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How Nanotechnology can Really Improve the Future of Orthopedic Implants and Scaffolds for Bone and Cartilage Defects

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Abstract

The osteointegration of the orthopaedic implants could improve the biocompatibility and the life span of the implants. The ideal implants should be made by materials easily colonized by bone-forming cells (osteoblasts), which can synthesize new bone matrix. Some implant materials are not often compatible with osteoblasts, but rather they promote the formation of soft connective tissue. There are a number of important reasons to explore the potential for the application of nanomaterials in orthopedic surgery. The use of nanotechnology has been tested on a wide range of materials (such as metals, ceramics, polymers, and composites), where either nanostructured surface features or constituent nanomaterials (including grains, fibers, or particles with at least one dimension from 1 to 100 nm) have been utilized. These nanomaterials have demonstrated superior properties compared with their conventional (or micron structured) counterparts, due to their distinctive nanoscale features and the novel physical properties that ensue. Aim of this paper is to explore how nanotechnology can really improve the future of orthopedic implants and scaffolds for bone and cartilage defects. Here we are showing the most relevant works about the use of nanotechnologies for the treatment of osteocondral defects.

Keywords: Nanotechnology; Nanoparticles; Nanomaterials; Bone defect; Cartilage defect; Orthopedic implant

Introduction

Due to the aging of the world's population, the market for orthopedic implants is growing rapidly. Each year, over 600.000 joint replacements are performed in the USA alone, with an estimated worldwide cost in excess of 3 billion dollars [1].

Extended bone defects, caused by trauma, tumor, infectious and periprosthetic osteolysis need to be surgically treated because of their low potential of repair. Currently, bone allograft and autograft represent 80% of all transplantation done in the world. Withal, this technique shows many disadvantages, such as the risk of infections, the immunological rejection, the low bone availability and high costs. Minor cartilage defects, that do not involve the subchondral bone layer, won't be repaired spontaneously. On the contrary, major defects are healed intrinsically by a fibro cartilaginous repair tissue, much poorer than the original hyaline articular cartilage. The goal, however, is to produce a repair tissue that has the same functional and mechanical properties of hyaline articular cartilage [2].

Today's orthopedic implant materials do not completely allow patients to return to their normal, daily active lifestyles they enjoyed prior to the implant; the average lifetime of orthopedic implants is, indeed, only 10-15 years [3]. Failed implants require several challenging revision surgeries, which drastically increase cost and recovery time and thus, especially in the case of young implant recipients, this means they will have to undergo several painful and expensive surgeries during their lifetime. For successful osteointegration, an orthopedic implant material must be inhabitable for bone-forming cells (osteoblasts), so that they can colonize the implant surface and synthesize new bone tissue [4,5]. The success of both orthopedic implants and tissue engineered construct is highly dependent on the interactions between the selected biomaterial and the host tissue. One of the key factors identified in the failure of both types of implants was insufficient tissue regeneration around the biomaterial immediately after implantation [6,7]. This has been attributed to poor surface interaction of biomaterials with the host tissue.

Several different materials have been proposed, studied and applied for the preparation of orthopedic implants, and among those, nanostructured materials are raising much interest among the scientific community because they possess important skills to resolve the main concerns orthopedic surgery has to face [8-10]. As natural tissues are nanometer in dimension, and cells interact directly with, and create, nanostructured extra-cellular matrices (ECM), the biomimetic features and physiochemical properties of nanomaterials play a key role in both stimulating cell growth and guiding tissue regeneration [1]. Natural tissues possess numerous nanometer features due to presence of collagen fibrils and other proteins less than 100 nm in one dimension [11]. Bone tissue, in particular, possesses both proteins (such as collagen) and ceramics (hydroxyapatite and other calcium phosphates) that have fundamental dimensions less than 100 nm at least in one direction. When examining the surface roughness of bone, it is clearly seen that it is a nanomaterial. The implants used today are in contrast with this, as they are smooth at the nanometer level and have average surface feature sizes closer to 10 to 100 microns. Nanometer or submicron surface structures have accelerated cellular responses by emulating the dimension, geometry, and arrangement of components

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of natural tissue [12]. The range below 100 nm is crucial because the classic laws of physics change, resulting in novel physical properties that enable researchers to produce new materials with exact properties such as size and strength beyond conventional limits.

The use of nanotechnology has been tested on a wide range of materials (such as metals, ceramics, polymers, and composites), where either nanostructured surface features or constituent nanomaterials (including grains, fibers, or particles with at least one dimension from 1 to 100 nm) have been utilized [13,14]. These nanomaterials have demonstrated superior properties compared with their conventional (or micron structured) counterparts, due to their distinctive nanoscale features and the novel physical properties that ensue [15]. Furthermore, nanomaterials have consistently been reported to decrease infection, reduce scar tissue growth, and promote bone growth. Interestingly, this has been observed through the use of both nanoparticles assembled as implants and as current implants that are modified to have nanostructured features [16,17]. The latter have received increased attention, as there is no concern over nanoparticles becoming loose through mechanical wear and potential associated toxicity, which has yet to be largely determined. There are numerous examples of implants with nano rough surface features that mimic those that natural tissue possess, which have been shown to better promote tissue growth than do flat or nano-smooth implants [12]. Regardless of chemistry, a better design strategy may be to fabricate orthopedic implants to have structures similar to the nanoscale features of natural human bone. In this context, modification of grain size, topography, pore, and/or particle size into the nanometer regime is simpler than developing novel chemistries which may or may not achieve improved osteo-integrative properties [4]. Another benefit is the significantly greater surface area that can be achieved through the use of nano-structured compared to micron structured materials. Surface area increases alone can be beneficial when promoting bone growth, or detrimental when promoting inflammation or infection. Therefore, it is the other properties related to greater surface area, such as higher surface roughness that allows nanomaterials to promote bone growth [12].

Nanosized carbon tubes, nanoparticulate metals (such as Ti, CoCr, Ti_6Al_4V), nanoparticulate ceramics (hydroxyapatite (HA), titania, alumina, zinc oxide, etc.), and composite implant materials thereof have all been shown to increase tissue regeneration by promoting the adsorption and bioactivity of certain proteins, such as fibronectin and vitronectin, which are contained in plasma and are important for mediating tissue-forming cell adhesion [11,18].

The development of bioinspired nanocomposites has great potential to improve the efficacy of current orthopedic implants and tissue engineering constructs. For organic/inorganic biocomposites, it is possible to obtain a wide range of mechanical and biological properties by modifying the type and distribution of inorganic phase in the organic matrix, and hence to optimize the performance of the biomedical devices and their interaction with the host tissues. Current research is as well exploring the potential use of mesenchymal stem cells as a source for tissue engineering and the combination of cells with biodegradable nanostructured scaffolds [19-21].

Importance of the Nanomaterials Surface

Although various definitions are in use for the word "nanomaterial" by different experts, the commonly accepted concept refers to materials with nanosized topography or composed of nanosized building components. Examples include materials with a basic structural unit in the range 1-100 nm (nanostructured), crystalline solids with grain sizes 1-100 nm (nanocrystals), individual layers or multilayer surface coatings in the range 1-100 nm (nanocoatings), extremely fine powders with an average particle size in the range 1-100 nm (nanoparticles), and fibers with a diameter in the range 1-100 nm (nanofibers) [22,23].

As mentioned, natural bone is a nanostructured composite composed of a polymer matrix (mainly collagen) reinforced with nanometer-sized ceramic particles (mainly carbonated HA). Recent researches in bone regeneration suggested that better osteoconductivity (the recruitment of mesenchymal stem and pluripotent osteoprogenitor cells to a bone healing site) and enhanced osteoinduction would be achieved if synthetic materials were fabricated to resemble bone in terms of its nano-scale features [6,24].

Metals are the most common materials used for total bone replacement or implant fixations, as the mechanical properties of metals meet the requirements for load bearing bone applications [25-27]. However, both metal and polymeric implants may fail due to several factors, such as: infection; inflammation; severe stress shielding and strain imbalances (due to differences in the mechanical properties of an implant and the surrounding bone), leading to implant loosening and eventual fracture; the generation of wear debris in articulating components of implants, which become lodged between the implant and surrounding tissue and lead to bone cell death [18,28,29]; and incomplete, or prolonged bone-integration (i.e., lack of bonding of an orthopedic implant to juxtaposed bone) [2].

