



3D scaffolds-specific cellular mechanoreponse as a pivotal regulating factor in tissue engineering

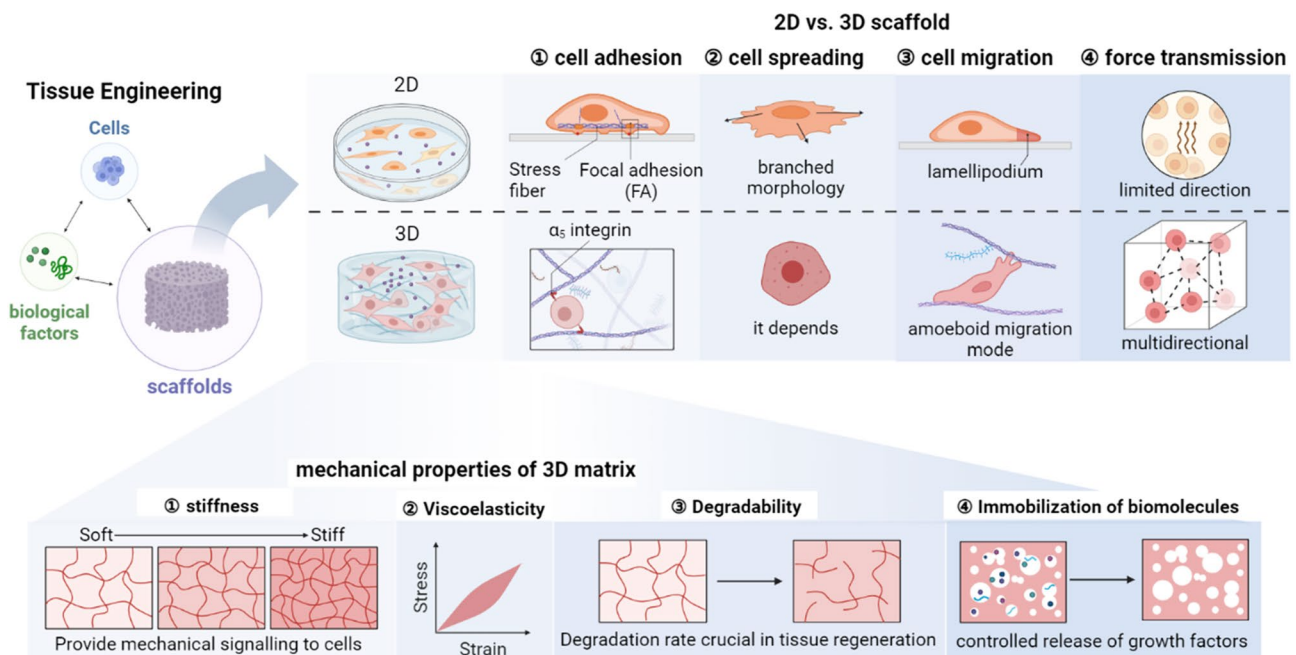
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Received: 1 April 2024 / Revised: 9 May 2024 / Accepted: 11 May 2024 / Published online: 29 May 2024
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Abstract

In tissue engineering, the mechanical properties of the extracellular matrix (ECM) or scaffolds have increasingly been considered to impact therapeutic efficacy by regulating cell behaviors, including differentiation, proliferation, migration, and adhesion. However, the understanding of how cells sense, integrate, and convert the mechanical cues from the ECM cues into biochemical signals to control certain cell behaviors is still elusive, especially in 3D, which more closely mimics the natural microenvironment than 2D systems. This review highlights the key differences between 2 and 3D in the contexts of mechanoregulative cell behaviors such as cell adhesion, spreading, migration, and force transmission. Furthermore, critical designing factors that needs to be considered for the fabrication of 3D tissue engineering scaffolds is discussed: stiffness, viscoelasticity, degradability, and the immobilization of biomolecules. Although mechanotransduction in 3D is actively being studied, understanding cellular mechanotransduction in 3D and designing of mechanoregulative 3D scaffolds still presents several challenges, including varying mechanical properties depending on different tissues, dynamic mechanical environments, and integration of multimodal cues. Interdisciplinary methodologies encompassing material engineering, cell biology, and mechanical engineering would serve to mitigate these challenges and augment our understanding of mechanoregulation governing cellular behaviors, thus fostering advancements in biomedical applications in the future.

Graphical Abstract



Extended author information available on the last page of the article

Keywords Mechanotransduction · Tissue engineering · Scaffold · Stem cell mechanobiology · Integrin

1 Introduction

It has become evident that intracellular biomolecular mechanisms alone are insufficient to elucidate all the cell behaviors, and the cell microenvironment can play a critical role in the cellular functions. In native tissues, cells are surrounded by extracellular matrix (ECM), which functions as a scaffold providing mechanical support and facilitating biological signaling within cells. This underscores the significance of ECM as an important element in the cell microenvironment that governs both cell behaviors and phenotype by directly interacting with cells.

In particular, mechanical properties of the ECM have increasingly been considered as crucial for regulating cell behaviors and physiological processes through mechanotransduction. Cells can detect and respond to various mechanical signals, such as substrate stiffness, viscoelasticity, porosity, fluid shear stress, and mechanical stretching, by activating specific genes and signaling pathways that allow the cells to adapt to their physical environment. These responses, often referred to as mechanoresponses, are integral to cellular behavior, tissue function, and overall physiological health. Consequently, understanding and manipulating these properties have broad implications for fields such as tissue engineering and regenerative medicine.

Most of the current understanding of cellular mechanoresponse come from 2D studies. Early research in this field focused on the impact of stiffness in 2D culture models on various processes like cell migration, proliferation, malignancy and differentiation. While these studies established the concept of mechanotransduction and have relevance to some *in vivo* contexts, a growing body of evidence indicates that mechanoresponse in 3D environments diverges from its 2D counterpart. Flat surfaces lack the complexity of the 3D *in vivo* environment. This leads to significant differences in various aspects such as cell morphology according to actin organization, integrin engagement, and activation of mechanosensitive ion channels. All of these contribute to overall mechanoresponse behaviors of cells. Therefore, it is crucial to understand how 3D environments differ from 2D and what additional factors need to be considered in designing 3D models for successful tissue engineering. Ultimately, this would provide improved insight for developing biomaterials that accurately mimic the native tissue environment and effectively guide cellular behaviors for tissue regeneration.

