# In Situ Tissue Engineering: A New Dimension

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**Abstract**

Tissue engineering has evolved to provide ways to construct tissues primarily aiming at replacing lost or damaged tissues or improving function. It has been classically developed using ex vivo means in which cells are generally cultured with biomaterials and subsequently engineered constructs are transplanted into the body. However, this approach is associated with several challenges that have limited its successful translation to the clinic. With in situ tissue engineering, it is possible to stimulate internal body regenerative potential by using biomaterials, biomolecules, and genes, which can reduce risks and challenges associated with

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ex vivo tissue engineering. In addition, in situ tissue engineering may potentially accelerate the clinical application of the technology and may lead to the development of more effective regenerative therapeutics through a collaborative multidisciplinary approach.

**Keywords**

Biomaterials · Guided tissue regeneration · In situ · Regeneration · Tissue engineering

## 13.1 Introduction

Millions of people suffer from tissue loss or organ failure (Langer and Vacanti 1993). Organ transplantation is needed to replace failed organs, but this is limited due to the shortage of organ donors (Giwa et al. 2017). Therefore, scientists have been seeking ways to create biological substitutes by using tissue engineering (Kurniawan 2019a; Ashammakhi et al. 2021a). Tissue engineering was developed to enable the production of functional tissue constructs for the purpose of providing graft-like structures that can be used to replace or treat tissue defects or failed organs (Rouwkema et al. 2011). It has conventionally relied on seeding scaffolds with cells (Zhang et al. 2016; Gunther et al. 2015) or recently developed three-dimensional (3D) bioprinting of cell-laden constructs (Potyondy et al. 2021; Tavafoghi et al. 2021; Davoodi et al. 2020; Erdem et al. 2020; Shao et al. 2020) ex vivo, followed by the transplantation of engineered constructs to target sites in the body. However, engineered constructs often fail because cells die following transplantation due to the lack of vascular supply (Ashammakhi et al. 2020a; Chandra et al. 2020). The need to use cells from various sources and expand and seed them outside the body is associated with increased risks and concerns (Belk et al. 2020; Marks et al. 2016). On the contrary, in situ tissue regeneration is reliant on stimulation of body innate potential for regeneration, which can be achieved without the need for ex vivo procedures, and it can be achieved using biomaterials, biomolecules, genes, or cellular products (Ashammakhi et al. 2021b; Sengupta et al. 2014; Abdulghani and Mitchell 2019). In addition to reduced challenges and risks of complications, costs can also be reduced, and the path to regulatory body approval and clinical translation can be accelerated (Yang et al. 2020; Wissing et al. 2017; Gaharwar et al. 2020; Xia et al. 2021). Various approaches to in situ tissue engineering are presented and discussed in this chapter. Current challenges and new ideas for future developments are also highlighted.

## 13.2 Tissue Engineering

### 13.2.1 Concepts and Evolution of Tissue Engineering

The term "tissue engineering" was first coined at a National Science Foundation (NSF)-sponsored meeting in 1988 (Skalak and Fox 1988). Later, in 1993, it was defined by Langer and Vacanti as an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes for the repair or regeneration of tissue or organ functions (Langer and Vacanti 1993). After that, the number of studies in this area has exponentially increased (Viola et al. 2003). For instance, a study conducted by Cao et al. in 1997 drew much attention to tissue engineering. In this study, a three-dimensional a polymer construct containing cartilage cells was implanted in the subcutaneous tissue of a mouse, and the cartilage tissue formed in the sahpe of human ear was reported (Cao et al. 1997). Moreover, the skin substitute TransCyte, made by culturing foreskin fibroblasts on nylon mesh to improve wound healing, was approved by the Food and Drug Administration (FDA) in 1997. Tissue engineering was considered as a promising strategy for the engineering of various organs and tissues, ranging from musculoskeletal tissues such as bone (Cao et al. 2020) and cartilage (Yang et al. 2020) to cardiovascular tissues such as heart valves and blood vessels (Wissing et al. 2017). FDA-approved treatments developed by tissue engineering approach have made significant progress in the past two decades (Ashammakhi et al. 2021a; Hoffman et al. 2019).

Tissue engineering has proposed a new concept for repair, regeneration, and replacement of tissues with constructs made using cells, scaffolds, and signaling molecules (Langer and Vacanti 1993). Because these components could be made available in laboratories, this concept potentially is a safe and reliable source for clinical use and can contribute to addressing organ deficiency (Smits and Bouten 2018; Kurniawan 2019b). Cells for tissue engineering can be autologous primary cells or stem cells (Khademhosseini et al. 2020; Bajada et al. 2008; Vapniarsky et al. 2015). The use of autologous primary cells helps to avoid the risk of developing an adverse immune response. However, they have disadvantages, such as the need for an invasive procedure for their retrieval, which can be more of a problem, especially in the elderly or people with underlying diseases (Fodor 2003; Koh and Atala 2004). The use of stem cells with very high proliferation capacity is an excellent option to circumvent these limitations. These cells respond to chemical and mechanical signals from their microenvironment and differentiate into various tissues. Complete differentiation or removal of all stem cells before transplantation should be ensured because of the risk of uncontrolled or abnormal growth and tumor formation (Bedel et al. 2017; Howard et al. 2008).

Signaling molecules that include growth factors, peptides, and small molecules are used to guide cell behavior and promote them to regenerate new tissue. Growth factors are polypeptide molecules that interact with cell surface receptors and direct cellular behavior, such as proliferation and differentiation, by activating signaling cascades. For instance, growth factors such as bone morphogenetic proteins (BMPs) secreted by osteoblasts, chondrocytes, and osteoprogenitor cells are critical not only

for regulating bone formation and repair but also for maintaining the bone mass during postnatal growth. By interacting with BMP receptors at the cell surface, they activate intracellular signal transduction and cause the expression of bone-specific genes (Wu et al. 2016). However, the main drawbacks of using growth factors are high cost, immunogenicity, short half-life, and need for supra-physiological doses (Subbiah and Guldberg 2019; Caballero et al. 2019; Ashammakhi 2018). Short peptides derived from therapeutic proteins are less immunogenic than growth factors due to their small size, but they are also not stable. Small molecules can be natural or synthetic non-peptide molecules with low molecular weight. Because of their small size, which is less than 1 kDa, they are stable and non-immunogenic. Moreover, they are often uncharged and hydrophobic. So they can easily penetrate the phospholipid bilayer cellular membrane and activate signaling pathways of transcription and gene expression, thus directing cellular behavior (Balmayor 2015).