The introduction of an implant into a living organism causes specific reactions in the biological environment [30]. The biomolecules and cells together with the intrinsic properties of the chosen biomaterials determine the biocompatibility and longevity of the implants. Since the interaction of those biomolecules and cells with the biomaterial surface is a vital element in the evaluation of the biomaterial, scientists have reexamined the pertinent host – cell interactions in order to design materials that facilitate favorable interactions and enhance tissue regeneration. Ultimately, improved symbiosis should result in an accelerated healing time, an increase in implant longevity, and a reduction in the necessity for revision surgery [1]. For example, fibrous soft tissue, as opposed to hard bone tissue, has been shown to improperly fix orthopedic implants to the surrounding bone, which leads to loosening under physiological loading conditions and eventually to implant failure. Excessive fibrous tissue formation also hinders the growth of osteoblasts and bone-resorbing cells (osteoclasts), resulting in less new bone regeneration between an implant and adjoining bones. Furthermore, the rapid formation of new bone tissue reduces the detrimental impact of wear debris generated from articulating components of orthopedic implants [4]. It is believed that the lack of attention thus far dedicated to understanding cellular recognition of the proteins initially adsorbed by biomaterial surfaces is one of the key factors limiting the lifespan of implants. In order to improve orthopedic implant materials, one must concentrate on the cellular processes that lead to efficient new bone growth. Positive responses from osteoblasts, including increased initial adhesion, proliferation, and differentiation from non-calcium-depositing to calcium-depositing cells are essential. In addition, coordinated activities between osteoblasts and osteoclasts, are needed to maintain healthy bone surrounding the implant. Poor communication between these cells leads to cell necrosis adjacent to the implant, which leaves the bone weaker and thus more prone to fracture [31]. Due to the importance of these specific cellular events, orthopedic research has been concentrated on understanding the cellular recognition of surfaces and on creating biomaterial surface properties to maximize such interactions, to promote the creation of more bone.

While often the focus has been on orthopedic implant chemistry (from metals to ceramics to polymers), recent discoveries have highlighted that nanotechnology may universally improve all materials used for regrowing bone. Nanomaterials have been used to modify the surface features of current implants and drug delivery devices to reduce infection, inhibit chronic inflammation and ultimately to increase appropriate tissue growth [11,32,33].

Cartilage and Bone Tissue Engineering

Bone is a living tissue, which continuously rebuilds its structure, and thus most of the common bone lesions, like fractures, heal well with conventional therapy. However, in case of large defects and osseous congenital deformities, or in the case of a separation of the articular surface from the bone layers or very large osteochondral defects, an artificial prosthesis is required [34-36].

Cartilage is an avascular tissue composed of chondrocytes entrapped in an ECM rich in proteoglycans and collagens. Chondral defects suppose a challenging clinical problem as the proportion of elderly people in the population increase [37]. Cartilage injuries lead to joint pain and loss of function with limited capacity for self-repair. Innate repair mechanisms in cartilage are limited due to the scarcity/absence of resident SCs and the lack of a vascular and lymphatic system. Clinical treatments for articular cartilage injury include physical therapy, arthroscopic drilling, debridement, autologous osteochondral grafts from non-weight-bearing body regions, or autologous cell injections. However, the donor site morbidity and the difficulty in trimming and grafting for the desired shape limit their clinical applications [38].

Conventional tissue replacements (such as autografts and allografts) have a variety of problems that cannot satisfy high performance demands necessary for today's patient. Consequently, tissue engineering (or regenerative medicine) emerged initially defined by Robert Langer and Joseph Vacanti as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" [39].

Over the past decade, one of the main goals of bone tissue engineering has been develop biodegradable materials as bone substitutes for filling large bone defects. In tissue engineering, tissue substitutes are constructed in the laboratory by combining living cells with artificial components such as biomaterials which are subsequently introduced into a patient to create, repair or replace natural tissue and/or organs.

Ideal scaffolds should be biodegradable and are designed as a temporary 3D mirror matrix, onto which cells grow and regenerate the needed tissues, and should have the same function of the ECM network, a scaffold furnishing cells with precisely-controlled cell-cell, cell-matrix, and cell-soluble factor signals which ultimately dictate activity [40,41]. Thus, understanding these interactions is of crucial importance [42]. The functional roles of the native ECM scaffold are structural: to support cells and provide a substrate for cell migration and survival; biochemical: to sequester growth factors and other chemical cues that regulate cell fate; and biological: to present bioactive peptide sequences that can directly bind receptors and activate intracellular signaling pathways [43]. Furthermore, another key characteristic of the ECM that dictates cell behavior has been recently identified namely the size and topographical features of its structural elements [44].

Many investigators are currently seeking to fabricate biomimetic nanostructured tissue engineering scaffolds encapsulating cells (such as progenitor cells and chondrocytes) for repairing and regenerating bone and cartilage tissues [45]. The scaffolds will resorb after fulfilling the template functions, and thus nothing foreign will be left in these patients. The scaffolds provide the necessary support for the cells to proliferate and differentiate and their architectures define the ultimate shapes of new bones [45]. In addition such scaffolds must allow for proper diffusion of oxygen and nutrients to cells embedded into the scaffold as well as proper diffusion of waste from the cells [46,47].

Successful design of scaffolds involves comprehensive consideration of macro and microstructural properties of the scaffolds and their interactions with natural tissue at nanoscale range [48]. Matrices can be classified according to their nature (proteic, polysaccharidic, synthetic and natural) or to their form (mass, mass porous, foam, viscous liquid and hydrogel). The ideal properties of a matrix are biocompatibility to prevent the inflammatory reactions to protect host tissue; three-dimensional shape allowing proliferation and cellular differentiation and porosity permitting migration of cells and diffusion of molecules, nutrients and oxygen. The matrix must also allow cell adhesion to facilitate the implantation of cells in the lesion and maintenance in the implant and can be bioactive, allowing the homogeneous and controlled release of growth factors or morphogens. The whole matrix has to adhere to the host tissue; maintain its mechanical integrity in order to avoid its flow after implantation and be degradable to integrate the physiological processes of tissue remodeling. Finally, pore size is a very important property because the scaffolds with large void volume and large surface-area to volume ratio maximize space to help cells, tissues, and blood vessels penetrate. To receive a high surface area per unit volume, however, smaller pores are preferable as long as the pore size is greater than the diameter of osteoblasts (10 μm). If the size is too small, pore occlusion by the cells may happen. This will prevent penetration and neovascularization of the inner areas of the scaffold [49]. The matrix must be also applicable by mini-invasive surgery thus if possible injectable, and should be reproducibly on a large scale from versatile processing techniques for a variety of shapes and sizes to match bone and cartilage defects in the patients. Furthermore, the scaffolds should be easily sterilizable to prevent infection: the sterilization process should not interfere with the bioactivity of the materials or alter their chemical structure [50].

Nanostructured Materials in Bone and Cartilage Tissue Engineering

By controlling surface properties, various nanophase ceramic, polymer, metal and composite scaffolds have been designed for bone/cartilage tissue engineering applications (Table 1) [18]. The selection of the most appropriate material to produce a scaffold used in bone tissue engineering applications is a very important step towards the construction of a successful tissue engineering product. So far, a wide variety of natural and synthetic biomaterials, such as polymers, ceramics, and a combination of them, have been studied for bone tissue engineering applications.

Natural matrices

Natural biomaterials can be in their native form, such as ECM from allografts and xenografts, or can be in the form of smaller building blocks, which include but not limited to inorganic ceramics such as calcium phosphates and organic polymers like proteins, polysaccharides, lipids and polynucleotides. Natural biomaterials usually have superb biocompatibility so that cells can attach and grow with excellent viability. However, one issue with natural materials is their limited physical and mechanical stability and therefore they