In this review, we focus on 3D environment-specific features during cell mechanotransduction compared to 2D and explore how these differences can be implemented

for potential use in tissue engineering. First, we start by reviewing cellular mechanotransduction process, followed by four models to understand mechanotransduction in 2D. Then, distinctive impact of ECM mechanics on various cell behaviors, such as cell adhesion, cell spreading, cell migration and force transmission will be covered. We describe the specific regimes that need to be considered when designing 3D scaffolds.

2 Mechanotransduction

The cellular mechanoresponses primarily involve the process by which cells convert mechanical signals or forces into biochemical responses, referred to as mechanotransduction. Cells sense the mechanical stimuli through transmitting the signals across the cell membrane and translating these into intracellular biochemical signals which results in variety of cellular responses (Fig. 1a). This process is initiated by specialized transmembrane proteins or structures on the cell membrane, such as integrins and ion channels acting as mechanosensors. Once mechanical signals are transduced across cell membrane, intracellular signaling pathways are activated. These pathways involve a series of biochemical events that transmit the mechanical information to the cell's interior. This complex process allows cells to respond to their mechanical environment and adapt their behavior accordingly, changing cell morphology, migration, proliferation, differentiation, and apoptosis. Consequently, mechanotransduction is considered as crucial for regulating various physiological processes, including tissue development, maintenance of tissue integrity, immune response, and wound healing. It is also implicated in pathological conditions such as development of cancer, cardiovascular diseases, and fibrosis.

In particular, much of our mechanistic knowledge about mechanotransduction is derived from studies conducted on rigid 2D substrates and can be explained by several representative models. First of all, focal adhesion model emphasizes the role of focal adhesions, which represents specialized structures where cells make contact with the ECM in a 2D environment. Integrins, cell surface receptors, bind to the ECM, forming focal adhesions that link the ECM to the cell's cytoskeleton. Mechanical forces transmitted through these focal adhesions activate signaling pathways, influencing various cellular processes. Tension-dependent model is based on the generation of intracellular tension by cytoskeleton as a key element in mechanotransduction. The

