Engineered Polymeric Materials/Nanomaterials for Growth Factor/Drug Delivery in Bone Tissue Engineering Applications

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Abstract

Accelerating number of bone fractures/disorders, increased global burden, high demand, and limited availability of traditional bone grafts have shifted the research interest toward development of alternatives strategies, including the development of growth factor/drug delivery systems. The conventional delivery methods are limited in application due to the poor local retention, half-life, stability, requirement of high dosage, and inactivation of growth factor and drugs in biological systems. Polymeric materials/nanomaterials have emerged as promising candidates for therapeutic delivery of growth factors and drugs in tissue engineering applications due to their functionality and highly porous structure, which is suitable for high drug loading and extracellular-mimicking properties that promote cell attachment and proliferation for tissue repair. Engineering of polymeric materials has resulted in advancement of polymer chemistry in drug delivery applications by providing stimuli-sensitive polymeric systems, which can respond to pH, temperatures, and the presence of biomolecules. Different polymeric structures, such as nanofibers, nanoparticles, hydrogels, and 3D-printed scaffolds, have been investigated to overcome the problem of low drug efficacy and burst release. The polymeric materials maximize the effectiveness of growth factors/drugs by providing sustained, controlled, and localized release. However, the selection of polymers and growth factors/drugs is significantly important for the optimization and development of drug release systems similar to the release of growth/osteogenic factors in natural bone healing. This chapter focuses on the design strategies being employed for the next-generation engineered polymeric material/nanomaterial-based advanced

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delivery systems for enhancing the bone repair and regeneration as well as their potential application in regenerative medicine.

13.1   Introduction

Bone fractures/injuries affect millions of people worldwide, posing a heavy global economic burden (Meling et al. 2009; Bonafede et al. 2013). Bone tissue forms an essential part of the body and serves as a structural scaffold and supports kinematic motion while protecting our vital organs. Skeletal injuries due to falls, accidents, trauma, infections, tumors, or other bone-related disorders are very common and often require surgical interventions (Meling et al. 2009; Einhorn and Gerstenfeld 2015; Amir et al. 2019). Musculoskeletal injuries are predicted to be one of the major causes of morbidity and mortality worldwide (Agarwal-Harding et al. 2016; Mattson et al. 2019). The bone is a complex tissue and it consists of about 30% organic (collagen and proteoglycans) and 70% inorganic components (hydroxyapatite and amorphous calcium phosphate) having inherent ability to remodel and heal to maintain the tissue integrity (Salgado et al. 2004). The natural fracture/bone healing is a slow and time taking process that relies on the biological responses. Several conditions, such as osteoporosis, diabetes, autoimmune disorders, trauma, infections, and fixation stability, may significantly affect the biological responses during the fracture or bone injury; thus, compromising the healing and leading to the poor clinical outcomes. These conditions may result in about 10% delayed union or nonunion cases requiring additional surgical procedures (Einhorn and Gerstenfeld 2015). The rapid acceleration in bone-related disorders, fractures, and heavy economic burden posed due to the high demand of bone substitutes/grafts has motivated researchers to work on the advancement in bone tissue engineering. Currently, natural grafts including autograft and allografts are considered as the gold standard for the bone substitute, but the limited supply, risks of infection, disease transmission, and host responses have limited their use; thus, generating the demand for novel treatment strategies (Einhorn and Gerstenfeld 2015; Lee et al. 2015; Wang and Yeung 2017). These current strategies generally include systemic administration of bone-forming drugs, hormones, or other biomolecules, the development of synthetic grafts, onsite delivery of drugs, growth factors, and cells from the graft to address the limitations associated with the current approaches of bone regeneration (Shi et al. 2019). The local delivery of drugs and growth factors from the biomaterials has shown promising results and several polymeric systems are being explored to enhance the bone tissue regeneration by on-site delivery of drugs and biomolecules along with providing the matrix/scaffold for the cell attachment and tissue integration (Luginbuehl et al. 2004; Kempen et al. 2009b; de Guzman et al. 2013, p. 2;

Peterson et al. 2014; Martino et al. 2015). Bone tissue possess unique material and biological properties and, therefore, designing an effective bone regeneration solution requires a greater understanding of the natural healing process, biomolecules, and molecular mechanisms involved in the healing process.

13.1.1 Fracture Healing

The fracture healing comprises of mainly three phases: inflammation, repair, and remodeling (Fig. 13.1). These phases of healing overlap and can be distinguished by cellular and molecular factors (Kolar et al. 2010; Einhorn and Gerstenfeld 2015). The role and timing of these factors in the bone-healing process may provide important insights in designing the new delivery strategies to enhance bone regeneration. Bone fracture results in the disruption of the blood supply, leading to the
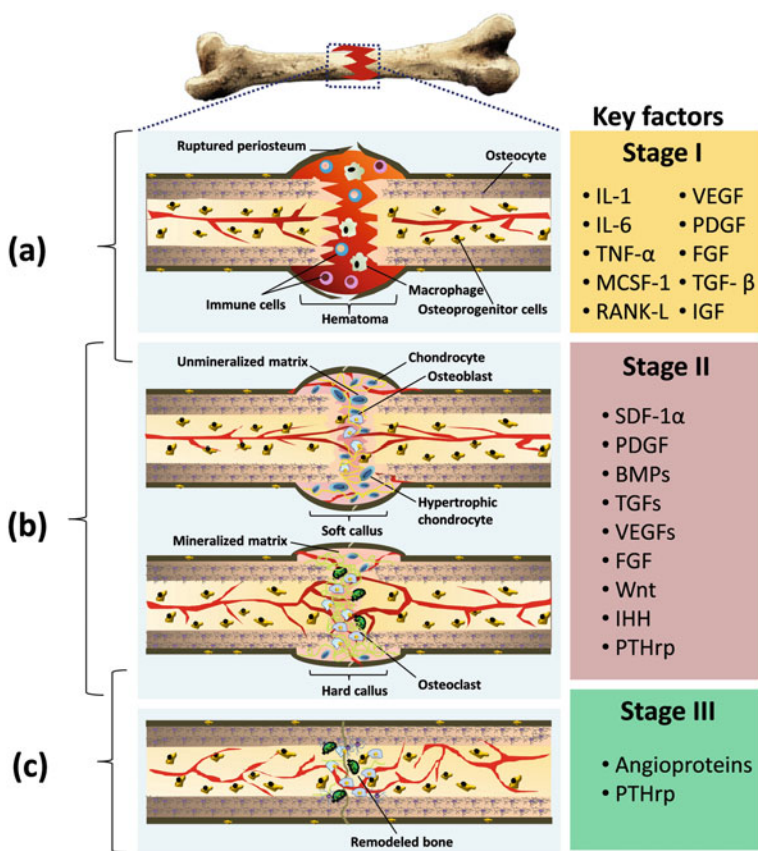

Fig. 13.1 Fracture healing phases and key factors involved at different stages of healing. (**a**) Inflammation, (**b**) repair, and (**c**) remodeling

formation of hematoma at the injury site. Initially, acute inflammatory responses can be seen within 24 h at the injury site with the expression of inflammatory markers initiating the repair process by stimulating angiogenesis process and recruiting mesenchymal stem cells at the site for the deposition of extracellular matrix (Kolar et al. 2010; Claes et al. 2012). Various proinflammatory cytokines, including interleukins (IL-1and IL-6), tumor necrosis factor-α (TNF-α), macrophage colony-stimulating factor 1 (MCSF-1), receptor activator of nuclear factor kappa-B ligand (RANKL), and transforming growth factor-β (TGF-β) can be seen in the early phases (Fig. 13.1a) (Marsell and Einhorn 2011; Claes et al. 2012; Einhorn and Gerstenfeld 2015). TNF-α and IL-6 play a very important role in tissue regeneration and their complete absence has shown to delay the mesenchymal stem cell differentiation (Gerstenfeld et al. 2003; Yang et al. 2007). However, prolonged inflammation period or chronic inflammation due to the conditions, such as infection and autoimmune diseases, may lead to the failed or impaired healing (Claes et al. 2012; Einhorn and Gerstenfeld 2015). In normal conditions, the inflammation phase is succeeded by the repair phase (Fig. 13.1b). The damage and rupture of blood vessels create the hypoxic environment triggering the release of proangiogenic factors, such as vascular endothelial growth factor (VEGF), angiopoetin-1, and platelet-derived growth factor (PDGF), promoting the vascularization of the newly formed bone tissues at the injury site (Grundnes and Reikerås 1992; Mayr-wohlfart et al. 2002; Hankenson et al. 2011).

The breakdown products are removed and the hematoma is replaced in a stepwise manner with the dense granulation tissues. Depending on the mechanical stability of the fracture site, the healing may take place directly or indirectly (Yu et al. 2010; Foster et al. 2020). In the direct healing, stability of the fracture site allows bridging of Haversian canals and results in osteoclasts infiltration, followed by the vascularization and recruitment of fibroblasts, MSCs, and osteoprogenitor cells by macrophages. The mineralization of long bones follows endochondral ossification route (Marsell and Einhorn 2011; Claes et al. 2012; Ghiasi et al. 2017). The mechanically unstable fractures lead to the indirect bone healing, both intramembranous and endochondral ossification (Claes et al. 2012; Ghiasi et al. 2017). The high mechanical strain in this case results in lesser new vessel formation, and the low oxygen level shifts the equilibrium of progenitor cells toward chondrogenic differentiation. The cartilaginous tissue formation takes place within 7–10 days of the injury (Grundnes and Reikerås 1992; Marsell and Einhorn 2011). SDF-1α is released from the periosteum and induces the phase of bone healing by inducing cell migration toward the bone formation site (Kitaori et al. 2009). Bone morphogenetic protein (BMP) and TGF-β help in bone regeneration by triggering the differentiation of chondrocytes (Benazet et al. 2009; Einhorn and Gerstenfeld 2015). BMP along with the Indian hedgehog (IHH) and parathyroid hormone-related protein (PTHrP) pathways stimulate the hypertrophy of chondrocytes toward bone, resulting in the formation of the mineralized bone matrix (Mak et al. 2008; Benazet et al. 2009; Haumer et al. 2018).

The final phase of fracture healing involves remodeling of the bone that may take place up to years to maintain the structural and mechanical integrity of the bones

(Fig. 13.1c). The hypertrophic chondrocytes become apoptotic and start releasing calcium after 2 weeks of bone fracture (Einhorn 1998, 2005; Thompson et al. 2015). The increase in the mechanical strength of the calcified callus reduces the tissue strain; thus, favoring the vessel formation and recruitment of MSCs and monocytes (Melnyk et al. 2008; Haumer et al. 2018). These differentiate into the osteoblasts and osteoclasts and the remodeling of bone starts (Einhorn 1998; Einhorn and Gerstenfeld 2015). This leads to the transformation of woven bone to the lamellar bone (Schindeler et al. 2008). These events are regulated by osteoblast-secreted cytokines MCSF and RANKL (Schindeler et al. 2008). The decrease in most inflammatory cytokines, except IL-1, TNF-α, and BMP-2, can be seen in this phase (Claes et al. 2012). As the gap is filled, the low tissue strain allows the intramembranous ossification after 4–6 weeks of bone fracture (Claes 2011).

13.1.2 Role of Growth Factors, Drugs, and Other Biomolecules in Bone Regeneration

Bone healing is a complex process that is accomplished by coordinated involvement of cells, bioactive molecules, and extracellular matrix. Cells release several growth factors at the injury site to induce and promote the bone regeneration and remodeling process. Growth factors are signaling molecules that induce and control various cell responses. Several inflammatory, angiogenic, osteogenic, and systematic factors are involved in bone repair and remodeling (Einhorn 1998; Einhorn and Gerstenfeld 2015). The inflammatory factors, including TNF-α, ILs, and prostaglandins, are responsible for the stimulation and differentiation of osteoblast and osteoclast cells and their release also activates the secondary signal cascade, resulting in enhanced angiogenesis (Kanzaki et al. 2002; Gerstenfeld et al. 2003; Ponte et al. 2007). Several anti-inflammatory and immunomodulatory factors, corticosteroids, and anti-inflammatory drugs have been reported to show the proregenerative or proresorptive effects. Angiogenesis factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF), have been explored in the bone regeneration as they promote the development of vascular network at the injury site; thus, supporting the osteogenic, chondrogenic, and mesenchymal stem cells (Schmidmaier et al. 2001; Keramaris et al. 2008; Wu et al. 2020). Among them VEGF has been extensively investigated and have been found to increase the vascularization and bone regeneration (Keramaris et al. 2008). However, the delivery of VEGF along with other osteogenic factors have shown more promising results in bone regeneration (Peng et al. 2002; Patel et al. 2008; Kempen et al. 2009b; Lee et al. 2020). Osteogenic growth factors, such TGF-β, BMPs, growth differentiation factor (GDF), and stromal-derived growth factor-1 (SDF1), have been also explored (Lieberman et al. 2002; Park et al. 2005; Kitaori et al. 2009; Kempen et al. 2009b; Yamano et al. 2014). TGF-β have shown bone induction but only up to endochondral bone formation (Ramoshebi et al. 2002; Ripamonti 2006). List of factors and drugs explored in bone regeneration applications is provided in Table 13.1.

Table 13.1 List of commonly used growth factors and drugs and their outcomes in bone regeneration (Aronin et al. 2010, p. 7; Donneys et al. 2012; Cattalini et al. 2012; Ibrahim et al. 2014; Einhorn and Gerstenfeld 2015; Yuasa et al. 2015; Shah et al. 2015; Gupta et al. 2019; Kuroda et al. 2019)

Growth factors/drugs		Effects/outcomes
Growth factors	TGF-β FGF-2 BMP-2 and BMP-7 VEGF GDF IGF PDGF	Induce bone formation Induce angiogenesis and promote bone regeneration Increase de novo bone formation Increase angiogenesis and bone regeneration Enhance bone repair Enhance tissue regrowth Support bone regeneration by enhancing angiogenesis
Hormones	PTH	Promote bone formation or resorption depending on the dose
Drugs and other molecules	Alendronate Ibandronate Pamidronate Zoledronate Clodronate	Reduce bone resorption and increase mineralization
	Dexamethasone	Inhibit osteoclastogenesis and promote osteoblastogenesis (dose dependent)
	FTY720	Promote angiogenesis and osteogenesis
	Deferoxamine (DFO)	Vascular response on bone regeneration
	Simvastatin Lovastatin	Anti-inflammatory and proangiogenic activities, promote osteogenesis

BMP isoforms, such as BMP-2, BMP-4, and BMP-7, have shown more promising results in de novo bone formation in ectopic and orthotopic sites (Kirker-Head 2000; Seeherman and Wozney 2005). Some systematic factors, such as parathyroid hormone (PTH), growth hormone, steroids, calcitonin, and Vitamin D, have also been investigated in bone tissue engineering and reported to promote bone formation (Weiss et al. 1981; Kempen et al. 2010; Hankenson et al. 2011; Abbassy et al. 2016). The growth factors may provide specific control over regeneration by manipulating the signaling processes. Although these molecules have shown good results in bone healing alone or in combination with other growth factors or drugs, the selection of growth factor is critical to maximizing the bone repair. Several drugs have also been explored in this field due to the high cost and low stability of growth factors. Bisphosphonates, such as alendronate, ibandronate, pamidronate, and zoledronate, have been explored in bone tissue regeneration and showed improved bone formation and prevention of bone resorption. Dexamethasone and FTY720 have shown increased osteogenesis.