Material	Origin	Processing	Properties
Collagen	Natural Tissue - acid treatments - alkali treatments - proteolysis	- Injectable hydrogels - Hydrogels - Membranes - Films - Sponges - Scaffolds - Microspheres - Nanospheres	- Biocompatibility - Biodegradability - Structural integrity - Cell adhesion - Neovascularization
Gelatin	Natural Tissue - Collagen hydrolisis	- Hydrogels - Scaffolds	- Biocompatibility - Biodegradability - No immunogenicity - No pathogen transmission
Fibrin	Natural - Blood clots	- Injectable hydrogels - Scaffolds	- Biocompatibility - Biodegradability - Enhanced tissue formation
Hyaluronic acid	Natural - Microbes	- Injectable hydrogels - Hydrogels - Scaffolds	- Biocompatibility - Biodegradability - No immunogenicity - Inhibition of chondrocytic chondrolysis, - Anti-inflammatory - Inhibitory of prostaglandin synthesis, - Release of proteoglycan
Chitosan	Natural - Crustaacean - Microbes	- Hydrogels - Scaffolds	- Biocompatibility - Bioresorbability - Antimicrobial - Cell adhesion
Alginate	Natural - Brown algae - Bacteria	- Hydrogel - Scaffolds	- Biocompatibility - Metal chelation - Low cost
Agarose	Natural - Algae	- Hydrogel - Scaffolds	- Biocompatibility - Cell adhesion
Chondroitin sulfate	Natural - Tissues	- Hydrogel - Scaffolds	- Biocompatibility - Anti-inflammatory
Calcium phosphate Bioceramics	- Synthesis	- Scaffolds	- Biocompatibility - Enhanced tissue formation
Silicate Bioceramics	- Synthesis	- Scaffolds	- Biocompatibility - Mechanical strength
Titania	- Synthesis	- Scaffolds - Implants	- Biocompatibility - Mechanical strength
PLA	- Synthesis	- Scaffolds	- Biocompatibility - Biodegradability - Non-immunogenicity - Chemical versatility
PGA	- Synthesis	- Scaffolds	- Biocompatibility - Biodegradability - Non-immunogenicity - Chemical versatility
PLGA	- Synthesis	- Scaffolds	- Biocompatibility - Biodegradability - Non-immunogenicity - Chemical versatility
PCL	- Synthesis	- Scaffolds	- Biocompatibility - Biodegradability - Elasticity
PGS	- Synthesis	- Scaffolds	- Biocompatibility - Elasticity
PEG	- Synthesis	- Hydrogel	- Biocompatibility - Excretion
Polymer/ Bioceramic composites	- Synthesis	- Scaffolds	- Properties of Polymer and Bioceramics
Nanofibers	- Synthesis	- Scaffolds	- Biocompatibility - Cells alignment
CNT	- Synthesis	- Scaffolds	- Biocompatibility - Mechanical strength - Electrical stimulation
CNF	- Synthesis	- Scaffolds	- Biocompatibility - Mechanical strength - Electrical stimulation

Table 1: Principal nano-sized materials in bone and cartilage tissue engineering.

may not be suitable for some load-bearing applications. This is the reason why researchers using natural biomaterials are prompted to develop technologies improving and rein- forcing the mechanical and shape stability of natural biomaterials. Examples include developing composites with synthetic material and crosslinking.

Another issue is the potential immunogenicity, because natural biomaterials from allogenic or xenogenic sources may be antigenic to the hosts. As a result, researchers are attracted to technologies such as removal of telopeptides in procollagen for reduction of immunogenicity [51]. Natural scaffolds offer a good biocompatibility for cell attachment and differentiation. They include carbohydrate-based hyaluronic acid, agarose, alginate, chitosan, and protein-based collagen or fibrin glue. Furthermore, advances in genetic modification and cloning technologies have been made to allow massive production of natural polymers with high purity [52].

Protein matrices: Collagens is one of the most common scaffold materials for cartilage tissue engineering (Table 1) [50,53,54]. Collagen is a fibrous protein and a major natural extracellular matrix component. It has very attractive biological properties desirable for bone tissue engineering applications; on the other hand, there are concerns over collagen because of poor handling and poor mechanical properties to support bone loading requirements.

The collagen most beneficial attributes include biocompatibility, biodegradability, structural integrity, cell infiltration and attachment, and neovascularization [55]. Type I collagen scaffolds meet most of these criteria. In addition, type I collagen binds integrins through RGD and non-RGD sites which facilitates cell migration, attachment, and proliferation. Type I collagen scaffolds can be used for bone tissue repair when they are coated with osteogenic proteins such as bone morphogenic protein (BMP) and bone sialoprotein (BSP). BSP, a small integrin-binding ligand N-linked glycoprotein (SIBLING), has osteogenic properties and plays an essential role in bone formation. BSP also mediates mineral deposition, binds type I collagen with high affinity, and binds αvβ3 and αvβ5 integrins which mediate cell signaling [56-58].

Generally, collagen molecules can be extracted and purified from tissues by a variety of techniques such as acid treatments (commonly, dilute acetic acid), alkali treatments (usually using NaOH solutions) or proteolytic procedures, followed by treatments with neutral salts, dialysis, precipitation and centrifugation. However, the technique that offers higher yields and, consequently, is commonly applied for the isolation and purification of soluble collagen from native materials involves a proteolytic treatment in acidic environment (e.g. pepsin) to cleave the collagen cross-links and telopeptides which store the major antigenic determinants [59]. In addition to collagen produced by decellularisation of extracellular collagenous tissues that preserves its native architecture, it can be produced by complete breakdown of collagenous tissues into collagen molecules which can later be reconstituted in vitro into their native fibrillar structure. The reconstituted collagen gel is very weak due to the presence of high percentages of fluid within its structure, and thus different approaches have been used to produce tissue-like three dimensional dense structures with mechanical properties suitable for proper handling and manipulation [60]. Nowadays, several different clinical situations including neural repair, bladder repair, skin substitute, ligament and tendon repair, and dental use [60], requires the use of either naturally or synthetically derived collagen, which can be used in form of injectable hydrogel, membranes and films, sponges and scaffolds, micro- an nano-spheres [59].

Many studies have demonstrated that a combination of collagens (such as type I and type II collagens) with chondrocytes and stem cells facilitated cartilage tissue growth in vitro and in vivo [50]. Positive preliminary results after implantation of grafts of collagen gel containing autologous chondrocytes and three-dimensional culture in vitro. Collagen gels containing MSC formed hyaline-like tissue in cartilaginous defects after 7 months and after 1 year, patients had recovered a normal activity. Despite immunoreactivity associated to its bovine origin, collagen gels therefore could appear as suitable matrices for cartilage tissue engineering [61].

Gelatin has also been processed into porous materials for bone tissue repair [62]. It is a hydrolyzed form of collagen extracted from skin, bone, tendon, ligament, and other connective tissues [63]. Since gelatin is a denatured biopolymer, the selection of gelatin as a scaffolding material can circumvent the concerns of immunogenicity and pathogen transmission associated with collagen. Gelatin contains integrin binding sites for cell adhesion and carboxylic acid groups that bind calcium ions present in HA [64].

Fibrin derived from blood clots and has been successfully employed in bone engineering (e. g. cranial implant) and showed complete bone healing. Since fibrin is enzymatically crosslinked to form a gel as adhesive glue, mixture of fibrin with bioactive molecules could be applied as an injectable setting [65].

Polysaccharides matrices: Several polysaccharide materials have been successfully employed by virtue of different factor including the critical role of saccharide units in cell signaling, the development of powerful new synthetic techniques with potential of automated synthesis of biologically active oligosaccharides, rapid expansion in tissue-engineering research and associated need for new materials with specific, controllable biological activity and biodegradability [66]. Among the polysaccharides, hyaluronic acid, chitosan, alginate, agarose, and chondroitin sulfate play the most important role.

Hyaluronic acid (HyA) is a naturally occurring hydrophilic, non-immunogenic glycosaminoglycan component of the cartilaginous ECM [67]. It is naturally produced in joints by chondrocytes in cartilage and synoviocytes to provide viscoelasticity to joint fluid and critical components to the extracellular matrix of articular cartilage [68]. It is degraded naturally by hyaluronidases but its degradation products are able to induce chondrolysis [67]. The advantage of HyA includes its inhibitory effect on fibronectic fragment-mediated chondrocytic chondrolysis, anti-inflammatory effects, inhibitory effects on prostaglandin synthesis, proteoglycan release, and degradation [67]. HyA has been shown to support bone growth in dog alveolar ridge defects, rabbit mid-tibial non-unions, and rat calvarial defects when mesenchymal stem cells (MSCs) are added [69-71].

Injections of HyA into the joint have been used by orthopedists, with good reported results, over the past few decades for the treatment of arthritis, predominately of the knee, but also with potential clinical indications for other large joints [72]. Injecting HyA into joints with osteoarthritis has been shown to augment the flow of synovial fluid, inhibit the degradation and normalize the synthesis of endogenous hyaluronic acid, in addition to relieving joint pain [73].

Chitosan is partially deacetylated chitin consisting of β (1-4)-linked D-glucosamine residues with a variable number of randomly located N-acetylglucosamine groups, showing specific interactions with many growth factors, adhesion proteins, and receptors. Cationic nature and high charge density in solution help chitosan to form insoluble ionic complexes or polyelectrolyte complexes with a wide variety of water-

soluble anionic polymers [66]. This natural polymer is biocompatible, bioresorbable, and bioactive and thus extremely attractive for tissue engineering applications because it promotes attachment, proliferation, and viability of mesenchymal stem cells [74].