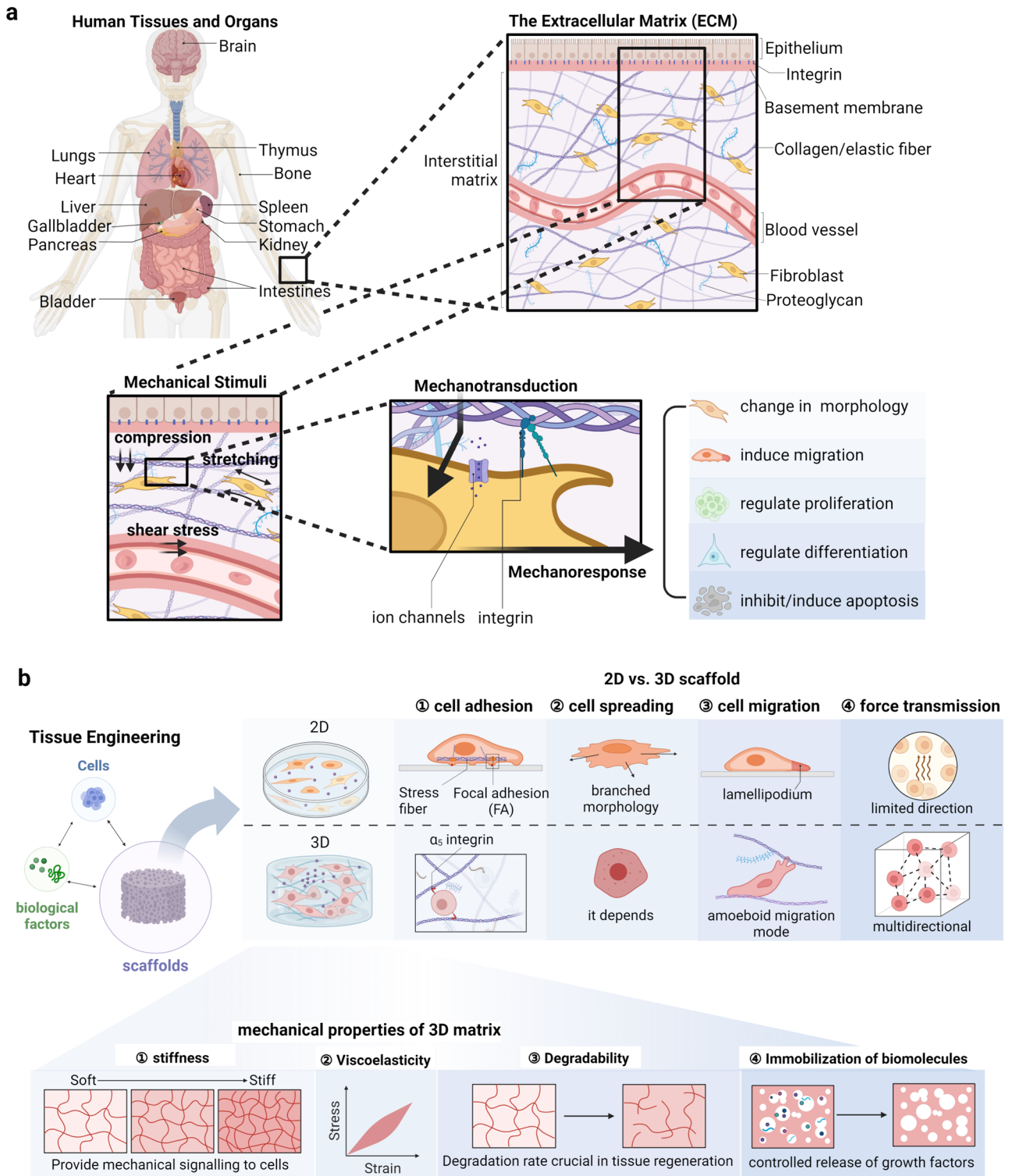
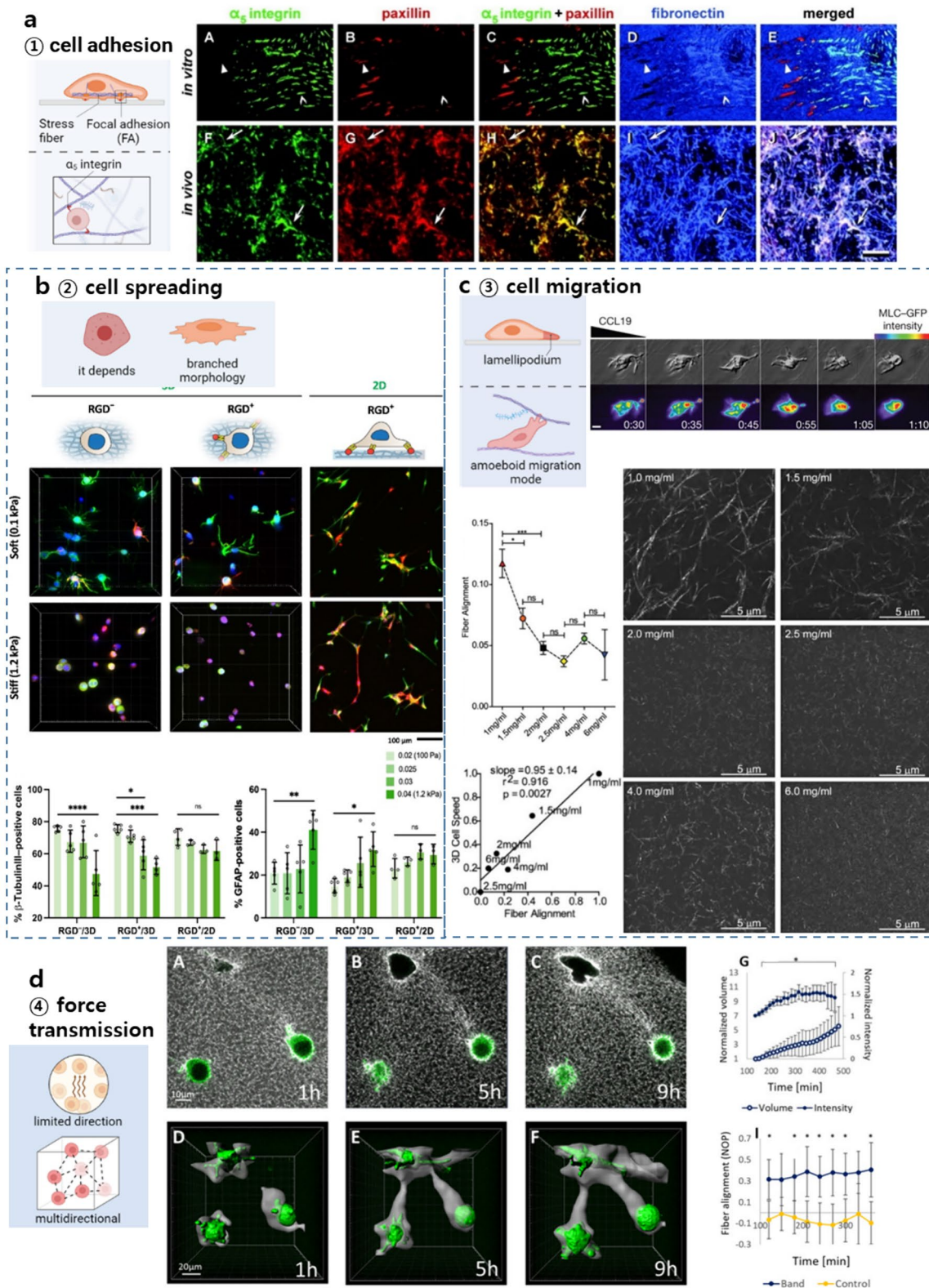


Fig.1 Cell-ECM mechanotransduction, cellular mechanoreponse in 2D vs. 3D scaffold, essential mechanical properties of 3D matrix. **a** Mechanotransduction in human in vivo. **b** Components of tissue engi-

neering, focusing on 2D vs. 3D scaffold about different cell-ECM interaction. (Created by biorender)



mechanical forces experienced by cells in a 2D matrix lead to tension within cytoskeleton components: the actin fibers (F-actin), microtubules (MTs), and intermediate filaments

(IFs) [1]. An integrative model proposes that multiple signaling pathways and components collaborate to coordinate cellular responses to mechanical cues. This considers the

Fig. 2 Cellular mechanoresponse in 2D vs. 3D scaffold. **a** Mouse fibroblast (NIH-3T3) in vitro 2D cover slip coated with fibronectin (A–E). Transverse cryostat craniofacial mesenchyme sections of an E13.5 mouse embryo (F–J). $\alpha 5$ integrin: green, paxillin: red, colocalized: yellow, fibronectin: blue, fibrillar structures: white. Focal adhesions: filled arrowheads, fibrillar adhesions: open arrowheads, 3D-matrix adhesions: arrows. Scale bar, 5 μm [2]. **b** Immunofluorescence images of cells in 3D and 2D hydrogels. β -tubulin III: green, GFAP: red, DAPI: blue. Scale bar, 100 μm (middle). Plots of β -tubulin III-positive cells and GFAP-positive cells in three types of hydrogels: RGD–/3D, RGD+/3D, and RGD+/2D (bottom) [13]. **c** Time-lapse images of GFP attached myosin light chain (MLC) in wild-type (WT) dendritic cell (DC) migrating towards CCL19 in 3D collagen gel (upper row). MLC–GFP intensity profiles, highest levels: red (top right). Differential interference contrast (DIC) microscopy images, time in min:s (lower row) [10]. Images of confocal microscopy of 3D collagen matrices. Scale bar 5 μm (lower right). Plot of FFT analysis and correlation plot of normalized 3D cell speed vs. alignment of fibers (left) [29]. **d** Confocal microscope images of fibrin gels (gray) with fibroblast cells (actin–GFP, green) in 1, 5, and 9 h post-cell seeding (A–C). Alignment band: gray, cell isosurfaces: green (D–F). Analysis of nematic order parameter (NOP) over time in the band and control area (right) [34] (color figure online)

integration of various signaling modules, including focal adhesions, cytoskeletal dynamics, and nuclear responses. It suggests that the overall cellular response is a result of the combined effects of these components. On the other hand, biophysical model integrates physical principles to explain mechanotransduction in 2D environments. It considers the physical properties of both cells and substrates including stiffness and viscoelasticity. These models are not mutually exclusive, and different aspects of each model may contribute to the overall understanding of mechanotransduction in 2D environments. Furthermore, the research in this field continues to evolve.