Scaffolds are artificial matrices that can provide a foundation for cells to adhere and perform their activities such as proliferation, migration, and protein synthesis. The main purpose of scaffold design is to produce structures similar to those of the extracellular matrix (ECM) of native tissue. These 3D constructs with interconnected pores can be in the form of hydrogels, fiber-based structures, sponges, or other structures that are made of natural, synthetic materials or hybrids. They may contain ceramics, polymers, or a combination of both. Scaffolds must be biocompatible and conventionally biodegradable. Besides, their degradation time must be proportional to the formation of new tissue. It has been proven that their physicochemical properties can be used to control and guide cellular behavior (Xia et al. 2021; Khademhosseini et al. 2020; Haider et al. 2020; Mabrouk et al. 2020).

### 13.2.2 Approaches: Ex Vivo Versus In Situ Tissue Engineering

The human body has an innate ability to repair, regenerate, and renew via stem cells that are resident or migrate to damaged sites (Fig. 13.1). However, this inherent regeneration mechanism is not always sufficient. Therefore, tissue engineering can be employed to improve the self-regeneration capacity of the body (Andreas et al. 2014). Ex vivo and in situ tissue engineering are two main strategies for the repair of damaged tissues, which are beyond repair.

In the ex vivo strategy, the process of tissue engineering is performed in the laboratory and outside the body in such a way that scaffolds are combined with cells with or without biological molecules in vitro. The starting point of this strategy is to obtain tissue-matched cells, either from the patient or from other individuals as stem cells. Then, the cells are expanded in vitro followed by their seeding into a scaffold. After that, the cells are stimulated to produce their own ECM. Eventually, these engineered tissue constructs are implanted into the body to produce desired tissues (Kurniawan 2019b). However, this strategy is limited due to major drawbacks such as donor tissue morbidity, lack of reliable and reproducible cell sources, the need for large quantities of immune-compatible cells, and challenges of in vitro cell culturing such as loss of cellular phenotype. Furthermore, these expensive engineered

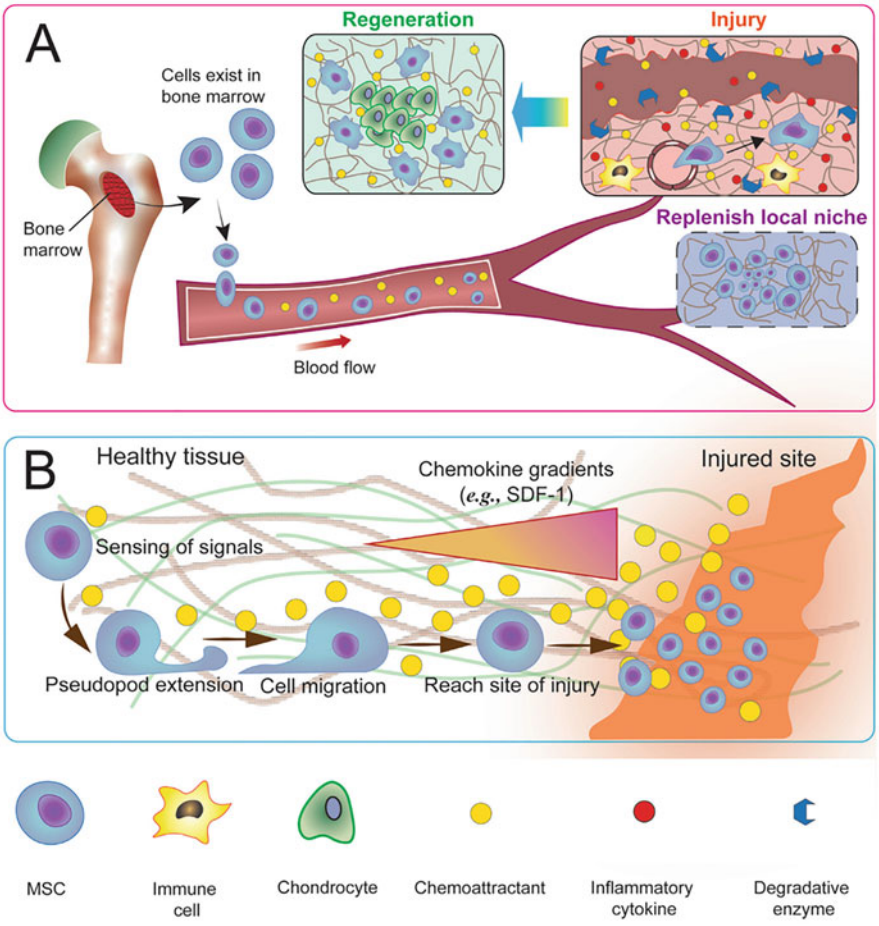


**Fig. 13.1** Schematic representation of two principal modes of endogenous stem/progenitor cell (ESPC) mobilization, migration, and recruitment (illustrations not to scale) in response to guidance gradients (e.g., chemokines and growth factors). (**a**) In general, ESPCs can mobilize from a central cell niche (e.g., bone marrow), disseminate throughout the body via the blood flow, and recognize and interact with microvascular endothelial cells in desired tissues or organs, where these cells replenish the local niches during homeostasis or reach the sites of injury and participate in tissue regeneration. (**b**) The second mode of ESPC mobilization, which is known as interstitial migration, requires that stem/progenitor cells migrate within the extracellular matrix and recognize and obey extravascular guidance gradients to reach the injured site and participate in tissue regeneration. In contrast to mode (**a**), this trafficking mode occurs independent of blood flow and requires active amoeboid movement. (Reproduced from Yang et al. 2020, with permission from Elsevier)

constructs may elicit an immune response after implantation. On the other hand, in in situ tissue engineering, the body itself forms the desired tissue with higher chances of success of the tissue engineering process eventually (Cao et al. 2020; Smits and Bouten 2018). Hence, in situ tissue engineering strategy or endogenous regeneration

**Table 13.1** Ex vivo vs. in situ tissue engineering

| Ex vivo | In situ |
|---|---|
| Requires a compatible cell source | Utilizes the body's inherent regenerative capacity |
| Complex cell culture processes | Improved shelf life |
| High cost | Low cost |
| Immune reaction | Fewer regulatory hurdles |
| Donor-site morbidity | Scalable and consistent quality |

was proposed by scientists to overcome the challenges associated with ex vivo tissue engineering (Barrilleaux et al. 2006). In situ tissue engineering eliminates the need for ex vivo cell manipulation and its complex culturing processes, with the consequent reduction in costs. Moreover, endogenous repair and regeneration are more compatible and reduce the risk of immune rejection. In situ tissue engineering could also induce neovascularization throughout the scaffolds by providing adequate stimuli. Because there is no need to use cells in this approach, regulatory hurdles are reduced, and as a result, the clinical translation of this strategy can be faster (Table 13.1) (Yang et al. 2020; Cao et al. 2020; Kurniawan 2019b; Andreas et al. 2014; Jakob et al. 2012).