In vitro studies indicate that matrices containing chitosan are able to improve cartilage repair, promote chondrogenic activity of human chondrocytes and synthesis of ECM proteins when used alone or in association with various other polymers, like alginate or hyaluronic acid [72].

Alginate is an unbranched binary copolymer of (1-4)-linked β-D-mannuronic acid and R-L-guluronic acid derived from brown algae or bacterial sources, able to chelate a variety of divalent metal ions, such as calcium, magnesium, and barium [75]. The ease of preparation, favorable cellular response, and low cost are advantages of alginate gels, making them attractive candidates for the development of tissue-engineering constructs [76]. Alginate gel has been widely used for studying the phenotype, organization, and turnover of chondrocytes or intervertebral disk cells and the differentiation of adipose-derived adult stem cells and bone marrow-derived mesenchymal stem cells [77,78].

Agarose is a linear polysaccharide and consists of agarobiose repeat units, which are comprised of alternating units of galactose and 3,6-anhydrogalactose. Gelation of agarose occurs when a homogeneous solution is cooled from 99 to 35°C, the temperature below which coilhelix transition takes place. Agarose gel is one of the most frequently used systems for in vitro studies, particularly those involving mechanical stimulation, and has advantages of low cost, effective cellular responses, and easy preparation [79]. Chondrocytes cultured in agarose gel preserve certain physiological features of chondrocyte behavior and have been used to investigate chondrocyte response to physical and chemical stimuli in a controlled manner. There are numerous studies to demonstrate the suitability of agarose scaffolds for differentiation of stem cells into chondrocyte [80,81].

Chondroitin sulfate is a sulfated polysaccharide composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid), stimulating the metabolic response of the tissue both in vitro and in vivo and showing anti-inflammatory properties. Several work report on its beneficiary aspects in preventing the prevalence of osteoarthritis [82,83].

Artificial matrices

Synthetic biomaterials have better controllable physical and mechanical properties and can be used to tailor for both soft and hard tissues. Nevertheless, for synthetic biomaterials, biocompatibility becomes the major issue because cells may have difficulties in attachment and growth on these materials. Therefore, many processes modifying the surface and bulk properties have been developed to improve their biocompatibility. Examples include surface laser engineering and coating with natural biomaterials such as collagen. Furthermore, with the development of composites materials, the combinations of biomaterials for making porous scaffolds have become enormous [21].

Bioactive ceramics: The main advantage of using ceramics lies in their high cytocompatibility with bone cells. For bone tissue engineering, alumina zirconia, titania and calcium phosphate (Tricalcium phosphate (TCP) TCP, $Ca_3(PO_4)_2$), hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) and its derivates, as well as their combinations) are the most common types of bioceramics that have been used to fabricate scaffolds for bone tissue regeneration [84,85]. These ceramics are widely considered to be osteoconductive because their surface properties support osteoblast adhesion, growth, and differentiation and are also reported to be osteoinductive as result of their capacity to bind and concentrate BMPs in vivo.

Calcium phosphate ceramics are known to have excellent biocompatibility and are bioactive as they bind to bone and enhance tissue formation. Alone they offer no osteogenic or osteoinductive properties. The structure and the Ca/P-ratio of the different ceramics (HAp 1,67, TCP 1,5) are similar to the mineral phase of natural bone. Therefore ceramics induce an interface mechanism which leads to a release of calcium and phosphate ions. This results in an indefinable connexion between the ceramic and the bone (bonding osteogenesis). Woven bone accumulates directly on the ceramic surface without a separating layer of connective tissue and is converted to lamellar bone in the course. The presence of interconnecting pores is essential to prevent blind alleys with low oxygen tension which can prevent the osteoblastic differentiation [86,87].

Moreover, selected ceramics such as HA and TCP, due to their chemical and structural similarity to the mineral phase of native bone, can react with physiological fluids and form tenacious binds to hard and soft tissues trough cellular activity, thus classifying the mass bioactive [45]. In addition the dissolution rate of HA depends on its crystallinity, and therefore, it can be controlled to be compatible with the rate of new bone growth. The dissolution of HA crystals has been observed to be much slower than amorphous HA [88].

The clinical applications of these bioactive ceramics in large bone defects repair have been limited because of their intrinsic brittleness, difficulty in deforming and shaping, and mechanical properties preventing them sustain the mechanical loading needed for bone remodeling [45]. Nanophase ceramics, especially nano-hydroxyapatite (HA, a native component of bone), are popular bone substitutes, coatings and other filler materials due to their documented ability to promote mineralization. The nanometer grain sizes and high surface fraction of grain boundaries in nanoceramics increase osteoblast functions (such as adhesion, proliferation and differentiation) [15]. The highest adsorption of vitronectin (a protein well known to promote osteoblast adhesion) was observed on nanophase ceramics, which may explain the subsequent enhanced osteoblast adhesion on these materials [89].

Although β-TCP ceramics have been regarded as biodegradable materials, their degradation kinetic tends to be slow, and it is generally accepted that conventional sintered CaP ceramics lack osteoinductivity [90]. For this reason, a new concept of "third generation biomaterials" in which bioactive materials should stimulate cell and tissue growth has been proposed [91]. Recently, bioactive glasses have been emerged as interesting materials, even if their major disadvantages are the high brittleness, low bending strength, fracture toughness and workability, which limit its clinical application [92]. Silicate bioceramics mainly include binary oxides ($CaO\text{-}SiO_2$, $MgO\text{-}SiO_2$), ternary oxides ($MgO\text{-}CaO\text{-}SiO_2$, $ZnO\text{-}CaO\text{-}SiO_2$), and quaternary oxides ($SrO–ZnO–CaO–SiO_2$). Compared to conventional phosphate-based bioceramics, silicate bioceramics have more broad chemical compositions, which may contribute to their adjustable physicochemical properties, such as mechanical strength, bioactivity and degradation [93,94].

Several research groups have focused on modifying the surface roughness of titania, the oxide layer that forms on titanium-one of the most widely used implant chemistries in orthopaedics [95]. Webster group manipulated titania surface roughness was manipulated with

formation of micron size particles, resulting in up to three times more calcium ion deposited by osteoblasts [89].

Biodegradable polymers: Just as natural polymers, synthetic polymers offer controllable biodegradability and ease of fabrication. Synthetic materials indeed provide excellent chemical and mechanical properties that natural polymers usually fail to possess. The great advantage of synthetic polymers is associated with their processibility and flexibility to tailor to have appropriate chemical and mechanical properties [96]. Furthermore, they pose less danger of immunogenicity or transmission of lethal diseases and currently elicit increasing interest from scientists who are investigating their potential as synthetic bone and cartilage tissue engineering scaffolds [97].

As before explained, since bone and articular cartilage is under continuously excessive loading environments, the mechanical mismatch between implanted scaffolds and surrounding tissues may frequently deteriorate cartilage regeneration at defect sites and then lead to implant failure. Thus, biomaterial scaffolds with both superior biocompatibility and suitable mechanical properties similar to bone and cartilage are desirable [20].

While natural polymers are high molecular weight macromolecules, which make it difficult to process, various synthetic routes for man-made polymers provide better opportunities to control molecular weights, functional groups, configurations, and conformations of polymer chains. Tailoring polymer structure can determine the length and degradation characteristics, which may be the most influential parameter dictating release behavior of growth factors [98]. Degradation of synthetic polymers may occur via a hydrolytic pathway and enzymatic cleavage. Disadvantages of several synthetic polymers include possible acute and/or chronic inflammatory response, potential localized pH decrease due to relative acidity of hydrolytically degraded by-products, retarded clearance rate, and limited biological function [96].

The most popular synthetic polymers for bone and cartilage tissue engineering scaffolds are poly lactic acid (PLA, which is present in both L and D forms), poly-glycolic acid (PGA), and their copolymer polylactic-co-glycolic acid (PLGA) [99].

PLGA was originally developed for use in resorbable surgical sutures and biodegradable drug delivery systems. PLGA gradually degrades into the endogenous natural metabolites lactic acid and glycolic acid by non-enzymatic hydrolisis of ester bonds in its backbone [100]. The use of this materials depends of many practical advantages, such as the possibility to precisly control the chemical composition (the lactide/glycolide ratio), the crystallinity, molecular weight, molecular weight distribution, as well as microstructure and macrostructure (including porosity). This allows adequate control of scaffold degradation rate and mechanical strength. The degradation products of these polymers can be removed by natural metabolic pathways [10].