13.1.3 General Requirements of Matrix/Scaffolds for Growth Factor/Drug Delivery

Bone is a very complex tissue and, therefore, designing the scaffolds require several considerations, including the choice of material, biocompatibility, biodegradation, osteoinductive/osteogenic nature, mechanical strength, porosity, and growth factor/drug release, to support the cell adhesion, proliferation, nutrient exchange, diffusion of biomolecules, and integration of scaffold. Several efforts have been made to incorporate growth factors/drugs within the matrix/scaffolds to provide their local release at the injury site to improve bone-healing outcomes. Among them, the extracellular matrix (ECM)-mimicking scaffolds provide the native-like environment to the cells for attachment and proliferation along with the release of growth factors/drugs to induce osteogenesis and enhance bone healing (Hudalla and Murphy 2011; Martino et al. 2015). The scaffolds working as a delivery carrier must fulfill some additional criteria, like high loading capacity, uniform distribution, targeted delivery, controlled/sustained release, and physical and chemical stability, and must protect the host molecules from losing their activity (Fig. 13.2). The optimization of drug loading and release from a scaffold requires certain considerations such as composition of scaffold, morphology, porosity, cross-linking, loading capacity, nature of interactions between the drug and the scaffold, binding affinities, stability of loaded molecule, and degradation of the matrix.

13.2 Challenges

Systemic delivery of growth factors and drugs is limited due to their short half-life, instability, and undesirable toxicity (Xinluan et al. 2015; Kuroda et al. 2019). Targeted delivery approaches are helpful in overcoming these limitations. There is a critical need to design bone substitutes providing local delivery of osteogenic agents. The drug delivery approaches have significantly advanced in the last few
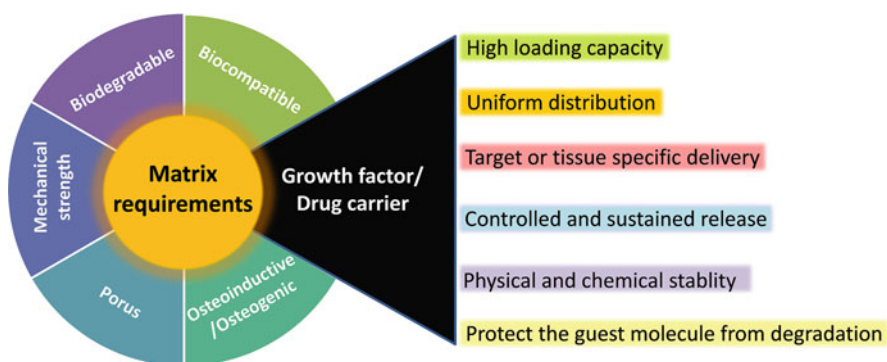

Fig. 13.2 General requirements of matrices/scaffolds for local delivery of growth factors/drugs for bone tissue engineering applications
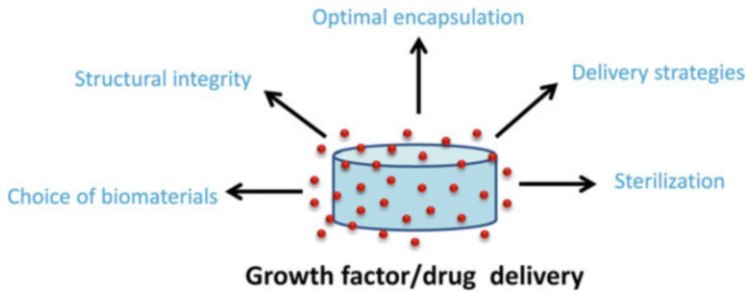
Fig. 13.3 Challenges in growth factor/drug delivery

years but designing an ideal delivery system suitable for all applications remains challenging as different shape, size, and structure of bone defects require different mode of delivery, mechanical, and degradation properties. Earlier permanent implants were employed for drug delivery but due to their poor integration and other limitations, degradable implants are now preferred (Vo et al. 2012). It is also challenging to design a scaffold releasing the growth factor/drug to restore to tissue at the same rate as that of implant degradation. The delivery systems for bone tissue engineering require long-term controlled release of growth factors/drugs and it is challenging to maintain its bioactivity throughout the course. Major challenges in growth factor delivery are listed in Fig. 13.3.

The optimization of drug loading also varies from one case to other and the interaction of different materials with the encapsulated growth factor/drug may lead to the loss of their integrity, thus resulting in low bioactivity. The modifications of growth factors and drugs for incorporation in the scaffolds and controlled release may also reduce their functionalities. Therefore, it is not easy to develop a single delivery strategy that can overcome all drawbacks of the carrier. It is also difficult to select a material having interconnected porous network required for cellular ingrowth along with providing the adequate mechanical support throughout the healing phase. Besides, the sterilization of growth factors encapsulated grafts also remains a major drawback.

13.3   Materials for Growth Factor/Drug Delivery

A wide range of materials have been explored as delivery systems for temporal and spatial control of growth factors/drugs/other bioactive molecules. Various metal, ceramic, and polymer-based implants have been evaluated as drug delivery carriers by physically or chemically loading the growth factor/drug molecules. A list of growth factor/drug-loaded biomaterials and polymeric systems available in market and undergoing clinical trials is provided in Tables 13.2 and 13.3.

Table 13.2 Growth factor/drug-loaded biomaterials for bone regeneration in market

Product	Composition	Application	Company	References
INFUSE® Bone graft	Collagen sponge loaded with BMP-2 titanium/PEEK cage	Replacement of autograft, degenerative disc diseases, anterior lumbar interbodyfusion (ALIF), spinal fusion, and open tibial shaft fractures	Medtronic, Inc.	https://global.medtronic.com/xg-en/e/response/infuse-bone-graft.html
Osigraft (OP–1® Implant & Putty)	BMP-7 containing type 1 bovine bone collagen matrix mixed with the putty additive carboxymethylcellulose sodium	Fracture healing of the long bones, and treating lumbar spine pseudoarthrosis	Olympus Biotech	https://www.strykermeded.com/media/1976/op-1-implant-msds.pdf (withdrawn)
Augment®	PDGF loaded in tricalcium phosphate matrix	Hindfoot and ankle fusion procedures	Wright Medical Group	https://www.wright.com/biologics_self/augment-injectable
GEM 21S®	PDGF with an osteoconductive matrix, ß-TCP	Dental problems such as intrabony, periodontal defects, furcation periodontal defects, and gingival recession associated with periodontal defects	Lynch Biologics	https://www.lynchbiologics.com/products/gem-21s/

13.3.1 Metals and Ceramics-Based Materials

Metal-based implants (mainly titanium and stainless steel) are highly used in orthopedic applications due to their high mechanical properties (Peterson et al. 2014; Prasad et al. 2017). These implants result in poor integration due to their inert nature and lack of osteoprogenitor function, which may lead to the formation of fibrous capsules around the implant, resulting in the loosening of implants and compromise in the long-term activity (Prasad et al. 2017; Kämmerer et al. 2020). Physical loading and chemical conjugation of growth factors, drugs, or other bioactive molecules have been explored on these implants to improve the bone-healing outcomes. BMP-2, FGF, VEGF, N-bisphosphonate, and dexamethasone incorporation into metallic implants have been carried out to improve the osseointegration (Kim et al. 2011; Peterson et al. 2014; Al-Jarsha et al. 2018; Kämmerer et al. 2020). Kim et al. immobilized the BMP-2 and heparin on titanium (Ti) surfaces and reported increased ALP activities, calcium deposition, and osteoblast function and decreased

Table 13.3 Growth factor/drug-loaded biomaterials for bone regeneration in clinical trials

Product	Composition	Phase	Application	Organization	References
KUR-111 KUR-112	Fibrin matrix+ HA/TCP loaded with PTH	2	Tibial plateau fractures Tibial shaft fractures	Kuros Biosurgery AG	NCT00533793 https://kurosbio.com/fibrin-pth/tibial-plateau-fractures/
KUR-113 bone graft	TGplPTH1-34 in fibrin	2	Degenerative disc disease, spinal fusion	Kuros Biosurgery AG	NCT04294004
FGF-2 gelatin hydrogels	rhFGF-2 in gelatin hydrogels	Preparatory	Tibial shaft fractures	–	Kawaguchi et al. (2010)
Rosuvastatin gel with 1:1 mixture of PRF and bone graft	Combination of rosuvastatin (RSV) in situ gel with the mixture of autologous platelet-rich fibrin (PRF) and HA bone graft	4	Mandibular degree II furcation defects	Government Dental College and Research Institute, Bangalore	NCT02369250
Simvastatin-methylcellulose gel	Simvastatin-methylcellulose	4	Alveolar bone in periodontitis	University of Nebraska	NCT03452891
rhFGF-2/HA	rhFGF-2 in sodium hyaluronate	1, 2	Human intrabony defects	Universidade Federal Fluminense	NCT02337166

inflammatory responses (Kim et al. 2011). Peterson et al. developed multilayer coatings of poly(methacrylic acid) and poly-L-histidine on anodized Ti surfaces immobilized with BMP-2 or FGF that provided sustained release of growth factors over 25 days with BMP-2 release being more effective than FGF for bone growth (Peterson et al. 2014). Poly(ethyl acrylate) (PEA) and fibronectin (FN)-coated Ti discs loaded with BMP-7 were developed and reported to promote potential osteodifferentiation of human mesenchymal stem cells (HMSCs) (Al-Jarsha et al. 2018). Although these efforts improved implants' interaction with cells, the surface adsorption of drugs or growth factors on metallic implants resulted in an initial burst release, resulting in adverse effects in several cases.

Kämmerer et al. chemically modified the titanium (TiO_2) surface with BMP-2, BMP-7, and an anti-osteoclastic drug (alendronic acid) and it showed significant improvement in cell growth and alkaline phosphatase (ALP) expressions at third and seventh day of bone-marrow-derived stem cell (BMSC) culture (Kämmerer et al. 2020). In order to improve the osteogenic potential and growth factor loading of bone implants, ceramics such as bone-derived minerals, tricalcium phosphates (TCP), hydroxyapatite (HA), and bioglass (BG) have also been used alone or in combination as osteoinductive coatings on metallic implants or scaffold carriers for growth factors and drugs. Teotia et al. reported that the nanohydroxyapatite and calcium sulfate bone substitutes functionalized with BMP-2 and zoledronic acid in low doses resulted in highest mineralization and neo-bone formation compared to ceramics loaded with zoledronic acid alone and without BMP-2 or zoledronic acid, when implanted in 8.5-mm critical size defect in calvarium of male Wistar rats for 8 weeks (Teotia et al. 2017). Calcium phosphates (CaP) have also been explored for FGF, hepatocyte growth factor (HGF), rhBMP-2, endothelial growth factor (EGF), FGF-2, and dexamethasone release and shown improvement in bone regeneration. Mesoporous bioactive glasses (MBG) are considered as osteoconductive and osteoinductive, and possess high specific surface area that makes them suitable for growth factor delivery applications. Different growth factors (BMP and VEGF) and drugs (dexamethasone, gentamicin, and ibuprofen) can be easily loaded and released from MBG particles, fibers, scaffolds, and composites (Wu et al. 2009, 2013; Dai et al. 2011; Wu and Chang 2012; Kim et al. 2016). Although the highly porous and osteoinductive nature of ceramics make them a suitable candidate for drug loading and cell activities, the burst release of growth factor/drug due to the electrostatic interactions or dissolution or degradation of ceramics limits their use as drug carriers, and usually polymer coating is employed to attain sustained release.

13.3.2 Polymeric Materials

Polymeric materials have been explored as drug carriers in bone tissue regeneration due to their versatile and tunable properties. Most polymeric materials closely mimic the extracellular matrix and, thus, provide the surface for cell attachment and growth. Biodegradable polymers can be tuned to resorb by the body after bone healing. The polymers contain variety of functional groups and can be functionalized easily as per

the requirement to load drugs physically or chemically. Polymers used in bone regeneration are classified as natural and synthetic polymers.

13.3.2.1 Natural Polymers

Proteins and polysaccharides extracted from plants, algae, animal, or human sources are considered as natural polymers. They have been proven to be useful material for tissue engineering due to their high biocompatibility, ECM-mimicking nature, and favorable degradation products (Mano et al. 2007). Collagen is a protein and most extensively used natural polymer, which forms a major organic component of the bones. Fibrinogen/fibrin is a fibrous protein, which forms a temporary matrix at the wound and have several binding sites for cells, growth factors, and ECM (Martino et al. 2014). These proteins have been used for biomolecule delivery but their proteolytic stability remains a major concern (Rajangam and An 2013). Other common natural polymers used for growth factor/drug delivery in bone tissue engineering are gelatin, keratin, silk fibroin (SF), alginate, chitosan, hyaluronic acid, heparin sulfate, pullulan, and dextran as they possess excellent biocompatibility and low immunogenicity (de Guzman et al. 2013). These polymers also possess functional groups that can be utilized for physical cross-linking and chemical modifications. The 3D networks formed by natural polymers can retain high water contents, are highly porous, and mimic ECM. Collagen-based scaffolds have been shown to promote osteogenic gene expression of MSCs and mineralization and, therefore, explored for the release of exogenous growth factors, such as FGF, PDGF, VEGF, IGF-1, HGF, BMP-2, TGF-β, and GDF-5 (Kanematsu et al. 2004; Yamano et al. 2014). Yamano et al. reported that the collagen membranes (CM) loaded with PDGF and GDF-5 showed significant bone regeneration compared to the control and CM alone, with more effectiveness in case of collagen membranes loaded with GDF-5 (Fig. 13.4) (Yamano et al. 2014).