## 13.3 In Situ Tissue Engineering

### 13.3.1 Concept and Approaches

In situ tissue engineering field has gained much attention due to its advantages such as being a minimally invasive treatment, ability to integrate to the native tissue, ability to treat complex defects, low cost, and ease of administration, which results in enhanced patient compliance (Yang et al. 2014; Di Bella et al. 2018; Avti et al. 2012; Hakimi et al. 2018). In situ-forming hydrogels involve the sol-gel transition of polymeric precursors containing a variety of polymers in the presence of various hydrogel crosslinking agents including nontoxic chemical crosslinking, physical ionic crosslinking, enzymatic crosslinking under physiological conditions, and supramolecular chemistry (Ghavami Nejad et al. 2020). This category of tissue engineering requires several factors, such as chemical stability, nontoxic networking agents, and controllable gelation time and kinetics (Yang et al. 2014). Numerous natural and synthetic polymers have been suggested for the synthesis of hydrogels. Natural polymers, including polysaccharides (such as alginate, chitosan, hyaluronic acid, and gums) and proteins (such as gelatin, collagen, and fibrin), have attracted much attention because of their inherent bioactivity, biocompatibility, biodegradability, non-toxicity, abundant sources, and low cost (Bao et al. 2020). However, their molecular weight inconsistency from batch-to-batch processes, low mechanical stability, and possible immunogenicity are their main drawbacks, which lead to preferring synthetic polymers such as polylactide (PLA), poly(lactide-*co*-glycolide) (PLGA), polycaprolactone (PCL), poly(ethylene glycol) (PEG), poly(vinyl alcohol)

(PVA), polyacrylamide (PAM), polypropylacrylamide (PNIPAM), and polymethacrylate (Abdulghani and Mitchell 2019; Li et al. 2015). Synthetic polymers are well-known for their high mechanical stability, low immunogenicity, and tailored structure but lack sufficient bio-functionality. To combine the advantages of natural and synthetic materials, hybrid hydrogels (Wang et al. 2015; He et al. 2017; Ashammakhi et al. 2019a), nanobiocomposite hydrogels (Olate-Moya et al. 2020; Yang and Yuan 2019), interpenetrating network (IPN) hydrogels, and slide-ring hydrogels were developed (Jiang et al. 2017; Bin Imran et al. 2014).

The development of biomaterials and polymer chemistries has enhanced our knowledge and use of various biomaterials and various material solidification approaches that employ local microenvironment pH, temperature, and ions (Hoare and Kohane 2008). The development of biomaterial-based approaches can utilize the immune response in a favored manner by using cellular reprogramming to direct cells toward tissue regeneration (Sadtler et al. 2016; Dziki et al. 2016). As a result, combinatorial transcriptional "code" can lead to the recruitment of endogenous stem cells. This approach can modulate the differentiation of immune cells and cytokines and enhance endogenous progenitor contribution to regeneration by the aid of chemotactic signaling. It can also help to mitigate immune rejection of exogenously delivered cells considerably. These immune-mediated approaches can be facilitated by designing smart and stimuli-responsive biomaterials (Piras et al. 2006; Ashammakhi and Kaarela 2017; Lu et al. 2016). These stimuli-responsive biomaterials can stimulate regeneration by interacting with the immune system and regulating the healing kinetics through their inherent biochemical and biophysical characteristics. These biochemical properties include the release of signaling factors, such as small biomolecules to direct cellular responses by activating specific genes or pathways. However, these signaling factors need to be considered and designed carefully based on the targeted tissue microenvironment. For example, in vascularized tissues such as bone and heart, biomolecules should facilitate angiogenesis, whereas in avascular tissues such as cornea and cartilage, they should suppress it. The release of growth factors and mineral ions can also promote cell differentiation toward specific cell lineages and modulate targeted tissue regeneration (Ashammakhi 2018). For example, calcium ions can activate calcium-sensing receptors to trigger chemotaxis and differentiation processes (Hofer and Brown 2003). On the other hand, the biophysical properties of biopolymers such as topography, degradation, structure, and stiffness can influence the local microenvironment by altering the number of cells and concentration of ions or enzymes by in situ injection therapy. The stiffness and topological features of the polymeric scaffold, microparticles, and hydrogels can alter cell adhesion, spreading, and fate (Reilly and Engler 2010; Engler et al. 2006; Wimpenny et al. 2012). The porosity of the biomaterial determines cellular infiltration and vascularization (Griffin et al. 2015). The rate of degradation of the in situ synthetic matrix should be synchronized with the rate of tissue regeneration. Because degradation alters other physical properties such as porosity, morphology, roughness, and 3D framework of the scaffold, new tissue should allow load transfer and provide mechanical integrity eventually to fully replace functions provided by the scaffold. To match scaffold degradation with the

neo-tissue generation, strategies such as varying structural parameters and biological and biochemical functionalization can be used (Gaharwar et al. 2020; Nikkola et al. 2015). In addition, magnetic nanoparticles can also be used to influence stem cells, and exerted physical effect can be used to direct their differentiation (Khademhosseini et al. 2020; Ashammakhi et al. 2018a; Du et al. 2017), which opens new avenues for the use of nanoparticles for in situ tissue engineering.

Several in situ tissue regeneration approaches, including those applied to the bone, cartilage, skin, tendons, and other tissues, can be categorized as top-down and bottom-up approaches. In both approaches, the selection of appropriate materials and processing techniques need to be carefully selected to fulfill the demands of simplicity, adaptability, portability, and ease of manipulation over a short period of time. Generally, in situ regenerative technologies can be divided into non-computer-aided and computer-aided approaches, which is also known as additive manufacturing.

#### 13.3.1.1 Non-computer-Aided Approach to In Situ Tissue Engineering

This approach aims to generate a non-predefined pattern of a construct that can be used to fill a tissue defect. For example, spinning and spraying technologies can be used to form nanofibers or droplets/particles to produce biomimetic scaffolds by employing polymers, ceramics, or their combinations (Nikkola et al. 2015, 2008; Yang et al. 2018; Ndreu et al. 2008; Will et al. 2016; Araujo et al. 2010). In electrospinning, for example, a polymer precursor is fed into a capillary and the extrusion of the spinning solution in the presence of high voltage is carried out. Upon reaching a desired value of electric field between the collector and the generated droplet at the tip of the nozzle, a jet stream of charged viscous solution is directed toward the collector and forms nano-scale non-woven fiber constructs. Several processing parameters such as applied voltage, solvent type, polymer concentration, feed rate, needle diameter, and distance between the tip of the nozzle and the collector influence the diameter, orientation, and structure of the forming fibers. Single nozzle, coaxial nozzle, and dual nozzle enable the production of simple fibers, core-shell fibers, and hybrid fibers. Furthermore, the incorporation of different collectors allows the control over fiber orientation in the resulting constructs (Ashammakhi et al. 2012, 2007a, 2006, 2007b) (SkinCare n.d.). The basic elements used in the electrospraying method are similar to those used in electrospinning, consisting of a high voltage supply, a metallic nozzle, a collector, and a syringe pump. Here, an electric charge between two electrodes makes the liquid jet breaks into fine droplets. The higher the electric charge, the smaller is the size of generated droplets. Several types of solvents, such as ethanol, water-ethanol, and organic solvents, have been used for producing particles based on their evaporation and polymer solubility (Boda et al. 2018). Consequently, in situ spraying and spinning are becoming promising approaches, and portable devices are being developed for the formation of fibrillar or aggregated particles onto tissue defect sites (Dias et al. 2020).

