The use of nanoscaffolds and dendrimers in tissue engineering

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To avoid tissue rejection during organ transplantation, research has focused on the use of tissue engineering to regenerate required tissues or organs for patients. The biomedical applications of hyperbranched, multivalent, structurally uniform, biocompatible dendrimers in tissue engineering include the mimicking of natural extracellular matrices (ECMs) in the 3D microenvironment. Dendrimers are unimolecular architects that can incorporate a variety of biological and/or chemical substances in a 3D architecture to actively support the scaffold microenvironment during cell growth. Here, we review the use of dendritic delivery systems in tissue engineering. We discuss the available literature, highlighting the 3D architecture and preparation of these nanoscaffolds, and also review challenges to, and advances in, the use dendrimers in tissue engineering.

Advances in the manufacturing of dendritic nanoparticles and scaffold architectures have resulted in the successful incorporation of dendritic scaffolds in tissue engineering.

Introduction

The self-repair mechanism of the body is a crucial critical process that maintains the integrity and function of injured cells, tissues and organs [1]. Following injury, mechanical trauma, or infection, different body tissues, including the liver, skin, and bone, can regenerate or repair through natural self-repair processes [2,3]. However, this process can take longer time and recovery becomes slower with increasing age, or the presence of disease or injury [4]. Furthermore, this repair process is limited to small, localized defects, while larger and/or severe

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http://dx.doi.org/10.1016/j.drudis.2016.12.007

reconstructions are more challenging, resulting in negative impacts on the quality of life of patients. When this natural process is ineffective, it can be possible to replace the damaged part of the body with a healthy one via organ transplantation [5]. However, this is not a simple treatment, given that various considerations need to be taken into account [6], in particular, the production of donor-specific antibodies because of the autoimmune response can result in the rejection of solid organ transplants [7].

Uncontrolled autoimmune suppression can amplify the chances of graft rejection, and can result in the death of the recipient [8]. However, the replacement of damaged organs with new and/or artificially reconstituted cells and/or organs using the patient's own cells is now possible as a result of the development of tissue engineering. Given its interdisciplinary nature, tissue engineering combines the fields of life sciences, bioengineering, materials sciences, medicine, and pharmaceutical sciences [9]. It can be used to manufacture complex biological substitutes that can be taken from the bench to the bedside to reestablish, preserve, or improve tissue and/or organ function [10,11]. An overview of tissue engineering will help us to understand the concept of tissue regeneration inside a scaffold microenvironment based on the patient's own cells. Thus, here we review the use of nanoscaffolds, with a special emphasis on dendritic delivery systems in tissue engineering.

Tissue engineering highlights

Tissue engineering can be used to develop functional constructs that can be used to reestablish, maintain, or improve the condition of injured body parts or tissues. This approach uses the patient's own cells to grow the tissue or organ required outside of the body using the patient's own cells; the engineered tissue or organ is then transplanted into the body, thus mimicking the native ECM binding sites and topography of the original tissue or organ [12,13].

Recent approaches toward tissue engineering have mainly focused on: (i) implanting newly created cells for the restoration of cellular structural network; (ii) exploring biomaterial scaffolds for endogenous cell infiltration, proliferation, and subsequent regeneration; (iii) a combination of (i) and (ii); and (iv) *in situ* tissue engineering by the incorporation of regenerative stimuli in the biomaterial scaffold [2]. A 3D scaffold is particularly important in tissue engineering because it provides a microenvironment for the cells being cultured to grow in so that a construct is grown that has the requisite cellular behaviors, specific structures, and cellular properties. Such tissue-engineered constructs are now available in the clinical and offer patients an alternative to medication for several diseases [14].

Since 2003, most tissue-engineered products have been marketed in the USA and European Union (EU) for the management of burns and wounds, and also for cartilage replacement [15] (Fig. 1).

FIGURE 1

Marketed tissue-engineering products for biological applications.

Research has also been performed into tissue engineering bone for treating craniofacial defects [16], cartilage for patients with osteoarthritis, and alveolar bone for patients with a cleft lip and palate, as well as for the regeneration of hair follicles, treatment for narrowing of the voice-box and upper windpipe, replacement corneas, and blood vessels for the treatment of cardiovascular conditions [17,18].

Research has also focused on the development of biological bandages with antimicrobial peptide dendrimers that can be used to improve the treatment of burns without inducing any cytotoxicity and without altering the gene profile of progenitor cells [19]. To establish the functionalized engineered tissue to recover the structural integrity of the damaged tissue or organ, three major components are necessary; the scaffold; the cells to be seeded; and the growth factors required in the growth medium. Below, we discuss each of these components and their importance in the regeneration and manipulation of connective tissues.

Cell types and tissue scaffolds

Given the advantages of tissue engineering using native cells of patients, this approach has resulted in more predictive and efficient organ regeneration following artificial organ transplantation. The type of cells used during tissue engineering experimentation is a major focus of research, with studies focusing on the use of embryonic stem cells (ESCs) [20] and mesenchymal stem cells (MSCs) [21] as an ideal cell sources. MSCs can be manipulated to produce harder tissues of human body, such as teeth [22] and bones [23], as well as vascular tissues [24] depending on the microenvironment available in the scaffold.

Imitating the 3D structure with a suitable ECM is crucial for the successful development of the artificial tissue and/or organ [25]. A biocompatible, biodegradable 3D scaffold that has the required mechanical integrity and elasticity will facilitate the growth of the desired cells in a cell-specific microenvironment.

An ideal scaffold contains an adequate surface area and directs the cells toward the development of the required tissue phenotype [26]. The selection of scaffolds depends on the type of cells to be seeded onto it. For example, higher concentrations of ECM are necessary for cells forming connective tissues, whereas cell-dense tissues require less ECM [27].

Cheung *et al.* [28] reported current trends in the scaffold materials used for skin and skeletal tissue engineering. The bio-mimicking natural materials used in the preparation of scaffolds include collagen, fibrin, hyaluronic acid, gelatin, chitosan, polyhydroxyalkanoates, and decellularized ECM (i.e. the separation of necessary proteins from the animal tissue following the removal of host cells) [29,30]. Collagen is the most common and preferred scaffold construction biomaterial because it is already present in connective tissues. Its highly porous spongy framework supports the growth of tissues by holding them in its lattice, while simultaneously maintaining a hydrostatic fluid pressure and a continuous supply of growth nutrients [31].

In addition to natural biopolymers, synthetic polymers are also used in the preparation of 3D scaffolds [32]. Materials used include polyesters [poly(glutamic acid), poly(lactic acid), poly(DL-lactide-co-glycolide), poly(ε-caprolactone)], poly(fumarate), and their copolymers with polyethylene glycol (PEG), poly(vinyl alcohol), poly(amido-amines), and their derivatives. Synthetic scaffolds are advantageous in that they are more flexible and can be manufactured in different sizes and shapes [33]. Some inorganic materials are preferred in the engineering of bone tissues, including calcium phosphate ceramics and cements, ceramic/polymer composites, and bioactive glass [34,35]. However, because of their brittleness and poor flexure strength, ceramics and cements are difficult to sculpt, leading to defects in the resulting tissue. Therefore, superior mechanical strength along with elasticity and biocompatibility of bioceramics enables the formation of different sculptures in tissue engineering applications [36].

