Chapter

Current Scenario of Regenerative Medicine: Role of Cell, Scaffold and Growth Factor

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Abstract

Impairment of the clinical tissue-implantation is due to the lack of a suitable organ donor and immunogenic rejection, which leads to the cause for the enormous loss of human life. The introduction of artificial regeneration of tissues by Langer and Vacanti in 1993, has revolutionized in the field of surgical organ transplantation, to alleviate the problem of tissue injury-related death. There is no doubt that the term "regenerative medicine" to open a new space of tissue reconstruction, but the complications that arise due to the proper machinery of the cell, supporting biomaterials and growth factors has yet to be resolved to expand its application in a versatile manner. The chapter would provide a significant overview of the artificial tissue regeneration while a triangular relationship between cells, matrixes, and growth factors should be established mentioning the necessity of biomedical tools as an alternative to organ transplantation.

Keywords: biomaterials, biocompatibility, extracellular matrix, therapeutic molecules, regenerative medicine

1. Introduction

Advances in biomaterials are implicated in the huge results as the artificial support matrices penetrate the barrier of all physicochemical properties in clinical tissue implantation, an artifact of regenerative tissue engineering applications. Unlike traditional tissue grafting, the artificial implanting process involves the replacement of any damaged tissues irrespective of age, diseases, and kinds of trauma. Based on the potential requirements, efforts in different fields that include material science, cell biology, medicine, theory and computational studies represent a versatile contribution in regenerative medicine to save thousands of lives. The preliminary idea of tissue engineering is to reconstruct the traditional surgical or mechanical device-related techniques; those though significantly prevented the untimely demises of lives. The time on demand of available organ donors and appropriate complimentary biological environments are implemented in the term 'Tissue Engineering' (TE) in 1933 by Langer and Vacanti [1]. TE is the versatile gift of scientists who have greatly remodeled and mimicked the *in vivo* biological niche through a combination of engineered biomaterials, cells of interest and biochemical factors, which are important factors for tissue development. The manufacture of the implantation of the desired shape is the key factor in TE, which could support

seeding of isolated cells, cell–cell interaction, and its proliferation and migration. The cell adhesion and secretion of extracellular matrixes (ECM) are switched through growth factor-mediated signaling pathways [2]. The growth factors are the class of cytokines, at the basis of cell attachment, proliferation and migration in tissue regeneration, remodeling and other various cellular functions [3, 4]. Epithelial growth factors (EGFs), platelet-derived growth factors (PDGFs), insulin-like growth factors (IGFs), and hematopoietic cell growth factors (HCGFs) are promising types of cytokines that have played a significant role in tissue engineering.

The new generation of biomaterials has revolutionized the fields of regenerative medicine while, the development of 3D architecture could enable us to mimic the ideal *in vivo* tissue organoid. Other important parameters of the supporting scaffolds include the porosity, biodegradability, biocompatibility and good carrier of the therapeutic molecules, immobilized onto the matrixes. Due to intrinsic biological characteristics, both synthetic and natural materials have been investigated to formulate the three-dimensional support structure, but the requirements related to microbial resistance, genotoxicity, and mechanical strength remain to be questioned. Collagen, gelatin, hyaluronic acid, alginate, guar gum, chitosan, polyhydroxyalkanoates are the well-known naturally originated, biocompatible and biodegradable polymers, which have emerged as a bio-mimicking matrixes in the development of artificial 3D construct, but their mechanical as well as hydrophilicity limited its unique usages. The drawback is significantly erased by the appearance of biosynthetic technology. In particular, the discovery of carbon compounds such as carbon nanotubes, graphene oxide nanoparticles, etc., has revolutionized the fields of tissue engineering. The additional biocompatibility, antimicrobial activity and mechanical stability of such compounds are considered to fabricate the bioimitating 3D construct. Furthermore, synthetic materials such as poly (lactide-coglycolide) and poly (ethylene glycol) play an important role in the construction of the ideal scaffold [5, 6].

The challenge in tissue regeneration is the seeding of cells. The risk factors such as *in vivo* immune-rejection, viral or microbial contaminations, processing of clean healthy cells are the main obstacles in choosing of the desired cells. Keeping in mind, the allogeneic cells, i.e., cells from a healthy person is considered for the several tissue regeneration systems. A variety of stem cells has been an essential and elementary option for the *in vitro* cell growth in the regeneration of cartilage tissues [7]. Nevertheless, an ideal scaffold with interconnected porous networks, proper mechanically and biologically appropriate engineering should lead to ECM secretion with prominent adhesion, and cell transduction and proliferation are best suited for tissue engineering applications.

This chapter will explore the sources of the 3D polymer construct and its validity in the biological niche, i.e. biocompatibility and cell motility through different growth factors.

2. Why is the 3D construct in tissue regeneration (TE)?

In tissue engineering, the assessment of cell compatibility is evaluated in a *"in vitro"* model of either 2D or 3D based on the results of optimal support arrays in which cells must continually increase their colonies as the native tissue environment as shown in **Figure 1**. The trafficking of therapeutic delivery in several cases is to get rid of all kinds of forthcoming issues. Therefore, it must circumvent these obstacles by taking into account various facts such as the extracellular matrix density, the nature of the cell-tissue interaction, and the penetration of nano carriers through

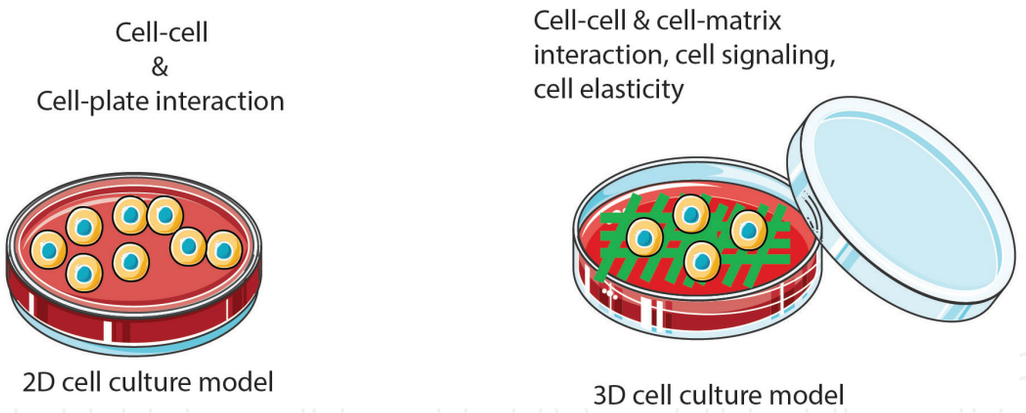

Figure 1.
Schematic comparison of 2D and 3D cell culture model.

tissue layers, etc. In 2D cell culture, the cells are grown, adhered to flat polystyrene surfaces in a thin monolayer's manner. The proliferation, migration, epigenetic and genetic expressions are varied as compared to *in vivo* tissue regeneration. Cell attachments and motility also constraint within monolayer frames and are exposed to unlimited nutrient sources. The two-dimensional culture is related to an easy and cost-effective process. However, it deprives the study of cell–cell interaction, cell-matrix interaction, cell signaling, and the nature of cell elasticity, which promotes the usefulness of the biological niche, i.e. the 3D construct.

Unlike 2D model, 3D constructs regulate the cell growth and proliferation in heterogeneity similar to *in vivo* tissue as the exposure of growth factors, oxygen, nutrients distributed unevenly in the scaffold or any other 3D construction. The cell morphology as well as the cell polarity is maintained because it greatly controls the cell-signaling, cell topology and various metabolic activities [8, 9]. However, culturing in 3D models is a lengthy and expensive process and is formulated in several ways: 3D spheroid where cells spend their survival time in layers, mimicking the correct cell–cell and cell-matrix interaction as in the environment of native tissue. The identification of biomarkers and the expression of genes that are involved in topological processes as well as the polarity changes of cancer cells are examined in the spheroid models [10]. Furthermore, it is expected that the adherence and proliferation of primary cells follow all the characteristics of *in vivo* tissue processes [11]. The 3D scaffolding systems, made of biodegradable and biocompatible materials, have demonstrated superior cell migration, proliferation through a network of porous 3D microenvironments [12]. In view of the significant advantages, the utility of bio-imitating 3D constructs is however encompassed in the niche of tissue regeneration though; 2D cell culture is still used as a reliable method before initiating the animal studies.