Investigation of in situ gelling matrices in the form of 3D polymeric networks has increased tremendously due to their capacity to carry biomolecules, oxygen, and nutrients and support cellular functions, namely, proliferation, migration, and

differentiation (Park et al. 2012). Crosslinking of polymers for in situ gelling matrices can be achieved via physical and chemical stabilization techniques (Ghavami Nejad et al. 2020). These networks may be composed of either conventional or smart biomaterials, which can be engineered to trigger regenerative processes while exhibiting stimuli-responsive, shape memory, and self-healing properties (Ashammakhi et al. 2021; Rammal et al. 2021; Mantha et al. 2019). Reversible and irreversible self-assembly of building blocks for the production of in situ-forming matrices can be achieved by physical mechanisms such as thermal, ionic, electrostatic, or peptide self-assembly crosslinking, or by chemical mechanisms such as click chemistry, Schiff-base reaction, enzyme-mediated crosslinking, and photo crosslinking (Fig. 13.2) (Yang et al. 2014).

Other methods of in situ tissue engineering include guided tissue engineering (GTR) (Hutmacher et al. 1996; Kellomäki et al. 2002), in which tissue regeneration can be achieved by protecting the tissue defect site from invasion by scar tissue (Fig. 13.3) (Elgali et al. 2017). Other theories behind the function of this method include the local concentration of important cytokines. This method has been widely investigated and applied to different tissue types that include guided nerve regeneration (Bell and Haycock 2012), guided periodontal ligament regeneration (Villar and Cochran 2010), and guided bone regeneration (Elgali et al. 2017; Ashammakhi et al. 1995a; Ashammakhi 1996; Vesala et al. 2002; Asikainen et al. 2005). Membranes made from biodegradable or non-degradable materials are used to protect such tissue defects. Biodegradable membranes need no subsequent removal procedure, and they guide the formation of material-native tissue hybrid neomembranes (Ashammakhi 1996; Ashammakhi et al. 1995b), and they are gradually replaced by native tissues. GTR membranes can be used alone or with additive elements such as osteoconductive materials (Kellomäki et al. 2000; Puumanen et al. 2005) in case of bone regeneration or drugs and cytokines such as growth factors. They can also be combined with tissue materials such as grafts (Ashammakhi et al. 1995a). With the rising interest in in situ tissue engineering, guided tissue regeneration will gain a new momentum, and accumulated literature should be of great benefit to support future research and advances in this area.

#### 13.3.1.2 Computer-Assisted Approach to In Situ Tissue Engineering

Additive manufacturing, also known as three-dimensional (3D) printing, is a state-of-the-art technique that relies mainly on printing artificial acellular constructs. Among the available options, light-based (infrared [IR], ultraviolet [UV], or visible light) and extrusion-based systems are more readily available, either in the form of portable devices or a robotic approach, controllable movements along three axes. BioPen is a good example of an extrusion-based portable device, which can be used with various inks to support rapid in situ gelation and consists of a bioink chamber, a multi-inlet nozzle, a motorized extruder, and a light source (Di Bella et al. 2018; Cathal et al. 2016). Gelatin methacryloyl (GelMA), which is a photocurable polymer, can be transited from sol to gel within 1 s upon exposure to light irradiation in the presence of photoinitiators and appropriate physiological conditions (Erdem et al. 2020; O'Connell et al. 2020). GelMA printability and mechanical properties

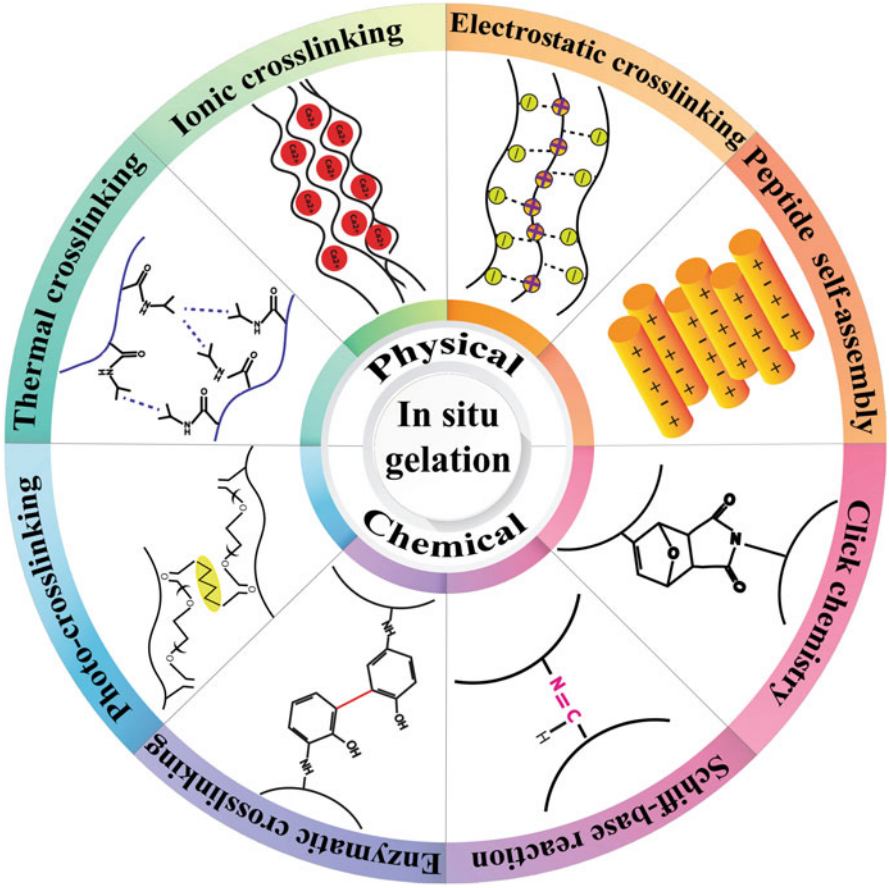


**Fig. 13.2** Various in situ gelling mechanisms, consisting of physical crosslinking including (**a**) thermal, (**b**) ionic, (**c**) electrostatic crosslinking and (**d**) peptide self-assembly, and chemical crosslinking including (**e**) click chemistry, (**f**) Schiff-base reaction, (**g**) enzyme-mediated crosslinking, and (**h**) photo crosslinking

