17

Cell-Biomaterial Interactions Reproducing a Niche

Silvia Scaglione[1], Paolo Giannoni[2] and Rodolfo Quarto[3]
[1]*CNR - National Research Council of Italy, IEIIT Institute, Genoa*
[2]*Advanced Biotechnology Center, Genoa*
[3]*DIMES, University of Genoa*
Italy

1. Introduction

During the last years, several major progresses and improvements have been introduced in orthopaedic surgery as innovative and attractive approaches for potentially solving many of the limitations of the current therapies. Natural processes of bone repair are sufficient to restore the skeletal integrity for most lesions. However, this auto-regenerative potential has dimensional limits which require manipulation of natural healing mechanisms to be overcome.

In bone tissue engineering, biomaterials have been therefore proposed as scaffolds to direct and guide bone regeneration and to deliver stem cells in anatomical sites where the regenerative process is defective. Although material science technology has resulted in clear improvements, no ideal bone substitute has been developed yet and hence large bone defects still represent a major challenge for orthopaedic and reconstructive surgeons.

In the past years, scaffolds design has evolved from the obsolete, first-generation "spare-part" concept, to second-generation bio-inert "cell carriers", where biomaterials had to provide mechanical strength, durability, and possibly operate as cell delivery vehicles to achieve the regeneration of the target tissue- up to a third-generation of bio-functional materials that seek to incorporate instructive signals into scaffold nanostructures to modulate cellular functions, direct cell fate, and finally govern tissue regeneration in vivo. They should finally be resorbed in vivo, as soon the neo-formed tissue is able to fully substitute the graft.

The most intriguing concept in modern biomaterials is thus obtaining materials able to mimic a specific pre-existing microenvironment and, therefore, inducing cells to differentiate in a predetermined manner and to regenerate by themselves the desired tissue (i.e. bone tissue) according to physiological pathways.

In order to reach this ambitious task, intelligent biomaterials should be properly designed and an informative microenvironment, mimicking a physiological niche, provided. Any material has to be considered informative in the sense that its intrinsic nature (i.e. chemical composition) and structure (i.e. macro- micro- nano-architecture) will anyway transmit a signal that will be read and decoded by colonizing cells. We still know very little of how to create local microenvironments, or artificial niches, that will govern stem cells behaviour

and their terminal fate. However, it has been highlighted in the last years that stem and progenitor cells are able to modify their behaviour and fate when loaded onto specific substrates (Hench and Polak 2002; Dalby, Gadegaard et al. 2007; Hunt 2008).

Among basic requirements for the design and generation of bone substitute materials, there is the development of biomimetic scaffolds with (i) an internal architecture able to favour cell migration and in vivo vascularisation, and (ii) a chemical composition permissive to cell attachment, selective differentiation and maintenance of cellular functions.

In this context, the bioengineering challenge become ambitious, since the complex cell-biomaterial interaction moves on multiple spatial and temporal scales. The micro-environmental cues, such as chemical environmental variables, are able to stimulate specific cellular responses at the molecular level already at early time points (Goshima, Goldberg et al. 1991; Ohgushi, Dohi et al. 1993; Fabbri, Celotti et al. 1995; Kon, Muraglia et al. 2000; Erbe, Marx et al. 2001; Endres, Hutmacher et al. 2003; Kasten, Luginbuhl et al. 2003; Livingston, Gordon et al. 2003; Niemeyer, Krause et al. 2003; Arinzeh, Tran et al. 2005; Kotobuki, Ioku et al. 2005; Kondo, Ogose et al. 2006; Fan, Ikoma et al. 2007; Mygind, Stiehler et al. 2007; Gigante, Manzotti et al. 2008; Ng, Tan et al. 2008; Bernhardt, Lode et al. 2009; Saldana, Sanchez-Salcedo et al. 2009). For example, ceramic scaffolds (i.e. hydroxyapatite) are able to induce a faster and more efficient cell adhesion. However, the cell-signalling pathways involved in the variation of gene expression are yet to be fully elucidated. Recently, it has been reported that cells loaded onto biomaterials are also able to decode the topographic cues of the scaffold, and respond to the shape of the micro- environment priming a specific cell differentiation pathway (Lenza, Vasconcelos et al. 2002; Dalby, Gadegaard et al. 2007; Huang, Lin et al. 2007; Wei and Ma 2008).

Tissue development and regeneration implies a spatio-temporal assembly of differentiating cells organized to create functional structures. This process is finely tuned, progressing gradually through cell-matrix and cell-cell interactions. Biomechanical forces generated by the contact among the differentiating cells within the tissue or with the ECM, have a profound impact on tissue growth, development, maintenance, and repair by providing the required metabolic support, strength, and endurance.

In this chapter, we will discuss about those biomaterials that are being designed and manufactured to gain the informative status necessary to drive proper molecular cross-talk and cell differentiation. In particular, we will explore: (i) how to develop intelligent informative scaffolds, (ii) how stem/progenitor cells decode biomaterials, (iii) promising bone substitutes in a tissue-engineering scenario.

2. How to develop informative scaffolds

Progress in biomaterials design and engineering are converging to enable a new generation of instructive materials to highlight as candidates for regenerative medicine. An emerging philosophy aims to overpass the traditional approach of recreating the complexity of living tissues ex vivo; in this context, the most ambitious strategy attempts to develop synthetic materials that establish key interactions with cells in ways that unlock the body's innate powers of organization and self-repair. The complex cell-biomaterial interaction moves on multiple spatial and temporal scales. Therefore, in order to influence effectively the cell behaviour, scaffolding materials must bear complex information, coded in their *physical* and *chemical* structures. In particular, bio-scaffolds must be properly designed to mimic the spatial organization of stem cells and their *niche* physiological structure.

Stem cell niche is defined as a dynamic microenvironment that balance stem cells activity to maintain tissue homeostasis and repair throughout the lifetime of the organism (Voog and Jones 2010). In principle, stem cells in their niche make decisions to either remain in a quiescent state, undergo self-renewal, or to exit the niche upon exposure to local or systemic stimuli. These signals are actively coordinated and presented in a temporally and spatially regulated manner. Proper microenvironmental cues given by the biomaterial may become "informative" for cells, stimulating specific cellular responses (Fig.1).

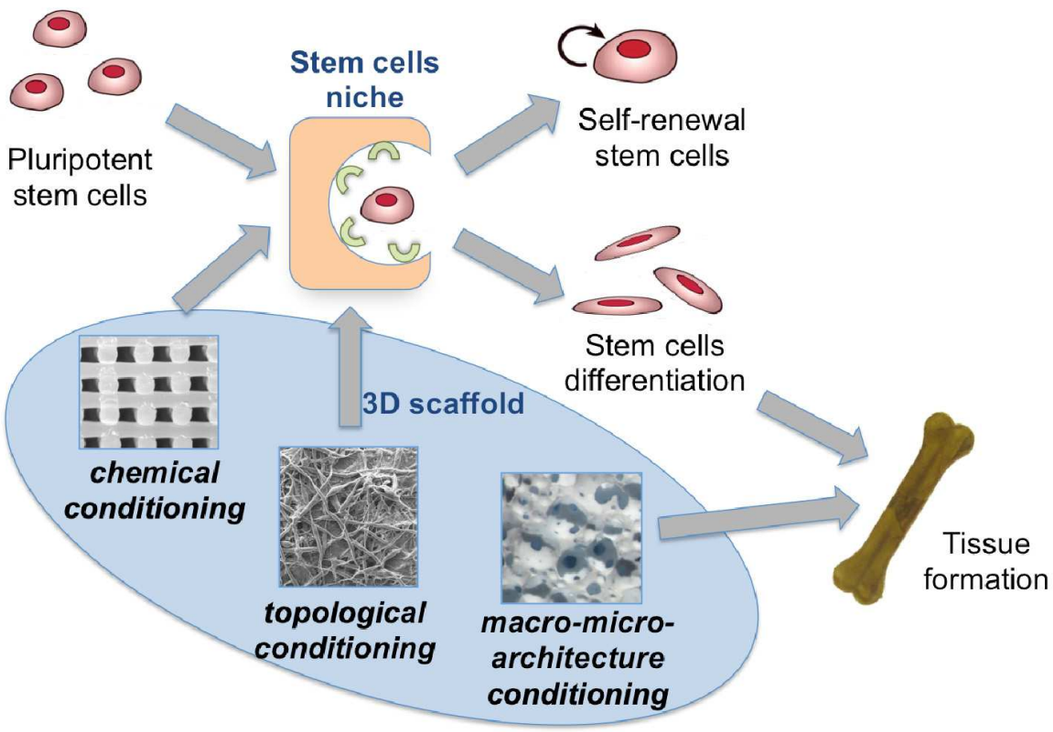


Fig. 1. Cell-biomaterial interaction.

The influence of chemical environmental variables on cell activity (i.e. *chemical conditioning*) was already probed (Goshima, Goldberg et al. 1991; Ohgushi, Dohi et al. 1993; Yuan, Yang et al. 1998; Yuan, Kurashina et al. 1999; Boo, Yamada et al. 2002; Kasten, Luginbuhl et al. 2003; Niemeyer, Krause et al. 2003; Arinzeh, Tran et al. 2005; Fan, Ikoma et al. 2007; Nakamura 2007; Guarino, Causa et al. 2008; Cheng, Ye et al. 2009; Saldana, Sanchez-Salcedo et al. 2009). For example, ceramic scaffolds (i.e. hydroxyapatite) allow a faster and more efficient cell adhesion (Goshima, Goldberg et al. 1991; Ohgushi, Dohi et al. 1993; Yuan, Yang et al. 1998; Yuan, Kurashina et al. 1999; Kasten, Luginbuhl et al. 2003; Arinzeh, Tran et al. 2005; Fan, Ikoma et al. 2007; Cheng, Ye et al. 2009; Saldana, Sanchez-Salcedo et al. 2009).
The synthesis of complex inorganic materials mimicking natural structures offers exciting avenues for the chemical construction of macrostructures and a new generation of biologically and structurally inspired scaffolds for tissue engineering.
Besides chemical conditioning, progenitor cells fate is also affecting by topographic cues of the scaffolds (i.e. *topological conditioning*). Recently, it has been reported that cells loaded

onto biomaterials are able to decode the topographic signals of the scaffold, and respond to the shape of the microenvironment priming a specific cell differentiation commitment (Dalby, Gadegaard et al. 2007). Thus, nanostructured biomaterials such as nanoparticles, nanofibers, nanosurfaces, and nanocomposites have gained increasing interest in regenerative medicine, since they offer a temporary ECM for regenerative cells (Hollister, Maddox et al. 2002; Balasundaram and Webster 2007; Wei and Ma 2008; Zhang and Webster 2009).

Recent studies have also shown that in the absence of adhesion peptides, cells interact with scaffolds by means of adsorbed protein, and in this regard topography and hydrophilicity are key considerations. For example, fibrous meshes with nanoscale fibre diameters have shown selective take-up of proteins relevant for cell attachment, such as fibronectin and vitronectin (Place, Evans et al. 2009). And whereas hydrophobic scaffolds tend to adsorb protein in sub-optimal configurations (with hydrophobic residues displaced towards the scaffold surface), hydrophilic polymers adsorb protein in a hydrated interfacial phase wherein the proteins are more likely to retain their native conformation (Place, Evans et al. 2009).