3. Choice of material in artificial supporting matrixes

The involvement of suitable materials, whether synthetic or naturally extracted in the fabrication of the artificial tissue environment, remains a challenge. The prerequisite in the development of 3D constructs includes biocompatibility, biodegradability, mechanical strength, interconnected and antimicrobial porous mechanical strength, antimicrobial and interconnected porous networks in which cellular activities could be performed analogously to their native tissue domicile. Taking into account the fact that biomaterials that actually contain the structural

component or similar biochemical and physicochemical identity of native tissue have been granted for the processing of the platform in support of artificial cells. Meanwhile, the involvement of any unique material may or may not be able to create the imitation of equivalent tissues requires a broad understanding of the cell-matrix interaction. Therefore, the role of the combination of materials is considered as the essential means to overcome all the barriers that include bioactivity, biodegradability, microbial contamination and maximum mechanical flexibility which contribute as a key to tissue engineering.

3.1 Impact of naturally sourced biomaterials on cell-cell cross-talk

Mimicking of biological tissue environment using collagen is an attractive theme, which is due to the inherent features such as fibrous structure, biocompatibility and low antigenicity. However, the improvement of mechanical stability and biodegradability requires additional treatment that includes cross-linking or chemical modification in the presence of second party molecules. The approaches of modification of different natural biopolymer and its tuning into desired artificial tissue architecture that support the adhesion, proliferation, and migration of cells in biological niche are discussed here.

3.1.1 Collagen as base material and its derivatives

Collagen is a key component in extracellular matrixes and composed of RGD (arginine–glycine–aspartic acid) domains that plays a potential role in cell adhesion, growth and motility through its interaction with cells. But, the drawback due to poor mechanical stability and biodegradation is overcome by the modification with various natural polymers or synthetic polymers. In one approach, collagen molecules were chemically conjugated with oxidized guar gum to immobilize platelet-derived growth factor [13]. The guar gum which is a water soluble and ionic polysaccharide was oxidized to poly(dialdehyade) guar gum in presence of sodium periodate. The resultant oxidized guar gum not only promoted the cross-linking of collagen molecules but also helped to immobilize the platelet-derived growth factor, enabling the formation of biologically active hybrid 3D scaffolds with excellent swelling, thermal and biodegradable properties. FTIR, SEM analysis was performed to confirm the synthesis of the hybrid structure. SEM morphology revealed the interconnected 3D porous honeycomb structure with an average pour size of 15 ± 7 μm. The hybrid scaffold was shown to promote the release of growth factors with the increase of NIH 3 T3 cell density and proliferation and was seen as a promising candidate for tissue engineering applications.

Recently, Diogo et al. developed a method of fabrication of '*in situ*' mineralized collagen based 3D printed hydrogel. As an alternative to various traditional approaches, Co-precipitation method is used to mineralize the collagen fiber in presence of calcium chloride ($CaCl_2$) and ammonium hydrogen phosphate [$(NH_4)_2HPO_4$]. To prepare the cell laden 3D printed hydrogel, variuos raio's of mineralized collagen and alginate (a biocompatible and degradable natural polymer) mixture was treated with incubated L929, mouse fibroblast cell line and printed using a bioprinter V1 (REGEMAT 3D, Granada, Spain), resulted the cell laden 3D printed bi-oink. The cell-laden scaffold was shown to support the adhesion, growth and survival of mouse fibroblast cell line [14]. The similar kind of the cell-laden-collagen core and alginate-polyethylene oxide shell based 3D porous structure was developed using microfluidic channel and at low temperature working condition for cryopreservation (**Figure 2A**) which is subjected to maintain the shortage of cells, tissue and organs. The *in vitro* assays of two days cryo-preserved

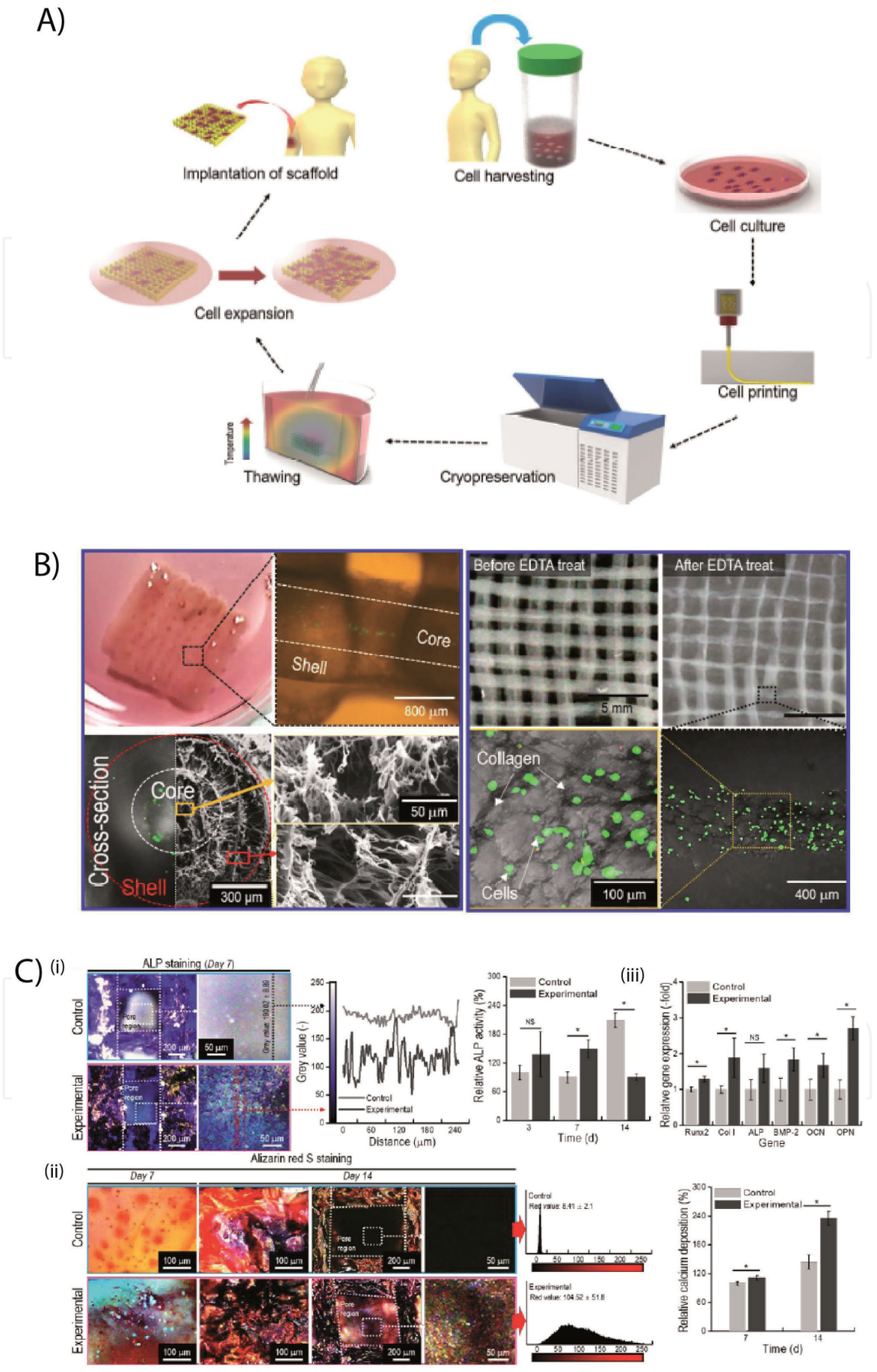

Figure 2.
(A) Application of cryopreservation/cell-printing process to tissue engineering processes. [Ref: [15], reproduced with permission from publishing authority]. (B): (a) Optical and SEM images of final scaffold with core/shell mesh structure. (b) Optical images of final scaffold before/after EDTA treatment and fluorescence images of live cells and collagen fiber in core strut after EDTA treatment. [Ref: [15], reproduced with permission from publishing authority]. (C): Osteogenic differentiation of human adipose-derived stromal cell (hASC)-laden