Gelatin and silk fibroin have emerged as alternatives of collagen. Silk fibroin is biocompatible, osteoconductive, and possesses excellent mechanical properties (Meinel et al. 2005). The scaffold developed using silk fibroin has been investigated as the carrier of BMP-2 and showed good osteogenic outcomes (Ma et al. 2016). Gelatin-based materials have also shown improvement in cell attachment (Chen et al. 2007). Chitosan is among the commonly used natural polymer derived from chitin. It is reported to promote cell adhesion and proliferation as well as osteoblast differentiation along with antibacterial and mucoadhesive properties (Levengood and Zhang 2014; Tao et al. 2020). Hyaluronic acid and pullulan have been reported to support attachment and proliferation of mesenchymal stem cells (Singh et al. 2016; Zhai et al. 2020). Different polymeric structures fabricated using these polymers have been utilized in growth factor/drug delivery. Several growth factors including BMPs, VEGF, FGF, PDGF, TGF-β, and drugs have been tested using natural polymers. Generally, these polymers are found to show burst release due to the rapid degradation in biological environment but modification in their physical or chemical structure or processing usually improves the control release kinetics. Although natural polymers show excellent biocompatibility and bioactivity, their rapid degradation and poor mechanical strength limit their use in bone regeneration.
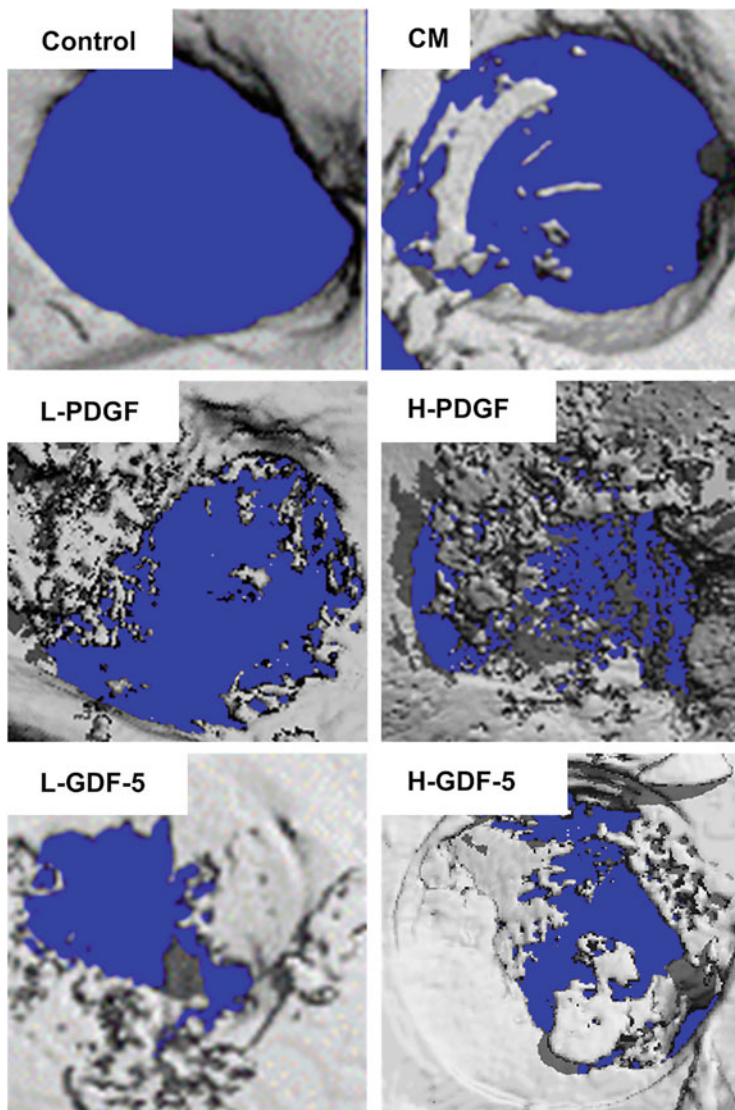
Fig. 13.4 Micro-CT images of new bone formation in rat mandible defects treated with collagen membrane (CM), and collagen membranes containing 0.5 μg PDGF (L-PDGF), 1 μg PDGF (H-PDGF), 20 μg GDF (L-GDF5), and 60 μg GDF-5 (H-GDF5) after 4 weeks of surgery. The blue color represents nonmineralized defect. (Reproduced with permission from Yamano et al. 2014)

13.3.2.2 Synthetic Polymers

Synthetic polymers have been extensively explored in growth factor/drug delivery in bone tissue engineering. They can be designed to overcome the limitations

associated with natural polymers. These polymers can be easily processed and tuned physically, chemically, and mechanically for delivery applications even though they are associated with disadvantages, such as acute or chronic immune response, bulk degradation, low clearance rate, and limited biological activities (Gunatillake and Adhikari 2003; Puppi et al. 2010). Poly(methyl methacrylate) (PMMA) polymers have been used as bone filler and cement (Freeman et al. 1982). It is a nondegradable polymer and impedes bone remodeling (Freeman et al. 1982; Maloney et al. 1990). Several polymers, such as poly(ethylene glycol) (PEG), polyurethanes (PU), poly (ε-caprolactone) (PCL), poly(anhydrides), poly(α-hydroxy acids), poly(propylene fumarate) (PPF), poloxamers, polyphosphates, and poly(phosphagens), have been explored in bone tissue engineering till date (Kempen et al. 2009a; Hudalla and Murphy 2011; Yu et al. 2015). Polyesters, such as polylactic acid (PLA), poly (glycolic acid), and poly(lactic-*co*-glycolic acid) (PLGA), have been used as growth factor carriers in bone regeneration. PLA nanosheets loaded with BMP-2 are reported to show constant and sustained release of BMP-2 for more than 2 months in vitro and induce bone regeneration in critical-sized mouse calvaria defects (Fig. 13.5) (Huang et al. 2017).

Different polymeric structures have been used to successfully encapsulate and retain the bioactivities of TGF-β1, BMP, and IGF. PLA, PGA, and PLGA showed limitations associated with acidic degradation products, resulting in tissue damage (Gunatillake and Adhikari 2003). PPF- and PCL-based scaffolds have shown high mechanical properties suitable for bone tissue engineering applications. PPF-based scaffolds have been explored to release BMP-2 (Kempen et al. 2009a). PCL nanofibers have been explored for the sustained release of dexamethasone for bone regeneration applications (Martins et al. 2010). Most synthetic polymers discussed here are hydrophobic, lack cell binding properties, and do not support cell attachment (Pierschbacher and Ruoslahti 1984; Lieb et al. 2003). PEG is a hydrophilic and biocompatible polymer widely used for the controlled and sustained release of growth factors, as it is known to increase the half-life and retain the bioactivity of growth factors. Its poor mechanical strength limits its use. Synthetic polymers can be mixed with natural polymer to improve their bioactivity.

13.3.3 Polymer Composites

Composite materials are formed by combining two or more different materials with different composition in such a way that the combinations result in specific biological, physical, chemical, and mechanical properties. Composites contain a continuous matrix and a dispersed phase. The choice of matrix and dispersed phase is critical to determining the properties of composite. Composites are made by different combinations of natural polymers with synthetic polymers and polymers with ceramics (Poh et al. 2020). As bone is made-up of organic and inorganic components, the composite materials fabricated using a combination of polymers/ proteins and ceramics are best suited for bone regeneration applications because their mechanical and biological properties can be modulated. Incorporation of ceramics,
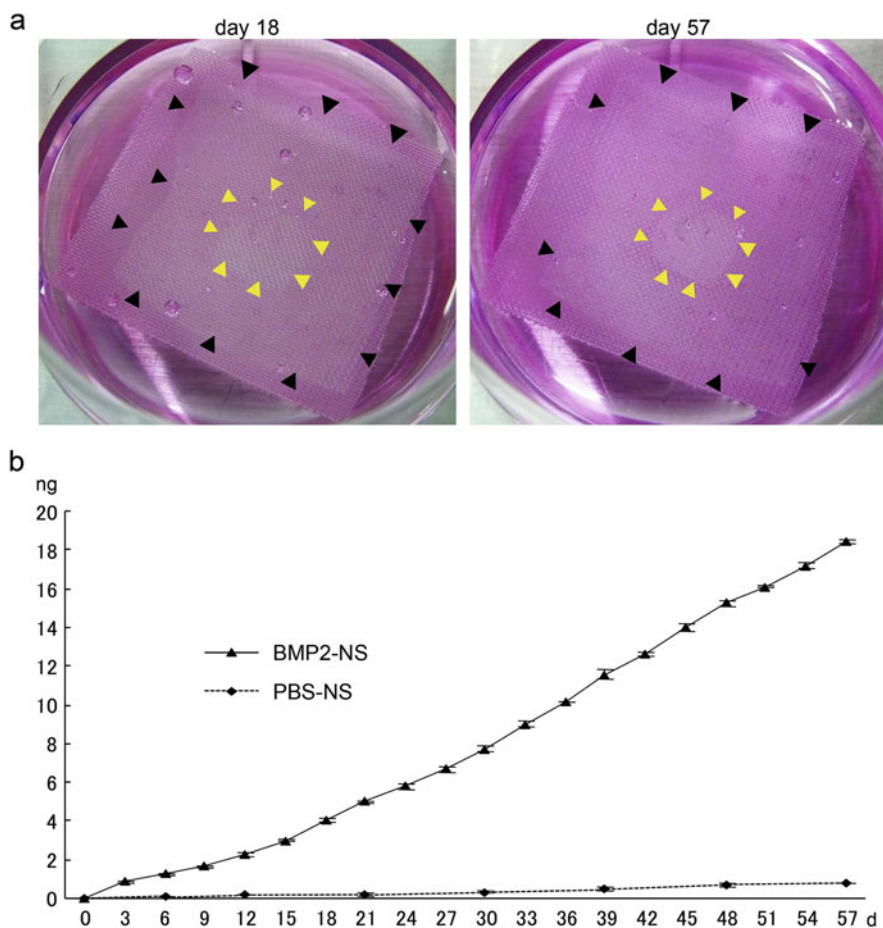
Fig. 13.5 In vitro release of BMP-2 from PLA nanosheets (reproduced with permission from Huang et al. 2017). (**a**) Sandwich-type PLA nanosheet soaked in DMEM at 37 °C at 18 and 57 days of incubation. Black arrows represent edges of large nanosheet and yellow arrows represent small nanosheet edges. (**b**) Cumulative release profiles of BMP-2 released from PLA nanosheets loaded with BMP-2 (BMP2-NS) or PBS (PBS-NS)

such as HA, bioglass, and β-TCP, to the polymer matrix, such as PLLA, PLGA, PEG, collagen, silk fibroin, dextran, pullulan, and chitosan, has shown enhanced mechanical strength (Barone et al. 2011; Poh et al. 2020). Ceramic materials impart the polymer composites with load bearing, retarded delivery, and osteoinductive properties. Several composite biomaterials have been investigated for growth factor/ drug delivery and showed promising outcomes in bone regeneration. Electrospun fibrous scaffolds of PCL-gelatin incorporated with bioactive glass nanoparticles (mBGn) loaded with dexamethasone showed almost linear release kinetics for up to 28 days and substantial osteogenic effects (El-Fiqi et al. 2015). PCL/nHA/BG
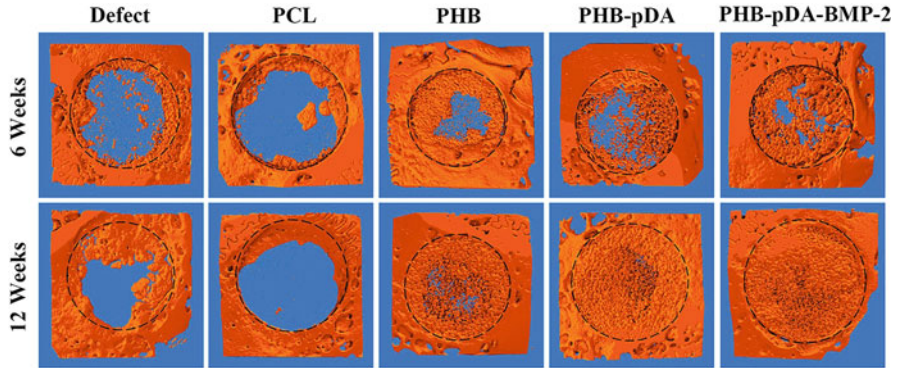
Fig. 13.6 Micro-CT images of calvaria bone defects showing new bone formation in control, PCL-, PHB-, PHB-pDA-, and PHB-pDA-BMP-2-treated groups after 6 and 12 weeks of implantation. (Reproduced with permission from Li et al. 2019)

(PHB) incorporated with polydopamine (pDA) and BMP-2 has shown long-term sustained release of BMP-2 and complete healing of calvaria bone defects in rabbits in 12 weeks (Fig. 13.6) (Li et al. 2019).

13.4 Encapsulation of Growth Factors/Drugs in Polymeric Materials and Nanomaterials

A wide range of delivery systems have been developed for local release of bioactive molecules. The growth factor/drug can be encapsulated in the polymeric matrix either physically or chemically. The encapsulation of growth factor prevents the loss of its bioactivity. Both physical and chemical strategies of loading growth factors/drugs are discussed in this section.