That being said, not all of the aforementioned phenomena may hold true in the native tissue microenvironment, which is based on 3D. While studying mechanotransduction in 2D provides valuable insights, it is recognized that cells may behave differently in a 3D environment, and understanding the differences between mechanotransduction in 3D and 2D environments would be important in various scientific and biomedical contexts.

3 Distinctive impact of ECM mechanics on cells

Cellular mechanoresponse can exhibit significant differences coming from a number of physical distinctions between 2 and 3D microenvironment. In 2D microenvironment, cells experience mechanical forces primarily in the plane of the culture substrate. The responses are often limited to lateral signaling. In contrast, in 3D context, cells experience mechanical forces not only in the horizontal plane but also in the vertical direction. Cells in 3D microenvironments

encounter spatial constraints, and their responses can be influenced by interactions with surrounding structures in multiple dimensions. Consequently, cells in 2D often exhibit a flattened morphology with a greater emphasis on lateral spreading, whereas 3D environments exhibit more rounded or branched cell morphology, depending on the interactions with the surrounding matrix. In particular, during the mechanotransduction, the stiffness of 2D substrates directly influence cell behavior. On the other hand, the cells in 3D environment sense the stiffness of the surrounding matrix, which includes not only the lateral stiffness but also the vertical stiffness, providing a more intricate mechanical landscape. This section will cover how 3D mechanics impact on various biological processes.

3.1 Cell adhesion

Cell adhesion is the process by which cells make physical contact with the ECM, contributing to numerous biological processes, including cell migration, differentiation, tissue morphogenesis and tumorigenesis. It is generally mediated through specific class of transmembrane adhesion receptors known as integrins. These integrins are linked to numerous structural proteins that act as scaffolding proteins that further enhance the cell adhesion by anchoring them to the actin stress fibers (Fig. 1b).

Due to the distinct spatial arrangements and structural characteristics, the cell adhesion in 3D environment differs from that observed in 2D environment. First, cells adhere to the 2D substrates on only one surface and the focal adhesions are typically concentrated at the cell periphery, while cells in 3D matrix can form adhesions in all directions, exhibiting more dispersed distribution throughout the cell, adapting to the irregularities and orientations within the 3D matrices. For instance, 3D fibrillar matrix adhesions of fibroblastic cells differed from focal and fibrillar adhesions characterized on 2D substrates, exhibiting distinct phosphorylation of adhesion complex proteins [2, 3]. Focal adhesion kinases (FAK) and paxillin served as major factors for forming focal adhesions on 2D substrates, whereas colocalization of the paxillin and $\alpha 5$ integrin rather than localizing separately to classical focal adhesions was observed in 3D (Fig. 2a). Considering that the integrin $\alpha 5 \beta 1$ binds directly to the synergy site of fibronectin, this greater association of $\alpha 5$ integrin in 3D matrices may indicate that the cell adhesion in 3D are under high tension [4] (Fig. 1b). Second, the size of adhesion in 3D differs from that in 2D. The phosphopaxillin focal adhesion structures of osteoblasts in the 3D nanofibrous gelatin scaffolds were less than on 2D substrates [5], of which typical focal adhesion structure formations are rare in vivo [2, 6]. Furthermore, cells within 3D hydrogel-based matrices did not exhibit large focal adhesions [7–9]. Third, for some cases, their functional contribution to the

regulation of cell behaviors is relatively attenuated in 3D than in 2D where integrin-mediated cell adhesion has been considered as essential part for the cellular interaction with ECM. It has been reported that leukocytes use integrin-mediated adhesion when moving over 2D surfaces, whereas the contribution of integrins during 3D movement of leukocytes relatively lower. Instead, the cells migrated by the sole force of actin-network expansion, which promotes protrusive flowing of leading edge as an alternative mechanisms of cell adhesion [10, 11]. Moreover, maintenance of neural progenitor cell (NPC) stemness in 3D degradable hydrogel was independent from arginine-glycine-aspartic acid (RGD)–integrin binding [12]. This suggests that the NPC adhesion to 3D matrix is not a key process for regulating stemness in response to degradability. Fate commitment of NPC in response to the mechanical property of 3D matrix has also been found to be independent on the RGD-integrin binding [13]. On the nanostructured 2D substrates, restricted cellular adhesion and less-polarized cell shape attenuated mechanosensing status of the neural stem cells thereby inducing a fate commitment biased to neurogenesis [14, 15].

3.2 Cell spreading

Cell spreading refers to the process by which a cell adheres to and extends its membrane over a substrate or surface, increasing its contact area with that surface. It is a fundamental cellular behavior and crucial for various physiological processes, including tissue development, wound healing, and immune response. The process of cell spreading typically involves cell adhesion, extension, and reorganization of cytoskeleton. Consequently, cell spreading transcends mere adhesion and becomes more integrated feature for tissue engineering. Here, the focus shifts towards the morphological transformations undergone by cells which leads to cell fate such as apoptosis.