Generally, polymers that undergo hydrolyitic cleavage tend to have more predictable degradation rates in vivo than polymers whose degradation is mediated predominantly by enzymes because the levels of enzymatic activity may vary widely not only among different patients but also among different tissue sites in the same patient. The complex degradation process indicates the difficulties in controlling the release rate. Firstly, a random chains scission of ester bonds starts, and secondly a differentiation between the surface and interior begins random. In the following, low molecular weight oligomers begin to diffuse through the thinning outer layer, and when the molecular weight of these oligomers is slow enough to allow the solubilization in the medium, weight loss begins. Importantly, the degradation rate of polymers such as PGA, PLA, and PLGA, even can be tailored to satisfy the requirements from several years by altering the ratio of polylactic to polyglycolic acid, molecular weight and its distribution, crystallinity, hydrophilicity, pH of the surrounding fluids, as well as specimen size, geometry, porosity, surface properties and sterilization methods. The degradation rate becomes higher as the molecular weight becomes higher. The lower the cristallinity is, the higher the chance of penetration of water molecules to initiate hydrolysis of the chains [101]. Polymer crystallinity is a measure of the alignment of polymeric chains along each other. In the same conditions hydrophilic PGA degrades faster in aqueous solutions or in vivo than the hydrophobic PLA because the adsorption of water molecules is higher into the chain of the former polymer, although the ester bonds in them have about the same chemical reactivity towards water. The extra methyl group in the PLA repeating unit (compared with PGA) makes it more hydrophobic, reduces the molecular affinity to water, and thus leading to a slower hydrolysis rate. Therefore, it seems that the higher the glycolic acid content, the faster the degradation rate. However the lifetime of PLGA becomes shortest at PLA/PGA ratio of 50/50, because the more crystalline domains of PGA form as the amount of glycolic acid in the copolymer increases [102]. In the crystalline state, the polymer chains are densely packed and organized to resist the penetration of water. Consequently, backbone hydrolysis tends to only occur at the surface of the crystalline regions, which takes a much longer time than hydrolysis in amorphous polymer or in the amorphous regions of semi crystalline polymer.

Degradation leads to a loss of mechanical properties and an increase in crystallinity as a result of content loss. The amorphous regions of semi crystalline polymers are subjected to degradation earlier than the crystalline regions, leading to an increase in crystallinity. PGA loses mechanical integrity between two and four weeks while PLA takes months or even years [103,104].

These materials are usually sterilized by exposure to ethylene oxide. Unfortunately, the use of ethylene oxide gas represents a serious safety hazard as well as potentially leaving residual traces in the polymeric devices, as they must be degassed for extended periods of time [100].

In particular, it has been shown that PGA improved proteoglycan synthesis when compared to collagen scaffolds. In addition, increasing chondrogenesis was observed in a chondrocyte/PGA/bioreactor system over 40 days of cultivation.

PLGA scaffolds have a controllable porosity and a suitable surface structure for cell attachment, proliferation, and differentiation. Studies demonstrated that they are suitable for the chondrogenesis of human adipose-derived stem cells. Additionally, PLGA scaffolds have been loaded with various chondrogenic factors like TGF-ß and dexamethasone to improve chondrogenic differentiations of bone marrow-derived MSCs [20].

Other aliphatic polyesters, such as poly ε-caprolactone (PCL) and poly propylene fumarate (PPF) or poly ethylene fumarate (OPF) are also used in bone tissue engineering applications [105,106].

PCL degrades at a significantly lower rate than PLA, PGA, and PLGA [107-109], and therefore it is less attractive for general tissue engineering applications, but more attractive for long-term implants an controlled drug release applications; nevertheless As when copolymerized with PGA or PLA, it provides elastic properties which are useful for cartilage regeneration [105].

Poly glycerol sebacate (PGS) is another interesting bioresorbable polymer, as it can degrade and further resorb in vivo, with the degradation products eliminated through natural pathways as it is the case with other polymers [110]. PGS is relatively inexpensive, exhibits thermoset elastomeric properties, and maybe tailored to achieve mechanical properties and degradation rates targeted to a particular application [111].

Poly ethylene glycol (PEG) is a linear polyether that is used extensively in biomedical applications due to its hydrophilic and highly biocompatible properities. Even though PEG is not biodegradable, lower molecular weight (below MW10,000) can be safely excreted by metabolism in body. The strong advantage of PEG is its ability to be crosslinked by chemical modification to conjugate acryl groups to be reactive by radical polymerization processes [112].

Hydrogels: The search for a minimal-invasive surgery has justified the development of injectable matrices for cartilage tissue engineering. These injectable matrices have to be able to solidify, once implanted, to gain the desired shape and present the mechanical properties of the tissue to repair [113]. Hydrogels are three-dimensional polymeric networks that are able to absorb and retain large volume of water [114-116]. Viscous polymers from various origins can be transformed in hydrogel by modifying their environment. Crosslinking of hydrogels can be initiated by physical stimuli like pH, temperature or ionic environment or chemical crosslink through cross slinking agent, photo polymerization or enzymatic reaction [117-119].

Hydrogels generally present good biocompatibility [120]; moreover, cells, growth factors or bioactives components can be homogeneously incorporated. Their high water content allowed rapid diffusion of nutrients and metabolites. Collagen and gelatin represent the main protein used for hydrogel production [121-123], while chitosan, hyaluronic acid and alginate are the widely employed polysaccharides [124-127].

Among synthetic polymers, polyvinyl alcohol, polyethylene glycols (PEG) and poly(lactide-coglycolide) represent the mostly used. Hydrogels therefore appeared as appealing materials for cartilage tissue engineering. Kisiday et al. [128] developed a self-assembling peptide hydrogel scaffold for cartilage repair, seeded with bovine chondrocytes and then allowed to self assemble into a hydrogel. The chondrocyte seeded hydrogels were then studied for their ability to support chondrocyte proliferation, ECM production, and phenotype maintenance. Their results demonstrated that the chondrocytes were able to produce cartilage-like ECM, which was rich in proteoglycan and type II collagen (phenotypic markers of chondrocytes). Further, the authors observed that the mechanical properties continuously increased with time, which was indicative of the continuous deposition of glycosaminoglycan-rich matrix by the chondrocytes [129].

Hydrogels lack the ability to mineralize because they prevent the formation of chemical bonds with bone and hard tissue in general [130]. Thus, different attempts, and different strategies have been developed to prepare hydrogels possessing the capacity to mineralize. Some strategies involves the functionalization of the polymeric hydrogel backbone with negatively charged groups, the soaking of hydrogels in solutions that are saturated with respect to calcium phosphate; the incorporation of enzymes that catalyze deposition of bone mineral, and the incorporation of synthetic analogues to matrix vesicles that are the initial sites of biomineralization [131,132].

Composite matrices

As single-material scaffolds offer disadvantages, either because of low cytocompatibility (synthetic materials) or because of limited mechanical stability (natural materials), there is a desire to design composite materials combining the respective advantages of synthetic and natural materials [133].

For tissue repair and regeneration, fibrin glue, alginate, and hyaluronan have been used to modify various PLGA, PGA, PCL scaffolds, and the results revealed that these composite scaffolds can stimulate the chondrogenesis of different chondrocytes or progenitor cells [15]. Nanophase ceramics, especially nano-hydroxyapatite, are popular bone substitutes, coatings and other filler materials due to their documented ability to promote mineralization. The nanometer grain sizes and high surface fraction of grain boundaries in nanoceramics increase osteoblast functions (such as adhesion, proliferation and differentiation). The highest adsorption of vitronectin, which promotes osteoblast adhesion, was observed on nanophase ceramics. This may explain the subsequent enhanced osteoblast adhesion on these materials [25]. Natural bone matrix is a typical example of organic/inorganic composite material consisting of collagen and mineral (apatite). This natural composite material has an excellent balance between strength and toughness, superior to either of its individual components.