13.4.1 Physical Immobilization

Growth factors/drugs can be physically encapsulated in polymeric carrier systems, such as microspheres, hydrogels, liposomes, and micelles, and the loading is governed by electrostatic interactions, hydrophobic interactions, and hydrogen bonding (Park et al. 2005; Mao et al. 2014; Li et al. 2015; Juhl et al. 2019). These molecules are physically adsorbed on the matrix and their encapsulation to the matrix prevents their denaturation. The polyionic complexes formed by the electrostatic interactions between the polymers and drugs have been explored for the drug delivery applications in bone regeneration (Kim et al. 2011; Ao et al. 2020). In physical loading, the release of growth factor/drug is driven by passive diffusion or material degradation. The quantity and degradation rate of material can be controlled to tune the release rate. The surface area and porosity of scaffolds also influence the
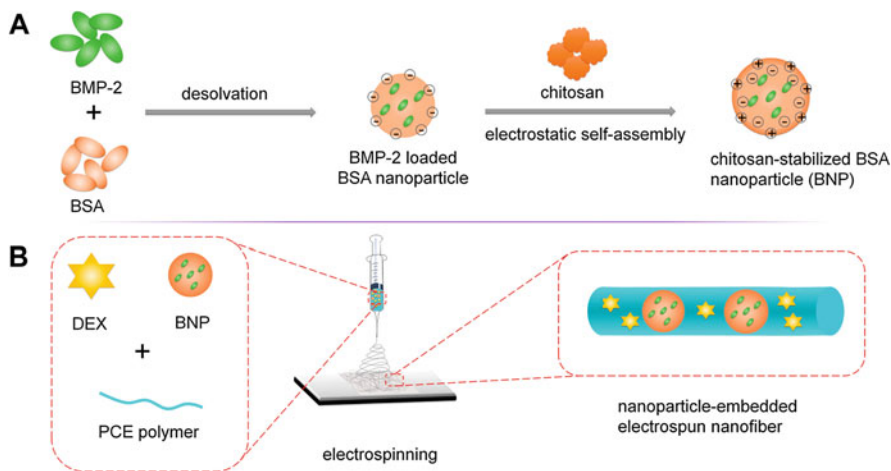
Fig. 13.7 Dual loading of BMP-2 and dexamethasone in BSA nanoparticle-embedded electrospun nanofibers. (Reproduced with permission from Li et al. 2015.) (**a**) Physical loading of BMP-2 in BSA nanoparticles stabilized with chitosan. (**b**) Electrospinning of dexamethasone and BNP-embedded PCE copolymer nanofibers

loading of growth factors/drugs. Collagen sponges have been extensively explored to deliver BMP-2 in bone fusion and fracture repair applications (Kanematsu et al. 2004). The materials showing high-affinity noncovalent interactions with the growth factor/drug have been shown to attain high encapsulation efficiency. Heparin-based scaffolds have been found to interact with BMP-2 through its N-terminal heparin-binding region, resulting in high adsorption (Kim et al. 2011; Gandhi and Mancera 2012; Ao et al. 2020). Physical encapsulation of drugs along with the growth factors has been used to enhance the osteogenic outcomes. Li et al. developed a nanoparticle-embedded electrospun nanofiber scaffold for physical encapsulation and dual release of BMP-2 and dexamethasone (Li et al. 2015). The encapsulation of BMP-2 in BSA nanoparticles was carried out to prevent BMP-2 from losing its bioactivity, and these BMP-2-loaded BSA nanoparticles were then blended with dexamethasone and poly(ε-caprolactone)-*co*-poly(ethylene glycol) (PCE) copolymer for coelectrospinning (Fig. 13.7). It preserved the bioactivity of BMP-2 along with a patterned release of dexamethasone within first 8 days and BMP-2 release lasting up to 35 days, resulting in significant repair of rat calvaria defects than that of the nanofiber scaffold without BMP-2 and dexamethasone. A nucleotide aptamer-functionalized fibrin hydrogel had been reported to improve the retention and release kinetics of the growth factor (Juhl et al. 2019). The fibrinogen (F) was functionalized with anti-VEGF aptamer using the thiol-acrylate chemistry, followed by VEGF addition and hydrogel fabrication. It provided the improved VEGF release, enhanced angiogenesis, and osteogenesis in vivo (Fig. 13.8).
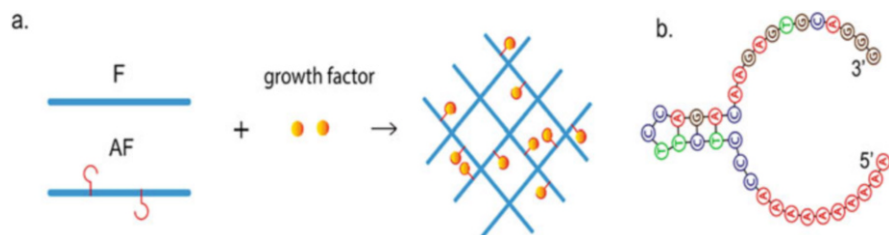
Fig. 13.8 Aptamer-functionalized fibrinogen hydrogel loaded with a growth factor. (Reproduced with permission from Juhl et al. 2019.) (**a**) Growth factor (VEGF) loading in hydrogels fabricated using native fibrinogen (F) and aptamer-functionalized fibrinogen (AF). (**b**) Secondary structure of anti-VEGF aptamer

13.4.2 Chemical Conjugation/Tethering

Covalent attachment of growth factors/drugs provide a better control over loading and release profiles and helps in designing stimuli-responsive delivery systems. Growth factors and polymers are chemically modified to obtain functional groups, such as amines, thiols, acrylates, hydrazides, aldehydes, and azides, to conjugate the growth factors/drugs to the polymeric materials (Bentz et al. 1998; DeLong et al. 2005; He et al. 2008; Chen et al. 2012; Koehler et al. 2013; Vogus et al. 2017). Initially, succinimidyl groups containing homobifunctional PEG linkers were employed to conjugate growth factors to the collagen-based biomaterials. The modified TGF-β and other growth factors resulted in improved outcomes compared to the unmodified growth factors (Bentz et al. 1998). NHS coupling is used to conjugate the growth factors with PEG and other polymers. In some studies, acrylated growth factors conjugated with PEG diacrylate through photopolymerization were also reported (DeLong et al. 2005). Click chemistry has emerged as a promising technique to conjugate bioactive molecules due to its high specificity and low toxicity (He et al. 2008). Click chemistry provides simultaneous tethering of growth factors/drugs and hydrogel fabrication. These strategies employing the use of amine group present in the lysine or at the N-terminal of growth factors were promising but may interfere with their bioactivity (Veronese 2001).

Thiol coupling strategies were used to conjugate bioactive molecules using the cysteines present in their backbone or by functionalization using additional cysteines. The C-terminus of VEGF was functionalized with cysteine and linked to vinyl sulfone groups on PEG linkers, using Michael-type addition (Zisch et al. 2003). Diels-Alder approach had been used for conjugating bioactive molecules with promising results (Koehler et al. 2013). In a study, tetrafunctional maleimide PEGs were cross-linked with tetrafunctional thiols through Michael addition and the furan-functionalized dexamethasone was conjugated in the polymeric matrix through Diels-Alder reaction between furan-functionalized dexamethasone and PEG maleimide (Fig. 13.9). It provided the long-term controlled release of drug, leading to an improved osteogenic differentiation and mineralization. Another study
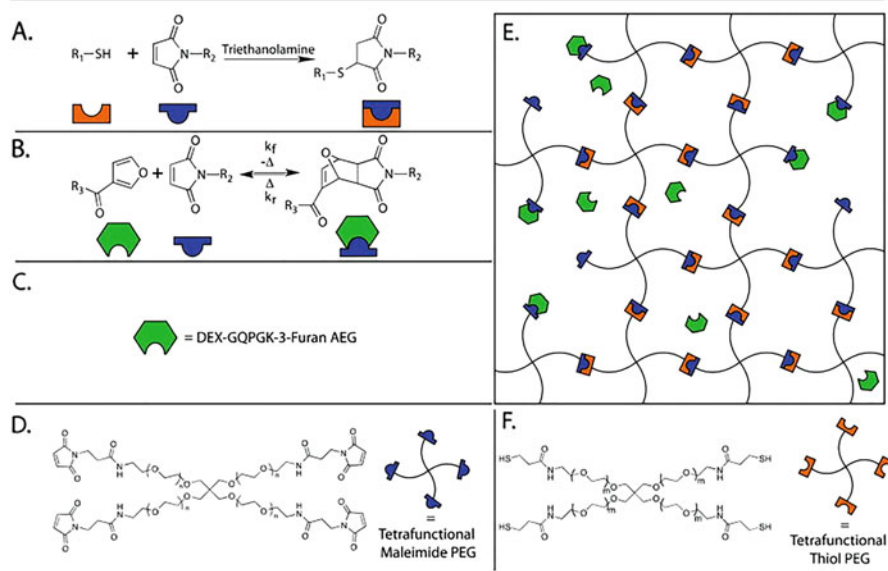
Fig. 13.9 Hydrogels with Diels-Alder modulated release of dexamethasone. (Reproduced with permission from Koehler et al. 2013.) (**a**) Michael addition reaction between thiol and maleimide groups of PEGs. (**b**) Diels-Alder reaction between furan on dexamethasone and maleimide groups on PEG. (**c**) Dexamethasone-labeled peptide with furan functionality. (**d**, **f**) Tetrafunctional maleimide and thiol PEG linkers. (**e**) Hydrogel network having Diels-Alder conjugated dexamethasone

reported that the bioactivity of the growth factors is influenced by the cross-linking chemistry. The activity of BMP-2 was higher in hydrazone cross-linked hyaluronic acid hydrogels than those with thiol-maleimide cross-links. Thiol-functionalized hyaluronic acid hydrogels incubated with BMP-2 showed diminished phosphorylation of Smad 1/5/8 (signal transducer of osteogenic differentiation), whereas no effect was seen in case of aldehyde-functionalized hyaluronic acid. Higher bone formation was observed in case of hyaluronic acid hydrogels with hydrazone cross-links (18 ± 4.25 mm^3) than with thiol-maleimide cross-links (1.25 ± 0.52 mm^3), after 8 weeks of implantation (Fig. 13.10).

13.5 Engineered Polymeric Materials/Nanomaterials for Growth Factor/Drug Delivery

Polymeric materials/nanomaterials have been investigated for growth factor/drug delivery. The conventional delivery methods, such as intravenous infusion, were employed for the delivery of bioactive molecules but they were associated with shortcomings, such as poor local retention and stability, high dosage requirements, low efficiency, and loss in their bioactivities. Engineered polymers are being used to
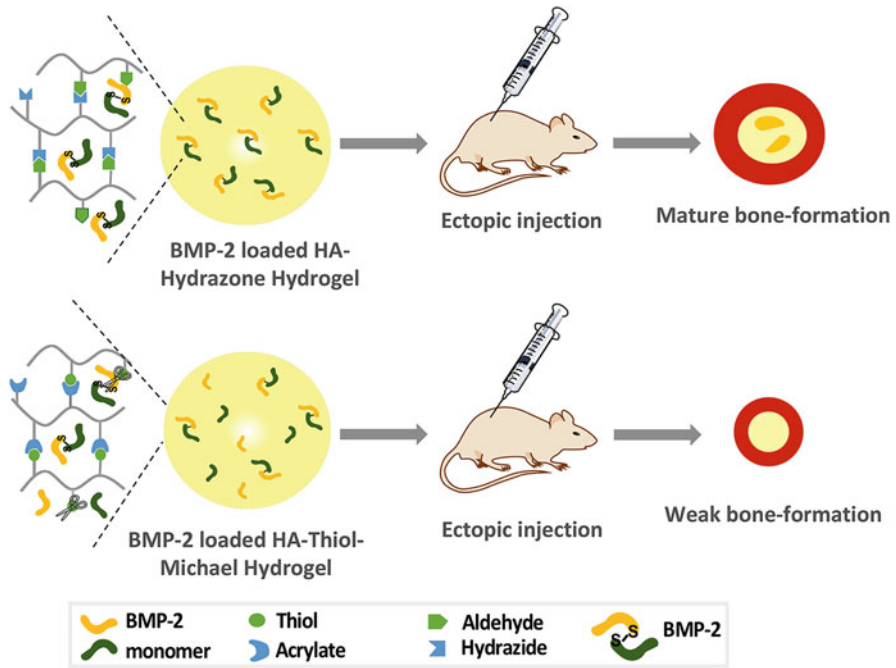
Fig. 13.10 BMP-2-loaded hyaluronic acid-hydrazone (HA-hydrazone) and HA-thiol-Michael hydrogel showing the difference in bone formation after ectopic implantation of hydrogels. (Reproduced with permission from Paidikondala et al. 2019)

tune the loading and release of bioactive molecules and it has resulted in the advancement in polymeric structures and development of smart delivery systems. Various engineered polymeric structures and stimuli-sensitive smart delivery systems will be discussed in this section.

13.5.1 Structure-Based Delivery Systems

Efforts have been made to develop different polymeric structures, such as nanofibers, nanoparticles, microparticles, films, hydrogels, and 3D-printed scaffolds, based on the requirements in order to overcome the limitations associated with the conventional delivery systems (Fig. 13.11).

13.5.1.1 Polymer-Growth Factor/Drug Conjugates and Nano/Microparticles

Polymeric macromolecules are conjugated with drugs and other bioactive molecules for the development of systematic or local delivery vehicles for bone tissue engineering applications. Conjugation of bioactive therapeutics to polymers improves the drug's solubility in water, increases their half-life, stability, and retention time in
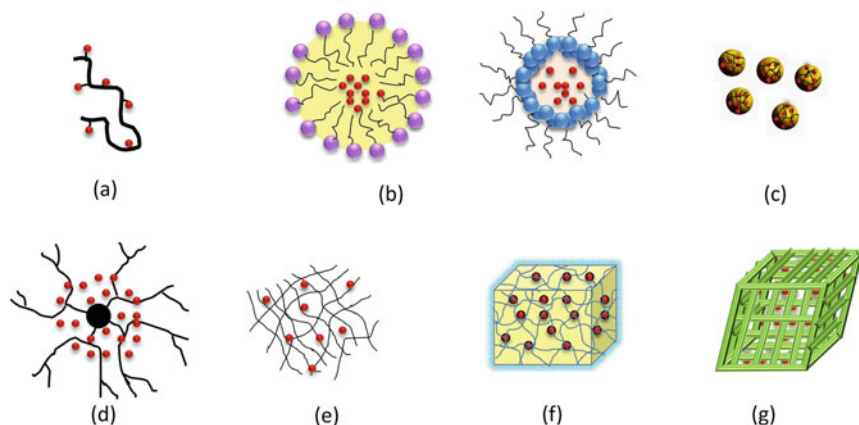
Fig. 13.11 Engineered polymeric structures for growth factor/drug delivery. (**a**) Polymer-drug/growth factor conjugates, (**b**) micelles and reverse micelles, (**c**) nanoparticles, (**d**) dendrimers, (**e**) nanofibers, (**f**) hydrogels, (**g**) 3D printed scaffolds

systemic circulation, and allows control over the release (Vogus et al. 2017). The delivery to the disease/injury site involves either noncovalent encapsulation of drugs or covalent conjugation to the polymer (Kaminskas et al. 2012). The noncovalent encapsulation of drugs is carried out in polymer assemblies, such as micelles or nano/microparticles, allow the utilization of unmodified drug/growth factor. The covalent conjugation of growth factors/drugs to polymers requires modification using specific linkers and it allows a preferential release in the presence of biological cues. PDCs have shown promising outcomes. Polyglutamates, bisphosphonates, and polyaspartates have been used as synthetic ligands to target bone tissues (Culpepper et al. 2013; Jiang et al. 2014; Cole et al. 2016). The conjugation of alendronate (a bisphosphonate drug used to treat osteoporosis) to poly(*N*-(2-hydroxypropyl) methacrylamide (HPMA) or poly(ethylene glycol) (PEG) or their combination with other polymers resulted in high accumulation in bone tissues (Wang et al. 2003; Chen et al. 2012; Karacivi et al. 2017). Dendrimers are branched polymers that have been conjugated to drugs and other bioactive molecules for delivery applications. One important aspect of growth factor or protein delivery is to protect its structure and bioactivity.