As mentioned above, biodegradability and biocompatibility are key prerequisites of the scaffold material. In addition, the scaffold must have an interconnected porous network and should allow cell adhesion, which favors cell attachment, colonization, and migration, resulting in growth [13,37]. It is believed that porous (>80–90%) and interconnected microstructure networks provide essential cell nutrients that lead to the successful growth of tissue without the formation of a necrotic core [38]. However, the optimal growth of the seeded cells is dependent on the diameter of the pore size and its porosity; thus, the latter two features have direct implications for the functionality of the 3D scaffold during tissue engineering applications [39].

Results of early studies showed that cellular necrosis can occur during the growth of cells inside the scaffold material because of the depletion of surface nutrients [38]. To develop suitable microenvironments for the growth and functionality of seeded cells, scaffolds are important both *in vitro* and *in vivo* [13]. Usually, the interconnected porous scaffolds assist in the continuous transportation of nutrients and removal of cell waste, thereby facilitate the proliferation and migration of cells [40]. An approach for encouraging the differentiation of cells into the microenvironment of the nanofiber mesh scaffolds is detailed in Fig. 2. Several techniques have been developed to control the porosity and pore size of the scaffolds to regulate the behavior of the cells. Although the porosity and pore size of the scaffolds need to be optimal for certain types of tissue engineering, it is believed that the porous mesh network of proteins and glycosaminoglycans in ECMs in the scaffolds undergoes continuous remodeling through cross linking to mimic the actual microenvironment needed for cell growth [39].

Cellular microenvironment: features supporting successful tissue regeneration

Early studies focused on achieving the structural integrity of the organ and/or tissue microscopically by replicating the mechanical properties of the desired tissue. More recent research has aimed to establish the physical structure of a specific tissue or organ to maintain and regulate the normal activity of the tissue or organ it is replacing. Facilitating the development of biomimetic environments to produce engineered tissue equivalents with desired functionality, seeded cells are fabricated with ECM within the scaffold. ECM has a crucial role in that it provides nutrients and growth factors that stimulate cell proliferation, migration, differentiation, and maturation, resulting in a functional tissue [41,42].

A prospective scaffold should be structured in such a way that the ECM with the required nutrients should always surround the cells. Thus, the architecture of the ECM within the scaffold should be engineered to supply the nutrients at a constant rate. Biomaterial, micro- and/or nanotechnology systems have made it possible to

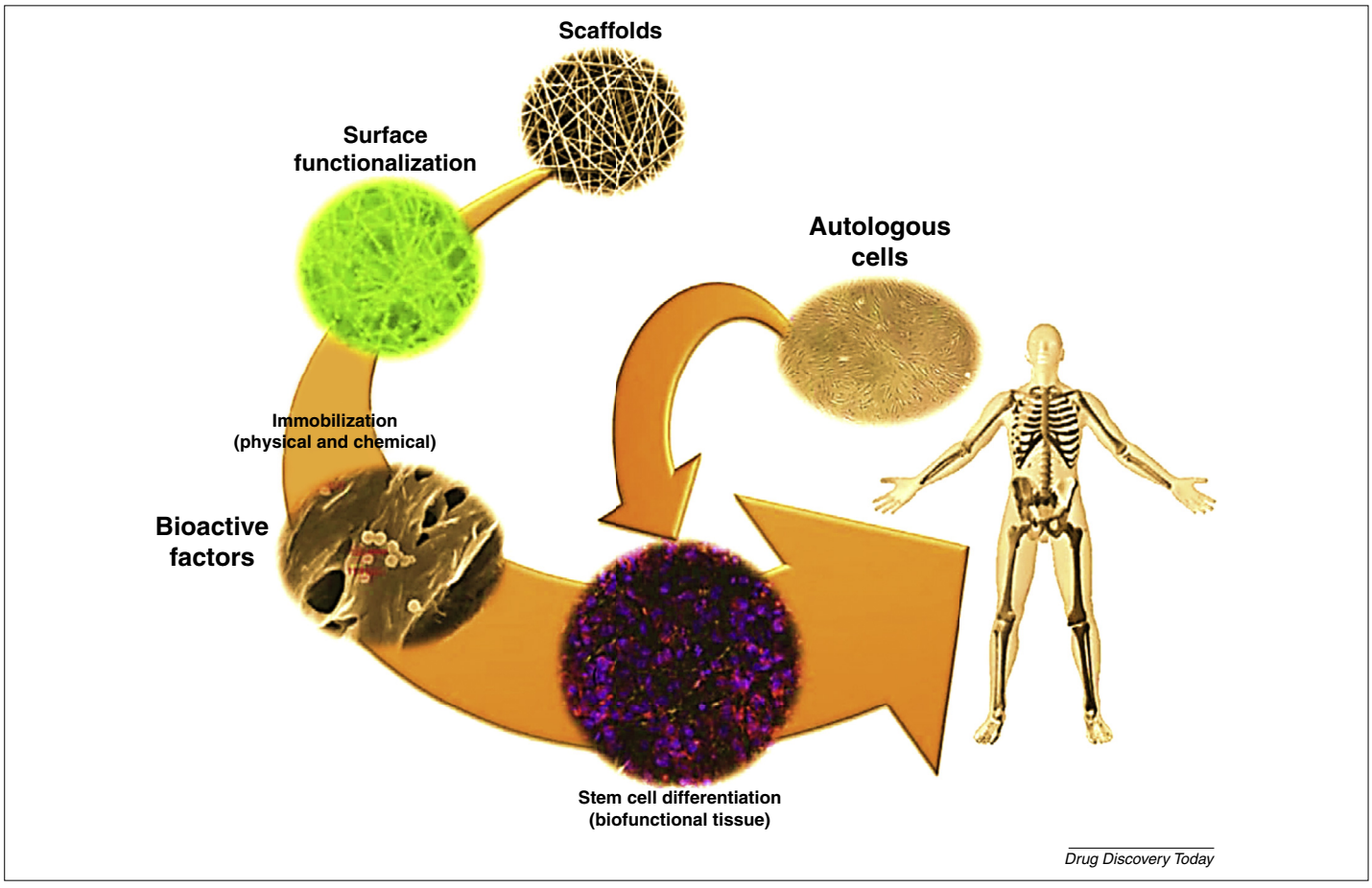

FIGURE 2

Approach to control cell differentiation in the microenvironment of electrospun nanofiber meshes [32].

develop, design, and fabricate such a biomimetic microenvironment. The incorporation of nanotechnologies in particular has made it possible to develop scaffolds with nanotopographic surfaces that release essential molecules (e.g. growth factors) from the nanocarriers to maintain cell growth at a controlled rate. Therefore, in tissue engineering, scaffolds also act as a reservoir of required nutrients, releasing them at a controlled rate for the growth of the seeded cells [43].

