Polymeric scaffolds as stem cell carriers in bone repair

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Abstract

Although bone has a high potential to regenerate itself after damage and injury, the efficacious repair of large bone defects resulting from resection, trauma or non-union fractures still requires the implantation of bone grafts. Materials science, in conjunction with biotechnology, can satisfy these needs by developing artificial bones, synthetic substitutes and organ implants. In particular, recent advances in polymer science have provided several innovations, underlying the increasing importance of macromolecules in this field. To address the increasing need for improved bone substitutes, tissue engineering seeks to create synthetic, three-dimensional scaffolds made from polymeric materials, incorporating stem cells and growth factors, to induce new bone tissue formation. Polymeric materials have shown a great affinity for cell transplantation and differentiation and, moreover, their structure can be tuned in order to maintain an adequate mechanical resistance and contemporarily be fully bioresorbable. This review emphasizes recent progress in polymer science that allows reliable polymeric scaffolds to be synthesized for stem cell growth in bone regeneration. Copyright © 2013 John Wiley & Sons, Ltd.

Received 6 June 2012; Revised 29 July 2013; Accepted 30 August 2013

Keywords biomaterials; bone; polymer; regenerative medicine; scaffolds; stem cells

1. Introduction

Nowadays, the needs to overcome diseases and provide worldwide medical care are high priorities (Atala et al., 2008; Langer, 2009). In this field, regenerative medicine seeks to devise new therapies for patients with severe injuries or chronic diseases in which the body’s own responses do not provide recovery of complete anatomy and functionality (Hutmacher et al., 2007; Sakurada et al., 2008). In order to address this task, research is mainly directed toward two strategies, not mutually exclusive and often indicated to be potentially joint solutions in regenerative medicine, (a) stem cell technologies (El Tamer and Reis, 2009; Fisher et al., 2010; Marolt et al., 2010) and (b) targeted delivery of growth factors and cytokines to induce autologous cell proliferation and tissue restoration (Hu and Ma, 2011; Mourino and Boccaccini, 2010; Tabata, 2005). Direct injection of in vitro cultured cells is one attractive alternative (Mourino and Boccaccini, 2010; Seong et al., 2010), but several concerns arise as trials are performed: injected cells very often leave the zone of injection, as they are not confined by any support, and easily extravasate into the circulatory torrent, migrating all over the body towards a rather uncertain fate (Santos et al., 2011). Hence, materials science, together with biotechnology and engineering, is considered to be a promising strategy for developing artificial bones and organ implants (Alarcon et al., 2005; Cordonnier et al., 2011; Kretlow and Mikos, 2008; Stevens, 2008). In this framework, three-dimensional (3D) biomaterial-made scaffolds were first developed as a temporary substrate to grow cells in an organized fashion, before performing the transplantation of such combined structures (Chen et al., 2010; Langer, 2009; Smith et al., 2009). Among the most investigated applications, regeneration of the musculoskeletal system is a primary target, due to both the increasing need for bone surgery and the relatively simple organic–functional organization of bone tissue. Although bone has an extremely high potential to regenerate itself after damage (Costa-Pinto et al., 2011; Jones and
Yang, 2011), the efficacious repair of large bone defects resulting from resections, traumas, large defects or non-union fractures still requires the implantation of bone grafts (Fassina et al., 2010; Kretlow and Mikos, 2007). Every year, hundreds of thousands of surgical cases of bone-grafting procedures are performed. Moreover, the demand for bone grafts continues to rise and it is expected to be even greater over the next decade, as the population ages (Zhang et al., 2012). The efforts to address these problems and limitations of the early developed devices have led to the development of new biomaterials and alternative therapies, among which a tissue-engineering approach holds great promise (Puppi et al., 2010; Woodruff and Hutmacher, 2010). Metals and ceramics are traditional materials employed in bone engineering, due to their mechanical properties and high biocompatibility (Cordonnier et al., 2011; Marelli et al., 2011). Moreover, many ceramics can be obtained with a microstructure that is very close to that of the bone porous mineral fraction. Recent technologies allow similarly structured metal devices to be obtained too, but, nevertheless, metal implants cannot perform as efficiently as a healthy bone, and metallic structures cannot remodel with time. Indeed, they are lacking in osteogenic regeneration capabilities and are not biodegradable, thus requiring subsequent surgical procedures in a wide set of clinical circumstances (Luyten et al., 2011). Recent advances in materials science have provided an abundance of innovations, underlining the leading role of polymers in this field (Puppi et al., 2010). In order to design substitutes with improved bone biocompatibility, tissue engineers seek to create synthetic, 3D bone scaffolds made from polymeric materials, incorporating cells or growth factors to induce the growth of normal bone tissue (Rezwan et al., 2006). In summary, four key elements are required from a polymeric graft to sustain the achievement of optimal bone formation (Yu et al., 2009): (a) mechanical stability; (b) osteoconductivity; (c) the ability to carry osteogenic cells; and (d) considerable osteoinductive potential for customization and adaptability via modification of the design parameters, including scaffold architecture, composition and biodegradability. Here, polymeric materials have shown a great affinity for cell transplantation and differentiation (Dawson and Oreffo, 2008; Pirraco et al., 2010) and their structure can be tuned in order to maintain an adequate mechanical resistance and be fully bioresorbable at the same time. In summary, the regenerative medicine approach is presented in Figure 1, from stem cells to differentiated bone lineages, using polymeric growth scaffolds, with final in vivo implantation. Accordingly, the following review describes the wide range of polymers use to manufacture stem cell scaffolds in bone regenerative medicine strategies. Special attention is focused on the stem cells chosen, specifically embryonic (Hwang et al., 2007; Jukes et al., 2010) or adult (Gothard et al., 2011; Kanczler et al., 2008; Oreffo et al., 2005; Salinas and Anseth, 2009; Undale et al., 2009).

2. Synthetic polymers

2.1. Polysters

Polysters (PEs) are a class of polymers that contain an aliphatic ester bond in their backbone. Although a number of polysters are commercially available and all of them are theoretically degradable, the hydrolytically stable nature of the ester bond underlines that only polysters with reasonably short aliphatic chains can be utilized as degradable polymers for biomedical applications (Valappil et al., 2006). In particular, they are mildly hydrophobic; ester bond stability causes them to undergo bulk erosion. They can be synthesized by ring-opening or condensation polymerization. In this framework, poly(lactic acid) (PLA), poly(glycolic acid) PGA, poly(lactide-co-glycolide) (PLGA) and poly(ε-caprolactone) (PLCL)
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(PCL) represent a versatile class of synthetic biodegradable materials suitable for bone tissue engineering, particularly in combination with bone morphogenetic proteins (BMPs) (Bessa et al., 2008a, 2008b). The advantages of these polymers are high purity, convenient processing and good mechanical properties, in addition to their biodegradability. Indeed, their degradation products can be resorbed through normal metabolic pathways (Hu and Ma, 2011; Yu et al., 2010a). These materials have been used for over 45 years as resorbable sutures and fixation devices, and they are already approved for human use in several forms and formulations worldwide. This class has been under intensive research in the development of osteosynthesis devices since the 1960s, with the successful fabrication of several 3D porous scaffolds, craniofacial devices (Habal, 1997) and orthopaedic implants, such as pins and screws.

2.1.1. PLA

PLA is commonly synthesized by ring-opening polymerization of dilactide, the dimerization product of lactic acid. As two enantiomeric isomers of lactide exist, PLA is available as a fully crystalline or a fully amorphous form, depending on the relative levels of the optical isomers present in the molecule. Tuning their relative amounts to mimic tissues mechanical properties is also possible. Poly(l-lactic acid) (PLLA) is a semi-crystalline polymer with a crystallinity of around 37% and a melting temperature around 175 °C, suitable for applications where high mechanical strength and toughness are required (e.g. for long lasting sutures and though orthopaedic devices). On the other hand, the polymerization of racemic dilactide leads to poly(δ,δ-lactic acid) (PDLLA), which is an amorphous polymer used in craniofacial fixation applications and drug delivery systems (Giardino et al., 2006; Maraldi et al., 2011). Further combinations have been employed to match stiffness and degradation kinetics, such as poly(l-lactide-co-δ,δ-lactide) (PLDL). Several studies with stem cells were conducted on PLA scaffolds in the past 10 years, revealing its suitability as a cell growth matrix (Griffon et al., 2011; Hu et al., 2009; Jager et al., 2005; Lee et al., 2012; Lim et al., 2010; McCullen et al., 2007; Rim et al., 2012; Sun et al., 2010). Even though the biocompatibility of this polymer was evaluated in both in vitro and in vivo models back in the 1970s, only in 2001 did Yang et al. (2001b) examine the mineralization of a PLA-based matrix that took place after 4 weeks, confirming the expression of the mature osteogenic phenotype (Figure 2). Successful adhesion and growth of human osteoprogenitors on protein and peptide-coupled PLLA polymer films have provided useful positional and environmental information on bone repair and healing mechanisms. In 2004 the same research group (Yang et al., 2004) examined the ability of the scaffolds to promote human osteoprogenitor differentiation and bone formation in vitro and in vivo.