Polymeric micro- and nanoparticles, including micelles and liposomes (Park et al. 2007), have been successful in the growth factor/drug delivery, as they can encapsulate them within their core shell while protecting it from degradation. Amphiphilic block copolymers have been used to encapsulate drugs because they form micelles due to self-aggregation in aqueous medium. Micelles and reverse micelles are employed in drug delivery applications due to their biocompatible nature and capability to protect encapsulated agent (Aliabadi and Lavasanifar 2006). Polymeric micelles have been employed for simvastatin, dexamethasone, and methotrexate delivery to the bone and have shown great potential in this field (Liu et al. 2013; Wang et al. 2016; Xu et al. 2018b). Wang et al. reported that a micelle system
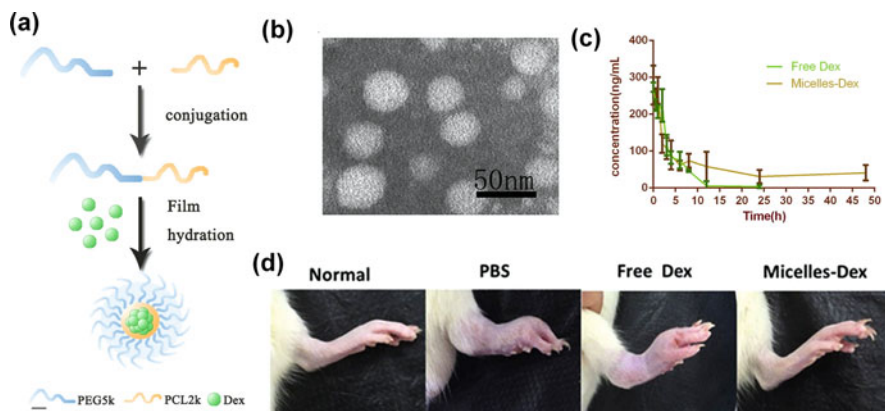
Fig. 13.12 Fabrication and characterization of dexamethasone-loaded micelles. (**a**) Schematic representation of drug-loaded micelle formation. (Reproduced with permission from Wang et al. 2016.) (**b**) TEM images of drug-loaded micelles. (**c**) Pharmacokinetics of free drug and drug-loaded micelles. (**d**) Photographs of hind legs from different treatment groups for swelling observations

fabricated from amphipathic poly(ethylene glycol)-block–poly-(ε-caprolactone) (PCL-PEG) polymer by film dispersion for dexamethasone loading and release showed relatively long, persistence present in the circulation and preferential accumulation in inflamed joints (Wang et al. 2016). The expression of TNF-α and IL-1β was significantly reduced in the groups treated with the drug-loaded micelles compared to the free drug, and the swelling in hind limbs of groups treated with the drug-loaded micelles was significantly reduced (Fig. 13.12). Micelles have been decorated with bisphosphonates for the targeted delivery of antibiotics to the bone (Cong et al. 2015; Kamble et al. 2020). Liposomes have been investigated for the growth factor/drug delivery to the bone (Metselaar et al. 2003; Park et al. 2007; Mao et al. 2014; Kroon et al. 2015; Crasto et al. 2016). The introduction of polymers, such as PEG, to liposomal formulations has provided the strategy to design biologically inert and safe platform for drug delivery by incorporating characteristics like reduced interactions with serum proteins, longer blood circulation time, and low immunogenicity. Mao et al. have developed a liposomal formulation showing enhanced targeted delivery of FTY720, with entrapment efficiency of ~85% (Mao et al. 2014, p. 720).

13.5.1.2 Polymeric Nanofibers

Polymeric nanofiber-based scaffolds have been found to be interesting candidates for bone repair as they possess ECM-mimicking structure, morphology, and superior ability to promote osteogenesis (Carbone et al. 2014; Chen et al. 2017). The 3D network of nanofibers resemble the extracellular matrix and provides a high surface area for drug loading. Electrospinning technique is a simple and most widely used technique for the preparation of nano- to microsized fibers. The therapeutic agents

can be incorporated into the interior or the surface of single nanofiber to obtain sustained drug delivery. Polymeric materials like silk fibroin (Li et al. 2006), PCL (Martins et al. 2010), chitosan-based (Tao et al. 2020), collagen/PCL, poly(DL-lactide-*co*-glycolide) (PLGA) (Lee et al. 2013), PLA (Ding et al. 2013; Cho et al. 2014; Madhurakkat Perikamana et al. 2015), hyaluronan/PLA (Wu et al. 2020), polycaprolactone (PCL)/gelatin (Wang et al. 2019), PCL/PVA (Mickova et al. 2012), SF/PCL/PVA (Cheng et al. 2019), and poly(L-lactide-*co*-caprolactone)/collagen/gelatin (Su et al. 2012) have been explored for the delivery of dexamethasone, BMP-2, BMP-7, VEGF, FGF, alendronate, and other therapeutics in bone regeneration. PLGA/PCL nanofibers were used to deliver FTY720, a targeted agonist of sphingosine-1-phosphate receptors 1 and 3, and reported to enhance vascularization and osseous tissue growth in critical-sized bone defects (Das et al. 2013). PEG/PCL-based nanofibers with core-shell structures have proved to be promising for the redox-sensitive delivery of BMP-2 to the bone (Gong et al. 2018). In a study, polydopamine (pDA)-mediated BMP-2 immobilization was carried out on PLA nanofibers for the guided bone regeneration (GTR) (Cho et al. 2014). The BMP-2 retained their activity on the nanofibers for up to 28 days and showed significant enhancement in ALP activities and calcium mineralization after 14 days of culture. These nanofibers showed good osteogenic outcomes at low dose of BMP-2. Another study reported that the incorporation of alendronate- and BMP-2-mimicking peptide-conjugated heptaglutamate moiety into mineralized nanofiber fragments of PLGA/collagen/gelatin nanofibers via calcium chelation resulted in significant enhancement in new bone formation compared to the unfilled defect control (Boda et al. 2020). The difference was not significant (alendronate) for single and coloaded (alendronate and BMP-2-mimicking peptide) bone grafts.

Coaxial electrospinning technique and layer-by-layer (LBL) assembly techniques have been used to incorporate growth factors/drugs into polymeric nanofibers having core-shell structure. Core-shell structure of nanofibers provides protection to growth factors and allows their sustained release. A core-shell SF/PCL/PVA nanofibrous mat was prepared by coaxial electrospinning, and LBL techniques were used to incorporate BMP-2 in the core along with the connective tissue growth factor (CTGF) on the surface of nanofibers (Cheng et al. 2019). These nanofibers provided coordinated temporal release of BMP-2 and CTGF, and showed increased vessel formation and improved bone tissue recovery. The transient release of CTGF resulted in proangiogenic effects on the bone healing, and sustained release of BMP-2 from these nanofibers showed 43% improvement in bone regeneration than the only BMP-2 release systems. Although these electrospun nanofibers mimic of ECM resulted in improved osteogenic outcomes, the smooth surface of these nanofibers did not mimic the nanotopography of collagen surface found in ECM, which is important to determine cell responses. Huang et al. investigated the natural collagen fibers mimicking drug-loaded core-shell nanofibers with hierarchical nanostructures in bone regeneration (Huang et al. 2020). The BMP-2-loaded PCL/PVA nanofibers were fabricated by coaxial electrospinning and their surface was decorated with nanoshish-kebab (SK) structures (Fig. 13.13), which were made up of nanofiber (shish) and disc-shaped lamellae (kebabs). These structures resulted
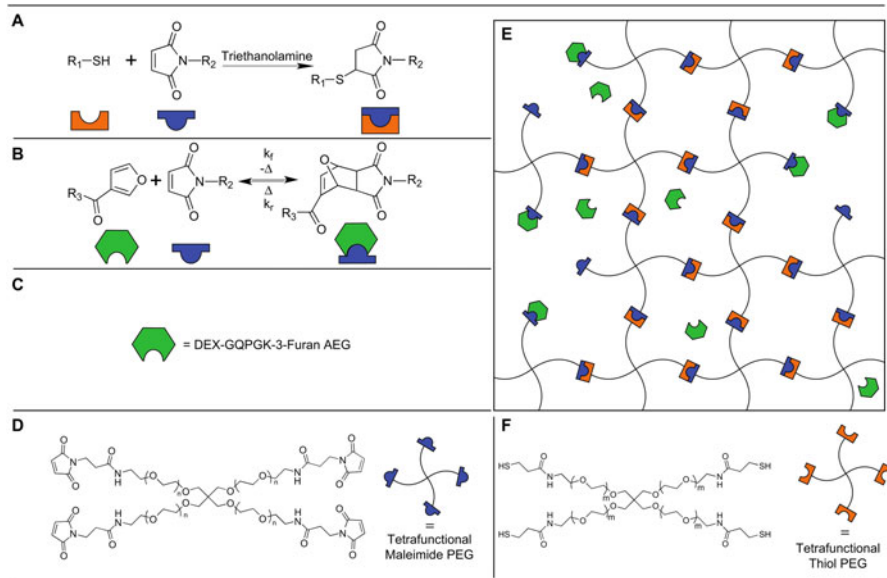
Fig. 13.13 Nanoshish-kebab (SK)-decorated BMP-2-loaded core-shell nanofibers and their role in bone regeneration. NB represents new bone. (Reproduced with permission from Huang et al. 2020)

in increased proliferation, attachment, and osteogenic activities of cells. The BMP-2 release from the nanofibers (SK-PCL/PVA-BMP-2) exhibited release kinetics similar to zero order. The SK-PCL/PVA group resulted in more bone formation (24.57 ± 3.81%) compared to the control group (1.21 ± 0.23%). The best results were obtained in case of SK-PCL/PVA-BMP-2 group (76.38 ± 4.13%), which showed more bone formation compared to PCL/PVA-BMP-2 group (39.86 ± 5.74%), suggesting that the hierarchical nanostructured surfaces on BMP-2-loaded core-shell nanofibers promoted osteointegration. In another study, collagen nanofibers were assembled in the pores of electrospun membranes of VEGF-loaded hyaluronan-PLA to obtain hierarchical micro/nanofibrous membranes providing the sustained release of VEGF for periosteal regeneration (Wu et al. 2020).

13.5.1.3 Gels/Hydrogels

Hydrogels are interesting candidates as carriers for growth factor/drug delivery in bone regeneration as they possess ECM-mimicking structure with 3D-interconnected porous networks and provide suitable environment for cell growth and sustained release of biomolecules/drugs (Bai et al. 2018). Both natural and synthetic polymers have been investigated as hydrogels or polymeric scaffolds for bone regeneration applications. Growth factors/drugs can be incorporated into hydrogels either by noncovalent interactions or covalent bonds to control the release kinetics. The hydrogel-based scaffolds can be tailored to improve and control various properties, like porosity, stiffness, drug encapsulation efficiency,

injectability, biodegradation, and stimuli-responsive nature (Bentz et al. 1998; Ham et al. 2016; Bai et al. 2018; Kim et al. 2020).

Glycosaminoglycans (GAGs) were modified with the pendent aldehyde groups to tune the degradation of hydrogels and release of growth factors from the matrix (Wang et al. 2013). The physical properties and release of BMP-2 from pegylated fibrinogen (PF) hydrogels containing encapsulated BMP-2 protein were controlled by varying the size and amount of PEG-diacrylate linker (Ben-David et al. 2013). Comparable PEG/albumin hydrogels were also investigated for BMP-2 release in bone defects (Kossover et al. 2020) and PVA/heparin-based hydrogels were prepared with hydrazone linkages for the pH-controlled release of VEGF (Roberts et al. 2015). Keratin hydrogels were tuned for erosion and controlled delivery of insulin-like growth factor-α. Mixtures of keratoses and keratein were cross-linked using disulfide chemistry to control the erosion of hydrogels and control the growth factor release (Ham et al. 2016). Injectable hydrogels have been explored as minimum-invasive drug delivery carriers in bone regeneration. Injectable CaSO$_4$/FGF-18 incorporated within chitin-PLGA hydrogels have been reported for the sustained release of FGF-18 and almost-complete bone healing of cranial bone defect (Sivashanmugam et al. 2017). Hydroxypropyl guar-graft-poly(N-vinylcaprolactam) copolymer was synthesized by graft polymerization to impart thermoresponsive properties in the hydrogels to control the drug delivery (Parameswaran-Thankam et al. 2018). Interpenetrating thiolated PEG-diacrylate hydrogel networks (IPN) incorporated with either polycation-based coacervates or gelatin microparticles were explored for maintaining the bioactivity and sustained release of BMP-2 to achieve enhanced skull bone regeneration (Kim et al. 2018c).