of the final hydrogel can be increased by the incorporation of rheological modifiers such as silicate particles and methylcellulose. In contrast to handheld devices, robotic-assisted bioprinting can better be used to produce complex architectures via computer-aided design (CAD), thus better mimicking complex native structures (Ma et al. 2020; Li et al. 2017). A study introduced the integration of wound imaging with additive manufacturing in order to improve personalized medicine and significantly reduce surgeon's intervention (Ding and Chang 2018; Ashammakhi et al. 2019b). However, clinical translation needs to be undertaken. Developing a suitable bioink formulation and gelation mechanism are two important steps that determine the performance of in situ-forming hydrogels. Therefore, attempts have been made to optimize bioink composition, crosslinking efficiency, gelation time, stiffness, and

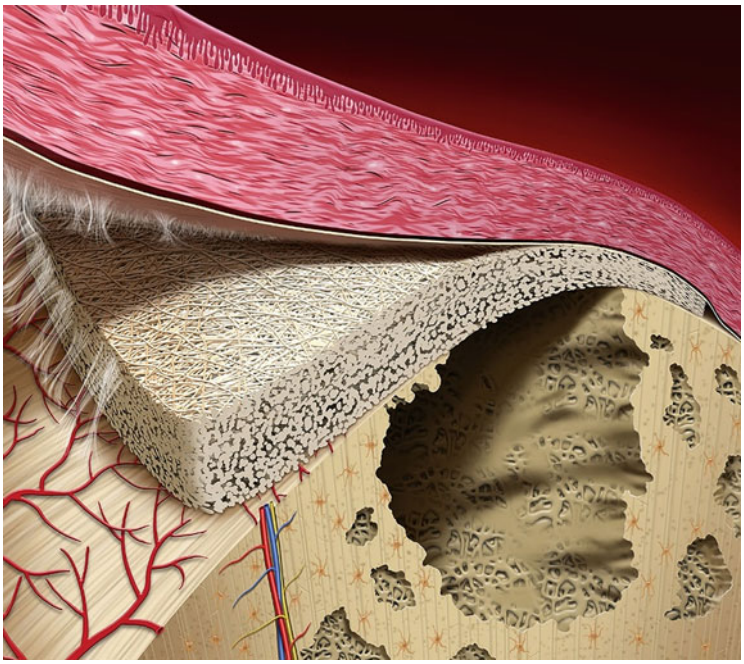

**Fig. 13.3** Schematic illustration of the principle of guided bone regeneration (GBR). (Reproduced from Elgali et al. 2017, under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution)

shape retention without postprocessing manipulation (Dias et al. 2020; Agostinacchio et al. 2021; Jang et al. 2018).

### 13.3.2 Biomaterial-Based In Situ Tissue Engineering

The elimination of the need to recapitulate the exact tissue microenvironment for engineering tissues might be the greatest advantage of in situ regenerative approaches. In situ tissue regeneration requires the selection of appropriate biomaterials. Biomaterials suitable for in situ tissue engineering could be divided into synthetic, natural, and hybrid materials (Murdock and Badylak 2017). In all cases, biomaterials must be biodegradable and nontoxic and do not lead to adverse immune responses (Cao et al. 2020; Murdock and Badylak 2017). In situ-forming hydrogels have recently received considerable attention due to their desirable features such as injectability, minimal invasiveness, and complete defect filling (Sontyana et al. 2018). In one study, silk fibroin-based injectable hydrogels were reported to be of great help for in situ bone regeneration. After 4 and 8 weeks of hydrogel injection, in situ bone regeneration in implanted hydrogel occurred at 220% faster than that seen in untreated cases (Fig. 13.4) (Shi et al. 2017).

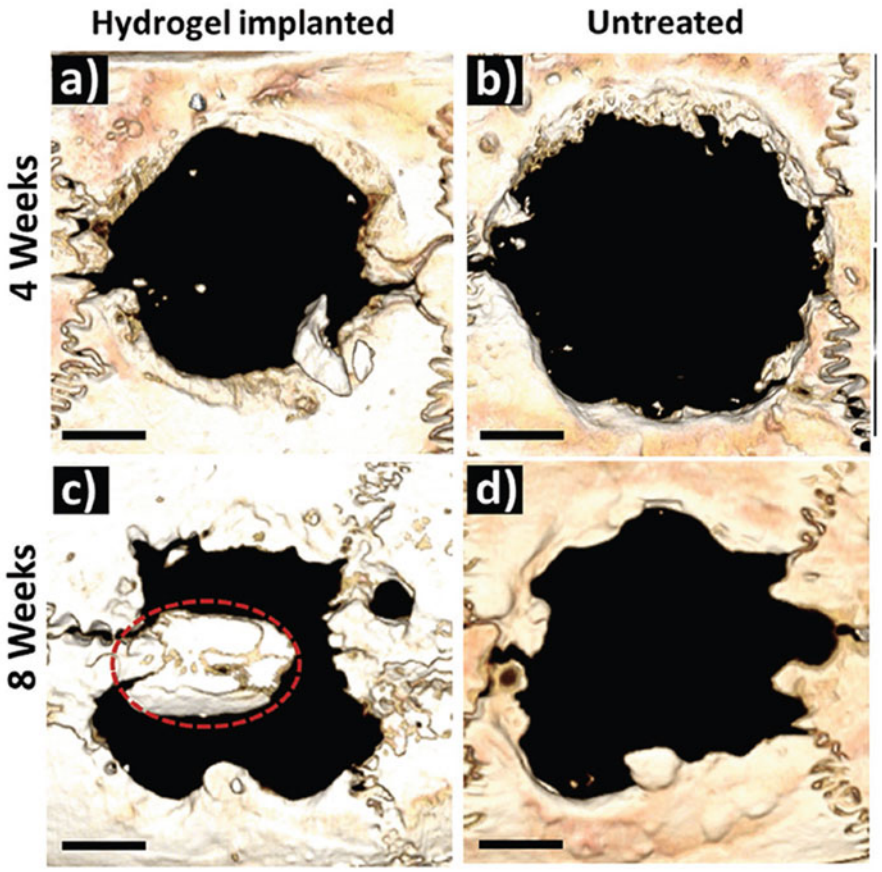


**Fig. 13.4** (**a**–**d**) Micro-CT showing accelerated bone repair in hydrogel implanted group, as compared to untreated group. Reproduced from Maiz et al. 2020, under the terms and conditions of the Creative Commons Attribution (CC BY) license, http://creativecommons.org/licenses/by/4.0/.