In this context, the use of BMP-2 encapsulated into polymer scaffolds showed morphological evidence of new bone matrix and cartilage formation after subcutaneous implantation. Enriched PLA scaffolds seeded with mesenchymal stem cells (MSCs) showed bone regeneration also in femur segmental (Dong et al., 2010; Kanczler et al., 2008) and critical-sized skull defects (Aydin et al., 2011; Di Bella et al., 2008a, 2008b), whereas the bare scaffold did not. Furthermore, scaffold performances could be enhanced modifying their structure with several active compounds, e.g. with poly(l-lysine) physically entrapped (Alvarez-Barreto et al., 2007) uniformly throughout the scaffold surface, in a controllable fashion, while arginine–glycine–aspartic acid–cysteine (RGDC) adhesion peptide was linked to the poly(l-lysine) by disulphide bonds. The presence of RGDC on the surface of PLAG scaffolds increased the cell surface area and the number of adherent MSCs. The seeding of rat MSCs on arginine–glycine–aspartic acid (RGD)-modified PLLA foams (Alvarez-Barreto and Sikavitas, 2007), using oscillatory flow perfusion, evidenced that cell adhesion is dependent on the applied flow rate and that cell attachment is strengthened at higher levels of RGD modification. The levels of RGD modification of PLLA scaffolds on MSCs osteogenesis were also evaluated (Alvarez-Barreto et al., 2011) under conditions of flow perfusion, considering that there is an RGD surface concentration optimal for differentiation and that it is flow rate-dependent. Functionalization of surfaces by grafting in the vapour phase with poly(l-lactide) films (Edlund et al., 2008) with heparin covalently bonded to the surface and an osteoinductive growth factor underlined the biocompatibility of the functionalized surfaces by a much improved attachment and proliferation of MSCs. Also, calcium phosphates could be added to PLA scaffolds: among them, hydroxyapatite [HA; Ca_{10}(PO_{4})_{3} (OH)] has gained attention because of its usage in bone grafting, resulting from its excellent osteoconductive and bioactive properties (Iafisco et al., 2011, 2012; Sun et al., 2011). HA has its most thermodynamically stable phase in physiological conditions and has the ability to be directly chemically bonded to the bone. Despite these advantages, HA exhibits quite limited mechanical properties that become scarce when highly porous constructs are considered. Owing to the poor mechanical properties of HA, the recent trend in bioceramic research has been focused on improving its mechanical and biological properties. In order to perform better osteointegration, PLLA/collagen I (CT1)- and HA-modified scaffolds (Liu et al., 2010) were assessed in terms of cell attachment, proliferation and differentiation: nano-hydroxyapatite (nHA) / collagen (C) / PLLA (P) reinforced with chitin fibers (CF) (nHACP/CF) seeded with cultured goat bone marrow MSCs could repair a segmental bone defect by 8 weeks after surgery. Moreover, transfected rabbit ADCs mixed with nano-hydroxyapatite (nHA) recombinant-human like collagen/PLA (Hao et al., 2010) cultured in osteoblast cell medium were implanted into 15 mm length critical-sized segmental radial defects in rabbits: the medullary cavity was recanalized and the bone rebuilt. Different PLLA/HA scaffold shapes were seeded with MSCs (Eslaminejad et al., 2008a, 2008b): MTT for viability and alizarin red for osteogenic differentiation after 21 days were used to investigate these, and needle shapes resulted in better performance than spheres or cylinders. Also the use of
PLLA/tricalcium phosphate (TCP)/tooth apatite scaffolds (Choi et al., 2010) were compared with each other, both in vitro and in vivo, using human dental pulp stem cells (hDPSCs): an inflammatory reaction in the polymer-bioceramic scaffolds, transplanted subcutaneously into the dorsal area of immunocompromised mice, was studied. Also the surface modifications of PLA scaffold with calcium phosphate (CP) (Kim et al., 2011) exhibited osteogenic differentiation of rat bone marrow MSCs (rBMMSCs) significantly higher than in standard conditions and with better osteodifferentiation. Not only scaffold modifications but also co-polymers of PLA have been deeply investigated (Abou Neel et al., 2010; Lv et al., 2009; Shin et al., 2008; Vertenten et al., 2009; Xue et al., 2010). In detail, two synthetic long-term degradable polymers, PLLA-co-trimethylene carbonate (Resomer® LT706) and polycaprolactone (PCL) (Neuss et al., 2011), were used. Both polymers enhance osteogenic differentiation compared to tissue culture polystyrene wells, and MSCs cultured on Resomer LT706 showed higher numbers of genes involved in skeletal development and bone formation. The generation of bio-functional polymer blends, using a high-throughput material formulation and micro-

Figure 2. Fluorescence photomicrographs of human bone marrow cells after 5 h (A, B) and 6 weeks (C, D) of culture on PLGA scaffolds coated with fibronectin (FN). Viable cells (green) were detected using Cell Tracker green and necrotic cells (red) using ethidium homodimer-1. Negligible cell adhesion was observed on unmodified PLGA (E): original magnifications = (A) ×50; (B) ×640; (C) ×500; (D) ×200; (E) ×200. (F–M) Expression of bone markers by human bone marrow fibroblasts in situ (F–I) and on paraffin sections (J–M), as detected by immunocytochemistry, on PLGA scaffolds coated with FN following 6 weeks of culture. (F,I) Alkaline phosphatase activity detected by histochemistry: original magnifications = (F) ×100; (J) ×200. (G,K) Type I collagen: original magnification = (G) ×100; (K) ×200. (H,L) Cbfa-1 expression (PEBP2αA): original magnification = (H) ×100; (L) ×450. (I) Osteocalcin expression: original magnification = ×100. (M) Cell ingrowth and mineralization, as detected by von Kossa and alkaline phosphatase histochemistry: original magnification = ×200. Mineralization can also be observed in (J) and (K). Reprinted with permission from Yang et al. (2001b). Copyright © 2001 Elsevier
array approach, was also recently studied (Khan et al., 2010): a blend of PLLA and PCL with appropriate physical properties was found to display remarkable bone-like architecture, providing a robust template for skeletal stem cell attachment and bone regeneration. The same co-polymeric architecture was recently also used to reinforce bovine mineral matrices (Pertici et al., 2010), obtaining 3D scaffolds that exhibited a very high cell viability and sustained in vitro differentiation of human ADGs (Bardelli et al., 2009). Furthermore the addition of natural polymers, such as CT1 (Schofer et al., 2009a, 2009b) and gelatin (Mattiil et al., 2008), could promote the cell growth and differentiation of human MSCs (hMSCs). Indeed, on the one hand, synthetic polymers can be tuned in terms of composition, rate of degradation and mechanical and chemical properties; on the other hand, naturally derived polymers provide structures extremely similar to living tissues, such as stimulating a specific cellular response, which sometimes supersedes the advantages of synthetic polymers (Perale et al., 2011a; Shoichet, 2010).

2.1.2. PGA

Poly(glycolic acid) (PGA), called also polyglycolide, is synthesized by ring opening from the dimer (glycolide). PGA of high molecular weight is a tough polymer, melting at about 225 °C, and because of its high crystallinity PGA, unlike PLA, is not soluble in most organic solvents. PGA synthesis is also possible by acid-catalysed polycondensation, which allows for longer chain synthesis. The resulting polymer has, nevertheless, a low molecular weight and often poor mechanical properties (Agrawal et al., 1997) that compromise its employment in load-bearing applications. For these reasons, its use as a stem cell scaffold is limited and, according to our best knowledge, only a few notable studies were done. Significant studies were conducted on scaffold imaging with multiphoton autofluorescence and second-harmonic generation (SHG) (Lee et al., 2006), extremely useful for following the progress of extracellular matrix (ECM) formation and underlining how PGA orientation and progressive induction alters scaffold conformation. Non-woven scaffolds, 2.3 mm thick, spun with fibres of PGA, were purchased from Synthecon (Houston, TX, USA): they were cut into 3.5 × 3.5 mm pieces and seeded with MSCs. Here, multiphoton autofluorescence is useful for imaging the PGA scaffold and stem cells, while SHG is useful for following the progress of ECM formation. The initial ECM formation was found to align with the PGA scaffold orientation, indicating that biomechanical stimuli and/or the chemical environment generated during chondrogenesis may be sufficient for scaffold reorganization. The possibility of synthesizing scaffolds with both natural and synthetic characteristics seemed to improve the number of adhering human bone marrow MSCs. In particular, the acid modification of small intestinal submucosa (SIS) could act as a potential scaffold to enhance MSCs proliferation and differentiation on PGA scaffolds (Ahn et al., 2007). Moreover, regenerative possibilities using PGA and stem cells harvested from adult skeletal were demonstrated in vivo on the reconstruction of calvarial defects in an adult rat model (Taub et al., 2009). Thus, the possibility of tailoring physical, chemical and mechanical properties is the main advantage of this class of materials. However, their possible premature degradation and their acidic products may cause low cell viability and immune response.