Impact of cross-linking chemistry was observed on bone formation and BMP-2 release, and hydrazone cross-linked hydrogels showed higher bone formation than thiol-Michael cross-linked hydrogels (Paidikondala et al. 2019). Ao et al. reported a fibrin glue/fibronectin/heparin-based hydrogel as a promising delivery system for BMP-2 to induce bone formation in calvaria critical-sized defects. The release of BMP-2 from these hydrogels was largely dependent on degradation (Ao et al. 2020). Several complex hydrogel-based scaffolds fabricated by incorporating nanoparticles or nanofibers have been investigated for the growth factor/drug delivery in bone regeneration. Lee et al. fabricated a patterned scaffold system containing PCL/gelatin fibers and PEG hydrogel micropatterns using a combination of electrospinning and photolithography techniques for the controlled and sequential release of multiple growth factors (Lee and Koh 2014). Firstly, glutaraldehyde-cross-linked PCL/gelatin nanofibers were fabricated to form electrospun scaffold, followed by drop casting of PEG-diacrylate and 2-hydroxy-2-methylpropiophenone (HOMPP) precursors, and placing a photomask array of microwells over them (Fig. 13.14). The scaffolds were exposed to UV light and unreacted precursors were removed by washing with water to obtain hydrogel-incorporated patterned nanofibrous scaffolds. FGF was adsorbed on fibers, while BMP-2 was entrapped in the hydrogel matrix for the sequential delivery of low doses of FGF initially and long-time sustained release of BMP-2 to promote osteogenic differentiation of hMSCs. Injectable hydrogel scaffolds fabricated by encapsulating chitosan
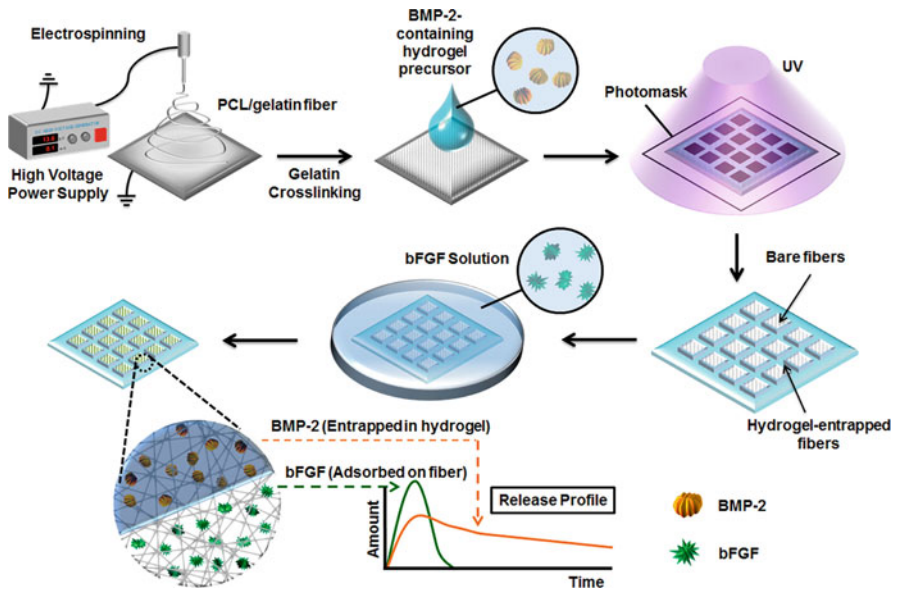
Fig. 13.14 Micropatterned hydrogel-nanofiber scaffolds for growth factor delivery in bone regeneration. (Reproduced with permission from Lee and Koh 2014)

microparticles as carriers of BMP-9 in VEGF-loaded thermosensitive hydrogels have also been explored for bone regeneration (Gaihre et al. 2019). The complex hydrogel system containing methylcellulose and alginate cross-linked with calcium and loaded with chitosan microparticles showed increased efficiency of loaded growth factors at the target site and resulted in enhanced osteogenic activities of hMSCs.

Subbiah et al. developed an injectable microparticle-incorporated hydrogel system for VEGF and BMP-2 delivery in a composite injury model (Subbiah et al. 2020). BMP-2-loaded heparin methacrylamide microparticles (HMP) were incorporated in VEGF-encapsulated Ca-alginate gels. This system containing VEGF encapsulated in alginate resulted in an early release by day 7, while maintaining the controlled release of BMP-2 from the HMPs. Although the system was capable to provide the tunable release of growth factors, the results showed that VEGF alone was not significant to enhance the bone-healing effects of low-dose BMP-2 irrespective of simultaneous or tunable release kinetics in a critical composite injury. Hydrogels hold a great promise so far as the encapsulation and sustained delivery of growth factors/proteins are concerned but their brittle structure and low cell infiltration capabilities have created a need to investigate other systems such as cryogels to overcome these challenges, as they possess highly interconnected macroporous structures (Kim et al. 2018b; Lee et al. 2020). Polymeric cryogels are formed at a subzero temperature by lyophilizing the ice crystals during cryogelation process. Lee et al. developed a double cryogel (DC) system for the dual delivery of growth factors with different release kinetics. The DC system was fabricated by
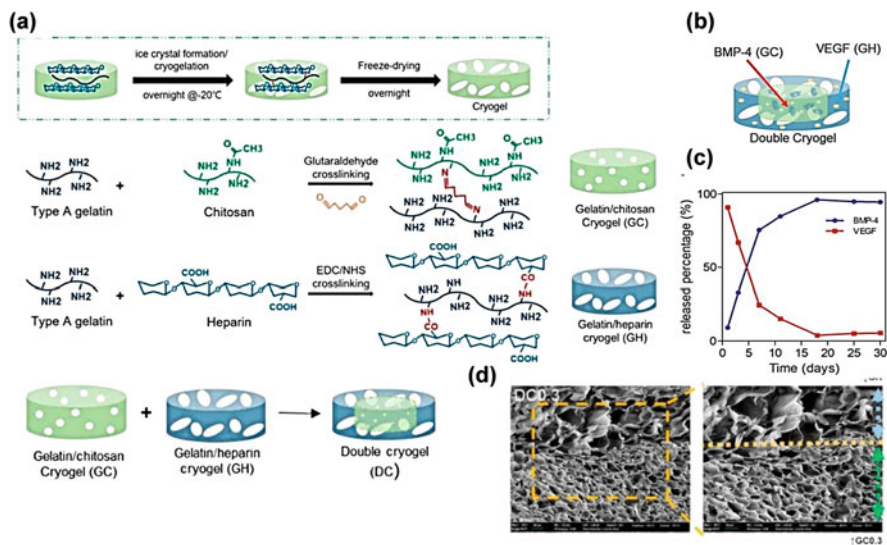
Fig. 13.15 Dual cryogel (DC) system for the sequential release of growth factors. (Reproduced with permission from Lee et al. 2020.) (**a**) Fabrication of gelatin/chitosan (GC) cryogel, gelatin/heparin (GH) cryogel, and double cryogel (DC). (**b**) Double cryogel (DC) incorporated with BMP-2 and VEGF. (**c**) Percent release from DC (fabricated using 0.3% w/v chitosan) for 30 days. (**d**) FESEM images of DC

surrounding BMP-2-loaded gelatin/chitosan (GC) cryogels with VEGF-loaded gelatin/heparin (GH) cryogel (Lee et al. 2020). The DC cryogels exhibited a highly porous network and the outer layer of DC resulted in the initial release of VEGF to stimulate angiogenesis and vascularization in the defect, whereas the inner GC layer led to sustained release of BMP-2 to induce osteogenesis (Fig. 13.15). The sequential and sustained release of growth factors from the DC gels showed good outcomes in bone regeneration in critical-sized cranial defect models.

13.5.1.4 3D-Printed Scaffolds

Three-dimensional (3D) printing is currently the most explored technique for the development of advanced scaffolds with appropriate functions to treat bone fractures or defects (Wang et al. 2020). The printed scaffolds provide several advantages over conventional scaffolds, such as customization of shape, pore size, and tunability of mechanically properties (Kim et al. 2010; Schüller-Ravoo et al. 2011; Bose et al. 2013). The scaffolds closely mimic the multiscale structure of tissues and provide the local and sustained release of growth factors/drugs. Various additive manufacturing approaches (AM) including 3D printing, rapid prototyping, solid freeform fabrication, direct digital manufacturing, selective laser sintering, stereolithography, and 3D plotting are used to fabricate such scaffolds (Bose et al. 2003, 2018; Chia and Wu 2015; Guvendiren et al. 2016). The advancement in 3D printing techniques has provided versatile scaffolds with new functions. These

techniques provide precise control over complex pore geometries, size, and inter-connectivity of pores, thereby providing control over overall mechanical strength of scaffold and cell infiltration (Bose et al. 2013; Wang et al. 2020). They also provide flexibility to prepare patient-specific predesigned scaffolds that can match the bone defect size and geometry (Bonda et al. 2015; Geven et al. 2015). The surface of scaffolds can be modified and growth factors/drugs can be incorporated by physical or chemical cross-linking.

Several 3D-printed polymeric scaffolds have been investigated. PCL-based 3D architectures were prepared through 3D plotting and wet spinning, and loaded with BMP-2/9 to investigate the effect of scaffold architecture on growth factor delivery and bone regeneration. The result indicated that the architecture did not affect the release kinetics (BSA) but the proliferation and osteogenic differentiation of MSCs (Yilgor et al. 2010). The 3D-printed alginate scaffolds cross-linked using $CaCl_2$ and incorporating BMP-2-loaded gelatin microspheres were investigated and these tissue-engineered bioprinted constructs were found effective in promoting osteo-genic differentiation in vitro and in vivo through controlled release of BMP-2 (Poldervaart et al. 2013). The internal vascularization of scaffolds with high osteoinductive properties closely mimicked the bone developmental stage. Using this approach, Yan et al. investigated a 3D-printed biodegradable scaffold fabricated using layer-by-layer assembly technique for the control release of deferoxamine (DFO) (Yan et al. 2019), which is an iron chelator and used as a hypoxia mimic to induce vascularization of scaffolds and activate HIF1-α, resulting in the activation of proangiogenic gene cascades. It may also induce osteogenic differentiation of preosteoblast cells. The 3D-printed PCL scaffolds were modified with amine groups followed by carboxymethyl chitosan (CCS) and loaded with DFO, as shown in Fig. 13.16. These scaffolds showed high biocompatibility and mechanical properties matching the cancellous bone, promoted vascularization, and enhanced bone forma-tion at the defect site. Several other 3D-printed PEG, PCL, and PLA scaffolds have been developed for the controlled/sustained release of growth factors and drugs and showed promising outcomes in bone regeneration (Li et al. 2018; Kim et al. 2018a; Kondiah et al. 2020; Yao et al. 2020).

The 3D-printed scaffolds have been employed for the dual delivery of growth factors and/or drugs. Teotia et al. fabricated alendronate (ZA) and BMP-loaded 3D scaffolds using layer-by-layer solidification of photo-cross-linkable poly (trimethylene carbonate) resin containing high ratio of ceramics. Next, the scaffolds were filled with microporous cryogels to enhance the overall surface area that resulted in the enhancement in overall surface area for cell infiltration. Their studies indicated the potential application of BMP and ZA-loaded 3D-printed scaffolds in critical-sized long bone and cranial defect healing (Teotia et al. 2020). Wang et al. have investigated the dual delivery of angiogenic and osteogenic peptides using cryogenic 3D printing of scaffolds (Wang et al. 2021). The scaffolds were fabricated by cryogenic 3D printing of osteogenic peptide containing water/PLGA/emulsion and β-tricalcium phosphate, followed by surface coating of angiogenic peptides. They showed hierarchical porous structure that was mechanically comparable to cancellous bone. Improved angiogenesis and osteogenic effects were obtained due to the quick release of angiogenic peptide and sustained release of osteogenic peptide.
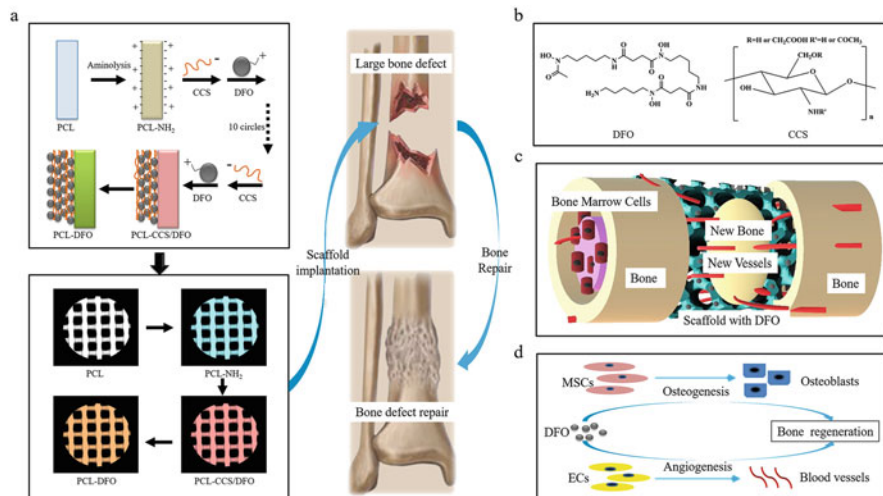
Fig. 13.16 Deferoxamine (DFO) loading on the surface of 3D-printed PCL scaffold and its effect on bone regeneration (reproduced with permission from Yan et al. 2019). (**a**) Fabrication of DFO-loaded PCL scaffolds by surface aminolysis and layer-by-layer assembly of carboxymethyl chitosan (CCS). (**b**) Chemical structure of DFO and CCS. (**c**) The angiogenic and osteogenic effects of PCL-DFO scaffold. (**d**) The mechanism of bone regeneration promoted by DFO in mesenchymal cells (MSCs) and vascular endothelial cells (ECs)

13.5.2 Stimuli-Responsive Delivery Systems

Polymer science and engineering has enabled scientists to overcome challenges associated with the polymeric biomaterials in biomedical applications. Advancement in this field has led to the design and development of a new class of polymers that changes their physical and/or chemical properties when exposed to an external stimuli (Hoffman 1995; Wei et al. 2017). These smart/intelligent/stimuli-responsive polymers have been explored for on-demand growth factor/drug in bone regeneration. Such polymers respond to change in pH (Kocak et al. 2017), temperature (Doberenz et al. 2020), magnetic field (Manouras and Vamvakaki 2017), and the presence of specific biomolecules (Xu et al. 2018a). The stimuli-responsive elements included in the delivery systems often mimic natural conditions (Korde and Kandasubramanian 2019). Various polymeric materials in combination with other materials have been engineered to provide smart drug delivery systems as per the requirements. Usually a sensitive/stimuli-responsive moiety is incorporated in polymeric backbones. Different types of smart polymeric systems under investigation for growth factor/drug delivery in bone tissue engineering will be discussed in this section.