Especially for anchorage-dependent cells, adhesive interaction with the surrounding ECM define cell shape and organization therefore make difference in cell spreading. Notably, in 2D, cell spreading is highly associated with the cell adhesion, consequently, directly influencing cell behavior because of the limited spatial cues. Since the cells in 2D surfaces spread freely in the horizontal plane but have no support for spreading in the vertical dimension, they exhibit a forced apical-basal polarity (Fig. 1b). This polarity is unnatural for most mesenchymal cells, which only polarize from front to rear during migration forming a stellate morphology [16]. Weaver et al. reported that the apical-basal polarity is likely to modulate the cellular sensitivity to apoptosis.

Mechanical properties of microenvironment differently impact the cell spreading in between 2D and 3D. First, the stiffness of the substrates effect cell spreading. It is generally

known that cells adhered on 2D substrates intend to spread out more when the stiffness of the substrates are increased. Cell spreading in 3D matrix is also altered with the change in stiffness of the matrices, but the trend is strongly dependent on cell types, gel types, and the stiffness range (Fig. 1b). Less contractile cells with low mechanical properties such as neural stem cells (NSCs) show increased cellular projection and spreading in 3D elastic soft hydrogels than stiff gels even though the stiffness of the ‘stiff gels’ is not higher than 2 kPa [13, 17, 18]. We have previously explored how the ratio of a cross-linker (azide) to HA monomer in the hydrogels affects cell development (Fig. 2b). These cells exhibit higher susceptibility to spatial constraints of 3D matrices. In contrast, cancer cells exhibit opposite trend in response to the stiffness of 3D matrices [8]. Depending on whether the 3D matrix has a nanoporous or microporous structure, there can be an opposite trend in cell spreading in response to the mechanical properties [19]. Second, the viscoelasticity of matrices influence the cell spreading differently in 2D and 3D. Fibroblasts embedded in 3D viscoelastic hydrogels exhibiting stress relaxation showed increased cell spreading than the cells in the gels with less stress relaxation [20]. This enhancement was attributable only to the altered stress relaxation, since the initial elastic modulus and RGD cell-adhesion-ligand density was constant. On the other hand, spreading of the cells cultured on 2D viscoelastic substrates with stress relaxation was greater than the cell spreading on elastic substrates with the same initial modulus, exhibiting opposite trend to that of 3D [21].

3.3 Cell migration

Cells migrate individually or collectively during morphogenesis and homeostasis. Cell migration also plays a crucial role in both disease progression and recovery, including wound healing, cancer metastasis, cell therapy, and immune response. In fact, lack of migration is a major cause of failure of potential regenerative therapies that misplaced cells can execute abnormal functions in foreign microenvironment [22]. Like cell spreading, cell migration highly depends on the status of cellular microenvironment since cells pull/push along the ECM fibers or squeeze through the pore structure of the network during migration.

Extensive studies have explored the molecular and biophysical mechanism of cell migration on flat, 2D substrates. Cells migrating on glass coverslips exhibit a specialized and stereotypical version of locomotion in a standard model of 2D migration [3]. It is generally known that the extension of lamellipodia and retraction of the back of the cell are representative feature of the migration on 2D substrates (Fig. 1b) [3, 23, 24].

On the other hand, much less is known about how cells migrate through 3D matrices. The cells in 3D

microenvironment use a wide variety of migratory modes, such as mesenchymal, amoeboid and lobopodial (Fig. 1b) [25]. Above all, since confinement from the 3D ECM can limit the extension of lateral protrusion, several cell-intrinsic processes influence the migration: cellular secretion of proteases that degrade the ECM, pulling of the matrices and transient or permanent remodeling of the ECM by cells [23]. Moreover, cell migration in 3D microenvironments is affected by a more diverse range of signaling cues from the ECM than that in 2D. Pre-existing pores or passageways in the ECM open the gateways for rapid cell penetration. In this context, ECM stiffness or the degree of crosslinking in the matrix will influence how much cells can expand such pores to infiltrate through or how much proteases the cells need to secrete to expand the opening [26–28]. Migration in 3D also can be guided by matrix architecture and the alignment of fibrous structures [29]. In Fig. 2c upper right, Lämmermann et al. showed activated myosin II, using time-lapse imaging of DCs expressing a myosin light chain–GFP fusion protein. The accumulation is seen at the cell rear when it contracts. Fraley et al. showed that the higher the collagen density, the faster the cell migrates (Fig. 2c). Notably, 3D cell migration does not share as many features regarding the contribution of cell adhesion in 2D cell migration as it does with cell migration in a 1D microenvironment along a line or a narrow linear structure. For instance, cells in both 1D and 3D fibrillar microenvironment show similar cell morphology and position of centrosome, and the migration speed in both is independent of ECM ligand density [30].

3.4 Force transmission

Cells constantly experience and generate forces that significantly influence their behavior and communication with their surrounding environment, the ECM. These forces can be external, such as shear stress generated by fluid flow and stresses which cells experience when they are compressed, extended, or stretched. In response to external forces, cells can generate internal forces through the dynamic assembly and contraction of their actin cytoskeleton thereby producing endogenous contractile forces [31]. These forces not only remodel surrounding ECM but can also influence other cells at distances exceeding several cell diameters.

The way forces are transmitted between cells and the ECM differs significantly between 2 and 3D environments. In 2D, forces are primarily exerted on the flat substrate, while 3D matrices allow for multidirectional force transmission, leading to more complex patterns and enabling long-range communication (Fig. 1b). This multidirectional transmission makes cells to sense and respond to their surroundings in a more comprehensive way compared to 2D cultures.