2.1.3. PLGA

Co-polymerization of different poly(ε-hydroxy acids) is a way to tune and blend the properties of the resulting polyester (Smith and March, 2007). By varying the ratio of lactide to glycolide in poly(lactide-co-glycolide) (PLGA) it is possible to achieve almost all desired physical, chemical, surface and degradation properties. The strength of PLGA co-polymer is maximal when the healing of bone is just beginning, and it becomes less in concomitance with bone healing and regeneration advance (Habal and Pietrzak, 1999). This continuous decrease in integrity could anyway be positive in some applications, e.g. in bioabsorbable devices for cranial growth in children (Antikainen et al., 1994), where compensatory bone lengthening (normalizing bone response) may take place, leading to a maintained overall skeletal morphology (Eppley and Sadove, 1992). For these purposes, more rapidly degrading materials, such as PLGA, are preferred because they soften with time and allow for device elongation without interfering with bone regrowth (Eppley and Sadove, 1994). The first stem cell experiments on PLGA scaffolds were conducted >10 years ago (Martin et al., 2001), with bone marrow MSCs expanded in monolayers and then cultured on 5 mm diameter, 2 mm thick PLGA foams, showing that cartilaginous and bone-like ECMs were uniformly distributed throughout the construct volume after 4-weeks of cultivation. PLGA used as a carrier and growth matrix for MSCs has been deeply studied in vitro (Gomi et al., 2004; Graziano et al., 2007, 2008; Koc et al., 2008; Kundu et al., 2009; Wang et al., 2009b, 2012; Yang et al., 2010). In other approaches, human osteoprogenitor cells were transfected with AxCAOBMP-2, a vector carrying the human BMP-2 gene, and seeded within PLGA scaffold (Partridge et al., 2002a, 2002b). PLGA can also allow the controlled release of ascorbate-2-phosphate and dexamethasone for osteogenesis purposes (Kim et al., 2003), with a significantly higher mineralization of MSC-seeded scaffolds with respect to control ones. Also the in vivo implantation of osteoprogenitor constructs demonstrated bone cell differentiation and production of bone tissue (Chastain et al., 2006; Hao et al., 2008; Morgan et al., 2007; Ren et al., 2005, 2007; So et al., 2008; Sun et al., 2007; Tanaka et al., 2009). Indeed, primary human bone marrow cells with osteogenic pleiotrophin (PNT) were cultured on PLGA (75:25) porous scaffolds (Yang et al., 2003); PNT-adsorbed constructs showed morphological evidence of new bone matrix formation after subcutaneous implantation into athymic mice. PNT in combination with PLGA has the ability to promote adhesion, migration, expansion.
and differentiation of human osteoprogenitor cells. Furthermore, PLGA modification was considered extremely important in order to enhance scaffold properties and also guarantee a natural environment for cell growth. In this framework, a class of modular peptides that include a biologically active sequence derived from the growth factor BMP-2 and a series of HAs were used with PLGA scaffold (Lee et al., 2010b): the BMP-2-derived sequence is biologically active, as measured by its ability to promote osteogenic differentiation of hMSCs, and the characteristics of this approach suggest that it can potentially be applied to a wide range of biomaterials. The bone-like HA coatings can be grown on a polymer surface using a biomimetic approach (Figure 3).

Moreover, small intestinal mucosa SIS-loaded PLGA scaffolds were prepared by solvent casting/particle leaching (Kim et al., 2006b); bone formation on an SIS–PLGA hybrid scaffold as a natural–synthetic scaffold was better than that on a PLGA-only scaffold. Moreover, the important role of HA was evaluated by seeding scaffolds with MSCs in vitro (Nazarpak et al., 2011; Yun et al., 2009; Zhang and Chen, 2010) and in vivo (He et al., 2010; Kim et al., 2008), showing a significant increase in bone formation in terms of mineralization and expression of bone-specific genetic markers. PLGA scaffold chemical modification could also be possible with a mixture of fibrin and hyaluronic acid (HY) (Kang et al., 2011): fibrin/HY used as a vehicle for drug delivery coating of the scaffold significantly enhanced initial cell attachment and in vitro release of BMP-2; the transplantation of undifferentiated ADCs inoculated on BMP-2-loaded, fibrin/HY-coated scaffolds resulted in a more improved bone formation and mineralization. Following the same rationale, the incorporation of CaSiO₃ (CS) into PLGA microspheres was studied to investigate how the phase structure of CS influences the in vitro and in vivo bioactivity of the microspheres (Wu et al., 2011), improving in vitro MSCs viability and de novo bone-formation ability in vivo. The high versatility of PLGA also allows the formation of nanoparticles (Schneider et al., 2008) that can be loaded with BMP-2 (Yilgor et al., 2010). Sequential delivery of BMP-2 provided slightly lower proliferation than did simultaneous delivery, but the highest ALP of all indicated a synergistic effect on the osteogenic differentiation. Also, the type of textured surface seemed to influence stem cells to exert a different response (Graziano et al., 2008): cells cultured on a concave textured surface had better cell–scaffold interactions, leading to osteodifferentiation, while less good cell performance was obtained using convex surfaces, due to scarce cell proliferation. Indeed convex substrates seem to be unsuitable for intimate cell interactions, and the protein profile pattern showed a diffuse decrease of bone-specific proteins. In view of the high degradability of PLGA scaffolds, attention should be paid to their acidic degradation products (Félix Lanao et al., 2011; Rhee and Lee, 2007; Sung et al., 2004). A comparative study (Rhee and Lee, 2007) of fast (PLGA)- vs slow (PCL)-degrading 3D scaffolds indicated that fast degradation negatively affects cell viability and migration into the scaffold in vitro and in vivo. This effect is likely due to the significant acidification of the local environment, due to the polymer degradation. Hence, the angiogenic response developed within the scaffolds implanted in vivo was related to the presence of an inflammatory response.

Figure 3. Bone-like HA coatings can be grown on PLGA substrates using a biomimetic approach. SEM images of a PLGA film (A) and a mineral-coated PLGA film (B): scale bars = 20 μm. (C) High-magnification SEM image of a HA-coated substrate, demonstrating plate-like nanoscale morphology: scale bar = 1 μm. (D) XRD pattern of PLGA and HA-coated PLGA substrates (denotes peaks associated with HA mineral phase). Reprinted with permission from Lee et al. (2010b). Copyright © 2010 Elsevier
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2.1.4. PCL

The most prominent and thoroughly investigated poly(lactone) polymer is poly(ε-caprolactone) (PCL), an aliphatic, semi-crystalline polyester with interestingly low glass transition (−60 °C) and melting (59–64 °C) temperatures. As a consequence, PCL is always in a rubbery state at room temperature and this unusual property undoubtedly contributes to the very high permeability of PCL for many therapeutic drugs (Lee et al., 1987; Woodruff and Hutmacher, 2010) and to its high thermal stability. PCL is prepared by ring-opening polymerization of the cyclic monomer ε-caprolactone, and it can be co-polymerized with numerous other monomers because of its high chemical affinity (Yu et al., 2010b). Microorganism-mediated biodegradability, together with possible hydrolysis under physiological conditions, led to the definition of PCL as a biodegradable material (Chasin and Langer, 1990). At high molecular weights, PCL retains its mechanical properties for 5–6 months and then gradually loses its physical properties until complete metabolization over a period of 2 years (Calvert et al., 2000), and for this reason is suitable for bone tissue engineering (Mountziasi et al., 2010). Indeed, PCL degrades at a much slower rate than PLA and is therefore most suitable for the development of long-term implantable systems. The superior rheological and viscoelastic properties over many of its aliphatic polyester counterparts render PCL easy to manufacture and manipulate into a large range of implants and devices (Woodruff and Hutmacher, 2010). Coupled with relatively inexpensive production routes and FDA approval, PCL represents a promising platform for the design and fabrication of longer-term degradable implants, which may be manipulated physically, chemically and biologically to possess tailorable degradation kinetics to suit a specific anatomical site (Martins et al., 2010). During osteogenic stimulation, MSCs proliferated inward and onto PCL (and PCL-HA) scaffold surfaces, showing increased metabolic activity (Arafat et al., 2011; Endres et al., 2003; Kumar et al., 2011; Oh et al., 2010; Salerno et al., 2010; Yilgor et al., 2008). Here, the presence of a differentiating agent, such as DEX (Thibault et al., 2010; Vergroesen et al., 2011), enhanced the proliferation and osteogenic differentiation of MSCs. In vivo studies of PCL seeded scaffolds showed high ability to repair damaged bones (Arorin et al., 2008; Dupont et al., 2010; Luong-Van et al., 2007; Rodrigues et al., 2011; Shin et al., 2004; Zhou et al., 2007). Improvement of osteogenic differentiability could also be possible by incorporating factors such as DEX into electrospun PCL nanofibres (Vergroesen et al., 2011). Several material parameters have been verified to be extremely important for cell growth. In particular, highest the MSCs differentiation was observed for cross-linked scaffolds, indicating the influence of scaffold structure on cellular activities. Indeed, the change of PCL surface properties strongly influences material and biological properties (Haslauer et al., 2011; Rentsch et al., 2009, 2010). Surface changes can be induced by ion beam irradiation (Marletta et al., 2007), forming He⁺-irradiated PCL scaffolds, and it is confirmed to improve the adhesion of MSCs and support their differentiation. Mineralization with CP or HA and CT1 was also shown to be suitable for bone tissue engineering (Chen and Chang, 2011; Edlund et al., 2008; Guarino et al., 2008; Phipps et al., 2012; Scaglione et al., 2010; Schantz et al., 2005; Shokrollahi et al., 2010; Weinand et al., 2007; Yu et al., 2009). Indeed, the effects of HA particle size and shape in the coating layer on the mechanical and biological properties of a biphasic calcium phosphate (BCP) scaffold were examined (Roohani-Esfahani et al., 2010): needle-shaped coated HA/PCL particles induced the differentiation of primary human bone-derived cells and possessed superior physical, mechanical, elastic and biological properties. Furthermore, combinations of the polymer with BMMSCs and TCP were tested in vivo in a unicortical tibial defect model in eight goats (Rai et al., 2010; Vertenent et al., 2009): biocompatibility and bone-healing characteristics were evaluated and the results demonstrated cell survival and proliferation in the polymer-substituted bone defects. PCL–TCP scaffolds with placenta-derived MSCs (PDMSCs) were also implanted in full-thickness mandibular defects (2 × 2 cm) in pigs (Lee et al., 2010a): the experimental groups had higher levels of regenerated volume fraction and surface than the control group, and histological examination revealed the presence of effective regeneration even in the central area of defect. Several strategies have been studied in order to improve PCL scaffold properties (Liao et al., 2010). The immobilization of hyaluronic acid (HY) on PCL scaffolds (HY–PCL) showed higher attachment, proliferation and differentiation of stem cells (Chen et al., 2011; Kim et al., 2006a). Also, the incorporation of collagen onto the surface of the fibres modulated the attachment and proliferation of pig bone marrow MSCs (Ekaputra et al., 2009): the combination with osteogenic cell sheets offers a novel and promising strategy for engineering the tubular bone analogues. The synthesis of PCL co-polymers has been deeply studied and analysed (Mohammadi et al., 2007). In particular, the co-polymerization of poly (hydroxymethyl glycolide) with PCL creates scaffolds consisting of phMGCL, produced by means of a rapid prototyping technique (3D plotting) with high porosity and interconnected pore structure (Seyednejad et al., 2011). Cells filled the pores of the pHMGCL scaffold within 7 days and displayed increased metabolic activity supporting osteogenic differentiation, as visible in Figure 4. Thus, easy processability together with high biocompatibility are the main advantages of PCL scaffolds. However, attention must be paid to their slow degradation rate and their high hydrophobicity.

