thermoplastic polymer is mixed with the porous agent and then the blend is poured in a mold with an appropriate shape. The mold is then heated above the glass transition temperature of the polymer and at last the obtained solid is immersed in a solvent to promote the dissolution of the porogens. The advantage of this methodology is the possibility to monitor the pore size and porosity (generally 80–84%) by varying the amount of porogenous [47]. A good variant of melt molding is extrusion or injection molding proposed by Gomes *et al.,* who replaced the porous agent with a blowing agent based on citric acid. During the heating process, the blowing agent degraded producing carbon dioxide which formed interconnected and well-shaped pores [48].

Gas foaming is an high pressure processing technique described by Mooney *et al* who produced sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents. Solid disks of the polymer are exposed to high pressure CO_2

(5.5 MPa) at room temperature followed by a decreasing of gas pressure to reduce its solubility in the polymer bulk. It brings CO_2 to abandon the polymer forming well-shaped pores [49].

Phase inversion/particulate leaching is a valid method to obtain polymeric scaffolds. After the polymer solubilization in a suitable solvent, the solution is dissolved in water that provokes the polymer precipitation. Obviously, it is possible to modulate the characteristics of the scaffolds obtained through this method by varying the polymer concentration but also the temperature of the solution. Holy *et al.* used this technique to develop porous PLGA scaffold with architecture similar to osseous trabecular for bone tissue engineering [50].

Another interesting method is the fiber bonding. It allows obtaining scaffolds containing a dense frame of synthetic fibers that form a sufficiently porous three-dimensional structure. This technique provides the alignment of the PGA fibers in the desired orientation and subsequently they are covered with a PLLA/methylene chloride solution and heated above the melting temperatures of both polymers. When PLLA is removed through a dissolution process, the PGA fibers remain attached to each other forming a thick net.

In the freeze-drying method, the polymer solution is first frozen rapidly at temperatures below 0°C followed by solvent removal by vacuum sublimation. It can be applied to obtain both natural and synthetic scaffolds [51]. At last, the progress of computer technology led to the development of new techniques like solid freeform fabrication (SSF) whose introduction has signed a new era for manufacturing industry. These techniques allow to produce layer-by-layer 3D objects starting from information generated by CAD system or computer-based medical imaging modalities. Obviously, the use of a computerized production system saves time and modulates with extreme precision parameters related to the micro and macro architecture of the scaffold.

The first SFF technique used for tissue engineering purpose was 3D printing. This technique uses a printer head that places a liquid binder onto thin layers of powder following the object shape generated by a CAD system. Using this technique, Kim *et al.* obtained porous PLGA scaffolds [52], while Zeltinger *et al.* created poly(l-lactic acid) disk shaped scaffolds with two different porosities (75% and 90%) and four different pore size distributions (<38, 38–63, 63–106, and 106–150 μm) [53].

Another interesting SFF methodology is fused deposition modeling (FDM). In this case, a filament of thermoplastic material is fed and melted inside a heated liquifier head and then it is forced out by an extruder and deposited on a platform. Layer by layer, the 3D object is then obtained. By varying the direction of material deposition for each layer, it is possible to change the pore size and interconnectivity of the scaffold. Using this methodology, Hutmacher et al. obtained polycaprolactone scaffolds with honeycomb-like structure and a porosity of 61 +/− 1% and proved their in vitro ability to promote proliferation of primary human fibroblasts and periosteal cells [54].

6. Biomaterials for bone tissue engineering: current applications and new perspectives

One of the current problems in orthopedic clinic is represented by bone lesions caused by traumas, cancer resection degenerative diseases, or nonunion of fractures, which do not heal spontaneously but require surgical procedures. Today, the gold standard for osseous replacement is the autologous bone graft. This technique employs cells of the same patient generally taken from different sites such as fibula or iliac crest that are implanted in bone defect to promote a rapid healing. Although it minimizes the risk of autoimmune response, which was the critical side

of xenogenic grafts, it presents some disadvantages such as donor site morbidity, infections, and post-surgery chronic pain [55–57]. In sight of this, science aims to find innovative solutions like application of biomaterials to orthopedics in order to develop medical implant useful to accelerate the healing, restoring the physiological functions of bone.