*structures with (experimental) or without (control) chicken bone marrow cell-conditioned medium. (i) Optical microscopy images of alkaline phosphatase (ALP) staining (at 7 days) and relative ALP activity (at 3, 7, and 14 days) of hASCs in the control and experimental structures. The experimental structure reached maximum ALP activities at 7 days (significantly higher than those of control) and then decreased at 14 days, whereas the ALP activities of the control group continued to increase at 14 days (n = 6, *p < 0.05). Decreased gray values of the magnified ALP staining, indicating that the proliferating cells in the pore were effectively mineralized. The image was captured in the pore of the experimental structure. (ii) Optical microscopy images of Alizarin Red S staining (at 14 days) and relative calcium deposition of the scaffolds (at 7 and 14 days). Calcium deposition levels of the experimental scaffolds were significantly higher than those of the control scaffolds (n = 6, *p < 0.05). Increased red staining was observed in the pore region in the experimental scaffold. (iii) Expression levels of runt-related transcription factor 2 (*Runx2*), collagen type I alpha chain 1 (*Col I*), Alp, bone morphogenic protein 2 (*Bmp-2*), osteocalcin (*Ocn*), and osteopontin (*Opn*) at 14 days of culture. Significantly increased expression levels of* Runx2, Bmp-2, Ocn, *and* Opn *were detected in the experimental structure (n = 6, *p < 0.05). All values are expressed as mean ± SD. [Ref. [20], reproduced with the permission from publishing authority]. [Ref: [16], reproduced with permission from publishing authority].*

osteoblast cells or human adipose stem cells demonstrated good cell viability and steady growth similar to conventional 3D scaffold based cell treatment as shown in **Figure 2B**, which would bring the potential application in tissue engineering [15]. In a report, the mixture of neonatal chicken bone marrow cells (cBMCs) derived bioactive component and collagen was used to prepare the cell supporting bioink, which was further printed with human adipose tissue-derived stromal cell (hASC) lines to formulate the hASC-laden 3D scaffold [16]. The '*in vitro*' study using 3D architecture was shown to promote the growth, proliferation and osteogenesis of hASC cells. The system was further implanted in a rat mastoid obliteration model to monitor the potential effect of cBMCs derived bioactive component on the osteogenic differentiation in new bone regeneration. After 12 weeks of post transplantation, experimental groups showed excellent bone formation as compared as shown in **Figure 2C**. The modification of collagen fiber with synthetic polymer such as polycaprolactone or polylactic acid also resulted the mechanically and biologically active 3D porous cell supporting materials and which was confirmed by SEM, FTIR and proton NMR studies. In skin tissue engineering, the 3D scaffold was shown to increase the adipose tissue derived mesenchymal stem cell (AT-MSC) adhesion and growth with the formation of a tissue environment compared to only PCL or PLA-based scaffolds [17].

3.1.2 Hyaluronic acid as base material and its derivatives

As like collagen, hyaluronic acid (HA) is also a part of extracellular matrix and shown to have a potential role in modulating inflammation, cell attachment, and migration as well as tissue morphogenesis, owing to the biodegradable, biocompatible, non-immunogenicity and anti-inflamatory properties. An attempt by Gao et al. was initiated to develop the self-crosslinked hyaluronic acid-grafted collagen-I hydrogel using EDAC/NHS reaction method. Further, chondrocytes was encapsulated into the hydrogel to verify the cell-matrix interaction and which had a significant effect on the secretion of cartilage-specific matrices to promote the migration, proliferation and gene expression of chondrocytes cells. The *in-vivo* cytocompatibility and biodegradation studies on Sprague–Dawley (SD) rats (~200 g) showed the gradual decrease of the assembled nanofibre bundle and weight of the hydrogel after prolonged subcutaneous implantation [18]. Therefore, the manufactured self-crosslinking hydrogel could find significant application in tissue engineering applications.

In an approach, the injectable HA-SH/peptide hybrid hydrogels was developed based on the covalent/noncovalent supramolecular interaction between thiolated hyaluronic acid (HA-SH) and BPAA-AFF-OH short peptide to regulate the chondrogenic expression both *in vitro* and *in vivo* environments in the cartilage tissue

engineering [19]. The prepared hydrogel confirmed by proton NMR, FTIR and SEM analysis was then employed to explore the cytocompatibility of chondrocyte cells. The chondrocyte cells laden hybrid scaffolds demonstrated the significant adhesion, proliferation of chondrocyte with the expression of chondrogenic specific genes such as Col II, Sox9 and AGG. The *in vivo* study based on the subcutaneous implantation of chondrocyte cells encapsulated hydrogel in New Zealand rabbit's models also revealed the abundant aggregation and proliferation of cells with the secretion of matrix after 4 weeks of post implantation period, resulting in the inhibition of hypertrophy trend of chondrocytes and tunable hyaline cartilage formation.

In order to improve the mechanical properties, methacrylated HA was modified with elastin-like polypeptide (ELP, consists of 70 repeats of the pentapeptide VPGVG) through free radical photopolymerization technique. The hydrogel made from the combination of MeHA/ELP revealed the tunable physicochemical and mechanical properties which is comparable to native tissue structure. Further, incorporation of zinc nanoparticles into the hydrogel had resulted an excellent antimicrobial platform for cell adhesion, growth, and proliferation phenomena (See **Figure 3A**). The '*in vivo*' cytocompatility experiment via subcutaneous implantation of MeHA/ELP hydrogel demonstrated that the weight of the hydrogel was significantly decreased with the generation and growth of autologous tissue without any inflammatory action [20]. This was due to the biodegradation of the transplanted hydrogel that led to the new space for the spreading and infiltration of the proliferated cells. The immunofluroescent staining study also exhibited the minor invasion and infiltration of lymphocyte and macrophages cells, assigning the potential application of the engineered hydrogel in various artificial tissue regeneration processes.

Like various tissue regeneration processes, utilizing of HAbased cell supporting materials explicated the significant output in artificial salivary gland repairing and remodeling. In an experiment, Lee et al. demonstrated the synthesis of hyaluronic acid–catechol (HACA) conjugates based platform named as NiCHE (nature-inspired catechol conjugated hyaluronic acid environment) to mimic the mesenchyme of embryonic submandibular glands (eSMGs) as shown schematically in **Figure 3B(i)** [21]. The NICHE was developed by the coating of HACA conjugates on the various polymeric scaffolds such as polycarbonate membrane, stiff agarose hydrogel, and polycaprolactone that led to cell adhesion and growth, vascular endothelial and proliferation of progeniotor of eSMGs cells isolated from ICR mice fetus [See **Figure 3B(ii&iii)**].

3.1.3 Gelatin as base material and its derivatives

Owing to the excellent biocompatibility, biodegradability and water solubility, gelatin has emerged tremendous interest for the formation of 3D hydrogel in tissue engineering application. Song et al. developed an injectable 3D printed gelatin hydrogel composed of continuous phase gelatin and gelatin microgels. The two-step cross-linked injectable gelatin was shown to exhibit the biocompatible lattice, cup-shaped, tube-shaped and rheological modified structure analogous to human anatomical features. The biocompatibility of the microgel led to the spread and expression of metabolic activities in mouse fibroblast cells, which can be attributed to the good cell-matrix interaction such as *in vivo* remodeling stages [22].

Due to the limited clinical success in repairing defective cartilage, unlike conventional surgery, the biopolymer-based tissue engineering approach such as the production of gelatin-linked electrospun, gelatin-polycaprolactone (gelatin-PCL) nanofiber-filled decellularized extracellular matrix has been investigated to monitor biological functions. The decellularized composite has shown to exhibit the