2.1.5. Other polyesters

The poly-b-aminoesters (PBAE) library were assessed as candidate materials that met design criteria for scaffolding in mineralized tissue repair and could be implanted subcutaneously in cranial defects (Brey et al., 2010). Samples in both locations displayed mineralized tissue.
formation in the presence of BMP-2, as evidenced by radio-
graphs, micro-computed tomography and histology. Polyhydroxyalkanoate (PHA) showed higher hydrophobic-
ity, surface energy and rougher surface than the other
well-studied polymers such as PLA (Wei *et al*., 2009). The
PHA materials tested were either less than or equal to TCPs
for supporting cell growth and poly(3-hydroxybutyrate-
co-4-hydroxybutyrate-co-3-hydroxyhexanoate) [P(3HB-
4HB-3HHx)] was the only PHA material that signi-
ficantly promoted cell proliferation compared to TCPs. Also, cells
cultured on poly(ethylene oxide terephthalate)-co-poly
(buthylene terephthalate) (PAX)–HA scaffolds presented a
significantly higher osteonectin and RUNX2 expression,
compared to those on PAX–HA–Collagen scaffolds
(Nandakumar *et al*., 2010a). BMP-2 and S100A4 expression
of PAX–Collagen and PAX–HA–Collagen constructs was
lower than the basal level expression of cells on PA
scaffolds, where bioactive composite scaffolds prepared
by electrospinning could find a potential use in bone-
regeneration applications. Electrospun scaffolds from a
block co-polymer-poly(ethylene oxide terephthalate)-poly
(buthylene terephthalate) with a CP layer was tested to im-
prove bioactivity (Nandakumar *et al*., 2010b), but no signif-
icant effect of CP coating was observed on the expression of
ALP *in vitro*, while implantation of scaffold–goat MSC
constructions subcutaneously in nude mice resulted in bone
formation. Moreover, the incorporation of polypropylene/
polyethylene terephthalate (PP/PET) fibres significantly
enhanced the compressive strength of the collagen sponge
(Mohajeri *et al*., 2010): this not only improves the mechanical
properties but also enables MSCs to positively improve
their proliferation and differentiation. The ability of
polyesters to support hMSC colonization and osteoblastic
differentiation *in vitro* was also investigated using polyprop-
ylene fumarate/polypropylene fumarate diacrylated PPF/
PPF-DA highly porous devices, shaped as partial ossicular
replacement prostheses (PORP)-like scaffolds (Danti *et al*.,
2010), that can be appropriately fabricated to allow both
the colonization of MSCs and osteoblastic maturation.
Here, high physical and mechanical properties are the main
characteristics of these scaffolds, while the adverse tissue
reactions caused by acidic degradation products, together
with problems with cell attachment, represent the
main inconvenience.

### 2.2. Polyethers (PEG)

Poly(ethylene glycol) (PEG) scaffolds are widely used in
tissue engineering, due to their hydrophilic nature and
controllable, reproducible chemistry (Ahn *et al*., 2009).
This versatility allows the control of particular properties,
such as molecular weight, local microstructure, degrada-
tion rate, crosslinking density, mechanical strength and
stiffness. The resulting scaffolds can be used alone or in
composite materials, and they usually represent a good
trade-off between cytocompatibility and mechanical
requirements. Bovine bone marrow MSCs could be
expanded in monolayers and cultured on 5 mm diameter,
2 mm thick foams made of PEG, where the cartilaginous
ECM (containing collagen type II) or bone-like ECM
(Martin *et al*., 2001) can be deposited. After 4 weeks of
cultivation, cartilaginous and bone-like ECMs were
uniformly distributed throughout the construct volume.

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**Figure 4.** Attachment and differentiation of MSCs on pHMGCL (top) and PCL (bottom) scaffolds. Actin filaments (green) and nuclear
staining (blue) show cell attachment after 1 (A, D) and 11 (B, C, E, F) days of culture. Only a few cells (green) were detected on the strands (bluish autofluorescence) of both scaffold types and only those cells that had attached to the outside of the scaffold could be visualized (A, D). After 11 days in culture, in both scaffold types (B, E), cells had divided to completely cover the strands of the PCL
scaffolds (E), while in the pHMGCL scaffolds cells had proliferated to span the scaffold pores (B). Note that the autofluorescence of the pHMGCL strands has increased and interferes with the detection of cells on the strands (B, C). Cellular alkaline phosphatase (red) production (C, F) demonstrates the osteogenic differentiation potential of MSCs in both scaffold types. Scale bars = 200 μm.
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The viability of encapsulated hMSCs up to 4 weeks in culture was investigated using a membrane integrity assay, and their gene expression was determined with reverse transcription–polymerase chain reaction (RT–PCR) (Nuttelman et al., 2004). hMSCs photoencapsulated in PEG hydrogels, and subjected to osteogenic media, are also able to differentiate into osteoblasts and mineralize the matrix. PEG was also used for co-polymerization with other monomers [e.g. poly(-caprolactone-co-lactide), forming PCLA–PEG–PLA constructs], where the resulting solution rapidly form a stable gel under physiological conditions (pH 7.4 and 37 °C) and can thus be injected subcutaneously into mice (Kim et al., 2009). Several in vivo studies revealed mineralized tissue formation and high levels of ALP activity in the mineralized tissue. Betz et al. (2010) proposed a tissue-engineering approach for orbital bone repair, based on a cyclic acetal biomaterial formed from 5-ethyl-5-(hydroxymethyl)-β,β-dimethyl-1,3-dioxiane-2-ethanol diacrylate (EHD) and poly(ethylene glycol) diacrylate (PEGDA): they hypothesized that EH–PEG hydrogel macroporosity facilitates intercellular signalling among hMSCs and, in order to investigate this phenomenon, hMSCs were loaded into EH–PEG hydrogels of varying pore size and porosity. The results demonstrated that macroporous EH–PEG hydrogels support hMSCs, promoting a dramatic increase in BMP-2 expression due to scaffold architecture. This upregulation of BMP-2 expression was associated with faster hMSCs differentiation, as measured by ALP expression. Following the increasing interest on carbon nanotubes, Nayak et al. (2010) proposed an alternative approach, studying the effects of a thin film of PEG–carbon nanotubes spray. This hybrid composite was supposed to influence MSCs proliferation, morphology and osteodifferentiation. Interestingly, cell differentiation occurred even in the absence of additional biochemical inducing agents, as evidenced by multiple independent criteria, at the transcriptional, protein expression and functional levels. Taken together, these findings suggest that functionalized carbon nanotubes represent a scaffold suitable toward a very selective differentiation into bone tissue. Moreover, incorporation of the RGD peptide sequence can further increase the functionality of PEG hydrogels, improving the cellular response. The formation of peptide-functionalized PEG hydrogels (Anderson et al., 2011) could enhance the growth and differentiation of hMSCs in the presence of osteogenic, chondrogenic or adipogenic differentiation media. Thus, on the one hand, PEG scaffolds exhibit mechanical properties not suitable for bone healing while, on the other hand, their high hydrophilicity is extremely useful for cell attachment and growth. For these reasons PEG is commonly used in blends with polyesters.