Polymer/Inorganic composites: Being similar to the major inorganic component of natural bone, the inorganic compound such as hydroxyapatite (HA) or calcium phosphate in a composite scaffold provides good osteoconductivity while the polymer provides the continuous structure and design flexibility to achieve the high porosity and high surface area, which are necessary for anchorage dependent cells such as osteoblasts to survive and differentiate [133]. By blending and phase separation techniques, polymer/inorganic composite (PLLA/HA and PLGA/HA) scaffolds have been developed with improved mechanical properties and osteoconductivity [134-136]. The HA-containing scaffolds improve osteoblastic cell seeding uniformity and show significantly enhanced expression of mature bone marker genes such as osteocalcin and bone sialoprotein over plain polymer scaffolds. HA in the composite scaffolds significantly improves the protein adsorption capacity, suppresses apoptotic cell death, and provides a more favorable microenvironment for bone tissue regeneration [137].

In addition to mimicking the organic/inorganic nature of the bone matrix, polymer/nano-HA scaffolds have also been developed to mimic the nano-sized features of natural bone mineral [138]. Considering that protein-scaffold and cell-scaffold interactions occur at the scaffold pore surfaces, a biomimetic approach has been developed to grow bone-like apatite nano particles on pre-fabricated porous polymer scaffolds in a simulated body fluid (SBF) to efficiently modify the internal pore wall surfaces with bone-like apatite without altering the bulk structures and properties of the scaffolds [139,140]. The apatite generated via the biomimetic process in an SBF is partially carbonated HA more similar to the natural bone apatite (calcium deficient Ca/P~1.5) than the stoichiometric HA crystals (Ca/P=1.67). The partially carbonated apatites should degrade faster than the stoichiometric HAP crystals and serve as a better scaffold component in terms of new bone tissue modeling and remodeling. The growth of apatite crystals is significantly affected by the polymer materials, porous structure, ionic concentration of the SBF, as well as the pH value [141,142].

In another study, Ramay and Zhand [86] used HA with ∝-tricalcium phosphate (∝-TCP) to develop biodegradable nanocomposite porous scaffolds. Incorporation of HA nanofibers as a second component in TCP (Tricalciumphosphate) significantly increased the mechanical strength of the porous composite scaffolds. This study introduced

nanocomposites with HA nanofibers as a promising scaffolding system for load bearing applications such as bone tissue engineering [85].

Nanofibers

The high surface area to volume ratio of the nanofibers combined with their microporous structure favors cell adhesion, proliferation, migration, and differentiation, all of which are highly desired properties for tissue engineering applications as they provide optimal conditions for cell attachment and growth [129]. For example the dense actin networks observed in the elongated hMSCs cultured on oriented nanofibrous scaffolds appear similar to the cytoskeleton observed in mature articular chondrocytes, especially at the articular surface, suggesting that the use of oriented nanofibrous polymeric scaffolds could be advantageous to better mimic articular cartilage tissue.

Nanofibers can be currently fabricated via the following manufacturing approaches: electrospinning, phase separation and self-assembly. Other applications used are solvent casting/particulate leaching (especially for ceramic and nanoporous polymer matrices), chemical etching (Coatings), 3D printing techniques. The resulting nanofibers have fiber diameters ranging in size from 50 nm to several microns [129].

Engineering an oriented ECM environment to regulate tissue alignment could be optimized by oriented electrospun nanofibers, and that specific tissue engineering applications, such as creating the superficial zone of articular cartilage, may be significantly improved by seeding cells on nanofibrous scaffolds. Oriented nanofibrous scaffolds can be used to guide cell alignment along the nanofibers, and aligned cells could be then used to remodel and modulate the regenerated ECM and microenvironment. The cell arrangement onto an oriented nanofibrous scaffold could be due to contact guidance and/or cytoskeletal reorganization. It has been shown that cell elongation induced by the aligned PCL nanofibers is expected to reorganize the cytoskeletal structures that regulate the cell morphology, adhesion, and locomotion. The level of collagen type II from nanofibrous scaffolds far exceeded any levels measured from the other scaffold types. This may indicate that such oriented nanofibrous PCL scaffolds may be better suited for engineering the superficial zone of articular cartilage, which naturally possesses a high content of collagen type II ECM [143]. The mechanism by which nanofibers enhance cell adhesion is not completely understood. One possible explanation is through the enhanced and selective adsorption of adhesion molecules to the nanofibers [129]. A study on nanofibrous scaffolds showed that they adsorbed four times more human serum proteins than the scaffolds with solid pore walls. These nanofibrous scaffolds tended to selectively adsorb fibronectin and vitronectin. Cell adhesion was increased almost two-fold on these nanofibrous scaffolds. Several recent findings suggest that cell shape and cytoskeletal organization might play a significant role in regulating cell phenotype. Cartilage specific gene and protein levels, such as collagens type II and IX, were upregulated in nanofibrous cultures compared to microfibrous cultures. This suggests that nanofibers are capable of maintaining the chondrogenic phenotype, and provides further evidence for a correlation between the morphological/cytoskeletal modulation and phenotypic control [144]. The underlying mechanisms have yet to be elucidated. It appears, however, that such environments may promote Rac activation, a GTPase important in cell adhesion and signal transduction, and F-actin assembly.

In the following described work Li et al. [144] fabricated a PCL (Poly Caprolactone)-based nanofibrous scaffold, by electrospinning, which was seeded with fetal bovine chondrocytes (FBC) and studied for their ability to maintain chondrocytes in a mature functional state. The results demonstrated that FBCs seeded on the PCL nanofibers were able to maintain their chondrocytic phenotype by expressing cartilage-specific extracellular matrix genes like aggrecan, collagen type II and IX, and cartilage oligomeric matrix proteins.

Further, FBCs exhibited a spindle or round shape on the nanofibrous scaffold in contrast to a flat, well-spread morphology as seen when cultured on tissue culture polystyrene. Another interesting finding was that serum-free medium produced more sulfated proteo-glycan-rich cartilaginous matrix when compared with the same cultured in monolayer on tissue culture polystyrene. These results demonstrated that the bioactivity of FBCs depends on the architecture of the scaffold and the composition of the culture medium. Furthermore PCL nanofibers in the presence of a member of the transforming growth factor- family caused the differentiation of MSCs to chondrocytes that was comparable to that caused by cell aggregates or pellets. However, since the PCL nanofibrous scaffolds possess better mechanical properties than cell pellets, they show potential to be developed as a scaffolding system for MSC delivery and hence cartilage tissue engineering [129].

Another important and interesting study for the treatment of large bone defects has been carried out designing peptide amphiphile (PA) materials capable of self-assembling into well-defined nanofibers that display specific bioactive epitopes on their surface to control cell behavior both in vitro and in vivo. The objective of the study was to determine the in vivo osteogenic potential of self-assembling PAs comprising bioactive epitopes specifically designed to promote bone regeneration. The main design feature of the PA material was the incorporation of phosphorylated serine residues S(P) segments within well-defined self-assembled nanofibers with ECM-like fibrous architectures. The objective of this approach was to generate a completely artificial bone-bioactive matrix that mimics elements of bone biomineralization. Nanofiber forming PA molecules contain a peptide segment with one domain that has a strong propensity to form extended ß-sheets and a second domain with amino acid residues important to bioactivity. The ß sheet domain promotes the assembly of molecules into fibrous aggregates and discourages aggregation into spherical nanostructures. The second segment, covalently grafted to the peptide, has greater hydrophobicity than any peptide and forms the core of fibers upon self-assembly, thus ensuring display of the peptide segments at an aqueous interface. The resulting self-assembled PA nanofibers are a few nanometers in diameter and can easily attain lengths of microns. Furthermore, several bioactive cues can be presented simultaneously by co-assembling multiple PA molecules bearing different signals.