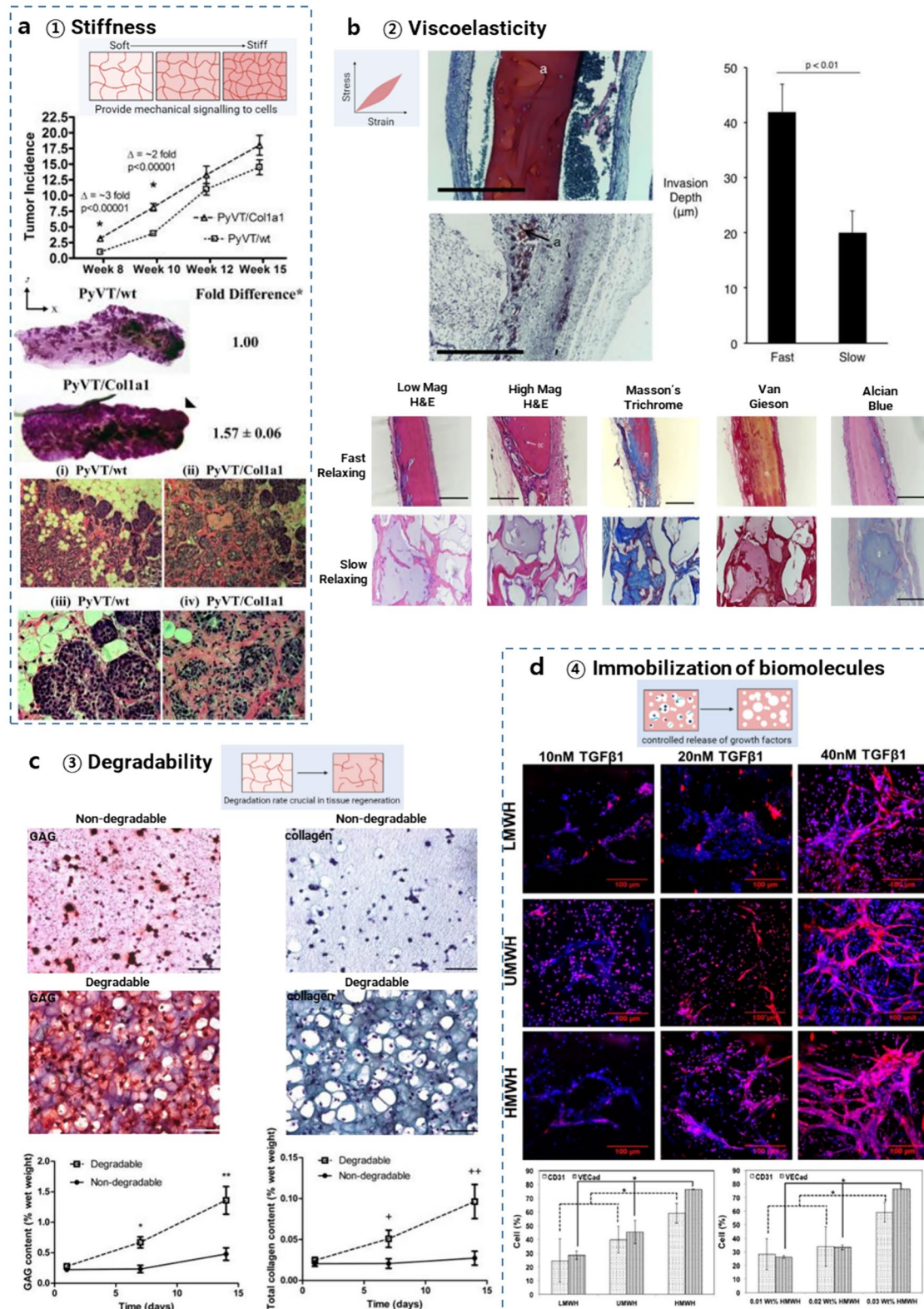
The long-range communication of cells through force transmission of ECM is facilitated by the alignment and densification of ECM fibers. Fiber alignment can guide the migration of specific cell types, such as myofibroblasts, contributing to the progression of fibrotic diseases [32, 33]. Natan et al. reported that in the context of fibrosis and tissue mechanics, these alignments are typically observed in 3D environments [34]. In Fig. 2d, density and alignment band get more visible in gels as incubation time increases. Within this system, significant increases in both normalized volume and intensity of fibrin fibers were observed after 500 min, compared to the beginning.

Interestingly, cellular forces in 3D ECM create complex mechanical stress fields impacting other cells and triggering feedback mechanisms. A positive feedback was reported in recent studies. For instance, endogenous contractile force on the matrix can cause ECM not only to align but also to stiffen. This creates a positive feedback loop further promoting cell force generation and potentially affecting the function within ECM. Liu et al. reported that cells embedded in 3D matrices adapt to their stiffness by stiffening their bodies and generating more force in response to stiffer environments [35]. In confining 3D nanoporous ECMs, cancer cells apply protrusive forces to deform the ECM, and in sufficiently plastic ECMs such forces lead to permanent matrix deformations, which in turn promote growth of protrusions and result in larger pores for cell migration [36]. When tumor cells proliferate in 3D spheroids, it has been reported that hydrostatic stresses are generated mechanically opposing tumour growth [37, 38].

While contractile forces are crucial in both 2D and 3D, the 3D environment allows for additional force-generating mechanisms. In 3D, cells use polymerized branched actin networks to create protrusive forces forming structures like filopodia and lamellipodia [25, 39–42] for cell migration and outward forces generated by microtubule-based spindles during mitosis [43, 44]. How these forces are generated in 3D remains an active area of study [36].

4 Mechanoresponse-related regimes for designing 3D scaffolds

Tissue engineering applications rely heavily on 3D scaffolds to provide a suitable microenvironment for cell growth and regeneration. These scaffolds play a crucial role in mimicking the actual in vivo microenvironment where cells interact and respond to various mechanical cues. Therefore, the material properties of these scaffolds are crucial for determining the cellular response and fate. Polymers that are available for cell binding and resistant to rapid enzyme degradation can be used as a base material for the scaffold. Additionally, specific cell-adhesion peptide motifs



or proteins can be incorporated to control how cells interact with the scaffold surface [45]. One of the most desired properties of 3D scaffolds is high porosity to improve cell

migration, infiltration, efficient nutrient/oxygen diffusion and waste removal [46], biocompatibility, and biodegradability [47]. In particular, tunable mechanical properties such