The fabricated scaffold is able to deliver nutrients to the attached cells directly and protects the bioactive agents from biodegradation by forming a highly regulated network that promotes the proliferation and migration of cells, and their differentiation into a functionalized tissue or organ. Several bottom-up and top-down fabrication technologies have been used to manufacture polymeric 3D scaffolds for tissue engineering applications to construct functionally augmented engineered tissue [44]. Below, we discuss the fabrication techniques used to develop a 3D microenvironment for cell growth.

Fabrication techniques in practice

The scaffold or extracellular environment in which the cells are to be grown can be synthesized using various nanofabrication techniques that allow the formation of porous scaffolds with underlying nanofeatures. Here, we discuss the nanotopographies that are currently being adopted for the fabrication of scaffolds, including assembly, freeze-drying, additive manufacturing, electrospinning using bottom-up technologies, and various imprinting techniques using top-down technologies.

Self-directed organization of components

Self-assembly is the autonomous organization of components into a 3D cellular environment with a final pattern without any external intervention [45]. Hydrogels of natural, synthetic, or peptide-derived polymers could serve as an architecture for cell growth, although collagen is the most widely accepted natural hydrogelling agent. Techniques adopted for the manufacturing of extruded collagen nanostructured microfibres include the extrusion of a collagen solution into a functionalized solution [46], extrusion into fine laboratory tubing [47], and the application of an electrical current [48] or magnetic field [49].

Freeze drying and additive manufacturing

Freeze drying is an inexpensive and reproducible nanotopographic technique that can utilize natural polymers, synthetic polymers, or a mixture thereof. The freeze dried nanocomposites can be manufactured by freezing the polymer solution and sublimation with controlled monitoring of the molecular weight of the polymer, pH of the solution, freezing rate, time, temperature, pressure [44,50], and, most importantly, the concentration of the cross-linkers [51].

Additive manufacturing, 3D printing, or rapid phototyping fabrication technologies create a scaffold through deposition of material, usually layer-by-layer, to obtain the specific 3D

morphological composition based on computer-aided designs [52]. Among the other methods, additive manufacturing has gained great interest in scaffold manufacturing because it can be used to obtain the precise shapes and microstructures needed based on the imaging data with good accuracy, reproducibility, and a high degree of automation. Variations of this technique include photopolymerization, selective layer sintering, 3D printing, extrusion-based technologies, and organ printing [53].

Electrospinning and imprinting technologies

Electrospinning is the process of integrating physical sciences into the life sciences. The advantages of implication electrospinning in tissue engineering were highlighted by Townsend-Nicholson and Jayasinghe [54] and Jayasinghe *et al.* [55] and include the ability to deposit a controlled number of cells in the 3D scaffold for use in cellular and developmental biology. To generate neo tissues in the scaffold, electrospinning is considered to be the simplest fabrication technique. This technique can result in the more successful alignment of the electrospun fibers in this tailored scaffold compared with other techniques [56]. This is possible via the use of a jetting and/or electrospraying technique to get the desired cell-bearing microenvironment and this avoids the problem of needle clogging. In addition, it is possible to achieve a high-throughput microenvironment using a coaxial arrangement of needles for cell suspension and a viscous polymer with the application of electrical conductivity [54,55,57,58]. By controlling blending of the biomaterial, fiber diameter, fiber alignment, geometry, and the incorporation of essential components, it should be possible to maintain the phenotypic and functional characteristics of such nanofibrous scaffolds [59]. This technique could be helpful in the generation of 3D scaffolds to recover or replace tissue-based anomalies [57]. In addition, the 3D scaffold could be further fabricated to improve its functionalization potential via the incorporation of micro- and nanoparticles [60,61].

To obtain a particular design of the scaffold for the growth of cells, several models of imprinting have been introduced, which involve molecular imprinting, photo-induced imprinting, nanoimprint lithography, and soft lithography [44]. It should be possible to introduce pillars, lines, nanotubes, grooves, ridges, capillary-like tubes [62], holes or pits, and other nano- to microstructured topographic fabrications in the scaffolds by using these techniques [63].

Such advances in nanofabrication have enabled researchers to investigate cell–nanotopographic interactions and have facilitated the modification of the morphology, signaling, orientation, attachment, migration, and differentiation of the seeded cells. Thus, as discussed below, scaffolds can be fabricated using various nanotechnologies to obtain structures with high mechanical strength, which can then be successfully used in tissue regeneration applications.

The emerging use of nanotechnology in tissue engineering

3D nanostructured scaffolds can be used to mimic the structure of the ECM to precisely control the arrangement of cells and bioactive nanomaterials in the microenvironment that is necessary for cell adhesion, proliferation, and differentiation [64]. Nanofibrous scaffolds are particularly relevant here because of their similarity to the physical structures of extracellular proteins [65]. In addition, the use of nanocomposite-based scaffolds [66] and carbon nanotubes [67] is gaining increasing attention for the preparation of 3D scaffolds. Furthermore, researches are also focusing on the nanotopographic modification of the microenvironment with the incorporation of groves and ridges, given their structural similarity to the ECM [68].

The presence of growth factors in the ECM is necessary for the proliferation, migration, and differentiation of the seeded cells to form the functionalized tissue. Given their poor absorption quality along with the self-aggregation and enzymatic degradation of growth factors, resulting in their short half-life, the bioavailability of these substances is low [69,70]. Moreover, the localized and controlled release of growth nutrients from the nanocarrier in the 3D scaffold surrounding the seeded cells will be necessary for the accurate regeneration of a functionalized tissue from the seeded cells [71].

Various novel approaches that incorporate growth factors into the nanocarrier system have been developed [72–74]. For example, vascularization to maintain a continuous blood supply to the engineered tissue could be possible if vascular endothelial and fibroblast growth factors are incorporated into the scaffolds for controlled release. Incorporation of nanocarrier-loaded growth nutrients into the 3D scaffolds have been shown to prevent the degradation of growth factors, thereby highlighting the possibility for, and flexibility of, the sustained release of growth factors, which would further prevent the necrosis of growing cells and reduce any adverse effects of the degraded growth factors [32,38]. Several strategies using micro- and nanocarrier systems have been introduced to deliver growth factors to the seeded cells.

The use of nanocarriers to deliver growth factors in tissue engineering as well as for the delivery of several pharmaceuticals in different diseased conditions depends on the size of the nanocarriers, which can result in a tremendous increase in surface area [75,76]. Nanosized carriers used in pharmaceutical delivery systems are usually 10–200 nm in size because such particles can easily pass through blood capillaries and become distributed throughout the human body [77–79]. Additionally, particles less than 10 nm in size are simply cleared by the renal system, whereas those greater than 200 nm are phagocytosed and removed by the spleen [80–82].