2.3. Polyamides

Polyamides (PAs) are prepared from acids and amines. The polymerization reaction takes place at a higher temperature than the melting points of the reactants and the polymer. In order to polymerize, this mixture is brought to a temperature of 255–265 °C under nitrogen. Generally it may be fabricated into a tough film by pressing or extruding in the form of filament. Both film and filament may be stretched over a hot place to give highly orientated crystalline products. The most-studied synthetic responsive polymer is poly(N-isopropylacrylamide) (PNIPAM), which undergoes a sharp coil–globule transition in water at 32 °C, changing from a hydrophilic state below this temperature to a hydrophobic state above it. The phase transition, and hence the origin of the ‘smart’ behaviour, arises from the entropic gain as water molecules associated with the side-chain isopropyl moieties are released into the bulk aqueous phase as the temperature increases past a critical point. The temperature at which this occurs (the lower critical solution temperature, or LCST) corresponds to the region in the phase diagram at which the enthalpic contribution of water hydrogen-bonded to the polymer chain becomes less than the entropic gain of the system as a whole, and thus is largely dependent on the hydrogen-bonding capabilities of the constituent monomer units. Accordingly, the LCST of a given polymer can be tuned, as desired, by variation in hydrophilic or hydrophobic co-monomer content: materials based on co-PNIPAM with a wide range of phase transition temperatures have now been reported. MSC–scaffold constructs were cultured for up to 7 days and the adhesion, proliferation and differentiation of MSCs into the osteoblastic phenotype were assessed (Wang et al., 2007). To investigate the in vivo biocompatibility and osteogenesis of the composite scaffolds, both pure n-HA–PA scaffolds and MSCs–scaffold constructs were implanted, underlining that the introduction of MSCs to the scaffolds enhanced the efficiency of new bone formation, especially at the initial stage after implantation in maxillofacial surgery. The osteogenic differentiation of MSCs was greatly influenced by the addition of growth factors, and the combination of an MSCs-seeded scaffold containing BMP-2 was a promising method to enhance in vitro osteogenic differentiation and in vivo ectopic bone formation (Na et al., 2007). The effect of strain, surface chemistry and topography on bone marrow MSCs was investigated: dynamic conditions improved cell proliferation but decreased osteogenic differentiation (Ozturk et al., 2009), while physically and chemically modified PNIPAM scaffold had a positive influence on the population of the scaffolds under dynamic culture conditions. Scaffolds of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) were prepared by the particulate-leaching method and modified by the introduction of polycrylamide (PAM) (Ke et al., 2010): sheep bone marrow MSCs were cultured on PHBV and modified PHBV. The scaffold modification did not influence BMSCs viability, but the initial cell adhesion at 1 h of culture was enhanced. Moreover, the surface of the polycrylamide scaffold was also modified with either collagen or plasma polymer coatings containing amino, carboxyl or phosphate moieties (Lanniel et al., 2011). Here, neurogenic differentiation, indicated by b-III tubulin expression, was seen to be greatest on the carboxyl surfaces and for the lowest surface stiffness substrates, resulting in bone nodules formation and matrix calcification. Thus,
PAs show high biocompatibility and a broad range of biodegradation and mechanical properties. As inconveniences, they have high costs compared to other synthetic polymers.

2.4. Polyacrylates

Polyacrylic acid (PAA), or Carbomer as commercially better known, is a type of anionic polymer synthesized from acrylic acid by controlled radical polymerization. In a water solution at neutral pH, many of the side-chains of PAA will lose their protons and acquire a negative charge that make PAA a polyelectrolyte. As a biomedical device it has several uses (Perale et al., 2011a, 2012; Santoro et al., 2011), such as a scaffold for tissue engineering and for controlled drug release in different targeted tissues. In addition, its acylated derivates, synthesized by esterification, play a key role in regenerative medicine. MSCs are able to grow on two plasma-deposited acrylic acid (pDAA) coatings (Mattioli-Belmonte et al., 2005). The in vitro behaviour was compared to that of MG-63 cells: an osteoblast-like cell line underlined that both studied coatings exhibited satisfactory compatibility and modulatory effects on MSCs. Moreover, poly(methylmethacrylate) (PMMA) can modulate the proliferation and osteogenic differentiation of early- and late-passage bone marrow-derived hMSCs (Wang et al., 2006b): the immobilized extracellular ascorbic acid and soluble ascorbic acid synergistically promote osteogenic differentiation of MSCs and their capacity to undergo osteogenic differentiation. Peptide modification of PMMA appears capable of enabling osteoblastic development from only a subpopulation of connective tissue progenitors (CTPs) in marrow, provides insights into the integrin-mediated behaviours of CTPs and highlights differences between freshly isolated marrow and culture-expanded cells (Au et al., 2007). The effect of surface electrostatic properties on osteogenic differentiation of MSCs was investigated (Guo et al., 2008): the cells adhered, spread and proliferated more quickly on the positively PAA-modified surface than on the negatively PAA-modified and standard surfaces. Positively charged, negatively charged and standard surfaces supported osteogenic differentiation and their effect required the presence of DEX. Hydrogels carrying biomimetic peptides were prepared, using methacyryloylated peptides and methacyryloyl-GGGYIGSR-OH in the polymerization mixture with poly (hydroxyethylmethacrylate) (PHEMA) (Paripovic et al., 2012; Studenovska et al., 2010): the effect of biomimetic modification of hydrogels with fibronectin (RGDS) and laminin (YIGSR) peptides affected the seeding efficiency of porcine MSCs. Hence, unmodified hydrogels showed very low cell adhesion, due to their highly hydrophilic nature, while the incorporation of peptides significantly improved the adhesion. PHEMA covalently and non-covalently anchored to HS exhibited a different effect on hMSCs proliferation (Calcaro et al., 2010): exposure to HS/fibroblast growth factor (FGF-2) during early growth, but not during post-confluence, is essential for hMSCs differentiation to the fibroblast lineage. The delivery platform here is a step towards the development of a new class of biomaterials that enables the prolonged, non-covalent binding and delivery of growth factors.

2.5. Other synthetic polymers

Synthetic polymers were some of the earliest biomaterials used as tissue-engineering scaffolds. This class of materials showed very important advantages in this field, such as easier large-scale production and highly tunable properties (Perale et al., 2011a; Shoichet, 2010). Both of these advantages contributed to the large number of formulations present in the literature. In contraposition to the advantages of the naturally derived polymers, synthetic polymers offered wider scope to design and control the characteristics of the material. Moreover, the possibility of reducing allergenic risks using a completely artificial biocompatible material devoid of animal proteins is evident. The most common classes of synthetic polymers were investigated in preceding paragraphs, while the remaining ones are discussed here.

Cell attachment, proliferation, ALP activity and calcium content were measured to evaluate the ability of MSCs to adhere and differentiate on polypyrrole (PPy) scaffolds (Castano et al., 2004): increasing monomer concentrations resulted in PPy films of increased thickness and surface roughness. Thin films demonstrated superior induction of MSCs osteogenicity, which was comparable to that of standard TCP dishes. The presence of CP nanoparticles comprising HA and TCP synthesized together with PVA was also studied (Guha et al., 2009), together with carbon nanoparticles (Rodrigues et al., 2012): in situ mineralization exerted a good control over the morphological features of biphasic nanoparticles, osteoblast division and differentiation. Increase of cell viability and attachment was achieved on PVA scaffolds functionalized with bioactive glass (Gomide et al., 2012) or PCL (Shaiee et al., 2011). Also, different PVA scaffold mobilities influence cell viability (Gonzalez-Garcia et al., 2012). The effects of surface nanotopography on in vitro osteogenesis in human MSCs were investigated in polyurethanes (You et al., 2010): surface nanotopography can enhance osteogenic differentiation synergistically with biochemical induction substance. Surface modification using poly-amino acid urethane co-polymer and collagen together with tissue-engineering technology might facilitate bone anchoring. MSCs cultured on these polymers for 1 week were then implanted at rat subcutaneous sites and harvested after 4 weeks (Matsumoto et al., 2011; Shtansky et al., 2011): in vitro as well as in vivo results confirmed the importance of polymer surface to support the osteogenic differentiation, which resulted in new bone formation. Thermosensitive poly(organophosphazene)–RGD conjugates were synthesized to enhance scaffold functionality by a covalent amide linkage (Chun et al., 2009): MSCs on polymer–peptide (GRGDS) conjugate constructs, using an
Polymeric scaffolds as stem cell carriers in bone repair

injection into a nude mouse, were tested to express mRNA markers. This scaffold promises to be a good cell-delivery material able to induce osteogenic differentiation of MSCs for enhancing ectopic bone formation. The advantages of thermoresponsive, peptide-containing hydrogels as a supportive matrix for genetically engineered stem cells were demonstrated (Garty et al., 2010), underlining the necessity for stable peptide–polymer conjugates for prolonged cell support. The unique polymer characteristics, combined with enhanced cell–cell interactions, suggested the potential use of these biomaterials. In Table 1 are summarized the synthetic polymers used as stem cell growth matrices in bone tissue engineering.

3. Natural polymers

The use of natural polymers in bone tissue engineering has been gaining widespread attention, owing to their favourable attributes of biodegradability, low toxicity and low manufacture and disposal costs (Imam et al., 1999; Kaplan, 1998). Moreover, they offer a wide range of advantages for tissue-engineering applications such as biological signalling, cell adhesion, cell responsive degradation and remodelling (Ko et al., 2010). Naturally derived polymers are typically composed of a polymeric network that can contain a higher water content (Ratner and Bryant, 2004). Polymeric hydrogels have the distinct advantage of being injectable, which allows the delivery of the construct to be less invasive and thereby reduces surgical risks (Peral et al., 2011b). However, the inadequate physical properties of natural polymers that are soluble or rapidly degrade, together with the possible loss of biological properties during formulation, often compromise their use as unique scaffold materials (Shoichet, 2010). Particularly, their poor mechanical properties represent the primary limitation in the development of tissue-engineered scaffolds. Indeed, the use of natural hydrogels is restricted to osteoinductive drug delivery systems and bone defect repairs, while load-bearing applications are out of their range. Furthermore, the risk of immunorejection and disease transmission makes proper screening and purification necessary (Puppi et al., 2010; Werner and Elisseef, 2006), although this problem also applies to current standard treatments, i.e. allografts and xenografts. Nevertheless, even though naturally derived polymers are characterized by having batch-to-batch variations and low structural strength (Barbosa et al., 2005; Popa et al., 2012; Yang et al., 2001a), several authors have investigated polysaccharides and proteins, the main species involved in osseous chemical regeneration, making them appealing choices for scaffolds synthesis for bone repair strategies.