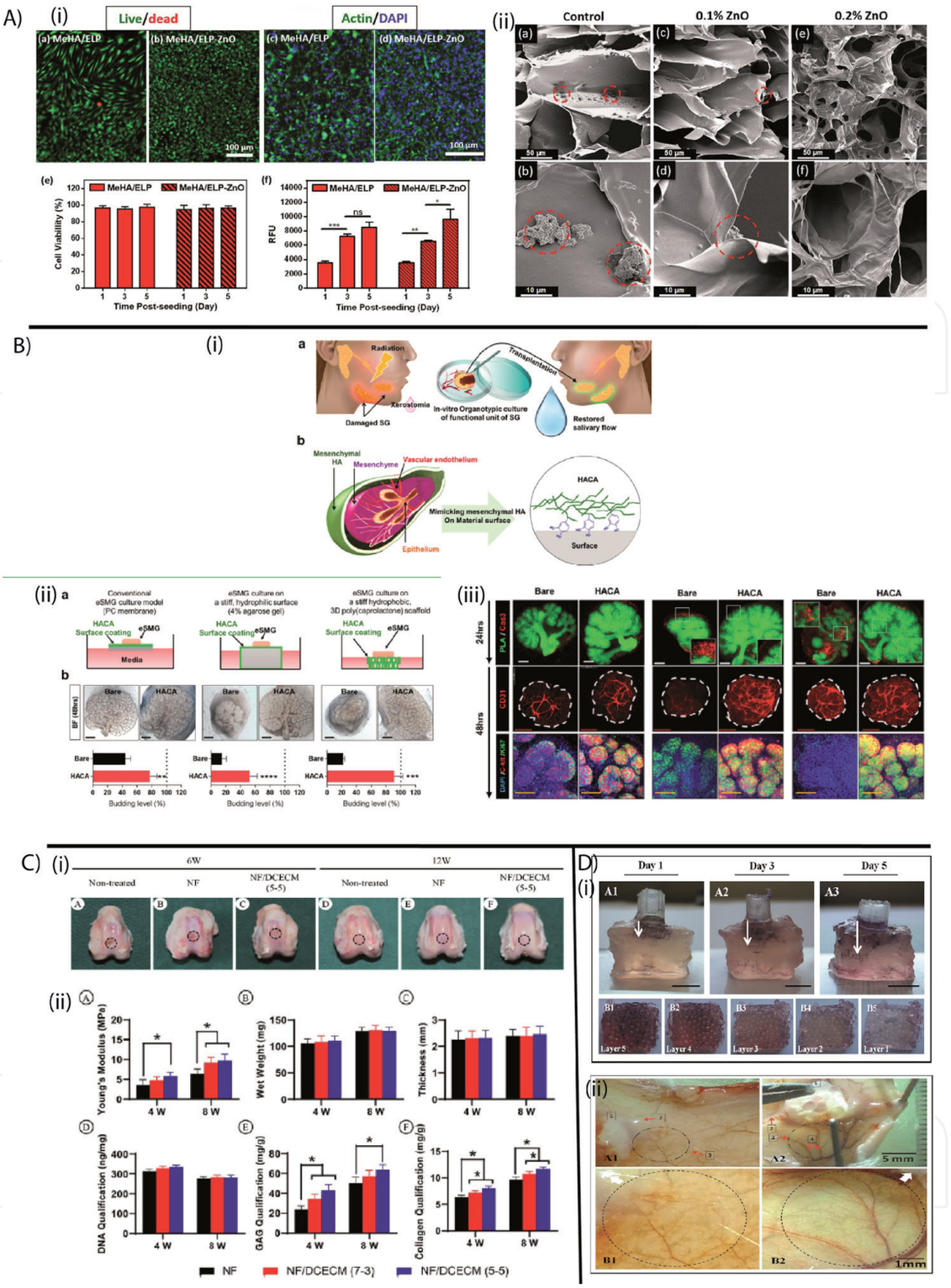

Figure 3.
(A) (i) In vitro cytocompatibility of MeHA/ELP and MeHA/ELP-ZnO hydrogels. Representative live/dead images from hMSCs seeded on (a) MeHA/ELP and (b) MeHA/ELP-ZnO hydrogels after 5 days of seeding. Representative phalloidin (green)/DAPI (blue) stained images from hMSCs seeded on (c) MeHA/ELP and (d) MeHA/ELP-ZnO hydrogels at day 5 post culture. Quantification of (e) viability and (f) metabolic activity of hMSCs seeded on hydrogels after 1, 3, and 5 days of culture. Hydrogels were formed by using 2% MeHA and 10% ELP with 0 and 0.2% (w/v) ZnO nanoparticles at 120 s UV exposure time ($p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). [Ref: [20], reproduced with permission from publishing authority]. (ii) In vitro antimicrobial properties of MeHA/ELP-ZnO hydrogels with different ZnO concentrations. Representative SEM images of methicillin-resistant* Staphylococcus aureus *(MRSA) colonization on hydrogels containing (a, b) 0% ZnO, (c, d) 0.1% ZnO, and (e, f) 0.2% ZnO. Clusters of bacteria are shown in dashed circles. [Ref: [20], reproduced with permission from publishing authority]. (B): (i/a) Schematic Diagram of Salivary Gland Damage and Tissue Engineering-Based Therapeutic Approach; (i/b) Schematic Diagram of Mimicking Mesenchymal HA of Developing eSMG on Material Surfaces by Using Adhesive HACA. [Ref: [21], reproduced with permission from publishing authority]. (ii) NiCHE coating platform enhances growth of eSMGs on various substrates.*

*(b) Bright field images and budding levels of eSMGs cultured either on bare or HACA-coated materials after 48 h-culture. Average bud count of eSMGs freshly isolated from TP15 mouse fetus is considered as 100%. Scale bar = 200 μm. (iii) Apoptotic activity, VE structure, and mitotic activity of progenitor cells of eSMGs cultured either on bare or HACA-coated materials after 24 and 48 h-culture, respectively. White scale bar = 200 μm, red scale bar = 500 μm, yellow scale bar = 100 μm. [Ref: [21], reproduced with permission from publishing authority]. (C) (i) Gross view and histological evaluation of repaired region at 6 and 12 weeks postsurgery. Macroscopic images of cartilage defects regions at 6 weeks (A–C) and 12 weeks (D–F) postsurgery. (ii) Biomechanical and biochemical analyzes of the engineered cartilage tissue in vivo. Young's modulus (A), wet weight (B), thickness (C), DNA qualification (D), GAG qualification (E), and collagen qualification (F) of the in-vivo-engineered cartilage tissue. Values are expressed as mean ± SD, n = 3, *p < 0.05. [Ref: [23], adapted with permission from publishing authority]. (D) (i): (A1-A3) Spatial distribution of the purple formazan crystal inside the 5 L implant at day1(A1), 3(A2) and 5(A3). The scale bars represent 5 mm. Distribution profile of purple formazancrystal indicates an in-growth of cells inside the implant. (B1–5) Presence and distribution of MTT crystals on each bead layer. (ii) Study of the osteogenic properties of the implant in vitro. The study was carried out using human mesenchymal stem cells (hMSC). (A) Time dependent variation of alkaline phosphatase expression. ALP activity was measured in the supernatant of the culture. (B) Study of the expression of osteogenic marker through RT-PCR. The study was carried out after culturing the hMSC on different substrates in presence of osteogenic media for 14 days. (C) Fluorescent micrographs of the replated partially differentiated hMSC stained with FITCPhalloidin(green) and DAPI (blue). The cells were initially cultured on implant and control substrates. They were then trypsinized and replated on tissue culture plate. Imaging was done after 3 days of replating. [Ref: [26], reproduced with permission from publishing authority].*

excellent mechanical property and promoted the cartilage regeneration with the secretion of collagen and glycosaminoglycan as shown in **Figure 3C** [23].

The development of gelatin methacrylate (GelMA) and poly (ethylene glycol) diacrylate (PEGDA) printed three layered scaffold, modified by lysine functionalized rosette nanotubes (RNTK) significantly improved the adhesion, growth and differentiation of adipose-derived mesenchymal stem cells (ADSCs). The RNTK not only acted as a potential biomimetic layer, its presence dramatically increased the secretion of collagen II, glycosaminoglycan, and total collagen as compared to native GelMA-PEGDA scaffolds, and have an potential impact on cartilage regeneration [24].

To improve wound repair caused by burns or accidental injuries, a versatile approach has been shown to fabricate the skin tissue analogue of a mechanically stable acellular elastomeric scaffold in the presence of biodegradable polyurethane and gelatin composite. The Gel-20%PU showed the best cell infiltration and biodegradation in a mouse *in vivo* experiment. Also, it reveals negligible immunogenicity and could be accepted as a substitute for new generation tissues [25].