If the chemical and topographical cues imprint the progenitor/stem cell fate at early time points, by reproducing a proper stem cell niche, the 3D architecture design (i.e. pore size, total porosity, surface area) of the scaffolds plays a pivotal role at prolonged time of cell-biomaterial interaction (i.e. *macro-micro architecture conditioning*). During this phase, as soon cells are addressed towards their differentiative fate, it may be beneficial for a biomaterial to provide adequate space (porosity) and appropriate surface to foster and direct new tissue formation.

In the bone tissue engineering, for instance, pores are necessary to allow migration and proliferation of osteoblasts and mesenchymal cells, as well as vascularization (Gauthier, Bouler et al. 1998; Boyde, Corsi et al. 1999; Lu, Flautre et al. 1999; Chang, Lee et al. 2000; Hollister, Maddox et al. 2002; De Oliveira, De Aguiar et al. 2003; Karageorgiou and Kaplan 2005; Mastrogiacomo, Scaglione et al. 2006). In addition, macroporosity has a strong impact on the amount of newly formed bone tissue; moreover, a porous surface may improve mechanical interlocking between the implant biomaterial and the surrounding natural bone.

During the newly tissue formation, the internal structure of the scaffolds at a micro-macro scale may still influence the pattern of newly tissue formed: whenever a graded and geometrically ordered scaffold is offered to the cells as template for bone tissue regeneration, lamellar bone tissue is newly formed. Conversely, if neither biomechanical cues nor geometrical rules are applicable a bone tissue lacking in structural organization will be deposited within the implanted scaffolds (Scaglione et al. 2011).

2.1 Design of the chemical structure

A range of responses, such as cell adhesion, viability and differentiation, can be differentially affected by particular natural/synthetic substrates. Ideally, the chemical structure design of scaffolds for tissue engineering should meet the following criteria: (1) the surface should permit cell adhesion, promote cell growth, and allow the retention of differentiated cell functions; (2) the scaffolds should be biocompatible, neither the material nor its degradation by-products should provoke inflammation or toxicity in vivo; (3) the scaffold should be biodegradable and eventually eliminated.

In the last years, the generation of biologically and structurally inspired scaffolds mimicking the chemical construction of natural structures has been proposed and carried out. In many cases, biomimetic strategies do not set out to copy directly the structures of biological materials but aim to abstract key concepts from the biological systems that can be adapted within a synthetic context. The simplest biomimetic approach involves the design of single component systems that mimic the chemistry of the targeted biological material.

In the field of bone tissue engineering, a wide range of biomaterials, whose composition is such that they mimic natural bone, has been tested to stimulate ossification and to improve the osteogenic potential of osteo-progenitor cells. Calcium and phosphate ions are important components during the mineralization phase of the ossification process. Materials composed of calcium phosphate such as hydroxyapatite (HA) and tricalcium phosphate (TCP) are attractive candidates for bone substitutes (Bruder, Kraus et al. 1998; Gauthier, Bouler et al. 1998; Boyde, Corsi et al. 1999; Flautre, Anselme et al. 1999; Marcacci, Kon et al. 1999; Kon, Muraglia et al. 2000; Dong, Kojima et al. 2001; Dong, Uemura et al. 2002; Livingston, Gordon et al. 2003; Gauthier, Muller et al. 2005). They are also particularly advantageous for bone tissue engineering applications as they induce neither immune nor inflammatory responses in recipient organisms (Erbe, Marx et al. 2001; Livingston, Ducheyne et al. 2002; El-Ghannam 2005).

HA is a natural component of bone tissue and therefore has been considered the ideal material to build bone substitutes. The ceramic performs as a mechanical support, an osteomimetic surface and as a template for the newly formed bone tissue. On the other hand, cells recognize the ceramic surface as pre-existing bone (osteo-mimesis) and differentiate into osteoblasts depositing bone extracellular matrix.

HA coatings have been also proposed to improve the outcome of prosthetic implants, improving the interaction between natural bone and implanted device. Porous HA ceramics support bone formation by marrow mesenchymal stem cells in vitro and in vivo. However, its brittleness and poor resorbability limits its application in the regeneration and repair of bone defects.

To avoid these limitations, polymer materials have received increasing attention and have been widely used for tissue engineering applications; in addition to biodegradability they also offer an eased processability. There are two kinds of polymer materials: synthetic polymer, and naturally derived polymers. The main biodegradable synthetic polymers include polyesters, polyanhydride, polyorthoester, polycaprolactone, polycarbonate, and polyfumarate. The polyesters such as polyglycolic acid (PGA), polylactic acid (PLA), and their copolymer of polylactic-co -glycolic acid (PLGA) are most commonly used for tissue engineering. The naturally derived polymers include proteins of natural extracellular matrices such as collagen and glycosaminoglycan, alginic acid, chitosan, and polypeptides.

Biocompatible polymers have also been regarded as candidates for bone substitutes (Ren, Ren et al. 2005; Williams, Adewunmi et al. 2005; Jiang, Abdel-Fattah et al. 2006; Wu, Shaw et al. 2006; Bonzani, Adhikari et al. 2007). However, a number of practical problems still persist, such as the difficulty in controlling the in vivo degradation of bio-resorbable polymers, low efficiency of cell seeding, cytotoxicity of the breakdown products produced during scaffold degradation, in addition to poor mechanical properties, incomparable with natural hard tissues.

To overcome these limitations, ceramic/polymer composite materials have been explored (El-Amin, Botchwey et al. 2006; Kim, Park et al. 2006; Leung, Chan et al. 2006; Kretlow and Mikos 2007; Ren, Zhao et al. 2007). When used in blends with other polymers, HA particles exposed on the surface of scaffolds favour focal contact formation of osteoblasts. A bone-like mineral film, consisting mainly of calcium apatite, when layered onto the surface of polymeric-based substrates, does not achieve the same effect as when HA is incorporated into the bulk material.

Interestingly, heterogeneous composite scaffolds consisting of two distinct, but integrated layers, have been proposed to induce cells towards different lineages, (i.e. cartilage and bone) and possibly generate heterogeneous tissues, such as osteochondral grafts (Sherwood, Riley et al. 2002; Martin, Miot et al. 2007; Grayson, Chao et al. 2008; Tampieri, Sandri et al. 2008; Harley, Lynn et al. 2010; Kon, Delcogliano et al. 2010; Lynn, Best et al. 2010). Within these informative biomaterials, cells may recognize the differently designed surfaces of the graft as pre-existing bone/cartilage tissue (biomimesis) and deposit bone/cartilage extracellular matrix accordingly to their specific localization in the scaffold. Experimental evidences confirmed that the proper design of layered scaffolds containing distinct compositional and structural features that reflect the functional environment of the native tissues is able to address progenitor cells to alternate differentiation pathways, thus inducing a simultaneous regeneration of multiple tissues (Sherwood, Riley et al. 2002; Tampieri, Sandri et al. 2008; Harley, Lynn et al. 2010; Kon, Delcogliano et al. 2010; Lynn, Best et al. 2010).

2.2 Design of the architectural structure

Porous three-dimensional (3D) scaffolds fabricated from synthetic and naturally derived materials have been widely used in different tissue engineering applications, such as cartilage, bone, skin, and ligament. Depending on the specific targeted tissue, the internal architecture has been intelligently designed and the density, pore shape, pore size and pore interconnection pathway of the material predetermined. A key structural parameter, which is also common for a wide number of tissue substitutes, is the total porosity, which is mandatory for a massive cellular induced tissue formation within the implanted scaffold.

In the last decade, several methods have been developed to prepare these kinds of porous 3D scaffolds, including gas foaming (Mooney, Mazzoni et al. 1996; Harris, Kim et al. 1998), three-dimensional printing (Hutmacher 2000), phase separation (Schugens, Maquet et al. 1996; Nam and Park 1999) and porogen leaching (Mikos, Sarakinos et al. 1993).

The gas-foaming technique uses high-pressure CO_2 gas processing and dissolved gas molecules create the macropores noted post processing. The porosity and pore structure is dependent on the amount of gas dissolved in the polymer/ceramic structure, the rate and type of gas nucleation and the diffusion rate of gas molecules through the material to the pore nuclei. The advantages of this method are a large surface area for cell attachment and a rapid diffusion of nutrients in favour of cell survival and growth. The drawback of this method might be a lack of structural stability of the final scaffold.

To improve the pore structure, a combination of different techniques may be carried out, such as gas foaming and particulate leaching. After expansion, the salt particulates are leached out to yield macropores within the scaffold. The overall porosity and level of pore connectivity can be regulated by the ratio of material/salt particulates and the size of the salt particulates.

3D printing is a solid free-form fabrication process, which produces components by inkjet printing a binder into sequential powder layers. The part is built sequentially in layers. The binder is delivered to the powder bed producing the first layer, the bed is then lowered to a fixed distance, powder is deposited and spread evenly across the bed, and a second layer is built. This is repeated until the entire part, e.g. a porous scaffold, is fabricated.

The phase-separation technique is based on thermodynamic demixing of a homogeneous polymer-solvent solution into a polymer-rich phase and a polymer-poor phase, usually by either exposure of the solution to another immiscible solvent or cooling the solution below a bimodal solubility curve. Solvent is removed by freeze-drying, leaving behind the polymer as foam. Morphology is controlled by any phase transition that occurs during the cooling step, i. e. liquid-liquid or solid-liquid.

The porogen leaching method involves the casting of a mixture of polymer solution and porogen in a mold, drying the mixture, followed by a leaching out of the porogen with water to generate the pores. Usually, water-soluble particulates such as salts and carbohydrates are used as the porogen materials. The pore structures can easily be manipulated by controlling the property and fraction of the porogen, and the process is reproducible. This technique provides easy control of the pore structure and has been well established.

Such hierarchical porous architectures not only define the mechanical properties of the scaffold, but also the initial void space that is available for regenerating cells to form new tissues (including new blood vessels) as well as the pathways for mass transport via diffusion and/or convection (Gauthier, Bouler et al. 1998; Boyde, Corsi et al. 1999; Lu, Flautre et al. 1999; Chang, Lee et al. 2000; Hollister, Maddox et al. 2002; De Oliveira, De Aguiar et al. 2003; Karageorgiou and Kaplan 2005; Mastrogiacomo, Scaglione et al. 2006). While interconnected macroporosity of a biomaterial is important to provide sufficient space for cellular activity and tissue deposition, interactions between cells and biomaterials occur at the interface, i.e., the entire internal pore walls of a 3D scaffold. Microporosity is thus another key parameter of the architectural structure design of the scaffolds. Moreover, an incomplete pore interconnection or a limiting calibre of the interconnections could represent an important constraint to the overall biological system by limiting blood vessels invasion (Mastrogiacomo, Scaglione et al. 2006).