Among the nanocarriers available for the controlled delivery of growth factors, lipid-based nanocarriers, dendrimer-based nanocarriers, inorganic nanomaterials [71] and graphene oxide [83] are the most commonly utilized. Some nanocarriers are able to encapsulate multiple agents along with cell-specific growth factors and can release them one by one in response to changes in the microenvironment (e.g. pH, temperature, light, or mechanical stress) [71].

The ECM also can be mimicked by a novel dendrimer-based hydrogel, which exhibits a highly interconnected porous network, enhanced mechanical stiffness, and a low swelling ratio. Well-defined building blocks could be made possible by incorporating dendrimers as a polymerizing agent in the hydrogel [84]. The hydrogel supports the proliferation and differentiation of MSCs without any cytotoxic effects. Thus, such a dendrimer-based hydrogel system can be considered as a model for developing innovative materials with applications in tissue engineering to simulate the native environment for cell growth [15,85]. Wang *et al.* successfully produced bony tissue in mice following the incorporation of two

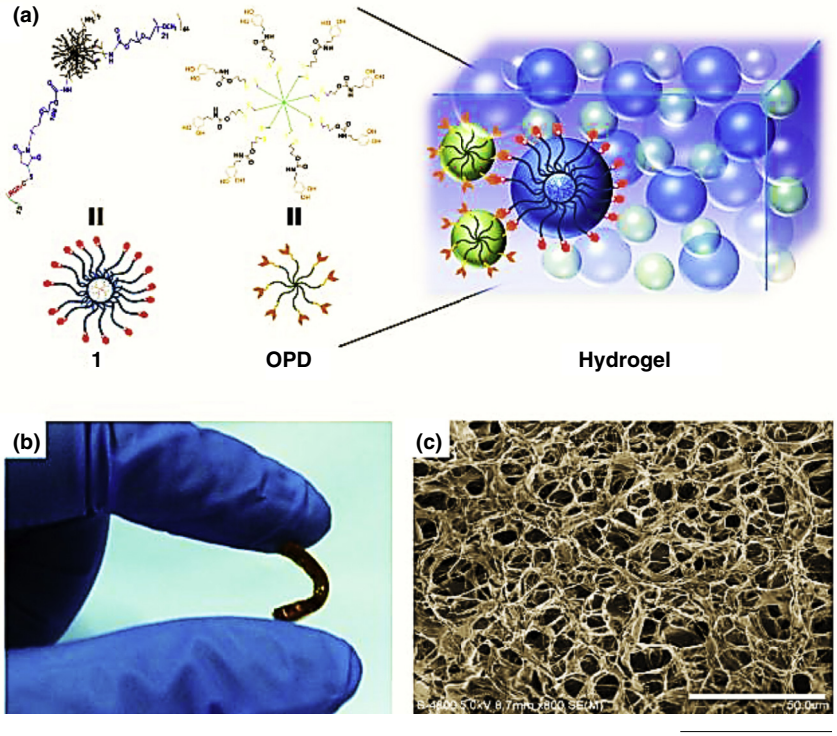

FIGURE 3

Biological applications of dendrimers. **(a)** Schematic presentation of a hydrogel based on a bioactive fragment-modified generation 4 poly (amido amine) dendrimer ('1') and a DOPA-terminated 8-armed PEG ('OPD'). **(b)** The hydrogel at room temperature. **(c)** Scanning electron microscope image of the hydrogel; scale bar = 50 mm [15].

different types of dendrimer within the mechanical integrity of the biodegradable porous hydrogel network (Fig. 3) [15].

Among the available nanocarrier systems, dendrimer-based nanocarriers have shown to be promising model systems for the controlled release of growth factors in the ECM of 3D scaffolds for developing new advanced materials for tissue engineering [85–87]. The use of dendrimers in the fabrication of the microenvironment of the scaffold is summarized below.

Use of dendrimers in tissue engineering

Dendrimers have a 3D branched topology with special physical and chemical properties, a well-defined globular shape, multifunctionalities, and potential application as materials, catalysts, and in biology [88]. There has been increasing research interest in the use of dendrimers in diagnostic applications, such as imaging agents in radiotherapy, X-ray, and magnetic resonance imaging (MRI), for gene delivery, pharmaceutical drug delivery, as carriers for vaccines, antimicrobial agents, and cancer treatment, and also as biomedical materials [89–91].

Dendrimers are tree-like synthetic macromolecules with a large number of branching points, a 3D globular architecture, monodispersity, and a nanolevel size [91,92]. In this well-defined structure, terminal units are on the globular surface and the dendritic units are internal [93]. Diverse applications of dendrimers are possible because such macromolecules serve as carriers for both hydrophilic and hydrophobic drugs or nutrients and can deliver them to a specific target [94].

The layers from the central core of a dendrimer resemble the layers of an onion and are often known as 'generations'. On the basis of the number of generations, the effects of a dendrimer can be negative or positive. However, most negative results are not published. Dendrimer effects are positive up to a certain number of generations and are negative thereafter [95]. In addition, dendrimers with up to six generations can efficiently penetrate cells, whereas the rate of penetration decreases in dendrimers with six to ten generations [96].

Tissue engineering and properties of dendrimers

Dendrimers are nanoparticles with improved physical and chemical properties. There are several unique characteristics that make dendrimers suitable nanocarrier drug delivery tools. These properties include: (i) reproducibility of uniform size, branching, and a well-defined globular shape; (ii) well-defined nano-sized structure, which makes them an ideal carrier in different biomedical applications because of their ability to cross cell membranes without premature elimination from the body; (iii) better biocompatibility with little or no toxicity (dendrimers with terminal neutral group) [97]; (iv) their surface charge can be manipulated to facilitate their interaction with the targeted biosystem; (v) aqueous solubility; and (vi) improved pharmacokinetic properties that deliver drug components to the target organ and/or tissue, improving the therapeutic effect and reducing any toxicity because of the decrease in the amount of free drug in the extravascular system [98]. Processes for manufacturing dendrimers are summarized below.

Production of dendrimers for scaffold development

Dendrimers used for scaffold development are symmetric, highly branched polymers that are spherical in shape with a diameter of

1–20 nm [98]. There are two major parts to a dendrimers: (i) a central poly-functional core; and (ii) the generations formed by the repetitive addition of monomers. The latter is facilitated by the functional groups in the core, which further facilitates an increase in generations. Following an increase in the number of generations, the dendrimer becomes tightly packed, reaching a maximum size. Two major methods have been used to synthesize dendrimers: the divergent (inward-out) method and the convergent (outward-in) method [98].