In the work authors investigated the impact of a matrix with biomimetic elements on bone regeneration within a defect. In addition to a collagen-like fibrilar architecture (cylindrical nanofibers), the biomimetic features of the matrix include its ability to nucleate in vivo hydroxyapatite crystals that resemble those in natural bone. Previous work demonstrated first in two-dimensional experiments the ability of peptide amphiphile nanofibers with phosphoserine residues near their surfaces to nucleate thin hydroxyapatite crystals with their c-axis parallel to nanofibers. This crystallographic relationship is observed in biology with respect to the long axis of collagen fibrils. Extending the study the authors obtained three-dimensional networks of similar nanofibers by promoting mineralization in well-established osteogenic media containing organophosphates and the enzyme alkaline phosphatase. Tests in vivo of the 3D-biomimetic system as a matrix to promote bone regeneration,were performed, using an orthopedic rat femoral critical-

size defect model. Using co-assembly of two PA molecules, the authors also tested the combined effect on bone bioactivity of the fibronectin epitope RGDS and the phosphoserine residues for hydroxyapatite nucleation. The central molecular feature of their strategy was to design PAs that could generate three dimensional, fibrous matrices that display high concentrations of phosphorylated serine residues on their surfaces. These nanofibers would not only introduce in vivo biomimetic nucleation of hydroxyapatite and its biological consequences, but would also help augment the overall deposition of mineral within the defect. In order to further enhance bioactivity of the artificial matrix, they took advantage of co-assembly of two molecules in these supramolecular systems and combined S(P)-PA molecules with RGDS-PA molecules. PA molecules with the RGDS fibronectin epitope were introduced to promote integrin-mediated adhesion of cells that participate in bone regeneration such as mesenchymal stem cells, osteoprogenitor cells, osteoblasts, and vascular tissue cells. The RGDS+S(P)-PA gel matrices led to the highest average amount of ossified tissue within the callus and at a level that was statistically equivalent to those treated with artificial matrices only containing the phosphorylated serine residues [S(P) PA] or the type of allogeneic demineralized bone matrix used clinically at the present time [56,57]. Furthermore, qualitative observations of the different PA gels revealed a similar mechanical stability among all the different groups. Therefore, it is possible that the S(P)-containing nanofibers are presenting a favorable hydroxyapatite nucleation environment within and around the fracture site, which is promoting earlier biomineralization compared to those of other treatments and controls and leading to a higher content of ossified tissue within the callus after 4 weeks. There are several physical and biological mechanisms through which the bioactive PA nanofiber matrix may be enhancing bone formation. First the PA gel tends to coat the surface of the bone, both over the periosteum and on the cross-section where the defect was created, including close to the medullary canal. This flow of the PA Matrix could promote its contact with osteoprogenitor and mesenchymal stem cells (present in the periosteum and bone marrow) and facilitate their migration towards the defect site. Non-collagenous proteins such as phosphophoryn or bone sialoprotein, which are rich in S(P) residues, not only play a role in nucleation of mineral but have been shown to stimulate gene expression and enhance osteoblast differentiation of MSCs in vitro. Furthermore, other groups have recently described an Osteoconductive and Osteoinductive effect of calcium phosphate minerals on mesenchymal stem cells. Thus an earlier presence of this mineralized matrix as a result of the highly concentrated S(P) residues on the surface of the nanofibers could stimulate local mesenchymal stem cell population into an osteoblastic phenotype. Furthermore, this HA-containing niche may also be stimulating osteoclast activity, which would subsequently stimulate osteoblasts to begin the formation of new bone [145].

Others interesting materials Among the various types of nanomaterials are carbon nanotubes (CNTs) and carbon nanofibers (CNF), which have attracted increasing attention due to their mechanical, electrical, thermal, optical, and structural properties [146]. Carbon nanotubes (CNTs) are well-ordered carbon nostructures consisting of carbon atoms bonded to each other via sp^2 bonds [147]. CNTs can be imagined as cylinder formed of rolled grapheme sheets. If only one graphene sheet in involved, rolled, thus single walled carbon nanotube (SWCNT) are formed, while in the case of more concentric sheets the formation of multi-walled carbon nanotube (MWCNT) occurs. CNFs are similar but have a less perfect arrangement of atoms [148,149].

Carbon nanotube based substrates have been shown to support the growth of osteoblastic cells [150]. Since carbon nanotubes are not biodegradable, they behave like an inert matrix on which cells can proliferate and deposit new living material, which becomes functional, normal bone.

In bone tissue engineering, CNT and CNF composite materials have been prepared and used [151], in the presence of bioceramics [152], as scaffolds (MWCNT/polycarbosilane fabricated by the spark plasma sintering (SPS) method [153], injectable hydrogels (SWNT/poly(propylene fumarate)/propylene fumarate-diacrylate [154]. Regarding CNFs, in a study by Price et al. CNFs were dispersed in polycarbonate urethane (PCU) and tested for the adhesion of osteoblasts, fibroblasts, chondrocytes, and smooth muscle cells on the composite scaffolds [155]. It was found that the composites with smaller scale (i.e., nanometer dimension) carbon fibers promoted osteoblast adhesion but did not promote the adhesion of other cells. More interestingly, smooth muscle cell, fibroblast, and chondrocyte adhesion decreased when carbon nanofiber surface energy increased.

Therapeutic Potential of Mesenchymal Stem Cells: Stem Cell Biology Meets Nanotechnology

The regeneration of bone is a key issue at the forefront of current tissue engineering applications, owing to the ease of use and accessibility of osteoprogenitor cells. The molecular mechanisms of human MSC regulation and the importance of specific growth factors during the different stages of osteogenic differentiation are subjects of intensive investigation [156]. Recent advances in the field of biomaterials have led to a transition from nonporous, biologically inert materials to more porous, osteoconductive biomaterials, and, in particular, the use of cell-matrix composites [157]. A number of delivery vehicles have been successfully used in cell-matrix composites in vivo, such as porous ceramics of hydroxyapatite and tricalcium phosphate loaded with autologous MSCs. These constructs were capable of healing critical sized segmental bone defects not capable of being healed by resident cells or by the addition of the osteoconductive device alone [158].

Although acellular approaches to bone reconstruction using scaffolds and osteogenic growth factors have shown moderate clinical success, the delivery of exogenous cells capable of forming bone tissue may be required for bone defects in patients with a limited local supply of responsive osteoprogenitor cells such as older patients, smokers, or patients with certain diseases. Site-specific delivery has the advantage of being able to deliver large numbers of cells directly to the required area. In tissue engineering strategies, this typically involves placing cells on a 3D scaffold, followed by implantation at the injury site [159]. However cell survival after delivery is a critical issue in the development of cell-based strategies, especially for thick tissues such as bone. The lack of initial vascularity in bone defects limits the transport of nutrients to and waste products from the center of the defect [160]. Therefore, if cells are seeded throughout a 3D scaffold and placed at the defect site, cells located at the center of the scaffold may not survive. An alternative is to deliver cells to the periphery of the defect via a thin membrane or scaffold. Delivery of cells on the periphery of bone defects via a tissue engineered periosteum may be an effective approach to enhance cell survival by the presence of a neighboring vasculature [161-163].

This delivery strategy may enhance cell survival by positioning the cells in proximity to the surrounding highly vascularized tissues, and thereby providing for nourishment and clearance of waste products. A cell source needs to be identified that is readily available, propagated easily, has high osteogenic potential, and will be accepted by the recipient

immune system. The identification of a cell source that may be easily harvested, expanded to large numbers, and controllably differentiated may be tremendously beneficial clinically for the reconstruction of damaged tissues. Bone marrow- derived mesenchymal stem cells (MSCs) have demonstrated a strong potential for differentiation into bone-forming cells, and have been shown to promote repair of critically sized bone defects in preclinical animal studies.

These cells are well suited for autologous transplantation, making them a feasible cell source for clinical deployment due to the lack of immunogenic issues associated with this transplantation modality. However, MSCs are associated with reduced mineralization capacity in older donors and following expansion to achieve therapeutic cell numbers [158,164,165]. Electrospinning has recently emerged as a technique to fabricate scaffolds for tissue engineering, with fiber diameters ranging from tens of nanometers to as large as 10 mm. The nanofiber mesh obtained by this process is a unique scaffold membrane that possesses structural features with a size scale similar to extracellular matrix (ECM) components, high porosity, and large surface-area-to volume ratios. These properties allow for enhanced cellular attachment and spreading, and therefore nanofiber meshes may serve as an effective delivery vehicle for cells to a defect site in vivo. A recent in vitro study comparing the biodegradable polymers poly-L-lactide (PLA) and poly-L- lactide co-glycolide (PLGA) on the basis of adherence and proliferation of seeded trabecular bone- derived osteoprogenitor cells showed that PLGA was the better substrate for the attachment and subsequent osteogenic differentiation of these progenitor cells [158].

Also for articular cartilage a potential resolution of disease states is the regeneration of cartilage tissue using autologous MSCs. The induction of chondrogenesis in MSCs depends on the coordinated activities of many factors, including parameters such as cell density, cell adhesion, and growth factors [156]. Adult chondrocytes have been isolated from various sources like articular cartilage, nasal septum, ribs or ear cartilage. Matrix-induced Autologous Chondrocyte Implant (MACI implant, Genzyme) has been developed. In this method, chondrocytes are expanded in a collagen membrane and then reimplanted into articular cartilage defects without suturing [6]. One of the main limits related to the use of chondrocytes, is their instability in monolayer culture resulting in the loss of their phenotype. This loss of the chondrocytic phenotype is accompanied by a phenotypic shift towards a fibroblastic one. This fibroblastic phenotype is characterized by an increased expression of collagen I, and the adoption of the spindle-shape characteristic of fibroblasts. This process of dedifferentiation is however reversible. Indeed, if dedifferentiated chondrocytes are placed in a three-dimensional environment, they retrieve their differentiated phenotype. It has been shown that cell adhesion and proliferation is significantly improved on oriented nanofibrous scaffolds.