13.5.2.1 pH Responsive

The polymers containing acidic or basic moieties are capable of accepting or releasing protons in response to the change in pH and, therefore, considered as
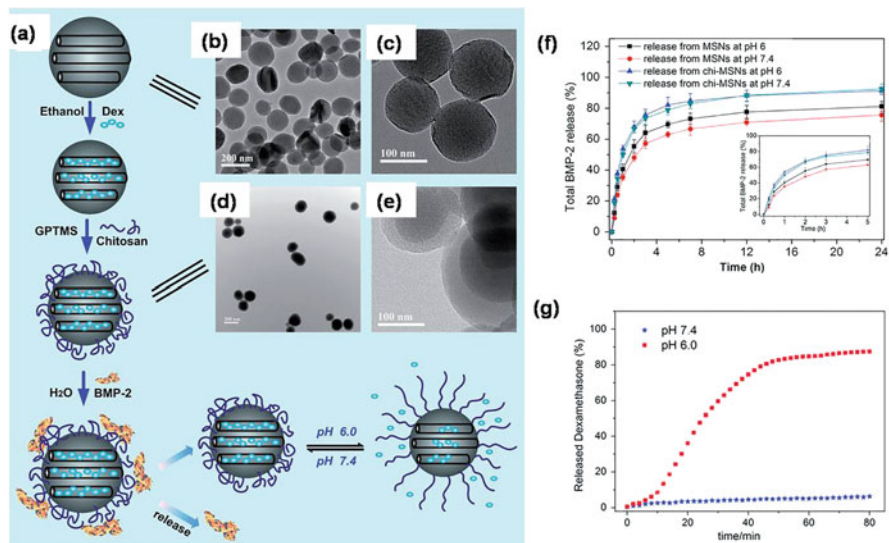
Fig. 13.17 (**a**) Dexamethasone/BMP-2@chi-MSNs synthesis for the controlled release of BMP-2 and pH-sensitive drug release. (**b**, **c**) TEM and HRTEM images of MSNs. (**d**, **e**) TEM and HRTEM images of chi-MSNs. (**f**) In vitro release profiles of BMP-2. (**g**) In vitro release profiles of drug. (Reproduced with permission from Gan et al. 2015a)

pH-responsive polymers (Kocak et al. 2017). Such materials are used in drug delivery applications. The accumulation of lactic acid at the fracture site due to interrupted blood supply may result in low pH (Zhang et al. 2017) and prolonged inflammatory responses due to the bone diseases, such as rheumatoid arthritis and infection, may also alter the pH of bone tissue. The resorption by osteoclasts also creates an acidic microenvironment (Arnett and Dempster 1986). Consequently, pH-responsive growth factor/drug delivery can be employed in such conditions. Various polymers, gels/hydrogels, microparticles, and polymeric coatings with pH-responsive functional groups and/or linkages have been investigated. Chitosan is among the most extensively investigated polymer because chitosan-based materials show pH-responsive properties due to the presence of free amine groups in the polymer structure. The noncovalent interactions with the functional groups of chitosan facilitate the loading of growth factors and drugs.

Mesocellular foam-based nanocarriers were fabricated by cross-linking glycidoxypropyltrimethoxysilane (GPTMS) with *N*-carboxymethyl chitosan for the pH-sensitive release of BMP-2, while maintaining its bioactivity (Gan et al. 2015b). Chitosan (Chi)-coated mesoporous silica nanoparticles (MSNs) were developed for the dual delivery of BMP-2 and dexamethasone and it resulted in significantly increased osteoblastic differentiation in vitro and in vivo (Gan et al. 2015a). The drug was loaded into the hydrophobic nanochannels of MSNs, which were coated with chitosan and cross-linked using GPTMS, followed by the physical coating of BMP-2 on chitosan surface (Fig. 13.17). BMP-2 was released immediately from the

surface of chitosan-MSNs (Chi-MSNs), whereas the drug was released after the endocytosis in cells. The low pH value resulted in change in the dispersing states of chitosan due to the protonation of amino groups; thus, resulting in the drug release into the cytosol. Luca et al. investigated the chitosan and hyaluronic acid-based hydrogels for BMP-2 delivery and observed that the nature of carrier and formulation pH influences the formation of bone volume and quality (Luca et al. 2010). Polyelectrolyte multilayer (PEM) systems have been explored as pH-responsive systems. PEM systems fabricated using layer-by-layer deposited poly(β-aminoester) (PBAE) and chondroitin sulfate (CS) for BMP-2 release resulted in enhanced preosteoblast differentiation than free BMP (Macdonald et al. 2011). In another study, combined VEGF and BMP-2 release resulted in 33% higher bone density in the de novo bone compared to BMP-2 alone (Shah et al. 2011). Poly-L-lysine (PLL)/ hyaluronic acid (HA) PEMs also released BMP-2 (Crouzier et al. 2011) while preserving its secondary structure in films (Gilde et al. 2012), and hydrated and dry coatings on Ti surfaces (Guillot et al. 2013). The BMP-2 adsorbed on anodized Ti surface followed by poly-L-histidine and poly(methacrylic acid) coatings on it showed high levels of protein release over several months while preserving its bioactivity (Salvi et al. 2016). The protection of growth factors and maintaining the concentrations in therapeutic window are critical to obtaining the desired outcomes. Polymeric nanocapsules protecting internally loaded growth factors for pH-sensitive release have been reported (Yan et al. 2010; Tian et al. 2016).

Deng et al. reported 3D-printed polyetheretherketone (PEEK) scaffolds for pH-responsive delivery of Ag^+ for in vivo antibacterial activity and Ca^{2+} release for bone in growth and osseointegration in infective bone defects (Deng et al. 2020). The Ag/apatite-integrated PEEK scaffolds were prepared by multilayer coatings of polydopamine (pDA), silver nanoparticles (AgNP), and apatite. In presence of acidic conditions, the solubility of apatite increased; thus, enhancing the delivery of Ca^{2+} and PO_4^{3-} ions, resulting in osteoinduction. The calibrated release of Ag^+ with these ions provided a synergistic antibacterial and osteogenic local environment and the attachment of bacteria to the implant surface resulted in the production of lactic or acetic acid, leading to a pH reduction; thus, providing the stimulus for ion release.

13.5.2.2 Temperature Sensitive

Thermoresponsive polymeric materials modulate their properties in response to change in temperature. Such hydrogels are promising in tissue engineering applications as they can show sol-to-gel conversion and change in their swelling and degradation behavior when subjected to a temperature change. Thermoresponsive polymers and cross-linkers allow injectability and in situ gelation of hydrogels. Most thermoresponsive polymers used in tissue engineering applications have lower critical solution temperature (LCST) (Schild and Tirrell 1990; Klouda 2015) and form hydrogels on increasing the temperature. Polymers like chitosan, poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN), hydroxypropyl guar-graft-poly(N-vinyl caprolactam) (HPG-gPNVCL), PCL, poly (ε-caprolactone-co-1,4,8-trioxa-[4.6]spiro-9-undecanone)-poly(ethyleneglycol)-poly(ε-aprolactone-co-1,4,8-trioxa[4.6]spiro-9-undecanone)(PECT), poly

(ε-caprolactone-co-lactide)-*b*-poly(ethyleneglycol)-*b*-poly(ε-caprolactone-co-lactide), poly(NIPAM-*co*-DMAEMA), and cellulose have been investigated as thermoresponsive systems for growth factor and drug delivery in bone regeneration (Liu et al. 2014; Morochnik et al. 2018; Müller et al. 2018; Parameswaran-Thankam et al. 2018; Chen et al. 2019; Gaihre et al. 2019; Maturavongsadit et al. 2020).

The macroporous hydrogels show good cell penetration but often lead to rapid burst release of drugs. Dexamethasone-glycidyl methacrylated dextran (Dex-GMA)/gelatin scaffolds containing microspheres loaded with BMP have been used for temperature-sensitive release (Chen et al. 2007). The incorporation of microspheres into hydrogels resulted in the sustained release. The thermoresponsive properties of matrix provided control over the growth factor/drug release. Besides growth factor delivery, the most challenging task in this field is to design and develop smart and minimally invasive scaffolds for irregular bone defects. Stimuli-responsive shape memory scaffolds/hydrogels have provided a solution to this problem. Liu et al. developed a PCL and hydroxyapatite-based porous scaffold loaded with BMP-2 that can be compressed to a small volume using hot compression, which can subsequently regain its original shape at body temperature. These scaffolds showed controlled release of BMP-2 and promoted new bone formation on implantation in rabbit mandibular bone defects (Liu et al. 2014). Gaihre et al. developed an injectable scaffold by using the combination of chitosan microparticles (MPs) with thermoresponsive methylcellulose (MC) gels loaded with BMP-2 and VEGF. MPs were loaded with BMP-9 and incorporated in the gel, whereas VEGF was introduced within the gel matrix (Fig. 13.18). The temperature-sensitive gelation at physiological temperature resulted in an injectable growth factor delivery system. The release of VEGF from these scaffolds was higher compared to BMP-9. The BMP-9-VEGF-loaded MP gels significantly enhanced the subcutaneous and cranial bone formation as observed from the micro-CT images at 6 and 12 weeks of implantation (Gaihre et al. 2019).

Kim et al. developed poly(ε-caprolactone-*co*-lactide)-*b*-poly(ethylene glycol)-*b*-poly(ε-caprolactone-*co*-lactide)- and *O*-phosphorylethanolamine-conjugated alginate hydrogels that can transform to hydrogels at physiological temperature (37 °C) (Kim et al. 2020). The thermoresponsive property of these bioconjugates enabled their subcutaneous administration as sols at lower temperature into the dorsal region of Sprague-Dawley rats and in situ hydrogel formation at 37 °C. These in situ hydrogels demonstrated the sustained release of BMP-2 and biomineralization in in vitro and in vivo. Thus, thermoresponsive hydrogels hold potential as injectable drug delivery carriers in bone regeneration.

13.5.2.3 Biomolecule Sensitive

Biomolecules, such as enzymes, play important roles in the biological activities of bone and other tissues with injury or disorders. Matrix metalloproteinases (MMPs) are found at the injury site and play a crucial role in ECM remodeling. A provisional fibrin-rich matrix containing growth factors is formed by platelets, neutrophils, and macrophages after the injury. It is degraded by MMPs and growth factors regulate vascularization and tissue regeneration in that region (Ravanti and Kähäri 2000).
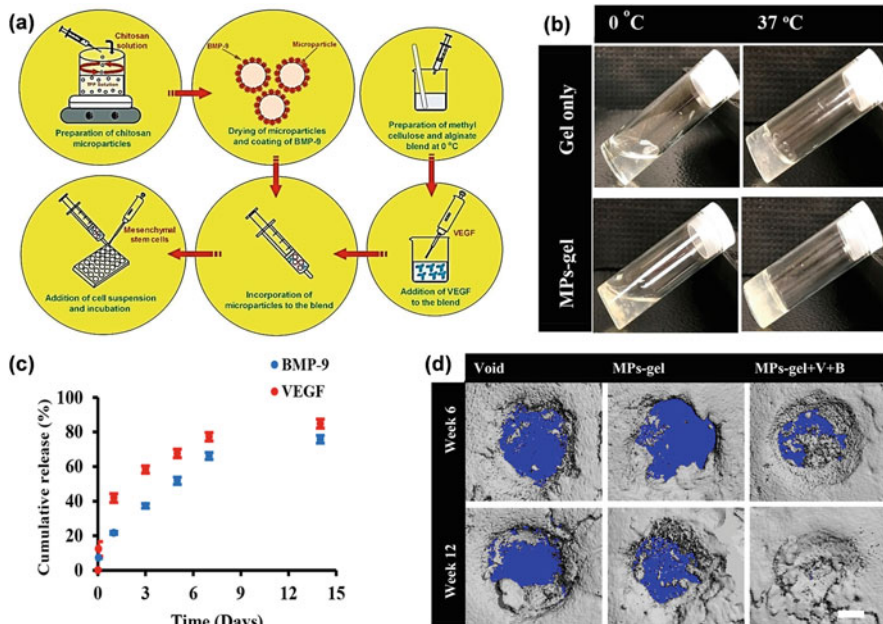
Fig. 13.18 Thermoresponsive MP gels for BMP-2 and VEGF delivery (reproduced with permission from Gaihre et al. 2019). (**a**) Fabrication of MP gels. (**b**) Thermoresponsive sol-to-gel conversion. (**c**) BMP-2 and VEGF release from MPs gel scaffolds. (**d**) Micro-CT analysis of cranial defects of rats after 6 and 12 weeks

MMP expressions are found to be high in bone and cartilage under several pathological conditions, like rheumatoid arthritis, osteoporosis, infection, and osteoarthritis. Enzyme-sensitive biomaterial matrix has been developed for the on-demand release of growth factors and drugs. Lutolf et al. synthesized a MMP-sensitive BMP-2-loaded PEG-based hydrogel to regenerate bone within an orthotopic model (Lutolf et al. 2003). The proteolytic degradation of gels by MMP-2 resulted in 100% BMP-2 release, which was only 10% in saline, demonstrating that the release kinetics was degradation dependent. These BMP-2-loaded MMP-sensitive hydrogels improved the bone-healing outcomes in comparison to hydrogels without MMP-sensitive activities or without BMP-2.

MMP-sensitive BMP-2-loaded polymeric delivery vehicles were fabricated using thiol-ene chemistry and showed improved bone formation in critical-sized bone defects in comparison to adsorbable collagen sponge (Mariner et al. 2013). In another approach, MMP-sensitive difunctional peptides were used for cross-linking maleimide-modified hyaluronic acid polymers to fabricate hydrogels capable of protease-mediated degradation and BMP-2 release (Holloway et al. 2014). BMP-2 release from these hydrogels increased with a decrease in cross-linking density of polymers or an increase in collagenase concentration. BMP-2-loaded hyaluronan
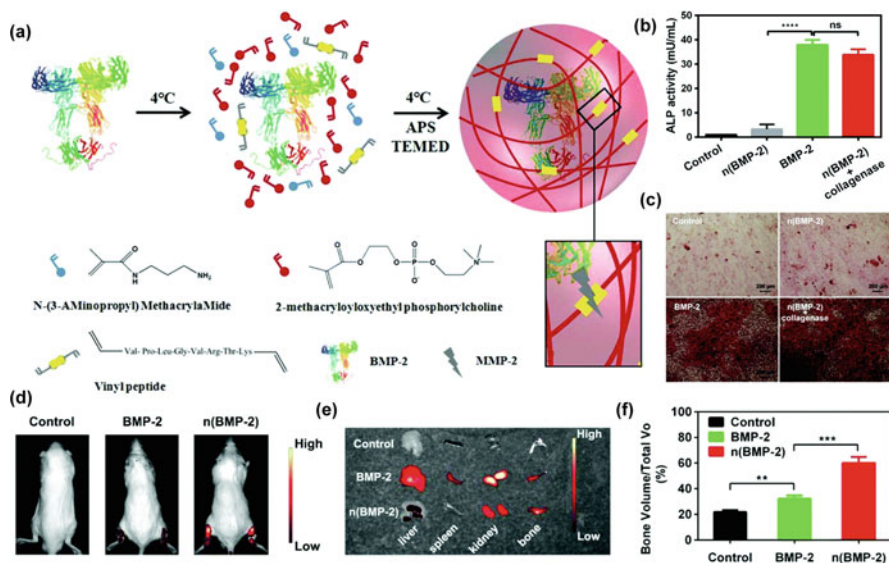
Fig. 13.19 MMP-sensitive polymeric nanocapsules for BMP-2 delivery at the fracture site. (Reproduced with permission from Qi et al. 2019.) (**a**) BMP-2-loaded nanocapsule formation and cleavage of MMP-sensitive peptide linker by MMPs. (**b**) ALP activities of MSCs incubated with native BMP-2 and n(BMP-2) (****$P < 0.0001$). (**c**) Alizarin red staining to visualize calcium nodule accumulation in MSCs at 21 days. (**d**) In vivo fluorescence images of tibial injury. (**e**) BMP-2 and n(BMP-2) distribution in main tissues and the tibial injury site after intravenous injection of native BMP-2 and n(BMP-2). (**f**) Semi-quantitative analysis of the bone tissue volume per total tissue volume (BV/TV) after 2 weeks of therapy (**$P < 0.01$, ***$P < 0.001$)

hydrogels covalently linked with bisphosphonate (BP) ligands have been investigated for proteolytic degradation-mediated enzyme-responsive release of BMP-2 (Hulsart-Billström et al. 2013). These hydrogels exhibited less than 10% BMP-2 release over 2 weeks and preserved its bioactivity, resulting in the induction of osteogenic differentiation. Chondroitin sulfate and PEG-based hybrid hydrogel system were incorporated with MMP-sensitive lysine peptides for MMP-sensitive degradation leading to tunable BMP-2 release and increased osteogenic activities (Anjum et al. 2016).