The design of an implant for skeletal defects may consider the main characteristics of bone tissue which is divided into two different forms: cortical bone, almost solid with less than 10% porosity and trabecular bone organized in a sponge-like pattern with a porosity of 50–60% [58]. According to the classification of Hanch and Navarro, the evolution of bone implant devices has marked three different generations: (a) bio-inert materials (first generation), (b) bioactive and biodegradable materials (second generation), and (c) biomaterials capable of inducing specific cellular responses by incorporating into a 3D scaffold bone progenitor cells and growth factors [59, 60].

The purpose of first generation of implants was the integration with host tissue without eliciting specific immune response. These implants include metals (Stainless steel, Ti-based, and Co–Cr-based alloys), ceramics (natural and synthetic HA), and polymers (silicone rubber, PE, acrylic resins, polyurethanes, polypropylene PP, and polymethylmethacrylate).

The second generation of implants was developed between 1980 and 2000 and intends to improve both the bioactivity and *in vivo* biodegradation. To this purpose, one of the possible strategies was to modify the first generation by strategic coating such as HA, *beta*-tricalcium phosphate (*b*-TCP), or bioactive glass. Another innovation was the use of natural or synthetic polymers like, poly(*p*-caprolactone), polylactide, polyglicolide, and chitosan with controlled *in vivo* biodegradation rate.

Third generation of implants combines biomaterials useful to develop 3D porous bioactive, biodegradable scaffolds with the integration of progenitor cells, and specific growth factors. This innovation laid the foundations for modern bone tissue engineering strategies. Even if an ideal combination of biomaterials for scaffold production has not been identified yet, recent studies have demonstrated the great efficiency of ceramics in mimic chemical-physic characteristics of bone tissue ECM. Also, our group tested *in vitro* and *in vivo* potential of collagen type-1/Ha-Mg combination to promote bone injury healing. We demonstrated that although biomimetic scaffolds are "*per sè*" able to promote tissue regeneration thanks to their high osteoinductivity, their combination with progenitor cells and growth factors would be more efficient [19–21]. Generally, osteogenic cells such as adult stem cells (ASC) isolated from adult tissues like bone marrow, adipose tissue, or muscle are good candidate to be transplanted in skeletal lesion together with an appropriate scaffold. These kinds of cells are characterized by high capacity of self-renewal and potential of osteogenic differentiation. Moreover, it has been suggested that ASC possess immunosuppressive effects, which make them particularly privileged for transplantation in vivo.

Growth factors are cytokines normally secreted by different cell types. Acting on their own receptors, they induce intracellular pathways, which promote proliferation, cellular adhesion, and differentiation. Bone tissue produces different growth factors such as bone morphogenetic proteins (BMPs), transforming growth factor beta (TGFb), fibroblast growth factors (FGFs), insulin growth factor I and II (IGF I/II), and platelet-derived growth factor (PDGF), which have been proposed for tissue engineering strategies. In particular, BMP2 and 7 have been cloned and are commercially available as recombinant proteins. The interest in them for bone regenerative practices has increased since 1965 when Urist discovered that demineralized bone transplanted in subcutaneous tissue induces bone formation [61]. This potential was later attributed to the presence of

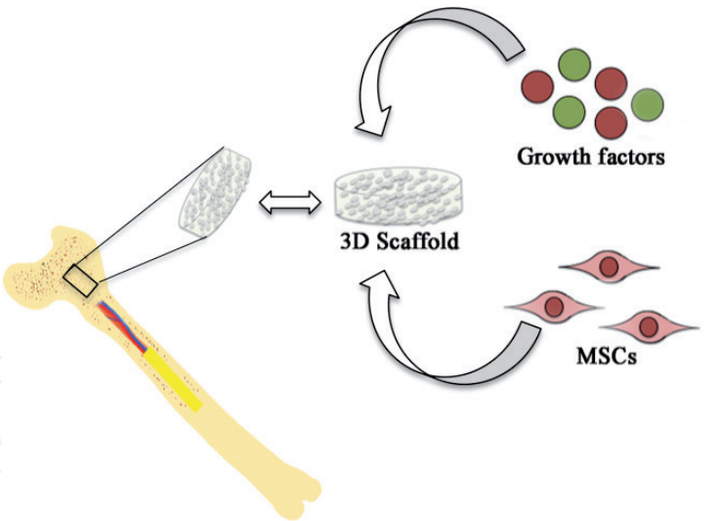

Figure 1.
Schematic representation of bone tissue engineering.