3.1. Polysaccharides

Polysaccharides are the most frequently employed natural polymers in biomedical applications. They consist of a large variety of polymers biosynthesized in wood, plants, algae and marine crustaceans, but also produced by bacteria and fungi. They are characterized from a wide range of glycosidic linked structures, based on about 40 different monosaccharides. Polysaccharides have some excellent properties, such as non-toxicity and stability to variations of pH, and can be both biologically and chemically functionalized.

3.1.1. Chitosan

One of the widely studied polymers for tissue-engineering application is chitosan, which is the co-polymer of D-glucosamine and N-acetyl-D-glucosamine. It is biodegradable, non-toxic and possesses antibacterial properties (Sellgren and Ma, 2012). Chitosan has a hydrophilic surface, promoting cell adhesion, proliferation and differentiation, and thus evokes a minimal foreign body reaction, with little or no fibrous encapsulation (Costa-Pinto et al., 2011; Dash et al., 2011). Unfortunately, these peculiarities depend strongly on the content of allergens, viruses and other contaminants, and the use of ultrapurified chitosan is hence a strict rule: material impurities affect not only the implant’s bioactivity, but influence properties such as viscosity, solubility and depolymerization kinetics, which are fundamental in tissue-engineering applications (Holme et al., 2008). Furthermore, chitosan can be moulded into various forms, with a rather well designed porous structure, by means of different techniques, such as freeze-drying, rapid prototyping and internal bubbling processes (Di Martino et al., 2005; Holme et al., 2008; Pertici et al., 2008), but nevertheless its mechanical weakness and instability, together with its incapacity to maintain a predefined shape, narrows its field of application (Shanmugasundaram et al., 2001; Zhao et al., 2010). Several studies have been performed on scaffold modification: Konjac glucomannan (KGM) presence contributed to improving the biocompatibility of chitosan materials (Nie et al., 2011). Moreover, scaffold modification with HA resulted in higher cell attachment and proliferation on the scaffold, indicating non-toxicity and cell biocompatibility in vitro and in vivo (Jiang et al., 2009). Human MSCs exhibited higher initial cell adhesion efficiency to 2D HA chitosan (HCG) membranes and maintained higher proliferation rates in 3D porous HCG than in classic chitosan scaffolds (Zhao et al., 2006). Differentiation assays indicated that the multilineage differentiation potential of human MSCs was preserved in 3D porous scaffolds. The effect of surface apatite nanostructure on HA-coated chitosan scaffold (Wang et al., 2011) was studied in terms of cell shape, cytoskeleton organization and osteogenic differentiation in vitro and the enhanced ability to differentiate, assessed on day 14 with alizarin red staining. Moreover, chitosan–alginate gel composite material (Park et al., 2005) was injected into the subcutaneous space on the dorsum of nude mice to investigate new bone tissue formation, confirming its ability to stimulate new bone formation. Human MSCs were either encapsulated in polyelectrolyte complexation (PEC) fibres and constructed
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into a fibrous scaffold or seeded on PEC fibrous scaffolds (Yim et al., 2006): higher cell proliferation and differentiation were observed on the hMSC-encapsulated scaffolds compared to the hMSC-seeded scaffolds, underlining that the reported PEC fibrous scaffold system has the potential to compose a multicomponent system for various tissue-engineering applications. Adding chitosan and fibres into calcium phosphate cement (CPC)–chitosan–fibre scaffolds, reinforcing the CPC role, did not compromise osteodifferentiation and mineral synthesis by the cells (Weiran and Xu, 2010a, 2010b). RhBMP-2 was immobilized onto the carboxylic groups on the surface of gelatin/β-chitosan porous scaffold (Bae et al., 2009) to improve cell viability and osteogenic differentiation. To improve mechanical properties while maintaining high biocompatibility, scaffolds obtained by melt blending between chitosan and synthetic polymers were developed as hyaluronic acid-γ-chitosan-γ-poly-(N-isopropylacrylamide) (Liao et al., 2011) and chitosan with poly(butylene succinate) (Costa-Pinto et al., 2009). hBMSCs were seeded on those structures and cultured for 3 weeks under osteogenic conditions, and the presence of mineralized ECM was demonstrated (Figure 5).

Thus, the advantages of chitosan scaffolds involve cell adhesion, highly promoted due to the high hydrophilicity of the surface and able so to address proliferation and differentiation. However, the disadvantages are poor mechanical properties and instability.

### 3.1.2. Hyaluronic acid

Another natural polymer that has been conjugated to alginate (Lindenhayn et al., 1999) is hyaluronan, which is also referred to as hyaluronic acid. Hyaluronan (HY) is a biomacromolecule having an anionic polysaccharide
chain made up of alternating N-acetyl-glucosamine and D-glucuronic acid repeat units. Hyaluronan, a key component of the ECM, is responsible for attaining a high degree of lubricity in different tissues, due to its high capacity to absorb and retain water. These characteristics and its presence as an exceptionally high molecular weight poly-electrolyte result in its extraordinary viscoelastic properties (Hulsart-Billstrom et al., 2011). Moreover, it directly affects tissue organization via interactions with cell-surface receptors, promoting the migration of cells and facilitating ECM remodelling (Allison and Grande-Allen, 2006). High molecular weight HY’s ability to promote regeneration is because it inhibits the differentiation of osteoclasts, cells specialized for bone resorption, by interacting with the TLR4 receptor present in them. HY is also implicated in the induction of MSC migration by stimulating the overexpression of the cell surface cluster determinant 44 (CD44) receptor (Martinez-Sanz et al., 2011). Thus, HY-based material is an interesting candidate for bone tissue-engineering applications where a localized treatment is desirable, e.g. bone defects, non-union fractures or around orthopaedic implants. Furthermore, it is easy degradable by enzymes called hyaluronidase without any visible inflammatory response. However, the hydrophilic, polyanionic surfaces of HY biomaterials do not thermodynamically favour cell attachment and tissue formation (Shu et al., 2003). Therefore, to enhance cell interactions, surfaces coated with ECM proteins, such as type collagen I and fibronectin, have been developed by creating physically or covalently linked functional domains (Ghosh et al., 2006; Ramamurthi and Vesely, 2002). The ability to support osteogenesis and chondrogenesis after subcutaneous implantation into nude mice underlined that HY-based delivery vehicles have the advantage of degradation characteristics that allow complete bone replacement. Human MSCs were seeded on the scaffold and osteogenesis was induced in the presence of FGF-2. Only at the highest hMSCs concentration tested (Manferdini et al., 2010) were the cells uniformly distributed inside and outside the scaffold, producing mineralized calcium-positive areas mainly present along the backbone of the scaffold. To enhance the properties of hyaluronan, it can be crosslinked with gelatin and implanted in situ into a defect, showing superior integration of the repaired tissue (Liu et al., 2006). In addition, bioglass–collagen–HY–phosphatidylserine (PS) (BCHP) composite porous scaffolds exhibited initial attachment, proliferation, migration and differentiation of the cells on the scaffold (Xu et al., 2009, 2010): once implanted, this composite scaffold showed a low inflammatory response and foreign body response within 3 weeks, together with a higher degree of healing, as visible from Figure 6.

HY scaffolds thus show several advantages, such as no immune response, the ability to be enzymatically degradable, and high biocompatibility. However, attention should be paid to HY’s low hydrophilicity and mechanical properties.

### 3.13. Alginate

Alginate is a linear co-polymer of β-D-mannuronic acid and α-L-guluronic acid. Depending on the weed source and growing conditions, the ratio of mannuronic and guluronic acid can vary. Because of its physicochemical properties, alginate is usually manufactured as a hydrogel. As it is able to crosslink under very mild conditions, at low temperatures and in the absence of organic solvents, alginate is a suitable scaffold which can be either injected into the site of interest or moulded and then implanted (Chang et al., 2001; Esalminejad et al., 2007). Moreover, the high swelling ratio of this polymeric matrix, together with other peculiar characteristics, such as the dissolution and biodegradation of the system under normal physiological conditions and the high tunable porosity, allow high diffusion rates of biomacromolecules and active moieties (Puppi et al., 2010). Alginate gels have therefore been widely studied for bone-regeneration applications as scaffolds and matrices and for controlled delivery of several therapeutic agents and cells (Alsborg et al., 2001; Eiselt et al., 2000). Particularly, alginate hydrogels have been deeply investigated as carriers of BMPs, alone or in combination with cells (Lopiz-Morales et al., 2013).

![Figure 6. X-rays of femurs with empty defects and those treated with either BCHP or BCHP/MSCs at 3 days, 3 weeks and 6 weeks postsurgery. BCHP/MSC-treated femurs exhibited the highest degree of healing from week 3 to week 6. Reprinted with permission from Xu et al. (2010). Copyright © 2010 Wiley](image-url)
Human osteoprogenitors (HOPs) (Grellier et al., 2009; Penolazzi et al., 2010) inside RGD-grafted alginate microspheres were studied in vitro and further implanted in a bone defect, producing high bone formation. Nucleation of a bone-like HA mineral (Suarez-Gonzalez et al., 2010) was assessed by incubating the alginate scaffold in modified simulated body fluids for 4 weeks: the ability to control nucleation on the surface or on the inner pore surfaces of scaffolds was demonstrated. Moreover, scaffold modification with gelatin (Petrenko et al., 2011) or fibrin (Zhou and Xu, 2011) provided higher adhesion and proliferation of human bone marrow MSCs.

Thus, the advantages of alginate include the possibility of injecting alginate hydrogels, so avoiding risks due to surgical procedures, while the disadvantage is that these hydrogels have low mechanical properties.