Wise et al conducted a study designed to demonstrate the feasibility of hMSCs to maintain viability, orientation, and proliferation when cultured for 35 days on aligned electrospun nano- and microfibrous PCL scaffolds. Viable and aligned hMSCs were also cultured in media containing TGF-ß1 and induced to chondrogenically differentiate on the nano- and microfibrous scaffolds. Results indicate that hMSCs were able to maintain cell alignment on both types of fibrous PCL scaffolds, but chondrogenically differentiated to a greater extent when cultured on the nanofibrous scaffold [143]. The level of collagen type II from nanofibrous scaffolds far exceeded any levels measured from the other scaffold types. Oriented nanofibrous PCL scaffolds may be better suited for engineering the superficial zone of articular cartilage, which naturally has a high content of collagen type II ECM. Engineering an oriented ECM environment to regulate tissue alignment could be optimized by oriented electrospun nanofibers, and that specific tissue engineering applications, such as creating the superficial zone of articular cartilage, was shown to be significantly improved by seeding cells on nanofibrous scaffolds. Unlike microfilaments found in typical spread hMSCs on 2D substrates that can be described as large actin stress fibers and terminate at focal contact sites on the cell membrane [166]. HMSCs cultured on aligned PCL scaffolds showed much different actin cytoskeletal organization. Thin fibrous tether-like actin structures connecting the hMSC with the neighboring aligned nanofiber bundle were also observed. Although it remains to be further studied, these findings could lead to the notion of unique hMSC binding characteristics to the nanofibers that may influence not only cell alignment but also improved cell differentiation. Not only similar fibrous connections are found in the hMSC–nanofiber interactions, but also actin filaments are likely involved in the development of these unique adhesion-like structures [167]. It is also interesting to note that the dense actin networks observed in the elongated hMSCs cultured on oriented nanofibrous scaffolds appear similar to the cytoskeleton observed in mature articular chondrocytes, especially at the articular surface [168], suggesting that the use of oriented nano-fibrous polymeric scaffolds could be advantageous to better mimic articular cartilage tissue [169-172]. The use of chondrogenic progenitors, in particular MSCs, will undoubtedly be of high potential for nano-engineering application. Nonetheless, more sophisticated approaches combining deliverable bioactive factors together with a chondroconductive scaffold will be required. Although some growth factors have been proposed (the most characterized factors which stimulate the anabolic activity in cartilage include Transforming Growth Factor (TGF)-ß, Bone Morphogenetic Protein (BMP), Fibroblast Growth Factors (FGF), Insulin Growth factor (IGF)-1, Hedgehog (hh) and Wingless (Wnt) proteins), none are capable to specifically induce the desired lineage and a timely regulated combination of factors is likely to be required for the obtention of a functional and stable chondrocyte phenotype. Currently, only one Phase I clinical trial in cartilage tissue engineering using MSCs is underway [10].

Conclusions

Currently orthopedic surgery is "macro". However, the introduction of nanotechnology to the practice of orthopedic surgery may begin a paradigm shift within the field (Figure 1). Much of the work of nanotechnology in orthopedic surgery is occurring in the laboratory

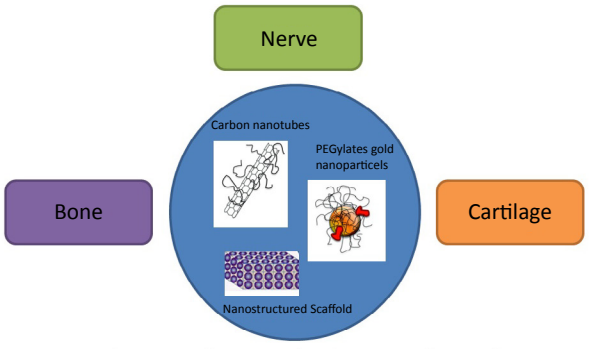

Figure 1: Main applications of nanotechnology in orthopaedic surgery.

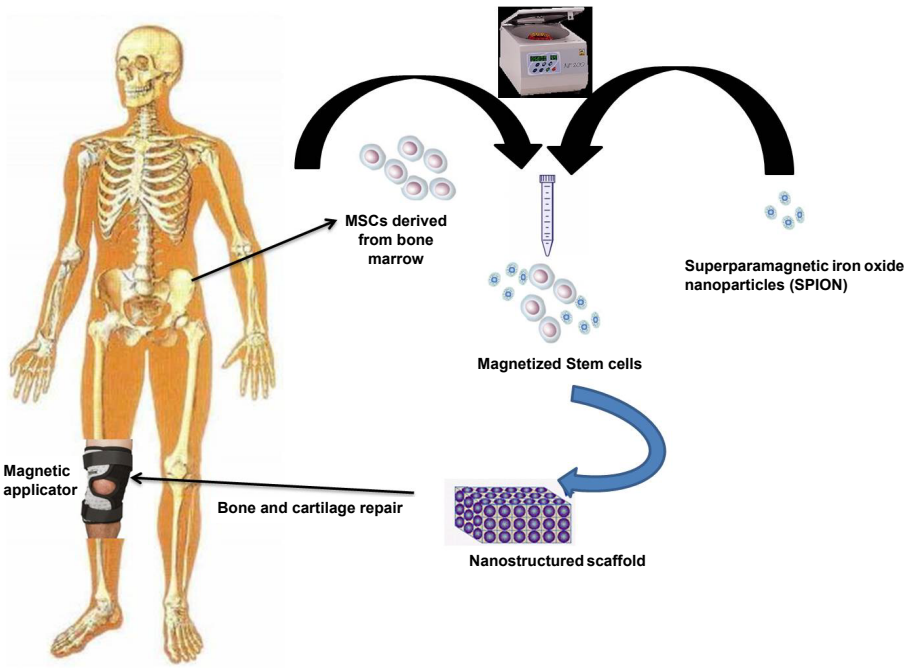

Figure 2: A future application of nanotechnologies for the treatment of osteo-chondral defects: MSCs can be magnetized by using iron nanoparticles in order to guide them towards the osteo-chondral defect through external magnetic fields. Moreover a nanostructured scaffolds can be applied as tridimensional support and it can guide the MSCs differentiation by using specific scaffold nanostructured patterns (Mechanotransduction).

setting or in early in vivo testing. Significant basic and translational research and development is needed, from basic science to practical applications, to realize its full clinical potential. Critical to the successful realization of the potential of nanotechnology in orthopedics will be a multidisciplinary effort between industry and medicine.

The proposal to utilize nanomaterials in the next generation of improved orthopedic implant materials, and as scaffolds for regenerative tissue engineering is directed at improving surface properties to create an environment more conducive to osteoblast function, bone ingrowth and regeneration of cartilage tissue (Figure 2). It is hoped this will resolve some of the main unanswered questions in orthopedics: the effective healing of cartilage damage and bone defects. As we saw, nanofibrous structures have been shown to favorably affect cell adhesion, proliferation, and phenotypic expression. Several techniques, most importantly electrospinning, phase separation, and self-assembly, have been developed to fabricate polymeric nanofibers for orthopedic applications. Among these, electrospinning has emerged as one of the most versatile techniques for developing nanofibrous structures from a wide range of polymers and composite materials.

The potential for novel treatments are apparent but the risk must be studied and fully understood.

The safety of nanoparticles that may become dislodged from implants during surgical manipulation, or from fragmentation of articulating components of a joint prosthetic composed of nanophase materials, once in the human body is largely unknown, both in manufacturing and when used as a component of an implantable device.

Understanding the long-term biological consequences of nanotechnology products is critical. In vitro and in vivo tests will need to be developed to predict human reactions to some nanotechnology products.

Therefore in addition to basic and translational research, the biological, manufacturing, and regulatory issues with respect to nanotechnology need to be addressed moving forward.

Nevertheless, nanotechnologies are considered to be the break-through event in applied medical sciences, with the worldwide market for nanotechnology expected to reach one trillion dollars by 2015 and their biomedical application represents a great business challenge. Orthopedic surgery is certainly one of the most important clinical domains likely to benefit from this revolution.

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