Besides macro-micro porosity, the design of a proper surface morphology/topography may directly and significantly affect cell-scaffold interactions and ultimately tissue formation and function (Woo, Chen et al. 2003; Smith and Ma 2004; Woo, Jun et al. 2007; Smith, Liu et al. 2008). Cells in vivo are exposed to adhesive contacts in all three-dimensions, thus bio-scaffolds must be organized to mimic the spatial organization of the stem cells niche. Extensive efforts have been therefore made to identify scaffolds that resemble the natural extracellular matrix (ECM). As well as requiring information from each other, cells derive a vast wealth of information from their environments, including the material that surrounds and separates them within tissues, the ECM. An informative material scaffold must take on this instructive role to some degree in order to maintain cell viability and control cell behaviour.

Advanced manufacturing techniques can be used to control the spatial sub-micrometric internal architecture in engineered tissues, manipulating the scaffold topography on the length scale of the stem cell niche and smaller. It has been demonstrated that nanofibrous polymeric scaffolds offer to the cells biomimetic configurations that resembles ECM collagen fibers in

their ability to support the differentiation of progenitor/stem cells along adipogenic, chondrogenic, and osteogenic lineages (Yang, Murugan et al. 2005; Badami, Kreke et al. 2006; Erisken, Kalyon et al. 2008; Bashur, Shaffer et al. 2009; Wise, Yarin et al. 2009).

Nanotechnology, or the use of nanomaterials, may help to realize materials mimicking surface properties (including topography, energy, etc) of natural tissues. For these reasons, different approaches toward the formation of nano-fibrous materials have emerged in the last years: self-assembly, electrospinning and phase separation (Jayaraman, Kotaki et al. 2004; Vasita and Katti 2006; Barnes, Sell et al. 2007; Smith, Liu et al. 2008; James, Toti et al. 2011). Each of these approaches is very different but has a unique set of characteristics which lends to its development as a scaffolding system with a potential to accommodate cells and guide their growth and subsequent tissue regeneration.

For instance, self-assembly can generate small diameter nano-fibers in the lowest end of the range of natural extracellular matrix collagen, while electrospinning has only generated large diameter nano-fibers on the upper end of the range of natural extracellular matrix collagen. Moreover, electro-spinning can be used to generate polymeric scaffolds with aligned nano-scale fibers that direct spatial adhesion and orientation of cells upon differentiation. Phase separation, on the other hand, has generated nano-fibers in the same range as natural extracellular matrix collagen and allows for the design of macropore structures.

In addition to the dimensional similarity to tissue compartments, nanomaterials also exibit unique surface properties due to their significantly increased surface area and roughness compared to conventional or micron structured materials. Material surface properties mediate specific proteins adsorption and bioactivity, further regulating cell behaviour and tissue regeneration (Sato and Webster 2004; Balasundaram and Webster 2006; Liu and Webster 2006).

3. How stem/progenitor cells decode biomaterials

Although the identification of mesenchymal stem cells (MSC) is currently a matter of discussion, these cells have become attractive targets for clinical applications and a large number of studies on use in regenerative medicine have been produced (Tonti and Mannello 2008). The classic paradigm for tissue engineering considers seeding an appropriate cell source, like MSC, on or within a scaffold that facilitates cells growth, organization and differentiation into a specific and functional tissue.

Regardless of the topography and of the chemistry of the scaffolds, the constructs must also provide some level of physical support from the moment of implantation, to assist cell attachment and provide room for the deposition of new matrix, if needed for tissue reconstitution. This clearly implies close contact between matrix proteins, either of endogenous (cellular) or exogenous (secreted) origin, and the scaffold, a “play-of-three” that is extremely relevant for the cell.

In living tissues the main extracellular matrix constituents are comprised within a few macromolecule classes, such as collagens, elastin, proteoglycans, hyaluronic acid -and its derivatives- and adhesion glycoproteins (among which fibrinogen and fibronectin, tenascins and thrombospondins). Alternative splicing and secretion of different proportions of the ECM components allow the generation of a wide range of matrices, ranging from basal lamina to bone. Often the prototypical scaffolds for the cell-based repair of mesenchymal tissues (mainly cartilage or bone, or both), whether composed of ceramic or biodegradable

polymers, can be tailored to support cell adhesion and to degrade at rates coincident with new tissue development. However, on both scaffold types, the mechanism of cell adhesion is indirect and relies onto the deposition of extracellular matrix proteins by the seeded cells (Murphy, Hsiong et al. 2005; Chastain, Kundu et al. 2006). As a result, ceramic or biosynthetic scaffolds may lack at first the specificity of the original tissues, i.e. the exogenous proteic signals that ease cell adhesion, undermining the recognition of the local microenvironment by the cells and the consequent repair processes. For engineered tissue repair, then, it is critical to understand the mechanisms by which cells first recognize the surfaces and structures on which they are seeded and how these, in turn, may govern cell functions and influence cell-mediated remodelling events at the interfaces between the cell-seeded constructs and the host tissues.

3.1 The role of cell adhesion molecules as active mechanosensors

Cells normally sense the microenvironment elasticity as they anchor and pull on their surroundings. These processes relay in part on specific adhesion proteins- myosin, integrins, cadherins- able to transmit forces to the substrates. Considerable attention has been posed in understanding the cell responsiveness to external forces, ranging from fluid flow to stretching and twisting (Alenghat and Ingber 2002). However the cells respond also to the sensed resistance, whether it comes from normal tissue matrix or from synthetic substrata, with cytoskeletal alterations. The most recent literature points out to the existence of a feedback loop, in which cell-exerted forces are coupled to microenvironmental elasticity able to induce subsequent and additional changes to cellular responses (Discher, Janmey et al. 2005). Typically four protein families are involved in the adhesion processes: IgCAM, selectins, cadherins and integrins. The first three interact with complementary proteins/ligands on the partner cells surfaces, whereas integrins bind prevalently to extracellular matrix proteins. The expression of restricted isoforms of each of these classes of proteins allows specific interactions among cells and extracellular matrix, an essential requisite for embryonic development, tissue regeneration and force transmission. However, in the light of scaffold surface recognition, integrins and cadherins are of paramount importance for sensing the microenvironment external to the cell. Integrins are heterodimeric receptors made of two transmembrane chains, α and β, both contributing to binding specificity. A combinatorial strategy allows the vertebrate cells to express several sub-sets of integrins by selective combinations of 18 different α chains and additional 8 different β chains. At least 24 different dimers are so far known. The cytoplasmic tails of the intergrins, extracellularly linked to ECM components, bind to actin filaments of the cytoskeleton to generate focal contacts, through the concerted action of talin and vinculin. Another adaptor protein, paxillin, binds the integrins to recruit Src and FAK (focal adhesion) tyrosin-kinases, (Critchley 2000; Turner 2000). This chain of events promotes, within minutes, a rise of the intracellular Ca2+ concentration and the development of mature focal contacts, named focal adhesion sites, anchoring the actin stress fibers to the cell membrane. The tension generated by the organized stress fibers on the adhesion sites is maintained during cell movement and migration. Cell adhesion onto a specific substrate depends, then, on the integrin density on the cell surface, on the ligand concentration on the substratum surface and on their reciprocal affinity. The rapid association/dissociation of the integrin/ligand complexes allows the cell to redefine the interactions with the ECM during anchoring, movement and migration (Bercoff, Chaffai et al. 2003).

The importance of sensing the mechanical properties of the ECM within the cell surroundings has been clearly established in studies with tumor cells and fibrobroblasts (Discher, Janmey et al. 2005; Paszek, Zahir et al. 2005). Indeed tumors (often detected as a rigid mass within softer tissues) display a peculiar rigidity, in part due to the interstitial pressure caused by a perturbed vascular structure, in part due to fibrosis, but in part also due to an increase of the elastic module of transformed cells as a consequence of an altered cyto-architecture (Beil, Micoulet et al. 2003). A current understanding is that cells take advantage of actinomyosin contractility for dual interactions with the matrix. Cell contraction at integrin-based adhesion sites is essentially resisted by the matrix, and is followed by the accumulation of additional molecules at the sites involved. This process comes to a balance when tension forces are equilibrated at the cell-matrix interface. Regardless of the non-malignant or malignant nature of the cells, the mechanotransducing functions of integrins represent a major focus of several researches (Bershadsky, Balaban et al. 2003), Integrins are known to regulate Rho- and growth factors-ERK (Extracellular signal-regulated kinase) dependent growth (Lee and Juliano 2004). At the same time ERK influence ROCK (Rho-associated protein kinase) and myosin activity (Huang, Kamm et al. 2004). Interestingly matrix stiffness modulates growth factor signalling and Rho GTPase activity (Wang, Weaver et al. 1998); moreover, Rho activity is elevated in stiff tumors and is linked to cell invasivness, although single members of the Rho family are differently involved in branching and lamellipodia broadening (Vega, Fruhwirth et al. 2011); instead ROCK activation contributes to cell contractility by inhibiting depolimerization of actin filaments (Paszek, Zahir et al. 2005). In epithelial morphogenesis, for example, matrix stiffness clusters integrins and these, in turn, enhance ERK activation and ROCK-generated contractility and focal adhesion. Indeed non-malignant mammary epithelial cells can be induced to form normal polarized and growth-arrested acinar structures in basal membrane, laminin-containing collagen I gels that match stiffness of normal mammary gland stroma. However even a small increase in matrix stiffness (by changing the gel cross-linker ratios) significantly compromises tissue organization, inhibits lumen formation and disrupts adherens junctions, as testified by a diffused β -catenin and non co-localized E-cadherin and β -catenin. Integrin α3 β1 and talin are involved in the normal adhesion machinery, both on soft and stiff substrata; however epithelial cells interacting with a soft matrix assemble focal complexes and express high amounts of total and active Src family kinase, whereas on a stiff matrix, cells spread and assemble stress fibers, activate more ERK in response to growth factors, and form focal adhesion sites with FAK^{pY397} and vinculin (Paszek, Zahir et al. 2005). These events are in compliance with altered levels of integrin expression in stiff tumors (Guo, Ma et al. 2009) as well as in rigid 2D substrata with respect to 3D matrices (Yeung, Georges et al. 2005). Interestingly a similar behaviour was also observed in fibroblasts, where a phosphorylated FAK^{pY397} and vinculin were recruited to α5 β1 adhesion sites on stiff gels, although in this case force-dependent integrin aggregation precedes the appearance of FAK^{pY397} (Nicolas, Geiger et al. 2004). Force-dependent integrin anisotropy, as a result of matrix stiffness sensing, was demonstrated to be of relevance using integrin mutants (V737N) that promoted self-association through enhanced hydrogen bonding in the transmembrane domain of the protein. Although integrin clustering was not induced on stiff substrata, on soft gels V737N integrin-expressing cells spread significantly more, formed larger adhesion sites, expressed FAK^{pY397} and activated more ERK in response to growth factors (Paszek, Zahir et al. 2005). A mechanoregulatory circuit, then, integrates physical cues from the extracellular matrix with focal adhesion sites, through ERK- and

Rho-dependent cytoskeletal contractility, and regulates cells and tissue phenotype. Unbalance in integrin expression was also detected when placent-derived mesenchymal stromal cells were induced toward angiogenesis; VEGF-mediated adhesion and migration of placental-derived MSC onto fibronectin correlated with enhanced expression of α5 β1; at the same time anti- α5 or anti- β 1 antibodies inhibited angiogenesis when cells were cultured on chick corioallantoic membranes (Lee, Huang et al. 2009).