BMP. Obviously, the choice to include a growth factor in the scaffold requires the use of biomaterials that can act as drug delivery systems protecting the cytokine from *in vivo* proteolysis and ensuring a progressive and controlled release over time. In this sense, a good alternative is the physical immobilization of the growth factor in a biodegradable hydrogel. In this case, the release will be controlled by the *in vivo* degradation of hydrogel cross-linked with generation of water-soluble hydrogel fragments [9] (**Figure 1**).

7. Conclusions

Recently, the interest in natural and synthetic biomaterials for medical devices production has increased, and more and more in-depth studies are carried out to better detect their possible applications linked to chemical-physical features and the extractive or synthetic methods, which do not alter their structural properties and biocompatibility. Moreover since tissue engineering strategies have become a valid alternative for body structure and function restoring, biomaterials are also used for the fabrication of 3D porous biomimetic bioactive scaffolds with controlled degradation rate *in vivo.*

As previously mentioned, the main classes of biomaterials for scaffold production are ceramics, natural and synthetic polymers, metals, composites, and hydrogels. *In vitro* and *in vivo* studies have showed the advantages related to their use in regenerative medicine field but they have also highlighted the possible negative sides.

Regarding the application of biomaterials to tissue engineering, the current aim of science is to find the natural or synthetic substance or the combination with the most satisfactory performance *in vivo,* able to promote cell proliferation and differentiation in a tissue lesion in order to restore the normal architecture of ECM.

In conclusion, tissue engineering strategies especially in orthopedic clinic field represent an effective and sophisticated alternative for the future, but their success strictly depends on an ever deeper knowledge about the characteristics of the biomaterials and the potentialities of their combinations.

Conflicts of interest

The authors declare no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.83839

Author details

Anna Dolcimascolo[1], Giovanna Calabrese[1*], Sabrina Conoci[2] and Rosalba Parenti[1]

1 Department of Biomedical and Biotechnological Sciences, Physiology Section, University of Catania, Catania, Italy

2 STMicroelectronics Stradale Primosole, Catania, Italy

*Address all correspondence to: soniacalabrese@hotmail.com

References

[1] O'Brien FJ. Biomaterials & scaffolds for tissue engineering. Materials Today. 2011;**14**(3):88-95. DOI: 10.1016/S1369-7021(11)70058-X

[2] Langer R, Vacanti JP. Tissue engineering. Science. 1993;**260**(5110): 920-926

[3] Salgado AJ, Coutinho OP, Rui L, Reis RL. Bone tissue engineering: State of the art and future trends. Macromolecular Bioscience. 2004;**4**:743-765. DOI: 10.1002/mabi.200400026

[4] Ravaglioli A, Krawjesky R. Bioceramics. Netherlands: Springer; 1992. ISBN 978-94-010-5032-6

[5] Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. Biomaterials. 2000:175-189. DOI: 10.1016/B978-008045154-1.50021-6

[6] Yu X, Tang X, Gohil SV, Laurencin CT. Biomaterials for bone regenerative engineering. Advanced Healthcare Materials. 2015;**4**(9):1268-1285. DOI: 10.1002/adhm.201400760

[7] Tirrell M, Kokkoli E, Biesalski M. The role of surface science in bioengineered materials. Surface Science. 2002;**500**:61-83. DOI: 10.1016/S0039-6028(01)01548-5

[8] Babensee JE, Anderson JM, McIntire LV, Mikos AG. Host response to tissue engineered devices. Advanced Drug Delivery Reviews. 1998;**33**:111-139

[9] Tabata Y. Biomaterial technology for tissue engineering applications. Journal of the Royal Society Interface. 2009;**6**:S311, S324. DOI: 10.1098/rsif.2008.0448

[10] Ratner B, Hoffman A, Schoen F, Jack Lemons J. Biomaterials Science, 3rd Edition. An Introduction to Materials in Medicine. Academic Press; 2012.