Divergent and convergent approaches

The divergent growth process comprises activation of the functional units of the dendrimer core, with subsequent radial addition of branching monomers [95,99,100]. The activated core is reacted with at least two protecting branching sites. The protecting sites are then removed and form the first generation of the dendrimer. This process is then repeated until the desired size or number of generations is reached. Figure 4a illustrates the development of a four-generation dendrimer [101]. Although this method requires

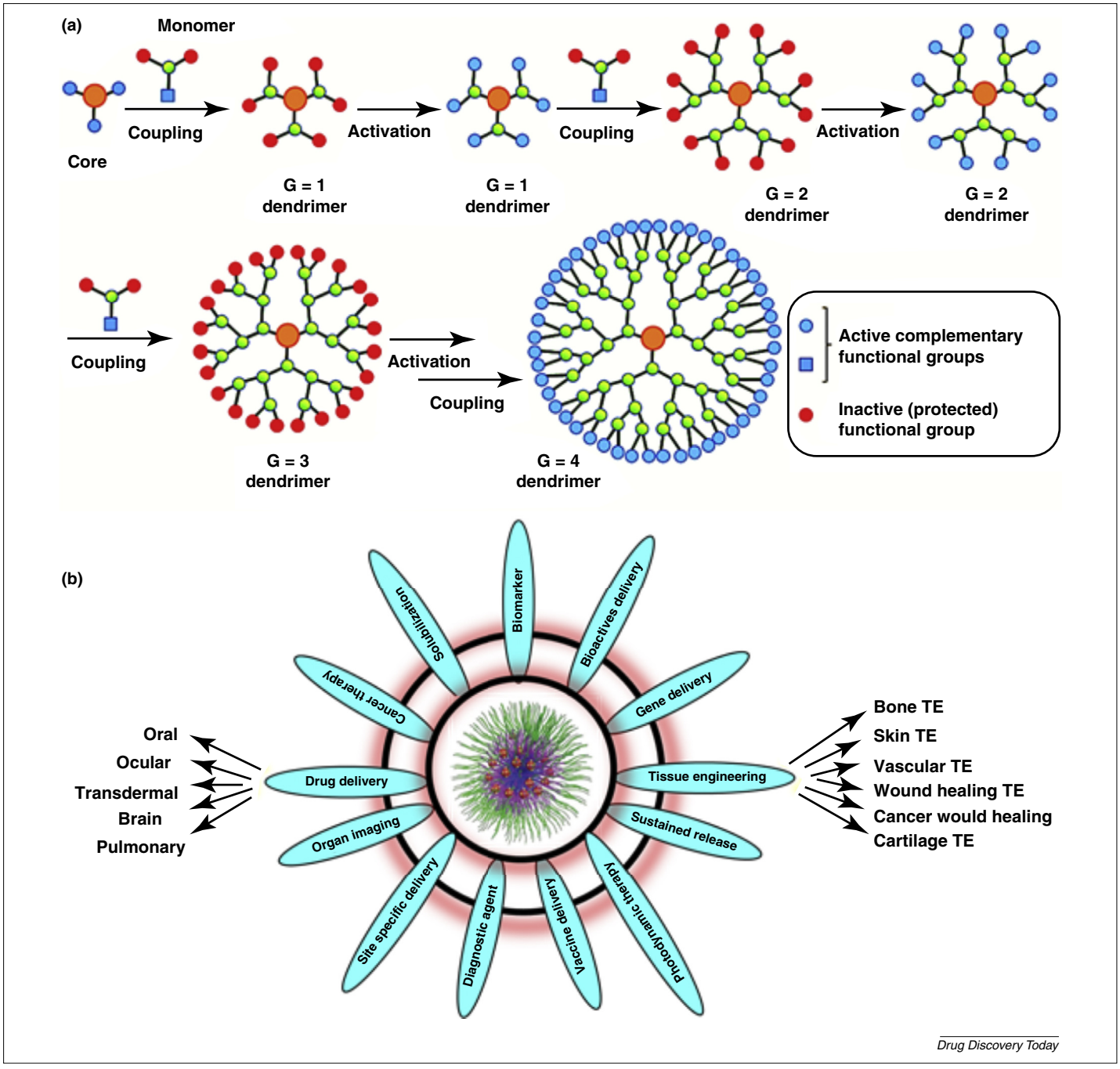

FIGURE 4

Optical microscopy and scanning electron microscopy of scaffolds seeded with rat bone marrow stromal cells. **(a)** Process for the activation and coupling of monomers to form a four-generation dendrimer using the divergent growth method [119]; **(b)** use of dendrimers for different biological applications. *Abbreviation*: TE, Tissue engineering.

extensive loading of monomers and chromatographic separation, it has the advantage that it is possible to change the groups of the outermost layer [102].

The convergent growth process also involves two steps: the linking of the outermost layer to produce a focal point functionalized dendron, followed by the attachment of the massive branches to the common core. The process has a lower yield because of the repeated reactions and the resulting lower-order dendrimers limit its use; however, it has the advantages of decreased side reactions, ability to control the molecular weight and position of the functional groups, and a simple purification process [98].

Auxiliary techniques in dendrimer production

Additional methods have also been developed for the synthesis of dendrimers. The hypercore and branched monomer method was developed to speed up the synthesis and also to increase the yield. It uses preassembled oligomers attached to the active attaching groups of the hypercore to form higher-generation dendrimers. The double exponential approach involves divergent and convergent growth from a single starting material. Products generated through these two methods are then reacted to form a trimer, which can be redeveloped for exponential growth. The Lego chemistry approach was developed to reduce the cost and time of dendrimer synthesis. In this method, a highly functionalized core and branched monomers are used. Refinement of the method resulted in the increase in the number of terminal surface groups from 48 to 250 in a single step. Furthermore, this method requires a minimum volume of solvent, resulting in an easy purification process with nontoxic byproducts [99,103]. The Click chemistry method has been adopted for its faster and reliable synthesis approach. This method results in an excellent yield of high-purity dendrimers [99,103].

Thus, the method of dendrimer preparation enables the reproducibility of the process, production time, and output of the product, including its purity, yield, desired terminal functional group, and so on. Below, we describe the application of dendritic macromonomer compositions in emerging biomedical and bioscience fields.

Biomedical applications of dendrimers

Given their unique architectural structure, molecular uniformity, specific properties resulting from multifunctional end groups, compatibility with pharmaceutical products, flexibility of drug-targeting options, the potential applications of dendrimers is a focus of research worldwide [104]. This nanoparticle-based drug delivery device has successfully been introduced for several therapeutic, biomedical, and diagnostic applications, as summarized in Fig. 4b.

There are a few dendrimer-based products currently awaiting approval for commercialization. For example, dendrimer-based nanomedicines have been patented for range of therapeutic application, including disease-based targeted treatments, MRI, DNA/RNA transfection vectors, pharmaceutical and metal delivery, antiviral and antimicrobial treatments, and as diagnostics [105]. The therapeutic application of a dendrimer as a topical antimicrobial gel (VivaGel® SPL7013) for the treatment of HIV, herpes simplex virus 2 (i.e. genital herpes, human papilloma virus) and bacterial vaginosis is now in a Phase 3 trial (Starpharma Pty Ltd., Melbourne, Australia).