In fibroblasts fluorescence imaging has shown that F-actin fibers and stress fibers become increasingly organized if cells are cultured on increasingly stiffer substrates (Discher, Janmey et al. 2005). Contractile myotubes, instead, display strong focal adhesion and stress fibers when cultured on stiff gels or on glass micropatterns; they will however display actomyosin striation if cultured on top of a first layer of muscle cells (Discher, Janmey et al. 2005), Similarly, heart cells pulling on equally stiff heart cells can generate a positive feedback on their cytoskeletal organization that may not occur when the substratum is a scaffold or a different cell type. However variations and differences between cell types imply active and regulated responses, rather than a universal need of cells to exert traction forces; differences may depend in part on the expression and engagement of adhesion molecules, like it happens in the generation of shell-to-core cell aggregates obtained when randomly mixing two different cell types. Such an event was detected when mixing cardiomyocytes and retinal cells and is currently explained by the generation of surface tension at the interfaces of cell layers originated from low and high N-cadherin expressing cells (Discher, Janmey et al. 2005).

Cadherin-mediated interactions are prevalently homophylic, Ca2+-dependent and are responsible of driving cells into close contact through the organization of adherent junctions and desmosomes. Their common structural characteristic is the CAD domain, a folded structure organized in 7 β-sheets. Calcium ions bind between two CAD adjacent domains rendering the structure stiff, but the domains are free to rotate if the if the ions are not bound. Several cadherins display more than 5 extracellular CAD domains, arranged for *trans* or *cis* binding with other cadherin partners on the opposite cell (He, Cowin et al. 2003).

Cadherins contribute to growth contact inhibition; their cytoplasmatic tails bind adaptor proteins of the catenin family, linking cell-to-cell recognition to signal transduction pathways (Hamidouche, Hay et al. 2008). Cadherin-mediated adhesion was in fact linked to GTPase-cytoskeleton signalling (Delanoe-Ayari, Al Kurdi et al. 2004). Interestingly this process presents similarities with the self-organizing condensing mesenchymal cells that drive the growth of the pre-cartilagineous anlage in limb bud development through growth factor diffusion-processes and aptotaxis, although contribution from the latter seems relative (Christley, Alber et al. 2007).

In vitro chondrogenesis of MSC offers additional cues to dissect the pathways involved in cell shape-mediated commitment to differentiation. Chondrogenesis of MSC is induced when cells are cultured in high-density micromass pellets and stimulated with transforming growth factor β (Mackay, Beck et al. 1998). Rac1, another small GTPase, displayed a much higher activity in MSC undergoing smooth-muscle cell differentiation if compared to the same cells induced to chondrogenesis. Rac1 further regulated N-cadherin expression, a known requirement for smooth-muscle cell differentiation (Gao, McBeath et al. 2010). However it should be remembered that a dominant negative Rac1 was not sufficient to inhibit N-cadherin upregulation in the presence of TGF β 3, thus suggesting the possible presence of additional contributor proteins. The cytoplasmic domain of N-cadherin is

indirectly linked to the cytoskeleton via the α-, β - and p120-catenin complex; deletion mutants of N-cadherin, lacking the β -catenin binding site, failed to support smooth-muscle cell differentiation of MSC, highlighting the role of these protein complexes in cell fate. Indeed cadherins are known to be implicated in mesenchymal condensation, although their expression is normally downregulated during chondrocytic differentiation (Oberlender and Tuan 1994). Possibly a concerted action of N-cadherin and Rac1 is necessary for myogenic differentiation of MSC, ensuring cell-to-cell contact; this may be a transient requirement that becomes unnecessary once cells get separated and encased in extracellular matrix during chondrogenic maturation.

3.2 Progenitor cells' lineage commitment: A matter of feeling

Transient signals, then, may be responsible of limited (time-wise and intensity-wise) cellular responses: other factors, such as soluble inducers in the growth medium, may also couple to matrix anchorage, as it was demonstrated for fibroblasts (Nakagawa, Pawelek et al. 1989), also derived from mesenchymal progenitors.

It is well known that differentiated cells of mesenchymal origin adhere and contract not only within soft tissues, but also on a variety of substrates in vitro, such as on collagen-coated acrylamide gels and glass (Engler, Sen et al. 2006). Such a wide range of possible adhesion substrates parallels a wide variation in matrix stiffness sensing, which in turns influences focal adhesion structures and the cell cytoskeleton (Cukierman, Pankov et al. 2001; Discher, Janmey et al. 2005). However, for tissue engineering purposes, pluripotent stem cells, rather than terminally differentiated ones, represent the gold standard for current and potential clinical applications (Peters, Schell et al. 2010; Ding, Shyu et al. 2011). In the last years it has been shown that stem cells or progenitor cells can be isolated from almost every tissue of the body (Bianco and Robey 2001), including menstrual blood (Ding, Shyu et al. 2011). Under the correct conditions, these cells can be stimulated to form new tissue, by using a simple biomaterials-based approach (Bianco and Robey 2001; Boo, Yamada et al. 2002; Cancedda, Bianchi et al. 2003; Barrilleaux, Phinney et al. 2006; Hutmacher, Schantz et al. 2007; Gigante, Manzotti et al. 2008; Hunt 2008)

In principle, stem cells in their niche undergo self-renewal, or exit the niche upon exposure to local or systemic stimuli. During tissue development and repair these signals are actively coordinated and are presented in a temporally and spatially regulated manner (Connelly, Garcia et al. 2008; Santiago, Pogemiller et al. 2009); for example the ECM surrounding osteogenically differentiating MSC is dynamically remodelled: biglycan is first detected in bone marrow surrounding MSC but not in unmineralized or in mineralized bone matrices; fibronectin and versican are observed in the regions of early mesenchymal condensation but they disappear in mature bone; decorin is present in unmineralized matrix but absent in mineralized bone (Hoshiba, Kawazoe et al. 2009). Indeed, bone marrow-derived mesenchymal stem cells (BMSC) represent a widely used class of progenitors, due to their ability to differentiate into several lineages (for ex, osteogenic, chondrogenic, myogenic or neurogenic), each of which is characterized by different matrix microenvironments and anchorage-dependent requirements (Goessler, Bieback et al. 2006; Djouad, Delorme et al. 2007; Boskey, Doty et al. 2008). At the tissue level, in fact, matrix stiffness accounts for distinctive ranges (Engler, Sen et al. 2006). The resistance that a cell feels when it deforms the ECM can be measured by the elastic constant of the matrix microenvironment, *E*, with values that range from 0.1-1.0 kPa (soft tissues, for example brain), to 1.0-20.0 kPa (muscle)

up to >25.0 kPa for bone. By controlling the matrix elasticity in polyacrylamide gels through the cross-linker (bis-acrylamide) concentration, and by providing adhesion by coating the gels with collagen I, known to support myogenic and osteogenic differentiation, Engler and co-workers (2006) demonstrated that matrix stiffness, in spite of the same culturing conditions and medium supplements, can specify MSC lineage differentiation.

Among the cell's cytoskeletal motors, the non-muscle isoforms of myosin II (NMM II) (Kim, Kovacs et al. 2005) are suitable candidate mechanotransducers, able to generate signals proportional to the matrix deformation. The three existing isoforms (non-myosin II A, B or C) are involved in tensioning cortical actin structures linked to focal adhesion sites. These actin bridges transmit the force from the cell inside to the elastic matrix (Tamada, Sheetz et al. 2004) and are associated with signalling molecules (Bershadsky, Balaban et al. 2003). A counterproof of the involvement of non-myosin II in sensing matrix stiffness in MSC derives from the use of a specific NMM II-inhibitor, blebbistatin, which does not exerts its function on any other form of MSC myosin, other than myosin VI (Limouze, Straight et al. 2004). The administration of this molecule during MSC plating on different matrices blocks cell branching, elongation and spreading, but it has no relevant effect if exposure is carried out after cells have already spread and adopted a specific morphology, or 24 hrs post-seeding. Within this time frame, in fact, blebbistatin, was shown to inhibit the actin-dependent activation of the NNM II ATPase activity; these results were also confirmed by the use of an additional inhibitor specific for the myosin light chain kinase (MLCK), known to activate NNM II (Dhawan and Helfman 2004). Results lead to the conclusion that indeed NNM II appears to be necessary for matrix-elasticity driven lineage specification in MSC.

Traction stresses also modify the surrounding matrix around the cells. Although larger tractions are exerted on stiffer surfaces, typical tractions ($\tau \sim 1$KPa) exceed by far the viscous traction exerted by culture fluid on the cells. Moreover if matrix strain is relatively constant, cells need to be less contractile on soft gels than on stiff ones. Thus their adhesion will not be as strong, as it has consistently been measured by reduced forces needed to peel off cells from gels versus glass (Engler, Griffin et al. 2004).

Local sensing of force and/or geometry are therefore transduced into biochemical signals that regulate cell growth, differentiation shape and even cell death (Vogel and Sheetz 2006). Stiffness sensitivity and consequent cytoskeletal reorganization, however, not only interest the membrane/cytoplasmic compartment: nuclear deformations also take place in response to cytoskeletal modifications, cell cycle and division. Chromatin and laminin B contribute to the viscoelastic properties of the somatic cell nucleus, with single contributions prevailing according to the swelling condition of the nucleus. The nucleus is stiff and resists distortion at short times, whereas it undergoes deformation at longer times, providing essentially an infinite spectrum of timescales for structural reorganization and genome expression kinetics (Dahl, Engler et al. 2005). Nuclear deformation was also reported in response to culture conditions of MSC; static or perfusion cultures on 3D poly(ethylene terephthalate) scaffolds affected the ability of MSC to synthesize and deposit and organized ECM network, but, at the same time, affected nuclear shape: only cells in perfusion cultures displayed uniform spherical nuclei. Interestingly, cells in perfusion systems down-regulated Rex-1 and Oct-4 stemness-related genes, implying that a less primitive stem cell phenotype was retained in the perfusion cultures (Zhao, Grayson et al. 2009). This is in accordance with a possible stiffness sensing-dependent lineage commitment previously described, which would couple a stiff scaffold with a loss of the pluripotency and with an osteogenic-oriented MSC differentiation.