[11] Leong KF, Cheah CM, Chua CK. Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. Biomaterials. 2003;**24**(13):2363-2378

[12] Hadjipanayi E, Mudera V, Brown RA. Close dependence of fibroblast proliferation on collagen scaffold matrix stiffness. Tissue Engineering and Regenerative Medicine. 2009;**3**(2): 77-84. DOI: 10.1002/term.136

[13] Lichte P, Pape HC, Pufe T, Kobbe P, Fischer H. Scaffolds for bone healing: Concepts, materials and evidence. Injury. 2011;**42**(6):569-573. DOI: 10.1016/j.injury.2011.03.033

[14] Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends in Biotechnology. 2012;(10):546-554. DOI: 10.1016/j.tibtech.2012.07.005

[15] Best SM, Porter AE, Thian ES, Huang J. Bioceramics: Past, present and for the future. Journal of the European Ceramic Society. 2008;**28**:1319-1327. DOI: DOI :10.1016/j.jeurceramsoc.2007.12.001

[16] Albee F, Morrison H. Studies in bone growth. Annals of Surgery. 1920;**71**:32-38

[17] Lagers RZ. Properties of osteoconductive biomaterials: Calcium phosphates. Clinical Orthopaedics and Related Research. 2002;**395**:81-98

[18] Ray RD, Ward AA. A preliminary report on studies of basic calcium phosphate in bone replacement. In: Surgical Forum, American College of Surgeons, 1951. Philadelphia: WB Saunders Co; 1952. pp. 429-434

[19] Calabrese G, Giuffrida R, Fabbi C, Figallo E, Furno DL, Gulino R, et al. Collagen-hydroxyapatite scaffolds

induce human adipose derived stem cells osteogenic differentiation in vitro. PLoS One. 2016;**11**(3). DOI: 10.1371/journal.pone.0151181

[20] Calabrese G, Giuffrida R, Forte S, Salvatorelli L, Fabbi C, Figallo E, et al. Bone augmentation after ectopic implantation of a cell-free collagen-hydroxyapatite scaffold in the mouse. Scientific Reports. 2016;**6**:36399. DOI: 10.1038/srep36399

[21] Calabrese G, Giuffrida R, Forte S, Fabbin C, Figallo E, Salvatorelli L, et al. Human adipose-derived mesenchymal stem cells seeded into a collagen-hydroxyapatite scaffold promote bone augmentation after implantation in the mouse. Scientific Reports. 2017;**7**(1):7110. DOI: 10.1038/s41598-017-07672-0

[22] Calabrese G, Forte S, Gulino R, Cefalì F, Figallo E, Salvatorelli L, et al. Combination of collagen-based scaffold and bioactive factors induces adipose-derived mesenchymal stem cells chondrogenic differentiation in vitro. Frontiers in Physiology. 2017;**8**(50). DOI: 10.3389/fphys.2017.00050

[23] Calabrese G, Gulino R, Giuffrida R, Forte S, Figallo E, Claudia Fabbi C, et al. In vivo evaluation of biocompatibility and chondrogenic potential of a cell-free collagen-based scaffold. Frontiers in Physiology. 2017;**8**:984. DOI: 10.3389/fphys.2017.00984

[24] Hench LL. The story of bioglass. Journal of Materials Science. Materials in Medicine. 2006;**17**(11):967-978. DOI: 10.1007/s10856-006-0432-z

[25] Rahaman MN, Day DE, Bal BS, Fu Q, Jung SB, Bonewald LF, et al. Bioactive glass in tissue engineering. Acta Biomaterialia. 2011;**7**(6):2355-2373. DOI: 10.1016/j.actbio.2011.03.016

[26] Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials. 2006;**27**(18):3413-3431. DOI: 10.1016/j.biomaterials.2006.01.039

[27] Vagaská B, Bacáková L, Filová E, Balík K. Osteogenic cells on bio-inspired materials for bone tissue engineering. Physiological Research. 2009;**59**(3):309-322

[28] Deponti D, Di Giancamillo A, Gervaso F, Domenicucci M, Domeneghini C, Sannino A, et al. Collagen scaffold for cartilage tissue engineering: The benefit of fibrin glue and the proper culture time in an infant cartilage model. Tissue Engineering Part A;**20**(5-6):1113-1126. DOI: 10.1089/ten.TEA.2013.0171

[29] Hoyer M, Drechsel N, Meyer M, Meier C, Hinüber C, Breier A, et al. Embroidered polymer-collagen hybrid scaffold variants for ligament tissue engineering. Materials Science & Engineering. C, Materials for Biological Applications. 2014;**43**:290-299. DOI: 10.1016/j.msec.2014.07.010