3.1.4 Sodium alginate as base material and its derivatives

The implementation of the osteogenic microenvironment loaded with therapeutic agents has emerged as the key pathway for bone tissue engineering in recent decades. Like various biopolymers, the utility of alginate, which is a polyionic-polysachharide comprising units of mannuronic acid and guluronic acid, has strengthened the field of next-generation polymer remodeling. The fabrication of calcium alginate bead based 3D implant made by the stacking of hexagonal closed pack (HCP) layers (**Figure 3D(i)**) in presence of glutaraldehyde crosslinker facilitated the spatiotemporal drug release in the artificial matrixes through the changes of the spatial coordinates of the drugs loaded layers. The supporting scaffold promoted the growth, progression and cytosketal reorganization of the osteoblast cells and triggered the expression of the alkaline phosphatase, runx2 and collagen type1 in human mesenchymal stem cells, attributed to the osteoconductive and osteogenic nature of the implant [26]. The *in vivo* assessment of the VEGF loaded implant was conducted in mice model and it revealed the regeneration of tissue with prominent existence of neovascularization as shown in **Figure 3D(iii)**, due to cohesive interaction between supportive implant and native tissue environment. A recent report [27] demonstrated the formation of mechanically stable alginate-gelatin

(ALG-GEL) hydrogels, resembling the comprehensive nonlinear and complex mechanical features of brain soft tissues. The rheology analysis also indicated that the stiffness of the hydrogel is solely dependent on the blending concentration and incubations times of the composites, assigning for the potential application in the fabrication of brain tissue supporting matrixes. Another report, where, (2, 2, 6, 6-Tetramethylpiperidin-1-yl)oxyl or (2,2,6,6-tetramethylpiperidin-1-yl) oxidanyl [TEMPO] oxidized cellulose nanofibre incorporated alginate scaffolds was investigated to enhance the biodegradability, sustainability and mechanically strengthened reactive surface area. The rheology study resulted in the recovery of 60% viscosity than that of native alginate scaffolds. The simulated body fluid (SBF) mediated mineralization evolved the nucleation of the hydroxyappetite into the hydrogel [28]. The combinatory efforts including direct writing 3D printing and freeze-drying techniques were carried out to develop the dual porous mechanically and dimensionally stable cellulose based 3D scaffolds. The dehydrothermal treatment displayed the increased surface hardness, indentation modulus and compression strength, should opened the new glimpse toward the decoration of bio-mimetic bone tissue engineering [29]. Furthermore, several studies have been conducted using cellulose or different derivatives to present an active tissue engineering tool and revealed the positive result in terms of cytocompatibility, biodegradability and flexibility of the scaffolds [30]. Similarly, Suneetha et al. [31] described the synthesis of mussel inspired polydopamine (PDA) filled sodium alginate (SA) – polyacrylamide (PDA – SA – PAM)-based hydrogel for skin tissue regeneration. The *in situ* synthesis process was conducted via two consecutive reaction steps. Initially, the dopamine molecules in the dopamine and sodium alginates blend was polymerized through alkali-induced polymerization and secondly, free radical polymerization technique was used to polymerize the acrylamide part in the processing of mechanically and biologically supporting adhesive hydrogel. The cytocompatibility assessment of the human skin fibroblasts (SFs) and keratinocytes (KTs) seeded PDA – SA – PAM-based hydrogel exhibited the higher cell adhesion, proliferation and spreading into the 3D microenvironment as compared PDA-free or 2D polystyrene plate and which is confirmed by fluorescence based live-dead assays or SEM morphology analysis as shown in **Figure 4A(i&ii)**. In addition, the effect of PDA molecules on the platelet adhesion was evaluated by the processing of porcine whole blood and it showed the higher adhesion of the platelet on the hydrogel as shown in SEM images, attributing to the potential effect of PDA in the regulation of fibrous network and adhesion of bioactive molecules.

3.1.5 Chitosan as base material and its derivatives

Chitosan, a polysaccharide with various functional groups has increased tremendous interest in biomedical applications such as tissue engineering. But, major problems due to poor solubility and biodegradability limit its monopoly use in the processing of cell supporting materials. This is avoided by the stacking or modifying with various synthetic or natural biomaterials. In a report, Li et al. developed the oxidized alginate hydrogel crosslinked with N, O-carboxymethyl chitosan with moderate swelling, degradation and porosity. The chitosan modified alginate scaffold revealed improved biocompatibility, as the number of free aldehyde groups in the oxidized alginate is reduced after crosslinking [32].

In another report [33], the methacrylated chitosan molecules were conjugated with lysozyme (an endo-carbohydrase) via riboflavin initiated photo-cross-linking to a constructed biodegradable and biocompatible hydrogel. The *in vitro* biodegradation study of the hydrogel revealed the increase of the pore size and larger fraction of outliers in cryo-SEM micrographs. Further, the mouse bone marrow

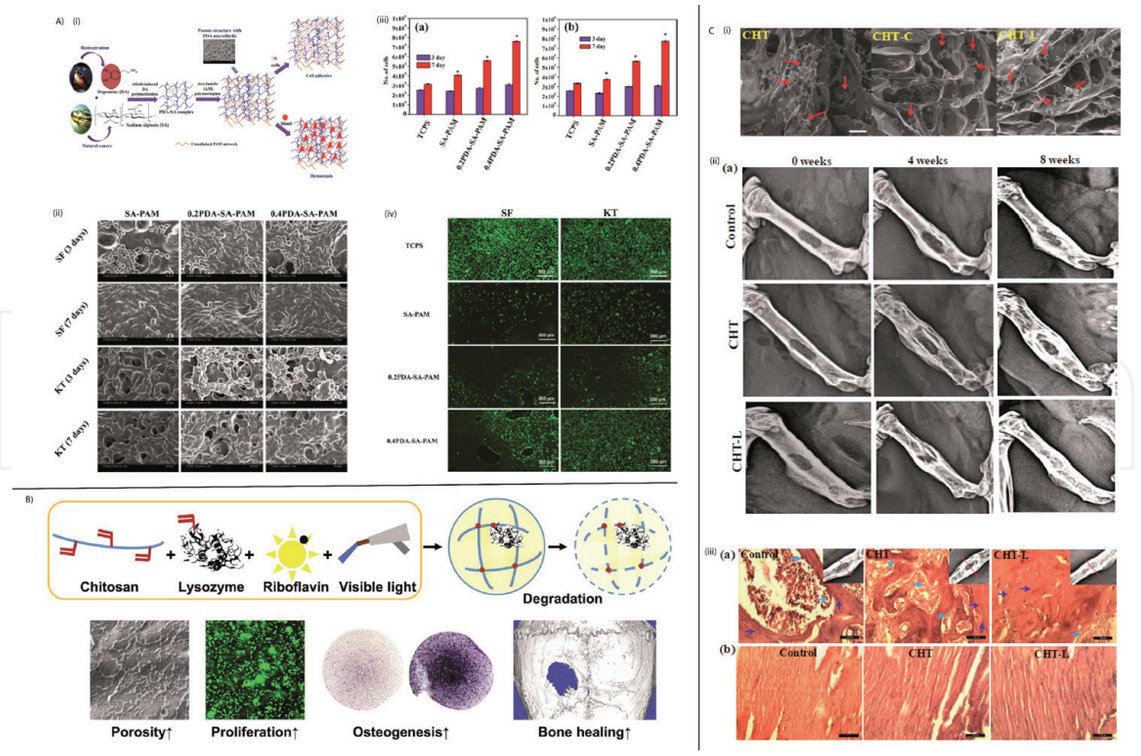

Figure 4.
*(A) (i) Schematic Representation of the Formation of Adhesive Hydrogels for Tissue Engineering Applications. (ii) SEM images of cell attachment of SF and KT cells on hydrogels in different culture conditions (3 and 7 days). (iii) MTT assay cell proliferation of hydrogels for (a) SF and (b) KT. (iv) live/dead assay fluorescence images of SF and KT cells on hydrogels under 10× magnification (scale bar 200 μm) (*p < 0.05). [Ref: [31], reproduced with permission from publishing authority]. (B) Schematic presentation of Chitosan-Lysozyme Conjugates for Enzyme-Triggered Hydrogel Degradation in bone Tissue Engineering Applications. [Ref: [33], reproduced with permission from publishing authority]. (C) (i) SEM images of the cell seeded scaffold of pure CHT and its nanohybrid. Red arrow indicates the position of the cells. Scale bar = 60 μm; (ii) (a) X-ray photographs of rat femur bone defects of control (devoid of any material), CHT (filled with pure CHT scaffold), and CHT-L (filled with nanohybrid scaffold, CHT-L) after one day, four weeks and eight weeks of implantation. Blue and cyan arrows indicate osteocyte and osteoblast cell, respectively; (iii/a) H & E stained histopathological section of the rat femur bone defects after eight weeks of implantation, inset figure of each image indicates that the section were taken from that part (indicated by dotted red lines). Scale bars represent 100 μm; (iii/b) Histopathological section of connective tissue attached with the bone, stained using H & E. Scale bar is 100 μm. [Ref: [35], reproduced with permission from publishing authority].*