Furthermore, clinical research (currently in Phase 1trials) on docetaxel dendrimers has been initiated to target advanced metastatic breast, prostate, lung, and ovarian carcinoma by Starpharma and AstraZeneca. In addition, the extensively branched structure, multivalence effect, and multistage release behavior of dendrimers could all be exploited as potential tools in tissue engineering. Below, we describe examples of the use of dendrimers in tissue engineering, where the dendritic structure in the scaffold has a crucial role in optimizing the intended biomedical application.

Emerging role of dendrimers in tissue engineering

The primary steps in developing dendrimers for use in tissue engineering include developing dendrimers with the required nontoxic constructs in the 3D scaffold surface where cells can grow in the provided native ECM with the necessary hormones, signaling molecules, and other growth factors. Therefore, developing dendrimers with a porous structure helps to provide the nutrients at a constant rate with simultaneous clearance of the generated wastes. Scaffolds provide mechanical strength to the growing cells and supporting tissues, and their biodegradable characteristic aid the synthesis of new ECM over a period of weeks or months [27].

Several nanocarriers have been shown to be effective in the release of growth factors from artificial scaffolds in a controlled manner. Studies on dendrimers have shown that they can be used to encapsulate growth factors and release the components of native ECM in a controlled manner to enable its regeneration. Thus, it is possible to maintain the microenvironment of the scaffold to complement the growth of the seeded cells. Thus, biomedical studies on the application of dendrimers for several tissue-engineering approaches are promising.

Dendrimers in bone tissue engineering

Desai *et al.* reported the preparation of highly branched and well-arranged dendrimer nanoparticles comprising different surface groups. The hydrogelling agent acrylate was incorporated into the outer surface groups of the dendrimer chains. Photoreactive PEGylated poly(amido amine) (PAMAM) dendrimers were structurally characterized and hypothesized for utilization in bone tissue engineering [106]. Structurally, PAMAM dendrimers contain an ethylene diamine or ammonia core and amidoamide branching [107]. An advantage of PAMAM dendrimers in tissue engineering was also highlighted by Jiang *et al.* [108] and Oliveira *et al.* investigated the incorporation of dendrimers in bone tissue engineering [109]. These authors utilized increased the levels of alkaline phosphatase in, and mineralization characteristics of, the modified cellular microenvironment using a dexamethasone carboxymethyl chitosan/PAMAM dendrimer, which was found to enhance the ectopic early osteogenic differentiation of rat bone marrow stromal cells in the scaffold. Calcium deposition leading to higher mineralization was reported to be greater in the scaffold constructs exposed to a dexamethasone carboxymethyl chitosan/PAMAM dendrimer environment (Fig. 5) [109].

Murugan and Arumugam recently reported that covalent bonding with the multi-walled carbon nanotubes and hydrophilic polypropylene imine dendrimers showed excellent biocompatibil-

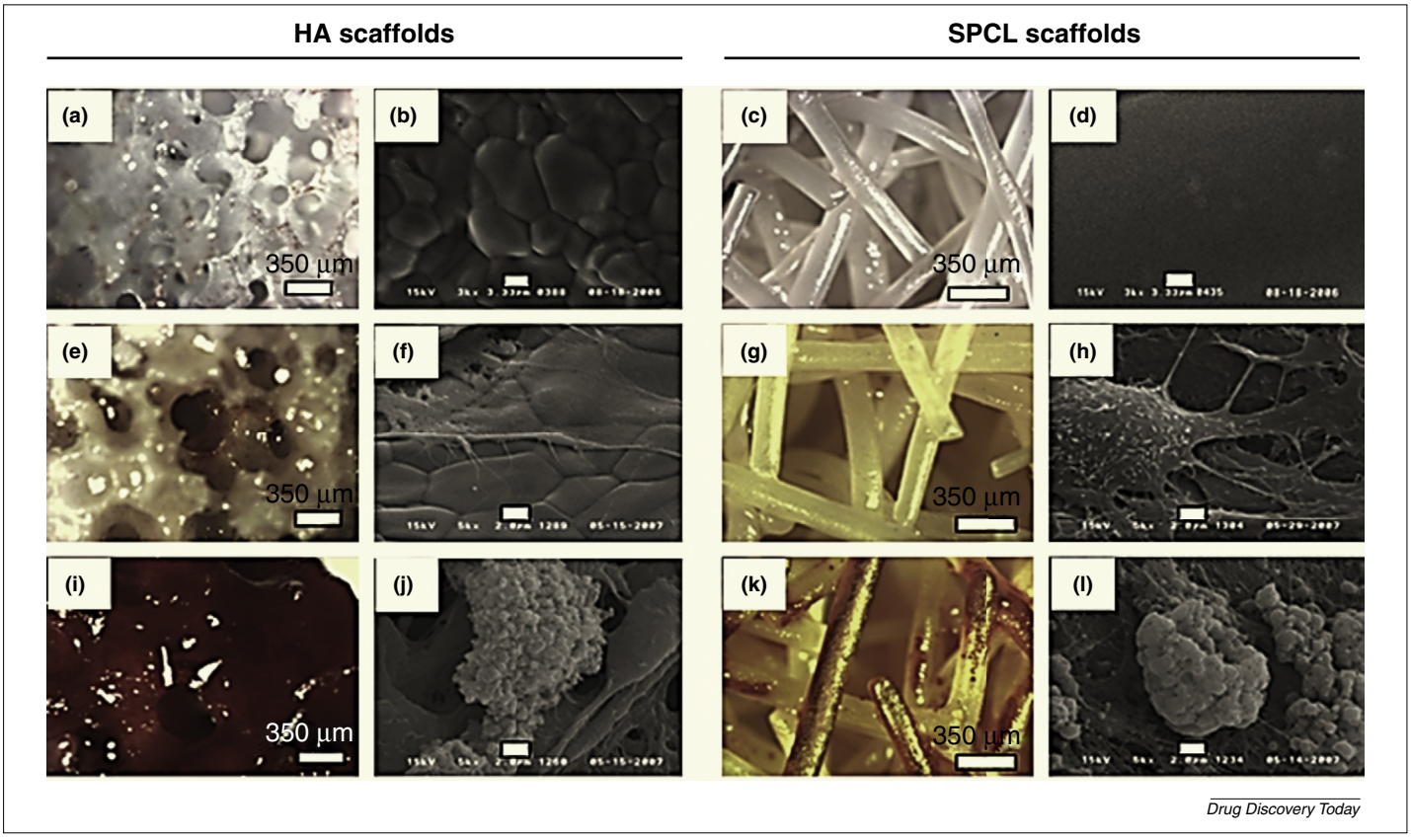

FIGURE 5

Optical microscopy and scanning electron microscope images of hydroxyapatite (HA) (left) and starch–polycaprolactone (SPCL) (right) scaffolds seeded with rat bone marrow stromal cells (RBMSCs), stained with Alizarin red (mineralization) after culturing in different culture media for 14 days: **(a–d)** controls (scaffolds without RBMSCs); **(e–h)** Complete Eagle's minimum essential medium; **(i–l)** MEM medium with Dex-loaded CMCht/PAMAM dendrimer nanoparticles [109].