To treat skin wounds, an electroconductive based on quaternized chitosan-g-polyaniline (QCSP) and benzaldehyde group functionalized poly(ethylene glycol)-co-poly(glycerol sebacate) (PEGS-FA) was developed (Zhao et al. 2017). Its use for the treatment of experimental skin defects in mice for 15 days indicated that hydrogels with optimal cross-linker concentration enhance wound healing (higher expression of EGF, TGF-β, and VEGF).

In situ tissue engineering using injectable hydrogels is also considered to be suitable for the regeneration of the heart tissue (Maiz et al. 2020). A recent study reported improved cell and tissue migration with the use of silk fibroin photo-lyogels containing microchannels as a platform for in situ tissue engineering (Baptista et al. 2020). In another study, an injectable and self-healing hydrogel based on

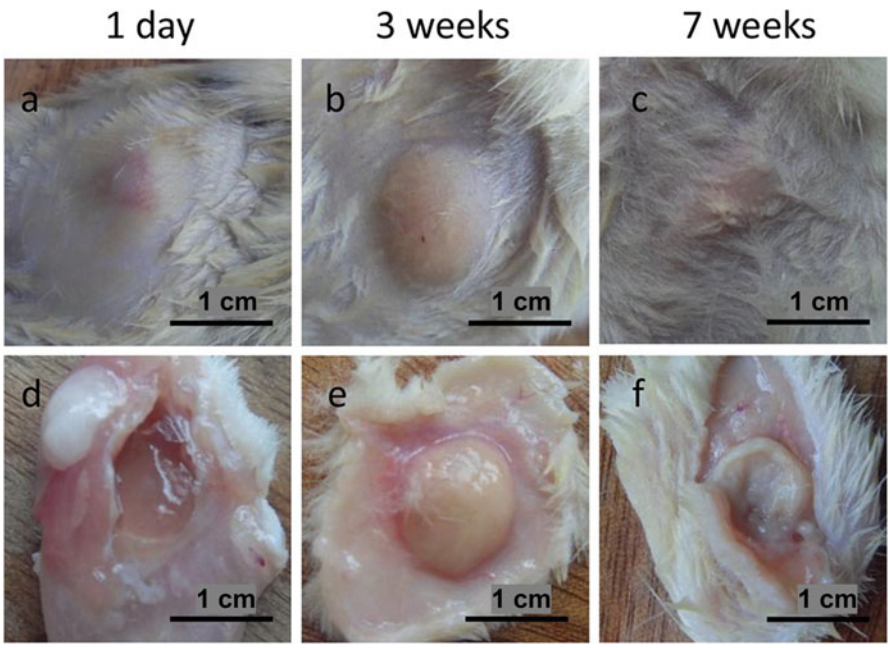


**Fig. 13.5** Carbohydrate-based hydrogel was subcutaneously injected in rats using a syringe for 1 day, 3 weeks, and 7 weeks. (**a**–**c**) The natural state of the hydrogel after injection; (**d**–**f**) the rats were sacrificed, and the injection site was carefully cut open. (Reproduced from Lu et al. 2015, with permission from the American Chemical Society)

carbohydrates was developed, and cells were encapsulated in the hydrogel. It was found that hydrogel was biodegradable and helped in tissue repair (Fig. 13.5) (Lu et al. 2015). Another important aspect of in situ tissue engineering is the need of proper vascularization in targeted tissue (Markowicz et al. 2005; Shahabipour et al. 2020).

### 13.3.3 Biomolecule-Based In Situ Tissue Engineering

Living systems are influenced by various complex biomolecules produced by cells and living organisms. Biomolecules have a wide range of structures and sizes with different functions in the body. They are normally classified according to their biochemistry into carbohydrates, proteins, lipids, and nucleic acids (NCERT 2019; Karp et al. 2016). Proteins are the most abundant biomolecules of living systems. They have many roles and virtually control all cell activities. As enzymes, they accelerate the rate of metabolic reactions. As structural elements, they provide mechanical support. As hormones, growth factors, and gene activators, they play essential signaling and regulatory functions in the body. As antibodies and as membrane receptors and transporters, they can be used for special targeting or

signaling purposes. For example, the use of peptide molecules such as anti-VEGF-receptor 2 (anti-VEGF-R2) aptamer and arginine-glycine-aspartic acid (RGD) adhesion peptide-incorporated hydrogel system was investigated for use in wound healing (Roy et al. 2020). The synergistic effect of RGD peptides and VEGF-R2 aptamer enhance cell attachment, migration, and survival, and they can; therefore, be useful for future application for in situ wound healing. The incorporation of RGD peptides and BMP has been frequently reported in other studies (Lin et al. 2016; Wang et al. 2017). Hyaluronic acid modified with bisphosphonate or hydrogel nanocomposites based on hyaluronic acid and dextran have been recently reported for in situ bone regeneration as noninvasive stem cells and BMP-2 delivery systems for bone regeneration. Results indicated that the bioactivity of BMP-2 is preserved for more than 4 weeks, resulting in better osteogenesis both in vitro and in vivo (Ensrud 2013; Zhang et al. 2020). To treat osteochondral defects, a biocompatible, injectable hydrogel based on dextran functionalized with ureido-pyrimidinone (UPy) was developed (Hou et al. 2015). In this study, two hydrogels were mixed together, where one hydrogel was loaded with chondrocytes for cartilage regeneration and the other with BMP-2 for bone regeneration. After 8 weeks of implantation, cartilage-bone tissue interface was successfully developed. The results of a platelet-derived growth factor-AB (PDGF-AB) and collagenase-loaded system on in situ meniscal defect repair were also reported to be successful (Qu et al. 2017). A pH-switchable super-molecular hydrogel (polyethylene glycol derivative functionalized with UPy) loaded with growth factors (hepatocyte growth factor [HGF], and insulin-like growth factor-1 [IGF-1]) was used for cardiac tissue regeneration (Bastings et al. 2014). The hydrogel is liquid in basic pH environment and rapidly forms a hydrogel when it is in tissue neutral pH environment. In vivo results in pigs demonstrated its ability to reduce scar collagen in a chronic myocardial infarction.