stromal cell line (BMSC) loaded hydrogel exhibited the adhesion, proliferation and spreading of BMSCs with the expression of the osteogenic-specific markers throughout various layers of hydrogel as compared to chitosan based hydrogel. This may be attributed to the lysozyme mediated breaking of chitosan chains, thereby helping the penetration of cells through the disintegrated hydrogel networks. Also, as shown in **Figure 4B,** the *in vivo* bone regeneration experiment demonstrated the significant recovery of the defected bone with new bone tissues after six weeks of hydrogel post-implantation in a nude mice model with the recruitment of cells to the damaged zone. Similarly, the mechanically stable and tunable porous graphene oxide incorporated alginate-chitosan-collagen (GO/SA-CS-Col) based composite scaffolds was fabricated via Ca^{2+} mediated crosslinking and freeze-drying techniques. The composition and surface morphology were confirmed by FTIR, Raman spectra, SEM and XRD analysis. *In vitro* study of the osteoblast cell encapsulated scaffold revealed the adhesion, proliferation of the cells and osteogenic differentiation [34]. In an approach, the sulfonated graphene oxide functionalized chitosan based hybrid scaffold was prepared and FTIR, SEM, TEM and XRD analysis confirmed the synthesis of the interconnected porous chitosan/GO nanohybrid scaffolds [35]. The *in vitro* drug release assay at phosphate buffer solution at 37°C exhibited the sustained release of the drug molecules, may be due

to the noncovalent interaction between drug and composites that triggering the slow diffusion of the drug molecules. The hybrid scaffold also revealed the high cell growth and spreading into the deep part of porous scaffold as compared to GO-free chitosan scaffold which was observed by fluorescence and SEM analysis as shown in **Figure 4C(i)**. Further, the *in vivo* experiment using cell laden scaffold in rat model was investigated and the 75 days of post-implantation result demonstrated the faster healing of the defected area with significant proliferation of the osteoblast cells as compared to pure chitosan scaffold (see in **Figure 4C(ii)**). Intertestingly, Zao et al., developed a glucono δ-lactone (GDL) incorporated and carboxymethyl chitosan (CMCh) stabilized calcium phosphate (ACP) (designated as CMCh-ACP hydrogel) bioactive hydrogel using freeze-drying process for mesenchymal stem cells [MSCs] based bone regeneration [36]. FTIR analysis exhibited the characteristics peaks at 1064and 547 cm^{-1} due to the presence of phosphate (PO_4^{3-}) group of spherical particles with average size of 80 nm. Next, the cytocompatability of the MCSs laden (iMAD cell line) CMCh-ACP hydrogel revealed the time dependent increase of cell density with negligible apoptotic cell morphology as in Hoechst 33258 stained, indicating the biocompatibility of the hydrogel for long-term cell friendly growth microenvironment. Like, *in vitro* assays, the *in vivo* bone regeneration experiment in presence of BMP9-(potent bone-forming factor) induced iMAD cells/CMCh-ACP hydrogel demonstrated the efficient new bone formation with the extensive vascularisation on the surface of the masses, attributing to the upregulation osteogenic-specific biomarkers and regulars, thereby enabling the BMP9 induced osteogenesis. In our several works, we demonstrated the modification of chitosan with different biomaterials such as montmorillonite clay (OMMT), hydroxyapatite, poly (ethylene glycol), polymethylmethacrylate-co-2-hydroxyethyl-methacrylate and polyvinyl alcohol. The formulated porous scaffolds were made with improved mechanical, antibacterial and biocompatible for application in bone tissue engineering [37–40].

3.1.6 Polyhydroxyalkanoates (PHA) as base material and its derivatives

Unlike different biomaterials, *polyhydroxyalkanoates (PHA)* are the class of biodegradable biopolymer extracted by the harvesting of microbial cells. Despite the cost-effective synthesis and ease of processing, the hydrophobicity, brittleness and lack of antibacterial limits it random uses in biomedical applications such drug delivery, surgical suture and supporting matrices for tissue regenerations. Efforts are being made to improve its biocompatibility, mechanical strength and antimicrobial properties by blending or modifying the surface with bioactive and high-strength nanomaterials. One approach was used to develop the biocompatible collagen-immobilized porous 3D scaffold based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [collagen/PHBV], which allows better growth and proliferation of the rat osteogenic cell line (UMR-106 cell line) than native PHBV scaffold [41]. A similar trend was also observed when hydroxyappetite was further incorporated into the collagen grafted PHBV composite. The modified scaffolds revealed better adhesion and growth of osteoblast cells [42]. In another study, a hyaluronic acid immobilized chitosan-grafted porous PHA membrane was fabricated and it exhibited better protein adsorption and improved adhesion and proliferation of L929 fibroblasts. The supportive matrix also showed excellent antibacterial activity against several bacterial strains [43]. The mechanically and biocompatibility challenged porous PHH-mixed PHB [PHBHHs] scaffolds showed significant growth and proliferation of chondrocytes isolated from rabbit articular cartilage (RAC) as compared to only PBHs or PHB scaffolds. With the increase of the PHBHH content in the mixture, both the mechanical and the cell compatibility increased

dramatically, they have a potential impetus in tissue engineering applications [44]. Recently, a group of researchers has potentially formulated the carbonaceous or conductive nonmaterial such as polyaniline, graphane oxide modified PHA based 3D porous scaffolds for tissue engineering applications. The antimicrobial and cell-stimulated active 3D constructs not only improved cell attachment, proliferation, but migratory behaviors were observed through interconnected porous networks. The magnetically active MRI scaffolds have tissue engineering applications controlled by significant bioimaging [45–47].

3.1.7 Silk as base material and its derivatives

Silk is a naturally occurring fibrous protein with biodegradability, biocompatibility, and mechanical durability that has utility in tissue engineering applications. In one study, silk fibroin-grafted polycaprolactone nanofibers were able to deliver dual growth factors such as bone morphogenetic protein-2 (BMP-2), transforming growth factor-beta (TGF-β), in the regeneration of bone tissue [48]. Li et al. also presented a similar type of biocompatibility while a PCL/silk 3D bioprinting scaffold was imposed to regenerate the meniscus tissue [49]. The computer-assisted 3D printed silk matrices have attracted significant attention and found to be improved the cell–cell and cell-matrix interaction and enable their activity in patient specific tissue architecture [50]. Similarly, the gelatin-silk composite was subjected to the fabrication of 3D bioprinting for cartilage tissue engineering in rabbit model [51].

The novel development of 4D printing hydrogel has gained significant attention in next generation biofabrication. The fabrication of 4D printing from 3D printing hydrogel was regulated by the modulation their interior or exterior properties with the proper controlled of expansion rate of the hydrogel in distilled water and salt water. The biocompatibility of assessment of the 4 D printing hydrogel was conducted in culture medium by shape change method as mentioned earlier. The results revealed the adhesion and growth of the PKH127 (green)-labeled human chondrocyte (hTBSCs) along with the deposition of cartilage extracellular matrix in the side of the construct. To verify the clinical applicability of the construct, the rabbit TBSC and chondrocytes-laden artificial 4D construct was implanted into the site of the rabbit trachea and the results of 8 weeks post-implantation revealed the regeneration of the respiratory epithelial layer and formation of neocartilage around the perichondrium. This findings proved the potential application of the cell laden 4D hydrogel in the recovery of respiratory organ, trachea regeneration [52]. A very recently, the approach of development of electrical simulation modulated polypyrrole/silk fibroin (PPy/SF) based conductive composite scaffold has been opened up the new avenue in the neuronal tissue regeneration [53]. The 3D printing electrospining method was used to fabricate for the alignment of silk fibrous, followed by the coating of polypyrrole (a mechanically stable conductive material) to get the desired silk fibroin (PPy/SF) composite scaffold as nerve guidance conduits (NGCs). Morphological tracking by SEM analysis exhibited the core-shell structure having interpenetrating PPy fibers on the smooth SF nanofibers with average diameter of 0.427 ± 0.083 μm. Resultant physicochemical properties such as mechanical stability (0.059 MPa) and conductivity (0.11446 ± 0.00145 mS/mm) of composite were comparable to ideal working in NGCs system, indicating the increase of mechanical property of the conduit by the coating of PPy. The ES controlled cell compatibility of the NGCs was evaluated with the seeding of Schwann cells (SCs) and it showed the significant growth, proliferation and migration of the cells with the expression of neurotrophic factors. Further, to investigate the effect of artificial NGCs on *in vivo* nerve tissue regeneration, the composite was implanted in defected sciatic nerve of rat and monitored for six months under ES regulation.