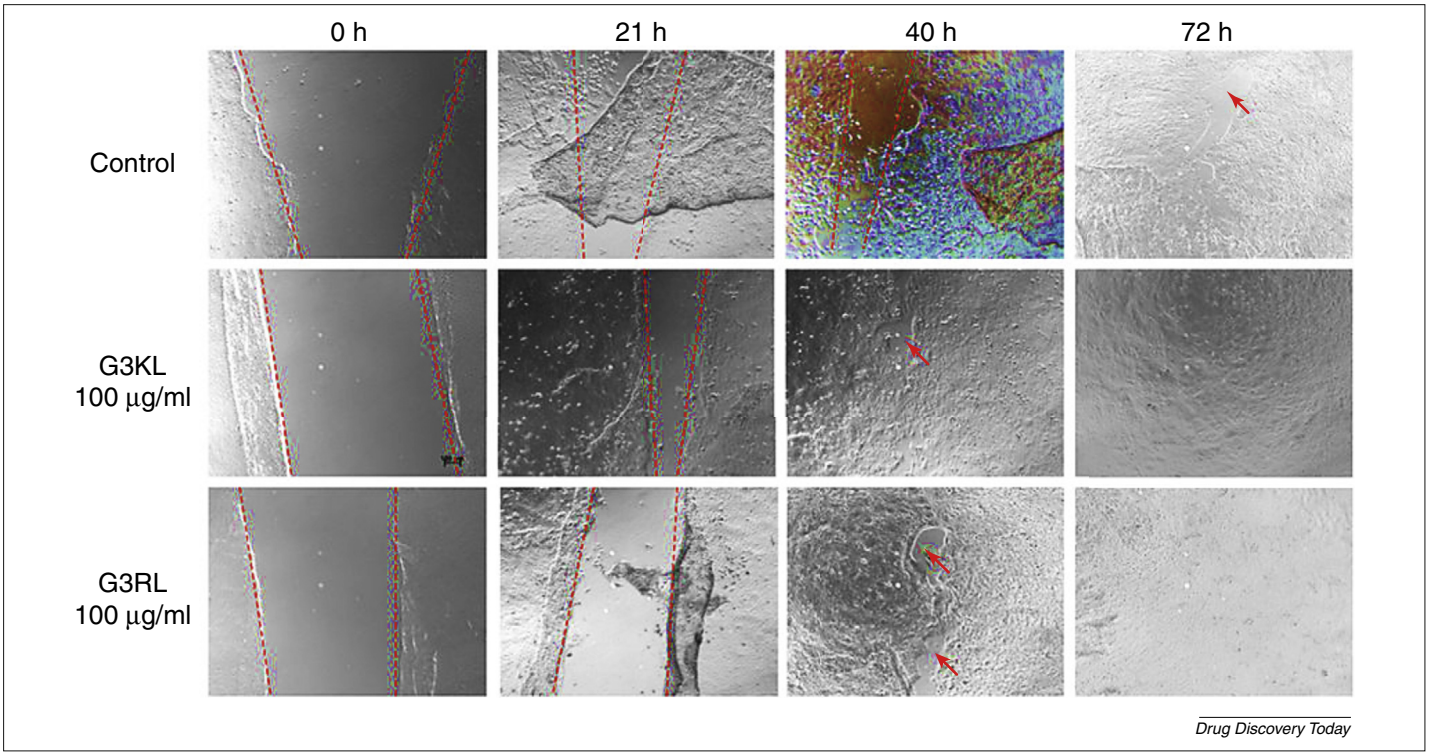

FIGURE 6

Scratch assay for visualizing keratinocyte migration for G3KL and G3RL dendrimers at 100 mg/ml over 72 hours of cell migration representing wound healing [19].