Once naive MSC are exposed to specific matrix stiffness, then, their gene transcription machinery up- or down-regulates specific gene subsets; immunostaining of cytoskeletal markers and transcription factors across the range of the tested matrix stiffnesses proved consistent with the lineage profiling in the experiments carried out by Engler and collaborators (2006): gene expression of markers for neurons, muscle or bone was induced 4- to 6-fold on the corresponding substrate with high specificity. Only stem cells grown on soft substrates with brain-like compliances expressed a phosphorylated form of neurofilament heavy chain. By converse myoD, a marker of muscle differentiation, and CBFα1, a transcription factor required for osteogenic differentiation, were expressed by cells grown on intermediate or rigid substrates, respectively. However it should be noted that expression levels for other markers was limited to only 50% of the standard level in terminally differentiated culture cells; muscle lineage-specific integrins expression was furthermore absent, evidencing that matrix stiffness compliance can drive the progenitor cells toward a developmental route but may not be sufficient *per se* to ignite terminal differentiation. Interestingly a bioinformatic approach also revealed that in mesenchymal stem cells genes regulated by high ECM stiffness included those indicative of the activation of two transcription factors downstream the Hippo signalling pathway, a highly conserved pathway involved in restraining cell proliferation and promoting apoptosis: YAP (Yes-associated protein) and TAZ (a transcriptional co-activator with a PDZ-binding motif, also known as WWTR1). Stiff surfaces induce nuclear translocation and activation of both factors, whereas their location was predominantly cytoplasmic and inactive in cells grown on soft matrices (Dupont, Morsut et al. 2011). In spite of the participation to the Hippo pathway, nuclear localization of the transcription factors was mainly due to the activity of the already cited Rho GTPase activity. Both factors are known to bind to Runx2, a transcription factor essential for osteogenic differentiation of mesenchymal cells. In a relevant set of experiments Dupont and co-workers (2011) demonstrated that depletion of YAP and TAZ prevented osteogenic differentiation of MSC cultured on stiff matrices and conversely promoted their adipogenic differentiation, thus elucidating the primary role of these factor in translating the cell mechanosensitivity from the cytoplasmic machinery to the nuclear/gene expression level.

3.3 Topography and surface chemistry: links to the mechanosensitivity of the cell

The optimization of the interactions between a scaffold matrix and the cell counterparts of the constructs can also be pursued by a specific biomimetic functionalization and/or nanostructuration of the interface. For prosthetic applications in orthopaedics, for example, cell attachment to grooved materials (Eisenbarth, Velten et al. 2007) and to nanocristalline coatings (Nicula, Luthen et al. 2007) has been documented since long. Indeed the interaction of the cells with the surrounding materials is within the nanometer scale. Thus nanoscaled topography of synthetic materials has attracted raising consideration because of its resemblance to in vivo surroundings, and mammalian cells were demonstrated to response to topographical surface variations (Silva, Czeisler et al. 2004; Dalby, McCloy et al. 2006; Dalby, McCloy et al. 2006). For articular chondrocytes, cell motility was increased when cells were cultured on 8 μm-deep grooved plastic-ware. Cells spread and oriented along the long axis of the groove. F-actin condensation was evident along the groove/ridge boundaries, correlated with a doubled velocity at which cells moved and was associated with a loss of the cell chondrogenic potential. Conversely 750 nm-deep grooves induced a reduced migratory capacity (Hamilton, Riehle et al. 2005; Hamilton, Riehle et al. 2005).

Although transdifferentiation of mesenchymal stem cells to neuronal lineages can be forced through specific induction media (Woodbury, Schwarz et al. 2000; Deng, Obrocka et al. 2001; Qian and Saltzman 2004) the mechanisms are not well understood. Nonetheless cultures of human MSC on nano-patterned plastic-ware, with gratings of 350 nm linewidth, an order of magnitude lower than the cells size, showed morphological changes in cell bodies and nuclei. A substantial confirmation of a new phenotype came from gene expression and microarray studies, in which microtubule associated protein 2 (MAP2) and β–tubulin III (Tuj1), both neuronal markers, were detected (Yim, Pang et al. 2007).

In the light of these results, it is reasonable to suppose that specific nano-patterning(s) may be compliant to or guide specific distribution(s) of the cell adhesion molecules within the cell surface. This distribution mirrors the one that cells would adopt in response to specific stiffness and elasticity of an underlying contact surface. The overall result is that nano-patterning may anticipate the cell response to a specific substratum and induce the consequences of cells adhesion onto it.

Clearly, once a cell has somewhat "decoded" its substrate and has ignited a new gene expression program in response to exogenous/endogenous stimuli, the secreted extracellular matrix protein will contribute to modify the microenvironment and to further drive the cell along a specific differentiation pathway. For example passive adsorption of two matrix protein like vitronectin (VN) and type collagen I (Col I) onto polymeric substrates were shown to mediate MSC adhesion and differently induced activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3-kinase (PI3K) signal transduction pathways (Kundu and Putnam 2006). Recent findings reveal that the *de novo* synthesis and deposition of ECM proteins by MSC alters the chemical identity of the polymeric substrate, stimulating changes in the integrin expression profiles. In turn these changes promote modifications in the MAPK and PI3K signalling pathways, therefore influencing the osteogenic differentiation of the seeded cells. Increasing amounts of fibronectin and Col I and decreased amounts of VN are in fact being deposited on poly(lactic) glycolic acid scaffolds over a 28-day period. The cell receptors pattern changed accordingly, providing higher levels for α5 β1 and α2 β1 integrins, (receptors for fibronectin and Col 1, respectively) and reduced levels for αV β3 integrin (VN receptor). Mechanistically, cell adhesion to Col I and fibronectin has been shown to induce the MAPK cascade, in particular the activation of the ERK1/2 system, critical for the activation of the osteogenic transcriptional factor Runx2 (Xiao, Jiang et al. 2000; Franceschi and Xiao 2003). Specific integrins then seem to be preferred or even required for the osteogenic differentiation of MSC. It should be remembered, though, that multiple integrins can bind a single ECM protein and that multiple ECM proteins can bind a single integrin (Miranti and Brugge 2002). Therefore a biofunctionalization of a scaffold surface should not focus on the presentation of a uniform coating to engage a single receptor, but rather identify the properties that control the presentation of integrin-specific epitopes within the coatings (Keselowsky, Collard et al. 2005).

Clearly several additional chemical modifications can be introduced and applied to almost any specific substrata, provided that the proper chemistry is used; indeed many strategies and approaches are currently being tested (Fu, Wang et al. 2011), ranging from simple coatings onto specific substrates (Uygun, Stojsih et al. 2009), to the contemporary use of genetic engineering and structural approaches (Benoit, Schwartz et al. 2008; Gorsline, Tangkawattana et al. 2010), to combinations of matrix-mimicking ligands and engineered structured nanomatrices (Anderson, Kushwaha et al. 2009). The same natural extracellular

matrix is *per se* able to induce specific cell commitment (Chen, Dusevich et al. 2007). Thus the combination of topographical and chemical cues may result in a synergistic effect, in some cases useful enough even to direct cell differentiation of adult MSC stem cells to non-canonical pathways, such as neuronal differentiation. Interestingly the effects of growth conditions onto a nano-patterned surface were stronger than the single biochemical induction on controls grown on un-patterned surfaces (Yim, Pang et al. 2007).

4. Promising bone substitutes in the tissue-engineering scenario

Ideal skeletal reconstruction depends on regeneration of normal tissues that result from initiation of progenitor cell activity. In this context, cells are considered as a key element to achieve the regeneration of the target tissue, since very few biomaterials are osteoinductive by themselves (Goshima, Goldberg et al. 1991; Ohgushi, Dohi et al. 1993; Boo, Yamada et al. 2002; Cancedda, Bianchi et al. 2003; Endres, Hutmacher et al. 2003; Livingston, Gordon et al. 2003; Derubeis and Cancedda 2004; Warren, Nacamuli et al. 2004; Arinzeh, Tran et al. 2005; Kimelman, Pelled et al. 2006; Bernhardt, Lode et al. 2009; Matsushima, Kotobuki et al. 2009).
The most intriguing concept in modern biomaterials is thus obtaining materials able to mimic a specific eventually pre-existing microenvironment and, therefore, inducing stem/progenitor cells to differentiate in a predetermined manner and to regenerate by themselves the bone tissue according to physiological pathways.
Several researches have been conducted using autologous bone marrow-derived osteo-progenitors to repair critical size segmental defects (Bianco and Robey 2001; Cancedda, Bianchi et al. 2003). The results of all these studies were in good agreement suggesting an important advantage in bone formation and, therefore, in the healing of the defect when cells were delivered together with a proper biomaterial scaffold. It is surprising that after the initial enthusiasm demonstrated by the flourishing of very encouraging large animal studies, only two pilot clinical studies have been performed (Quarto, Mastrogiacomo et al. 2001; Vacanti, Bonassar et al. 2001). Although material science technology has resulted in clear improvements in the field of regenerative medicine, no ideal bone substitute has been developed yet and hence large bone defects still represent a major challenge for orthopaedic and reconstructive surgeons. We are now aware, though, that the intended clinical use defines the desired properties of engineered bone substitutes. Anatomical defects in load bearing long bones, for instance, require devices with high mechanic stability whereas for craniofacial applications, initially injectable or moldable constructs are favorable. Therefore, the most intriguing concept is obtaining materials able to mimic a specific eventually pre-existing microenvironment, thus priming the natural processes of bone regeneration driven by cells.

5. Conclusion

In summary, a suitable scaffold for tissue engineering applications must have a structure correctly designed at different spatial scales to mimic the complex SC niche (Dellatore, Garcia et al. 2008). While it will probably not be necessary to mimic all aspects of the niche to enhance stem cells self-renewal and differentiation, it will almost certainly be necessary to simultaneously mimic multiple components of the niche (chemical and multi-scale architectural cues) to induce a specific cell differentiation and tissue ingrowth.
Proper surface sensing, then, has raised as a new requirement for progenitor cells lineage differentiation. Indeed precommitment of MSC grown on a specific matrix cannot be

overcome by the addition of soluble factors to the growth medium. The osteogenic differentiation of MSC seeded onto electrospun poly(ε-caprolactone)/ECM scaffolds is maintained even in the absence of dexamethasone in the culture medium, a molecule normally required in standard osteogenic induction of plastic-adherent MSC cultures (Thibault, Scott Baggett et al. 2010). This observation is therefore of paramount relevance for tissue engineering applications of MSC, considering that specific tissue repair applications, such as bone reconstruction, often lead cell-based applications to relevant rounds of ex-vivo cell duplications, normally performed on standard disposable culture plastic-ware. In this respect, the most recent literature brings new insights onto the sensitivity of stem cells to the mechanical microenvironment, but also raises relevant questions regarding the induction strategies and the physical environments of in vivo and ex vivo microenvironments. Significantly recent findings have also raised the possibility that an injured microenvironment may lose compliance due to insufficient sensitivity and remodelling options of stem cells once in a non-inducing environment such as a fibrotic scar (Berry, Engler et al. 2006). Moreover the current paradigm implies that tissue homeostasis is favoured by a compliant matrix and a relative low integrin-mediated cytoskeletal tension, whereas an elevated integrin-ERK-Rho activity favours a tumorigenic/proliferating behaviour, although an excessively stiff matrix or integrin-dependent activity would promote the generation of stable focal adhesion sites, ultimately antagonizing cell spreading.

Whether all these approaches and specific aspects, (scaffold stiffness compliance, surface topography and tridimensionality, scaffold chemistry) can be integrated into scaffold engineering to properly foster tissue regeneration remains to be seen; these aspects, however, have become even more relevant if the same pluripotent progenitor cells are used within tissue engineered composites proposed for multiple tissue repair, such as in the case of osteochondral defects. Microenvironmental changes may indeed influence the repair outcomes of the different tissues (Djouad, Delorme et al. 2007): the challenge, then, is to provide the proper cell "pre-commitment" in vitro to partially overcome an inappropriate pathological in vivo microenvironment.