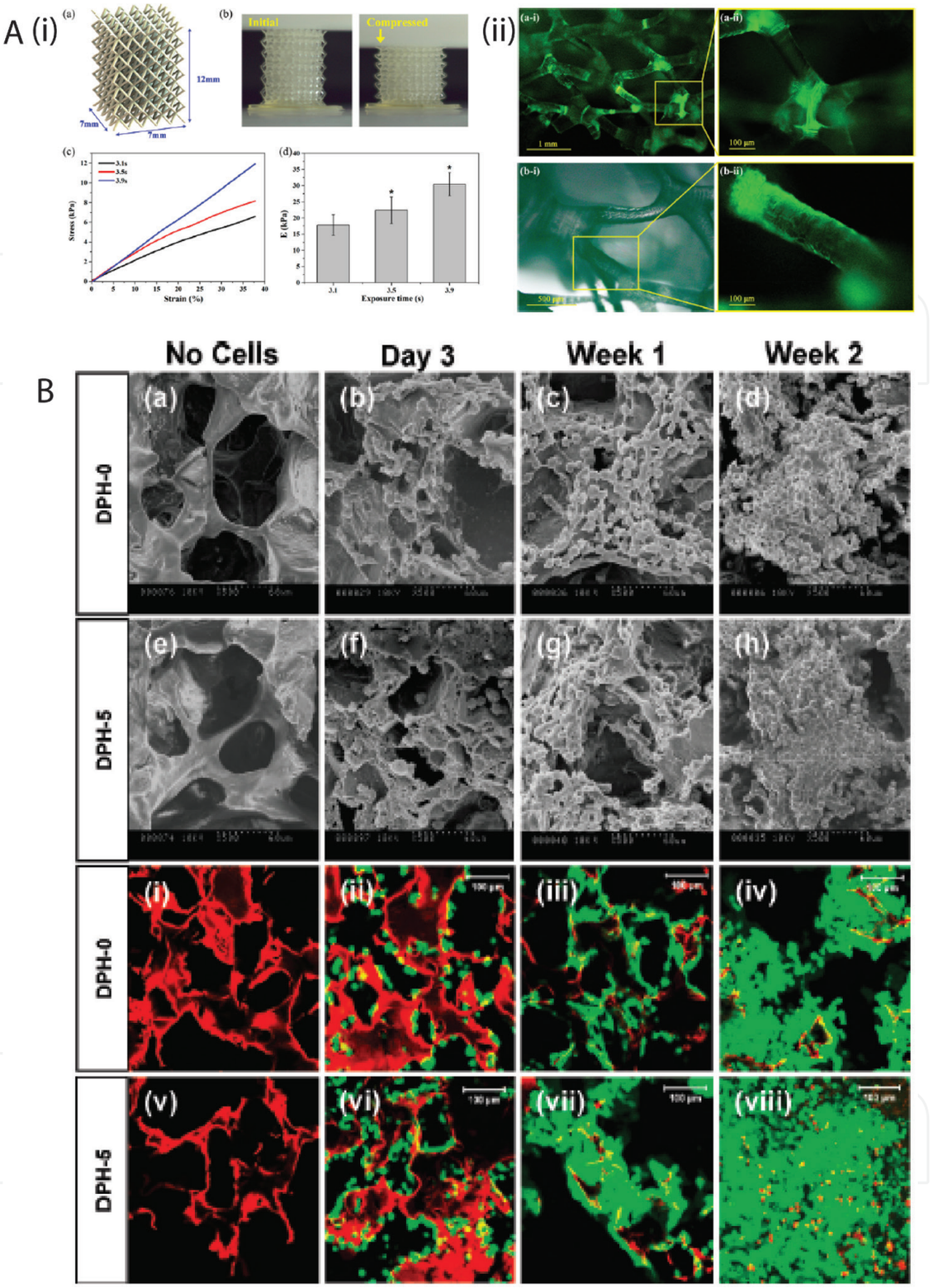

Figure 5.
(A) (i) (a) 3D model of the designed octahedral type of scaffold with marked dimensions; (b) scaffold was loaded on the compression platform; (c) strain–stress curves; (d) elastic moduli for three groups of homogeneous scaffolds; (ii) (a-i) Fluorescent microscope images taken with a 4 × objective to show the 3 T3 fibroblasts distribution in the scaffold. (a-ii) Interaction of 3 T3 fibroblasts with the joint of scaffold. (b-i) optical and fluorescent microscope images taken with a 10× objective. (b-ii) Fibrous-like cell layer formed on the frame surface of scaffold. [Ref: [54], reproduced with permission from publishing authority]. (B) Interaction of A10 VSMCs with D-PHI porous scaffolds. SEM (a-h) and live-dead (i-viii) images of D-PHI-0 (a-d and i-iv) and D-PHI-5 (e-h and v-viii) porous scaffolds in the absence of cells and following a 3 day, 1 week, and 2 week culture period with A10 VSMCs at 0 μm depth (scaffold surface). Cell-free scaffolds (i and v) fluoresce red under the microscope. All SEM images were taken at 500 × original magnification. [Ref: [55], reproduced with permission from publishing authority].

Histochemical and microscopic analysis revealed the densely regenerated myelinated fibers and mylinated axon dispersed in the fibrous networks that promoted the regeneration of the pheripheral injured nerve. Since, the cellular activities in nervous system are regulated by the expression of various neurotrophic factors (BDNF and NT-4/5) and signaling pathways. The ES modulation may activate the MAPK in the cell microenvironment and promoted the growth of the axon, correlated with nerve regeneration. Therefore, although several reports have been undergone using silk for the construction of different tissue architectures but intensive research must be conducted to find potential validity in clinical trials.

3.1.8 Different synthetic materials and their combination

The synthetic materials based 3D hydrogel have also shown to mimic the native tissue stiffness while the optimum conditions for the 3D constructions are digitally controlled. The digital light processing (DLP) based printed poly(ethylene glycol) diacrylate (PEGDA) hydrogels exhibited nearly 60% of enhanced elastic modulus, suited for the support of 3 T3 cells adhesion and proliferation as shown in **Figure 5A** [54]. In one study, degradable, polar hydrophobic and ionic porous polyurethane scaffolds were synthesized using a lysine-based crosslinker. The scaffolds demonstrated (see **Figure 5B**) the comprehensive mechanical, swelling and biocompatible properties that support the adhesion and growth of muscle cells in vascular tissue engineering [55]. Apsite et al., reported the design and fabrication of polycaprolactone and poly(N-isopropylacrylamide) based multilayerd porous electrospun mats. The self-folding 4D bio-fabrication was found to act as good cells adhesion and viability, assigning as a new perspective in new generation tissue engineering [56]. In a paper, Kutikov explained how the integration of hydrophilic polyethylene glycol into hydrophobic polyester block copolymers changes the physicochemical properties of 3D matrices. The incorporation also demonstrated the different types of cell adhesion, growth, and tissue regeneration both *in vitro* and *in vivo* experiment [57]. Therefore, the deviations in the fabrication of 3D artificial support matrices using natural biopolymer or synthetic materials individually must be compensated by the chemistry of piling or surface modification to increase any physical properties that would satisfactory fill the gaps to improve the clinical applications.

4. Effect of growth factors on cell-matrix interaction

Enormous studies have been thoroughly investigated on the interaction between cellular and bio-mimetic 3D matrices *in vitro* and *in vivo* tissue generation experiments that demonstrated the phenomena of adhesion, growth and differentiation of different cells. But, most of the study doesn't meet the pre-requisite for the successful clinical application due to the insufficient secretion of protein molecules that responsible for the biological and biochemical signaling between cell–cell and cell matrix cross-talk. The prominent small proteins that induce cell growth, proliferation, differentiation and regulate angiogenesis are encoded as growth factors. The emergence of versatility of different growth factors related to the reported mediated repair of damaged tissue tends to fall into various categories based on their functionality in tissue engineering. EGFs, NGFs, IGF, FGFs, PDGFs, interleukins etc. are the class of growth factors mainly disclosed for the cell–cell medicated trafficking of proliferation and actin-cytoskeleton in living tissue regeneration process.