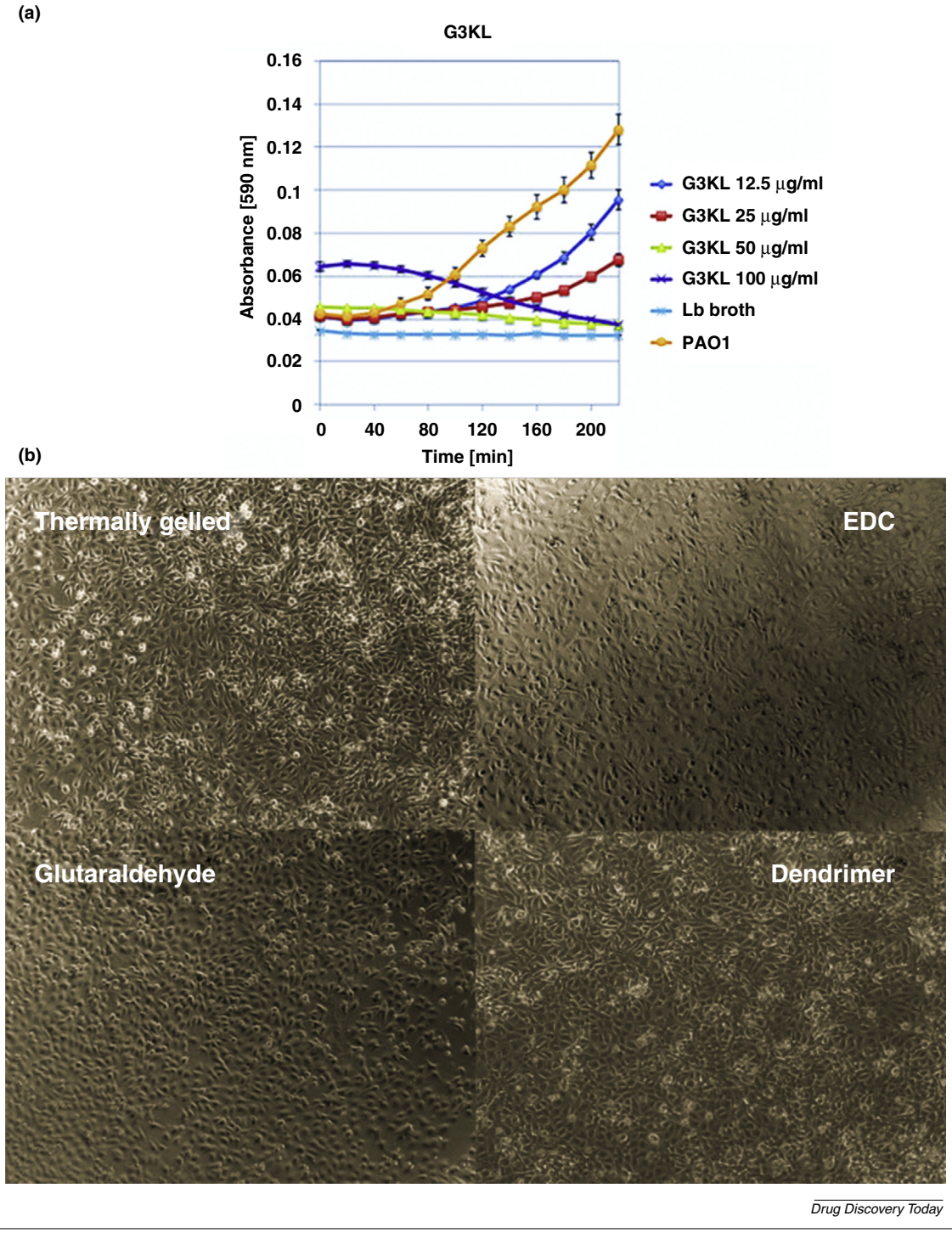

FIGURE 7

Bacterial growth kinetics in the presence of antimicrobial dendrimer. **(a)** Bacterial growth kinetics in the presence of a G3KL antimicrobial dendrimer [19]; **(b)** photomicrographs of human corneal epithelial cells on collagen gels after 4 days of culture [112]. *Abbreviation*: EDC, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride.

ity and dispersion ability in an aqueous system, and this could be successfully applied in bone tissue engineering. Bonding of hydroxyapatite (which is chemically similar to natural bone) with this complex promoted the development and proliferation of normal human osteoblast cells, followed by bone formation by mimicking the requisites of the ECM. The authors further reported the biocompatibility, biodegradability, bioactivity, and biosafety of the complex in cancer cells, highlighting its potential in both bone tissue engineering and cancer treatment [110].

Dendrimers as burn treatments

Multidrug-resistant nosocomial pseudomonas infections in third-degree burn injuries are common and can lead to severe physiological stress, economic burden, and even death [19]. These

third-degree burns involve damage to the epidermal and dermal layers and also destroy the vasculature, thus hindering the spontaneous self-regeneration ability [111]. To investigate the effectiveness of dendrimers in treating burns, Abdel-Sayed *et al.* performed an *in vitro* scratch assay with keratinocytes and analyzed the resulting cell migration. The presence of antimicrobial dendrimers in the biological bandage encouraged keratinocyte migration (Fig. 6), leading to the rapid closure of the *in vitro* wound within 72 hours [19].

Furthermore, antimicrobial dendrimers embedded within biological membranes were incubated *in vitro* with multiresistant *Pseudomonas aeruginosa* PAO1 in lysogenic broth to determine their antimicrobial potential. Bacterial inhibition kinetics in the bacterially favorable environment was determined by measuring the transmission of light through the dendrimer embedded biological membranes (Fig. 7a), showing the antimicrobial effects of the dendrimers and, thus, such a system could be effective in treating burn wound exudates [19].

Dendrimers in corneal tissue engineering

The cornea is the clear, transparent barrier of the eye, separating the external environment from the internal elements of this organ. Most of vision impairments result from corneal disease [112]. Currently, the only treatment available to recover vision and address blindness resulting from corneal damage is the allograft transplantation of cornea [113].

The use of collagen, the major building material of cornea, in the scaffold material combined with dendrimers could help in the regeneration of corneal tissue. The degree of crosslinking, as measured by Young's modulus of collagen gels, was observed to be over tenfold higher with a poly(propylene)imine octaamine dendrimer than with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride, accompanied by improved optical and mechanical properties [112] (Fig. 7b). Distinctive differentiation of the number of cells highlighted the growth of corneal cells in glutaraldehyde crosslinked collagens and dendrimer crosslinked collagen gels. Although there was a decrease in the number of cells in glutaraldehyde crosslinked collagens from Day 3 to Day 4 because of the release of toxic glutaraldehyde metabolites, dendrimer crosslinked collagen gels supported human corneal epithelial cell growth and adhesion without any toxicity [112].

Results from *in vitro* cell adhesion and growth culture studies suggest that dendrimer crosslinking shows better biological compatibility of crosslinked collagens along with improved biological interactions. The collagen gel was found to be more transparent and improved the permeability to glucose of *in vitro* corneal cells, paving the way to the use of this scaffold for corneal tissue engineering [112].

The use of cell-specific dendrimer synthesis for dendritic scaffolds is a new trend in tissue engineering, and is one that is likely to improve the patient's quality of life by overcoming problems of organ transplantation. Thus, dendrimers are currently in use in several fields, including: healing wounds by enhancing angiogenesis through the incorporation of antimicrobial activity [19]; altering the tissue environment in inflammation and cancer [114] by delivering non-viral genes [115]; exploiting the controlled release properties of bioactive agents in bone and cartilage generation [32]; in vascular generation to prevent clot formation at the surface by incorporating antithrombotic agents [116]; in the development of ultrviolet light-activated bioadhesive hydrogel formulations with PAMAM dendimers to replace materials of low adhesion strength and to avoid resultant toxic reactions with existing adhesives [117]; in enhancing antitumor efficacy in lung metastasis through the localization of drug administeration at the site of action; and reducing cardiac load [118]. Such applications can be further explored through the development of cell and/or tissue-specific microenvironments to address current challenges in tissue engineering.

Concluding remarks and prospects

Tissue engineering has emerged as a clinically viable option to regenerate required tissues or organs that avoid tissue rejection during organ transplantation. In this context, the biomedical application of hyperbranched, multivalent, structurally uniform, and biocompatible dendrimers has gained importance in tissue engineering, particularly for mimicking natural ECMs in the 3D scaffold microenvironment. The improvement over earlier convergent/divergent methods in terms of simplified and low-cost production using the Lego or Click production methods and the resulting high level of control over shape, size, generation, and surface functionality of the synthesized dendrimer have expanded the reach of dendritic architectural research. Thus, dendrimers can incorporate a variety of biological and/or chemical substances in their 3D architecture to actively support the scaffold microenvironment during cell growth.

Successful incorporation of dendritic scaffolds in tissue engineering has resulted from significant advances in the manufacturing of dendritic nanoparticles and improvements in the scaffold architecture. Several biomedical components utilizing dendrimers are now in preclinical and clinical trials, and it is clear from the research discussed here that the future for dendrimers and their associated scaffolds in tissue engineering and beyond is certainly bright.

Acknowledgements

R.K.T. acknowledges support from the Fundamental Research Grant (FRGS) scheme of the Ministry of Higher Education, Malaysia to support research on gene delivery. The authors would like to acknowledge the International Medical University, Malaysia for providing research support for their cancer and arthritis research. They also acknowledge internal grants to R.K.T. from the IMU–JC for providing start-up financial support to his research group.

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