[30] Aravamudhan A, Ramos DM, Nip J, Harmon MD, James R, Deng M, et al. Cellulose and collagen derived micro-nano structured scaffolds for bone tissue engineering. Journal of Biomedical Nanotechnology. 2013;**9**(4):719-731

[31] Schneider RK, Puellen A, Kramann R, Raupach K, Bornemann J, Knuechel R, et al. The osteogenic differentiation of adult bone marrow and perinatal umbilical mesenchymal stem cells and matrix remodelling in three-dimensional collagen scaffolds. Biomaterials. 2010;**31**(3):46780. DOI: 10.1016/j.biomaterials.2009.09.059

[32] Lu H, Kawazoe N, Kitajima T, Myoken Y, Tomita M, Umezawa A,

et al. Spatial immobilization of bone morphogenetic protein-4 in a collagen-PLGA hybrid scaffold for enhanced osteoinductivity. Biomaterials. 2012;**33**(26):6140-6146. DOI: 10.1016/j.biomaterials.2012.05.038

[33] Costa-Pinto AR, Correlo VM, Sol PC, Bhattacharya M, Charbord P, Delorme B, et al. Osteogenic differentiation of human bone marrow mesenchymal stem cells seeded on melt based chitosan scaffolds for bone tissue engineering applications. Biomacromolecules. 2009;**10**(8):2067-2073. DOI: 10.1021/bm9000102

[34] Bi L, Cheng W, Fan H, Pei G. Reconstruction of goat tibial defects using an injectable tricalcium phosphate/chitosan in combination with autologous platelet rich plasma. Biomaterials. 2010;**31**:3201-3211. DOI: 10.1016/j.biomaterials.2010.01.038

[35] Eğri S, Eczacıoğlu N. Sequential VEGF and BMP-2 releasing PLA-PEG-PLA scaffolds for bone tissue engineering: I. Design and in vitro tests. Artificial Cells, Nanomedicine, and Biotechnology. 2017;**45**(2):321-329. DOI: 10.3109/21691401.2016.1147454

[36] Wohlfahrt JC, Monjo M, Rnold HJ, Aass AM, Ellingsen JE, Lyngstadaas SP. Porous titanium granules promote bone healing and growth in rabbit tibia peri-implant osseous defects. Clinical Oral Implants Research. 2010;**21**:165. DOI: 10.1111/j.1600-0501.2009.01813.x

[37] Zuchuat J, Berli M, Maldonado Y, Decco O. Influence of chromium-cobalt-molybdenum alloy [ASTM F75] on bone ingrowth in an experimental animal model. Journal of Functional Biomaterials. 2018;**9**(1):2. DOI: 10.3390/jfb9010002

[38] Qian J, Xu W, Yong X, Jin X, Zhang W. Fabrication and in vitro biocompatibility of biomorphic PLGA/nHA composite scaffolds for bone tissue engineering. Materials Science & Engineering. C, Materials for Biological Applications. 2014;**1**(36):95-101. DOI: 10.1016/j.msec.2013.11.047

[39] Sekiya N, Ichioka S, Terada D, Tsuchiya S, Kobayashi H. Efficacy of a poly glycolic acid [PGA]/collagen composite nanofibre scaffold on cell migration and neovascularization in vivo skin defect model. Journal of Plastic Surgery and Hand Surgery. 2013;(6):47, 498-502. DOI: 10.3109/2000656X.2013.788507

[40] Wu C, Zhou Y, Xu M, Han P, Chen L, Chang J, et al. Copper-containing mesoporous bioactive glass scaffolds with multifunctional properties of angiogenesis capacity, osteostimulation and antibacterial activity. Biomaterials. 2013;**34**(2):422-433. DOI: 10.1016/j.biomaterials.2012.09.066

[41] Oughlis S, Lessim S, Changotade S, Bollotte F, Poirier F, Helary G, et al. Development of proteomic tools to study protein adsorption on a biomaterial titanium grafted with poly(sodium styrene sulfonate). Journal of Chromatography B. 2011;**879**:3681-3687. DOI: 10.1016/j.jchromb.2011.10.006

[42] Wichterle O, Lim D. Hydrophilic gels for biological use. Nature. 1960;**185**:117-129. DOI: 10.1038/185117a0