Mechanically, the function of growth factors is to drive progenitor cells to its damaged target tissues by extracellularly mediated signaling pathways. In fact,

therapeutic molecules bind to the cell surface transmembrane receptor and then to the internalized receptor-protein complex through phosphorylation-mediated signal transduction that triggers down-regulation of cells, followed by reduction of overwhelming responses and stimulation at the cellular level to carry out biological functions. Furthermore, the non-diffusible method leads to the binding of growth factor to the cell surface without any major internalization or downregulation in the results of long-term biological activities, as shown in **Figure 6A(i&ii)** [58]. Mimicking the *in vivo* tissue environment, various approaches have been implicated using growth factors loaded 3D biocomposite for sustained release without any dysfunction of the protein molecules. Bone morphogenesis protein-2 and 7 (BMP-2 & BMP-7) are the part of transforming growth factors enable the proliferation and osteogenic differentiation of bone marrow derived mesenchymal stem cells. The removal of bio-signaling molecules has demonstrated the deregulation of cell proliferation, differentiation and alteration of bone tissue formation [59, 60]. Co-administration of TGF-b3 and BMP-2 via alginate-based scaffolds revealed a tendency for increased osteogenesis in *in vivo* bone formation tests. A similar type of output has been observed while TGF-1 and IGF-1 are used simultaneously for bone tissue engineering [61, 62]. In a study, Kim et al., demonstrated that the inhibition of epithelial growth factor predominantly affects the cell–cell and contact based cell proliferation. In addition, over-expression of cadhreine, a transmembrane-type cell surface protein limited cell-to-cell contact with the arrest of cell cycle, resulted in spatial cell rearrangement tuned to tumor formation. Therefore, the epithelial growth factor plays an active function towards the formation of epithelial cells in tissue engineering [63].

It has been a challenge to meet the need to develop a bio-mimetic tool for vascular tissue engineering. In contrast to various soft tissues, vascular tissue controls the supply of oxygen, essential cellular nutrients, the transport of waste products and stem cells as well as progenitor cells. Therefore, it is urgent to reconstruct the

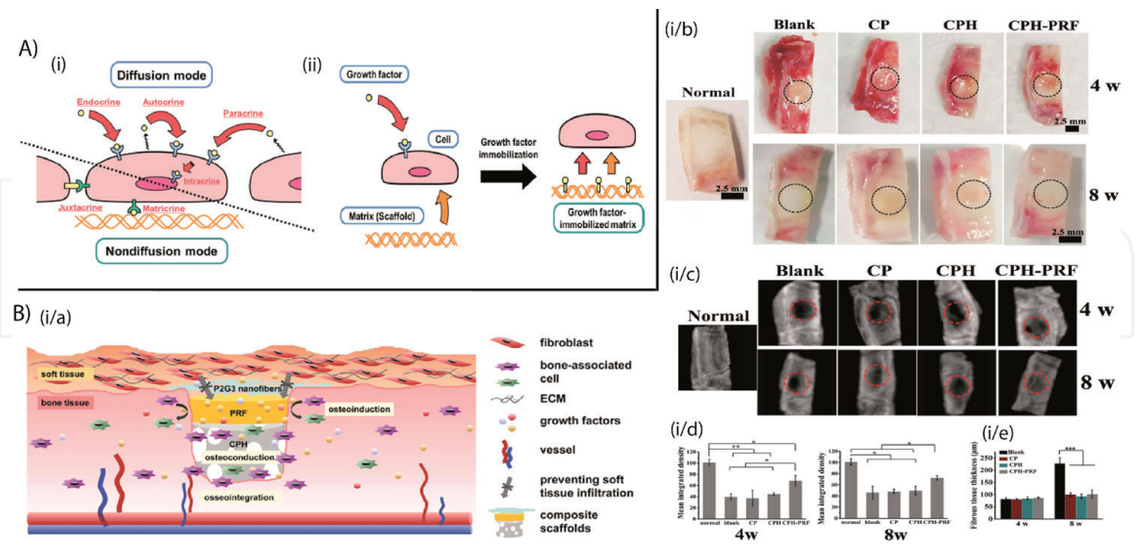

Figure 6.
*(A) (i) Modes of action of growth factors. Growth factors interact with their receptors in a diffusible manner (e.g., by endocrine, paracrine, autocrine and intracrine pathways) or in a non-diffusible manner (e.g., by juxtacrine and matricrine pathways). Some growth factors are known to act in both ways. (A) (ii) Three main factors in tissue engineering: cells, growth factors and matrices (scaffolds). The conjugation of growth factors and matrices provides a new approach for generating biofunctional substrates for regenerative medicine [Ref-58, reproduced with the permission from publishing authority]. (B) (i/a): Schematic representation of proposed mechanisms for enhanced bone regeneration in vivo. (i/b): Optical images of retrieved specimens from representative critical-sized bone defects at 4 and 8 weeks. (i/c) Micro-CT analysis. (i/d) Quantitative analysis of bone regeneration. (i/e) Fibrous tissue thickness at the defect site at 4 and 8 weeks (*P < 0.05; **P < 0.01; ***P < 0.001). [Ref. [66], adapted with permission from publishing authority].*

network of the neovascularization process with the initiation of adhesion, growth and differentiation of cells as a native tissue environment in artificial tissue engineering. Cao et al. demonstrated the therapeutic approaches of growth factors and their signal cascade that control neovascularization and the formation of neovessels using the spatiotemporally controlled 3D construct both *in vitro and in vivo* pathways. The literature also explained how vascular epithelial growth factor (VEGF) plays a critical role in vasculogenesis in stages of embryonic development from pre-existing blood vessels through consecutive signaling pathways [64, 65]. The multifunctional triple layered chitosan/poly (gamma-glutamic acid)/hydroxyapatite (CPH) hydrogels was formulated to regulate the release of platelet-rich fibrion (PRF) which was extracted from rat abdominal arota, into the site of damaged bone tissues. The PRF entrapped composite hydrogel was prepared through noncovalent electrostatic interaction and lyophilization technique to promote the osteoconductive mediated new bone tissue formation. The PRF entrapped composite hydrogel was prepared through noncovalent electrostatic interaction and lyophilization technique to promote the osteoconductive process in the formation of new bone. PRF is a combination of different growth factors such as TGF-β, PDGF, and IGF that have an abiity to induce the mineralization and the upregulation of various osteogenetic biomarkers in order to activate the osteoblast as seen in vitro and in vivo experiments. As a part of '*in vivo*' tissue regeneration study, rat calvarial defect models demonstrated the superior healing of the calvarial defect after 8 weeks post implantation period in presence of PRF/CPH composite than that of control experiment (see **Figure 6B**) [66]. In summary, the combined growth factors entrapped 3D supporting matrixes would bring a new avenue towards the cell–cell cross-talk mediated tissue generation with the advancement of bio-mimetic tools in finding the arrays of the artificial tissue engineering in the future.

5. Conclusions

Understanding the mechanism and basic criteria in the process of tissue regeneration has unveiled the secret of the communication involved in cell–cell, cell-matrix interaction that enables healing in an artificial tissue environment such as native tissue repair processes. In fact, the biocompatibility of any fabricated 3D architecture plays an important role in adhesion, proliferation, migration, and differentiation of the cells of interest to biologically mimic the signaling cascade that triggers cellular activities. Several investigations have shown that 3D constructs comprising naturally extracted and synthetic materials having a porous and mechanically stable geometry promoted integrin ligand-mediated differentiation and tailored actin-cytoskeletal cell morphology in a better way. The studies also explained how the biological and biochemical performances of cells are influenced by the different growth factors mediated signaling pathways and the active function of the ECM components. Therefore, present review provided the core thinking behind the physicochemical features of supporting matrixes that significantly control the cell–cell and cell-matrix interaction towards the implementation of clinically approved artificial 3D biocomposite for the successful clinical tissue engineering applications.

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Conflict of interest

No conflict of interest is a declaration.

Acronyms and abbreviations

TE	Tissue Engineering
ECM	Extracellular matrixes
EGFs	Epithelial growth factors
PDGFs	Platelet derived growth factors
IGFs	Insulin like growth factors
HCGFs	Hematopoietic cell growth factors
AT-MSCs	Adipose tissue-derived mesenchymal stem cells
GelMA	Gelatin methacrylate
PEGDA	Poly (ethylene glycol) diacrylate
HCP	Hexagonal closed pack
TEMPO	(2, 2, 6, 6-Tetramethylpiperidin-1-yl)oxyl or (2,2,6,6-tetramethyl-piperidin-1-yl)oxidanyl
SBF	Simulated body fluid
hBMSCs	Human bone marrow derived mesenchymal stem cells
OMMT	Montmorillonite clay
HA	Hyaluronic acid
IPN	Interpenetrating network based
BMP-2	Bone morphogenetic protein-2
PEGDA	Poly(ethylene glycol)diacrylate
VEGF	Vascular epithelial growth factor

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