6. Acknowledgment

This work was partially funded by the Italian Ministry of Education, Universities and Research (MIUR) (grant no. RBPR05RSM2; RBIP068JL9).

7. References

Alenghat, F. J. and D. E. Ingber (2002). Mechanotransduction: all signals point to cytoskeleton, matrix, and integrins *Sci STKE* 2002(119): pe6.

Anderson, J. M., M. Kushwaha, et al. (2009). Osteogenic differentiation of human mesenchymal stem cells directed by extracellular matrix-mimicking ligands in a biomimetic self-assembled peptide amphiphile nanomatrix *Biomacromolecules* 10(10): 2935-44.

Arinzeh, T. L., T. Tran, et al. (2005). A comparative study of biphasic calcium phosphate ceramics for human mesenchymal stem-cell-induced bone formation *Biomaterials* 26(17): 3631-8.

Badami, A. S., M. R. Kreke, et al. (2006). Effect of fiber diameter on spreading, proliferation, and differentiation of osteoblastic cells on electrospun poly(lactic acid) substrates *Biomaterials* 27(4): 596-606.

Balasundaram, G. and T. J. Webster (2006). Nanotechnology and biomaterials for orthopedic medical applications. *Nanomedicine* (Lond) 1(2): 169-76.

Balasundaram, G. and T. J. Webster (2007). An overview of nano-polymers for orthopedic applications. *Macromol Biosci* 7(5): 635-42.

Barnes, C. P., S. A. Sell, et al. (2007). Nanofiber technology: designing the next generation of tissue engineering scaffolds. *Adv Drug Deliv Rev* 59(14): 1413-33.

Barrilleaux, B., D. G. Phinney, et al. (2006). Review: ex vivo engineering of living tissues with adult stem cells. *Tissue Eng* 12(11): 3007-19.

Bashur, C. A., R. D. Shaffer, et al. (2009). Effect of fiber diameter and alignment of electrospun polyurethane meshes on mesenchymal progenitor cells. *Tissue Eng Part A* 15(9): 2435-45.

Beil, M., A. Micoulet, et al. (2003). Sphingosylphosphorylcholine regulates keratin network architecture and visco-elastic properties of human cancer cells. *Nat Cell Biol* 5(9): 803-11.

Benoit, D. S., M. P. Schwartz, et al. (2008). Small functional groups for controlled differentiation of hydrogel-encapsulated human mesenchymal stem cells. *Nat Mater* 7(10): 816-23.

Bercoff, J., S. Chaffai, et al. (2003). In vivo breast tumor detection using transient elastography. *Ultrasound Med Biol* 29(10): 1387-96.

Bernhardt, A., A. Lode, et al. (2009). In vitro osteogenic potential of human bone marrow stromal cells cultivated in porous scaffolds from mineralized collagen. *J Biomed Mater Res A* 90(3): 852-62.

Berry, M. F., A. J. Engler, et al. (2006). Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. *Am J Physiol Heart Circ Physiol* 290(6): H2196-203.

Bershadsky, A. D., N. Q. Balaban, et al. (2003). Adhesion-dependent cell mechanosensitivity. *Annu Rev Cell Dev Biol* 19: 677-95.

Bianco, P. and P. G. Robey (2001). Stem cells in tissue engineering. *Nature* 414(6859): 118-21.

Bonzani, I. C., R. Adhikari, et al. (2007). Synthesis of two-component injectable polyurethanes for bone tissue engineering. *Biomaterials* 28(3): 423-33.

Boo, J. S., Y. Yamada, et al. (2002). Tissue-engineered bone using mesenchymal stem cells and a biodegradable scaffold. *J Craniofac Surg* 13(2): 231-9; discussion 240-3.

Boskey, A. L., S. B. Doty, et al. (2008). Modulation of extracellular matrix protein phosphorylation alters mineralization in differentiating chick limb-bud mesenchymal cell micromass cultures. *Bone* 42(6): 1061-71.

Boyde, A., A. Corsi, et al. (1999). Osteoconduction in large macroporous hydroxyapatite ceramic implants: evidence for a complementary integration and disintegration mechanism. *Bone* 24(6): 579-89.

Bruder, S. P., K. H. Kraus, et al. (1998). The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects. *J Bone Joint Surg Am* 80(7): 985-96.

Cancedda, R., G. Bianchi, et al. (2003). Cell therapy for bone disease: a review of current status. *Stem Cells* 21(5): 610-9.

Chang, B. S., C. K. Lee, et al. (2000). Osteoconduction at porous hydroxyapatite with various pore configurations. *Biomaterials* 21(12): 1291-8.

Chastain, S. R., A. K. Kundu, et al. (2006). Adhesion of mesenchymal stem cells to polymer scaffolds occurs via distinct ECM ligands and controls their osteogenic differentiation. *J Biomed Mater Res A* 78(1): 73-85.

Chen, X. D., V. Dusevich, et al. (2007). Extracellular matrix made by bone marrow cells facilitates expansion of marrow-derived mesenchymal progenitor cells and prevents their differentiation into osteoblasts. *J Bone Miner Res* 22(12): 1943-56.

Cheng, L., F. Ye, et al. (2010). Osteoinduction of hydroxyapatite/beta-tricalcium phosphate bioceramics in mice with a fractured fibula. *Acta Biomater* 6(4):1569-74.

Christley, S., M. S. Alber, et al. (2007). Patterns of mesenchymal condensation in a multiscale, discrete stochastic model. *PLoS Comput Biol* 3(4): e76.

Connelly, J. T., A. J. Garcia, et al. (2008). Interactions between integrin ligand density and cytoskeletal integrity regulate BMSC chondrogenesis. *J Cell Physiol* 217(1): 145-54.

Critchley, D. R. (2000). Focal adhesions - the cytoskeletal connection. *Curr Opin Cell Biol* 12(1): 133-9.

Cukierman, E., R. Pankov, et al. (2001). Taking cell-matrix adhesions to the third dimension. *Science* 294(5547): 1708-12.

Dahl, K. N., A. J. Engler, et al. (2005). Power-law rheology of isolated nuclei with deformation mapping of nuclear substructures. *Biophys J* 89(4): 2855-64.

Dalby, M. J., N. Gadegaard, et al. (2007). The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nat Mater* 6(12): 997-1003.

Dalby, M. J., D. McCloy, et al. (2006). Osteoprogenitor response to semi-ordered and random nanotopographies. *Biomaterials* 27(15): 2980-7.

Dalby, M. J., D. McCloy, et al. (2006). Osteoprogenitor response to defined topographies with nanoscale depths. *Biomaterials* 27(8): 1306-15.

De Oliveira, J. F., P. F. De Aguiar, et al. (2003). Effect of process parameters on the characteristics of porous calcium phosphate ceramics for bone tissue scaffolds. *Artif Organs* 27(5): 406-11.

Delanoe-Ayari, H., R. Al Kurdi, et al. (2004). Membrane and acto-myosin tension promote clustering of adhesion proteins. *Proc Natl Acad Sci U S A* 101(8): 2229-34.

Dellatore, S. M., A. S. Garcia, et al. (2008). Mimicking stem cell niches to increase stem cell expansion. *Curr Opin Biotechnol* 19(5): 534-40.

Deng, W., M. Obrocka, et al. (2001). In vitro differentiation of human marrow stromal cells into early progenitors of neural cells by conditions that increase intracellular cyclic AMP. *Biochem Biophys Res Commun* 282(1): 148-52.

Derubeis, A. R. and R. Cancedda (2004). Bone marrow stromal cells (BMSCs) in bone engineering: limitations and recent advances. *Ann Biomed Eng* 32(1): 160-5.

Dhawan, J. and D. M. Helfman (2004). Modulation of acto-myosin contractility in skeletal muscle myoblasts uncouples growth arrest from differentiation. *J Cell Sci* 117(Pt 17): 3735-48.

Ding, D. C., W. C. Shyu, et al. (2011). Mesenchymal stem cells. *Cell Transplant* 20(1): 5-14.

Discher, D. E., P. Janmey, et al. (2005). Tissue cells feel and respond to the stiffness of their substrate. *Science* 310(5751): 1139-43.

Djouad, F., B. Delorme, et al. (2007). Microenvironmental changes during differentiation of mesenchymal stem cells towards chondrocytes. *Arthritis Res Ther* 9(2): R33.

Dong, J., H. Kojima, et al. (2001). In vivo evaluation of a novel porous hydroxyapatite to sustain osteogenesis of transplanted bone marrow-derived osteoblastic cells. *J Biomed Mater Res* 57(2): 208-16.

Dong, J., T. Uemura, et al. (2002). Promotion of bone formation using highly pure porous beta-TCP combined with bone marrow-derived osteoprogenitor cells. *Biomaterials* 23(23): 4493-502.

Dupont, S., L. Morsut, et al. (2011). Role of YAP/TAZ in mechanotransduction. *Nature* 474(7350): 179-83.

Eisenbarth, E., D. Velten, et al. (2007). Biomimetic implant coatings. *Biomol Eng* 24(1): 27-32.

El-Amin, S. F., E. Botchwey, et al. (2006). Human osteoblast cells: isolation, characterization, and growth on polymers for musculoskeletal tissue engineering. *J Biomed Mater Res A* 76(3): 439-49.

El-Ghannam, A. (2005). Bone reconstruction: from bioceramics to tissue engineering. *Expert Rev Med Devices* 2(1): 87-101.

Endres, M., D. W. Hutmacher, et al. (2003). Osteogenic induction of human bone marrow-derived mesenchymal progenitor cells in novel synthetic polymer-hydrogel matrices. *Tissue Eng* 9(4): 689-702.

Engler, A. J., M. A. Griffin, et al. (2004). Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. *J Cell Biol* 166(6): 877-87.

Engler, A. J., S. Sen, et al. (2006). Matrix elasticity directs stem cell lineage specification. *Cell* 126(4): 677-89.

Erbe, E. M., J. G. Marx, et al. (2001). Potential of an ultraporous beta-tricalcium phosphate synthetic cancellous bone void filler and bone marrow aspirate composite graft. *Eur Spine J* 10 Suppl 2: S141-6.

Erisken, C., D. M. Kalyon, et al. (2008). Functionally graded electrospun polycaprolactone and beta-tricalcium phosphate nanocomposites for tissue engineering applications. *Biomaterials* 29(30): 4065-73.

Fabbri, M., G. C. Celotti, et al. (1995). Hydroxyapatite-based porous aggregates: physico-chemical nature, structure, texture and architecture. *Biomaterials* 16(3): 225-8.

Fan, H., T. Ikoma, et al. (2007). Surface structural biomimetics and the osteoinduction of calcium phosphate biomaterials. *J Nanosci Nanotechnol* 7(3): 808-13.

Flautre, B., K. Anselme, et al. (1999). Histological aspects in bone regeneration of an association with porous hydroxyapatite and bone marrow cells. *J Mater Sci Mater Med* 10(12): 811-4.

Franceschi, R. T. and G. Xiao (2003). Regulation of the osteoblast-specific transcription factor, Runx2: responsiveness to multiple signal transduction pathways. *J Cell Biochem* 88(3): 446-54.