Polymeric nanocapsules have emerged as another interesting candidate for enzyme-sensitive release of growth factors. BSA-based nanocapsules have been reported for the enzyme-responsive release of VEGF (Wen et al. 2011; Qi et al. 2019). Qi et al. reported the effectiveness of enzyme-responsive release of BMP-2 from systematically administered polymeric nanocapsules for promoting bone regeneration (Qi et al. 2019). The monomer 2-(methacryloyloxy)ethyl phosphorylcholine (MPC) and the MMP-sensitive bisacryloylated VPLGVRTK peptide along with BSA were used for the fabrication of these capsules by in situ free radical polymerization method in presence of ammonium persulfate (APS) and 10% N,N,N',N'-tetramethylethylenediamine (TEMED) (Fig. 13.19). These nano-

BMP capsules (n(BMP)) retained the structure and function of BMP-2 during the circulation time (~48 h) and accumulated at the fracture site through malformed blood vessels. The accumulation of native BMP-2 was very less, as they were rapidly eliminated (~30 min) from the circulation. It was due to the reduced adsorption of serum proteins on nanocapsules that the uptake by macrophages decreased in comparison to native BMPs. At the fracture site, the degradation of n (BMPs) by MMPs led to the release of BMP-2, resulting in efficient repair of bone. The in vitro studies showed increased ALP activities and calcium deposition at 21 days suggesting the efficiency of these nanocapsules in growth factor release. The biodistribution of native BMP and n(BMP) showed that the nanocapsules reduced the elimination of BMP-2 by liver, spleen, and kidney and in vivo studies showed significantly increased bone formation by n(BMP). Overall, these nanocapsules provide a promising approach for the systemic delivery of growth factors for fracture healing.

Beside enzymes, glucose has been used as a stimulus for the growth factor delivery. Xiao et al. developed glucose-sensitive core cell nanofibers for BMP-2 release to treat mandible defects in diabetic rats (Xiao et al. 2019). The inner core layer of fibers was formed using BMP-2-loaded polyethylene oxide, whereas the outer shell layer comprised of polyvinyl alcohol (PVA) cross-linked *N*-(2-hydroxyl) propyl-3-trimethylammonium chitosan chloride (HTCC). The immobilization of glucose oxidase on these nanofibers imparted glucose sensitivity to the material. It reacts with glucose and converts it into gluconic acid resulting in a decreased pH. HTCC can sense the pH, leading to swelling or shrinkage of the matrix for growth factor release.

13.5.2.4 Redox Responsive

Reactive oxygen species (ROS) are generally produced during inflammation in response to the immune system against the pathogenic microbes and foreign agents (Kim and Wong 2013). Elevated levels of ROS have been detected in bone-related disorders, leading to tissue damage and impaired healing (Porto et al. 2015).

Redox-responsive systems have been investigated for the growth factor/drug release in bone tissue engineering. A study reported that injectable hydrogels fabricated from thiolated PEG precursors via disulfide bond were able to release the bioactive molecules upon encountering a reductant, such as glutathione (GSH). GSH is commonly present in the ECM and secreted in excess, when the level of ROS becomes high at the injury microenvironment. These hydrogels were able to provide sustained release of BMP-2, resulting in an increased ectopic bone formation (Yang et al. 2014). Gong et al. fabricated redox-sensitive poly(ethylene oxide) (PEO)-PCL nanofibers with a core-shell structure for BMP-2 delivery in the bone defect (Gong et al. 2018). PEO loaded with BMP-2 forms the inner core region of the nanofiber, whereas 6-arm PEG-PCL/6-arm PEG-PCL-sulfhydryl nanogels (c-6A PEG-PCL/6A PEG-PCL-SH NGs) containing disulfide linkages form the outer shell of the nanofibers. The outer shell of nanofibers responds to GSH concentration leading to nanogel degradation and increased permeability of shell, resulting in the release of BMP-2 from the inner core region in vitro and in vivo (Fig. 13.20). BMP-2 release
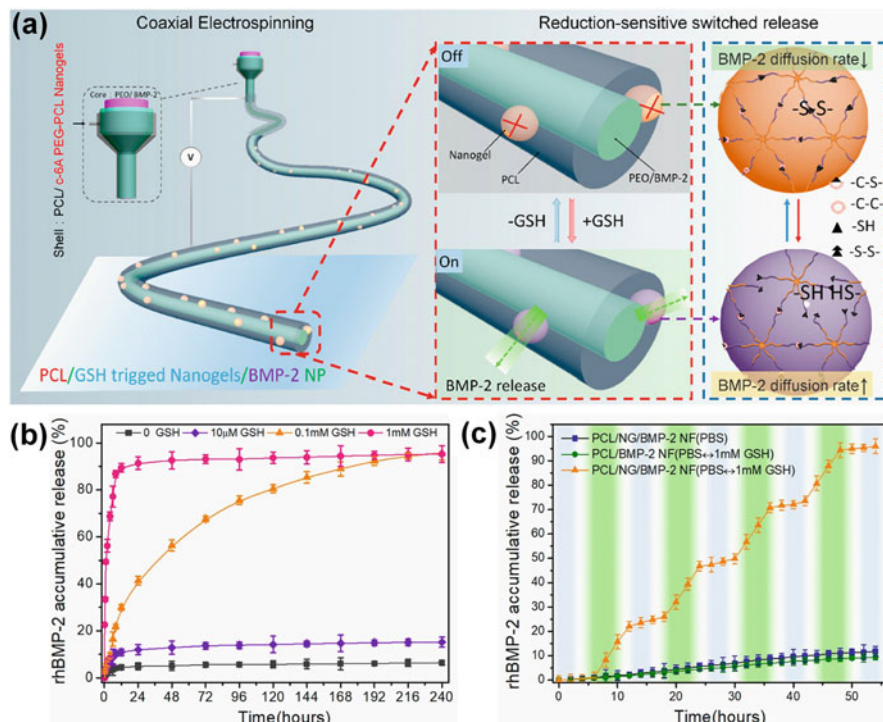
Fig. 13.20 Redox-sensitive BMP-2 release from nanofibers with core-shell nanostructure. (Reproduced with permission from Gong et al. 2018.) (**a**) Fabrication of nanogel-in-nanofiber device via coaxial electrospinning and redox-responsive release of BMP-2 mediated via outer shell degradation. (**b**) Release profiles of BMP-2 from the nanofibers at different concentrations of GSH. (**c**) BMP-2 release from nanofibers on increasing GSH at fixed intervals

from the inner core of nanofibers increased with an increase in GSH concentration- 10 μM, 0.1 mM, and 1 mM GSH, which resulted in 13 ± 1.5, 55 ± 2.5, and $90 \pm 2.9\%$ of BMP-2 release after 48 h of incubation. The redox switched behavior of nanofibers was monitored by incubating in GSH for 8 h, PBS for 8 h, and GSH for four cycle tests, and resulted in a sharp increase in BMP-2 concentration in GSH and slight increase in PBS for all cycles. The nanogels released $10\% \pm 3.2\%$ BMP-2 after four cycles, whereas the cumulative release from the PCL/NG/BMP-2 NF was observed to be $94.5 \pm 2.19\%$.

13.5.2.5 Magnetic/Electromechanical/Light Responsive

The polymeric biomaterials have been incorporated with external physical stimuli, like magnetic field, electric field, light, mechanical strain, and ultrasound, to remotely trigger the release of therapeutic payloads (Bansal and Zhang 2014; Linsley et al. 2015; Manouras and Vamvakaki 2017; Wang et al. 2018; Madani et al. 2020; Lanier et al. 2020). A study reported near-infrared (NIR) light-triggered release of Sr^{2+} ions from PLGA loaded with $SrCl_2$ and black phosphorous
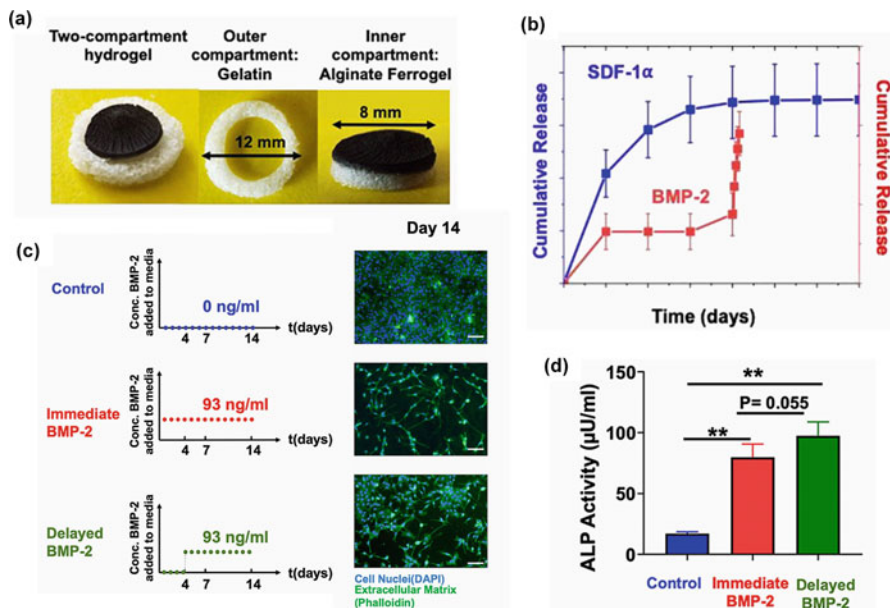
Fig. 13.21 Magnetic stimuli-responsive delivery of BMP-2 and SDF-1α from two compartment hydrogel system to recruit mouse mesenchymal stem cells (mMSCs). (Reproduced with permission from Madani et al. 2020.) (**a**) Images of two compartment magnetic-responsive gels. (**b**) Cumulative SDF-1α release from outer compartment of gel. (**c**) Magnetically triggered delayed delivery of BMP-2 from inner compartment of gels and its effect on 2D cell culture at 14 days. (**d**) ALP activities at 14 days. **Indicates statistically significant difference ($p < 0.01$)

(BP) nanosheets for excellent bone regeneration capabilities (Wang et al. 2018). BP was incorporated in BP-SrCl$_2$/PLGA microspheres for the NIR-responsive behavior. The rapid increase in local temperature by NIR radiation leads to an increase in glass transition temperature (Tg) of PLGA, causing the collapse and cracks in microspheres, resulting in Sr^{2+} release. Magnetically responsive two-compartment biomaterial systems were investigated for on-demand control over sequential delivery of BMP-2 and SDF-1α to optimize the bone regenerative outcomes (Madani et al. 2020). The two-compartment biomaterial consisted of an outer compartment made-up of hollow cylindrical gelatin gels (outer diameter of scaffold: 12 mm) and inner compartment made-up of 8 mm alginate ferrogels (Fig. 13.21). The outer compartment was loaded with SDF-1α and inner compartment with BMP-2. The release of SDF-1α from outer compartment resulted in the recruitment of cells, whereas the release of BMP-2 from inner compartment was magnetically controlled. This system was capable of releasing BMP-2 in a delayed manner in response to magnetic field applied; thus, providing a control over the timing between bone progenitor recruitment by SDF-1α and release of BMP-2 for osteodifferentiation. The ALP activities of cells provided with delayed release of BMP-2 at 14th day were significantly higher than that of cells with immediate release of BMP-2 and control, suggesting the potential of this system in bone regeneration.

13.6 Conclusions

Polymeric materials and nanomaterials fabricated from both natural and synthetic polymers have been employed as drug delivery carriers to enhance bone regeneration. Advancement in polymer engineering has resulted in the development of diverse polymeric structures and smart drug delivery systems capable of tunable local delivery of growth factors/drugs to the defect site along with providing an ECM-mimicking structure to support cell growth and infiltration to enhance the osteogenic outcomes. Growth factor/drug-loaded polymeric systems, like polymer-drug bioconjugates, nanoparticles, microparticles, micelles, nanofibers, hydrogels, polymeric scaffolds, multicomponent polymeric systems, and 3D-printed scaffolds, have shown promising results both in vitro and in vivo. The smart polymeric systems have been also used for the on-demand release of growth factors/drugs upon exposure to a stimulus. Advanced drug-loading techniques, like core-shell nanofibers, microparticles in hydrogel systems, and layer-by-layer loading, have been employed using different polymer-based systems to control the drug concentration and release kinetics. Dual/multiple bioactive factors and their sequential release have been investigated in order to mimic the natural microenvironment and phases of bone healing. Although multifunctional growth factor/drug carriers have been developed to overcome the challenges associated with bone regeneration, the design and development of a carrier mimicking the natural bone healing remains a challenge. Bone healing is a complex process that requires the involvement of multiple bioactive molecules at various phases of healing and, therefore, it is highly desirable to investigate multifunctional and multiresponsive hybrid materials mimicking the bone-healing process to overcome the challenges of bone regeneration. The stability and cost of growth factors also remains another major concern and, therefore, several combinations of angiogenic and osteogenic factors/drugs should be investigated using the biomaterials that can enhance the osteogenic outcomes while reducing the side effects of drugs by providing the controlled/sequential and sustained release. Difference in size and structure of grafts required for different patients or fracture sites require patient-specific customized grafts. Multifunctional and multiresponsive 3D-printed scaffolds must be investigated further for desired outcomes in bone regeneration.

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