Furthermore, there are other small or macro-biomolecules, which are used in tissue engineering. For example, kartogenin (KGN) is a small molecule that induces chondrogenesis and can be used for in situ cartilage tissue engineering (Dehghan-Baniani et al. 2020). Dendrimers, also known as synthetic macromolecules, represent another group of small molecules. For example, a synthetic dendritic polyglycerol sulfate-based in situ-forming hydrogel system was investigated, and it was proposed as a promising tool for future in situ cartilage tissue engineering (Dey et al. 2016). Another in situ injectable hydrogel system containing thiol functionalized poly(amido-amine) dendrimer and oxidized dextran was also investigated and proposed for potential use for in situ tissue engineering (Li et al. 2016a).

### 13.3.4 Genetics-Based In Situ Tissue Engineering

#### 13.3.4.1 Approach

**Polynucleotide-Based In Situ Tissue Engineering**

Nucleic acids represent an important category of biomolecules used for in situ tissue engineering. They can be classified into two types: deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Since they are long-chain polymers of nucleotides, they are also called polynucleotides. Gene-activated scaffolds for in situ bone regeneration and vascularization were reported to be successful (Lin et al. 2020; Sun et al. 2020). The activation of SOX-5, SOX-6, and SOX-9 transcription factors using gene-activated scaffolds for in situ chondrogenesis and inhibition of endochondral ossification was also reported (Raftery et al. 2020; Madry et al. 2020). In situ release of recombinant adeno-associated viral vectors on cartilage regeneration was also investigated for effective gene therapy and cartilage repair (Madry et al. 2020). Among important RNAs is RNA interference (RNAi), which silences the expression of specific genes and can be obtained through the delivery of microRNAs (miRNAs) or small interfering RNAs (siRNAs). However, the delivery of RNAi molecules is not easy. Moreover, RNAi biomolecules can be degraded under some circumstances. Although viral delivery vectors are mostly used for the expression of RNAi, it is risky due to mutagenesis and immunogenicity. Recently, improvement of the stability of mRNA by chemical modifications has attracted interest in developing mRNA-based therapies (Gaharwar et al. 2020).

**Transcription Factors**

By the delivery of lineage-determining transcription factors, cell state can be reprogrammed, and lineage-specific differentiation is induced. However, challenges associated with this approach include difficulties in preserving integrity and protein activity. These challenges can be overcome using retroviruses, lentiviruses, adenoviruses, and plasmids by integrating transgenes into the genome of the host. For instance, reprogramming of somatic cells into pluripotent cells can be performed by using four important transcription factors, including OCT4, SOX2, KLF4, and MYC expression using retroviral transduction (Gaharwar et al. 2020).

#### 13.3.4.2 Mechanism

For in situ regenerative engineering, genetic material can be delivered directly into the target site. Target genes integrate into the host genome of endogenous stem cells and transform the transfected cells to boost tissue regeneration. Target genes are included in vectors to protect them from DNase and lysosomal digestive enzymes. Two types of vectors are commonly used, viral and nonviral vectors (Ji et al. 2011).

**Viral Delivery**

The use of viral vectors is the usual method to introduce a therapeutic gene into cells. There are different kinds of viral delivery systems that can be used for gene transfection (Santos et al. 2011; Cucchiarini et al. 2016; Partridge and Oreffo

2004). DNA-based viral vectors used for gene delivery are usually more durable and integrating into the genomes. DNA-based viral vectors include lentivirus, poxvirus, adenovirus, adeno-associated virus, retrovirus, human foamy virus (HFV), and herpesvirus. RNA-based viral vectors for gene delivery are able to directly transcribe the infectious RNA transcripts. RNA-based gene delivery is not constant, and it is not permanent. For RNA-based gene delivery, human foamy virus, oncoretroviral vectors, and lentiviral vectors can be used (Sung and Kim 2019).

### Retrovirus

Retroviruses are widely used for DNA delivery into cells, such as hematopoietic marrow cells, meniscal cells, hepatocytes, keratinocytes, and endothelial cells (Partridge and Oreffo 2004). By integrating retroviral vectors into the host cell genome, transgenes of the host cell can be maintained for much longer times. Yet such integration may cause mutagenesis and activation of tumor genes. These vectors are used in the transduction of dividing cells and have low efficacy (Cucchiarini et al. 2016).

### Adenovirus

Adenoviruses are capable of producing a high-titer virus and great efficiency of transfection both in dividing and nondividing cell lines. Helper-dependent adenoviral vectors (HDAds) and adenoviral–retroviral hybrids result in long-term expression. The disadvantage of this type of gene delivery is the immune response and inflammation it induces (Partridge and Oreffo 2004).

### Lentiviral Vectors

The benefits of lentiviral vectors are greater than other viral approaches due to their integration into the genome of nondividing cells and high transduction effect. However, there still remain concerns regarding their mutagenesis potential (Cucchiarini et al. 2016). The viral transfection methods, including lentiviral and adenoviral vectors, are demonstrated in Fig. 13.6.

### Recombinant Adeno-Associated Virus (rAAV) Vectors

rAAV vectors have less immunogenicity than adenoviral vectors and are more efficient than nonviral and retro-/lentiviral vectors for transducing both dividing and nondividing cells and have long-lasting transgene expression. They remain stable in their host and have the ability of gene transfer in situ through the extracellular matrix because of their small size. Due to these reasons, rAAV vectors have been widely used in clinical applications (Cucchiarini et al. 2016).

### Herpes Simplex Virus (HSV) Vectors

HSV vectors deliver long transgenes into various cell types, including nondividing cells, but these kinds of vectors are toxic and have a temporary expression of the transgene (Cucchiarini et al. 2016).

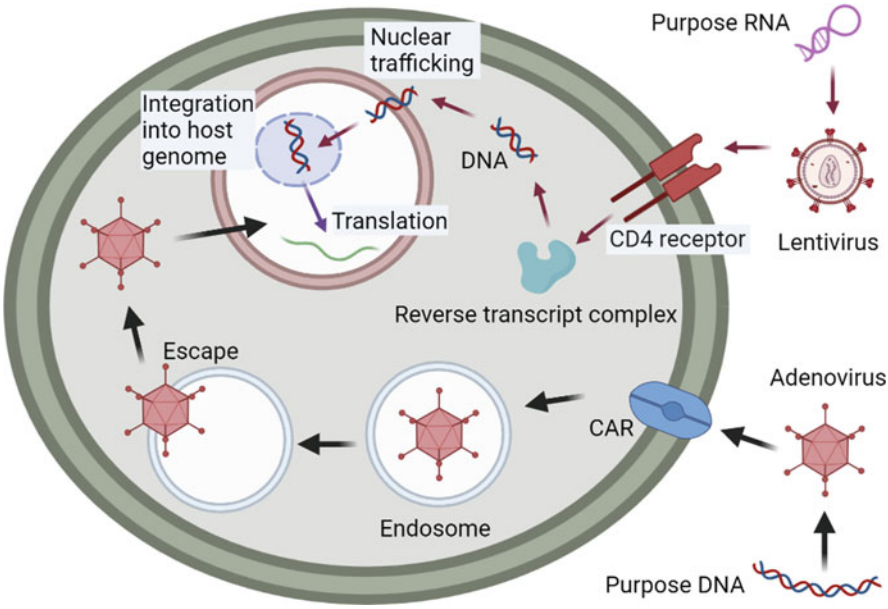


**Fig. 13.6** Lentiviral and adenoviral based transfection methods. This is an open access article distributed under the Creative Commons Attribution License, which allows unrestricted use, distribution, and reproduction. (Redrawn from Chen et al. (2010), using Biorender.com)

## Nonviral Delivery

Because of host immune reactions, toxicity, and risk of mutagenesis, the use of nonviral vectors has been increasing. These vectors can be used repeatedly, but they have low and transient transgene expression (Partridge and Oreffo 2004). Nonviral gene delivery is usually performed by using plasmid DNA, which includes the protein-gene encoding under the transcriptional control (Santos et al. 2011).