### 3.2. Proteins: collagen and polypeptides

Collagen represents a group of naturally occurring proteins which compose most of the connective tissue. Among the different forms, collagen I (CT1) is the most abundant in the human body. It is found in tendons, skin, fibrocartilage, artery walls and the organic parts of bones and teeth. In its native state, CT1 is biodegradable, low-antigenic and has valuable properties, such as the stimulation or inhibition of angiogenesis (Zhao et al., 2010) and the promotion of cellular proliferation and differentiation. For the above-mentioned reasons, collagen has become a favourite substrate for many tissue-engineering and regeneration applications (Uemura et al., 2003). Collagen scaffolds, for example, have been reported to promote cell and tissue attachment and growth (Kakudo et al., 2008; Kundu and Putnam, 2006; Ngiam et al., 2011; O’Brien et al., 2005) and to enhance bone formation by promoting the differentiation of osteoblasts (Seol et al., 2004). CT1 provides considerable mechanical strength in its natural polymeric state, which can be further improved by means of chemical manipulation to achieve the desired mechanical properties for orthopaedic applications (Curtin et al., 2012; Marelli et al., 2011). Certain processing methods, however, can alter the physical properties of the materials and may negatively affect cell-binding properties and tissue remodelling. For instance, exposure to chemical crosslinking agents can change a biocompatible collagen-based material into a form that incites a host foreign-body response (Atala et al., 2008). Unfortunately, the high degradation rate is the main disadvantage of using CT1 as a biomaterial, as it leads rapidly to loss of mechanical properties (Angele et al., 2004) and makes vain almost any chemical/mechanical method employed. Many attempts have been made to overcome this problem, allowing the scaffold to remain insoluble for a critical period, for instance, by adding mineral crystals or by combining collagen with either natural or synthetic polymers (Lee et al., 2009), or by applying various crosslinking methods (Glowacki and Mizuno, 2008). Collagen could also induce activation by contact and, once subjected to osteogenic differentiation and biosynthetic activity, MSCs were accompanied by the ultrastructural appearance of HA/calcium crystals and osteogenic gene induction (Schneider et al., 2010). To enhance scaffold properties, cells were also differentiated towards the osteogenic lineage on mineralized collagen sponges with TCP (Niemeayer et al., 2004; Weinand et al., 2006) and HA (Dawson and Oreffo, 2008; Teixeira et al., 2010; Thein-Han and Xu, 2011): bone tissue can be successfully formed (Gigante et al., 2008). In addition, increased mineralization with collagen covalently bonded with RGD sequences was observed (Meinel et al., 2004a, 2004b).

As a naturally occurring fibrous protein produced by silkworms and spiders, silk has many favourable properties, such as mechanical strength, flexibility, permeability and thermal conductivity. Since native silks were discovered in ancient China, they have long been used as fabric materials for textile industry and as sutures for surgery (Wang et al., 2006a; Yang et al., 2011). Silk fibroin (SF) is derived from raw silks after the removal of the contaminating sericin coating, thereby showing low immunogenicity and specific biocompatibility (Wang et al., 2006a). To date, SF has become an emerging biomaterial of natural origin and has been finding rapidly increasing applications in tissue engineering and other biomedical areas. 3D aqueous-derived silk fibroin scaffolds provide improved bone-related outcomes (Gomes et al., 2011; Kim et al., 2005), both alone and once the silk is covalently bonded with RGD sequences (Meinel et al., 2004a, 2004b): RGD peptide could improve the adhesion of MSCs to the silk scaffold, but it showed no impact on their proliferation (Wang et al., 2009a). Osteogenic differentiation of hMSCs seeded on these scaffolds resulted in extensive mineralization, and the ability to direct bone morphology via scaffold design suggested new options in the use of biodegradable scaffolds to control in vitro bone tissue (Uebersax et al., 2006). Once implanted to bridge a 10 mm gap in rat sciatic nerve, the scaffold promotes effects on peripheral nerve regeneration (Yang et al., 2011). The coexistence of BMP-2 and HA in electrospun silk fibroin fibres resulted in the highest calcium deposition and upregulation of BMP-2, underlining that they are potential candidates for bone tissue engineering (Li et al., 2006a). Attachment, proliferation and osteogenic differentiation of MSCs could also be influenced by self-assembling peptide–amphiphile molecules; the ALP activity and osteocalcin (OC) content of MSCs cultured in peptide–amphiphile nanofibres significantly increased compared with the static culture method (Hosseinkhani et al., 2006a, 2006b). MSCs can also differentiate into mature osteoblasts to form mineralized matrices within the polypeptide hydrogel scaffold: high ALP and OC contents were detected at both the protein and gene expression levels during culture periods within the scaffold (Hamada et al., 2008; Yoshimi et al., 2009). Moreover, in the huge field of polypeptides (Lutolf and Hubbell, 2005), complexes of zein scaffolds and rabbit MSCs were investigated on ectopic bone formation in nude mice and implanted into radius defects (Tu et al., 2009).
Gelatin or fibrin glue are also investigated as good cell delivery vehicles for transplanting therapeutic cells towards regeneration tissue, due to the outstanding performance shown by injectable shell-structure cell microcarriers endowed with favourable microstructure, desirable cytocompatibility and enhancing cell affinity (Breen et al., 2009; Hussain et al., 2012; Kang et al., 2010; Petrenko et al., 2011; Pierce et al., 2012; Ratanavaraporn et al., 2011; Su et al., 2010; Zandi et al., 2010).

Thus, the high biocompatibility of collagen and polypeptides is their main advantage, as they can easily allow cell attachment and growth. Their disadvantage is that their low mechanical properties underline the necessity to use them with synthetic polymers. In Table 2 are summarized the natural polymers used as stem cell growth matrices in bone tissue engineering.

4. Scaffold preparation methods

Controlling the microscale environment is a challenging but necessary goal (Norman et al., 2008). In this way, scaffold preparation should be done with attention paid to a microstructure that facilitates cell attachment, growth and differentiation (Dash et al., 2011; Puppi et al., 2010; Salgado et al., 2004; Yang et al., 2001a). Moreover, the processing methodology must not adversely affect the materials' properties, i.e. their biocompatibility or chemical properties. Also, the mechanical properties of scaffolds should be considered and compared with those of bone.

In the framework of 3D porous scaffolds, solvent casting/particulate leaching is probably the most common method used (Koc et al., 2008; Lim et al., 2010): this technique allows for an increase in pore interconnectivity; however, it also presents some disadvantages, such as the necessity to use toxic solvents and the limitation of producing only thin wafers up to 3 mm thick (Yang et al., 2001b). Another strategy is fibre bonding (Mikos et al., 1993); it can guarantee high porosity but no accuracy in microstructure control. Polymer fibres in the form of meshes have also been utilized as scaffolds, the individual fibres either woven or knitted into 3D patterns. On the one hand, they guarantee a large surface area, a key point for cell growth, while on the other they lack structural stability (Rodrigues et al., 2011). Another conventional method is phase-separation technology, in which liquid–liquid or solid–liquid separation is induced by lowering the solution temperature. One advantage of this that the activity of the incorporated molecules is maintained, while the difficulty of controlling scaffold morphology, together with solvent removal, are the main disadvantages (Morgan et al., 2007). In order to overcome the disadvantage of solvent removal, which could compromise biocompatibility, supercritical-fluid technology has gained increasing interest (Woodruff and Hutmacher, 2010). The major drawback in using supercritical CO₂ is that it yields a non-porous external surface, not optimal for cell attachment, and the cost is high. Polymeric scaffolds can be also prepared by freeze-drying, involving a thermally induced phase separation with the removal of the solvent (usually water) (Mohajeri et al., 2010; Puppi et al., 2010). The main disadvantages are the low mechanical stability and a pore size distribution in the resulting scaffold in the range of 100 μm. A different strategy to produce scaffolds is to use melt-based technologies, where the polymer mould is heated above the glass transition temperature and then immersed in solvents (Guarino et al., 2012). Here, the possibility of independently controlling porosity and pore size, together with macro shape control, are very important. There are problems in this method regarding non-amorphous polymers, which require a high temperature. In recent years, advances in computer technology have favoured new methodologies such as solid free-form fabrication (Costa-Pinto et al., 2011; Woodruff and Hutmacher, 2010). These techniques allow high control of microstructure and anisotropy to be obtained; however, the high temperature needed, the presence of solvent and low mechanical properties should also be considered. An alternative to porous scaffolds is represented by hydrogels, which seem to be suitable in bone repair because of their properties (Amini and Nair, 2012): the ability to retain water, thus mimicking living tissues, their high biocompatibility, as well as the possibility of precisely controlled release rates of drugs loaded within them (Endres et al., 2003; Perale et al., 2011a). Furthermore, their injectability reduces risks due to surgery and increases patient compliance. Hydrogels can be designed as temporary structures having the desired geometry and physical, chemical and mechanical properties adequate for implantation into the chosen target tissue. The various preparation techniques used are physical crosslinking (Hennink and Nostrum, 2002), chemical crosslinking (Barbucci et al., 2004), grafting polymerization (Said et al., 2004), and radiation crosslinking (Fei et al., 2000). The advantages of these systems are the final high injectability, degradability and possibility to sustain the delivery of drugs into the target tissue. However, the low control in porosity and microstructure, together with the mechanical properties, are the main drawbacks.