Fig. 3 Essential mechanical properties of 3D matrix. **a** Analysis of whole mammary glands dissected from the fourth inguinal area of PyVT/wt and PyVT/Col1a1 10-week-old mice. H&E stained histology sections (i)–(iv) [55]. **b** Safranin O stained collagen and bone of slow-relaxing (first) and fast-relaxing (second) cases cells at 2 weeks post-implantation. Residual alginate: red, marked “a”. Scale bars, 1 mm. Histological staining of calvarial wound sites months post-injury. Low-mag scale bar 360 μm , high-mag scale bar 180 μm , Masson’s trichrome scale bar 360 μm , Van Gieson, alcian blue scale bar 360 μm (fast-relaxing case), 720 μm (slow-relaxing case) [64]. **c** Glycosaminoglycan (GAG) and collagen distribution and production in non-degradable and degradable 8:1 co-cultured cartilage-specific matrix, sectioned at day 14. GAGs: red, nuclei: black, collagen: blue, nuclei: black or violet. Scale bars 100 μm [70]. **d** Confocal microscopy of CPCs and differentiated endothelial cells in heparin-containing HyA hydrogels (0.03 wt.% heparin). Ac-LDL: red, cell nuclei: blue. Flow cytometry shows percentage of endothelial cells expressing markers: CD31 and VE-cadherin (bottom left). Weight percentage of specific type of heparin (HMWH at 40 nm TGF β 1) (bottom right) [84] (color figure online)

as stiffness, viscoelasticity, and degradability of the scaffold play a significant role in mechanotransduction. These mechanical properties vary across different tissues, with soft tissues like brain having lower stiffness compared to stiffer tissues like muscle [48, 49]. As cells exert force on the surrounding environment, the ECM can stiffen, relax its resistance over time, and even deform permanently. These mechanical properties of the cells impact intracellular signaling, transcription and phenotype through cell–ECM mechanotransduction. Thus, it has become clear that stiffness, viscoelasticity, and degradability are key parameters regulating cell behaviors in 3D. Immobilization of biomolecules is also a highly applicable property in tissue engineering as well, therefore this section will cover these four key considerations for designing 3D scaffolds especially to get desired cell mechanoresponses [36].

4.1 Stiffness

The rigidity of the ECM profoundly influences the resistance encountered during cell growth and migration, thereby shaping the cell’s morphology. Notably, cell shapes in 2D and 3D environments differ significantly, playing different roles in the regulation of signal transduction and gene expression [50]. For instance, when mesenchymal stem cells (MSCs) were cultured under various stiffness conditions, a higher stiffness led to increased cell proliferation and osteogenesis, with observed morphology displaying spread-out polygonal shapes [51]. Upon controlling the shape of MSCs through micropatterning and observing cell differentiation, it was found that flat and spread-out cells differentiated into osteoblasts, whereas round cells differentiated into adipoblasts [52]. Thus, cell shape is important in proliferation and commitment, with matrix stiffness being a key property that regulates it.

Furthermore, increased stiffness in both 2D and 3D correlates with the emergence of malignant tumors [53, 54]. This phenomenon arises due to the activation of fibroblasts and macrophages, leading to increased cross-linking and density of collagen I. In breast cancer, a stiff ECM environment fosters cancer cell proliferation and an invasive phenotype [55–59]. Provenzano et al. investigated the link between mammary tumors and collagen density in mice. Mice with a specific genetic modification (PyVt/Col1a1) leading to stiffer collagen in their mammary glands developed significantly increased tumors compared to control mice (PyVT/wt). To quantify hyperplasia, they used three pairs of glands, calculated from a common threshold value set with density slicing in Image J software (Fig. 3a). It revealed statistically significant increase in the area of hyperplasia within the mammary glands with increased stromal collagen. Figure 3a shows that the collagen (red) is more visible in (ii), (iv) than (i) and (iii), and images (ii) and (iv) suggest more invasive phenotype.

While natural ECM components like collagen, fibrin, hyaluronic acid, and fibronectin closely mimic natural cellular ECM for research, their softness relative to actual tissues prompts recent investigations into the use of synthetic matrices such as alginate and or hybrid matrices combining them with natural ECM [60]. Additional crosslinkers can be incorporated to increase crosslinking density, which generally increase the stiffness. These hybrid matrices allow for the fine-tuning of stiffness, facilitating a nuanced exploration of their impact on cellular behavior [61, 62].

4.2 Viscoelasticity

In conjunction with stiffness, viscoelasticity emerges as a vital factor influencing ECM remodeling [50]. When cells grow and traverse, they dynamically engage with the ECM, generating mechanical forces that act in diverse directions [63]. This process allows for the modulation of cell function through alterations in cell shape and volume (Fig. 3b) [64]. Darnell et al. did histological staining and quantification of calvarial wound site remodeling of injury after 3 months. The histological images shown in Fig. 3b suggests the fast-relaxing case exhibits better healing with new bone formation and organized tissue structure compared to the slow-relaxing case.

The inherent viscoelasticity of the natural ECM results in enduring deformations in response to applied forces. Faster stress relaxation speed in viscoelastic matrices promotes spreading of anchorage-dependent cells such as muscle cells and fibroblasts, as well as the progression of cell cycle in individual cancer cells [20, 44, 65]. Similarly, the stemness of neuronal precursor cells is maintained in hydrogels with fast stress relaxation, whereas it is suppressed in hydrogels with covalent crosslinks [12].

Specially, in stress stiffening matrices, mechanical stress triggers increased matrix resistance and empowers breast cancer cells to generate augmented forces, thereby establishing a positive feedback loop. This case induces the alignment of fibers in response to mechanical forces, fostering cell shape deformation and movement [66].

The majority of tissues constituting the human body are viscoelastic, and also viscoelasticity of tissues is widely utilized to distinguish tissue boundaries or analyze diseases. Particularly, enhanced viscoelasticity in brain tissue is deeply associated with diseases such as multiple sclerosis and the progression of aging [67]. Additionally, viscoelastic matrices exhibit a wide range of changes in porosity and network structure upon stress-induced remodeling, yet understanding in this area remains largely insufficient. Future research that comprehensively observes factors such as viscoelasticity, degradability, porosity, and others in the matrix remodeling over time is needed.