### Chemical Methods

Chemical methods involve the use of natural or synthetic materials for genetic material delivery into the cell. Nonviral vectors have high structural and chemical versatility; they can be manipulated and adjusted to improve gene delivery and expression efficiency (Santos et al. 2011).

### Physical Methods

Physical methods represent an ineffective way of gene delivery into cells, including naked DNA. They also include cell manipulation, enabling improved gene delivery, like nano- and microinjection, electroporation, and molecular vibration (Santos et al. 2011).

#### 13.3.4.3 Advantages and Limitations

Ease of production and use, the durability of expression of therapeutic genes, and the simplicity and safety are the most important advantages of the gene activation method. Viral vectors have high efficiencies in gene delivery and transduction, and they allow prolonged expression of genes. The function of the viral vector is dependent on the characteristics of the parental viral vector, which are not yet well understood. Also, for producing viral vectors, the development of complex procedures based on cell culture and infection must be undertaken to obtain virions that contain therapeutic nucleic acids. Some viral vectors persuade inflammation and immune response after injection into the host (Giacca 2010). Clinical use of viral vectors is limited because of disadvantages such as transformation, mutagenesis, inflammation, and immune reactions. Furthermore, they have limitations in the delivery of exogenous DNA and large and big productions (Santos et al. 2011; Partridge and Oreffo 2004). On the other hand, nonviral gene delivery methods have less immunogenicity and risk of infectious disease transmission. They are flexible in the molecular size of DNA and are very cheap. However, chemical methods have shown lower transfection efficiency, temporary expression of the gene, and high toxicity compared to viral vectors (Santos et al. 2011).

### 13.3.5 Cell-Product-Based In Situ Tissue Engineering

More recently, it was thought that the therapeutic effect of stem cells might also be attributed to their secreted extracellular vesicles (EVs) (Lamichhane et al. 2015). Therefore, studies that explore the use of stem cell-derived EVs have been increasingly published (Lamichhane et al. 2015; Tsiapalis and O'Driscoll 2020; Liu and Holmes 2021; Kanada and Ashammakhi 2021). With the help of EVs, the need for cell delivery and attending risks and complications can be eliminated (Kanada and Ashammakhi 2021; Allan et al. 2020). In one study, exosomes were loaded into a methylcellulose-chitosan hydrogel and injected in a wound, where they resulted in enhanced cell proliferation and skin remodeling in diabetic mice (Wang et al. 2020).

## 13.4 Current Challenges and Future Directions

In situ tissue engineering has shown significant progress over the recent years by using biomaterials, biomolecules, genetics-based platforms, and cellular products. In particular, pre-vascularized tissue constructs, acellular organs, and microfluidics can be introduced as novel approaches in this field (Sengupta et al. 2014). Further, dynamic four-dimensional (4D) printing (Ashammakhi et al. 2018b) can be considered for further research and application for in situ tissue engineering in the future (Momeni et al. 2017; Ashammakhi et al. 2019c). The promising therapeutic outcome of the in situ toolbox has led to the development of a new paradigm to mitigate the current limitations of tissue engineering. However, there are still challenges in using

in situ platforms that need to be addressed to enhance its successful clinical translation in the future.

Advances in in situ tissue engineering require profound insight into the tissue healing dynamics, which involve biophysical and biomechanical factors acting at the defect site in order to establish an efficient tissue engineering tool and achieve successful regeneration. A full understanding of regeneration processes and the fate of endogenous stem cells and their migration is needed (Li et al. 2016b). Therefore, cell labeling followed by their long-term tracking would be a useful strategy to monitor engineered platforms and cell homing. This also requires the development of appropriate animal models (Zhang et al. 2017; Jahed et al. 2020). Also, novel in vitro models that investigate the use of human macrophages to stimulate the immune response should be considered. This can be achieved by using organ-on-a-chip (OoC) models, which will obviate the need for the use of other species that have different physiology (Elmusrati and Ashammakhi 2018; Ashammakhi et al. 2018c, 2019d, 2020b). It was proved that many factors such as scaffold morphology and stiffness and biochemical cues that are released following degradation affect the immune response, which should be taken into consideration in vitro (Guo et al. 2019), and new methods can also be useful in this regard (Ashammakhi et al. 2020c; Jiang et al. 2021; Tanataweethum et al. 2020; Bhatia and Ingber 2014). Providing appropriate conditions that can simulate the body environment with specific biophysical and biomechanical features can help to regenerate tissues in a more controlled and efficient fashion. This requires that advanced and intelligent designs of bioreactors be developed. Such approaches will help to predict and guide the formation of new tissues in the native microenvironment using in situ regenerative approaches, which can be fine-tuned at a later stage (Ashammakhi et al. 2019e). Since in situ tissue engineering relies on the body's inherent ability to regenerate, interindividual, tissue, and site variations in regenerative potential should be considered and investigated. Besides, the body regeneration capacity is affected by the immune system, which can also vary with age, health status, and medications, factors which should also be considered.

## 13.5 Conclusions

The use of in situ regeneration relies on leveraging body innate regenerative potential by using different tools to stimulate and enhance this ability, such as biomaterials, biomolecules, and cell products. In contrast to the ex vivo, the need for an external source of cells for transplantation is not required. In situ tissue engineering can be an effective method to advance regenerative therapeutics and enhance their clinical translation. Indeed, stimulating and enhancing specific pro-regenerative microenvironment will lead to the development of more effective and minimally invasive therapeutics. Although much progress has been made in the field of in situ regeneration of some tissues, many other tissues still need investigation to develop novel therapeutics. To make impactful advances in in situ tissue engineering a multidisciplinary approach based on the cooperation between

biochemists, biologists, biomedical engineers, and clinicians, as well as sustained funding are required.

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