5. Future directions

The similarity to the biological environment, together with the reduced likelihood of toxicity and inflammatory reactions, gives materials of natural origin a distinct advantage over synthetic ones. Despite this advantage, naturally derived polymers possess very poor mechanical properties. In contrast, synthetic biodegradable materials are easily formed into desired shapes with good mechanical strength, but their hydrophobic properties inhibit cell-binding properties and prevent scaffold biomimetics. Thus, these two kinds of biodegradable polymers have been hybridized to combine the advantageous properties of both constituents. Composite polymers are expected to be physically and biologically superior to single material-based scaffolds, as the properties of a composite may
<table>
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<tr>
<th>Polymer</th>
<th>Polymer repeat unit</th>
<th>Acronym</th>
<th>Cell line</th>
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<tr>
<td>Alginate</td>
<td></td>
<td>Human bone marrow MSCs</td>
<td>(Eslaminejad et al., 2007; Grellier et al., 2009; Petrenko et al., 2011; Suarez-Gonzalez et al., 2010)</td>
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<td>Chitosan</td>
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<td>Human umbilical cord MSCs</td>
<td>(Penolazzi et al., 2010; Zhou and Xu, 2011)</td>
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<td>Dog bone marrow MSCs</td>
<td>(Liao et al., 2011)</td>
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<td>Hyaluronic acid</td>
<td>HY</td>
<td>Human bone marrow MSCs</td>
<td>(Costa-Pinto et al., 2009; Nie et al., 2011; Sellgren and Ma, 2012; Weir and Xu, 2010a, 2010b; Yim et al., 2006; Zhao et al., 2006, 2010)</td>
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<td>Human dental pulp SCs</td>
<td>(Bae et al., 2009)</td>
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<td>Rat bone marrow MSCs</td>
<td>(Mohammadi et al., 2007; Park et al., 2005; Wang et al., 2011)</td>
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<td>Polypeptides</td>
<td></td>
<td>Human bone marrow MSCs</td>
<td>(Chen et al., 2011; Griffon et al., 2011; Kim et al., 2006a; Manferdini et al., 2010; Solchaga et al., 1999)</td>
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<td>Human iliac-derived MSCs</td>
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<td>Rabbit bone marrow MSCs</td>
<td>(Liu et al., 2006)</td>
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<td>Rat bone marrow MSCs</td>
<td>(Loken et al., 2008; Xu et al., 2010)</td>
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<td>Dog bone marrow MSCs</td>
<td>(Yoshimi et al., 2009)</td>
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<td>Silk fibroin</td>
<td>SF</td>
<td>Human bone marrow MSCs</td>
<td>(Kakudo et al., 2008)</td>
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<td>Human amniotic fluid-derived SCs</td>
<td>(Dawson and Oreffo, 2008; Eslaminejad et al., 2007; Gigante et al., 2008; Hosseinkhani et al., 2006a; Kundu and Putnam, 2006; Li et al., 2006b; Meinel et al., 2004a, 2004b; Niemeyer et al., 2004; Petrenko et al., 2011)</td>
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<td></td>
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<td>Human bone marrow MSCs</td>
<td>(Schneider et al., 2010; Thein-Han and Xu, 2011)</td>
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<td>Porcine bone marrow MSCs</td>
<td>(Kang et al., 2010; Weinand et al., 2006)</td>
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<td>Rat bone marrow MSCs</td>
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<td>Porcine skin-derived MSCs</td>
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<td>Rat bone marrow MSCs</td>
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be programatically varied by mixing different materials in various ratios. Ceramic nanoparticles, carbon nanotubes, hydroxyapatite and calcium phosphates may be further components that can be incorporated to improve the structural properties of these polymers, and have a key role in future developments of tissue-engineered composites. Mechanical properties and osteoconductivity are of crucial importance for the regeneration of load-bearing tissues such as bone, to withstand stresses, to avoid scaffold fracture and to maintain the structure to define the shape of the regenerated tissue. Inorganic nanoparticle fillers have been shown to add tensile strength, stiffness, abrasion resistance, crack resistance and stability to polymer networks. Furthermore, the presence of an osteoinductive mineral phase, e.g. bone-like apatite, provides the further benefit of increased stiffness and also enhances and accelerates new bone formation. The significantly superior mechanical properties of these scaffolds create a better environment for bone healing and new tissue formation within the defect. Nevertheless, although the ceramic particles increase material stiffness and enhance creep behaviour, the higher the particle content, the higher the number of interfaces between the polymer and the ceramic, which has to be taken into account because failure can preferentially occur at the interface when the scaffold is under mechanical loading. High-density polyethylene (HDPE) reinforced with hydroxyapatite particles, for example, showed no enhancement of structural behaviour, due to the low degree of chemical interaction between the polymer matrix and the ceramic fillers. It appears that this issue has been neglected in the context of bone engineering, although much effort has been made in the enhancement of the interfacial adhesion in conventional polymer matrix composites. The development of coupling methodologies that increase the adhesion of the ceramic particles to the polymeric matrix is believed to be a possible route for the improvement of the mechanical performance of these composites. From a materials science point of view, a clear trend of the mechanical performance of these composites can be detected. Furthermore, the idea of using natural macromers, such as fibronectin, laminin or agarose, to coat synthetic polymers to favour cell attachment and viability has been suggested in tissue-engineering concepts from its very first description. In recent years, great importance has been given to synthetic–natural composite scaffolds. They could be the result of a block co-polymerization between synthetic and natural macromers, or just an interpolymer complex bonded by physical interactions. The goal of this approach should be to combine the biocompatibility of natural gels with the possibility of tuning the mechanical and physical properties of the synthetic components. Hence, it can be predicted that composites will be the second and third generations of scaffold materials that will enter the clinical arena in bone-engineering applications. To improve the osteoinductive properties of grafting materials, they have been combined with growth factors and different cells types, with variable results depending on the host regenerative capability. Therefore, osteoinductive approaches are crucial when the bone regenerative ability is diminished or lost. Unfortunately, many of the above-mentioned composites, when employed as growth factor carriers, show limitations in terms of biodegradability, inflammatory reactions, immunological rejection, disease transmission and, most importantly, the inability to provide a sustained therapeutic factor level. The repair and regeneration of musculoskeletal tissues, particularly bone, therefore remains a demanding application. Scaffolds should be designed in order to carry stem cells into the target tissue and then degrade, with a proper kinetic providing adequate space for tissue regrowth.

**Abbreviations**

AA, acrylic acid; ADC, adipose-derived stem cell; AFSC, amniotic fluid-derived stem cells; ALP, alkaline phosphatase; BCP, biphasic calcium phosphate; BMP, bone morphogenic protein; CHA, carbonated hydroxyapatite; CHS, chondroitin sulphate; CP, calcium phosphate; CPC, calcium phosphate cement; CS, calcium silicate; CT1, collagen I; CTP, connective tissue progenitors; DEX, dexamethasone; DPSC, dental pulp stem cell; DSC, dura mater stem cell; ECM, extracellular matrix; EHD, 5-ethyl-5-(hydroxymethyl)-β,β-dimethyl-1,3 Dioxane-2-ethanol diacrylate; ERK, extracellular signal-regulated kinase; ESC, embryonic stem cell; FAK, focal adhesion kinase; FGF, fibroblast growth factor; HA, hydroxyapatite; HCG, hydroxyapatite–chitosan; HOP, human osteoprogenitor; HS, heparan sulphate; HY, hyaluronic acid; KGM, konjac glucosamine; LCST, low critical solution temperature; MAPK, mitogen-activated protein kinase; MSC, mesenchymal stem cell; OC, osteogenic cell; P(3HB-4HB-3HHx), poly(3-hydroxybutyrate-co-4-hydroxybutyrate-co-3-hydroxyhexanoate); PA, polyamide; PAM, polyacrylamide; PAA, poly(acrylic acid); PAX, poly(ethylene oxide terephthalate)-co-poly(buthylene terephthalate); PBAB, poly-b-aminoester; PCL, poly(caprolactone); PDDA, plasma-derived acrylic acid; PDLLGA, poly(l-lactide-co-glycolide); PDMSC, placenta-derived mesenchymal stem cell; PE, polyethylene; PEC, polyelectrolyte complexation; PEG, poly(ethylene glycol); PEGDA, poly(ethylene glycol) diacrylate; PET, poly(ethylene terephthalate); PGA, poly(glycolic acid); PHA, poly(hydroxalkanoate); PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PHHEMA, poly(hydroxethyl methacrylate); PHMGCL, poly(hydroxymethylglycolide-co-caprolactone); PI3K, phosphatidylinositol-3 kinase; PLA, poly(lactic acid); PLAGA, poly(lactic acid glycolic acid); P(L-Co-PG-L), poly(lactide-co-propylene glycol-co-lactide); PLCL, poly(l-lactide-co-3-caprolactone); PLDL, poly(l-lactide-co-β-lactide); PLLA, poly(l-lactide); PMMA, poly(methyl methacrylate); PNIPAM, poly(N-isopropylacrylamide); PNT, osteoplectinophrin; PP, poly(propylene); PPF, poly(propylene fumarate); PPF-DA, poly(propylene fumarate) diacrylated; PPy, polypyrrole; PS, phosphatidylserine; PVA, poly(vinyl alcohol); RGDC, arginine–glycine–aspartic
Polymeric scaffolds as stem cell carriers in bone repair

acid–cysteine peptide; RGD, arginine–glycine–aspartic acid peptide; SF, silk fibroin; SIS, small intestinal submucosa; SPCl, blend of starch and poly(caprolactone); TCP, tricalcium phosphate; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

Conflict of interest

The authors state no conflict of interest and they haven't received any payment in preparation of this manuscript.

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Polymeric scaffolds as stem cell carriers in bone repair

Poly(caprolactone) in the 21st century. 


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