4.3 Degradability

3D scaffolds used as temporary structures that support cell growth and guide new tissue formation in tissue engineering need to be degraded as desired. To act as an effective wound healing material, the hydrogel must degrade at the same rate as tissue regeneration [68]. If degradation is too slow, it hinders cell migration and nutrient diffusion, while overly rapid degradation compromises the scaffold's structural integrity. For example, in bone repair, scaffolds need to degrade at a rate matching bone formation to maintain stability and allow bone growth [69]. On the other hand, rapid degradation within specific rate range can improve the matrix functions. Some studies show that faster-degrading hydrogels promote the formation of cartilage-like ECM by chondrocytes [70] (Fig. 3c) and mesenchymal stem cells (MSCs) [45, 71]. Sridhar et al. utilized two types of scaffolds including non-degradable and degradable matrices based on distribution of glycosaminoglycans (GAGs) and collagen. In non-degradable gels, GAGs and collagen were limited to the pericellular space but in degradable gels, they were spread out throughout the gel and connected and interacted with other molecules from nearby cells, forming extensive network. Both type of gels showed an increase in the amount of cartilage-specific ECM molecules production, the degradable gels had significantly higher production than non-degradable gels (Fig. 3c). Furthermore, it has been shown that the degradation rate of the matrix can affect chromatin organization in neural progenitor cells. Specifically, increased degradability leads to enhanced chromatin accessibility [72]. In addition, it has been reported that the degradability of 3D matrices increase cell–cell interaction thereby activating β -catenin signaling pathway for maintaining neural progenitor cell stemness [12]. In particular, Callari et al. showed that

dimensionality of matrices can exhibit different impacts of degradability to cell behaviors. MSCs displayed decreased spreading and YAP/TAZ nuclear localization when cultured atop more degradable 2D substrates, whereas the opposite trend was observed when MSCs were encapsulated within degradable hydrogels [73]. However, more investigation is needed to understand how this is regulated in different contexts and why effects might differ from those in 2D studies [36].

Research are increasing in the role of the 3D matrix degradability as an important factor for regulating morphogenesis in organoid models. Degradable gels promote apical-basal polarization and lumen formation in Madin–Darby canine kidney cysts [74], supporting cell viability and the formation of budded intestinal organoids via symmetry-breaking mechanisms [53, 54].

Researchers are developing “smart” scaffolds with adjustable degradation rates to accommodate individual patient needs according to age, diet, healing rates, and lifestyle. Post-fabrication tuning is crucial for adapting to dynamic changes in cell behavior, migration, and differentiation [75, 76]. One approach to achieving tunable and on-demand functionalities in scaffolds is through the use of photostimulus. Photodegradable hydrogels allow light-triggered degradation which has advantage in real-time external control. However, challenges like long degradation times with certain wavelengths and potential cytotoxicity need addressing [47].

As an application, biodegradable polymers have been used for growth factor delivery. Materials like PLGA (Poly(lactic-co-glycolic acid)) are widely used as nanocarriers for encapsulation and release for biomedical applications, due to their biocompatibility [77]. The fact that PLGA is one of the few polymers that Food and Drug Administration (FDA) has approved for human administration gives positive perspective [78]. Degradation of the polymer controls the release of embedded growth factors for targeted tissue regeneration [79]. This concept will be focused on the next section.

4.4 Immobilization of biomolecules

The technique of immobilizing biomolecules, such as enzymes, proteins, and antibodies, onto a solid support while preserving their biological activity is important in tissue engineering. Controlled release of growth factors or signaling molecules enables localized and sustained delivery within scaffolds, guiding cell differentiation and promoting tissue regeneration [80, 81]. For example, immobilized human epidermal growth factor (hEGF) on 3D scaffolds provides localized release for treating skin-related disorders like burns [82].

Studies have utilized various strategies for controlled release and delivery of GF as the microparticles break down from the supportive matrix. Degradable PLGA microspheres loaded with basic Fibroblast Growth Factor (bFGF) [83] embedded within a scaffold release the GF, promoting cardiac tissue regeneration and vascularization [77]. In another study, heat shock protein 27 (HSP27), fused with transcriptional activator (TAT) derived from human immunodeficiency virus (TAT-HSP27), was loaded in PLGA microspheres within alginate hydrogel successfully inhibited apoptosis of cardiomyoblasts which were cultured under hypoxic conditions. For enhanced bone regeneration, biodegradable chitosan scaffolds containing nanocapsules loaded with different growth factors such as BMP-4, PDGF, and IGF-I achieved customized release profiles through varying degradation rates, leading to optimal cell proliferation and mineralization [79]. Immobilized heparin on hydrogels can influence cell differentiation, such as endothelial cell differentiation and network formation within the hydrogel [84]. Cardiac progenitor cells (CPCs) were differentiated into endothelial cells in hyaluronic acid (HyA) hydrogels containing heparin (0.03wt.% heparin), which serves as a carrier for controlled release of biomolecules. They found that molecular weight of heparin affects the differentiation, and the network formation is prominently visible especially in hydrogels containing High Molecular Weight Heparin (HMWH) and 40 nM TGF β 1 than in Unfractionated Molecular Weight Heparin (UMWH), Low Molecular Weight Heparin (LMWH) (Fig. 3d).

5 Conclusion

This review has highlighted the distinctive mechanical properties in 3D ECM which significantly influence cell behavior through mechanotransduction in the context of tissue engineering. While 2D models have provided valuable insights, the complex 3D microenvironment, which better mimic natural cell microenvironment, presents unique challenges, including multidirectional interactions with surrounding structures.

Despite aforementioned advancements in understanding 3D-specific cellular mechanoresponses, several knowledge gaps still remain. For instance, elucidating 3D migration patterns and understanding long-range force transmission through the matrix are crucial for further progress. In addressing these gaps, exploring novel materials with tunable mechanical properties and improved biocompatibility holds significant promise.

Ultimately, by mimicking the complexities of the natural ECM through optimized 3D scaffolds, we could pave the way for significant advancements in tissue engineering. This not only allows for more accurate studies of various diseases

like cancer but also facilitates the discovery of biomarkers and the development of targeted therapies.

Acknowledgements This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2023-00213047).

Data availability No new data were created or analysed in this study. Data sharing is not applicable to this article.

Declarations

Conflict of interest The authors declare no conflict of interest.

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