Fu, R. H., Y. C. Wang, et al. (2011). Differentiation of stem cells: strategies for modifying surface biomaterials. *Cell Transplant* 20(1): 37-47.

Gao, L., R. McBeath, et al. (2010). Stem cell shape regulates a chondrogenic versus myogenic fate through Rac1 and N-cadherin. *Stem Cells* 28(3): 564-72.

Gauthier, O., J. M. Bouler, et al. (1998). Macroporous biphasic calcium phosphate ceramics: influence of macropore diameter and macroporosity percentage on bone ingrowth. *Biomaterials* 19(1-3): 133-9.

Gauthier, O., R. Muller, et al. (2005). In vivo bone regeneration with injectable calcium phosphate biomaterial: a three-dimensional micro-computed tomographic, biomechanical and SEM study. *Biomaterials* 26(27): 5444-53.

Gigante, A., S. Manzotti, et al. (2008). Adult mesenchymal stem cells for bone and cartilage engineering: effect of scaffold materials. *Eur J Histochem* 52(3): 169-74.

Goessler, U. R., K. Bieback, et al. (2006). In vitro analysis of integrin expression during chondrogenic differentiation of mesenchymal stem cells and chondrocytes upon dedifferentiation in cell culture. *Int J Mol Med* 17(2): 301-7.

Gorsline, R. T., P. Tangkawattana, et al. (2010). Accelerated chondrogenesis in nanofiber polimeric scaffolds embedded with BMP-2 genetically engineeered chondrocytes. *Journal Biomedical Science and Engineering* 3: 908-916.

Goshima, J., V. M. Goldberg, et al. (1991). The osteogenic potential of culture-expanded rat marrow mesenchymal cells assayed in vivo in calcium phosphate ceramic blocks. *Clin Orthop Relat Res*(262): 298-311.

Grayson, W. L., P. H. Chao, et al. (2008). Engineering custom-designed osteochondral tissue grafts. *Trends Biotechnol* 26(4): 181-9.

Guarino, V., F. Causa, et al. (2008). The role of hydroxyapatite as solid signal on performance of PCL porous scaffolds for bone tissue regeneration. *J Biomed Mater Res B Appl Biomater* 86B(2): 548-57.

Guo, H., Y. Ma, et al. (2009). Pivotal Advance: PKCzeta is required for migration of macrophages. J *Leukoc Biol* 85(6): 911-8.

Hamidouche, Z., E. Hay, et al. (2008). FHL2 mediates dexamethasone-induced mesenchymal cell differentiation into osteoblasts by activating Wnt/beta-catenin signaling-dependent Runx2 expression. *FASEB J* 22(11): 3813-22.

Hamilton, D. W., M. O. Riehle, et al. (2005). Articular chondrocyte passage number: influence on adhesion, migration, cytoskeletal organisation and phenotype in response to nano- and micro-metric topography. *Cell Biol Int* 29(6): 408-21.

Hamilton, D. W., M. O. Riehle, et al. (2005). The response of primary articular chondrocytes to micrometric surface topography and sulphated hyaluronic acid-based matrices. *Cell Biol Int* 29(8): 605-15.

Harley, B. A., A. K. Lynn, et al. (2010). Design of a multiphase osteochondral scaffold III: Fabrication of layered scaffolds with continuous interfaces. *J Biomed Mater Res A* 92(3): 1078-93.

Harris, L. D., B. S. Kim, et al. (1998). Open pore biodegradable matrices formed with gas foaming. *J Biomed Mater Res* 42(3): 396-402.

He, W., P. Cowin, et al. (2003). Untangling desmosomal knots with electron tomography. *Science* 302(5642): 109-13.

Hench, L. L. and J. M. Polak (2002). Third-generation biomedical materials. *Science* 295(5557): 1014-7.

Hollister, S. J., R. D. Maddox, et al. (2002). Optimal design and fabrication of scaffolds to mimic tissue properties and satisfy biological constraints. *Biomaterials* 23(20): 4095-103.

Hoshiba, T., N. Kawazoe, et al. (2009). Development of stepwise osteogenesis-mimicking matrices for the regulation of mesenchymal stem cell functions. *J Biol Chem* 284(45): 31164-73.

Huang, H., R. D. Kamm, et al. (2004). Cell mechanics and mechanotransduction: pathways, probes, and physiology. *Am J Physiol Cell Physiol* 287(1): C1-11.

Huang, J., Y. W. Lin, et al. (2007). Development of nano-sized hydroxyapatite reinforced composites for tissue engineering scaffolds. *J Mater Sci Mater Med* 18(11): 2151-7.

Hunt, J. A. (2008). Regenerative medicine: Materials in a cellular world. *Nat Mater* 7(8): 617-8.

Hutmacher, D. W. (2000). Scaffolds in tissue engineering bone and cartilage. *Biomaterials* 21(24): 2529-43.

Hutmacher, D. W., J. T. Schantz, et al. (2007). State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. *J Tissue Eng Regen Med* 1(4): 245-60.

James, R., U. S. Toti, et al. (2011). Electrospun nanofibrous scaffolds for engineering soft connective tissues. *Methods Mol Biol* 726: 243-58.

Jayaraman, K., M. Kotaki, et al. (2004). Recent advances in polymer nanofibers. *J Nanosci Nanotechnol* 4(1-2): 52-65.

Jiang, T., W. I. Abdel-Fattah, et al. (2006). In vitro evaluation of chitosan/poly(lactic acid-glycolic acid) sintered microsphere scaffolds for bone tissue engineering. *Biomaterials* 27(28): 4894-903.

Karageorgiou, V. and D. Kaplan (2005). Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials* 26(27): 5474-91.

Kasten, P., R. Luginbuhl, et al. (2003). Comparison of human bone marrow stromal cells seeded on calcium-deficient hydroxyapatite, beta-tricalcium phosphate and demineralized bone matrix. *Biomaterials* 24(15): 2593-603.

Keselowsky, B. G., D. M. Collard, et al. (2005). Integrin binding specificity regulates biomaterial surface chemistry effects on cell differentiation. *Proc Natl Acad Sci USA* 102(17): 5953-7.

Kim, K. Y., M. Kovacs, et al. (2005). Disease-associated mutations and alternative splicing alter the enzymatic and motile activity of nonmuscle myosins II-B and II-C. *J Biol Chem* 280(24): 22769-75.

Kim, S. S., M. S. Park, et al. (2006). Accelerated bonelike apatite growth on porous polymer/ceramic composite scaffolds in vitro. *Tissue Eng* 12(10): 2997-3006.

Kimelman, N., G. Pelled, et al. (2006). Applications of gene therapy and adult stem cells in bone bioengineering. *Regen Med* 1(4): 549-61.

Kon, E., M. Delcogliano, et al. (2010). Orderly osteochondral regeneration in a sheep model using a novel nano-composite multilayered biomaterial. *J Orthop Res* 28(1): 116-24.

Kon, E., A. Muraglia, et al. (2000). Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones. *J Biomed Mater Res* 49(3): 328-37.

Kondo, N., A. Ogose, et al. (2006). Osteoinduction with highly purified beta-tricalcium phosphate in dog dorsal muscles and the proliferation of osteoclasts before heterotopic bone formation. *Biomaterials* 27(25): 4419-27.

Kotobuki, N., K. Ioku, et al. (2005). Observation of osteogenic differentiation cascade of living mesenchymal stem cells on transparent hydroxyapatite ceramics. *Biomaterials* 26(7): 779-85.

Kretlow, J. D. and A. G. Mikos (2007). Review: mineralization of synthetic polymer scaffolds for bone tissue engineering. *Tissue Eng* 13(5): 927-38.

Kundu, A. K. and A. J. Putnam (2006). Vitronectin and collagen I differentially regulate osteogenesis in mesenchymal stem cells. *Biochem Biophys Res Commun* 347(1): 347-57.

Lee, J. W. and R. Juliano (2004). Mitogenic signal transduction by integrin- and growth factor receptor-mediated pathways. *Mol Cells* 17(2): 188-202.

Lee, M. Y., J. P. Huang, et al. (2009). Angiogenesis in differentiated placental multipotent mesenchymal stromal cells is dependent on integrin alpha5beta1. *PLoS One* 4(10): e6913.

Lenza, R. F., W. L. Vasconcelos, et al. (2002). Surface-modified 3D scaffolds for tissue engineering. *J Mater Sci Mater Med* 13(9): 837-42.

Leung, V. Y., D. Chan, et al. (2006). Regeneration of intervertebral disc by mesenchymal stem cells: potentials, limitations, and future direction. *Eur Spine J* 15 Suppl 3: S406-13.

Limouze, J., A. F. Straight, et al. (2004). Specificity of blebbistatin, an inhibitor of myosin II. *J Muscle Res Cell Motil* 25(4-5): 337-41.

Liu, H. and T. J. Webster (2006). Nanomedicine for implants: A review of studies and necessary experimental tools. *Biomaterials* 28(2): 354-369.

Livingston, T., P. Ducheyne, et al. (2002). In vivo evaluation of a bioactive scaffold for bone tissue engineering. *J Biomed Mater Res* 62(1): 1-13.

Livingston, T. L., S. Gordon, et al. (2003). Mesenchymal stem cells combined with biphasic calcium phosphate ceramics promote bone regeneration. *J Mater Sci Mater Med* 14(3): 211-8.

Lu, J. X., B. Flautre, et al. (1999). Role of interconnections in porous bioceramics on bone recolonization in vitro and in vivo. *J Mater Sci Mater Med* 10(2): 111-20.

Lynn, A. K., S. M. Best, et al. (2010). Design of a multiphase osteochondral scaffold. I. Control of chemical composition. *J Biomed Mater Res A* 92(3): 1057-65.

Mackay, A. M., S. C. Beck, et al. (1998). Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng* 4(4): 415-28.

Marcacci, M., E. Kon, et al. (1999). Reconstruction of extensive long-bone defects in sheep using porous hydroxyapatite sponges. *Calcif Tissue Int* 64(1): 83-90.

Martin, I., S. Miot, et al. (2007). Osteochondral tissue engineering. *J Biomech* 40(4): 750-65.

Mastrogiacomo, M., S. Scaglione, et al. (2006). Role of scaffold internal structure on in vivo bone formation in macroporous calcium phosphate bioceramics. *Biomaterials* 27(17): 3230-7.

Matsushima, A., N. Kotobuki, et al. (2009). In vivo osteogenic capability of human mesenchymal cells cultured on hydroxyapatite and on beta-tricalcium phosphate. *Artif Organs* 33(6): 474-81.

Mikos, A. G., G. Sarakinos, et al. (1993). Laminated three-dimensional biodegradable foams for use in tissue engineering. *Biomaterials* 14(5): 323-30.

Miranti, C. K. and J. S. Brugge (2002). Sensing the environment: a historical perspective on integrin signal transduction. *Nat Cell Biol* 4(4): E83-90.

Mooney, D. J., C. L. Mazzoni, et al. (1996). Stabilized polyglycolic acid fibre-based tubes for tissue engineering. *Biomaterials* 17(2): 115-24.

Murphy, W. L., S. Hsiong, et al. (2005). Effects of a bone-like mineral film on phenotype of adult human mesenchymal stem cells in vitro. *Biomaterials* 26(3): 303-10.