[43] Pasqui D, Torricelli P, De Cagna M, Milena Fini M, Barbucci R. Carboxymethyl cellulose—Hydroxyapatite hybrid hydrogel as a composite material for bone tissue engineering applications. Journal of Biomedical Materials Research. 2014;**102a**(5):1568-1579. DOI: 10.1002/jbm.a.34810

[44] Kinard LA, Dahlin RL, Lam J, Lu S, Lee EJ, Kasper FK, et al. Synthetic

biodegradable hydrogel delivery of demineralized bone matrix for bone augmentation in a rat model. Acta Biomaterialia. 2014;**10**(11):4574-4582. DOI: 10.1016/j.actbio.2014.07.011

[45] Lu L, Mikos AG. The importance of new processing techniques in tissue engineering. MRS Bulletin. 1996;**21**(11):28-32

[46] Mikos AG, Thorsen AJ, Czerwonka LA, Bao Y, Langer R, Winslow DN, et al. Preparation and characterization of poly(l-lactic acid) foams. Polymer. 1994;**35**:1068-1077. DOI: 10.1016/0032-3861(94)90953-9

[47] Thomson RC, Yaszemski MJ, Powers JM, Mikos AG. Fabrication of biodegradable polymer scaffolds to engineer trabecular bone. Journal of Biomaterials Science. Polymer Edition. 1995;**7**(1):23-38

[48] Gomes ME, Ribeiro AS, Malafaya PB, Reis RL, Cunha AM. A new approach based on injection moulding to produce biodegradable starch-based polymeric scaffolds: Morphology, mechanical and degradation behaviour. Biomaterials. 2001;**22**(9):883-889

[49] Mooney DJ, Baldwin DF, Suh NP, Vacanti JP, Langer R. Novel approach to fabricate porous sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents. Biomaterials. 1996;**17**(14):1417-1422

[50] Holy CE, Shoichet MS, Davies JE. Engineering three-dimensional bone tissue in vitro using biodegradable scaffolds: Investigating initial cell-seeding density and culture period. Journal of Biomedical Materials Research. 2000;**51**(3):376-382

[51] Yang S, Leong KF, Du Z, Chua CK. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Engineering. 2001;**7**(6):679-689. DOI: 10.1089/107632701753337645

[52] Kim SS, Utsunomiya H, Koski JA, Wu BM, Cima MJ, Sohn J, et al. Survival and function of hepatocytes on a novel three-dimensional synthetic biodegradable polymer scaffold with an intrinsic network of channels. Annals of Surgery. 1998;**228**(1):8-13

[53] Zeltinger J, Sherwood JK, Graham DA, Müeller R, Griffith LG. Effect of pore size and void fraction on cellular adhesion, proliferation, and matrix deposition. Tissue Engineering. 2001;**7**(5):557-572. DOI: 10.1089/107632701753213183

[54] Hutmacher DW, Schantz T, Zein I, Ng KW, Teoh SH, Tan KC. Mechanical properties and cell cultural response of polycaprolactone scaffolds designed and fabricated via fused deposition modeling. Journal of Biomedical Materials Research. 2001;**55**(2):203-216

[55] Banwart JC, Asher MA, Hassanein RS. Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. Spine. 1995;**20**:1055-1060

[56] Arrington ED, Smith WJ, Chambers HG. Complications of iliac crest bone graft harvesting. Clinical Orthopaedics. 1996;**329**:300-309

[57] Ahlmann E, Patzakis M, Roidis N, Shepherd L, Holtom P. Comparison of anterior and posterior iliac crest bone grafts in terms of harvest-site morbidity and functional outcomes. The Journal of Bone and Joint Surgery. American Volume. 2002;**84A**:716-720

[58] Sikavitsas VI, Temenoff JS, Mikos AG. Biomaterials and bone mechanotransduction. Biomaterials. 2001;**22**(19):2581-2593

[59] Hench LL, Polak JM. Third-generation biomedical materials.

Science. 2002;**295**(5557):1014-1017. DOI: 10.1126/science.1067404

[60] Navarro M, Michiardi A, Castaño O, Planell JA. Biomaterials in orthopaedics. Journal of the Royal Society Interface. 2008;**5**(27):1137-1158. DOI: 10.1098/rsif.2008.0151

[61] Urist MR. Bone: Formation by autoinduction. Science. 1965;**5**(27):1137-1158