Mygind, T., M. Stiehler, et al. (2007). Mesenchymal stem cell ingrowth and differentiation on coralline hydroxyapatite scaffolds. *Biomaterials* 28(6): 1036-47.

Nakagawa, S., P. Pawelek, et al. (1989). Extracellular matrix organization modulates fibroblast growth and growth factor responsiveness. *Exp Cell Res* 182(2): 572-82.

Nakamura, T. (2007). Biomaterial osteoinduction. *J Orthop Sci* 12(2): 111-2.

Nam, Y. S. and T. G. Park (1999). Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation. *J Biomed Mater Res* 47(1): 8-17.

Ng, A. M., K. K. Tan, et al. (2008). Differential osteogenic activity of osteoprogenitor cells on HA and TCP/HA scaffold of tissue engineered bone. *J Biomed Mater Res A* 85(2): 301-12.

Nicolas, A., B. Geiger, et al. (2004). Cell mechanosensitivity controls the anisotropy of focal adhesions. *Proc Natl Acad Sci U S A* 101(34): 12520-5.

Nicula, R., F. Luthen, et al. (2007). Spark plasma sintering synthesis of porous nanocrystalline titanium alloys for biomedical applications. *Biomol Eng* 24(5): 564-7.

Niemeyer, P., U. Krause, et al. (2003). Mesenchymal stem cells for tissue engineering of bone: 3D-cultivation and osteogenic differentiation on mineralized collagen. *Z Orthop Ihre Grenzgeb* 141(6): 712-7.

Oberlender, S. A. and R. S. Tuan (1994). Spatiotemporal profile of N-cadherin expression in the developing limb mesenchyme. *Cell Adhes Commun* 2(6): 521-37.

Ohgushi, H., Y. Dohi, et al. (1993). Osteogenic differentiation of marrow stromal stem cells in porous hydroxyapatite ceramics. *J Biomed Mater Res* 27(11): 1401-7.

Paszek, M. J., N. Zahir, et al. (2005). Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8(3): 241-54.

Peters, A., H. Schell, et al. (2010). Standard bone healing stages occur during delayed bone healing, albeit with a different temporal onset and spatial distribution of callus tissues. *Histol Histopathol* 25(9): 1149-62.

Place, E. S., N. D. Evans, et al. (2009). Complexity in biomaterials for tissue engineering. *Nat Mater* 8(6): 457-70.

Qian, L. and W. M. Saltzman (2004). Improving the expansion and neuronal differentiation of mesenchymal stem cells through culture surface modification. *Biomaterials* 25(7-8): 1331-7.

Quarto, R., M. Mastrogiacomo, et al. (2001). Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* 344(5): 385-6.

Ren, J., P. Zhao, et al. (2008). Poly (D: ,L: -lactide)/nano-hydroxyapatite composite scaffolds for bone tissue engineering and biocompatibility evaluation. *J Mater Sci Mater Med* 19(3):1075-82.

Ren, T., J. Ren, et al. (2005). The bone formation in vitro and mandibular defect repair using PLGA porous scaffolds. *J Biomed Mater Res A* 74(4): 562-9.

Saldana, L., S. Sanchez-Salcedo, et al. (2009). Calcium phosphate-based particles influence osteogenic maturation of human mesenchymal stem cells. *Acta Biomater* 5(4): 1294-305.

Santiago, J. A., R. Pogemiller, et al. (2009). Heterogeneous differentiation of human mesenchymal stem cells in response to extended culture in extracellular matrices. *Tissue Eng Part A* 15(12): 3911-22.

Sato, M. and T. J. Webster (2004). Nanobiotechnology: implications for the future of nanotechnology in orthopedic applications. *Expert Rev Med Devices* 1(1): 105-14.

Scaglione, S., P. Giannoni, et al. (2011). Order versus Disorder: in vivo bone formation within osteoconductive scaffolds. *Science* submitted.

Schugens, C., V. Maquet, et al. (1996). Polylactide macroporous biodegradable implants for cell transplantation. II. Preparation of polylactide foams by liquid-liquid phase separation. *J Biomed Mater Res* 30(4): 449-61.

Sherwood, J. K., S. L. Riley, et al. (2002). A three-dimensional osteochondral composite scaffold for articular cartilage repair. *Biomaterials* 23(24): 4739-51.

Silva, G. A., C. Czeisler, et al. (2004). Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science* 303(5662): 1352-5.

Smith, L. A., X. Liu, et al. (2008). Tissue Engineering with Nano-Fibrous Scaffolds. *Soft Matter* 4(11): 2144-2149.

Smith, L. A. and P. X. Ma (2004). Nano-fibrous scaffolds for tissue engineering. *Colloids Surf B Biointerfaces* 39(3): 125-31.

Tamada, M., M. P. Sheetz, et al. (2004). Activation of a signaling cascade by cytoskeleton stretch. *Dev Cell* 7(5): 709-18.

Tampieri, A., M. Sandri, et al. (2008). Design of graded biomimetic osteochondral composite scaffolds. *Biomaterials* 29(26): 3539-46.

Thibault, R. A., L. Scott Baggett, et al. (2010). Osteogenic differentiation of mesenchymal stem cells on pregenerated extracellular matrix scaffolds in the absence of osteogenic cell culture supplements. *Tissue Eng Part A* 16(2): 431-40.

Tonti, G. A. and F. Mannello (2008). From bone marrow to therapeutic applications: different behaviour and genetic/epigenetic stability during mesenchymal stem cell expansion in autologous and foetal bovine sera *Int J Dev Biol* 52(8): 1023-32.

Turner, C. E. (2000). Paxillin and focal adhesion signalling. *Nat Cell Biol* 2(12): E231-6.

Uygun, B. E., S. E. Stojsih, et al. (2009). Effects of immobilized glycosaminoglycans on the proliferation and differentiation of mesenchymal stem cells. *Tissue Eng Part A* 15(11): 3499-512.

Vacanti, C. A., L. J. Bonassar, et al. (2001). Replacement of an avulsed phalanx with tissue-engineered bone. *N Engl J Med* 344(20): 1511-4.

Vasita, R. and D. S. Katti (2006). Nanofibers and their applications in tissue engineering. *Int J Nanomedicine* 1(1): 15-30.

Vega, F. M., G. Fruhwirth, et al. (2011). RhoA and RhoC have distinct roles in migration and invasion by acting through different targets. *J Cell Biol* 193(4): 655-65.

Vogel, V. and M. Sheetz (2006). Local force and geometry sensing regulate cell functions. *Nat Rev Mol Cell Biol* 7(4): 265-75.

Voog, J. and D. L. Jones (2010). Stem cells and the niche: a dynamic duo. *Cell Stem Cell* 6(2): 103-15.

Wang, F., V. M. Weaver, et al. (1998). Reciprocal interactions between beta1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: a different perspective in epithelial biology. *Proc Natl Acad Sci U S A* 95(25): 14821-6.

Warren, S. M., R. K. Nacamuli, et al. (2004). Tissue-engineered bone using mesenchymal stem cells and a biodegradable scaffold. *J Craniofac Surg* 15(1): 34-7.

Wei, G. and P. X. Ma (2008). Nanostructured Biomaterials for Regeneration. *Adv Funct Mater* 18(22): 3566-3582.

Williams, J. M., A. Adewunmi, et al. (2005). Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials* 26(23): 4817-27.

Wise, J. K., A. L. Yarin, et al. (2009). Chondrogenic differentiation of human mesenchymal stem cells on oriented nanofibrous scaffolds: engineering the superficial zone of articular cartilage. *Tissue Eng Part A* 15(4): 913-21.

Woo, K. M., V. J. Chen, et al. (2003). Nano-fibrous scaffolding architecture selectively enhances protein adsorption contributing to cell attachment. *J Biomed Mater Res A* 67(2): 531-7.

Woo, K. M., J. H. Jun, et al. (2007). Nano-fibrous scaffolding promotes osteoblast differentiation and biomineralization. *Biomaterials* 28(2): 335-43.

Woodbury, D., E. J. Schwarz, et al. (2000). Adult rat and human bone marrow stromal cells differentiate into neurons. *J Neurosci Res* 61(4): 364-70.

Wu, Y. C., S. Y. Shaw, et al. (2006). Bone tissue engineering evaluation based on rat calvaria stromal cells cultured on modified PLGA scaffolds. *Biomaterials* 27(6): 896-904.

Xiao, G., D. Jiang, et al. (2000). MAPK pathways activate and phosphorylate the osteoblast-specific transcription factor, Cbfa1. *J Biol Chem* 275(6): 4453-9.

Yang, F., R. Murugan, et al. (2005). Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. *Biomaterials* 26(15): 2603-10.

Yeung, T., P. C. Georges, et al. (2005). Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion. *Cell Motil Cytoskeleton* 60(1): 24-34.

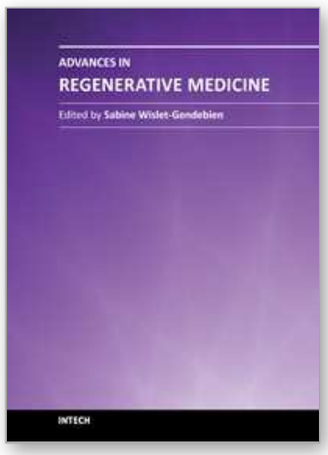
Yim, E. K., S. W. Pang, et al. (2007). Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. *Exp Cell Res* 313(9): 1820-9.

Yuan, H., K. Kurashina, et al. (1999). A preliminary study on osteoinduction of two kinds of calcium phosphate ceramics. *Biomaterials* 20(19): 1799-806.

Yuan, H., Z. Yang, et al. (1998). Osteoinduction by calcium phosphate biomaterials. *J Mater Sci Mater Med* 9(12): 723-6.

Zhang, L. and T. J. Webster (2009). Nanotechnology and nanomaterials: Promises for improved tissue regeneration. *Nano Today* 4: 66-80.

Zhao, F., W. L. Grayson, et al. (2009). Perfusion affects the tissue developmental patterns of human mesenchymal stem cells in 3D scaffolds. *J Cell Physiol* 219(2): 421-9.



Advances in Regenerative Medicine

Edited by Dr Sabine Wislet-Gendebien

ISBN 978-953-307-732-1
Hard cover, 404 pages
Publisher InTech
Published online 21, November, 2011
Published in print edition November, 2011

Even if the origins of regenerative medicine can be found in Greek mythology, as attested by the story of Prometheus, the Greek god whose immortal liver was feasted on day after day by Zeus' eagle; many challenges persist in order to successfully regenerate lost cells, tissues or organs and rebuild all connections and functions. In this book, we will cover a few aspects of regenerative medicine highlighting major advances and remaining challenges in cellular therapy and tissue/organ engineering.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Silvia Scaglione, Paolo Giannoni and Rodolfo Quarto (2011). Cell-Biomaterial Interactions Reproducing a Niche, Advances in Regenerative Medicine, Dr Sabine Wislet-Gendebien (Ed.), ISBN: 978-953-307-732-1, InTech, Available from: http://www.intechopen.com/books/advances-in-regenerative-medicine/cell-biomaterial-interactions-reproducing-a-niche

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen