Chemical Engineering Journal 505 (2025) 159295

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Review

Test and tune: evaluating, adjusting and optimising the stiffness of hydrogels to influence cell fate

Peiqi Yang [a,b,c], Gareth Boer [a,b], Finn Snow [c,d], Alysha Williamson [c,d], Samuel Cheeseman [e,f,j], Rasika M. Samarasinghe [a,b], Aaqil Rifai [a,b], Ayushi Priyam [a,b], Roey Elnathan [a,b], Roseanne Guijt [k], Anita Quigley [c,d,g,h,i], Rob Kaspa [c,d,g,h,i], David R. Nisbet [c,e,f,h,i,*], Richard J. Williams [a,b,c,d,e,*]

[a] School of Medicine, Deakin University, Geelong, VIC 3220, Australia
[b] Institute for Mental and Physical Health and Clinical Translation, School of Medicine, Deakin University, Geelong, VIC 3220, Australia
[c] Aikenhead Centre for Medical Discovery, St Vincent's Hospital, Fitzroy, VIC 3065, Australia
[d] Biomedical and Electrical Engineering, School of Engineering, RMIT University, Melbourne, VIC 3000, Australia
[e] The Graeme Clark Institute, The University of Melbourne, Parkville, Melbourne, VIC 3010, Australia
[f] Department of Biomedical Engineering, Faculty of Engineering and Information Technology, The University of Melbourne, Parkville, Melbourne, VIC 3010, Australia
[g] Department of Medicine, The University of Melbourne, Fitzroy, Melbourne, VIC 3065, Australia
[h] Department of Clinical Neurosciences, St Vincent's Hospital Melbourne, Fitzroy, Melbourne, VIC 3065, Australia
[i] Melbourne Medical School, Faculty of Medicine, Dentistry and Health Science, The University of Melbourne, Melbourne 3010 VIC, Australia
[j] School of Science, STEM College, RMIT University, Melbourne 3001 VIC, Australia
[k] School of Engineering, Deakin University, Geelong, VIC 3220, Australia

ARTICLE INFO

Keywords:
Biomaterials
Stiffness
Tissue engineering
Mechanical properties
Cell Fate

ABSTRACT

The use of bioequivalent hydrogels in tissue engineering (TE) is enabling 3D tissue-like scaffolds capable of reproducing the sophistication of natural cell–matrix interactions. Alongside the common concerns of chemical function, it is crucial that hydrogels have suitable mechanical properties, particularly stiffness, to create a complete biomimetic environment for cell development. Non-covalent biocompatible hydrogels are often too soft, while stiffer, covalently crosslinked materials may have challenging microenvironments in which porosity and residual chemicals can be problematic. If the potential of hydrogel-based TE to be realised, design strategies need to be carefully considered to achieve desirable end-use biomechanical properties. This review is intended for a cross-disciplinary readership; we discuss recent successes in bioengineering hydrogel stiffness, where materials that are responsive to cell inputs are used to explore the relationship between substrate stiffness and cellular fate commitment. We discuss the most popular measurements for mechanical studies, and outline optimal substrate stiffness for different cells. We summarise recent advanced studies on tuning stiffness and highlight future challenges challenges to address.

1. Introduction

The extracellular matrix (ECM) is a naturally produced, three-dimensional (3D) scaffold with biochemical (e.g., ligands, immobilized soluble factors, charge) and biophysical (e.g., viscoelasticity, degradability, dimensionality surface topography) signals that are constantly remodelled to modulate cell activity, tissue homeostasis, and disease pathogenesis [1–3]. Restoring the damaged and disordered ECM to ensure cell-mediated regeneration in human tissues after injury is a significant obstacle. To overcome this challenge, TE uses stem cells or differentiated cells embedded within bioscaffolds to generate specific tissues, which are often functionalized with growth factors to improve lineage commitment and integration. Furthermore, truly ECM mimicking scaffolds must contain microporous interconnected networks that maintain structural integrity and mechanical strength while permitting cell migration and tissue rejuvenation [4,5].

* Corresponding authors at: The Graeme Clark Institute, The University of Melbourne, Parkville, Melbourne, VIC 3010, Australia (DRN) and School of Medicine, Deakin University, Geelong, VIC 3220, Australia (RJW).
E-mail addresses: david.nisbet@unimelb.edu.au (D.R. Nisbet), richard.williams@deakin.edu.au (R.J. Williams).

https://doi.org/10.1016/j.cej.2025.159295
Received 24 October 2024; Received in revised form 30 December 2024; Accepted 4 January 2025
Available online 5 January 2025

Stiffness is a key mechanical property of biomaterials that influences cellular response to TE scaffolds [6]. The traditional understanding of TE was that the bioscaffolds should have consistent mechanical properties, shape and structure to match the host tissue. Recent research, however, has highlighted cells are able to effectively sense and respond to biomechanical stimuli, regardless of the shape and composition of the substrate [7,8]. While matching the stiffness of the interstitial matrix in which the cells reside is critically valued for its role in both biophysical support and mechanobiology, it is important to recognise that, in certain contexts, softer or stiffer matrices may outperform those attempting to precisely replicate *in vivo* matrix mechanics. This nuanced understanding highlights the complex influence of matrix stiffness on cellular behaviour.

Increasingly, it is becoming apparent that the surface properties of the tissues are key in the presentation of viscosity and elasticity experienced by the cell [9]. These mechanical cues such as stiffness, surface topographies, and the viscoelasticity of the interstitial biofluids are translated into biochemical signals by mechanotransduction [10]. These biophysical cues are tightly correlated to affect cell fate. In this review, we have somewhat simplified the complexity of the viscoelastic spectrum; we have identified substrate stiffness for emphasis, since it is amongst the most commonly reported values, has been effectively controlled by distinctive manufacturing methods, and is utilized to manipulate the differentiation of cells into new tissue types.

Increasing attention for bioscaffolds is via the design and optimisation of Hydrogels, as they generally consist of polymeric chains with hydrophilic components that can stock vast volumes of water and possess the ability to imitate the 3D biophysical and biochemical structure of ECM [11]. Hydrogels support cell survival by retaining substantial amounts of water, have a macro-, micro, and nanoporous structure, enable the diffusion soluble factors and dissolved gases, and tend to be held within a material that has a 'soft' consistency [11]. They can also be designed to be biodegradable, enabling the natural ECM deposition. Hydrogels are considered one of the most suitable and promising biomaterials for wide applications in TE, cell encapsulation, organ-on-a-chip, drug delivery and wound dressing [12–14]. The design strategies need to be carefully considered to ensure desirable biomechanical properties are achieved for the potential of hydrogel-based TE to be realised. One consideration of adopting hydrogels is their low mechanical strength; whilst it enables biomaterials that are simultaneously degradable, injectable and printable, it also results in an easily compromised structure and integrity, and inadequate support to cells or the capacity for retained drugs, placing application dependant limitations on their widespread adoption [15].

Consequently, defining the hydrogels' mechanical properties whilst maintaining their designed biofunctionality and fabricating them into a robust construct can be a huge hurdle, and this review aims to provide a helpful reference for further exploration. Firstly, we introduce the concept of stiffness and summarize the most popular measurements for mechanical studies, whilst considering how hydrogel stiffness controls and affects cellular functions. We then outline optimal substrate stiffness for different cells and summarize the stiffness of commonly used hydrogels. Finally, we systematically categorize recent advanced studies on tuning stiffness and identify future challenges that are urgently needed.

2. Why do we talk in terms of 'stiffness'?

The natural ECM is an inherently viscoelastic structure, presenting time-dependant mechanical behaviours such as creep and stress relaxation, and synthetic analogues can be limited to undergo permanent, unrecoverable deformation, in the form of viscoplasticity. The interplay between two factors is the function of the mechanisms used to form the hydrogels; as we will discuss later, parameters such as the choice of polymer network and composition, and crosslinking strategy (with covalent bonds or divalent cations) have a significant role here, creating a rigid or brittle system.

As such a discussion on mechanical properties of synthetic materials that reproduce the ECM, factors such as stiffness, viscoelasticity, and the structure and topography of hydrogels are extensively deliberated. Notably, there is a predominant emphasis on hydrogel stiffness compared to viscoelasticity and topography in current published reports, particularly with regards to mechanotransduction of transcriptional cellular pathways [16–23]. This propensity for a single metric may arise from the fact that most techniques used to measure mechanical properties are typically limited to testing elastic modulus (E) or shear modulus (G), and a lack of assessing viscosity, viscoelasticity, viscoplasticity or strength.

Our understanding of the intertwined relationship between these mechanical parameters extends from mathematical models. Stiffness is measured as distinctive modulus (E or G) based on Neo-Hookean model, which restricts the material description to be elastic, isotropic and incompressible [24]. Viscoelasticity describes the ability of hydrogels to exhibit characteristics of both viscosity and elasticity, typically assessed using rheological methods to measure stored energy (shear storage modulus, G') and dissipated energy (loss modulus, G''). Additionally, the topography or structure of hydrogel significantly influences stiffness, where mathematical frameworks such as rubber-like elasticity theory [25] and the Mooney-Rivilin model [26] connect hydrogel structural parameters to stiffness properties. Swelling in hydrogels, due to their elastic and hydrophilic attribute, typically absorbs the solvent, expands the polymer network, then alters their structure and mechanical properties. This swelling process generates elastic tension within the polymer network, preventing the material from fully dissolving. Theoretical frameworks, such as those developed by Peppas and Merrill [27,28], Flory and Rehner [29], Treloar [30], Tanaka [31], and Richbourg and Peppas [25,32], provide insights into how swelling affects the stiffness of hydrogels by modifying the structure of the polymer network. Therefore, as the inherent parameters that determine the overall mechanical properties, these factors are closely interrelated.

In this review therefore, our primary focus is on tuning stiffness to manipulate the mechanical behaviour and hence affecting cell fate.

3. The concept of stiffness

Stiffness is measured as elastic modulus, which is the resistance to a certain deformation while returning to its original shape. Elastic modulus is characterized as the stress (applied force per unit area) divided by the strain (deformation induced by stress). Elastic modulus and stiffness are often considered to be synonymous. However, stiffness is an intrinsic characteristic of structure, while moduli are used to define a property of the material that the structure is comprised of [7].

Young's modulus (E) defines the relationship between normal stress and strain under uniaxial loading, such as tension or compression, while the shear modulus (G) quantifies the material's resistance to deformation under shear stress. Elastic stress and strain are both perpendicular to the cross-sectional surface, whereas shear stress and strain are parallel to the surface, resulting in an angular motion (Fig. 1). However, E and G are correlated through the following equation in isotropic materials.

$$E = 2G(1+\nu)$$

Poisson's ratio (υ) describes how much lateral deformation responds to axial loading. The Poisson's ratios of most common solids including glass, concrete, iron, and sand are within the range of 0.2 to 0.3. For highly elastic materials such as rubber or incompressible solids, υ is deemed as 0.5, which means the volume remains the same by possessing proportionally increased length and decreased width when stretched. In most cases, researchers assume that hydrogels and cells have a Poisson's ratio of 0.5, even though it hardly occurs in nature. As such, E is roughly estimated as 3G [7,33].

However, in the real world, hydrogels and tissues are viscoelastic,

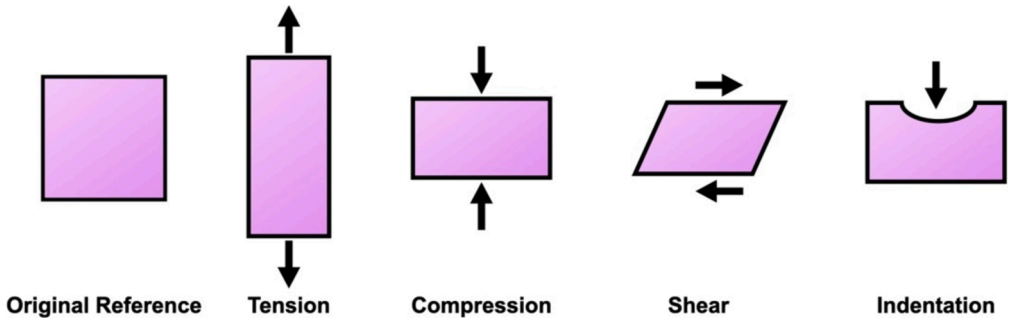

Fig. 1. Different types of stress act on tissues, including tension, compression, shear and indentation.

meaning they are elastic and viscous at the same time, and gradually respond to applied forces due to the temporal connections among adjacent long polymers [34]. Time-dependent tests such as stress relaxation, creep tests or oscillatory tests are often used to dynamically analyse this behaviour. Storage and loss modulus derived from the dynamic mechanical analysis under uniaxial forces (expressed as E' and E'') or shear stress (expressed as G' and G") are two significant parameters relevant to biological tissues which mostly have viscoelastic features (Table 1). Storage modulus refers to the stored energy in materials, characterizing the elastic responses. The loss modulus is regarded as the dissipated energy, indicating the viscous components. Gelation point refers to the critical condition (frequency or strain) at which the storage modulus and loss modulus become equal, marking a pivotal transition in the hydrogel's rheological behaviour. This crossover point is significant because it indicates the shift from a predominantly liquid-like state (sol) to a solid-like state (gel) during the gelation process. It is commonly used to identify the onset of gelation, where the material begins to form a 3D network structure, transitioning from fluidity to elasticity.

Therefore, multiple moduli associated with the inherent elastic characters of materials are manifested in the stiffness of the eventual form [35].

4. Stiffness measuring Tools

Stiffness is measured by analysing the resulting deformation under applied force. There are several commonly used systems to quantify stiffness in hydrogels or hydrogels-encapsulated cells based on the different type, size and location of the applied force (Table 2). Other testing systems such as optical coherence elastography, ultrasonic shear weave elastography, optical stretching have been deeply discussed in other reviews [36–38].

Stiffness results from different measuring systems can vary greatly. Wu et al. [36] compared seven strategies to measure the elastic/storage modulus of MCF-7 human breast cancer cells in the consistent culture medium and discovered that the reported E values differed substantially between different techniques. For example, when measured by AFM, the E values ranged from 0.53 ± 0.52 kPa to 13.5 ± 7.0 kPa depending on the probe (cantilever tip) size, testing temperature and indentation speed; while when analysed by a parallel-plates rheometer, the E' was 0.95 ± 0.15 kPa and E'' was 0.34 ± 0.04 kPa at a frequency of 1 Hz. Similar observations have been found by researchers studying the human cornea, with the reported elastic modulus values varying from 2.9 kPa to 19 MPa when measured by different methods including AFM, tonometry, or inflation testing [49]. In addition to tissue and cells, the different testing systems result in different values for the mechanical properties of hydrogels. For instance, Megone et al. [50] demonstrated that the shear modulus of the elastomer polydimethylsiloxane (PDMS) was 270 Pa when tested by rheology, while AFM provided much higher G values up to 13 kPa.

The results of these rheological experiments should primarily be correlative, although variations of up to three orders of magnitude may exist among different techniques. These discrepancies are likely caused by different contact methods, force application, sample loading principles, and configuration setup (i.e., different rheology geometry sizes, and AFM probes), etc. A significant study by Richbourg et al. [24] conducted a cross-evaluation of the accuracy and precision of stiffness measurements for PVA hydrogels using five different instruments: tensile testing, compression testing, rheometer, macro-indentation, and nano-indentation. Their findings indicated that tensile testing and macro-indentation yielded consistent results across the entire range of

Table 1
Different modulus related with stiffness and their internal relationship.

Moduli	Definition	Application	Assumption	Relationship to other moduli
Shear modulus (G)	Ratio of shear stress (τ) to shear strain (γ)	Elastic hydrogel, i.e. covalently crosslinked hydrogel	Neo-Hookean model: the behaviour of incompressible hyperelastic materials undergoing large deformations, with the stress–strain relationship derived from a simple strain energy function based on the deformation gradient.	For most hydrogels in TE applications, G serves as a more accurate and fundamental descriptor of stiffness $\tau = G(\gamma^2 - 1/\gamma)$
Young's modulus/ Elastic modulus (E)	Ratio of normal stress (σ) to normal strain (ε)	Linear tension or compression experiments	Hooke's law: the stress applied to a material is directly proportional to the strain produced, within the material's elastic limit.	$E = 2G(1 + v)$
Shear storage modulus (G') and loss modulus (G'')	Frequency and strain dependent elastic response to shear deformations	Viscoelastic hydrogel	Linear viscoelastic range (LVE): the stress and strain are proportional at which a material behaves in a linearly viscoelastic manner, or G' remains relatively constant over a specific frequency range at a fixed shear strain.	G' could be estimated as G when hydrogels are effectively elastic (G"< 0.05* G')
Complex modulus (E* or G*)	Combine the elastic and viscous components under cyclic loading. Its magnitude represents the overall stiffness of the hydrogel, and its phase angle (δ) indicates the relative contributions of elasticity and viscosity.	Viscoelastic hydrogel	Linear viscoelastic range (LVE): the stress and strain are proportional at which a material behaves in a linearly viscoelastic manner, or G' remains relatively constant over a specific frequency range at a fixed shear strain.	E*=E'+iE" G*=G'+iG" $\mathrm{Tan}(\delta)=\frac{G''}{G'}$

Table 2
Common mechanical testing methods (modified from ref [39]).

Mechanical technique	Applied force	Principle	Targeted sample	Resolution	Measured modulus	Limitations	Ref
Compression and tensile test	Compression and tension	Record the displacement response by a controlled uniaxial force	Metal and polymers	Centimetres to millimetres	E in Pascals (Pa) or gigapascals (GPa); Ultimate tensile strength in megapascals (MPa)	Sample must be prepared into specific shape. Strain rate sensitivity. Challenge to do with soft hydrogel.	[40]
Rheology	Shear	The small torque applied to geometry leads to the sample shear deformation response that is translated and analysed by a digital system.	Hydrogels and soft tissue, especially with viscoelastic properties	Millimetres to micrometres	G' and G'' in Pa; Viscosity (η) in Pa·s	Low resolution.	[41]
Magnetic resonance elastography (MRE)	Shear	The displacement propagation of the sample from the non-invasive shear waves with the set frequency is measured by analysing the MR picture.	In vivo organisms	Millimetres	G in Pa	High skill required. Expensive. In vivo only.	[42]
Magnetic twisting cytometry (MTC)	Shear	Magnetic microbeads apply the known twisting forces to living cells and the resulting bead rotation will be measured by a magnetometer and quantified as mechanical responses.	Living cells	Micrometres to nanometres	G* in Pa	High skill required. Expensive. Modulated magnetic signal, but the limited methods to demodulation.	[43]
Micro-indentation	Indentation	The sample is indented with a certain-forced probe and the change of depth and hardness of the sample will be evaluated.	Ex vivo tissues	Micrometres	E in Pa; Hardness in MPa	Only solid tissues. Low sensitivity.	[44]
Nano-indentation	Indentation	Same principle as micro-indentation but at a much smaller scale	Thin films and coatings, single phases in heterogeneous materials	Nanometres	E in Pa; Hardness in GPa	Highly sensitive to noise, complex sample preparation	[45]
Atomic force microscopy (AFM)	Indentation	Utilizes a nanoscale tip on a cantilever controlled by a piezoelectric element, that when it contacts with the sample causes a deflection in the photodetector, enabling measurement of the force	Live cells and hydrogels	Nanometres	E in Pa or GPa	High skill required. Expensive. E must be > 100 Pa.	[46]
Microrheology	Thermal fluctuation or active force	Tracks the motion of embedded tracer particles to probe local environments in an active or passive manner.	Soft materials (hydrogel and tissue) in small quantity	Micrometres	G' and G'' in Pa; η in mPa·s	Expensive and high skill required. The tracer particles might alter the local rheological properties.	[47]
Brillouin Microscopy	Acoustic force	The shift in the light's frequence due to the interaction between thermal acoustic phonons and light fraction introduced by a laser beam. This shift is related to the speed of sound that could be detected by spectrometer. Then the stiffness in the spatial and temporal modulation could be inferred by the sound speed.	Living cell, tissue, hydrogel with homo- or heterogenous properties	Micrometres to nanometres	Longitudinal modulus (M) in Pa	High mathematical skill required. Expensive. M of highly hydrated hydrogels does not correlate with E	[48]

stiffness values, while stiffness measurements from tensile and compression tests showed a high correlation (with a high R^2 value). Expanding the applicability of this model to other hydrogel systems in future research would greatly enhance our understanding of hydrogel stiffness.

The selection of an optimal strategy for measuring a sample's stiffness must take into account the material's properties (such as cells, tissues, or hydrogels), the required resolution (nano, micro, or macro), and the specific experimental questions being addressed.

5. The biological importance of stiffness

In 1942, Thompson first proposed the idea that mechanical signals could stimulate cells and tissues to behave differently in response to their surroundings [51]. Since then, numerous observations have confirmed various types of cells adapt themselves to being on soft or firm culture surfaces with an observable change in their activities [52]. The publication by Pelham and Wang in 1997 is considered a significant milestone in mechanobiology [53]. They developed a tenable polyacrylamide (PA) hydrogel system, where the ratio of acrylamide to bisacrylamide crosslinker could be adjusted to create hydrogels of variable stiffness. This enabled researchers to consistently analyse cellular reactions to different substrate stiffnesses. This simple regulative system has assisted researchers in proving the correlations between substrate stiffness and cellular regulations, including adhesion, migration, differentiation, etc [54–56].

Integrins are major adhesion receptors that regulate cellular processes from probing substrate stiffness to signalling [55]. Integrins can connect internally to the cell's cytoskeleton and simultaneously anchor cells to the ECM by binding to certain ligands, such as the ubiquitous

Arg-Gly-Asp (RGD) motif [57,58]. Clustered integrins dynamically stimulate the secretion of multiprotein complexes to form focal adhesions (FAs). The FA complex is able to mechanically bind the cell and the ECM, while simultaneously acting as an indicator, assembling actin filaments into stress fibres that affect cytoskeleton tension, as well as a regulatory signalling hub directing and transducing external signals that trigger a variety of cellular responses [59–61].

Compared to matrices with low stiffness, stiffer matrices typically encourage the maturation, turnover, and stabilization of FAs, leading to the formation of stress fibres and the recruitment of more signalling molecules (e.g., vinculin and talin), which promote cell adhesion, enhance mechano-signalling, and influence cellular functions such as motility and differentiation [55]. To elucidate the process, one view in mechanotransduction explicitly explains that actin can work with myosin by forming the molecular clutch system to probe the stiffness change in surrounding environment (Fig. 2) [62]. In this system, actin filaments, driven by polymerization at the cell's leading edge and myosin II-induced contractions, generate retrograde flow towards the cell's rear. This flow is resisted by integrins and linking proteins (i.e., vinculin and talin) between actin bundles and the ECM, transmitting forces and slowing down actin flow via a 'friction' mechanism. ECM stiffness is thought to be primary parameter to affect this mechanism: On rigid substrates, integrins resist displacement due to the high resistance of the ECM, thereby enhancing force transmission and stabilizing adhesions; in contrast, on compliant substrates, integrins move in response to ECM deformation, which reduces the effectiveness of the clutch system [63]. Gong et al. [64] pointed that beyond stiffness, viscoelasticity also affects the cell spreading especially where substrate relaxation time matches the clutch binding timescale on the soft substrates; however, it does not influence cell spreading for stiffer substrates, as the increased stiffness already saturates the bound clutches.

Substrate stiffness also plays an important role in the Rho signalling pathway, leading to active Rho GTPases (e.g., RhoA, Rac1, Cdc42) and Rho-associated kinase (ROCK), trigger actomyosin contractility, which in turn enhances focal adhesion (FA) formation and stress fibre assembly [65–67]. If the matrix is soft, the cells will detect less resistance, reduce Rho/ROCK activity, and release signals with different phenotypical results [68]. Moreover, YAP/TAZ, as transcriptional co-activators, can respond to physical cues and regulate mechanotransduction through their translocation from the cytoplasm to the nucleus, thereby activating transcriptional programs [69]. However, while YAP/TAZ strongly correlate with substrate stiffness in 2D cultures, this relationship remains unclear in 3D cultures [70,71].

Thus, at the molecular and genetic scale, substrate stiffness plays a pivotal role in regulating cellular functions via integrins-mediated FAs, Rho/ROCK and YAP/TAZ signalling pathways.

Increasingly, it is becoming clear that varying substrate stiffness affects the effectiveness and control of cell reprogramming. In one such example, Gerardo et al. [72] found that reprogramming efficiency significantly increases when somatic cells are cultured on substrates with a stiffness of approximately 100 kPa, compared to softer substrates (around 1 kPa) or stiffer ones (around 1.3 MPa). It could be that substrates that match the terminal differentiation goal improve the efficiency of attainment. Tissue homeostasis and disease progression are profoundly affected by cellular responses to substrate stiffness. Tissue homeostasis, a process of continual tissue renewal involving cellular growth and differentiation, is influenced by the physical, chemical, and biological stimuli from the ECM [55]. For example, bone can balance the ongoing process of resorption and formation and modify its structure to withstand mechanical forces, indicating that the behaviours of bone cells are regulated by the release of cytokines induced by substrate stiffness [73–75]. The Osteocyte system, the primary sensor detecting deformation caused by the matrix stiffness, relays signals to other bone cells and initiates remodelling in response to the amplitude of substrate stiffness [75,76]. If substrate stiffness is lower than the threshold, this will result in the apoptosis of osteocytes related to resorption triggered by a lack of repressive signals [77,78]. In contrast, the presence of deformation recognized by osteocytes could prevent their death and stimulate signals to inhibit the formation of osteoclasts which is responsible for bone erosion [79–81]. Hence, the substrate with mechanical properties, in particular stiffness, which resembles the local ECM is vital for tissues to maintain normal activities and homeostasis via induced signals to the cells.

Imbalanced tissue homeostasis often occurs after acute trauma. Abrupt fluctuations in mechanical loadings perceived by cells can cause tissue function impairment due to the absence of mechanical regulation required to modulate cell reformation [39,55,82,83]. In severe diseases, prolonged instability of homeostasis will have physical consequences on cells and tissues. For instance, traumatic brain injury interrupts the distribution of ECM and leads to the degradation of hyaluronic acid,

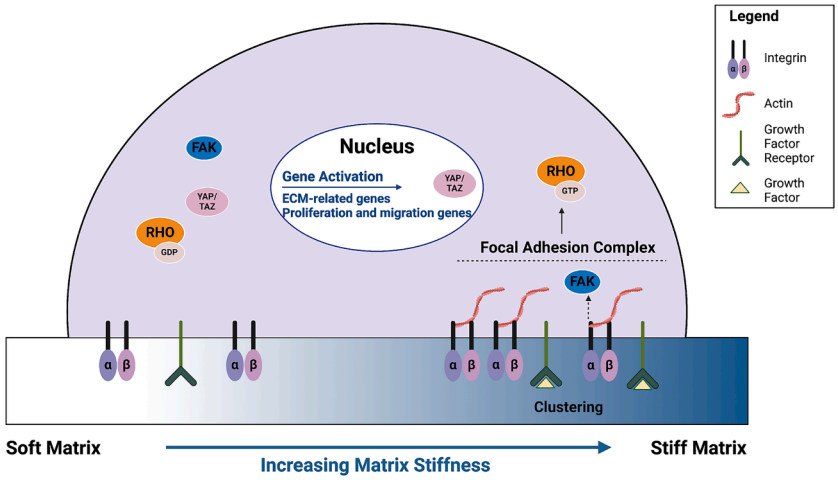

Fig. 2. The stiffer matrix induces the generation of focal adhesions and activation of the Rho pathway, thereby regulating cellular activities. The increase in ECM stiffness could stimulate the activation of integrins and growth factors. Activated integrins connect to the actin cytoskeleton and facilitate the recruitment of focal adhesion kinase (FAK) to form the FA complex. Concurrently, growth factors such as transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) undergo activation, binding with their receptors and clustering with integrins to augment biochemical signals. The synergy between activated integrins and growth factors plays a vital role in regulating Rho pathways. Additionally, FAK activation can also indirectly influence Rho/ROCK signalling. YAP/TAZ can be activated and translocated to the nucleus, thereby influencing the activation and transcription of ECM-related genes, as well as proliferation and migration genes. Created with BioRender.com.

followed by the breakdown of proteoglycans and various fibrous proteins [83,84]. Research has been conducted on the cerebral cortex of mice with a controllably injured brain, which has shown a considerable reduction in stiffness of the impaired tissues right after the trauma and this reduction continued for 28 days [85]. During the wound healing process, the injured extracellular matrix (ECM) is replaced by fibrous tissue, forming scar tissue. The increased stiffness of scar tissue compared to the original ECM is attributed to disruptions in normal ECM regeneration/degradation and an excess of ECM protein crosslinking [86]. Similarly, scarring in other parts of the body, such as the skin, would not significantly affect patient health, assuming that the other aspects of recovery are intact [55]. However, the scar tissue formation in the central nervous system can severely perturb normal function. Glial scar growth in the brain forms a physical barrier that restrains signal transmission and healing [87]. Along with mechanical damage, aging also disturbs the original homeostasis state [55,83,88–90]. A biological phenomenon is that muscle stiffness greatly increases in older people because of a higher degree of collagen crosslinking [89].

A long-term stiffness imbalance may compromise tissue and organ balance, leading to progressive diseases, such as fibrosis. Tumour development is a typical fibrotic progression with a continuously remodelled microenvironment, resulting in the dramatic excess in stiffness that is a distinguishing feature in diagnosing a tumour state [55]. Scientists have ascertained that cancerous breast tissue (1.5–9.3 kPa) is much firmer than healthy breast tissue (0.15–0.53 kPa) [91–93]. As a result of excessive fibronectin and collagen type I accumulation, the stiffness is almost ten times greater, resulting in a firm malignant growth adjacent to the local site of the lesions [94].

Stiffness is also one of the critical mediators to trigger the healing process that repairs damaged tissue and recovers impaired function. Angiogenesis and vascularisation are primary functions of endothelial progenitor cells (EPCs), which are triggered by matrix stiffness by the Rho signal pathway to secrete matrix metalloproteinases (MMPs) that help degrade the original ECM and form new tissues [95–97]. Therefore, mechanical cue, in terms of stiffness, regulates cell activity, thus affecting tissue homeostasis and disease progression. Hence, from an engineer's perspective, it is important to mimic local stiffness to ensure cellular behaviour matches the targeted purpose.

6. Optimal substrate stiffness

There are 11 major organ systems in the human body, presenting a wide range of stiffnesses. The elastic moduli spectrum spans from the minimum value of 11 Pa of intestinal mucus to the maximum value of 25 GPa of bone in the human body (Fig. 3) [98]. Nervous tissues are among the most flexible, followed by a majority of abdominal organs (such as kidneys and livers) and muscles, and then cartilage, ligaments, tendons, and bones. Cells adapt to varying stiffness with E ranging from near 50 Pa in the brain to over 2 GPa in the bone [92], but also varying viscoelasticity in the body, with G' and G" values up to kPa in the brain compared to $\sim 10^2$ GPa for G' and $\sim$ 1 GPa for G" in the bone [22].

Physiological functions and mechanisms determine the stiffness of each organ, tissue and cells. For instance, despite the fact that muscle and bone are anatomically and mechanically linked, the muscle is much more compliant than the bone. Physiologically, muscle is required to respond to mechanical loading and modulative signals by contraction and relaxation activities to mechanically move the body and secure bone in place, while bone provides stability to assist muscular functions and organ protection thus needing a stiffer structure.

The local ECM regulates cellular responses in functionally dynamic or static tissue through mechanical loadings and signalling communications between adjacent cells. Moreover, specific cells are particularly tailored to the distinct tissue at resident sites (Table 3). For example, neural and muscle cell behaviours such as growth, proliferation, differentiation, and death are favoured in more compliant substrates, while bone cells require a rigid matrix to maintain normal activities [92].

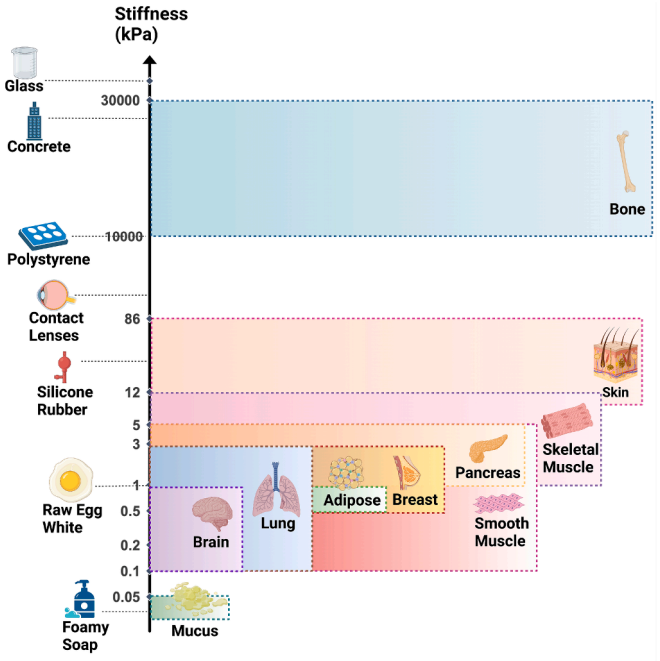

Fig. 3. Stiffness of human tissues. The stiffness of human tissue is usually quantified in a range. The elastic moduli (E) of these living tissues are presented in the figure as reported in the literature, in comparison with some common materials in our daily lives. Tissues are arranged in the ascending order of E values, spanning from the softest mucus (0.05 kPa) to the stiffest bone (30 mPa). Created with BioRender.com.

While some cell types can benefit from variable stiffnesses dependent on the desired tissue design, for instance, endothelial cells seeded in stiffer gels develop into vessels with larger lumens, conversely soft matrices promote the formation of branched capillary-like structures [92,99]. This further highlights modulating matrix stiffness as an important tool for engineers to customise cellular responses.

It has been generally accepted that mimicking the intrinsic stiffness of local tissue could provide suitable mechanical stimulus and cues for cells from this tissue and regulate cellular activities. However, the stiffness of human tissue is usually quantified in a range instead of a fixed value, i.e., human skin (60 to 850 kPa) and skeletal muscle (5–170 kPa) [7]. Further, a discrepancy is found between *in vivo* stiffness value and *in vitro* matrix stiffness for maximum biological responses. Compared to the elastic modulus for *in vivo* normal vascular smooth muscle cells (over 400 kPa), Nagayama et al. [100,101] reported that optimal substrate stiffness for vascular smooth muscle cells (VSMCs) differentiation (40 kPa) might be more dependent on VSMCs in contractile activation stage (90 kPa). Therefore, optimal substrate stiffness should always be carefully considered when designing scaffolds for different cells (Table 3).

7. Commonly used hydrogels

A hydrogel is a 3D network of interlinked polymers with hydrophilic properties that are capable of retaining large amounts of water. In healthcare and biomedical engineering, hydrogels are widely utilised in wound dressings, biosensors, TE, and drug delivery due to their biocompatibility and functionality [12,125–130]. The physical and mechanical properties of the gels can be customized to mimic the role of the local ECM and provide a congenial scaffold for cells (Fig. 4). The final gelation network is formed by combining monomers via physical, chemical, or enzymatic crosslinking methods (Fig. 5). In TE, hydrogels are categorized into two main groups, referred to as natural and synthetic hydrogels [11], each containing their own limitations. Natural

Table 3
Reported substrate stiffness for targeted cellular responses across different tissues.

Organ/ tissue	Cells	Cell Culture Conditons	Measuring Technique	Modulus	Reported stiffness for target response (kPa)	Behaviour Change with Low Stiffness	Behaviour Change with High Stiffness	Ref
Brain and nerve	Patient-derived glioblastoma xenograft cells	3D in PEG gel	Compression test	Elastic modulus	0.24	Cancer cell proliferation and spreading enhancement.	Increase drug resistance.	[102]
	Adult neural stem cell	2D on peptide-modified interpenetrating polymer network	Rheometer	Elastic modulus	0.5	Neuron formation (self-renewal).	Glial cultures (differentiation).	[103]
	Human iPSCs	2D on tilapia collagen gel	Rheometer	Shear storage modulus	1.5	Not clear.	The development of dorsal cortical neurons at an early stage.	[104]
	Schwann cell	2D on polyacrylamide gel	Compression test	Elastic modulus	7.45	Cell clustered.	Cell elongated. More random motility. Higher expression on adhesion proteins.	[105]
Blood and vessels	individual bovine aortic endothelial cells and spheroids	3D in glycated collagen gel	Compression test	Elastic modulus	0.515	Spheroids had fewer extension.	Enhance spheroid extension outgrowth and cellular spreading.	[106]
	Rat vascular smooth muscle cells	2D on polyacrylamide gel	AFM	Elastic modulus	40	Low cell spreading area and weak adhesion.	The elongated shape and thick actin stress fibres.	[100]
	Human umbilical cord-derived endothelial cells and mesenchymal stem cells	2D on Materigel coated hydrogel	Unknown	Unknown	17	Form the larger clusters and maximize condensation.	Smaller clusters and less condensation.	[107]
Skeletal muscle	Human muscle stem cells	3D in collagen hydrogel	AFM	Elastic modulus	1–2	Most cells maintained quiescent and proned to apoptosis.	Less cells were quiescent. Cells tended to activate and differentiate.	[108]
	Dental-derived gingival mesenchymal stem cells	3D in peptide coupled alginate	Compression test	Elastic modulus	10–16	Less capacity for myogenic differentiation.	10–16 kPa of stiffness range was the best for myogenic differentiation capacity.	[109]
	C2C12 myoblasts	2D on polyacrylamide gel with collagen coat	AFM	Elastic modulus	12	No striation.	Less differentiation when substrate stiffness over 12 kPa.	[110]
Kidney	Human renal progenitor cells	2D on polyacrylamide gel with collagen coating	Unknown	Elastic modulus	4	Isolated cells exhibited thin sheet-like morphology. High and stable circularity and cell spreading area. Stay quiescence.	Clustered cod-like morphology. Low circularity and cell spreading area. Proliferation.	[111]
	Murine inner medullary collecting duct	3D in micropatterned polydimethylsiloxane or alginate molds	Nanoindentation	Elastic modulus	439.9	Failed to form tubular structure.	Promoted tubular formation.	[112]
Immune system	Jurkat E6 T lymphocytes	2D on glass coverslip with polydimethylsiloxane coating	AFM	Elastic modulus	5	Less spreading	More spreading	[113]
	Bone marrow-derived mesenchymal stem cells and macrophages coculture	3D in transglutaminase cross-linked gelatin	Compression test	Elastic modulus	60.5	MSC proliferation. Positive effect on osteogenesis with cell coculture.	MSC osteogenic differentiation. M1-type macrophages. Negative effect on osteogenesis with cell coculture.	[114]
Heart	Rat cardiac fibroblasts	3D in hybrid collagen-alginate gel with dynamic stiffness modulation	Rheometer	Elastic modulus	5–30 (dynamically changed with time)	(5 kPa: normal condition) Potentially reverse the differentiation state, and slow the fibrotic process	(30 kPa: disease condition) Accelerate the fibrosis. Form mature FA complexes.	[115]
	Rat neonatal cardiomyocytes	2D on polyacrylamide gel with collagen coati	Compression test	Elastic modulus	22–50	Low excitation, fewer cells, reduced contraction force and cell elongation.	Higher fibroblast density and poor electrical excitability.	[116]
Skin	NIH-3 T3 mouse fibroblasts	2D on polyacrylamide gel	Rheometer	Shear storage modulus	10	Round shape.	Large spread cells. Actin fibres formation.	[117]

(*continued on next page*)

Table 3 (*continued*)

Organ/ tissue	Cells	Cell Culture Conditons	Measuring Technique	Modulus	Reported stiffness for target response (kPa)	Behaviour Change with Low Stiffness	Behaviour Change with High Stiffness	Ref
	Human epithelial cells	2D on PEGDA gel with protein coat	AFM	Elastic modulus	40	Maintain a rounded shape. Low YAP expression.	More actin stress fibres and vinculin clusters formation. Initially high YAP in nucleus and decreased gradually.	[118]
	Primary human dermal neonatal fibroblasts	3D in stiffness-gradient decellularized ECM hydrogel from porcine skin	Compression test	Elastic modulus	120	Randomly spread. After 5 days, cells aligned with 45° in regions.	Oriented elongation. After 5 days, cells decreased stiffness before the alignment.	[119]
Cartilage	Canine chondrocytes	2D and 3D on chitosan gel	Compression test	Elastic modulus	19.9	Flattered and spread morphology.	Round shape. Growth and proliferation promotion.	[120]
	Human mesenchymal stem cells	3D in peptide hydrogel	Rheometer	Shear storage modulus	21	Less chondrogenic differentiation.	Promote chondrogenic differentiation.	[121]
Bone	Human mesenchymal stem cells	3D in peptides-alginate-agarose hybrid gel	Compression	Elastic modulus	22	Inhibite osteogenesis and enhanced adipogenesis.	Osteogenic commitment and osteogenesis enhancement.	[122]
	Bone marrow mesenchymal stem cells	3D in GelMA	Compression	Elastic modulus	39–45	Enhance adipogenic/ chondrogenic differentiation	Promote osteogenesis.	[123]
	Rat calvarial osteoblasts	2D on polydimethylsiloxane	Compression	Elastic modulus	134	Small and round shape. Less actin fibres.	Polygonal shape. Osteoblastic differentiation.	[124]

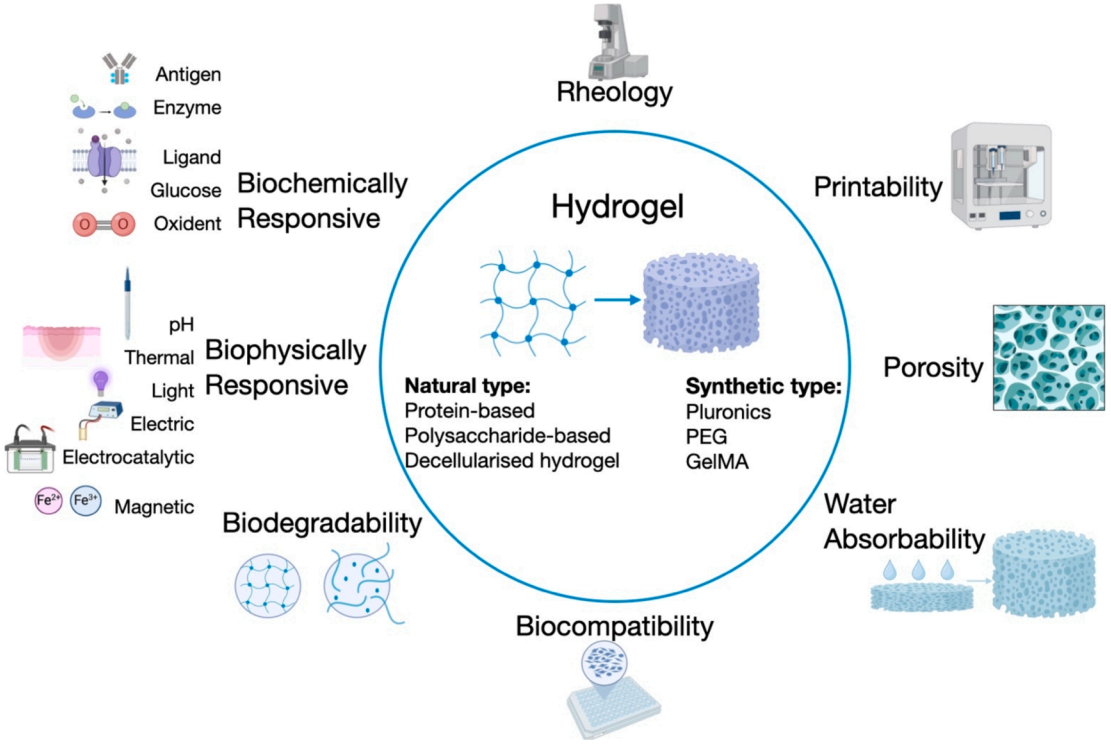

Fig. 4. The main characteristics of hydrogels in TE. To achieve practical implementation in TE, it is imperative for natural hydrogels involving protein-based, polysaccharide, synthetic or decellularized hydrogels, to meet essential criteria. These criteria include adequate mechanical strength, appropriate biodegradability, enhanced printability, and optimal preservation of cell viability. Created with Biorender.com.

hydrogels such as collagen, chitosan, fibrin, elastin, etc., usually have weak mechanical characteristics, whereas synthetic polymers such as Pluronics, poly(ethylene glycol) (PEG), gelatine methacryloyl (GelMA), etc., provide stronger mechanical properties at the expense of biological function. Synthetic polymers' absence of major biological molecules, such as growth factors, anchorage sites, and bioactive cryptic peptides, leads to a delay or absence of cell adhesion or migration [131]. We will discuss the mechanical properties of representative hydrogels by

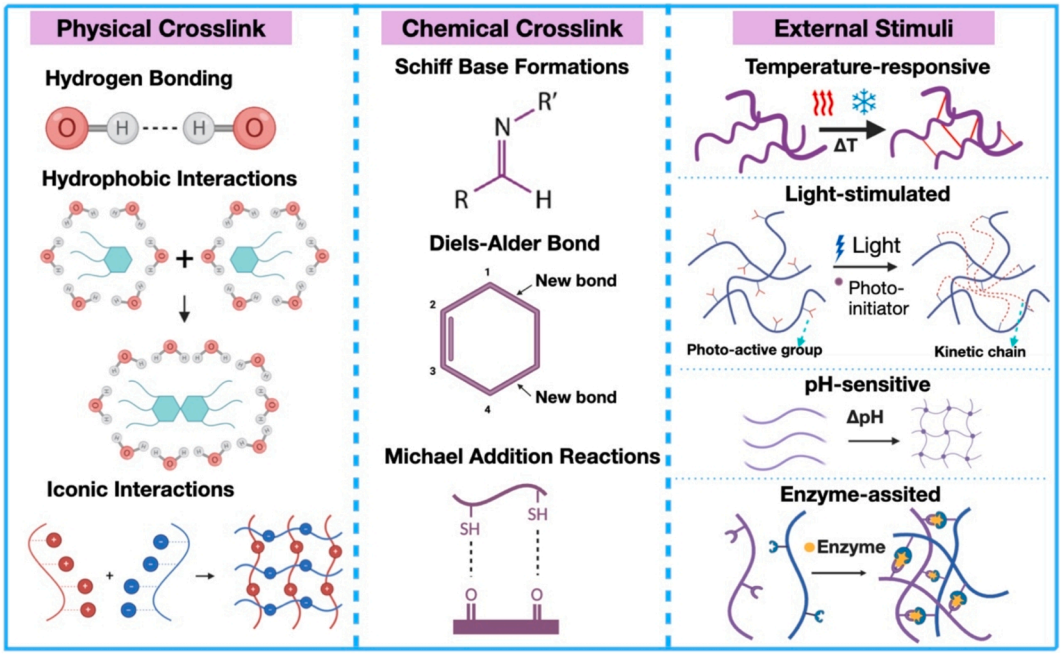

Fig. 5. Crosslinking mechanisms in hydrogels: physical, chemical, and stimulus-driven effects. Physical crosslinking involves non-covalent interactions, such as hydrogen bonds, hydrophobic association or ionic interactions, which stabilize the hydrogel network. Chemical crosslinking relies on covalent bonds formed through chemical reactions, enhancing the gel's structural integrity. External stimuli such as pH, temperature, light, enzyme and other environmental factors can modulate both physical and chemical crosslinking processes, affecting the gel's properties and functionality. Created with BioRender.com.

category (Table 4).

7.1. Natural hydrogels

Natural hydrogels are mostly derived from protein polymers, polysaccharide polymers, and decellularised tissues [131]. They preserve biologically active molecules such as growth factors and glycans that can promote vital cellular functions like cell growth, differentiation and death thus tuning or maintaining physiological or psychological functions of body systems, nonetheless they are generally biocompatible and biodegradable [140]. To counteract the inferior mechanical properties of naturally derived hydrogels, composite natural-synthetic or natural-natural hydrogels may be incorporated [131,141,142], in which the degree of crosslinking may be optimised. However, due to high batch-to-batch variations, manipulating and altering the sequence distribution of natural hydrogels is difficult since slight modifications can radically change the responses and fate of the embedded cells [141,143,144].

7.2. Protein-based hydrogels

Most protein hydrogels for TE utilise fibrous proteins that are already constituents of the ECM structure, such as collagen, elastin, and fibrin, which are employed as bioscaffolds to encapsulate cells. The source of these hydrogels is usually from animal extraction or biosynthetic routes [140]. For example, collagen is primarily derived from porcine, bovine, and fish skin tissues, whereas fibrin comes from autologous blood and from allogeneic or recombinant sources. Proteins can be treated under mild biocompatible conditions to fabricate hydrogels, and these hydrogels are biodegradable in the human body by proteolytic enzymes [145]. Therefore, protein-based hydrogels have versatile applications in the field of TE.

7.3. Collagen

In mammalian animals, collagen constitutes around one-third of the total protein and is mostly found in skin, bone, and connective tissues [146]. The structure of collagen is comprised of a triple helix including a duplicate set of amino acid sequences of −Glycine-X-Y-, where X stands for proline and Y refers to hydroxyproline. Collagen I is the most widely investigated and employed among 29 types of collagens [131]. In spite of the fact that this type of hydrogel has the ability to resist applied force and prevent plastic deformation, the lack of covalent bonding results in poor mechanical strength. To improve mechanical performance, different crosslinking methods have been employed [131]. The general preparation of collagen I hydrogel requires adding NaCl into the solution to strengthen mechanical characteristics, then raising the pH to the isoelectric point of collagen as the relatively weak ionic strength enables better linear viscoelastic behaviours and transparency [147]. Transparent hydrogels in TE could offer visualisation of encapsulated cells' behaviours and facilitate oxygen and nutrient exchange due to their microscale porous structure.

Yang et al. [148] tested the shear modulus of dry fibrils of collagen I ($G = 33 \pm 2$ MPa) via AFM. They also found that the hydration of these fibrils will decrease G by a factor of 10 (2.9 ± 0.3 MPa), which confirms the anisotropic performances of collagen fibrils. Moreover, carbodiimide-mediated chemical crosslinking of the collagen fibrils significantly raised G (74 ± 7 MPa at dehydrated state). Nevertheless, moderate crosslinking reinforcement is preferred to form mechanically supported collagen fibrils. Too high of a crosslink density can result in brittle structures that cannot dissipate excessive energy [149].

The disadvantage of using collagen as a hydrogel in the human body is that collagen degrades into amino acids, which can lead to thromboembolism and coagulation [131]. Moreover, collagen's high cost makes it unsuitable for mass production and widespread biomedical use [150].

7.4. Fibrin

Another representative of protein-based hydrogels is fibrin. Fibrin, procured from the blood, consists of two parts, fibrinogen and thrombin. When activated, fibrinogen transforms into fibrin and forms a fibrous network, which is a key step in tissue recovery and the coagulation

Table 4
The influence of selected hydrogel's stiffness on cellular response.

Hydrogels	Measuring Technique	Stiffness (E, kPa)	Biological Response	Ref
Collagen	Rheometer	15.4–31.8	Stiffer collagen gels support greater cell viability and cardiac lineage differentiation.	[132]
Fibrin	Compression test	3.4–10.9	Stiffer fibrin hydrogels support neural cell growth and differentiation. Softer gels support capillary-like structure formation from endothelial cells.	[133,134]
HA	Compression test	1.5–8	Crosslinked HA improves cell viability and the chondrogenesis potential of human adipose-derived stem cells.	[135]
Chitosan	Compression test	Below 1 −greater 30	Soft gels (E < 10 kPa) support neural stem progenitor cell proliferation and maximum proliferation was achieved on surfaces with a stiffness of 3.5 kPa. Stiffer gels (E > 7 kPa) improve oligodendrocyte differentiation, while gels with E < 1 kPa promote greater oligodendrocyte maturation and myelination.	[136]
dECM	Compression test	3.233–6.998	Regulate TGF-β1 induced differentiation and vascular network formation.	[137]
PEG	Compression test	129–3170	The stiffer matrix supports osteogenic and adipogenic differentiation (at a high cell density) with the assistance of cell adhesive RGD.	[138]
GelMA	Tensile test	49.9–139.1	The viabilities of human dermal fibroblasts are not affected by GelMA's stiffness. Cell proliferation and differentiation are inversely related to GelMA's stiffness.	[139]

cascade [131]. Due to its easy access from the blood, fibrin-based gels have been proposed as a collagen replacement [151]. Moreover, these hydrogels are primarily used in cardiac TE; however, poor mechanical properties and rapid degradation are major obstacles [152].

The mechanical behaviours of fibrin are controlled by the density of fibrinogen and thrombin. Fibrinogen is soluble; however, in the presence of thrombin, it can be converted into fibrin gel or a blood clot [153]. Kniazeva et al. [154] proved that fibrinogen concentration control can alter substrate mechanical cues, thus affecting angiogenesis and implanted endothelial cell viability. Additionally, fibrin is a "self-restoring" biomaterial [155]. When fibrin clots are continuously stretched during creep tests, their stiffness does not change, which is most likely due to reversible interactions between the N-terminal Gly-Pro-Arg sequence and complementary binding pockets, which hold the structure together and resist stress deformation [156–158].

7.5. Elastin

Elastin, a key ECM protein in connective tissue, confers resilience, strength and elasticity to different organs and tissues. In stretchable tissues, such as the skin, lungs, and aorta, elastin dominates due to its strength and flexibility [131,159]. This has made elastin a versatile hydrogel component when building a matrix for skin and vascular implants. Elastin must be purified before grafting into the body, but this process does not remove all immunogenic contaminants [160]. Moreover, elastin is not often chosen as a scaffold material due to its extensively crosslinked structure and insolubility [159,160]. Other soluble forms of elastin are therefore developed, such as tropoelastin, elastin-like peptides (ELPs), elastin-like recombinant (ELRs), and elastin-mimetic hybrid polymer (EMHP) [161–164].

By cleaving some signal peptides, tropoelastin can be converted to elastin, but the yield is extremely low [159]. According to the final application, amino acid sequences or peptide concentrations can be adjusted to control the tuneable mechanical properties. Stiffness can range from 1.6 to 1200 kPa and their extensibility (strain at failure) is up to 150 %, which emphasizes the versatility of elastin-based polymers [165].

7.6. Polysaccharide based polymers

Polysaccharides consist of repeating units of monosaccharides, such as glucose, mannose, and galactose, linked by glycosidic bonds, and exhibit superior water absorption and retention due to their abundant hydroxyl groups.

7.7. Glycosaminoglycans

In all mammalian tissues, glycosaminoglycans (GAGs) are long chains of negatively charged polysaccharides [166]. Four categories of GAGs are heparan sulfates (HS)/heparin, chondroitin sulphate (CS), keratan sulphate (KS), and hyaluronic acid (HA) [167]. Since HA is the only non-sulfated GAG, it interacts with other GAGs and proteoglycans (PGs) physically rather than through covalent bonds with core proteins [167]. In the ECM, GAGs are able to interact with functional biomolecules such as growth factors, core proteins, and proteases, dependently on their structure and compounds [167–169]. Additionally, GAGs are greatly polar and hydrophilic, allowing them to increase water retention, further making a partial contribution to mechanical stability of tissue by resisting extensive loading [167]. GAG/PG interactions with other matrix proteins, notably collagen, are also supportive of a mechanically stable ECM [170–172].

There is a remarkable number of applications for HA, as opposed to other GAGs, in TE, including wound healing, cartilage development and neural tissue regeneration [131,173,174]. HA plays a significant role in the development of early embryos and cell matrix with tuneable physical and rheological properties, despite its slow gel formation and fragile structure [175]. The crosslinking density and molecular weight determine how HA behaves mechanically. For example, Ren et al. [176] adjusted the molecular weight of HA from 4 kDA to 90 kDA to control the storage modulus of an HA hydrogel from 0.2 to over 1 kPa. HA with lower molecular weight (4 kDA) displayed a stress-relaxing tendency, indicating the way of deformation and stress change under constant strain. As a result of stress relaxation behaviour, a hydrogel's mechanical strength is also crucial for cellular spreading and proliferation

[176–178]. The improvements of mechanics of HA can also be carried out via crosslinking modifications, including physical (temperature control and ionic crosslinking), chemical (thiol-modification, Schiff-base strategy, Diels-Alder click reaction, photo-crosslinking) and enzymatic reactions [179].

7.8. Chitosan

Chitin, as the second most abundant polysaccharide, is insoluble and rigid with high amounts of acetylated groups [180]. After partial deacetylation, chitosan (CS) can be obtained with more amino groups and enhanced solubility. Since CS can form films, it shares structural similarities with GAGs and is capable of covalent immobilization, making it suitable for many TE applications [181,182]. However, the serious limitation of CS is its weak mechanical stability [180]. Mechanics of CS are directly regulated by molecular weight and deacetylation degree [183,184]. The Young's modulus of nanostructured CS films with different molecular weights has been found to differ in wet or dry conditions from 1.8 ± 0.4 to 2782 ± 500 MPa [185]. Aryaei et al. [186] compared the average elastic modulus of uncrosslinked and crosslinked CS films which were approximately 1.5 GPa and 4.7 GPa, respectively.

7.9. Agarose

A naturally derived polysaccharide from algae, agarose gels at 32 °C and melts at 40 °C, making it suitable for *in vitro* and *in vivo* applications [187,188]. Due to its thermosensitive properties, agarose can form a gel without crosslinking [187]. The gelation process of agarose consists of three steps: initiation, nucleation and pseudoequilibrium [189,190]. Despite its brittle characteristics in the solid state, agarose has the ability to maintain its structure at a wide spectrum of temperatures for an extended time period. The primary purpose of agarose is to function as a sacrificial bioink, supporting printing structures, as it is poor at supporting cell proliferation and synthesis[191]. It can be blended with other hydrogels (i.e., collagen) to ameliorate biocompatibility and cell viability [192,193]. It is also noteworthy that agarose can combine with synthetic hydrogels to build interconnecting mechanisms to covalently immobilize some biomolecules such as RGD or aggrecan [194]. In order to discover the viscoelastic and mechanical properties of the agarose hydrogel, Ed-Daoui et al. [195] adjusted the mass concentration of agarose from 25 to 50 g/l, and found that the corresponding elastic modulus increased from 274.6 to 869.5 kPa. They exploited Voigt's analogue model to prove the delayed elasticity behaviour of agarose hydrogel.

7.10. Decellularization

During decellularization, the process strives to preserve the architecture and bioactive functions of the ECM to create a bioscaffold that mimics native tissue by removing cells while maintaining the surrounding ECM [196]. However, in practice, achieving a perfect balance between effective cell removal and ECM preservation is challenging due to inherent trade-offs. In the process of decellularizing ECM (dECM) hydrogels, several critical factors must be considered, such as host tissue, matrix preparation, crosslinking systems, sterilization, and decellularization efficiency [131,197]. For instance, some sterilization processes, such as ethylene oxide or radiation (gamma or electron beam) and homogenization process may jeopardize the structure of dECM hydrogels and degrade their mechanical integrity [11,198]. Yao et al. [199] showed that the tensile strength of the dECM hydrogel from rabbit uterine tissue is almost two-fold lower than the host tissue (110.1 kPa and 225 kPa, respectively), which demonstrated that the decellularization process was detrimental to mechanical stability. The key parameters of local tissue including thickness, density and shape affect the results of decellularization. For example, thicker tissues with denser cells, such as the dermis will escalate the complexity of cell removal and may aggravate the damage from decellularization agents due to extended exposure time [197].

Concentration, time, and temperature significantly influence the mechanical properties of dECM hydrogels [200]. Massensini et al. [200] indicated that the maximum storage modulus and loss modulus of the dECM hydrogel derived from porcine urinary bladder increased eight times higher and six times higher respectively when the concentration was doubled from 4 mg/ml (G' 76.6 ± 10.4 Pa and G'' 11.0 ± 1.5 Pa) to 8 mg/ml (G' 460.4 ± 62.5 Pa and G'' 66.4 ± 9.3 Pa), which also confirmed that the storage modulus dominates the viscoelastic behaviour and that the hydrogels were in a solid state. They emphasized that the high concentration of dECM (larger than 3 mg/ml) is favored to offer a robust and feasible hydrogel. Since dECM hydrogels' weak mechanical properties and fast degradation rate hinder their use in regenerative engineering, further improvements must be made before they can be clinically applied.

7.11. Synthetic hydrogels

Although natural polymers may offer a better environment for the growth and recovery of cells and tissues, synthetic hydrogels can be modified according to the requirements of practical applications with reinforced mechanical and structural properties. Since Wichterle and Lím [201] introduced the first synthetic hydrogels, synthetic hydrogels now can be engineered with a variety of functional domains to achieve anticipated biological activities, such as crosslinkable blocks, cell adhesive groups, or external stimulus (e.g., light or electric) responsive domains.

7.12. Pluronics

Poloxamers, also known by their commercial name Pluronics, comprise hydrophobic polypropylene oxide (PPO) and hydrophilic polyethylene oxide (PEO) blocks arranged in triblock PEO-PPO-PEO sequence, which are thermo-responsive synthetic bioinks. There is a wide range of Pluronics with different molecular weights and PEO/PPO ratios, such as commercially available Pluronic F68, F108, and F127. When Pluronics are below critical micellar temperature and critical micellar concentration, they behave as unimers in the solution. When the critical micellar temperature and critical micellar concentration are exceeded, they form micelles. Apart from employing higher molar mass polymers with long PPO blocks to increase the mechanical strength, 'reverse' structured Pluronics PPO-PEO-PPO can also alleviate insufficient mechanical robustness [202]. Markus et al. [203] inferred that a reverse composition with high molecular weight enables Pluronics with a tenfold enhanced storage modulus (25–30 kPa) than 'normal' Pluronic F127 (3.5 kPa).

7.13. Poly(ethylene glycol)

Poly(ethylene glycol) (PEG) hydrogels have been widely used in TE due to their superior biocompatibility under proper polymerization conditions and tuneable physicochemical properties, including rheological properties, swelling rate, water content, permeability, etc. Physical, ionic, and covalent interactions are all possible ways in which PEG can generate stable covalently crosslinked gels [204]. Common fabrication methods of PEG gels are categorized into three polymerization mechanisms: step-growth, chain-growth and mixed-growth. Anseth's group [204,205] compared various polymerization mechanisms in their review paper and also highlighted that the mechanical properties of PEG hydrogels are primarily influenced by the molecular weight and concentration of PEG monomers, which in turn affect the morphology of chondrocytes. Nguyen et al. [206] concluded that as molecular weight increased from 508 Da to 10 kDa, stiffness and tensile modulus were also enhanced from 0.055 to 42.9 MPa and 0.02 to 3.5

MPa, respectively. This indicated that PEG hydrogels can be applied to mimic both soft tissue and hard tissue by adjusting the PEG molecular weight and concentration. A co-polymerization of RGD peptides with PEG can enhance PEG's bioactivity and promote the survival of cells [207,208]. Although PEG hydrogels are highly permeable to allow nutrients to diffuse, excellent permeability also hinders the controlled delivery of bioactive molecules, which is a huge obstacle in the design of PEG delivery systems.

7.14. Gelatin-methacryloyl (GelMA)

Since Van Den Bulcke et al. [209] modified gelatin with methacrylamide (MA), gelatin methacryloyl (GelMA) has been recognized as a versatile hydrogel for TE applications because of its tunable mechanical and biochemical properties. GelMA was designed to maintain stable structures without support from additional materials due to its low gelation temperature (below 30 °C) [210,211]. The introduction of MA groups does not affect the cell binding RGD motifs and matrix metalloproteinase (MMP) degradable motifs in gelatin. This implies that GelMA preserves the gelatin's biofunctions to support cellular attachment and growth functions and manipulates cell enzymatic degradation, concurrently possesses the advantages stemming from MA groups to form a 3D structure [212]. The side MA groups can be crosslinked using a photoinitiator and UV radiation to give a rigid gel. Anseth et al. [213] were the first to use GelMA to encapsulate cells and induced the differentiation of fibroblasts into myofibroblasts with the assistance of growth factors. The most outstanding advantage of GelMA is its tuneable mechanical properties, which could be controlled by several factors, i.e., concentration, UV crosslinking conditions, and degree of methacrylation [212,214–218].

8. Methods of tuning the stiffness

Researchers have studied the role of stiffness in modulating cellular and tissue functions or achieving a certain stiffness for specific biomedical applications by altering the concentration of hydrogel matrix, changing polymer structure and distribution, designing the molecular weight, controlling the swelling or adjusting the crosslinking density, in order to achieve the desired stiffness (Table 5).

8.1. Polymer concentration

Adjusting the density or concentration of hydrogels is a facile approach to modifying gel stiffness, but it is also detrimental to naturally fundamental structures [106]. To be specific, the polymer concentration strongly affects the porosity and topography of hydrogels, proven by variations in pore sizes and pore wall thickness [230]. Moreover, the higher concentration of polymers also increases the number of binding sites in close proximity to the cells and regulates the capability of molecules, oxygen and other biofunctional signals to diffuse through the hydrogel [231,232].

8.2. Macroscopic polymer architecture

Many hydrogels are amorphous, meaning they lack a long-range ordered structure. These hydrogels have a random network of polymer chains that are highly flexible and can swell significantly when they absorb water. The amorphous nature allows for a uniform distribution of water within the gel. Creating a hierarchical and fibrous network can increase stiffness by providing additional structural support. Modern techniques such as 3D printing and lithography can fabricate a hydrogel with a layered or patterned structures to realise a more precise control of designed mechanics [233–235]. In the microscale or nanoscale level, mechanical behaviours of hydrogel could be influenced by fibre size, fibre alignment, and distribution of individual polymer chain. These parameters could be tuned by fabrication techniques such as electrospinning to achieve a fibrous and mechanically controllable network through adjusting voltage, flow rate, distance between needle and collector, as well as the mechanics of employed polymer precursors [236]. Shin et al. [237], Sun et al. [238], and Wang et al. [239] all reported that the reduction in diameter of electrospun fibres significantly amplifies their stiffness exponentially. Tonsomboon et al. [240] reinforced elastic modulus by two orders of magnitude through infiltrating electrospinning gelatin nanofiber into alginate hydrogel. Especially, aligned fibres in those reinforced gels were three times mechanically stronger (tensile strength) than hydrogels with more random-oriented fibres due to the compliance by the isotropic nature. Main obstacles in current electrospinning to obtain 3D structure hydrogel is the limitation on materials, which is restricted into specific polymer precursors (such as uncrosslinked GelMA [241–243], HA variants [244,245], PVA [246–248]) with the right viscoelastic properties when in solution.

Acoustic holography is a novel noncontact technique that enables the creation of complex cell patterns and controlled stiffness in hydrogels, effectively mimicking biological tissues. This method generates intricate acoustic fields to spatially arrange cells, manipulating them along the nodes or antinodes of the acoustic field. As a result, it allows for the formation of arbitrary shapes rather than just regular patterns, with physiological stiffness that aligns with the properties of the cell patterns [249].

Microfluidics is another promising alternative method to obtain hydrogel with hierarchical structure into a desired final construct with the assistance of 3D bioprinting [250]. Burdick group [251] recently proposed to employ microfluidics to form a microgel bioink via packing the densely "jammed" microparticles which are compacted by physical interactions. They added crosslinker and photoinitiator to further enhance the mechanical properties of jammed material without decreasing the cell viability and maintained the inherent shear-thinning property from interparticle adhesion, highlighting the possibility of microfluids for hierarchical materials formation in 3D printing.

8.3. Nanoscale polymer engineering

Engineering the physical structure of polymers in nanoscale dimensions is a feasible approach to tuning the mechanical properties of hydrogels while maintaining their biofunctionality and biocompatibility [252].

Godbe et al. [253] reported the fabrication of supramolecular peptide hydrogels formed from the co-assembly of oligo-lysine compounds (K_4, K_{10}, K_{15}, and $K_{120–140}$) and an anionic peptide amphiphile. Changing the oligo-L-lysine gelator length linearly increased the storage modulus by 10.5 Pa for each individually added lysine on the nanofibers. They also found that decreasing stiffness on the order of 70 Pa notably improved cell (dopaminergic neurons derived from induced pluripotent stem cells) viability, neuronal growth and differentiation. Rodriguez et al. [254] used a self-assembling hydrogel with a laminin peptide sequence (Fmoc-DDIKVAV) and fabricated a counterpart with lysine (K) at the C terminal. The mechanical study showed that the Fmoc-DDIKVAVK (G' = 10 500 Pa) was almost 1.6 times stiffer than Fmoc-DDIKVAV (G' = 6500 Pa) due to the strengthened ionic interactions.

In addition to applying the strategy of engineering the chain length of single polymers, Boothroyd et al. [255] designed a multicomponent system to tune the stiffness by mixing the self-assembling peptide FEFEFKFK with its double length derivatives FEFEFKFK-GG-FEFFKFK, showing that the addition of double length peptides could intensify 30 times of storage modulus than pure short peptide (80 ± 5 Pa). Inspired by this work, Scelsi et al. [256] used another pair of peptides (SFFSF-NH_2 and its counterpart) to achieve the tuneable stiffness with a higher magnitude of up to 10^5 Pa for hard tissue scaffolds. The system with two homologous peptides exhibited the same extent of biocompatibility and low cytotoxicity as single peptides but with a mechanically reinforced strength to support cellular activities.

Table 5
Current Literature on Hydrogels with Tuned Stiffness Impacting Cell Fate.

Factor	Relationship with stiffness	How it affects stiffness	Recent reports on tuned stiffness impacting cell fate
Polymer concentration	Generally positive	Increase chain entanglement and crosslink density, leading to greater resistance to deformation; however, affecting the porosity and topography	Alshehri et al. [219] achieved a 2.5-fold increase in matrix stiffness by doubling the concentration of self-assembling tetrapeptides. Their results demonstrated that a low-stiffness matrix (20 kPa) supported greater bone cell differentiation compared to a high-stiffness matrix (50 kPa).
Macroscopic polymer architecture	Fibre diameter: generally negative Fibre density/ordered structure: positive	The denser and ordered structures by offering additional structural support and impact on water absorption.	Yi et al. [220] developed a highly aligned fibres hydrogel in the shell-core structure using stable jet coaxial electrospinning technique with the achievement of remarkably broad stiffness spectrum from 90 kPa to 13.18 MPa but maintaining the unchanged topographical properties. Stiffer electrospun fibres had less cells attachment (human vascular smooth muscle cells) at early stage but enhanced proliferation and migration and improved F-actin fibre assembly compared to compliant fibres. Their gene expression results implied that the increasing stiffness of hydrogel would shift cells toward proliferative and pathological state, which subsequently had an adverse effect on the proliferation and migration ability of human umbilical vein endothelial cells (hUVECs).
Nanoscale polymer engineering	Depends	Altering the nano-composition of polymers can change the packing and intermolecular interactions (van der Waals force, hydrogen bonds and π-π interaction)	He et al. [221] fabricated low molecular weight gels by using the different length of the alkyl chain (n-heptadecyl, n-undecyl and nonyl) to obtain moduli from 33 kPa to 6 kPa. Low stiffness gel tended to stimulate the mesenchymal stem cells into the chondrocytic differentiation, while higher stiffness gel motivated osteogenic differentiation.
Molecular weight	Generally positive	Longer polymer chains lead to more entanglement, and molecular weight distribution affects the physical properties as well	Park et al. [222] regulated the hydrolysis duration of silk fibroin to modulate the molecular weight of the hybrid silk fibroin/gellan gum hydrogel, with negligible impact on cell (human bone marrow mesenchymal stem cells) viability. It was observed that prolonged hydrolysis weakens the bonds between polymer chains, leading to a reduction in molecular weight. This decrease in molecular weight through hydrolysis tended to lead to larger pore sizes, a lower elastic modulus, and extended relaxation times.
Water diffusion	Generally negative	The water in the hydrogel system affects the intermolecular hydrogel bonds and entropic variation	Navarro et al. [223] tuned stiffness of the elastin-like protein hydrogel (100–1000 Pa) by modulating its hydrophilicity, achieved by elevating its transition temperature due to its LCST properties. Increased hydrophilicity resulted in higher storage moduli, which promoted the spreading of human mesenchymal stromal cells (hMSCs) and hUVECs, while inhibiting the spreading and neurite outgrowth of human neural progenitor cells.
Crosslinking conditions	crosslinker concentration: positive	Increase crosslink density leading to greater resistance to deformation	Lavrentieva et al. [224] fabricated stiffness gradients (1–1500 Pa) in GelMA hydrogel by varying the crosslinker concentration. Their results showed decreased cellular spreading and migration of human adipose tissue-derived MSCs and hUVECs with increased stiffness, displaying an opposite trend compared to Navarro et al. [223].
	Crosslinker structure: depends	Change the crosslink density and hierarchical structure	Morton et al. [225] compared three conformations of a peptoid crosslinker, all with the same length and sequence: random (0.6 kPa of G'), non-helical (3.2 kPa of G'), and helical (8.0 kPa of G'). The helical structure of the crosslinker likely enhances stiffness by altering chirality and helicity which increases molecular rigidity, and shortening the polymer's end-to-end distances due to its spiral configuration. They found that the softer gel enhanced hMSC proliferation, while the stiffer gel promoted cell spreading and differentiation.
	Physical/chemical crosslinking: depends	Chemical bonds are usually strong, permanent and irreversible compared with physical bonds; while physical bonds	Dodero et al. [226] evaluated chitosan nanofibrous membranes via both physical and chemical crosslinking methods. Physical crosslinking (phosphate ions) yielded smooth and highly homogeneous nanofibers, while chemical crosslinking (PEO) produced rougher and thicker fibres, likely due to the slower chemical reaction in the latter process that causes the fibres to swell. Their results unexpectedly showed that physical crosslinking method nearly doubled the E and tensile strength compared to chemical crosslinking. This may be due to the enhanced homogeneity and more ordered 3D conformation achieved through physical crosslinking, which provides superior mechanical support. Additionally, the physically crosslinked hydrogel demonstrated excellent biological compatibility, promoting cell adhesion and exhibiting low toxicity for L929 fibroblasts, HaCaT human keratinocytes, and Saos-2 osteoblasts.
	External stimuli (Temperature, light, pH, enzyme)	Affect the physical or chemical crosslinking of hydrogels with specific functional groups	Li et al. [227] designed a dynamic hydrogel platform using gelatin functionalized with hydroxyphenyl propionic acid (hpa) and glycidyl methacrylate, which underwent sequential enzyme and

(*continued on next page*)

Table 5 (*continued*)

Factor	Relationship with stiffness	How it affects stiffness	Recent reports on tuned stiffness impacting cell fate
			light crosslinking modifications to achieve further stiffening (threefold increase in G' and compressive modulus). The dynamic hydrogels enhanced cell proliferation, spreading, and volume expansion in long-term culture compared to static, stiff hydrogels. This highlights the importance of an initially soft microenvironment for cell spreading, while the later stiffening of the scaffold is more favourable for MSC osteogenesis.
Incorporation of other hydrogels	Physically crosslinked hybrid hydrogel	Add extra physical bond between different polymers	Dromel et al. [228] reported an *in situ* IPN hydrogel consisting of hpa-functionalized gelatin and tyramine-modified HA, with tenable stiffness (resulting in a 2–5 fold change in G). This hydrogel was designed to support the viability of human retinal ganglion cells (hRGCs) both *in vitro* and *in vivo*. Notably, hRGCs encapsulated in the stiffer hydrogels demonstrated improved attachment to the inner limiting membrane of the retina, potentially promoting optic nerve regeneration.
	Chemical crosslinked hybrid hydrogel	Add extra chemical bond between different polymers	Delplace group [229] proposed utilizing the alkyne-azide SPAAC method to mechanically strengthen HA, increasing its stiffness from 0.5 to 45 kPa with negligible swelling, while achieving rapid gelation within 15 min. The SPAAC-HA promoted L929 fibroblasts viability over 7 days and can be easily modified with polymer or adhesive peptides to meet specific cellular requirements.

8.4. Molecular weight

Generally, increasing the molecular weight (MW) of the polymer in a hydrogel system often leads to increased stiffness. For instance, Browne et al. [257] investigated the mechanical properties of HA based materials with three different MW (60kDA, 500kDA, and 1MDA) and reported that an increase in MW of the HA macromer resulted in a reduction of crosslinking time from 11 to 3 min, with the significant elevation of viscosity (by three orders of magnitude) and G' (from 287 Pa to 1450 Pa). This can be attributed to the formation of longer chain lengths resulting from higher MW, which creates a more extensive network upon crosslinking, which typically enhances the mechanical strength (tensile strength and stiffness) of the hydrogel. However, the physical properties of polymers are influenced not only by MW, but also the overall MW distribution of chain lengths. Increase the dispersity of high and low MW while maintaining the overall MW constant has been demonstrated to increase the viscosity of polymers at low shear frequencies and improve the shear thinning behaviour at high frequencies [258]. Kong et al. [259] used γ-irradiation and oxidation methods to obtain low MW alginate from high MW alginate, then mix the various ratio between low and high molecules to modify stiffness and degradation. They demonstrated that increasing the fraction of low molecular weight (MW) alginate molecules dramatically reduced viscosity. Additionally, the elastic modulus (E) initially rose with the addition of low MW alginate up to a certain value (50 % low MW), after which it fell. Their results with alginate hydrogel in accordance with the literature in polyethylenes bimodal polymers models, the combination of low MW fraction would lead to a higher young's modulus and yield strength with the increase in crystallinity. When the more dominance rising from low MW, the overall reduction of MW started to impact the network [260–262]. This implied creating a bimodal hydrogel network should be able to enhance the mechanical properties.

8.5. Water diffusion

The water diffusion in the hydrogel system contributes to the stiffness of hydrogel in three consequences, the compliance of polymer network due to the reduction of intermolecular hydrogel bonds density by absorbing additional liquid with negligible stiffness, the compliance due to the increase in entropy with extra water molecules in the intermolecular hydrogen bonds, and entropic stiffening caused by the stretching of polymer chain for water molecules [263,264]. The interplay among these three mechanisms dictates the total stiffness of the hydrogel during swelling. Many present studies have established and advanced the mathematic predictions (rubberlike elasticity theory and equilibrium swelling model) for this phenomenon based on foundational works of Flory [265], Treloar [30] and Nissan [264].

Lately, Brighenti et al. [263] derived a microstructurally motivated model to understand hydrogel's stiffness that accounts for water diffusion, dissociation among hydrogen bonds and entropic stiffening. Due to the important relationship between stiffness and water content in the mechanism, adjusting the water content in the system by dehydration or swelling could tune the mechanical properties. Meijer et al. [266] developed hydrophobic moieties (phenyltriazine groups) into hydrophilic system (ureidopyrimidinone) to concurrently maintain the hydrogen bonds and offer hydrophobic associations. Zhang et al. [267] used the similar principle to synthesise the hydrogels with hydrophobic motif (methyl) to achieve the tunability on water content (60–50 %) and mechanical properties (40–160 MPa of E and 1.8–3.7 MPa of tensile stress). Recently, owing to imitate the nature of epidermis with low water content and sufficient mechanic strength, Shen et al. [268] engineered a composite hydrogel network by controlling the ratio of hydrophobic/hydrophilic components to achieve water content below 12 % water and up to 1 MPa of modulus (not specified E or G). These works indicate that adjusting the swelling ratio by introducing the hydrophilic system could effective the mechanical properties.

8.6. Crosslinking

Crosslink density is a critical parameter related to hydrogels' mechanical properties. Lin & Gu [269] utilized computational models of randomly distributed fibres to examine the role of crosslink on Type 1 collagen stiffness. They demonstrated that as the inter-spacing halved and crosslink density doubled, the gel stiffness was improved around 40 times with the same gel concentration. Thus, the modulation of material's mechanical properties could be achieved by controlling crosslinking density. In general, there are two ways to adjust crosslink density, including controlling crosslinking conditions or mixing a second hydrogel. Crosslinking conditions, including different kinds of crosslinker, crosslinker concentration, or external stimulation intensity, etc., affect the kinetics of hydrogel crosslinking. However, some hydrogels are inert to the change of crosslinking conditions, or the maximum stiffness they can achieve is too low to support tissue regeneration. Incorporating another hydrogel that is sensitive to external conditions

and able to provide sufficient mechanical strength, can enable the scaffold to more closely mimic the ECM, in terms of mechanical properties independently of concentration.

8.7. Crosslinker concentration/density

An increase in crosslinker concentration will increase the number of monomers that covalently bind to each other and subsequently reduce pore size [270]. Polyacrylamide provides a versatile platform to tune stiffness by controlling acrylamide content and crosslinker concentration. With constant acrylamide concentration, an increased crosslinker concentration corresponds to a higher crosslinking density. Denisin and Pruitt [271] reported that the stiffness was enhanced with increasing crosslinker density up to an inflection point after which the stiffness started to decrease; this trend was observed in all polyacrylamide gels with constant acrylamide concentration. Other studies [272,273] also reported a functional relationship between polyacrylamide stiffness and crosslinker density. Schoenmakers et al. [274] discovered that this trend is also applicable to fibrous hydrogels. The occurrence of the inflection point can be explained by the transition from an ideal polyacrylamide gelation network to a clustered gel with heterogeneities [271,272].

8.8. Crosslinker structure

As previously mentioned, since the structural conformation of the polymer backbone influences stiffness, it is also possible for changes in the crosslinker structure of a hydrogel to affect crosslinking density and thereby alter hydrogel's stiffness. For instance, the hydroxyl groups on PEG allow for easy functionalization, enabling the creation of crosslinkers with different architectures. De Miguel-Jiménez et al. [275] compared the gelation kinetics of PEG crosslinkers in star (−4SH) or linear (−dithiols) structures with the same thiol density. They demonstrated that the star shape of crosslinking bonds accelerated the polymerization and enhanced the stiffness compared to linear connections since precisely specified conformation provides less dispersities and a more homogeneous structure. Additionally, the Hammond group [276,277] designed N-carboxyanhydride polymerized polypeptides, specifically poly(γ-propargyl L-glutamate) (PPLG) macromonomers, which were crosslinked with 4-arm PEG to form a tuneable 3D hydrogel. The mechanical behaviour of this hydrogel could be controlled by modulating the structure of PPLG, transitioning from a helical to a random coil conformation. Recent work by Morton et al. [225] showed that another crosslinker peptoid in the random, non-helical conformations significantly reduced the stiffness (0.6 kPa and 3.2 kPa of G' respectively) compared to helical conformations (8.0 kPa) under the same length, which agrees the Miguel-Jimenez's results that a hierarchical structure could contribute to hydrogel mechanics. Followed up by Castilla-Casadiego et al. [278] who compared peptoid crosslinkers with helical, non-helical, and unstructured conformations to control stiffness, it was found that soft substrates, when using unstructured crosslinkers, reduced YAP/TAZ activity in hMSCs. This, in turn, led to an increased secretion of immune-modulating factors through the activation of the NF-κB pathway, which may be important for understanding how mechanical cues, such as substrate stiffness, influence immune responses in stem cells.

Stimuli-responsive crosslinkers used in hydrogel formation also can change their conformation in response to external cues. For example, Liu et al. [279] designed a PEG derivates hydrogel using a "click" reaction by incorporating a phototuneable azide-functionalised light, oxygen and voltage-sensitive domain 2 (LOV2) protein crosslinker, enabling a dynamic modulation of hydrogel's stiffness in a spatiotemporal manner. Upon exposure to blue light (470 nm), the LOV2 induced a structural displacement of the Jα domain, resulting in a shortened length and reduced hydrogel's stiffness. Conversely, when returned back to a dark environment, the crosslinker restored its original compact conformation.

8.9. Spatial crosslinking presentation

The spatial presentation of mechanical information is a key parameter for regulating cell behaviours. Gradient hydrogels offer a powerful platform to investigate the effects of spatially varying stiffness [280,281]. For example, Hadden et al. [282] developed a new approach for creating stiffness gradients in hydrogels, by controlling the diffusion of cross-linkers and monomers in pre-polymerized hydrogels. The research achieved a range of hydrogel stiffness gradients from 0.5 to 8.2 kPa/mm, mirroring both physiological and pathological conditions. The significance of this work lies in its potential to enhance the study of mechanobiology, providing a straightforward, cost-effective approach for creating control stiffness gradients in hydrogels.

8.10. Physical crosslinking

The formation of physically crosslinked hydrogels involves molecular entanglements, and/or hydrophobic, electrostatic or hydrogen forces between polymer chains (Fig. 6). The sol–gel state could be reversed because all of these interactions are purely physical, weak and less stable. Although physical crosslinking bonds are fragile and easy to break under deformation and stress, they can dissipate strain energy and achieve a higher toughness than their covalent counterparts [283]. Physically reversible crosslinking also has been proven to induce a self-healing process and possess stimuli-responsive abilities [284]. For instance, gels that use hydrogen bonding between 2-ureido-4-primidone moieties to form a 3-D scaffold also have improved self-healing and shape memory capabilities [285].

8.11. Chemical crosslinking

Hydrogels that rely on chemical crosslinking are polymerized by covalent linkages (Fig. 7). Covalent crosslinks are typically strong, permanent and irreversible, thus leading to high stiffness and elasticity [286]. The formation of covalent bonds could be incorporated with small-crosslinker particles, polymer–polymer conjugation, light-sensitive constituents or by an enzymatic reaction as shown in Table 6. However, based on Lake Thomas theory, a higher chemical crosslinking density will reduce the partial chain length between crosslinks, ultimately weakening the hydrogel's mechanical toughness [287,288].

8.12. External stimuli

External stimuli such as temperature, light or pH can affect the either physical or chemical crosslinking of hydrogels with specific functional groups, eventually resulting in different mechanical behaviours [289].

8.13. Temperature

When exposed to temperature alterations, the solubility of the crosslinked thermos-sensitive network would be affected by the interaction shift between their hydrophilic and hydrophobic components. The resultant sol–gel transition would lead to different mechanical output.

The stiffness of certain polymers, namely low critical solution temperature (LCST) polymers, can be tuned by changes in temperature [290]. Common bioinks that can respond to temperature include Pluronic and its derivatives, PEO or PEG, and polyacrylamide [291,292]. For example, Pluronic F127 will be in the form of single chains at room temperature, while escalating temperature to 37 °C, the micelles inside Pluronic F127 will be packed together to reach sol to gel state and the stiffness will be increased. Another one of the most important thermos-responsive hydrogels is poly(N-isopropylacrylamide) (PNIPAM), due to its well-defined and sharp LCST and biocompatibility. Akimoto et al. [293] applied thermal stimulation (cycling between 33 °C and 37 °C) to

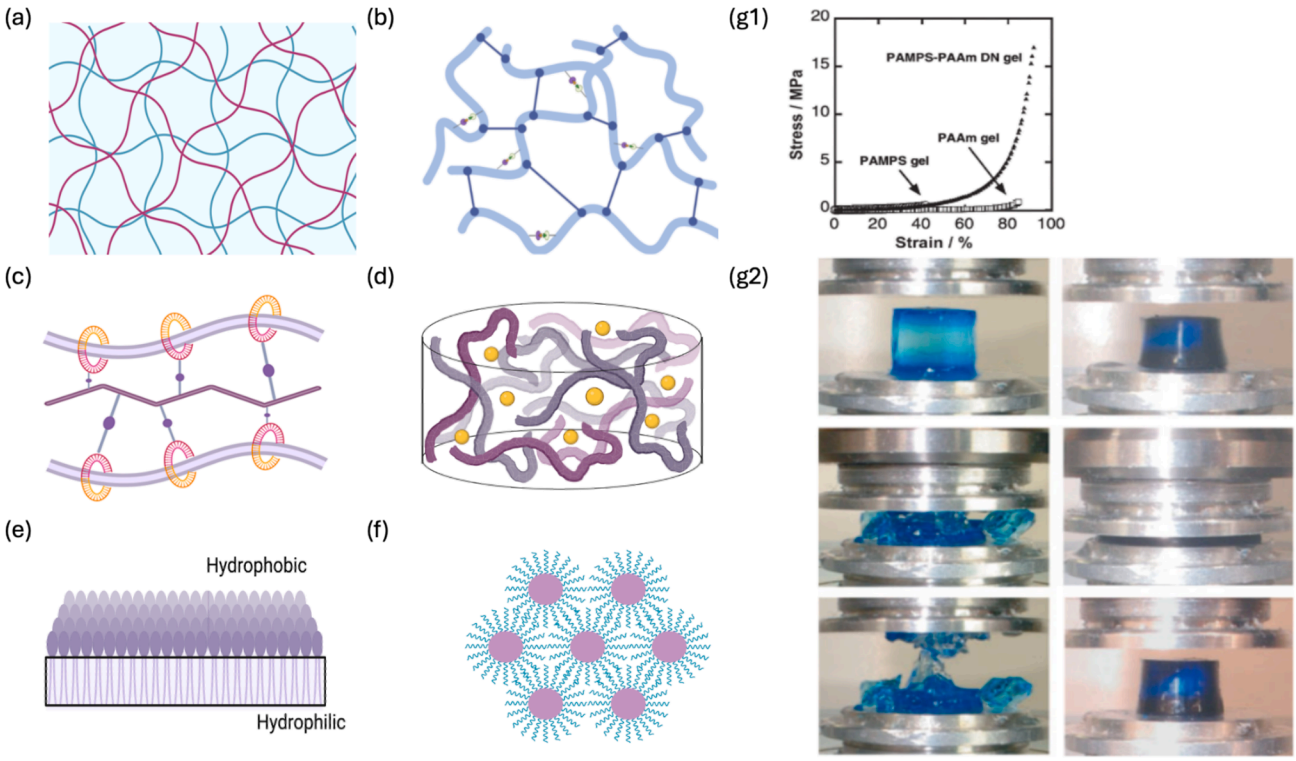

Fig. 6. Physical crosslinking approaches to enhance the stiffness in hybrid hydrogel synthesis. Schematic of physical crosslinking methods including (a) IPN, (b) dual-crosslinked networks, (c) slide-ring, (d) nanocomposite incorporation, (e) hydrophobic modification, and (f) MMC. Created with Biorender.com. DN hydrogels is listed here as a special example of IPN hydrogels as shown in (g1) stress–strain curves for hydrogels under uniaxial compression. DN gel could sustain up to 22 times higher stress (17.2 MPa) than single network gels [315] and (g2) DN structure of PAMPS −PAAm gel can support the integrity and sustain high compression [315].

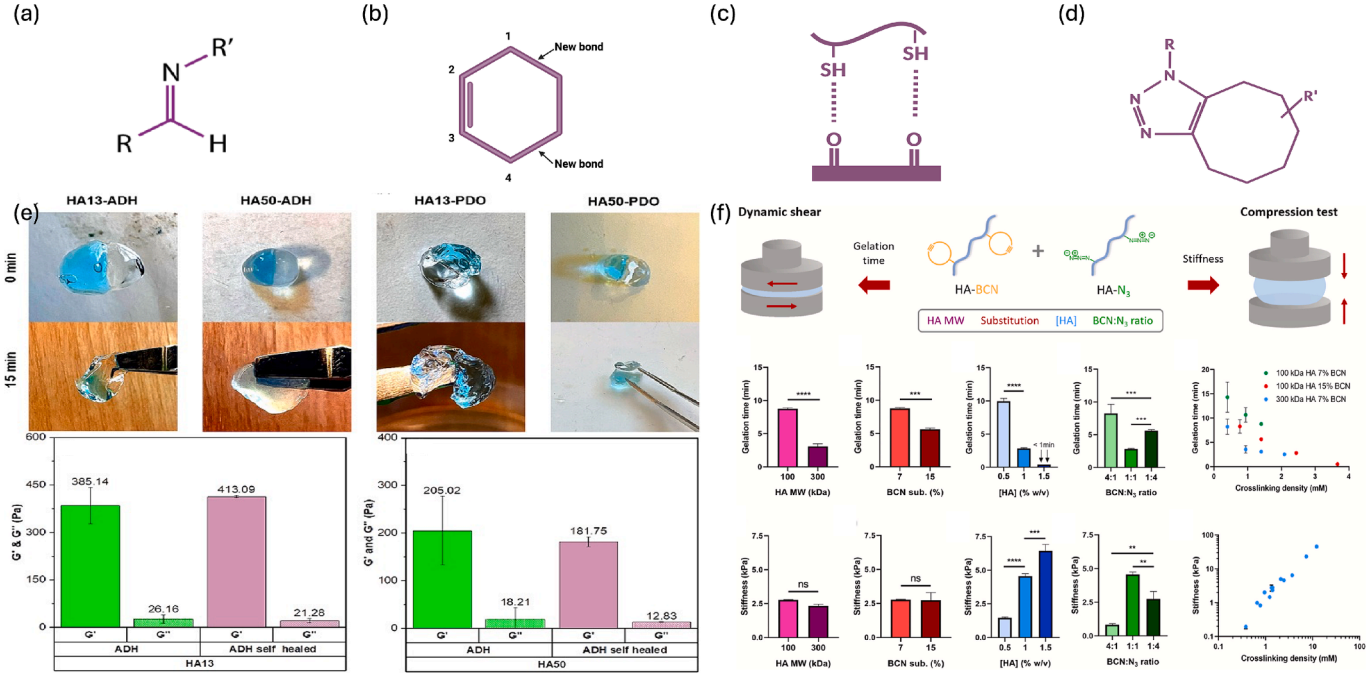

Fig. 7. Chemical crosslinking approaches to enhance the stiffness in hybrid hydrogel synthesis. Schematic of chemical crosslinking methods including (a) Schiff-base, (b) DA, (c) thiol-Michael addition, and (d) SPAAC. Examples of stiff hybrid HA-based hydrogels from(g) Schiff-base bonds and (e) SPAAC reactions. In (e), Visualised comparison of self-healing ability of different HA hybrid gel formed via 13% and 50% degree of substitution (DS) aldehyde-functionalized HA (HA-13 and HA-50), and crosslinked with adipic acid dihydrazide (ADH), and propanediylbishydroxylamine dihydrochloride (PDO) respectively. HA-ADH gels self-recovered in 15 min with better mechanical stability under the compression from the tweezer, conversely HA-PDO gels were not observed self-recovery capacity [414]. In (f), gelation time and stiffness were measured for SPAAC HA-based hydrogels with varying conditions involving molecular weight, DS, concentration and functional group ratio [229].

Table 6
The summary of crosslinking methods.

Crosslinking methods	Interactions between polymers	Applicable hydrogels	Benefits	Limitations	Ref
Physical (non-covalent interaction)	Polymeric interactions involve ionic, hydrophobic, and hydrogen bonds.	Agarose, alginate, chitosan, collagen, gelatin, Matrigel, Pluronic, and self-assembling peptides.	Self-healing and reversibility. Avoid chemical contamination or toxicity. A more liveable microenvironment for biological targets.	Weak mechanical stability.	[310]
Chemical (covalent bonding)	Reactions without external stimulus include condensation reactions, Schiff base formations, Michael addition reactions, azide-alkyne cycloadditions, and Diels-Alder bonding.	Gelatin, collagen, chitosan, and fibrin	Better mechanical stability. The range of modulus can be from MPa to GPa.	Unwanted side-reactions. High possibility of cytotoxicity.	[311]
	The photoinitiators induce the curing of monomers or oligomers when exposed to radiation.	Methacrylated gelatin (GelMA) and PEG	The speed and extent of photo-crosslinking are controllable. The rate and degree of crosslinking by photocrosslinking can be used to modulate the mechanical properties of hydrogels.	DNA damage by long-term UV exposure. Most photoinitiators are toxic and decrease biocompatibility. Strict condition requirements such as UV exposure duration, water solubility and choice of different radiation. Some photoinitiators (especially aromatic compounds) cause yellowing.	[312]
	The transamidation-mediated reaction to form isopeptide bonds is catalysed by $Ca2^{+}$-dependent enzymes, such as transglutaminase and thrombin.	Fibrin	Mild physiological conditions Self-healing. Water retention.	Low yield. Unproductive side reactions. Highly restricted applications.	[313]

modulate the stiffness of a PNIPAM hydrogel from 500 Pa to 580 Pa. They observed that this well-controlled stiffness modulation induced elongation and pseudopodia formation in human bone marrow-derived MSCs in PNIPAM hydrogel, an effect not seen in the control poly(N,N-dimethylacrylamide) hydrogel. In contrast, some other polymers, such as PVA, will be at the gelation state as a result of the formation of hydrogen bonds while the temperature decreases below the boundary upper critical solution temperature (UCST) [294]. These thermo-responsive characteristics allow control over material stiffness by adjusting temperature.

8.14. Light

Light (ultraviolet radiation (UV) or visible) in combination with a photoinitiator is also a promising method of modulating the stiffness of photoreactive polymers with chromophore moieties such as methacrylate or acrylate groups. Annabi et al. [295] developed an *in vivo* sealant by utilising modified human tropoelastin and methacrylic anhydride, which, in conjunction with photoinitiators, can form an elastic hydrogel under UV light. Compared to traditional and commercial sealants, the advancement of controllable mechanical and biodegradable properties from the light-sensitive hydrogel presents promising opportunities for clinical applications.

A higher photoinitiator concentration has been determined to generate numerous shorter polymer chains leading to a material with lower stiffness, while decreasing photoinitiator concentration contributes to forming longer polymer chains with greater molecular weight polymers resulting in a higher stiffness [296,297]. For example, Sheth et al. [297] found that there was a 1.3-fold increase in Young's modulus when the photoinitiator concentration was dropped from 0.5 % to 0.1 % and an additional 1.2-fold rise in Young's modulus when the concentration continued to decline from 0.1 % to 0.01 % under UV light. Interestingly, both Sheth et al. [297] and O'Connell et al. [214] did not report a statistically significant relationship between UV intensity and stiffness, while they both found that extending exposure time under a light source will initially increase stiffness, before reaching a threshold value. Although photocrosslinking is a common method to stiffen hydrogels, exposure to UV light can cause destructive damage to proteins and cell apoptosis, which is an essential consideration for applications for TE [298].

8.15. pH

Hydrogels with ionizable functional groups (i.e. carboxylic acid –COOH, amino –NH2, and sulfonic acid (−SO3H) groups) on the polymer backbone can gain or lose protons in accordance with the variation in environmental pH [299]. This process often comes with the swelling or shrinking of hydrogel via the electrostatic repulsion or attraction between ionized groups. Yoshikawa et al. [300] achieved 40-fold enhancement of E by adjusting the pH from 7 to 8 via binding the pH-responsive moieties poly-(2-(diisopropylamino)ethyl methacrylate to the hydrogel, and the mechanical properties could be easily reversed by reducing the pH back to 7. The stiffening of hydrogel due to the alteration of pH affected the mouse myoblasts (C2C12 cells) to form more stress fibres and flattened morphology. However, the endogenous tissues are far more sensitive, reacting to even minor changes in pH with a similar level of biological reactions [301]. Wang et al. [302] tailored the phenyl acrylate hydrogel with a weak acid group (acrylic acid) to endow it with pH-tuneable properties. Their results revealed that a marginal variation in pH of 0.06 could trigger a profound change in E, leading to a 50-fold softening. This is because a subtle rise in pH could induce more ionization and weaken the hydrogen bonds, ultimately resulting in an exceptionally significant softening effect. Wang's work may inspire the future development of stimuli-responsive hydrogel with a more ECM-level sensitivity.

8.16. Enzyme

Enzymatic crosslinked hydrogels utilize specific enzymes to catalyze covalent crosslinking reactions to rapidly create the 3D structure. This process is highly compatible with cells as it uses endogenous enzymes in the human body and occurs under physiological conditions such as pH 7.4, temperature 37°C, etc., without inducing toxicity [303–305]. The catalytic enzymes that facilitate fast gelling in hydrogel systems with

controlled mechanical properties achieved by adjusting enzymatic activities and concentration, make them promising for generating injectable hydrogels and bioinks [306–308]. However, the use of enzymes also imposes limitations on environmental conditions (including pH, temperature, external forces, and chemical denaturants) to prevent enzyme denaturation, which poses a hurdle to manufacture and leads to high production costs [303,309].

8.17. Incorporation of other hydrogels

Synthetic hydrogels possess controllable mechanical properties for facile synthesis of bioscaffolds [106,314]. However, synthetic hydrogels can struggle to promote intrinsic cellular activities due to inert biological properties and the lack of native fibre structures to adhere to cells. Thus, the hybridization of hydrogels with other polymers, nanoparticles or nanofibers has been developed to overcome the limitations of stiffness and the lack of biofunctionality.

8.18. Physically crosslinked hybrid hydrogels

There are several ways to incorporate mechanically enhanced second hydrogel/hydrogels via physically crosslinking methods, such as interpenetrating polymer networks (IPN), double networks (DN), dual-crosslinked networks, the slide-ring (SR) method, nanocomposite incorporation, hydrophobic modification, and macromolecular microsphere composite (MMC) hydrogels.

8.19. Interpenetrating polymer network (IPN)

Interpenetrating polymer networks (IPNs) contain two or more hydrogels with similar kinetics, at least one of them being crosslinked in the immediate existence of the other without the formation of covalent bonds. IPNs are often regarded as 'alloys' of crosslinked polymers with no apparent layer separation between different polymers, which are stable and irreversible until chemical bonds are disrupted [316,317]. IPN strategy can also generate relatively dense hydrogel networks with enhanced mechanical properties and more controllable physical structures. IPNs can either exist as a fully crosslinked network of polymers or as a semi-network (semi-IPN or pseudo-IPN) where linear polymers are embedded non-covalently within a covalent network.

Da Silva et al. [318] designed a fully crosslinked GelMA, collagen and elastin IPN (full GCE-IPN, the presence of Irgacure 2959 and Genipin as the photoinitiators) and a semi-GCE-IPN hydrogel (the presence of Irgacure 2959 only). The semi-GCE-IPN reached the highest shear storage modulus G' (ca. 9.20 kPa), which was approximately 1.18 times and 1.46 times higher than the single network GelMA polymer (ca. 7.80 kPa) and the full GCE-IPN (ca. 6.32 kPa), respectively. This result indicated that the semi-IPN structures would form native networks with the combination of proteins and develop a more intricate scaffold to mimic the ECM, while the lower G' value for the full IPN hydrogel might be caused by the higher crosslinking density, thus increasing the brittleness and lowering elasticity. In addition to providing a platform to build stiff hydrogels, IPN systems could also be modulated to meet the requirements for some soft tissues. Aprile and Kelly [319] produced two collagen-alginate IPN systems to tune matrix stiffness to achieve the regulation of the chondrogenesis of mesenchymal stem/marrow stromal cells. They controlled crosslinking time from 40 min to 150 min to obtain a soft IPN (5.2 ± 0.7 kPa) and a stiff IPN (17.5 ± 1.8 kPa), which also demonstrated that the soft IPN would be more supportive of the differentiation of marrow stromal cells. In recent years, IPN hydrogels with viscoelastic behaviours have been developed. For example, Sinha et al. [320] combined collagen I with hydrazone-bonded PEG to create an IPN with a tuneable stress relaxation response independently of the change of other conditions such as stiffness. They also demonstrated that the increased viscoelasticity promoted glioblastoma multiforme cell adhesion, proliferation, and migration.

A special type of mechanical improved IPN system, also known as "double networks" (DN), proposed by Gong et al. [315], has been of great interest to researchers due to their tuneable mechanical behaviours in biomaterials application, mainly as implanting materials for injured cartilage. DN hydrogels consist of two networks with contrasting structural properties: the first densely crosslinked polyelectrolyte network and the second neutral and loosely network (see Fig. 6) [321,321]. The first network as a minor component in DN aims to disperse the stress and raise fracture strength, while the second hydrogel plays a major role in hidden length, which could undergo large deformation. There are two key parameters, including the molar ratio of the first to the second network and their crosslinking density, that tune the mechanical properties of the DN hydrogel [321]. Despite that the DN hydrogels comprising of 90 % water under the optimal structure, it is considerably stiff and can serve as a replacement for cartilage, bone and tendon tissue due to their appreciable stiffness (elastic modulus of 0.1–1.0 MPa) and high fracture strength (failure tensile stress 1–10 MPa) [321–324].

Gong et al. [315] initially designed DN hydrogels by a two-step continuous free-radical polymerization to crosslink poly-2-acrylamide-2-methylpropanesulfonic acid (PAMPS) (the first network) and polyacrylamide (PAAm) (the second network). They prepared PAMPS and then soaked PAMPS into the second monomer (acrylamide) solution to crosslink the two networks. Although the conventional method could produce mechanically strong and stiff DN hydrogels, this method requires the first network to be a rigid polyelectrolyte with excellent swelling ability, which restricts the application of general hydrogels that are known for their soft mechanical properties [325]. The "molecular stent" method inserted ionic micelles or linear polyelectrolytes into a neutral DN system, thus maintaining the mechanical properties of DN hydrogels and broadening the possibilities of involving more kinds of gels [326]. Other DN synthesis methods including the void-DN method [327], liquid crystalline method [328], lamellar bilayers method [329] and soaking method [330] have been developed, while these methods still confront the challenges caused by the time-consuming process. Another noticeable DN fabrication method is the one-pot method which can significantly shorten the processing time and enhance DN hydrogels' mechanical behaviours [325,331,332]. Chen et al. [333] synthesized substantially stiff and reversible agar/polyacrylamide (Agar/PAM) DN hydrogels via a one-pot method which achieved a maximum compression stress of 38 MPa (633 times and 10 times mechanically exceed single network agar (0.06 MPa) and PAM (3.8 MPa), respectively). The elastic modulus for Agar/PAM DN gels was 123 kPa (1.46 times and 3.62 times higher than agar (84 kPa) and PAM (34 kPa) gels, respectively), which evidently emphasized that DN structures would far augment the mechanical properties of hydrogels than single network hydrogel.

8.20. Dual-crosslinked network

Although the traditional DN structure endows hydrogels with ultrahigh mechanical strength, they usually do not possess self-recovery and shape memory properties. The coordinate bonds via the attachment of metal ions and the specific ligands are used to prepare hydrogels with high strength, toughness, self-healing and shape memory due to their easy energy dissipation ability and reversible network [334–338]. Ca^{2+} crosslinked hydrogels with recovery abilities can have an elastic modulus of up to 29 kPa and a tensile strength of ca. 160 kPa, but they require one day to recover [339]. A dual-crosslinked hydrogel was reported by Lin et al. [340] composed of poly(acrylamide-co-acrylic acid) with Fe^{3+} coordination bonds acting as the secondary crosslink. The elastic modulus of this dual-crosslinked hydrogel can reach around 17 MPa when the molar ratio increases to 25 %, which is almost 300-fold larger than a single chemical crosslinked network (57.47 ± 4.1 kPa). Other mechanical properties, such as recovery time (4 h at room temperature to 87.6 %) and tensile strength (10 MPa), also indicated the feasibility of this novel method to obtain excellent mechanical

properties, which is achieved as the covalent bonds maintain unbroken under loading to support the hydrogel's integrity, while the ionic Fe^{3+} crosslinking network among the polymer chains fracture to dissipate energy and then reform.

There are many studies that report on the applicability of dual-crosslinking methods to other hydrogel systems [340–343]. Zhong et al. [344] proposed to use the one-pot free radical polymerisation method to rapidly fabricate a poly(acrylic acid) based gel with Fe^{3+} coordination crosslinking, which maintained the superior mechanical properties (fracture stress 0.3–1.38 MPa, storage modulus ca 10 kPa, stretchability above 2000 %) and concurrently alleviate the limitations from traditional Zhou's method to obtain excellent swelling capacity (swelling ratio up to 1800 g g^{-1}) and preserve reasonable water content (70–90 %). Debertrand et al. [345] compared two common fabrication methods to produce dual-crosslink gels with two kinds of metals (Ni^{2+} and Zn^{2+}) ligand coordination bonds. One method is the one-pot synthesis as used in Zhong's study [344], which is facile and time-saving. Another method involves first fabricating the chemically covalent hydrogel network in water and subsequently diffusing the secondary crosslinkers into the system. The storage moduli and loss moduli of two poly(acrylamide-co-1-vinylimidazole) P(AAm-co-VIm)-Ni^{2+} and P (AAm-co-VIm)-Zn^{2+} dual-crosslink gels could arrive at the level of 10 MPa, due to the existence of the dually crosslinking system. At the same frequency, P(AAm-co-VIm)-Ni^{2+} gels had slightly larger G' and G'' than P(AAm-co-VIm)-Zn^{2+} gels, while the difference in the values of the gels moduli in the same metal incorporated dual-crosslinked gels was small, indicating that the stiffness could be tuned via the incorporation of different metals, rather than fabrication method.

Apart from ionic coordination bonding, other noncovalent interactions can also be applied as a secondary crosslinking network in preparing mechanically reinforced hydrogels. For example, Chang et al. [346] reported poly(ureidopyrimidone methacrylate-co-stearyl acrylate-co-acrylic acid) [P(UPyMA-co-SA-co-AA)] hydrogels with the hydrophobic interactions between alkyl chains of SA and the quadruple hydrogen bonds between UPy motifs as a dually crosslinked network. The Young's modulus of this gel with different compositions ranged from 0.14 ± 0.01 to 10.01 ± 0.50 MPa, which increased with the molar ratio of UPyMA and SA responding to the increase of crosslinking density. Potiwiput et al. [347] introduced carboxymethyl chitosan (CMC) into the alginate-Ca^{2+} system to develop dual crosslinked alginate/CMC-based gels by ionic coordination bonding between alginate and Ca^{2+} ions and electrostatic crosslinking between the amine groups on CMC and the carboxyl group on alginate. The dual-crosslinked gels with different concentrations (0.5 %, 1 % and 2 % w/v) of CMC had a higher storage modulus than alginate-based hydrogels at the same angular frequency. The 1 % dual-crosslinked alginate/CMC had the maximum storage modulus above 5 kPa, larger than the values (both ca. 3 kPa) of 0.5 % and 2 % gels. The rheological results confirmed that instead of the maximum level, the equilibrium level of crosslinking density in the hydrogel system was more crucial to gaining optimal and peak viscoelasticity.

8.21. Slide-ring (SR)

Slide-ring (SR) crosslinked hydrogels are characterized by mobile polymer chains capable of sliding relative to one another within the network. Ito et al. [348,349] initially created SR gels by crosslinking polyrotaxane (PR) which consisted of multiple α-cyclodextrin (α-CD) molecules on the linear PEG. The crosslinking junctions can flexibly travel along the polymer chains between neighbouring CDs, while some uncrosslinked CD molecules still exist in the network. This specific structure contributes to distinctive mechanical properties, including weak stiffness and superior toughness, which is due to the molecular network of entropic elasticity [350,351]. Liu et al. [352] synthesized 1) three types of SR gels from polyrotaxane which comprises CD and different molecular weights of axial PEG (35 k, 60 k, and 100 k), and 2) fixed crosslinked (FC) gels consisting of pullulan with similar molecular weight as SR 35 k gel. Their rheological results showed that different molecular weights did not change the elastic modulus of SR gels (~15 kPa), while crosslinking density governed the stiffness of both SR gels and FC gels. Additionally, SR gels have a higher toughness but lower stiffness than FC gels, which is caused by the flexible movement of SR gels.

Preliminary SR gels were very soft (from 20 pa to few tens of kPa) compared to some hydrogels used for cartilage and bone replacement (MPa) [283,351–354]. Jiang et al. [355] innovated a way of fabricating SR gels with low CDs (2 % coverage) via a one-pot strategy which could stimulate the enzymatic end-capping reaction and then offer SR gels with high stretchability (strain 110 %-1600 % with the stress of ca. 1 MPa), improved stiffness (elastic modulus 20–50 kPa) and excellent toughness. Ito group [356] attempted to mechanically strengthen the SR gels by crosslinking divinyl sulfone (DVS) with CDs to reinforce their stiffness up to 130 kPa, which was higher than referenced fixed gels (110 kPa). Zheng et al. [357] were inspired by the dual-crosslink method and combined SR polymers with a rigid carboxyl-Fe^{3+} coordination polymer to construct a dually crosslinked SR gel with tuneable stiffness. This advanced SR gel achieved a broad elastic modulus range from 9.5 kPa to 8.3 MPa due to the synergistic effect of two crosslinks and maintained remarkable recovery properties. However, the process of increasing stiffness was accompanied by the water content loss from 82.7 % to 55.4 %. Although Zheng et al. did not test cell compatibility test on this SR gel, the discovery of the water content loss from 82.7 % to 55.4 % due to the increased stiffness may affect encapsulated cells' health since animal cells prefer to stay in an isotonic environment.

8.22. Nanocomposite hydrogels

Nanocomposite hydrogels are a particular class of hybrid hydrogels that modify the hydrogel with specific reinforced properties at the nanoscale (<100 nm). The nanoparticles with high surface-to-volume scattered within the crosslinked networks of the hydrogel can introduce specific properties such as electronic conductivity and magnetic sensitiveness and more importantly, provide pure hydrogels with reinforced mechanical performance [358–362]. The nanocomposite hydrogels can be modified to acquire exceptional properties due to synergistic effects from nanofillers and develop biofunctional tissue implants. The four most common types of nanofillers are organic nanoparticles (carbon nanotubes or CNTs, graphene oxide or GO, reduced graphene oxide or rGO, and nanodiamonds), inorganic nanoparticles (ceramic, hydroxyapatite, clay, calcium phosphate, silicates, metal and metal oxide), and polymeric nanoparticles (liposomes, dendrimers, polylactic-coglycolic acid and polycaprolactone) [363–365]. For instance, Liu et al. [366] reported that the cofacial alignment of negatively charged unilamellar titanate nanosheets could maximize the electrostatic repulsion and result in a structured ordering of the sheets, which gives rise to the anisotropic mechanical properties of the hydrogel system. The nanosheets within the hydrogel could act as a reinforced structure to resist compressive forces applied orthogonally, while enhancing flexibility when shear forces are applied parallel to the nanosheet alignment.

Additionally, graphene groups as the nanofiller have been compelling due to their superior stiffness (2.4 ± 0.4 TPa Young's modulus for single graphene and 2.0 ± 0.5 TPa Young's modulus for bilayer graphene), fracture strength (ca. 125 GPa for single graphene), thermal conductivity (ca. 5 kW/(m·k) for single graphene) and specific surface area (ca. 2630 m^2/g for single graphene sheet) [367,368]. Instead of graphene, GO is favoured due to its lower agglomeration tendency and better solubility [363]. Pereira et al. [369] loaded different concentrations of GO into poly(2-hydroxyethyl methacrylate) (pHEMA) to obtain the 8.3-fold and 3.1-fold increase in the stiffness (Young's modulus 6.5 MPa for 5 % GO, 2.1 MPa for 0.1 % GO, respectively) compared to pure pHEMA hydrogels (0.74 MPa). The tensile strength was also enhanced to 1.14 MPa, 7.4-fold higher than pure pHEMA.

Two basic principles to fabricate nanocomposite hydrogels include 1) the physical mixture method, in which nanoparticles are simply dispersed in the gel before gelation; and 2) the conjugated composite method, in which the nanoparticles serve as a crosslinker to covalently bind with the polymeric matrix and straight adjust the mechanical properties [370–374]. Kouwer group [375,376] demonstrated that the physically crosslinked method had negligible effects on the hydrogel stiffness, especially for fibrous hydrogels, due to unstable physical interactions. Thus, a recent trend is to discover a feasible method to tune the mechanical properties of nanocomposite hydrogels independently of the polymer concentration.

Chen and Kouwer [377] demonstrated that the incorporation of iron oxide nanoparticles in the poly-isocyanide (PIC)-based hydrogel could increase stiffness (shear storage modulus) from 20 pa to 200 pa when changing the state of nanocomposite from physical mixture to covalent crosslinking. The maximum stiffness could achieve 1 kPa when tuning the crosslinking density corresponded by adjusting the linker concentration on the nanoparticles and meanwhile, the nanoparticles and polymers at the minimal concentration (both 1 % wt). Additionally, Jaiswal et al. [378] also reported that with the constant concentration of collagen-based polymers and nanoparticles (5 μg/mL), controlling the size of Fe_3O_4 nanoparticles (4, 8, 12 nm) could be an effective approach to tuning the stiffness from 0.2 to 200 kPa. Another uniquely inorganic nanoparticle, namely hydroxyapatite (HAp), would enhance the mechanical properties of the hydrogels via constructing the secondary hydrogen bonds without altering the polymeric matrix structural integrity, the swelling ratio, and more importantly, sustain high cell viability and growth [363,379,380]. Sadat-Shojai et al. [381] showed that increasing the concentration of HAp from 0.1 to 0.5 mg/mL causes the increase in stiffness of nanocomposite hydrogels from ~ 20 to 28 kPa, which is higher than bare hydrogel (gelatin, 14 kPa). Meanwhile, their SEM results showed that the higher concentration only decreased average pore size marginally from 50 ± 15 μm for pure gelatin to 41 ± 8 μm and 42 ± 10 μm for 0.1 mg/mL HAp and 0.5 mg/mL HAp respectively, which also proved that HAp particles cause no influence on the structural integrity.

8.23. Hydrophobic modification

Hydrophobic interactions are one of the most critical noncovalent gelation techniques and have been exploited to tune the mechanical properties of synthetic hydrogels via manipulating the configuration, interaction site or density of the hydrophobic group [382]. The hydrophobic side chains within the hydrophilic polymeric matrix can aggregate together to form a hydrophobic micro-domain, and a bunch of these micro-domains function as physical crosslinking points in the network to efficiently dissipate energy and consequently ameliorate the mechanical properties [383–385]. Sufficient surfactants are required to solubilize the hydrophobic monomers in aqueous media (sodium dodecyl sulfate or SDS solution) during the process of polymerization [386,387]. These surfactants would cause undesirable impacts, such as toxicity, chain transfer reactions and complicated post-treatment procedures, however the lack of surfactants will result in disrupting the structure of the hydrogels and making them soft and brittle [387].

A surfactant monomer (surfmer) has been designed to resolve the issues, such as associative properties and solubility caused by the conventional surfactant [386]. Surfmers were improved to maintain the original function as surfactants and form a micelle to copolymerize with hydrophobic monomers with covalent interactions. Gao et al. [386] first prepared sodium 2-acrylamido-dodecane sulfonate ($NaAMC_{12}S$) as a surfmer to a hydrophobic monomer N-dodecylacrylamide and the hydrophilic backbone polyacrylamide to form a more stable hydrogel. Later, other surfmers such as alkylphenol polyoxyethylene ethers (OP-4 or OP-10) and 9 or 10-acrylamidostearic acid (NaAAS) were also introduced [387]. Jiang et al. [388] manifested that the mechanical properties of hydrophobic association hydrogels including elastic modulus (1.34–6.55 kPa), tensile strength (62.00–212.79 kPa), fracture strain (982.01–1828.36 %) responded to the change of composition of hydrophilic acrylamide, hydrophobic octylphenol polyoxyethylene acrylate and the SDS.

Despite that the incorporation of hydrophobic association into the polymeric network improves the mechanical behaviours, the improvement cannot satisfy the need for specific hard TE. Thus, hydrophobic association hydrogels are usually combined with latex particles [389–391], nanoparticles [392,393], electrostatic interactions [394], double network structure [395–397] or hybrid crosslinking [310,398,399] to achieve the targeted mechanical properties. For example, Qin et al. [400] fabricated an acrylamide-acrylic acid-octadecyl methacrylate hybrid hydrogel through the conventional micelle copolymerization processes and meantime introduced the second crosslinking network by adding Fe^{3+} to form a coordination bond with the carbonyl group. They evaluated this double crosslinked hydrogel was observed to have an elastic modulus of 8.0 MPa, and mechanic strength up to 6.8 MPa. Zhang et al. [401] proposed semi-crystalline poly(ε-caprolactone) hydrogels with hydrophobically associative interactions (N,N-dimethylacrylamide and 2-methoxyethyl acrylate) and heterogeneously thermoresponsive polymer network, which exhibited an excellent compressive elastic modulus of 1.76 MPa and mechanic strength of 7.57 MPa.

8.24. Macromolecular microsphere composite (MMC) hydrogels

The macromolecular microsphere composite (MMC) technique has become a novel hydrogel synthesis method to construct a well-defined polymeric structure with superior mechanical strength [402]. The MMC hydrogels are also named microgels (sizes from 100 μm to 100 nm) or nanogels (sizes below 100 nm). Wang's group [403–405] first developed MMC hydrogels by using a peroxidized microsphere (MMS) that serves as a multifunctional initiator and a crosslinker. In the fabrication process, ^{60}Co γ-rays irradiated MSSs induce peroxidization which distributes peroxy groups onto the MMS surface and then a peroxidized MMS can serve as a multifunctional initiator and a crosslinker. The subsequent step is the grafting polymerization in which the initiated MMSs covalently interact with grafted polymer chains. The evenly distributed uniform polymer chains could efficiently dissipate the energy from the applied stress, resulting in reinforced mechanical properties of the MMC gels. The MMC hydrogel with Poly(N-isopropylacrylamide) (PNIPAAm) made by Wang's group [404] exhibited excellent tensile stress (2.0–8.0 MPa) and elastic modulus (0.2 to 1.0 MPa) which are controlled by compositions and temperature. Huang et al. [403] prepared poly(acrylic acid)-MMC hydrogels and compared them with normally structured poly(acrylic acid). Their study showed that the normal hydrogel (water content 87. 5 wt%) fractured at a stress of 0.08 MPa and tensile strain of 45.5 %. In contrast, the MMC hydrogel (water content 89 %) kept the structure integrity even at high compression stress up to 10.2 MPa and a corresponding strain of 97.9 %, thus manifesting that the MMC structure could contribute to enhancing the mechanical strength compared to the normal structure.

Unlike most other reinforcement methods, MMC could also employ natural hydrogels to enhance their mechanical properties and biocompatibility. Duan et al. [406] developed a novel electronic skin biomaterial with self-wrinkled surface properties using chitosan microspheres *in situ* polymerized with aniline and acrylamide. The microsphere-structured hydrogels showed above 30-fold higher tensile strength compared to pure chitosan or polyacrylamide and the maximum fracture stress and strain of 0.879 MPa and 626 % for tensile test, and 10.02 MPa and 90 % for the compression test, respectively. More detailed rheological and mechanical measurements on chitosan microspheres functionalized with poly(acrylamideco-1-benzyl-3-vinylimidazolium bromide) were recently conducted by Zhang et al. [407]. They fabricated the MMC hydrogels through in situ copolymerisations of acrylamide and cucurbit-uril (CB) monomers. The results indicated that the

storage modulus of the microsphere was approximately 3.10 kPa, which was almost five times higher than that of any previously published CB-based hydrogels. Moreover, it was noted that multiplying the content of chitosan microspheres (0–1.9 g) in the hydrogel increased the elastic modulus (no exact data provided), tensile fracture strain (720 % to 1300 %) and fracture stress (90 to 490 kPa). Other MMC hydrogels such as starch-based [408–410] and gelatin-based [411–413] also showed outstanding mechanical properties compared to normally structured hydrogels, mainly because the microspheres served as the physical crosslinkers to fast dissipate the energy caused by tensile stress.

8.25. Chemical crosslinking modification

The introduction of additional crosslinking in the hydrogel system through the formation of chemical linkages via Schiff-base, Diels-Alder (DA), Thiol-based Michael addition, strain-promoted azide-alkyne cycloaddition (SPAAC) reactions can help regulate the mechanical properties of hydrogel.

8.26. Schiff-base crosslinking

The basic principle of incorporating Schiff-base linkages into hydrogels is to modify hydrogels with functional amine (–NH_2) and carbonyl (–CO-) groups and then covalently crosslink hydrogels via forming the dynamic imine bonds (–C=N–) which is biodegradable through control of pH and hydrolysis [179]. Aldehyde groups can be formed using $NaIO_4$-mediated oxidative cleavage of vicinal diols; the aldehyde groups can then be reacted with amino groups on another polymer to form the Schiff base linkages [415]. Due to the mild conditions, short reaction time, low cytotoxicity and biodegradability, the Schiff base reactions are one of the most commonly applied approaches to chemically crosslink hydrogels [416–419]. Wei et al. [420] bonded carboxyethyl-modified chitosan via using acrylic acid and periodate-oxidized sodium alginate together via the Schiff base reaction. The crosslinked chitosan-alginate had a storage modulus from 77.9 ± 3.9 Pa to 1961 ± 178 Pa with the increased concentration of modified chitosan from 0.01 to 0.03 g/ml, which met the stiffness of brain tissues (0.1–1 kPa). Another study accomplished by Liu et al. [421] showed that the dynamic imine groups formed by oxidized debranched starch and chitosan could reinforce the stiffness (storage modulus) to arrive the maximum value of 4.9 kPa at 50 °C at the amino-to-aldehyde molar ratio of 1.4, which is much higher than that of other chitosan-based hydrogels, especially another Schiff base chitosan-cellulose (maximum 0.5 kPa) [422].

Additionally, aromatic Schiff base bondings are ideal because of their ability to sustain the dynamic functions and boost the mechanical properties of hydrogels, thus being preferred over aliphatic Schiff base bondings [423,424]. For instance, Zhang et al. [425] demonstrated that the stiffness of the aromatic Schiff base crosslinked chitosan-PEG (benzaldehyde groups modified) was tuneable via controlling the ratio of functional groups CHO/NH_2 and the maximum stiffness (storage modulus) was around 20 kPa when CHO/NH_2 was 0.72 even the mass content was comparatively low (5.8 %). Meanwhile, the gelation time in this study was within 1 min, which indicates that the Schiff base modification is a facile and fast crosslinking approach to refine the mechanical properties. However, the largest barrier against the development of Schiff modification hydrogels is that the Schiff base bonds are prone to hydrolyse under acidic conditions, while the inflamed tissues are usually mildly acidic during wound healing process which restricts the use of Schiff gels for *in vivo* studies and clinical applications [179,426,427].

8.27. Diels-Alder (DA) crosslinking

The Diels-Alder (DA) reaction is a common 'click' chemistry method to fabricate chemically crosslinked hydrogels via the [4 + 2] cycloaddition between conjugated dienes and dienophiles with outstanding characteristics including high yields and near-zero by-products [428–433]. The DA system can arrive at a chemical equilibrium due to its dynamically thermoresponsive properties, where the DA linkages can occur under 90 °C while being cleaved with a higher temperature [434,435]. Recently, the most commonly used diene/dienophile linkages in hydrogels are produced through furan (and its derivatives, i.e., furfural)/maleimide modifications. A remarkably tough polymeric platform was fabricated by using neopentyl glycol diglycidyl ether (NGDE) and furfurylamine (FA) based on DA reactions [436]. The rheological study demonstrated the superior tunability of stiffness (elastic modulus) from 8.4 MPa to 1.2 Gpa with roughly three orders of magnitude increase and excellent tensile strength from 3 to 30 Mpa by controlling the mole ratio of maleimide/furan groups.

González et al. [437] developed another dual-crosslinking hydrogel system consisting of DA-crosslinked furan-starch derivative/maleimide-PEG with reinforced nanoparticles (cellulose nanocrystals). Due to the stable DA linkages, the storage moduli for all hydrogels were over 1 kPa. It is also worth noting that the integration of nanoparticles enhanced the G' from 1.38 kPa (0 wt% cellulose nanocrystals) to 2.01 kPa (5 wt% cellulose nanocrystals), as the nanoparticles formed hydrogen bonding with macromolecules into the polymeric matrix. One of the limitations that restricts normal DA based hydrogels in the field of cytobiology is the over-extended gelation time [415].

To accelerate the reaction rate of Diels-Alder (DA) cycloaddition, catalyst-independent inverse electron-demand DA (IEEDA) reactions were developed using an electron-deficient diene (tetrazine, Tz) and an electron-rich dienophile (norbornene, Nb). Koshy et al. [438] combined gelatin with Tz and Nb groups to create IEEDA-crosslinked gelatin within a few minutes. They demonstrated that the mechanical properties of IEEDA-crosslinked gelatin could be adjusted by varying the polymer concentration and the Tz:Nb ratio in the system. The maximum storage modulus (G') was observed at a Tz:Nb ratio of 1, reaching over 4 kPa for a 10 % w/v polymer concentration and below 400 Pa for a 5 % w/v polymer concentration. No significant difference in 3 T3 cell proliferation was observed between the 5 % and 10 % gels; however, cell viability was notably higher compared to GelMA at the same concentration. Despite these advancements, the traditional Tz group in IEDDA reactions unexpectedly degrades under physiological conditions, affecting the swelling and stiffness of the hydrogel [439]. To address this issue, Delplace et al. [440] substituted methylphenyltetrazine (MeTz) into the system. The phenyl group in MeTz allows MeTz-Nb HA to form a stable gel with minimal swelling within minutes, supporting cell viability for over 7 days. To control the degradation in IEDDA reactions, Dimmitt et al. [441] restructured the Nb group using carbic anhydride, resulting in accelerated and easily adjustable IEDDA PEG gels. These gels achieve a higher elastic modulus with a lower macromer concentration compared to thiol-Nb crosslinking gels.

8.28. Thiol-based Michael addition crosslinking

The Michael addition reactions are a facile system to crosslink hydrogels via the conjugate (1,4) addition that forms the linkages between a nucleophile (mostly a thiol group) and an electron withdrawing functional group (commonly acrylate, methacrylate, vinyl sulfone, or maleimide groups) [442,443]. The tuneable stiffness of thiol-related hydrogels could be achieved by manipulating the gelling conditions such as temperature, pH or concentration. Liu et al. [444] adjusted the stiffness (elastic modulus) of methacrylate modified dextran crosslinked with dithiothreitol from 10.9 ± 1.8 kPa to 29.6 ± 3.7 kPa by increasing the pH from 7.0 to 7.8, indicating that higher pH could boost the reaction rate between thiol functional pairs and form a more tightly combined polymeric matrix. Another study demonstrated that controlling the concentration of hyaluronic acid (3–5 %) and crosslinking densities (0.25–0.5) could adjust the stiffness of thiol-crosslinked hydrogels from 177.1 ± 0.5 to the maximum value of 1920.0 ± 12.1 Pa [445]. Godesky

and Shreiber [446] investigated the mechanical properties of thiol-modified hyaluronic acid (HA) crosslinked diacrylate PEG (PEGDA). In this work, the rheological results exhibited that the first gelation could be finished in one day and the stiffness is statistically significant linear to PEGDA concentration, as a result of the level of thiol-acrylate polymerization. However, the remaining thiol groups on hyaluronic acid could be oxidized over time and form disulfide bonds that stiffen the hydrogels [443,447,448]. Their results [446] showed that the storage modulus reached the maximum value of approximately 1200 Pa, 1000 Pa, and 700 Pa for 1.0 % HA + 0.2 %PEGDA, 0.8 % HA + 0.6 %PEGDA, and 0.8 % HA + 0.2 %PEGDA, respectively after 30 days, which also suggests that the disulfide oxidation of thiol groups on hyaluronic acid plays a critical role in the level of stiffness over a more extended period.

As one of the common Michael addition reactions, Thiol-maleimide linkage possesses the highest crosslinking efficiency, resulting in hydrogels with a wide range of stiffness [443,449,450]. Jansen et al. [451] investigated the elevated content of maleimide-PEG (3–20 wt%) which changed the elastic modulus of thiol-maleimide PEG hydrogels from around 1 to 4 kPa. Thiol-ene crosslinking causes rapid gelation however, these polymeric systems can also be heterogeneous since there is less polymerization time to form a homogenous gel than individually mixed components [452]. There are several methods to increase the homogeneity of thiol-maleimide gels, including managing the reaction temperature, pH, and polymer content to decelerate the reaction speed/kinetics. Guo et al. [453] proposed adding short peptides into the hydrogel system was less cytotoxic for cell encapsulation. They crosslinked maleimide-PEG and thiol-PEG with different peptides and found that the existence of phenylalanine-arginine-glycine (FRG) could most greatly raise the stiffness from 95.1 kPa (pure thiol-maleimide PEG hydrogels) to 108.9 kPa because of the effective crosslinking of thiol and maleimide and the mechanical homogeneity.

8.29. Strain-promoted azide-alkyne cycloaddition (SPAAC)

Modifying hydrogels with azide-alkynes click reactions is a current trend in chemical biology due to its bioorthogonal and cytocompatible reactivity. Sharpless et al. [454,455] addressed the temperature and pressure limitations of the original Huisgen [3 + 2] azid-alkyne cycloaddition [456] by introducing the copper(I) catalyst into the reaction, yet this catalyst is cytotoxic. Bertozzi and colleagues [457] then developed an alternative catalyst-free approach known as strain-promoted azide-alkyne cycloaddition (SPAAC). SPAAC was inspired by Wittig and Krebs' work [458] on cyclooctyne and phenyl azide which accelerates the reaction through the ring strain with 18 kcal/mol energy stored from bond angle deformation under physiological conditions. In addition to the aforementioned application of SPAAC in PEG hydrogel with an excellent spatiotemporal controlled mechanics described by DeForest et al. [279], Anseth et al. [459] also designed a PEG hydrogel incorporating azide groups and dibenzocyclooctyne (DBCO) instead of simple alkynes. The excess DBCO groups in their hydrogel construct enable photocrosslinking, allowing the stiffness to be adjusted in situ from 2 to 32 kPa of E'. The stiffening mechanics spectrum of DBCO-

Box 1
. The methods to tune other mechanical properties.

Viscosity

Various cell responses to alteration in hydrogel viscosity, such as adhesion, spreading and differentiation have been investigated [461–464]. Shear thinning, the reduction of viscosity under elevated shear stress, is a key characteristic of hydrogels used in bioprinting and *in vivo* injections. Shear-thinning hydrogels typically rely on physical bonds or dynamic covalent interactions, which can be broken under shear stress, allowing the gels to be extruded. Once shear is reduced, these interactions quickly reform, enabling the gel to recover its structure. Uman et al. [465] reviewed the interactions that contribute to shear-thinning, including physical bonds (e.g., hydrogen bonds, nanocomposite-based systems, guest–host interactions, metal–ligand coordination, and biorecognition motifs) as well as dynamic covalent chemistry (e.g., reversible DA reactions and Schiff base formation). For instance, Loebel et al. [466] modified HA with CD or adamantante to enhance shear thinning via physical host–guest association, and introduced secondary photo-crosslinking mechanism, methacrylated HA to support mechanical strength.

Viscoelasticity

Hydrogels show viscoelastic behaviour at high frequencies due to their water content. But only the hydrogels with predominantly non-covalent crosslinking display viscoelasticity within physiological frequency ranges (0.01–10 Hz), since their weak bonds allow for more liquid-like behaviour. For fully covalently crosslinked hydrogels, introducing a secondary non-covalent network (IPN, SR, or hydrophobic association) [467–469], incorporating entangled polymers [470,471], or adding loose ends [471] can modify their viscoelastic properties. In hydrogels with weak bonds, tuning viscoelasticity involves varying the ratio of weak to covalent bonds [472], adjusting the affinity of weak bonds [473], and incorporating inert molecular spacers (such as small PEG molecules into alginate [71]). Recently, Liu et al. [474] proposed using 3,4-dihydroxybenzaldehyde (DB) to create dynamic bonds with gelatin and GelMA, enabling adjustable viscoelasticity by independently controlling the storage modulus (G') and loss modulus (G''). DB, a small molecule, diffuses easily into the network; this, promotes rapid formation and breaking of noncovalent bonds, resulting in a viscous gel with G'' greater than G'. The viscoelasticity, influenced by the density of covalent crosslinks and the number of dynamic bonds, can be adjusted simply by varying the concentration of each component in this double-crosslinked system.

Strength and toughness

Strength refers to a hydrogel's ability to resist permanent deformation or failure, whereas toughness measures the total amount of absorbed energy before the breakage. Often there is a trade-off between strength and toughness, where increased strength usually leads to reduced deformability and increased brittleness, resulting in lower toughness. To enhance the toughness of hydrogels, it is crucial to increase the number of sacrificial bonds in the network, facilitating energy dissipation in the network. One approach to modifying a single-network hydrogel involves introducing physical interactions, such as hydrogen bonds and ionic interactions, into the system [475–477]. An alternative strategy is to form a dual crosslinking network that combines covalent crosslinking, which provides stiffness and structural stability, with non-covalent crosslinking, which acts as the energy dissipation mechanism to improve toughness [478,479]. For example, Zheng et al. [480] developed a dual physical crosslinking network hydrogel composed of hydrophobically crosslinked polyacrylamide (PAM) and iron-crosslinked sodium alginate, featuring high levels of stiffness, strength, and toughness, along with excellent self-repairing and recovery capabilities. Their results showed that the amount of iron in the double network positively affects stiffness, brittleness, and self-healing, as it can influence the degree of crosslinking and freely diffuse into the system, subsequently reforming breaks into a complete network. Conversely, a higher concentration of sodium alginate impairs self-healing by restricting the movement and hydrophobic interactions of the PAM component.

modified PEG in response to the range of moduli (from 5 to 22.1 kPa of E') measured from skeletal muscle 28 days post injury promoted C2C12 proliferation and myofiber hypertrophy. A recent study by Lagneau et al. [229] demonstrated a single-step method for synthesising HA only hydrogels via SPAAC from bicyclononyne (BCN) and azide groups. This approach yielded a broad spectrum of stiffness (0.5–45 kPa) with a long stability and minimal swelling. They further improved cell viability and adhesion by mixing azide-modified RGD peptides with the HA-BCN. Despite challenges such as the difficulty of synthesising cyclooctyne and potential regioisomeric distribution of triazoles [460], SPAAC remains a promising method for developing crosslinked bioorthogonal hydrogels with ambient stiffness and precise spatiotemporal control.

9. Conclusion and future Perspectives

Engineers continue to discover and improve methods of precisely controlling biological responses at micro/nanoscale by using ECM-mimic materials. Hydrogels are used to mimic the complexity and intricacy of the native ECM to support cell behaviour and fate. Since resident cells in different tissues can respond in their own way to mechanical cues from the living environment, the stiffness of hydrogel matrices has been studied in depth. It has been established that substrate stiffness is closely linked with cellular responses through membrane receptors, actin cytoskeleton networks, to the nucleus [481]. A comprehensive grasp of the complex relationship between matrix stiffness and cellular responses is essential for developing suitable hydrogels for TE applications. Considering and synthesizing hydrogels with optimal substrate stiffness for the desired cells can substantially improve tissue regeneration.

There is considerable scope for the systematic evaluation of engineered tissue performance using materials with varying moduli. However, a significant challenge lies in the disparity of modulus values determined by different measurement techniques, which complicate the comparisons of stiffness. Therefore, it is essential to establish community-driven standards for the measurement and analysis of stiffness across macro, micro and nanoscales. Such standards would not only address the complexities associated with the complexity with comparing current systems, but also provide a unified framework for evaluating mechanical properties, enhance reproducibility, and streamline the regulatory approval process, thereby fostering the efficient translation of engineered tissues into clinical applications.

Despite the fact that soft bioscaffolds (modulus up to kPa) can improve biological response even to reconstructed stiff tissue, problems in reaching the required optimal substrate stiffness remain to be resolved. Given that most hydrogels are deficient in mechanical strength, recent studies have contributed to our ability to tune substrate stiffness through adjusting concentrations or crosslinking, combining fillers, or adjusting chemical configurations at the nanoscale. But tuning the stiffness of hydrogels is often involved with inevitable but unwanted changes in porosity and nanotopography that also influence biological responses through the biophysical and biochemical regulation mechanisms. Furthermore, methods such as IPN, DN, and SR that massively enhance stiffness (from kPa to MPa) have a strict prerequisite of candidate hydrogels and are unsuitable for most hydrogels. So there is a need to develop a further feasible and effective methods for tuning hydrogel stiffness, addressing issues such as changes in pore size and nanotopography, as well as the limited hydrogels availability.

The interrelation among mechanical properties increases the difficulty of determining the effect of individual mechanical cues on cells or tissues. In this context, computational approaches and predictive models can significantly aid in optimizing the mechanical properties of hydrogel for specific cell types by simulating the interactions between the hydrogel matrix and cells under various mechanical conditions. These models can incorporate factors such as cell mechanotransduction, material properties (e.g., elasticity, viscoelasticity, swelling and topography), and environmental cues to predict how different stiffness levels influence cell behaviours, such as proliferation, differentiation, and migration. By integrating experimental data with computational simulations, these models can identify optimal stiffness ranges that promote desired cellular responses, ultimately accelerating the design of tailored hydrogels for specific tissue engineering applications.

In addition to the discrepancies associated with hydrogel stiffness characterization, the goal of replicating time-dependent properties of native tissue growth is a significant challenge that may need to wait for the evolution of 4D bioprinting. In 4D bioprinting, the printed hydrogel constructs can undergo changes in shape, mechanical properties or biofunctionalities over time in response to environmental stimuli, including temperature, pH, light, and oxygen levels [482]. The current hurdle of developing 4D bioprinting is to precisely control the spatial and temporal changes in hydrogel structures and mechanical properties as requested by natural physiological processes.

The development of hydrogels with multifunctionality, particularly those characterized by both mechanical (pressure, tension, or deformation) and electrical (conductivity or piezoelectricity) responsiveness, is also highly needed in bioelectronics and tissue engineering due to their ability to mimic the dynamic variation of the natural ECM and enhance biological responses. These hydrogels are usually incorporated with conductive polymers (e.g., polypyrrole [483], PEDO:PSS [484], and silk fibroin [485]), nanomaterials (e.g., GO and carbon nanotubes [486]), or ionic liquids [487] that possess both electroactive and mechanically responsive abilities. One major issue is achieving a balance between mechanical strength and electrical conductivity, as these properties often conflict with each other [488]. Furthermore, maintaining the biocompatibility and long-term stability of multifunctional hydrogels *in vivo* is an ongoing area of research.

Fabricating scaffolds from uniform biomaterials limits the application toward heterogeneous and anisotropic engineered tissue. To compensate for this, hierarchical gradients in stiffness, porosity, and viscoelasticity may be achieved by controlling polymer concentration, crosslinking density, or component redistribution through photolithography or microfabrication to spatially define regions of interest [489]. In doing so, heterogeneous scaffolds give the greatest hope for engineering heterogeneous tissues such as tissue interfaces.

Traditional static tissue cultures have limitations such as size, nutrient depletion, and waste accumulation and management. Bioreactors alleviate such limitations by continuously circulating culture media through porous hydrogel scaffolds, ensuring uniform cell viability within the structures. Bioreactors also facilitate tight control of cell distribution through the design of bioreactor 3D cell constructs [490]. For example, perfusion bioreactors are widely used in bone and cartilage tissue engineering because they provide a continuous flow of culture medium through the scaffold, enhancing nutrient and oxygen delivery while promoting the removal of metabolic waste [491,492]. This dynamic flow creates a physiologically relevant environment, particularly for tissues that are avascular or require precise nutrient gradients. Likewise, rotating wall bioreactors are often used in the cultivation of 3D cell cultures, including vascular and soft tissues. These bioreactors maintain a low-shear environment while suspending the construct in a nutrient-rich medium, promoting uniform cell growth and extracellular matrix deposition [493]. Additionally, uniaxial strain-inducive bioreactors can act as biomimetic stimulators of contractile (muscle) cell types to achieve (appropriately functional) contractility; but they potentially expose tissue constructs to plastic behaviours whereby the control of stiffness properties becomes distorted. To mediate this risk, hydrogel scaffolds' yield strain must be determined prior to bioreactor implantation to arrive at a viable strain regimen in which a scaffold's stiffness, and thus cellular response is predictable and reproducible. Additional properties such as the effects of strain rate, fatigue, hysteresis, and material degradation should also be characterized before initiating mechanical stimuli. To receive real-time feedback, biosensors may be incorporated to determine the growth rate of tissue constructs. Ideally, biosensors are capable of (i) detecting a stimulus output, (ii)

comparing the stimulus to the pre-defined regenerative milestone, and (iii) automatically triggering a response in the form of automated media exchange, agitation rate, and/or mechanical exposure.

To further replicate the dynamic mechanical environment of human tissue, advanced strategies can be employed. One such approach is the incorporation of mechanosensitive proteins, such as talin or vinculin, which play key roles in transducing mechanical signals into biochemical responses. By embedding these proteins or their functional domains into hydrogel scaffolds, it is possible to create materials that respond dynamically to applied mechanical strain, promoting cellular alignment, differentiation, and maturation [494,495]. Another promising strategy involves the use of piezoelectric materials within hydrogels. These materials generate electrical signals in response to mechanical deformation, mimicking the bioelectric cues that occur during muscle contraction. For example, hybrid hydrogels containing piezoelectric nanoparticles, such as barium titanate or zinc oxide, have been shown to enhance myogenic differentiation when subjected to cyclic mechanical loading [496,497]. These bioelectric signals can complement the mechanical cues provided by uniaxial strain bioreactors, creating a more physiologically relevant microenvironment for skeletal muscle regeneration.

In conclusion, a comprehensive understanding of how a hydrogel behaves over time under dynamic culture environments is imperative for analysing resultant cellular responses in future research on 4D bioprinting, gradient stiffness scaffold, and bioreactors in Tissue Engineering.

CRediT authorship contribution statement

Peiqi Yang: Writing – review & editing, Writing – original draft, Visualization, Resources, Conceptualization. **Gareth Boer:** Writing – review & editing. **Finn Snow:** Writing – review & editing. **Alysha Williamson:** Writing – review & editing. **Samuel Cheeseman:** Writing – review & editing. **Rasika M. Samarasinghe:** Supervision. **Aaqil Rifai:** Writing – review & editing. **Ayushi Priyam:** Writing – review & editing, Supervision. **Roey Elnathan:** Writing – review & editing. **Roseanne Guijt:** Writing – review & editing. **Anita Quigley:** Writing – review & editing, Supervision, Funding acquisition. **Rob Kaspa:** Writing – review & editing, Supervision, Funding acquisition. **David R. Nisbet:** Writing – review & editing. **Richard J. Williams:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rob Kaspa reports financial support was provided by National Health and Medical Research Council. David Nisbet reports financial support was provided by Australian Research Council. Roey Elnathan reports financial support was provided by Australian Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors acknowledge funding via the NHMRC Ideas Grant (Project 2002723), the ARC Future Fellowships (FT230100220 and FT220100749). P.Y. is also supported by the Deakin University Postgraduate Research Scholarship.

Data availability

No data was used for the research described in the article.

References

[1] R.J. Williams, T.E. Hall, V. Glattauer, J. White, P.J. Pasic, A.B. Sorensen, L. Waddington, K.M. McLean, P.D. Currie, P.G. Hartley, The in vivo performance of an enzyme-assisted self-assembled peptide/protein hydrogel, Biomaterials 32 (2011), https://doi.org/10.1016/j.biomaterials.2011.03.078.
[2] V. Penna, N. Moriarty, Y. Wang, K.C.L. Law, C.W. Gantner, R.J. Williams, D. R. Nisbet, C.L. Parish, Extracellular Matrix Biomimetic Hydrogels, Encapsulated with Stromal Cell-Derived Factor 1, Improve the Composition of Foetal Tissue Grafts in a Rodent Model of Parkinson's Disease, Int. J. Mol. Sci. 23 (2022), https://doi.org/10.3390/ijms23094646.
[3] K. Firipis, D.R. Nisbet, S.J. Franks, R.M.I. Kapsa, E. Pirogova, R.J. Williams, A. Quigley, Enhancing peptide biomaterials for biofabrication, Polymers 13 (2021), https://doi.org/10.3390/polym13162590.
[4] D. Sivaraj, K. Chen, A. Chattopadhyay, D. Henn, W. Wu, C. Noishiki, N. J. Magbual, S. Mittal, A.M. Mermin-Bunnell, C.A. Bonham, A.A. Trotsyuk, J. A. Barrera, J. Padmanabhan, M. Januszyk, G.C. Gurtner, Hydrogel Scaffolds to Deliver Cell Therapies for Wound Healing, Front. Bioeng. Biotechnol. 9 (2021), https://doi.org/10.3389/fbioe.2021.660145.
[5] M.T. Wolf, C.L. Dearth, S.B. Sonnenberg, E.G. Loboa, S.F. Badylak, Naturally derived and synthetic scaffolds for skeletal muscle reconstruction, Adv. Drug Deliv. Rev. 84 (2015), https://doi.org/10.1016/j.addr.2014.08.011.
[6] R. Langer, J.P. Vacanti, Tissue Engineering, Science 260 (1993), https://doi.org/10.1126/science.8493529.
[7] C.F. Guimarães, L. Gasperini, A.P. Marques, R.L. Reis, The stiffness of living tissues and its implications for tissue engineering, Nat. Rev. Mater. 5 (2020) 351–370, https://doi.org/10.1038/s41578-019-0169-1.
[8] D.E. Discher, P. Janmey, Y.L. Wang, Tissue cells feel and respond to the stiffness of their substrate, Science 310 (2005) 1139–1143, https://doi.org/10.1126/science.1116995.
[9] M.S. Abdul Halim, J.M. Dyson, M.M. Gong, M.K. O'Bryan, R. Nosrati, Fallopian tube rheology regulates epithelial cell differentiation and function to enhance cilia formation and coordination, Nat. Commun. 15 (2024) 7411, https://doi.org/10.1038/s41467-024-51481-9.
[10] M. D'Angelo, E. Benedetti, M.G. Tupone, M. Catanesi, V. Castelli, A. Antonosante, A. Cimini, The role of stiffness in cell reprogramming: A potential role for biomaterials in inducing tissue regeneration, Cells 8 (2019), https://doi.org/10.3390/cells8091036.
[11] I.T. Ozbolat, Bioink, in: 3D Bioprinting Fundam. Princ. Appl., Academic Press, 2016: pp. 41–92.
[12] S. Mantha, S. Pillai, P. Khayambashi, A. Upadhyay, Y. Zhang, O. Tao, H.M. Pham, S.D. Tran, Smart hydrogels in tissue engineering and regenerative medicine, Materials 12 (2019), https://doi.org/10.3390/ma12203323.
[13] Q. Chai, Y. Jiao, X. Yu, Hydrogels for biomedical applications: Their characteristics and the mechanisms behind them, Gels 3 (2017), https://doi.org/10.3390/gels3010006.
[14] C.M. Leung, P. de Haan, K. Ronaldson-Bouchard, G.A. Kim, J. Ko, H.S. Rho, Z. Chen, P. Habibovic, N.L. Jeon, S. Takayama, M.L. Shuler, G. Vunjak-Novakovic, O. Frey, E. Verpoorte, Y.C. Toh, A guide to the organ-on-a-chip, Nat. Rev. Methods Primer 2 (2022), https://doi.org/10.1038/s43586-022-00118-6.
[15] H. Lin, C. Yin, A. Mo, G. Hong, Applications of hydrogel with special physical properties in bone and cartilage regeneration, Materials 14 (2021) 1–20, https://doi.org/10.3390/ma14010235.
[16] L. Xu, C. Wang, Y. Cui, A. Li, Y. Qiao, D. Qiu, Conjoined-network rendered stiff and tough hydrogels from biogenic molecules, Sci. Adv. 5 (2019), https://doi.org/10.1126/sciadv.aau3442.
[17] A. Gandin, Y. Murugesan, V. Torresan, L. Ulliana, A. Citron, P. Contessotto, G. Battilana, T. Panciera, M. Ventre, A.P. Netti, L. Nicola, S. Piccolo, G. Brusatin, Simple yet effective methods to probe hydrogel stiffness for mechanobiology, Sci. Rep. 11 (2021), https://doi.org/10.1038/s41598-021-01036-5.
[18] L. Li, M.E. Griebel, M. Uroz, S.Y. Bubli, K.A. Gagnon, B. Trappmann, B.M. Baker, J. Eyckmans, C.S. Chen, A Protein-Adsorbent Hydrogel with Tunable Stiffness for Tissue Culture Demonstrates Matrix-Dependent Stiffness Responses, Adv. Funct. Mater. 34 (2024), https://doi.org/10.1002/adfm.202309567.
[19] Z. Chen, M. Ezzo, B. Zondag, F. Rakhshani, Y. Ma, B. Hinz, E. Kumacheva, Intrafibrillar Crosslinking Enables Decoupling of Mechanical Properties and Structure of a Composite Fibrous Hydrogel, Adv. Mater. 36 (2024), https://doi.org/10.1002/adma.202305964.
[20] J. Visser, F.P.W. Melchels, J.E. Jeon, E.M. Van Bussel, L.S. Kimpton, H.M. Byrne, W.J.A. Dhert, P.D. Dalton, D.W. Hutmacher, J. Malda, Reinforcement of hydrogels using three-dimensionally printed microfibres, Nat. Commun. 6 (2015), https://doi.org/10.1038/ncomms7933.
[21] Z. Wang, Y. Zhao, H. Bai, F. Chang, X. Yang, X. Wang, J. Liu, M. Wu, Q. Lin, J. Wang, H. Liu, Bioactive prosthesis interface compositing variable-stiffness hydrogels regulates stem cells fates to facilitate osseointegration through mechanotransduction, Int. J. Biol. Macromol. 259 (2024), https://doi.org/10.1016/j.ijbiomac.2023.129073.
[22] O. Chaudhuri, J. Cooper-White, P.A. Janmey, D.J. Mooney, V.B. Shenoy, Effects of extracellular matrix viscoelasticity on cellular behaviour, Nature 584 (2020), https://doi.org/10.1038/s41586-020-2612-2.
[23] N. Gjorevski, N. Sachs, A. Manfrin, S. Giger, M.E. Bragina, P. Ordóñez-Morán, H. Clevers, M.P. Lutolf, Designer matrices for intestinal stem cell and organoid culture, Nature 539 (2016), https://doi.org/10.1038/nature20168.
[24] N.R. Richbourg, M.K. Rausch, N.A. Peppas, Cross-evaluation of stiffness measurement methods for hydrogels, Polymer 258 (2022), https://doi.org/10.1016/j.polymer.2022.125316.

[25] N.R. Richbourg, N.A. Peppas, The swollen polymer network hypothesis: Quantitative models of hydrogel swelling, stiffness, and solute transport, Prog. Polym. Sci. 105 (2020), https://doi.org/10.1016/j.progpolymsci.2020.101243.

[26] K. Upadhyay, G. Subhash, D. Spearot, Hyperelastic constitutive modeling of hydrogels based on primary deformation modes and validation under 3D stress states, Int. J. Eng. Sci. 154 (2020), https://doi.org/10.1016/j.ijengsci.2020.103314.

[27] N.A. Peppas, E.W. Merrill, Development of semicrystalline poly(vinyl alcohol) hydrogels for biomedical applications, J. Biomed. Mater. Res. 11 (1977), https://doi.org/10.1002/jbm.820110309.

[28] N.A. Peppas, E.W. Merrill, Crosslinked poly(vinyl alcohol) hydrogels as swollen elastic networks, J. Appl. Polym. Sci. 21 (1977), https://doi.org/10.1002/app.1977.070210704.

[29] P.J. Flory, J. Rehner, Statistical mechanics of cross-linked polymer networks II. Swelling, J. Chem. Phys. 11 (1943), https://doi.org/10.1063/1.1723792.

[30] L.R.G. Treloar, The Physics of Rubber Elasticity (2005), https://doi.org/10.1093/oso/9780198570271.001.0001.

[31] T. Tanaka, D.J. Fillmore, Kinetics of swelling of gels, J. Chem. Phys. 70 (1979), https://doi.org/10.1063/1.437602.

[32] N.R. Richbourg, M. Wancura, A.E. Gilchrist, S. Toubbeh, B.A.C. Harley, E. Cosgriff-Hernandez, N.A. Peppas, Precise control of synthetic hydrogel network structure via linear, independent synthesis-swelling relationships, Sci. Adv. 7 (2021), https://doi.org/10.1126/sciadv.abe3245.

[33] R.G. Wells, Tissue mechanics and fibrosis, Biochim. Biophys. Acta - Mol. Basis Dis. 2013 (1832) 884–890, https://doi.org/10.1016/j.bbadis.2013.02.007.

[34] W. Norde, Rheology, with special attention to dispersions and interfaces, in: Colloids Interfaces Life Sci. Bionanotechnol., 2020: pp. 335–356. https://doi.org/10.1201/9781439873038-20.

[35] F. Baumgart, Stiffness - An unknown world of mechanical science? Injury 31 (2000) https://doi.org/10.1016/s0020-1383(00)80040-6.

[36] P.H. Wu, D.R.B. Aroush, A. Asnacios, W.C. Chen, M.E. Dokukin, B.L. Doss, P. Durand-Smet, A. Ekpenyong, J. Guck, N.V. Guz, P.A. Janmey, J.S.H. Lee, N. M. Moore, A. Ott, Y.C. Poh, R. Ros, M. Sander, I. Sokolov, J.R. Staunton, N. Wang, G. Whyte, D. Wirtz, A comparison of methods to assess cell mechanical properties, Nat. Methods 15 (2018), https://doi.org/10.1038/s41592-018-0015-1.

[37] M.D.A. Norman, S.A. Ferreira, G.M. Jowett, L. Bozec, E. Gentleman, Measuring the elastic modulus of soft culture surfaces and three-dimensional hydrogels using atomic force microscopy, Nat. Protoc. 16 (2021) 2418–2449, https://doi.org/10.1038/s41596-021-00495-4.

[38] C. Verdier, J. Etienne, A. Duperray, L. Preziosi, Review: Rheological properties of biological materials, Comptes Rendus Phys. 10 (2009), https://doi.org/10.1016/j.crhy.2009.10.003.

[39] J.M. Barnes, L. Przybyla, V.M. Weaver, Tissue mechanics regulate brain development, homeostasis and disease, J. Cell Sci. 130 (2017), https://doi.org/10.1242/jcs.191742.

[40] J. Nafar Dastgerdi, J.T. Koivisto, O. Orell, P. Rava, J. Jokinen, M. Kanerva, M. Kellomäki, Comprehensive characterisation of the compressive behaviour of hydrogels using a new modelling procedure and redefining compression testing, Mater. Today Commun. 28 (2021), https://doi.org/10.1016/j.mtcomm.2021.102518.

[41] H. Sundaram, B. Voigts, K. Beer, M. Meland, Comparison of the rheological properties of viscosity and elasticity in two categories of soft tissue fillers: Calcium hydroxylapatite and hyaluronic acid, Dermatol. Surg. 36 (2010), https://doi.org/10.1111/j.1524-4725.2010.01743.x.

[42] Y.K. Mariappan, K.J. Glaser, R.L. Ehman, Magnetic resonance elastography: A review, Clin. Anat. 23 (2010), https://doi.org/10.1002/ca.21006.

[43] Y. Zhang, F. Wei, Y.C. Poh, Q. Jia, J. Chen, J. Chen, J. Luo, W. Yao, W. Zhou, W. Huang, F. Yang, Y. Zhang, N. Wang, Interfacing 3D magnetic twisting cytometry with confocal fluorescence microscopy to image force responses in living cells, Nat. Protoc. 12 (2017), https://doi.org/10.1038/nprot.2017.042.

[44] J.G. Jacot, S. Dianis, J. Schnall, J.Y. Wong, A simple microindentation technique for mapping the microscale compliance of soft hydrated materials and tissues, J. Biomed. Mater. Res. - Part A 79 (2006), https://doi.org/10.1002/jbm.a.30812.

[45] A. Karimzadeh, S.S.R. Koloor, M.R. Ayatollahi, A.R. Bushroa, M.Y. Yahya, Assessment of Nano-Indentation Method in Mechanical Characterization of Heterogeneous Nanocomposite Materials Using Experimental and Computational Approaches, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-51904-4.

[46] N. Gavara, A beginner's guide to atomic force microscopy probing for cell mechanics, Microsc. Res. Tech. 80 (2017), https://doi.org/10.1002/jemt.22776.

[47] W. Liu, C. Wu, Rheological Study of Soft Matters: A Review of Microrheology and Microrheometers, Macromol. Chem. Phys. 219 (2018), https://doi.org/10.1002/macp.201700307.

[48] R. Prevedel, A. Diz-Muñoz, G. Ruocco, G. Antonacci, Brillouin microscopy: an emerging tool for mechanobiology, Nat. Methods 16 (2019), https://doi.org/10.1038/s41592-019-0543-3.

[49] C.T. McKee, J.A. Last, P. Russell, C.J. Murphy, Indentation versus tensile measurements of young's modulus for soft biological tissues, Tissue Eng. - Part B Rev. 17 (2011), https://doi.org/10.1089/ten.teb.2010.0520.

[50] W. Megone, N. Roohpour, J.E. Gautrot, Impact of surface adhesion and sample heterogeneity on the multiscale mechanical characterisation of soft biomaterials, Sci. Rep. 8 (2018), https://doi.org/10.1038/s41598-018-24671-x.

[51] D.W. Thompson, On growth and form, Cambridge University Press, 1942.

[52] J.T. Emerman, D.R. Pitelka, Maintenance and induction of morphological differentiation in dissociated mammary epithelium on floating collagen membranes, In Vitro 13 (1977), https://doi.org/10.1007/BF02616178.

[53] R.J. Pelham, Y.-L. Wang, Cell locomotion and focal adhesions are regulated by substrate flexibility, 1997. www.pnas.org.

[54] A.J. Steward, D.J. Kelly, Mechanical regulation of mesenchymal stem cell differentiation, J. Anat. 227 (2015) 717–731, https://doi.org/10.1111/joa.12243.

[55] A.M. Handorf, Y. Zhou, M.A. Halanski, W.J. Li, Tissue stiffness dictates development, homeostasis, and disease progression, Organogenesis 11 (2015) 1–15, https://doi.org/10.1080/15476278.2015.1019687.

[56] J. Vitte, A.M. Benoliel, A. Pierres, P. Bongrand, A.S.G. Curtis, Is there a predictable relationship between surface physical-chemical properties and cell behaviour at the interface? Eur. Cell. Mater. 7 (2004) https://doi.org/10.22203/eCM.v007a06.

[57] U. Hersel, C. Dahmen, H. Kessler, RGD modified polymers: Biomaterials for stimulated cell adhesion and beyond, Biomaterials 24 (2003), https://doi.org/10.1016/S0142-9612(03)00343-0.

[58] S.P. Massia, J.A. Hubbell, An RGD spacing of 440 nm is sufficient for integrin αvβ3-mediated fibroblast spreading and 140 nm for focal contact and stress fiber formation, J. Cell Biol. 114 (1991), https://doi.org/10.1083/jcb.114.5.1089.

[59] F. Karimi, A.J. O'Connor, G.G. Qiao, D.E. Heath, Integrin Clustering Matters: A Review of Biomaterials Functionalized with Multivalent Integrin-Binding Ligands to Improve Cell Adhesion, Migration, Differentiation, Angiogenesis, and Biomedical Device Integration, Adv. Healthc. Mater. 7 (2018), https://doi.org/10.1002/adhm.201701324.

[60] V. Bolós, J.M. Gasent, S. López-Tarruella, E. Grande, The dual kinase complex FAK-src as a promising therapeutic target in cancer, OncoTargets Ther. 3 (2010), https://doi.org/10.2147/ott.s6909.

[61] J.L. Guan, Integrin signaling through FAK in the regulation of mammary stem cells and breast cancer, IUBMB Life 62 (2010), https://doi.org/10.1002/iub.303.

[62] C.E. Chan, D.J. Odde, Traction dynamics of filopodia on compliant substrates, Science 322 (2008), https://doi.org/10.1126/science.1163595.

[63] J.D. Humphrey, E.R. Dufresne, M.A. Schwartz, Mechanotransduction and extracellular matrix homeostasis, Nat. Rev. Mol. Cell Biol. 15 (2014), https://doi.org/10.1038/nrm3896.

[64] Z. Gong, S.E. Szczesny, S.R. Caliari, E.E. Charrier, O. Chaudhuri, X. Cao, Y. Lin, R. L. Mauck, P.A. Janmey, J.A. Burdick, V.B. Shenoy, Matching material and cellular timescales maximizes cell spreading on viscoelastic substrates, Proc. Natl. Acad. Sci. U. S. A. 115 (2018), https://doi.org/10.1073/pnas.1716620115.

[65] A.B. Jaffe, A. Hall, Rho GTPases: Biochemistry and biology, Annu. Rev. Cell Dev. Biol. 21 (2005), https://doi.org/10.1146/annurev.cellbio.21.020604.150721.

[66] K. Burridge, K. Wennerberg, Rho and Rac Take Center Stage, Cell 116 (2004), https://doi.org/10.1016/S0092-8674(04)00003-0.

[67] H. Sreenivasappa, S.P. Chaki, S.M. Lim, J.P. Trzeciakowski, M.W. Davidson, G. M. Rivera, A. Trache, Selective regulation of cytoskeletal tension and cell-matrix adhesion by RhoA and Src, Integr. Biol. U. K. 6 (2014), https://doi.org/10.1039/c4ib00019f.

[68] F. Grinnell, Fibroblast-collagen-matrix contraction: growth-factor signalling and mechanical loading, Trends Cell Biol. (2000), https://doi.org/10.1016/S0962-8924(00)01802-X.

[69] S.C. Wei, L. Fattet, J.H. Tsai, Y. Guo, V.H. Pai, H.E. Majeski, A.C. Chen, R.L. Sah, S.S. Taylor, A.J. Engler, J. Yang, Matrix stiffness drives epithelial-mesenchymal transition and tumour metastasis through a TWIST1-G3BP2 mechanotransduction pathway, Nat. Cell Biol. 17 (2015), https://doi.org/10.1038/ncb3157.

[70] K.E. Scott, S.I. Fraley, P. Rangamani, A spatial model of YAP/TAZ signaling reveals how stiffness, dimensionality, and shape contribute to emergent outcomes, Proc. Natl. Acad. Sci. U. S. A. 118 (2021), https://doi.org/10.1073/pnas.2021571118.

[71] O. Chaudhuri, L. Gu, D. Klumpers, M. Darnell, S.A. Bencherif, J.C. Weaver, N. Huebsch, H.P. Lee, E. Lippens, G.N. Duda, D.J. Mooney, Hydrogels with tunable stress relaxation regulate stem cell fate and activity, Nat. Mater. 15 (2016), https://doi.org/10.1038/nmat4489.

[72] H. Gerardo, A. Lima, J. Carvalho, J.R.D. Ramos, S. Couceiro, R.D.M. Travasso, R. Pires das Neves, M. Grãos, Soft culture substrates favor stem-like cellular phenotype and facilitate reprogramming of human mesenchymal stem/stromal cells (hMSCs) through mechanotransduction, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-45352-3.

[73] J. Klein-Nulend, R.G. Bacabac, A.D. Bakker, Mechanical loading and how it affects bone cells: The role of the osteocyte cytoskeleton in maintaining our skeleton, Eur. Cell. Mater. 24 (2012), https://doi.org/10.22203/eCM.v024a20.

[74] S.J. Mellon, K.E. Tanner, Bone and its adaptation to mechanical loading: A review, Int. Mater. Rev. 57 (2012), https://doi.org/10.1179/1743280412Y.0000000008.

[75] P. Bergmann, J.J. Body, S. Boonen, Y. Boutsen, J.P. Devogelaer, S. Goemaere, J. Kaufman, J.Y. Reginster, S. Rozenberg, Loading and Skeletal Development and Maintenance, J. Osteoporos. 2011 (2011), https://doi.org/10.4061/2011/786752.

[76] J. Klein-Nulend, A.D. Bakker, R.G. Bacabac, A. Vatsa, S. Weinbaum, Mechanosensation and transduction in osteocytes, Bone 54 (2013), https://doi.org/10.1016/j.bone.2012.10.013.

[77] J.I. Aguirre, L.I. Plotkin, S.A. Stewart, R.S. Weinstein, A.M. Parfitt, S. C. Manolagas, T. Bellido, Osteocyte apoptosis is induced by weightlessness in mice and precedes osteoclast recruitment and bone loss, J. Bone Miner. Res. 21 (2006), https://doi.org/10.1359/jbmr.060107.

[78] G. Gu, M. Mulari, Z. Peng, T.A. Hentunen, H.K. Väänänen, Death of osteocytes turns off the inhibition of osteoclasts and triggers local bone resorption, Biochem. Biophys. Res. Commun. 335 (2005), https://doi.org/10.1016/j.bbrc.2005.06.211.

[79] L. You, S. Temiyasathit, P. Lee, C.H. Kim, P. Tummala, W. Yao, W. Kingery, A. M. Malone, R.Y. Kwon, C.R. Jacobs, Osteocytes as mechanosensors in the inhibition of bone resorption due to mechanical loading, Bone 42 (2008), https://doi.org/10.1016/j.bone.2007.09.047.

[80] S.D. Tan, T.J. de Vries, A.M. Kuijpers-Jagtman, C.M. Semeins, V. Everts, J. Klein-Nulend, Osteocytes subjected to fluid flow inhibit osteoclast formation and bone resorption, Bone 41 (2007), https://doi.org/10.1016/j.bone.2007.07.019.

[81] S.D. Tan, A.D. Bakker, C.M. Semeins, A.M. Kuijpers-Jagtman, J. Klein-Nulend, Inhibition of osteocyte apoptosis by fluid flow is mediated by nitric oxide, Biochem. Biophys. Res. Commun. 369 (2008), https://doi.org/10.1016/j.bbrc.2008.03.007.

[82] J.F. Eichinger, L.J. Haeusel, D. Paukner, R.C. Aydin, J.D. Humphrey, C.J. Cyron, Mechanical homeostasis in tissue equivalents: a review, Biomech. Model. Mechanobiol. 20 (2021), https://doi.org/10.1007/s10237-021-01433-9.

[83] C.E. Keating, D.K. Cullen, Mechanosensation in traumatic brain injury, Neurobiol. Dis. 148 (2021), https://doi.org/10.1016/j.nbd.2020.105210.

[84] N. George, H.M. Geller, Extracellular matrix and traumatic brain injury, J. Neurosci. Res. 96 (2018), https://doi.org/10.1002/jnr.24151.

[85] T. Boulet, M.L. Kelso, S.F. Othman, Long-term in vivo imaging of viscoelastic properties of the mouse brain after controlled cortical impact, J. Neurotrauma 30 (2013), https://doi.org/10.1089/neu.2012.2788.

[86] M. Xue, C.J. Jackson, Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring, Adv. Wound Care 4 (2015), https://doi.org/10.1089/wound.2013.0485.

[87] F.M. Maynard, M.B. Bracken, G. Creasey, J.F. Ditunno, W.H. Donovan, T. B. Ducker, S.L. Garber, R.J. Marino, S.L. Stover, C.H. Tator, R.L. Waters, J. E. Wilberger, W. Young, International standards for neurological and functional classification of spinal cord injury, Spinal Cord 35 (1997), https://doi.org/10.1038/sj.sc.3100432.

[88] J. Huynh, N. Nishimura, K. Rana, J.M. Peloquin, J.P. Califano, C.R. Montague, M. R. King, C.B. Schaffer, C.A. Reinhart-King, Age-related intimal stiffening enhances endothelial permeability and leukocyte transmigration, Sci. Transl. Med. 3 (2011), https://doi.org/10.1126/scitranslmed.3002761.

[89] L.K. Wood, E. Kayupov, J.P. Gumucio, C.L. Mendias, D.R. Claflin, S.V. Brooks, Intrinsic stiffness of extracellular matrix increases with age in skeletal muscles of mice, J. Appl. Physiol. 117 (2014), https://doi.org/10.1152/japplphysiol.00256.2014.

[90] W.J. Choi, C.M. Russell, C.M. Tsai, S. Arzanpour, S.N. Robinovitch, Age-related changes in dynamic compressive properties of trochanteric soft tissues over the hip, J. Biomech. 48 (2015), https://doi.org/10.1016/j.jbiomech.2014.12.026.

[91] J. Chang, E.M. Pang, K. Adebowale, K.M. Wisdom, O. Chaudhuri, Increased stiffness inhibits invadopodia formation and cell migration in 3D, Biophys. J. 119 (2020), https://doi.org/10.1016/j.bpj.2020.07.003.

[92] D.T. Butcher, T. Alliston, V.M. Weaver, A tense situation: Forcing tumour progression, Nat. Rev. Cancer 9 (2009), https://doi.org/10.1038/nrc2544.

[93] L. Kass, J.T. Erler, M. Dembo, V.M. Weaver, Mammary epithelial cell: Influence of extracellular matrix composition and organization during development and tumorigenesis, Int. J. Biochem. Cell Biol. 39 (2007), https://doi.org/10.1016/j.biocel.2007.06.025.

[94] P.P. Provenzano, D.R. Inman, K.W. Eliceiri, J.G. Knittel, L. Yan, C.T. Rueden, J. G. White, P.J. Keely, Collagen density promotes mammary tumor initiation and progression, BMC Med. 6 (2008), https://doi.org/10.1186/1741-7015-6-11.

[95] E. Miller-Kasprzak, P.P. Jagodziński, Endothelial progenitor cells as a new agent contributing to vascular repair, Arch. Immunol. Ther. Exp. (Warsz.) 55 (2007), https://doi.org/10.1007/s00005-007-0027-5.

[96] Q. Chen, M. Jin, F. Yang, J. Zhu, Q. Xiao, L. Zhang, Matrix metalloproteinases: Inflammatory regulators of cell behaviors in vascular formation and remodeling, Mediators Inflamm. 2013 (2013), https://doi.org/10.1155/2013/928315.

[97] D. Hanjaya-Putra, J. Yee, D. Ceci, R. Truitt, D. Yee, S. Gerecht, Vascular endothelial growth factor and substrate mechanics regulate in vitro tubulogenesis of endothelial progenitor cells, J. Cell. Mol. Med. 14 (2010), https://doi.org/10.1111/j.1582-4934.2009.00981.x.

[98] J. Sotres, S. Jankovskaja, K. Wannerberger, T. Arnebrant, Ex-Vivo Force Spectroscopy of Intestinal Mucosa Reveals the Mechanical Properties of Mucus Blankets, Sci. Rep. 7 (2017), https://doi.org/10.1038/s41598-017-07552-7.

[99] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, Blood vessels and endothelial cells, in: Mol. Biol. Cell, Garland Science, 2002.

[100] K. Nagayama, K. Nishimiya, Moderate substrate stiffness induces vascular smooth muscle cell differentiation through cellular morphological and tensional changes, Biomed. Mater. Eng. 31 (2020), https://doi.org/10.3233/BME-201087.

[101] K. Nagayama, S. Saito, T. Matsumoto, Multiphasic stress relaxation response of freshly isolated and cultured vascular smooth muscle cells measured by quasi-in situ tensile test, Biomed. Mater. Eng. 25 (2015), https://doi.org/10.3233/BME-151276.

[102] C. Wang, S. Sinha, X. Jiang, L. Murphy, S. Fitch, C. Wilson, G. Grant, F. Yang, Matrix stiffness modulates patient-derived glioblastoma cell fates in three-dimensional hydrogels, Tissue Eng. - Part A 27 (2021), https://doi.org/10.1089/ten.tea.2020.0110.

[103] K. Saha, A.J. Keung, E.F. Irwin, Y. Li, L. Little, D.V. Schaffer, K.E. Healy, Substrate modulus directs neural stem cell behavior, Biophys. J. 95 (2008), https://doi.org/10.1529/biophysj.108.132217.

[104] M. Iwashita, H. Ohta, T. Fujisawa, M. Cho, M. Ikeya, S. Kidoaki, Y. Kosodo, Brain-stiffness-mimicking tilapia collagen gel promotes the induction of dorsal cortical neurons from human pluripotent stem cells, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-018-38395-5.

[105] Y. Gu, Y. Ji, Y. Zhao, Y. Liu, F. Ding, X. Gu, Y. Yang, The influence of substrate stiffness on the behavior and functions of Schwann cells in culture, Biomaterials 33 (2012), https://doi.org/10.1016/j.biomaterials.2012.06.006.

[106] B.N. Mason, A. Starchenko, R.M. Williams, L.J. Bonassar, C.A. Reinhart-King, Tuning three-dimensional collagen matrix stiffness independently of collagen concentration modulates endothelial cell behavior, Acta Biomater. 9 (2013) 4635–4644, https://doi.org/10.1016/j.actbio.2012.08.007.

[107] T. Takebe, M. Enomura, E. Yoshizawa, M. Kimura, H. Koike, Y. Ueno, T. Matsuzaki, T. Yamazaki, T. Toyohara, K. Osafune, H. Nakauchi, H. Y. Yoshikawa, H. Taniguchi, Vascularized and complex organ buds from diverse tissues via mesenchymal cell-driven condensation, Cell Stem Cell 16 (2015), https://doi.org/10.1016/j.stem.2015.03.004.

[108] M. Quarta, J.O. Brett, R. DiMarco, A. De Morree, S.C. Boutet, R. Chacon, M. C. Gibbons, V.A. Garcia, J. Su, J.B. Shrager, S. Heilshorn, T.A. Rando, An artificial niche preserves the quiescence of muscle stem cells and enhances their therapeutic efficacy, Nat. Biotechnol. 34 (2016), https://doi.org/10.1038/nbt.3576.

[109] S. Ansari, C. Chen, X. Xu, N. Annabi, H.H. Zadeh, B.M. Wu, A. Khademhosseini, S. Shi, A. Moshaverinia, Muscle Tissue Engineering Using Gingival Mesenchymal Stem Cells Encapsulated in Alginate Hydrogels Containing Multiple Growth Factors, Ann. Biomed. Eng. 44 (2016), https://doi.org/10.1007/s10439-016-1594-6.

[110] A.J. Engler, M.A. Griffin, S. Sen, C.G. Bönnemann, H.L. Sweeney, D.E. Discher, Myotubes differentiate optimally on substrates with tissue-like stiffness: Pathological implications for soft or stiff microenvironments, J. Cell Biol. 166 (2004), https://doi.org/10.1083/jcb.200405004.

[111] M.E. Melica, G.L. La Regina, M. Parri, A.J. Peired, P. Romagnani, L. Lasagni, Substrate stiffness modulates renal progenitor cell properties via a ROCK-mediated mechanotransduction mechanism, Cells 8 (2019), https://doi.org/10.3390/cells8121561.

[112] M. Hamon, Y. Chen, P. Srivastava, H.M. Chang, V. Gupta, L. Jin, N. Yanagawa, P. V. Hauser, Matrix Stiffness Influences Tubular Formation in Renal Tissue Engineering, Appl. Sci. Switz. 13 (2023), https://doi.org/10.3390/app13074510.

[113] A. Wahl, C. Dinet, P. Dillard, A. Nassereddine, P.H. Puech, L. Limozin, K. Sengupta, Biphasic mechanosensitivity of T cell receptor-mediated spreading of lymphocytes, Proc. Natl. Acad. Sci. U. S. A. 116 (2019), https://doi.org/10.1073/pnas.1811516116.

[114] X.T. He, R.X. Wu, X.Y. Xu, J. Wang, Y. Yin, F.M. Chen, Macrophage involvement affects matrix stiffness-related influences on cell osteogenesis under three-dimensional culture conditions, Acta Biomater. 71 (2018), https://doi.org/10.1016/j.actbio.2018.02.015.

[115] Y. Han, Z. Shao, Y. Zhang, H. Zhao, Z. Sun, C. Yang, H. Tang, Y. Han, C. Gao, 3D matrix stiffness modulation unveils cardiac fibroblast phenotypic switching, Sci. Rep. 14 (2024) 17015, https://doi.org/10.1038/s41598-024-67646-x.

[116] B. Bhana, R.K. Iyer, W.L.K. Chen, R. Zhao, K.L. Sider, M. Likhitpanichkul, C. A. Simmons, M. Radisic, Influence of substrate stiffness on the phenotype of heart cells, Biotechnol. Bioeng. 105 (2010), https://doi.org/10.1002/bit.22647.

[117] T. Yeung, P.C. Georges, L.A. Flanagan, B. Marg, M. Ortiz, M. Funaki, N. Zahir, W. Ming, V. Weaver, P.A. Janmey, Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion, Cell Motil. Cytoskeleton 60 (2005), https://doi.org/10.1002/cm.20041.

[118] A. Guo, B. Wang, C. Lyu, W. Li, Y. Wu, L. Zhu, R. Bi, C. Huang, J.J. Li, Y. Du, Consistent apparent Young's modulus of human embryonic stem cells and derived cell types stabilized by substrate stiffness regulation promotes lineage specificity maintenance, Cell Regen. 9 (2020), https://doi.org/10.1186/s13619-020-00054-4.

[119] F. Zhao, M. Zhang, M. Nizamoglu, H.J. Kaper, L.A. Brouwer, T. Borghuis, J. K. Burgess, M.C. Harmsen, P.K. Sharma, Fibroblast alignment and matrix remodeling induced by a stiffness gradient in a skin-derived extracellular matrix hydrogel, Acta Biomater. 182 (2024) 67–80, https://doi.org/10.1016/j.actbio.2024.05.018.

[120] A. Subramanian, H.Y. Lin, Crosslinked chitosan: Its physical properties and the effects of matrix stiffness on chondrocyte cell morphology and proliferation, J. Biomed. Mater. Res. - Part A 75 (2005), https://doi.org/10.1002/jbm.a.30489.

[121] M. Mohammed, T.S. Lai, H.C. Lin, Substrate stiffness and sequence dependent bioactive peptide hydrogels influence the chondrogenic differentiation of human mesenchymal stem cells, J. Mater. Chem. B 9 (2021), https://doi.org/10.1039/d0tb02008g.

[122] N. Huebsch, P.R. Arany, A.S. Mao, D. Shvartsman, O.A. Ali, S.A. Bencherif, J. Rivera-Feliciano, D.J. Mooney, Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate, Nat. Mater. 9 (2010), https://doi.org/10.1038/nmat2732.

[123] M. Yue, Y. Liu, P. Zhang, Z. Li, Y. Zhou, Integrative Analysis Reveals the Diverse Effects of 3D Stiffness upon Stem Cell Fate, Int. J. Mol. Sci. 24 (2023), https://doi.org/10.3390/ijms24119311.

[124] T. Zhang, S. Lin, X. Shao, Q. Zhang, C. Xue, S. Zhang, Y. Lin, B. Zhu, X. Cai, Effect of matrix stiffness on osteoblast functionalization, Cell Prolif. 50 (2017), https://doi.org/10.1111/cpr.12338.

[125] C.Y. Su, L.K. Yeh, C.C. Lai, K.Y. Li, C.L. Tseng, H.W. Fang, Effects of lysosomal deposition on the friction coefficient of hydrogel contact lenses, Contact Lens Anterior Eye 43 (2020), https://doi.org/10.1016/j.clae.2019.09.007.

[126] R.C. Opt Veld, X.F. Walboomers, J.A. Jansen, F.A.D.T.G. Wagener, Design Considerations for Hydrogel Wound Dressings: Strategic and Molecular Advances, Tissue Eng. - Part B Rev. 26 (2020), https://doi.org/10.1089/ten.teb.2019.0281.

[127] S. Tavakoli, A.S. Klar, Advanced hydrogels as wound dressings, Biomolecules 10 (2020), https://doi.org/10.3390/biom10081169.

[128] J. Tavakoli, Y. Tang, Hydrogel based sensors for biomedical applications: An updated review, Polymers 9 (2017), https://doi.org/10.3390/polym9080364.
[129] M. Vigata, C. Meinert, D.W. Hutmacher, N. Bock, Hydrogels as drug delivery systems: A review of current characterization and evaluation techniques, Pharmaceutics 12 (2020), https://doi.org/10.3390/pharmaceutics12121188.
[130] A.S. Hoffman, Hydrogels for biomedical applications, Adv. Drug Deliv. Rev. 64 (2012) 18–23, https://doi.org/10.1016/j.addr.2012.09.010.
[131] M.C. Catoira, L. Fusaro, D. Di Francesco, M. Ramella, F. Boccafoschi, Overview of natural hydrogels for regenerative medicine applications, J. Mater. Sci. Mater. Med. 30 (2019), https://doi.org/10.1007/s10856-019-6318-7.
[132] Y.L. Lin, C.P. Chen, C.M. Lo, H.S. Wang, Stiffness-controlled three-dimensional collagen scaffolds for differentiation of human Wharton's jelly mesenchymal stem cells into cardiac progenitor cells, J. Biomed. Mater. Res. - Part A 104 (2016), https://doi.org/10.1002/jbm.a.35762.
[133] R. Mooney, B. Tawil, M. Mahoney, Specific fibrinogen and thrombin concentrations promote neuronal rather than glial growth when primary neural cells are seeded within plasma-derived fibrin gels, Tissue Eng. - Part A 16 (2010), https://doi.org/10.1089/ten.tea.2009.0372.
[134] B. Vailhé, M. Lecomte, N. Wiernsperger, L. Tranqui, The formation of tubular structures by endothelial cells is under the control of fibrinolysis and mechanical factors, Angiogenesis 2 (1998), https://doi.org/10.1023/A:1009238717101.
[135] B. Teong, S.C. Wu, C.M. Chang, J.W. Chen, H.T. Chen, C.H. Chen, J.K. Chang, M. L. Ho, The stiffness of a crosslinked hyaluronan hydrogel affects its chondro-induction activity on hADSCs, J. Biomed. Mater. Res. - Part B Appl. Biomater. 106 (2018), https://doi.org/10.1002/jbm.b.33881.
[136] N.D. Leipzig, M.S. Shoichet, The effect of substrate stiffness on adult neural stem cell behavior, Biomaterials 30 (2009), https://doi.org/10.1016/j.biomaterials.2009.09.002.
[137] G.R. Liguori, T.T.A. Liguori, S.R. de Moraes, V. Sinkunas, V. Terlizzi, J.A. van Dongen, P.K. Sharma, L.F.P. Moreira, M.C. Harmsen, Molecular and Biomechanical Clues From Cardiac Tissue Decellularized Extracellular Matrix Drive Stromal Cell Plasticity, Front. Bioeng. Biotechnol. 8 (2020), https://doi.org/10.3389/fbioe.2020.00520.
[138] K. Ye, L. Cao, S. Li, L. Yu, J. Ding, Interplay of Matrix Stiffness and Cell-Cell Contact in Regulating Differentiation of Stem Cells, ACS Appl. Mater. Interfaces 8 (2016), https://doi.org/10.1021/acsami.5b09746.
[139] M.Y. Shie, J.J. Lee, C.C. Ho, S.Y. Yen, H.Y. Ng, Y.W. Chen, Effects of gelatin methacrylate bio-ink concentration on mechano-physical properties and human dermal fibroblast behavior, Polymers 12 (2020), https://doi.org/10.3390/POLYM12091930.
[140] R. Panahi, M. Baghban-Salehi, Protein-based hydrogels, in: Cellul.-Based Superabsorbend Hydrogels, Springer, 2019: pp. 1562–1589. https://doi.org/10.1007/978-3-319-77830-3_52.
[141] E. Jabbari, J. Leijten, Q. Xu, A. Khademhosseini, The matrix reloaded: The evolution of regenerative hydrogels, Mater. Today 19 (2016), https://doi.org/10.1016/j.mattod.2015.10.005.
[142] E.M. Ahmed, Hydrogel: Preparation, characterization, and applications: A review, J. Adv. Res. 6 (2015), https://doi.org/10.1016/j.jare.2013.07.006.
[143] S.B. Lowe, V.T.G. Tan, A.H. Soeriyadi, T.P. Davis, J.J. Gooding, Synthesis and High-Throughput Processing of Polymeric Hydrogels for 3D Cell Culture, Bioconjug. Chem. 25 (2014), https://doi.org/10.1021/bc500310v.
[144] E. Meco, K.J. Lampe, Impact of Elastin-like Protein Temperature Transition on PEG-ELP Hybrid Hydrogel Properties, Biomacromolecules 20 (2019), https://doi.org/10.1021/acs.biomac.9b00113.
[145] R. Silva, B. Fabry, A.R. Boccaccini, Fibrous protein-based hydrogels for cell encapsulation, Biomaterials 35 (2014), https://doi.org/10.1016/j.biomaterials.2014.04.078.
[146] M.D. Shoulders, R.T. Raines, Collagen structure and stability, Annu. Rev. Biochem. 78 (2009), https://doi.org/10.1146/annurev.biochem.77.032207.120833.
[147] J. Lian, B.W. Mansel, B. Ingham, S. Prabakar, M.A.K. Williams, Controlling chain flexibility in collagen networks to produce hydrogels with distinct properties, Soft Mater. 15 (2017), https://doi.org/10.1080/1539445X.2016.1268626.
[148] L. Yang, K.O. Van Der Werf, C.F.C. Fitié, M.L. Bennink, P.J. Dijkstra, J. Feijen, Mechanical properties of native and cross-Linked type i collagen fibrils, Biophys. J. 94 (2008), https://doi.org/10.1529/biophysj.107.111013.
[149] M.J. Buehler, Nature designs tough collagen: Explaining the nanostructure of collagen fibrils, Proc. Natl. Acad. Sci. U. S. A. 103 (2006), https://doi.org/10.1073/pnas.0603216103.
[150] N. Thottappillil, P.D. Nair, Scaffolds in vascular regeneration: Current status, Vasc. Health Risk Manag. 11 (2015), https://doi.org/10.2147/VHRM.S50536.
[151] D.G. Seifu, A. Purnama, K. Mequanint, D. Mantovani, Small-diameter vascular tissue engineering, Nat. Rev. Cardiol. 10 (2013), https://doi.org/10.1038/nrcardio.2013.77.
[152] H.G. Sundararaghavan, J.A. Burdick, 5.509 - Cell encapsulation, in, Compr. Biomater., Elsevier (2011) 115–130, https://doi.org/10.1081/e-ebpp-120052545.
[153] J.W. Weisel, R.I. Litvinov, Fibrin formation, structure and properties, Subcell. Biochem. 82 (2017), https://doi.org/10.1007/978-3-319-49674-0_13.
[154] E. Kniazeva, A.J. Putnam, Endothelial cell traction and ECM density influence both capillary morphogenesis and maintenance in 3-D, Am. J. Physiol. - Cell Physiol. 297 (2009), https://doi.org/10.1152/ajpcell.00018.2009.
[155] R.I. Litvinov, J.W. Weisel, Fibrin mechanical properties and their structural origins, Matrix Biol. 60–61 (2017), https://doi.org/10.1016/j.matbio.2016.08.003.
[156] R.I. Litvinov, M. Pieters, Z. de Lange-Loots, J.W. Weisel, Fibrinogen and Fibrin, Subcell. Biochem. (2021), https://doi.org/10.1007/978-3-030-58971-4_15.
[157] I.N. Chernysh, C. Nagaswami, P.K. Purohit, J.W. Weisel, Fibrin clots are equilibrium polymers that can be remodeled without proteolytic digestion, Sci. Rep. 2 (2012), https://doi.org/10.1038/srep00879.
[158] G.W. Nelb, G.W. Kamykowski, J.D. Ferry, Rheology of fibrin clots. v. shear modulus, creep, and creep recovery of fine unligated clots, Biophys. Chem. 13 (1981), https://doi.org/10.1016/0301-4622(81)80020-8.
[159] J.H. Kristensen, M.A. Karsdal, Elastin, in: Biochem. Collagens Laminins Elastin Struct. Funct. Biomark., 2016. https://doi.org/10.1016/B978-0-12-809847-9.00030-1.
[160] C.M. Halabi, R.P. Mecham, Elastin purification and solubilization, Methods Cell Biol. (2018), https://doi.org/10.1016/bs.mcb.2017.08.012.
[161] J. Ozsvar, S.M. Mithieux, R. Wang, A.S. Weiss, Elastin-based biomaterials and mesenchymal stem cells, Biomater. Sci. 3 (2015), https://doi.org/10.1039/c5bm00038f.
[162] J.F. Almine, D.V. Bax, S.M. Mithieux, L.N. Smith, J. Rnjak, A. Waterhouse, S. G. Wise, A.S. Weiss, Elastin-based materials, Chem. Soc. Rev. 39 (2010), https://doi.org/10.1039/b919452p.
[163] J.C. Rodriguez-Cabello, I. Gonzalez De Torre, M. González-Pérez, F. González-Pérez, I. Montequi, Fibrous Scaffolds From Elastin-Based Materials, Front. Bioeng. Biotechnol. 9 (2021), https://doi.org/10.3389/fbioe.2021.652384.
[164] I. Gonzalez de Torre, M. Alonso, J.C. Rodriguez-Cabello, Elastin-Based Materials: Promising Candidates for Cardiac Tissue Regeneration, Front. Bioeng. Biotechnol. 8 (2020), https://doi.org/10.3389/fbioe.2020.00657.
[165] J.B. Leach, J.B. Wolinsky, P.J. Stone, J.Y. Wong, Crosslinked α-elastin biomaterials: Towards a processable elastin mimetic scaffold, Acta Biomater. 1 (2005), https://doi.org/10.1016/j.actbio.2004.12.001.
[166] N. Afratis, C. Gialeli, D. Nikitovic, T. Tsegenidis, E. Karousou, A.D. Theocharis, M. S. Pavão, G.N. Tzanakakis, N.K. Karamanos, Glycosaminoglycans: Key players in cancer cell biology and treatment, FEBS J. 279 (2012), https://doi.org/10.1111/j.1742-4658.2012.08529.x.
[167] M.I. Neves, M. Araújo, L. Moroni, R.M.P. Da Silva, C.C. Barrias, Glycosaminoglycan-inspired biomaterials for the development of bioactive hydrogel networks, Molecules 25 (2020), https://doi.org/10.3390/molecules25040978.
[168] S. Mizumoto, D. Fongmoon, K. Sugahara, Interaction of chondroitin sulfate and dermatan sulfate from various biological sources with heparin-binding growth factors and cytokines, Glycoconj. J. 30 (2013), https://doi.org/10.1007/s10719-012-9463-5.
[169] P.D. Mosier, C. Krishnasamy, G.E. Kellogg, U.R. Desai, On the Specificity of Heparin/Heparan Sulfate Binding to Proteins. Anion-Binding Sites on Antithrombin and Thrombin Are Fundamentally Different, PLoS ONE 7 (2012), https://doi.org/10.1371/journal.pone.0048632.
[170] K.M. Sepuru, K. Rajarathnam, Structural basis of a chemokine heterodimer binding to glycosaminoglycans, Biochem. J. 478 (2021), https://doi.org/10.1042/BCJ20200927.
[171] N.K. Karamanos, Z. Piperigkou, A.D. Theocharis, H. Watanabe, M. Franchi, S. Baud, S. Brézillon, M. Götte, A. Passi, D. Vigetti, S. Ricard-Blum, R. D. Sanderson, T. Neill, R.V. Iozzo, Proteoglycan chemical diversity drives multifunctional cell regulation and therapeutics, Chem. Rev. 118 (2018), https://doi.org/10.1021/acs.chemrev.8b00354.
[172] M. Raspanti, M. Viola, A. Forlino, R. Tenni, C. Gruppi, M.E. Tira, Glycosaminoglycans show a specific periodic interaction with type I collagen fibrils, J. Struct. Biol. 164 (2008), https://doi.org/10.1016/j.jsb.2008.07.001.
[173] C. Chircov, A.M. Grumezescu, L.E. Bejenaru, Hyaluronic acid-based scaffolds for tissue engineering, Rom. J. Morphol. Embryol. 59 (2018).
[174] D. Pankajakshan, D.K. Agrawal, Scaffolds in tissue engineering of blood vessels, Can. J. Physiol. Pharmacol. 88 (2010), https://doi.org/10.1139/Y10-073.
[175] W.F. Marei, F. Ghafari, A.A. Fouladi-Nashta, Role of hyaluronic acid in maturation and further early embryo development of bovine oocytes, Theriogenology 78 (2012), https://doi.org/10.1016/j.theriogenology.2012.03.013.
[176] Y. Ren, H. Zhang, Y. Wang, B. Du, J. Yang, L. Liu, Q. Zhang, Hyaluronic Acid Hydrogel with Adjustable Stiffness for Mesenchymal Stem Cell 3D Culture via Related Molecular Mechanisms to Maintain Stemness and Induce Cartilage Differentiation, ACS Appl. Bio Mater. 4 (2021), https://doi.org/10.1021/acsabm.0c01591.
[177] A. Bauer, L. Gu, B. Kwee, W.A. Li, M. Dellacherie, A.D. Celiz, D.J. Mooney, Hydrogel substrate stress-relaxation regulates the spreading and proliferation of mouse myoblasts, Acta Biomater. 62 (2017), https://doi.org/10.1016/j.actbio.2017.08.041.
[178] S. Nam, R. Stowers, J. Lou, Y. Xia, O. Chaudhuri, Varying PEG density to control stress relaxation in alginate-PEG hydrogels for 3D cell culture studies, Biomaterials 200 (2019), https://doi.org/10.1016/j.biomaterials.2019.02.004.
[179] S. Khunmanee, Y. Jeong, H. Park, Crosslinking method of hyaluronic-based hydrogel for biomedical applications, J. Tissue Eng. 8 (2017), https://doi.org/10.1177/2041731417726464.
[180] M.I. Wahba, Enhancement of the mechanical properties of chitosan, J. Biomater. Sci. Polym. Ed. 31 (2020), https://doi.org/10.1080/09205063.2019.1692641.
[181] Z.H. Zhang, Z. Han, X.A. Zeng, X.Y. Xiong, Y.J. Liu, Enhancing mechanical properties of chitosan films via modification with vanillin, Int. J. Biol. Macromol. 81 (2015), https://doi.org/10.1016/j.ijbiomac.2015.08.042.
[182] S. Prateepchanachai, W. Thakhiew, S. Devahastin, S. Soponronnarit, Mechanical properties improvement of chitosan films via the use of plasticizer, charge

modifying agent and film solution homogenization, Carbohydr. Polym. 174 (2017), https://doi.org/10.1016/j.carbpol.2017.06.069.
[183] F. Ahmadi, Z. Oveisi, M. Samani, Z. Amoozgar, Chitosan based hydrogels: Characteristics and pharmaceutical applications, Res. Pharm. Sci. 10 (2015).
[184] D. Elieh-Ali-Komi, M.R. Hamblin, Chitin and Chitosan: Production and Application of Versatile Biomedical Nanomaterials, Int. J. Adv. Res. 4 (2016).
[185] A. De Masi, I. Tonazzini, C. Masciullo, R. Mezzena, F. Chiellini, D. Puppi, M. Cecchini, Chitosan films for regenerative medicine: fabrication methods and mechanical characterization of nanostructured chitosan films, Biophys. Rev. 11 (2019), https://doi.org/10.1007/s12551-019-00591-6.
[186] A. Aryaei, A.H. Jayatissa, A.C. Jayasuriya, Nano and micro mechanical properties of uncross-linked and cross-linked chitosan films, J. Mech. Behav. Biomed. Mater. 5 (2012), https://doi.org/10.1016/j.jmbbm.2011.08.006.
[187] Z.M. Jessop, N. Gao, S. Manivannan, A. Al-Sabah, I.S. Whitaker, 3D bioprinting cartilage, in: 3D Bioprinting Reconstr. Surg. Tech. Appl., 2018. https://doi.org/10.1016/B978-0-08-101103-4.00034-X.
[188] T.K. Merceron, S.V. Murphy, Hydrogels for 3D bioprinting applications, in: Essent. 3D Biofabrication Transl., 2015. https://doi.org/10.1016/B978-0-12-800972-7.00014-1.
[189] M. Tako, S. Nakamura, Gelation mechanism of agarose, Carbohydr. Res. 180 (1988), https://doi.org/10.1016/0008-6215(88)80084-3.
[190] J.Y. Xiong, J. Narayanan, X.Y. Liu, T.K. Chong, S.B. Chen, T.S. Chung, Topology evolution and gelation mechanism of agarose gel, J. Phys. Chem. B 109 (2005), https://doi.org/10.1021/jp044473u.
[191] N.E. Fedorovich, J.R. De Wijn, A.J. Verbout, J. Alblas, W.J.A. Dhert, Three-dimensional fiber deposition of cell-laden, viable, patterned constructs for bone tissue printing, Tissue Eng. - Part A 14 (2008), https://doi.org/10.1089/ten.a.2007.0158.
[192] D.F.D. Campos, A. Blaeser, A. Korsten, S. Neuss, J. Jäkel, M. Vogt, H. Fischer, The stiffness and structure of three-dimensional printed hydrogels direct the differentiation of mesenchymal stromal cells toward adipogenic and osteogenic lineages, Tissue Eng. - Part A 21 (2015), https://doi.org/10.1089/ten.tea.2014.0231.
[193] Y.B. Lee, S. Polio, W. Lee, G. Dai, L. Menon, R.S. Carroll, S.S. Yoo, Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture, Exp. Neurol. 223 (2010), https://doi.org/10.1016/j.expneurol.2010.02.014.
[194] G.C. Ingavle, S.H. Gehrke, M.S. Detamore, The bioactivity of agarose-PEGDA interpenetrating network hydrogels with covalently immobilized RGD peptides and physically entrapped aggrecan, Biomaterials 35 (2014), https://doi.org/10.1016/j.biomaterials.2014.01.002.
[195] A. Ed-Daoui, M. Benelmostafa, M. Dahmani, Study of the viscoelastic properties of the agarose gel, in: Mater. Today Proc., 2019. https://doi.org/10.1016/j.matpr.2019.04.036.
[196] D.E. Heath, A Review of Decellularized Extracellular Matrix Biomaterials for Regenerative Engineering Applications, Regen. Eng. Transl. Med. 5 (2019), https://doi.org/10.1007/s40883-018-0080-0.
[197] M. Kawecki, W. Łabuś, A. Klama-Baryla, D. Kitala, M. Kraut, J. Glik, M. Misiuga, M. Nowak, T. Bielecki, A. Kasperczyk, A review of decellurization methods caused by an urgent need for quality control of cell-free extracellular matrix' scaffolds and their role in regenerative medicine, J. Biomed. Mater. Res. - Part B Appl. Biomater. 106 (2018), https://doi.org/10.1002/jbm.b.33865.
[198] C.L. Dearth, T.J. Keane, C.A. Carruthers, J.E. Reing, L. Huleihel, C.A. Ranallo, E. W. Kollar, S.F. Badylak, The effect of terminal sterilization on the material properties and in vivo remodeling of a porcine dermal biologic scaffold, Acta Biomater. 33 (2016), https://doi.org/10.1016/j.actbio.2016.01.038.
[199] Q. Yao, Y.W. Zheng, H.L. Lin, Q.H. Lan, Z.W. Huang, L.F. Wang, R. Chen, J. Xiao, L. Kou, H.L. Xu, Y.Z. Zhao, Exploiting crosslinked decellularized matrix to achieve uterus regeneration and construction, Artif. Cells Nanomedicine Biotechnol. 48 (2020), https://doi.org/10.1080/21691401.2019.1699828.
[200] A.R. Massensini, H. Ghuman, L.T. Saldin, C.J. Medberry, T.J. Keane, F.J. Nicholls, S.S. Velankar, S.F. Badylak, M. Modo, Concentration-dependent rheological properties of ECM hydrogel for intracerebral delivery to a stroke cavity, Acta Biomater. 27 (2015), https://doi.org/10.1016/j.actbio.2015.08.040.
[201] O. Wichterle, D. Lím, Hydrophilic Gels for Biological Use, Nature 185 (1960), https://doi.org/10.1038/185117a0.
[202] A. Sosnik, D. Cohn, Reverse thermo-responsive poly(ethylene oxide) and poly (propylene oxide) multiblock copolymers, Biomaterials 26 (2005), https://doi.org/10.1016/j.biomaterials.2004.02.041.
[203] F. Markus, J.R. Bruckner, S. Naumann, Controlled Synthesis of "Reverse Pluronic"-Type Block Copolyethers with High Molar Masses for the Preparation of Hydrogels with Improved Mechanical Properties, Macromol. Chem. Phys. 221 (2020), https://doi.org/10.1002/macp.201900437.
[204] C.C. Lin, K.S. Anseth, PEG hydrogels for the controlled release of biomolecules in regenerative medicine, Pharm. Res. 26 (2009), https://doi.org/10.1007/s11095-008-9801-2.
[205] S.J. Bryant, K.S. Anseth, D.A. Lee, D.L. Bader, Crosslinking density influences the morphology of chondrocytes photoencapsulated in PEG hydrogels during the application of compressive strain, J. Orthop. Res. 22 (2004), https://doi.org/10.1016/j.orthres.2004.02.001.
[206] Q.T. Nguyen, Y. Hwang, A.C. Chen, S. Varghese, R.L. Sah, Cartilage-like mechanical properties of poly (ethylene glycol)-diacrylate hydrogels, Biomaterials 33 (2012), https://doi.org/10.1016/j.biomaterials.2012.06.005.
[207] C.N. Salinas, K.S. Anseth, The influence of the RGD peptide motif and its contextual presentation in PEG gels on human mesenchymal stem cell viability, J. Tissue Eng. Regen. Med. 2 (2008), https://doi.org/10.1002/term.95.
[208] S. Vedaraman, D. Bernhagen, T. Haraszti, C. Licht, A. Castro Nava, A. Omidinia Anarkoli, P. Timmerman, L. De Laporte, Bicyclic RGD peptides enhance nerve growth in synthetic PEG-based Anisogels, Biomater. Sci. 9 (2021), https://doi.org/10.1039/d0bm02051f.
[209] A.I. Van Den Bulcke, B. Bogdanov, N. De Rooze, E.H. Schacht, M. Cornelissen, H. Berghmans, Structural and rheological properties of methacrylamide modified gelatin hydrogels, Biomacromolecules 1 (2000), https://doi.org/10.1021/bm990017d.
[210] C.G.Y. Ngan, A. Quigley, R.J. Williams, C.D. O'Connell, R. Blanchard, M. Boyd-Moss, T.D. Aumann, P. McKelvie, G.G. Wallace, P.F.M. Choong, R.M.I. Kapsa, Matured Myofibers in Bioprinted Constructs with In Vivo Vascularization and Innervation, Gels 7 (2021), https://doi.org/10.3390/gels7040171.
[211] I. Pepelanova, K. Kruppa, T. Scheper, A. Lavrentieva, Gelatin-methacryloyl (GelMA) hydrogels with defined degree of functionalization as a versatile toolkit for 3D cell culture and extrusion bioprinting, Bioengineering 5 (2018), https://doi.org/10.3390/bioengineering5030055.
[212] L. Xiang, W. Cui, Biomedical application of photo-crosslinked gelatin hydrogels, J. Leather Sci. Eng. 3 (2021), https://doi.org/10.1186/s42825-020-00043-y.
[213] J.A. Benton, C.A. Deforest, V. Vivekanandan, K.S. Anseth, Photocrosslinking of gelatin macromers to synthesize porous hydrogels that promote valvular interstitial cell function, Tissue Eng. - Part A 15 (2009), https://doi.org/10.1089/ten.tea.2008.0545.
[214] C.D. O'Connell, B. Zhang, C. Onofrillo, S. Duchi, R. Blanchard, A. Quigley, J. Bourke, S. Gambhir, R. Kapsa, C. Di Bella, P. Choong, G.G. Wallace, Tailoring the mechanical properties of gelatin methacryloyl hydrogels through manipulation of the photocrosslinking conditions, Soft Matter 14 (2018), https://doi.org/10.1039/c7sm02187a.
[215] C.H. Lin, J.J.M. Su, S.Y. Lee, Y.M. Lin, Stiffness modification of photopolymerizable gelatin-methacrylate hydrogels influences endothelial differentiation of human mesenchymal stem cells, J. Tissue Eng. Regen. Med. 12 (2018) 2099–2111, https://doi.org/10.1002/term.2745.
[216] M. Boyd-Moss, K. Firipis, A. Quigley, A. Rifai, A. Cichocki, S. Whitty, C. Ngan, C. Dekiwadia, B. Long, D.R. Nisbet, R. Kapsa, R.J. Williams, Hybrid Self-Assembling Peptide/Gelatin Methacrylate (GelMA) Bioink Blend for Improved Bioprintability and Primary Myoblast Response, Adv. NanoBiomed Res. (2021), https://doi.org/10.1002/anbr.202100106.
[217] N. Celikkin, S. Mastrogiacomo, J. Jaroszewicz, X.F. Walboomers, W. Swieszkowski, Gelatin methacrylate scaffold for bone tissue engineering: The influence of polymer concentration, J. Biomed. Mater. Res. - Part A 106 (2018), https://doi.org/10.1002/jbm.a.36226.
[218] M. Boyd-Moss, K. Firipis, C.D. O'Connell, A. Rifai, A. Quigley, G. Boer, B.M. Long, D.R. Nisbet, R.J. Williams, Shining a light on the hidden structure of gelatin methacryloyl bioinks using small-angle X-ray scattering (SAXS), Mater. Chem. Front. 5 (2021), https://doi.org/10.1039/d1qm01010g.
[219] S. Alshehri, H.H. Susapto, C.A.E. Hauser, Scaffolds from self-assembling tetrapeptides support 3D spreading, osteogenic differentiation, and angiogenesis of mesenchymal stem cells, Biomacromolecules 22 (2021), https://doi.org/10.1021/acs.biomac.1c00205.
[220] B. Yi, Y. Shen, H. Tang, X. Wang, B. Li, Y. Zhang, Stiffness of Aligned Fibers Regulates the Phenotypic Expression of Vascular Smooth Muscle Cells, ACS Appl. Mater. Interfaces 11 (2019), https://doi.org/10.1021/acsami.9b00293.
[221] J. He, Y. Hu, F. Wu, B. He, W. Gao, Control of MSC Differentiation by Tuning the Alkyl Chain Length of Phenylboroinc Acid Based Low-molecular-weight Gelators, J. Bionic Eng. 15 (2018), https://doi.org/10.1007/s42235-018-0056-2.
[222] S. Park, S.I. Kim, J.H. Choi, S.E. Kim, S.H. Choe, Y. Son, T.W. Kang, J.E. Song, G. Khang, Evaluation of Silk Fibroin/Gellan Gum Hydrogels with Controlled Molecular Weight through Silk Fibroin Hydrolysis for Tissue Engineering Application, Molecules 28 (2023), https://doi.org/10.3390/molecules28135222.
[223] R.S. Navarro, M.S. Huang, J.G. Roth, K.M. Hubka, C.M. Long, A. Enejder, S. C. Heilshorn, Tuning Polymer Hydrophilicity to Regulate Gel Mechanics and Encapsulated Cell Morphology, Adv. Healthc. Mater. 11 (2022), https://doi.org/10.1002/adhm.202200011.
[224] A. Lavrentieva, T. Fleischhammer, A. Enders, H. Pirmahboub, J. Bahnemann, I. Pepelanova, Fabrication of Stiffness Gradients of GelMA Hydrogels Using a 3D Printed Micromixer, Macromol. Biosci. 20 (2020), https://doi.org/10.1002/mabi.202000107.
[225] L.D. Morton, D.A. Castilla-Casadiego, A.C. Palmer, A.M. Rosales, Crosslinker structure modulates bulk mechanical properties and dictates hMSC behavior on hyaluronic acid hydrogels, Acta Biomater. 155 (2023), https://doi.org/10.1016/j.actbio.2022.11.027.
[226] A. Dodero, S. Scarfi, S. Mirata, A. Sionkowska, S. Vicini, M. Alloisio, M. Castellano, Effect of crosslinking type on the physical-chemical properties and biocompatibility of chitosan-based electrospun membranes, Polymers 13 (2021), https://doi.org/10.3390/polym13050831.
[227] X. Li, S. Liu, S. Han, Q. Sun, J. Yang, Y. Zhang, Y. Jiang, X. Wang, Q. Li, J. Wang, Dynamic Stiffening Hydrogel with Instructive Stiffening Timing Modulates Stem Cell Fate In Vitro and Enhances Bone Remodeling In Vivo, Adv. Healthc. Mater. 12 (2023), https://doi.org/10.1002/adhm.202300326.
[228] P.C. Dromel, D. Singh, E. Andres, M. Likes, M. Kurisawa, A. Alexander-Katz, M. Spector, M. Young, A bioinspired gelatin-hyaluronic acid-based hybrid interpenetrating network for the enhancement of retinal ganglion cells replacement therapy, Npj Regen. Med. 6 (2021), https://doi.org/10.1038/s41536-021-00195-3.
[229] N. Lagneau, P. Tournier, B. Halgand, F. Loll, Y. Maugars, J. Guicheux, C. Le Visage, V. Delplace, Click and bioorthogonal hyaluronic acid hydrogels as an

ultra-tunable platform for the investigation of cell-material interactions, Bioact. Mater. 24 (2023), https://doi.org/10.1016/j.bioactmat.2022.12.022.
[230] F. Nindiyasari, L. Fernández-Díaz, E. Griesshaber, J.M. Astilleros, N. Sánchez-Pastor, W.W. Schmahl, Influence of gelatin hydrogel porosity on the crystallization of CaCO 3, Cryst. Growth Des. 14 (2014), https://doi.org/10.1021/cg401056t.
[231] M. Ahearne, Introduction to cell-hydrogel mechanosensing, Interface Focus 4 (2014), https://doi.org/10.1098/rsfs.2013.0038.
[232] L. Figueiredo, R. Pace, C. D'Arros, G. Réthoré, J. Guicheux, C. Le Visage, P. Weiss, Assessing glucose and oxygen diffusion in hydrogels for the rational design of 3D stem cell scaffolds in regenerative medicine, J. Tissue Eng. Regen. Med. 12 (2018), https://doi.org/10.1002/term.2656.
[233] X.N. Zhang, Q. Zheng, Z.L. Wu, Recent advances in 3D printing of tough hydrogels: A review, Compos. Part B Eng. 238 (2022), https://doi.org/10.1016/j.compositesb.2022.109895.
[234] F. Valente, M.S. Hepburn, J. Chen, A.A. Aldana, B.J. Allardyce, S. Shafei, B. J. Doyle, B.F. Kennedy, R.J. Dilley, Bioprinting silk fibroin using two-photon lithography enables control over the physico-chemical material properties and cellular response, Bioprinting 25 (2022), https://doi.org/10.1016/j.bprint.2021.e00183.
[235] A.D. Lantada, N.M. Picazo, M. Guttmann, M. Wissmann, M. Schneider, M. Worgull, S. Hengsbach, F. Rupp, K. Bade, G.R. Plaza, Soft-lithography of polyacrylamide hydrogels using microstructured templates: Towards controlled cell populations on biointerfaces, Materials 13 (2020), https://doi.org/10.3390/ma13071586.
[236] J.W. Lee, K.H. Song, Fibrous hydrogels by electrospinning: Novel platforms for biomedical applications, J. Tissue Eng. 14 (2023), https://doi.org/10.1177/20417314231191881.
[237] M.K. Shin, S.I. Kim, S.J. Kim, S.K. Kim, H. Lee, G.M. Spinks, Size-dependent elastic modulus of single electroactive polymer nanofibers, Appl. Phys. Lett. 89 (2006), https://doi.org/10.1063/1.2402941.
[238] L. Sun, R.P.S. Han, J. Wang, C.T. Lim, Modeling the size-dependent elastic properties of polymeric nanofibers, Nanotechnology 19 (2008), https://doi.org/10.1088/0957-4484/19/45/455706.
[239] J. Wang, B. Yuan, R.P.S. Han, Modulus of elasticity of randomly and aligned polymeric scaffolds with fiber size dependency, J. Mech. Behav. Biomed. Mater. 77 (2018), https://doi.org/10.1016/j.jmbbm.2017.09.016.
[240] K. Tonsomboon, A.L. Butcher, M.L. Oyen, Strong and tough nanofibrous hydrogel composites based on biomimetic principles, Mater. Sci. Eng. C 72 (2017), https://doi.org/10.1016/j.msec.2016.11.025.
[241] K. Zhang, H. Lv, Y. Zheng, Y. Yao, X. Li, J. Yu, B. Ding, Nanofibrous hydrogels embedded with phase-change materials: Temperature-responsive dressings for accelerating skin wound healing, Compos. Commun. 25 (2021), https://doi.org/10.1016/j.coco.2021.100752.
[242] B. Wang, T. Xin, L. Shen, K. Zhang, D. Zhang, H. Zhang, J. Liu, B. Chen, W. Cui, Y. Shu, Acoustic transmitted electrospun fibrous membranes for tympanic membrane regeneration, Chem. Eng. J. 419 (2021), https://doi.org/10.1016/j.cej.2021.129536.
[243] C. Chen, J. Tang, Y. Gu, L. Liu, X. Liu, L. Deng, C. Martins, B. Sarmento, W. Cui, L. Chen, Bioinspired Hydrogel Electrospun Fibers for Spinal Cord Regeneration, Adv. Funct. Mater. 29 (2019), https://doi.org/10.1002/adfm.201806899.
[244] F. Qu, J.L. Holloway, J.L. Esterhai, J.A. Burdick, R.L. Mauck, Programmed biomolecule delivery to enable and direct cell migration for connective tissue repair, Nat. Commun. 8 (2017), https://doi.org/10.1038/s41467-017-01955-w.
[245] M.D. Davidson, M.E. Prendergast, E. Ban, K.L. Xu, G. Mickel, P. Mensah, A. Dhand, P.A. Janmey, V.B. Shenoy, J.A. Burdick, Programmable and contractile materials through cell encapsulation in fibrous hydrogel assemblies, Sci. Adv. 7 (2021), https://doi.org/10.1126/sciadv.abi8157.
[246] J.S. Lee, K.H. Choi, H.D. Ghim, S.S. Kim, D.H. Chun, H.Y. Kim, W.S. Lyoo, Role of molecular weight of atactic poly(vinyl alcohol) (PVA) in the structure and properties of PVA nanofabric prepared by electrospinning, J. Appl. Polym. Sci. 93 (2004), https://doi.org/10.1002/app.20602.
[247] M. Koosha, M. Raoufi, H. Moravvej, One-pot reactive electrospinning of chitosan/PVA hydrogel nanofibers reinforced by halloysite nanotubes with enhanced fibroblast cell attachment for skin tissue regeneration, Colloids Surf. B Biointerfaces 179 (2019), https://doi.org/10.1016/j.colsurfb.2019.03.054.
[248] G.J. Kim, K.O. Kim, Novel glucose-responsive of the transparent nanofiber hydrogel patches as a wearable biosensor via electrospinning, Sci. Rep. 10 (2020), https://doi.org/10.1038/s41598-020-75906-9.
[249] Y. Gu, C. Chen, J. Rufo, C. Shen, Z. Wang, P.H. Huang, H. Fu, P. Zhang, S. A. Cummer, Z. Tian, T.J. Huang, Acoustofluidic holography for micro- To nanoscale particle manipulation, ACS Nano 14 (2020), https://doi.org/10.1021/acsnano.0c03754.
[250] N. Weigel, Y. Li, A. Fery, J. Thiele, From microfluidics to hierarchical hydrogel materials, Curr. Opin. Colloid Interface Sci. 64 (2023), https://doi.org/10.1016/j.cocis.2022.101673.
[251] C.B. Highley, K.H. Song, A.C. Daly, J.A. Burdick, Jammed Microgel Inks for 3D Printing Applications, Adv. Sci. 6 (2019), https://doi.org/10.1002/advs.201801076.
[252] J. Kopeček, Hydrogels: From soft contact lenses and implants to self-assembled nanomaterials, J. Polym. Sci. Part Polym. Chem. 47 (2009), https://doi.org/10.1002/pola.23607.
[253] J.M. Godbe, R. Freeman, L.F. Burbulla, J. Lewis, D. Krainc, S.I. Stupp, Gelator Length Precisely Tunes Supramolecular Hydrogel Stiffness and Neuronal Phenotype in 3D Culture, ACS Biomater. Sci. Eng. 6 (2020) 1196–1207, https://doi.org/10.1021/acsbiomaterials.9b01585.
[254] A.L. Rodriguez, T.Y. Wang, K.F. Bruggeman, R. Li, R.J. Williams, C.L. Parish, D. R. Nisbet, Tailoring minimalist self-assembling peptides for localized viral vector gene delivery, Nano Res. 9 (2016), https://doi.org/10.1007/s12274-015-0946-0.
[255] S. Boothroyd, A. Saiani, A.F. Miller, Controlling network topology and mechanical properties of co-assembling peptide hydrogels, Biopolymers 101 (2014), https://doi.org/10.1002/bip.22435.
[256] A. Scelsi, B. Bochicchio, A. Smith, V.L. Workman, L.A. Castillo Diaz, A. Saiani, A. Pepe, Tuning of hydrogel stiffness using a two-component peptide system for mammalian cell culture, J. Biomed. Mater. Res. - Part A 107 (2019) 535–544, https://doi.org/10.1002/jbm.a.36568.
[257] S. Browne, S. Hossainy, K. Healy, Hyaluronic Acid Macromer Molecular Weight Dictates the Biophysical Properties and in Vitro Cellular Response to Semisynthetic Hydrogels, ACS Biomater. Sci. Eng. 6 (2020), https://doi.org/10.1021/acsbiomaterials.9b01419.
[258] R.J. Sifri, O. Padilla-Vélez, G.W. Coates, B.P. Fors, Controlling the Shape of Molecular Weight Distributions in Coordination Polymerization and Its Impact on Physical Properties, J. Am. Chem. Soc. 142 (2020), https://doi.org/10.1021/jacs.9b11462.
[259] H.J. Kong, D. Kaigler, K. Kim, D.J. Mooney, Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution, Biomacromolecules 5 (2004), https://doi.org/10.1021/bm049879r.
[260] C. Long, Z. Dong, X. Liu, F. Yu, Y. Shang, K. Wang, S. Feng, X. Hou, C. He, Z. R. Chen, Simultaneous enhancement in processability and mechanical properties of polyethylenes via tuning the molecular weight distribution from unimodal to bimodal shape, Polymer 258 (2022), https://doi.org/10.1016/j.polymer.2022.125287.
[261] A. Moyassari, T. Gkourmpis, M.S. Hedenqvist, U.W. Gedde, Molecular dynamics simulation of linear polyethylene blends: Effect of molar mass bimodality on topological characteristics and mechanical behavior, Polymer 161 (2019), https://doi.org/10.1016/j.polymer.2018.12.012.
[262] C. Long, Z. Dong, K. Wang, F. Yu, C. He, Z.R. Chen, Molecular weight distribution shape approach for simultaneously enhancing the stiffness, ductility and strength of isotropic semicrystalline polymers based on linear unimodal and bimodal polyethylenes, Polymer 275 (2023), https://doi.org/10.1016/j.polymer.2023.125936.
[263] R. Brighenti, M. Montanari, S. Monchetti, T. Hanuhov, A. Spagnoli, N. Cohen, Dependence of stiffness on water content in hydrogels: A statistical mechanics-based framework, Int. J. Solids Struct. 300 (2024) 112920, https://doi.org/10.1016/j.ijsolstr.2024.112920.
[264] A.H. Nissan, H-Bond Dissociation in Hydrogen Bond Dominated Solids, Macromolecules 9 (1976), https://doi.org/10.1021/ma60053a026.
[265] P.J. Flory, In Principles of polymer chemistry, 1953.
[266] R.P. Sijbesma, E.W. Meijer, Quadruple hydrogen bonded systems, Chem. Commun. (2003), https://doi.org/10.1039/b205873c.
[267] X.N. Zhang, Y.J. Wang, S. Sun, L. Hou, P. Wu, Z.L. Wu, Q. Zheng, A Tough and Stiff Hydrogel with Tunable Water Content and Mechanical Properties Based on the Synergistic Effect of Hydrogen Bonding and Hydrophobic Interaction, Macromolecules 51 (2018), https://doi.org/10.1021/acs.macromol.8b01496.
[268] Z. Shen, J. Ma, Y. Cai, S. Li, D. Ruan, S. Dai, Z. Sheng, J. Bai, D. Yin, J. Ping, Y. Ying, C. Yang, S. Qu, Z. Jia, Low-water-content polyelectrolyte hydrogels inspired by human epidermal stratum corneum, Cell Rep. Phys. Sci. 4 (2023), https://doi.org/10.1016/j.xcrp.2023.101741.
[269] S. Lin, L. Gu, Influence of crosslink density and stiffness on mechanical properties of type I collagen gel, Materials 8 (2015), https://doi.org/10.3390/ma8020551.
[270] H. Chavda, C. Patel, Effect of crosslinker concentration on characteristics of superporous hydrogel, Int. J. Pharm. Investig. 1 (2011), https://doi.org/10.4103/2230-973x.76724.
[271] A.K. Denisin, B.L. Pruitt, Tuning the Range of Polyacrylamide Gel Stiffness for Mechanobiology Applications, ACS Appl. Mater. Interfaces 8 (2016), https://doi.org/10.1021/acsami.5b09344.
[272] D. Calvet, J.Y. Wong, S. Giasson, Rheological monitoring of polyacrylamide gelation: Importance of cross-link density and temperature, Macromolecules 37 (2004), https://doi.org/10.1021/ma049072r.
[273] L.M. Lira, K.A. Martins, S.I.C. de Torresi, Structural parameters of polyacrylamide hydrogels obtained by the Equilibrium Swelling Theory, Eur. Polym. J. 45 (2009), https://doi.org/10.1016/j.eurpolymj.2008.12.022.
[274] D.C. Schoenmakers, A.E. Rowan, P.H.J. Kouwer, Crosslinking of fibrous hydrogels, Nat. Commun. 9 (2018), https://doi.org/10.1038/s41467-018-04508-x.
[275] A. de Miguel-Jiménez, B. Ebeling, J.I. Paez, C. Fink-Straube, S. Pearson, A. del Campo, Gelation Kinetics and Mechanical Properties of Thiol-Tetrazole Methylsulfone Hydrogels Designed for Cell Encapsulation, Macromol. Biosci. 23 (2023), https://doi.org/10.1002/mabi.202200419.
[276] A.M. Oelker, S.M. Morey, L.G. Griffith, P.T. Hammond, Helix versus coil polypeptide macromers: Gel networks with decoupled stiffness and permeability, Soft Matter 8 (2012), https://doi.org/10.1039/c2sm26487k.
[277] C.C. Ahrens, M.E. Welch, L.G. Griffith, P.T. Hammond, Uncharged Helical Modular Polypeptide Hydrogels for Cellular Scaffolds, Biomacromolecules 16 (2015), https://doi.org/10.1021/acs.biomac.5b01076.
[278] D.A. Castilla-Casadiego, L.D. Morton, D.H. Loh, A. Pineda-Hernandez, A. P. Chavda, F. Garcia, A.M. Rosales, Peptoid-Cross-Linked Hydrogel Stiffness Modulates Human Mesenchymal Stromal Cell Immunoregulatory Potential in the Presence of Interferon-Gamma, Macromol. Biosci. 24 (2024), https://doi.org/10.1002/mabi.202400111.
[279] L. Liu, J.A. Shadish, C.K. Arakawa, K. Shi, J. Davis, C.A. DeForest, Cyclic Stiffness Modulation of Cell-Laden Protein–Polymer Hydrogels in Response to User-

Specified Stimuli Including Light, Adv. Biosyst. 2 (2018), https://doi.org/10.1002/adbi.201800240.
[280] N. Zinkovska, J. Smilek, M. Pekar, Gradient hydrogels-The state of the art in preparation methods, Polymers 12 (2020), https://doi.org/10.3390/POLYM12040966.
[281] N. Zinkovska, M. Pekar, J. Smilek, Gradient Hydrogels—Overview of Techniques Demonstrating the Existence of a Gradient, Polymers 14 (2022), https://doi.org/10.3390/polym14050866.
[282] W.J. Hadden, J.L. Young, A.W. Holle, M.L. McFetridge, D.Y. Kim, P. Wijesinghe, H. Taylor-Weiner, J.H. Wen, A.R. Lee, K. Bieback, B.N. Vo, D.D. Sampson, B. F. Kennedy, J.P. Spatz, A.J. Engler, Y.S. Cho, Stem cell migration and mechanotransduction on linear stiffness gradient hydrogels, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 5647–5652, https://doi.org/10.1073/pnas.1618239114.
[283] K. Mayumi, C. Liu, Y. Yasuda, K. Ito, Softness, elasticity, and toughness of polymer networks with slide-ring cross-links, Gels 7 (2021), https://doi.org/10.3390/gels7030091.
[284] T. Murakami, B.V.K.J. Schmidt, H.R. Brown, C.J. Hawker, Structural Versatility in Slide-Ring Gels: Influence of Co-Threaded Cyclodextrin Spacers, J. Polym. Sci. Part Polym. Chem. 55 (2017), https://doi.org/10.1002/pola.28490.
[285] J. Yan, M. Li, Z. Wang, C. Chen, C. Ma, G. Yang, Highly tough, multi-stimuli-responsive, and fast self-healing supramolecular networks toward strain sensor application, Chem. Eng. J. 389 (2020), https://doi.org/10.1016/j.cej.2019.123468.
[286] R. Pethrick, Polymer physics. Edited by Michael Rubinstein and Ralph H Colby Oxford University Press, Oxford, 2003. ISBN 019852059X. pp 440, Polym. Int. 53 (2004). https://doi.org/10.1002/pi.1472.
[287] A.N. Gent, R.H. Tobias, Threshold tear strength of elastomers, J. Polym. Sci. Polym. Phys. Ed. 20 (1982) 2051–2058, https://doi.org/10.1002/pol.1982.180201107.
[288] G.J. Lake, A.G. Thomas, The strength of highly elastic materials, Proc. R. Soc. Lond. Ser. Math. Phys. Sci. (1967), https://doi.org/10.1098/rspa.1967.0160.
[289] J. Kopeček, J. Yang, Hydrogels as smart biomaterials, Polym. Int. 56 (2007), https://doi.org/10.1002/pi.2253.
[290] J. Xu, V. Abetz, Nonionic UCST–LCST Diblock Copolymers with Tunable Thermoresponsiveness Synthesized via PhotoRAFT Polymerization, Macromol. Rapid Commun. 42 (2021), https://doi.org/10.1002/marc.202000648.
[291] N. Yeredla, T. Kojima, Y. Yang, S. Takayama, M. Kanapathipillai, Aqueous Two Phase System Assisted Self-Assembled PLGA Microparticles, Sci. Rep. 6 (2016), https://doi.org/10.1038/srep27736.
[292] K.S. Burts, T.V. Plisko, A.V. Bildyukevich, A.V. Penkova, S.A. Pratsenko, Modification of polysulfone ultrafiltration membranes using block copolymer Pluronic F127, Polym. Bull. 78 (2021), https://doi.org/10.1007/s00289-020-03437-4.
[293] A.M. Akimoto, E.H. Niitsu, K. Nagase, T. Okano, H. Kanazawa, R. Yoshida, Mesenchylmal stem cell culture on poly(N-isopropylacrylamide) hydrogel with repeated thermo-stimulation, Int. J. Mol. Sci. 19 (2018), https://doi.org/10.3390/ijms19041253.
[294] T. Congdon, P. Shaw, M.I. Gibson, Thermoresponsive, well-defined, poly(vinyl alcohol) co-polymers, Polym. Chem. 6 (2015), https://doi.org/10.1039/c5py00775e.
[295] N. Annabi, Y.N. Zhang, A. Assmann, E.S. Sani, G. Cheng, A.D. Lassaletta, A. Vegh, B. Dehghani, G.U. Ruiz-Esparza, X. Wang, S. Gangadharan, A.S. Weiss, A. Khademhosseini, Engineering a highly elastic human protein-based sealant for surgical applications, Sci. Transl. Med. 9 (2017), https://doi.org/10.1126/scitranslmed.aai7466.
[296] N.S. Allen, J. Segurola, M. Edge, E. Santamari, A. McMahon, S. Wilson, A comparative kinetic study of commercial photoinitiators for UV/visible curable acrylate clear coatings, JOCCA - Surf. Coat. Int. 82 (1999), https://doi.org/10.1007/BF02692626.
[297] S. Sheth, E. Jain, A. Karadaghy, S. Syed, H. Stevenson, S.P. Zustiak, UV Dose Governs UV-Polymerized Polyacrylamide Hydrogel Modulus, Int. J. Polym. Sci. 2017 (2017), https://doi.org/10.1155/2017/5147482.
[298] T.B. Dorsey, A. Grath, A. Wang, C. Xu, Y. Hong, G. Dai, Evaluation of photochemistry reaction kinetics to pattern bioactive proteins on hydrogels for biological applications, Bioact. Mater. 3 (2018), https://doi.org/10.1016/j.bioactmat.2017.05.005.
[299] S. Bashir, M. Hina, J. Iqbal, A.H. Rajpar, M.A. Mujtaba, N.A. Alghamdi, S. Wageh, K. Ramesh, S. Ramesh, Fundamental concepts of hydrogels: Synthesis, properties, and their applications, Polymers 12 (2020), https://doi.org/10.3390/polym12112702.
[300] H.Y. Yoshikawa, F.F. Rossetti, S. Kaufmann, T. Kaindl, J. Madsen, U. Engel, A. L. Lewis, S.P. Armes, M. Tanaka, Quantitative evaluation of mechanosensing of cells on dynamically tunable hydrogels, J. Am. Chem. Soc. 133 (2011), https://doi.org/10.1021/ja1060615.
[301] M. Zarur, A. Seijo-Rabina, A. Goyanes, A. Concheiro, C. Alvarez-Lorenzo, pH-responsive scaffolds for tissue regeneration: In vivo performance, Acta Biomater. 168 (2023), https://doi.org/10.1016/j.actbio.2023.07.025.
[302] W. Wang, Y. Liu, Y. Liu, X. Yang, X. Wang, Highly sensitive smart hydrogels with pH-tunable toughness via signaling cascade amplification, Giant 16 (2023), https://doi.org/10.1016/j.giant.2023.100197.
[303] R. Naranjo-Alcazar, S. Bendix, T. Groth, G. Gallego Ferrer, Research Progress in Enzymatically Cross-Linked Hydrogels as Injectable Systems for Bioprinting and Tissue Engineering, Gels 9 (2023), https://doi.org/10.3390/gels9030230.
[304] J. Herlet, P. Kornberger, B. Roessler, J. Glanz, W.H. Schwarz, W. Liebl, V. V. Zverlov, A new method to evaluate temperature vs. pH activity profiles for biotechnological relevant enzymes, Biotechnol. Biofuels 10 (2017), https://doi.org/10.1186/s13068-017-0923-9.
[305] L.S. Moreira Teixeira, J. Feijen, C.A. van Blitterswijk, P.J. Dijkstra, M. Karperien, Enzyme-catalyzed crosslinkable hydrogels: Emerging strategies for tissue engineering, Biomaterials 33 (2012), https://doi.org/10.1016/j.biomaterials.2011.10.067.
[306] Y. Liu, R. Weng, W. Wang, X. Wei, J. Li, X. Chen, Y. Liu, F. Lu, Y. Li, Tunable physical and mechanical properties of gelatin hydrogel after transglutaminase crosslinking on two gelatin types, Int. J. Biol. Macromol. 162 (2020), https://doi.org/10.1016/j.ijbiomac.2020.06.185.
[307] M.A. Da Silva, F. Bode, A.F. Drake, S. Goldoni, M.M. Stevens, C.A. Dreiss, Enzymatically cross-linked gelatin/chitosan hydrogels: Tuning gel properties and cellular response, Macromol. Biosci. 14 (2014), https://doi.org/10.1002/mabi.201300472.
[308] Z. Hai, J. Li, J. Wu, J. Xu, G. Liang, Alkaline phosphatase-triggered simultaneous hydrogelation and chemiluminescence, J. Am. Chem. Soc. 139 (2017), https://doi.org/10.1021/jaci.6bll041.
[309] L. Valot, J. Martinez, A. Mehdi, G. Subra, Chemical insights into bioinks for 3D printing, Chem. Soc. Rev. 48 (2019), https://doi.org/10.1039/c7cs00718c.
[310] X. Lv, C. Liu, Z. Shao, S. Sun, Tuning physical crosslinks in hybrid hydrogels for network structure analysis and mechanical reinforcement, Polymers 11 (2019), https://doi.org/10.3390/POLYM11020352.
[311] W.E. Hennink, C.F. van Nostrum, Novel crosslinking methods to design hydrogels, Adv. Drug Deliv. Rev. 64 (2012), https://doi.org/10.1016/j.addr.2012.09.009.
[312] M. Kaur, A.K. Srivastava, Photopolymerization: A review, J. Macromol. Sci. - Polym. Rev. 42 (2002), https://doi.org/10.1081/MC-120015988.
[313] T. Heck, G. Faccio, M. Richter, L. Thöny-Meyer, Enzyme-catalyzed protein crosslinking, Appl. Microbiol. Biotechnol. 97 (2013), https://doi.org/10.1007/s00253-012-4569-z.
[314] A.M. Jonker, S.A. Bode, A.H. Kusters, J.C.M. Van Hest, D.W.P.M. Löwik, Soft PEG-Hydrogels with Independently Tunable Stiffness and RGDS-Content for Cell Adhesion Studies, Macromol. Biosci. 15 (2015), https://doi.org/10.1002/mabi.201500110.
[315] J.P. Gong, Y. Katsuyama, T. Kurokawa, Y. Osada, Double-network hydrogels with extremely high mechanical strength, Adv. Mater. 15 (2003), https://doi.org/10.1002/adma.200304907.
[316] R. Parhi, Cross-linked hydrogel for pharmaceutical applications: A review, Adv. Pharm. Bull. 7 (2017), https://doi.org/10.15171/apb.2017.064.
[317] E.S. Dragan, Advances in interpenetrating polymer network hydrogels and their applications, Pure Appl. Chem. (2014), https://doi.org/10.1515/pac-2014-0713.
[318] K. Da Silva, P. Kumar, S.F. Van Vuuren, V. Pillay, Y.E. Choonara, Three-Dimensional Printability of an ECM-Based Gelatin Methacryloyl (GelMA) Biomaterial for Potential Neuroregeneration, ACS Omega 6 (2021), https://doi.org/10.1021/acsomega.1c01903.
[319] P. Aprile, D.J. Kelly, Hydrostatic Pressure Regulates the Volume, Aggregation and Chondrogenic Differentiation of Bone Marrow Derived Stromal Cells, Front. Bioeng. Biotechnol. 8 (2021), https://doi.org/10.3389/fbioe.2020.619914.
[320] S. Sinha, M. Ayushman, X. Tong, F. Yang, Dynamically Crosslinked Poly(ethylene-glycol) Hydrogels Reveal a Critical Role of Viscoelasticity in Modulating Glioblastoma Fates and Drug Responses in 3D, Adv. Healthc. Mater. 12 (2023), https://doi.org/10.1002/adhm.202202147.
[321] J.P. Gong, Why are double network hydrogels so tough? Soft Matter 6 (2010) https://doi.org/10.1039/b924290b.
[322] J.P. Gong, Materials both tough and soft, Science 344 (2014), https://doi.org/10.1126/science.1252389.
[323] H. Tsukeshiba, M. Huang, Y.H. Na, T. Kurokawa, R. Kuwabara, Y. Tanaka, H. Furukawa, Y. Osada, J.P. Gong, Effect of polymer entanglement on the toughening of double network hydrogels, J. Phys. Chem. B 109 (2005), https://doi.org/10.1021/jp052419n.
[324] M.A. Haque, T. Kurokawa, J.P. Gong, Super tough double network hydrogels and their application as biomaterials, Polymer 53 (2012), https://doi.org/10.1016/j.polymer.2012.03.013.
[325] X. Huang, J. Li, J. Luo, Q. Gao, A. Mao, J. Li, Research progress on double-network hydrogels, Mater. Today Commun. 29 (2021), https://doi.org/10.1016/j.mtcomm.2021.102757.
[326] T. Nakajima, H. Sato, Y. Zhao, S. Kawahara, T. Kurokawa, K. Sugahara, J.P. Gong, A universal molecular stent method to toughen any hydrogels based on double network concept, Adv. Funct. Mater. 22 (2012), https://doi.org/10.1002/adfm.201200809.
[327] T. Nakajima, H. Furukawa, Y. Tanaka, T. Kurokawa, J.P. Gong, Effect of void structure on the toughness of double network hydrogels, J. Polym. Sci. Part B Polym. Phys. 49 (2011), https://doi.org/10.1002/polb.22293.
[328] W. Yang, H. Furukawa, J.P. Gong, Highly extensible double-network gels with self-assembling anisotropic structure, Adv. Mater. 20 (2008), https://doi.org/10.1002/adma.200801396.
[329] M.A. Haque, T. Kurokawa, G. Kamita, J.P. Gong, Lamellar bilayers as reversible sacrificial bonds to toughen hydrogel: Hysteresis, self-recovery, fatigue resistance, and crack blunting, Macromolecules 44 (2011), https://doi.org/10.1021/ma201653t.
[330] Y. Zhang, H. Qin, M. Alfred, H. Ke, Y. Cai, Q. Wang, F. Huang, B. Liu, P. Lv, Q. Wei, Reaction modifier system enable double-network hydrogel electrolyte for flexible zinc-air batteries with tolerance to extreme cold conditions, Energy Storage Mater. 42 (2021), https://doi.org/10.1016/j.ensm.2021.07.026.
[331] Z. He, W. Yuan, Adhesive, Stretchable, and Transparent Organohydrogels for Antifreezing, Antidrying, and Sensitive Ionic Skins, ACS Appl. Mater. Interfaces 13 (2021), https://doi.org/10.1021/acsami.0c18405.

[332] H. Sun, M. Zhang, M. Liu, Y. Yu, X. Xu, J. Li, Fabrication of Double-Network Hydrogels with Universal Adhesion and Superior Extensibility and Cytocompatibility by One-Pot Method, Biomacromolecules 21 (2020), https://doi.org/10.1021/acs.biomac.0c00822.
[333] Q. Chen, L. Zhu, C. Zhao, Q. Wang, J. Zheng, A robust, one-pot synthesis of highly mechanical and recoverable double network hydrogels using thermoreversible sol-gel polysaccharide, Adv. Mater. 25 (2013), https://doi.org/10.1002/adma.201300817.
[334] B.V.S. Iyer, V.V. Yashin, T. Kowalewski, K. Matyjaszewski, A.C. Balazs, Strain recovery and self-healing in dual cross-linked nanoparticle networks, Polym. Chem. 4 (2013), https://doi.org/10.1039/c3py00075c.
[335] Z. Wei, D.M. Lewis, Y. Xu, S. Gerecht, Dual Cross-Linked Biofunctional and Self-Healing Networks to Generate User-Defined Modular Gradient Hydrogel Constructs, Adv. Healthc. Mater. 6 (2017), https://doi.org/10.1002/adhm.201700523.
[336] Q. Chen, Y. Shi, K. Sheng, J. Zheng, C. Xu, Dynamically Cross-Linked Hydrogel Electrolyte with Remarkable Stretchability and Self-Healing Capability for Flexible Electrochromic Devices, ACS Appl. Mater. Interfaces 13 (2021), https://doi.org/10.1021/acsami.1c15432.
[337] Y. Hu, Z. Du, X. Deng, T. Wang, Z. Yang, W. Zhou, C. Wang, Dual Physically Cross-Linked Hydrogels with High Stretchability, Toughness, and Good Self-Recoverability, Macromolecules 49 (2016), https://doi.org/10.1021/acs.macromol.6b00584.
[338] Y. Qin, J. Wang, C. Qiu, X. Xu, Z. Jin, A Dual Cross-Linked Strategy to Construct Moldable Hydrogels with High Stretchability, Good Self-Recovery, and Self-Healing Capability, J. Agric. Food Chem. 67 (2019), https://doi.org/10.1021/acs.jafc.8b05147.
[339] J.Y. Sun, X. Zhao, W.R.K. Illeperuma, O. Chaudhuri, K.H. Oh, D.J. Mooney, J. J. Vlassak, Z. Suo, Highly stretchable and tough hydrogels, Nature 489 (2012), https://doi.org/10.1038/nature11409.
[340] P. Lin, S. Ma, X. Wang, F. Zhou, Molecularly engineered dual-crosslinked hydrogel with ultrahigh mechanical strength, toughness, and good self-recovery, Adv. Mater. 27 (2015), https://doi.org/10.1002/adma.201405022.
[341] D. Wu, W. Wang, D. Díaz-Dussan, Y.Y. Peng, Y. Chen, R. Narain, D.G. Hall, In Situ Forming, Dual-Crosslink Network, Self-Healing Hydrogel Enabled by a Bioorthogonal Nopoldiol-Benzoxaborolate Click Reaction with a Wide pH Range, Chem. Mater. (2019), https://doi.org/10.1021/acs.chemmater.9b00769.
[342] R. Long, K. Mayumi, C. Creton, T. Narita, C.-Y. Hui, Rheology of a dual crosslink self-healing gel: Theory and measurement using parallel-plate torsional rheometry, J. Rheol. 59 (2015), https://doi.org/10.1122/1.4915275.
[343] M. Liu, J. Guo, C.Y. Hui, A. Zehnder, Crack tip stress based kinetic fracture model of a PVA dual-crosslink hydrogel, Extreme Mech. Lett. 29 (2019), https://doi.org/10.1016/j.eml.2019.100457.
[344] M. Zhong, Y.T. Liu, X.Y. Liu, F.K. Shi, L.Q. Zhang, M.F. Zhu, X.M. Xie, Dually cross-linked single network poly(acrylic acid) hydrogels with superior mechanical properties and water absorbency, Soft Matter 12 (2016), https://doi.org/10.1039/c6sm00242k.
[345] L. Debertrand, J. Zhao, C. Creton, T. Narita, Swelling and mechanical properties of polyacrylamide-derivative dual-crosslink hydrogels having metal–ligand coordination bonds as transient crosslinks, Gels 7 (2021), https://doi.org/10.3390/gels7020072.
[346] X. Chang, Y. Geng, H. Cao, J. Zhou, Y. Tian, G. Shan, Y. Bao, Z.L. Wu, P. Pan, Dual-Crosslink Physical Hydrogels with High Toughness Based on Synergistic Hydrogen Bonding and Hydrophobic Interactions, Macromol. Rapid Commun. 39 (2018), https://doi.org/10.1002/marc.201700806.
[347] S. Potiwiput, H. Tan, G. Yuan, S. Li, T. Zhou, J. Li, Y. Jia, D. Xiong, X. Hu, Z. Ling, Y. Chen, Dual-crosslinked alginate/carboxymethyl chitosan hydrogel containing in situ synthesized calcium phosphate particles for drug delivery application, Mater. Chem. Phys. 241 (2020), https://doi.org/10.1016/j.matchemphys.2019.122354.
[348] K. Mayumi, K. Ito, Structure and dynamics of polyrotaxane and slide-ring materials, Polymer 51 (2010), https://doi.org/10.1016/j.polymer.2009.12.019.
[349] Y. Okumura, K. Ito, The polyrotaxane gel: A topological gel by figure-of-eight cross-links, Adv. Mater. 13 (2001), https://doi.org/10.1002/1521-4095(200104)13:7<485::AID-ADMA485>3.0.CO;2-T.
[350] K. Mayumi, M. Tezuka, A. Bando, K. Ito, Mechanics of slide-ring gels: Novel entropic elasticity of a topological network formed by ring and string, Soft Matter 8 (2012), https://doi.org/10.1039/c2sm25508a.
[351] K. Ito, Novel entropic elasticity of polymeric materials: Why is slide-ring gel so soft, Polym. J. 44 (2012), https://doi.org/10.1038/pj.2011.85.
[352] C. Liu, H. Kadono, K. Mayumi, K. Kato, H. Yokoyama, K. Ito, Unusual Fracture Behavior of Slide-Ring Gels with Movable Cross-Links, ACS Macro Lett. 6 (2017), https://doi.org/10.1021/acsmacrolett.7b00729.
[353] K. Minato, K. Mayumi, R. Maeda, K. Kato, H. Yokoyama, K. Ito, Mechanical properties of supramolecular elastomers prepared from polymer-grafted polyrotaxane, Polymer 128 (2017), https://doi.org/10.1016/j.polymer.2017.02.090.
[354] K. Kato, Y. Okabe, Y. Okazumi, K. Ito, A significant impact of host-guest stoichiometry on the extensibility of polyrotaxane gels, Chem. Commun. 51 (2015), https://doi.org/10.1039/c5cc07122d.
[355] L. Jiang, C. Liu, K. Mayumi, K. Kato, H. Yokoyama, K. Ito, Highly Stretchable and Instantly Recoverable Slide-Ring Gels Consisting of Enzymatically Synthesized Polyrotaxane with Low Host Coverage, Chem. Mater. 30 (2018), https://doi.org/10.1021/acs.chemmater.8b01208.
[356] C. Liu, N. Morimoto, L. Jiang, S. Kawahara, T. Noritomi, H. Yokoyama, K. Mayumi, K. Ito, Tough hydrogels with rapid self-reinforcement, Science 372 (2021), https://doi.org/10.1126/science.aaz6694.
[357] S.Y. Zheng, C. Liu, L. Jiang, J. Lin, J. Qian, K. Mayumi, Z.L. Wu, K. Ito, Q. Zheng, Slide-Ring Cross-Links Mediated Tough Metallosupramolecular Hydrogels with Superior Self-Recoverability, Macromolecules (2019), https://doi.org/10.1021/acs.macromol.9b01281.
[358] Z. Cao, D. Wang, Y. Li, W. Xie, X. Wang, L. Tao, Y. Wei, X. Wang, L. Zhao, Effect of nanoheat stimulation mediated by magnetic nanocomposite hydrogel on the osteogenic differentiation of mesenchymal stem cells, Sci. China Life Sci. 61 (2018), https://doi.org/10.1007/s11427-017-9287-8.
[359] J. Huang, Y. Liang, Z. Jia, J. Chen, L. Duan, W. Liu, F. Zhu, Q. Liang, W. Zhu, W. You, J. Xiong, D. Wang, Development of Magnetic Nanocomposite Hydrogel with Potential Cartilage Tissue Engineering, ACS Omega 3 (2018), https://doi.org/10.1021/acsomega.8b00291.
[360] Z. Deng, T. Hu, Q. Lei, J. He, P.X. Ma, B. Guo, Stimuli-Responsive Conductive Nanocomposite Hydrogels with High Stretchability, Self-Healing, Adhesiveness, and 3D Printability for Human Motion Sensing, ACS Appl. Mater. Interfaces 11 (2019), https://doi.org/10.1021/acsami.8b20178.
[361] C. Lim, Y. Shin, J. Jung, J.H. Kim, S. Lee, D.H. Kim, Stretchable conductive nanocomposite based on alginate hydrogel and silver nanowires for wearable electronics, APL Mater. 7 (2019), https://doi.org/10.1063/1.5063657.
[362] W. Xing, Y. Tang, On mechanical properties of nanocomposite hydrogels: Searching for superior properties, Nano Mater. Sci. (2021), https://doi.org/10.1016/j.nanoms.2021.07.004.
[363] M.A. Sakr, K. Sakthivel, T. Hossain, S.R. Shin, S. Siddiqua, J. Kim, K. Kim, Recent trends in gelatin methacryloyl nanocomposite hydrogels for tissue engineering, J. Biomed. Mater. Res. - Part A 110 (2022), https://doi.org/10.1002/jbm.a.37310.
[364] R.R. Wakaskar, General overview of lipid–polymer hybrid nanoparticles, dendrimers, micelles, liposomes, spongosomes and cubosomes, J. Drug Target. 26 (2018), https://doi.org/10.1080/1061186X.2017.1367006.
[365] S. Rafieian, H. Mirzadeh, H. Mahdavi, M.E. Masoumi, A review on nanocomposite hydrogels and their biomedical applications, IEEE J. Sel. Top. Quantum Electron. 26 (2019), https://doi.org/10.1515/secm-2017-0161.
[366] M. Liu, Y. Ishida, Y. Ebina, T. Sasaki, T. Hikima, M. Takata, T. Aida, An anisotropic hydrogel with electrostatic repulsion between cofacially aligned nanosheets, Nature 517 (2015), https://doi.org/10.1038/nature14060.
[367] J.D. Mangadlao, R. Huang, E.L. Foster, K.D. Pangilinan, C. Danda, A. Advincula, J. M. Maia, R.C. Advincula, Graphene Oxide-Poly(ethylene glycol) methyl ether methacrylate Nanocomposite Hydrogels, Macromol. Chem. Phys. 217 (2016), https://doi.org/10.1002/macp.201500356.
[368] J.U. Lee, D. Yoon, H. Cheong, Estimation of young's modulus of graphene by Raman spectroscopy, Nano Lett. 12 (2012), https://doi.org/10.1021/nl301073q.
[369] A.T. Pereira, P.C. Henriques, P.C. Costa, M.C.L. Martins, F.D. Magalhães, I. C. Gonçalves, Graphene oxide-reinforced poly(2-hydroxyethyl methacrylate) hydrogels with extreme stiffness and high-strength, Compos. Sci. Technol. 184 (2019), https://doi.org/10.1016/j.compscitech.2019.107819.
[370] Z.K. Cui, S. Kim, J.J. Baljon, B.M. Wu, T. Aghaloo, M. Lee, Microporous methacrylated glycol chitosan-montmorillonite nanocomposite hydrogel for bone tissue engineering, Nat. Commun. 10 (2019), https://doi.org/10.1038/s41467-019-11511-3.
[371] Y.H. Kim, X. Yang, L. Shi, S.A. Lanham, J. Hilborn, R.O.C. Oreffo, D. Ossipov, J. I. Dawson, Bisphosphonate nanoclay edge-site interactions facilitate hydrogel self-assembly and sustained growth factor localization, Nat. Commun. 11 (2020), https://doi.org/10.1038/s41467-020-15152-9.
[372] D.M. Nascimento, Y.L. Nunes, M.C.B. Figueirêdo, H.M.C. De Azeredo, F. A. Aouada, J.P.A. Feitosa, M.F. Rosa, A. Dufresne, Nanocellulose nanocomposite hydrogels: Technological and environmental issues, Green Chem. 20 (2018), https://doi.org/10.1039/c8gc00205c.
[373] S.K. Das, T. Parandhaman, M.D. Dey, Biomolecule-assisted synthesis of biomimetic nanocomposite hydrogel for hemostatic and wound healing applications, Green Chem. 23 (2021), https://doi.org/10.1039/d0gc03010d.
[374] K.J. De France, K.J.W. Chan, E.D. Cranston, T. Hoare, Enhanced Mechanical Properties in Cellulose Nanocrystal-Poly(oligoethylene glycol methacrylate) Injectable Nanocomposite Hydrogels through Control of Physical and Chemical Cross-Linking, Biomacromolecules 17 (2016), https://doi.org/10.1021/acs.biomac.5b01598.
[375] W. Chen, Y. Zhang, J. Kumari, H. Engelkamp, P.H.J. Kouwer, Magnetic Stiffening in 3D Cell Culture Matrices, Nano Lett. 21 (2021), https://doi.org/10.1021/acs.nanolett.1c00371.
[376] M. Jaspers, S.L. Vaessen, P. Van Schayik, D. Voerman, A.E. Rowan, P.H. J. Kouwer, Nonlinear mechanics of hybrid polymer networks that mimic the complex mechanical environment of cells, Nat. Commun. 8 (2017), https://doi.org/10.1038/ncomms15478.
[377] W. Chen, P.H.J. Kouwer, Combining Mechanical Tuneability with Function: Biomimetic Fibrous Hydrogels with Nanoparticle Crosslinkers, Adv. Funct. Mater. 31 (2021), https://doi.org/10.1002/adfm.202105713.
[378] M.K. Jaiswal, J.R. Xavier, J.K. Carrow, P. Desai, D. Alge, A.K. Gaharwar, Mechanically stiff nanocomposite hydrogels at ultralow nanoparticle content, ACS Nano 10 (2016), https://doi.org/10.1021/acsnano.5b03918.
[379] B.Y.S. Kumar, A.M. Isloor, K. Perisamy, G.C.M. Kumar, Structure and rheology of chitosan-nanohydroxyapatite composite hydrogel for soft tissue regeneration, AIP Conf. Proc. (2020), https://doi.org/10.1063/5.0003867.

[380] M. Sadat-Shojai, M.T. Khorasani, A. Jamshidi, A new strategy for fabrication of bone scaffolds using electrospun nano-HAp/PHB fibers and protein hydrogels, Chem. Eng. J. 289 (2016), https://doi.org/10.1016/j.cej.2015.12.079.
[381] M. Sadat-Shojai, M.T. Khorasani, A. Jamshidi, 3-Dimensional cell-laden nano-hydroxyapatite/protein hydrogels for bone regeneration applications, Mater. Sci. Eng. C 49 (2015), https://doi.org/10.1016/j.msec.2015.01.067.
[382] H. Jiang, L. Duan, X. Ren, G. Gao, Hydrophobic association hydrogels with excellent mechanical and self-healing properties, Eur. Polym. J. 112 (2019), https://doi.org/10.1016/j.eurpolymj.2018.10.031.
[383] G. Jiang, C. Liu, X. Liu, G. Zhang, M. Yang, F. Liu, Construction and properties of hydrophobic association hydrogels with high mechanical strength and reforming capabilitya, Macromol. Mater. Eng. 294 (2009), https://doi.org/10.1002/mame.200900160.
[384] S. Abdurrahmanoglu, V. Can, O. Okay, Design of high-toughness polyacrylamide hydrogels by hydrophobic modification, Polymer 50 (2009), https://doi.org/10.1016/j.polymer.2009.09.042.
[385] V. Can, Z. Kochovski, V. Reiter, N. Severin, M. Siebenbürger, B. Kent, J. Just, J. P. Rabe, M. Ballauff, O. Okay, Nanostructural Evolution and Self-Healing Mechanism of Micellar Hydrogels, Macromolecules 49 (2016), https://doi.org/10.1021/acs.macromol.6b00156.
[386] B. Gao, H. Guo, J. Wang, Y. Zhang, Preparation of hydrophobic association polyacrylamide in a new micellar copolymerization system and its hydrophobically associative property, Macromolecules 41 (2008), https://doi.org/10.1021/ma701967b.
[387] W. Li, H. An, Y. Tan, C. Lu, C. Liu, P. Li, K. Xu, P. Wang, Hydrophobically associated hydrogels based on acrylamide and anionic surface active monomer with high mechanical strength, Soft Matter 8 (2012), https://doi.org/10.1039/c2sm07200a.
[388] G. Jiang, C. Liu, X. Liu, Q. Chen, G. Zhang, M. Yang, F. Liu, Network structure and compositional effects on tensile mechanical properties of hydrophobic association hydrogels with high mechanical strength, Polymer 51 (2010), https://doi.org/10.1016/j.polymer.2010.01.061.
[389] W. Zhao, L. Duan, B. Zhang, X. Ren, G.H. Gao, Tough and ultrastretchable hydrogels reinforced by poly(butyl acrylate-co-acrylonitrile) latex microspheres as crosslinking centers for hydrophobic association, Polymer 112 (2017), https://doi.org/10.1016/j.polymer.2017.02.032.
[390] J. Xu, X. Ren, G. Gao, Salt-inactive hydrophobic association hydrogels with fatigue resistant and self-healing properties, Polymer 150 (2018), https://doi.org/10.1016/j.polymer.2018.07.045.
[391] J. Xu, X. Liu, X. Ren, G. Gao, The role of chemical and physical crosslinking in different deformation stages of hybrid hydrogels, Eur. Polym. J. 100 (2018), https://doi.org/10.1016/j.eurpolymj.2018.01.020.
[392] J. Yang, F.K. Shi, C. Gong, X.M. Xie, Dual cross-linked networks hydrogels with unique swelling behavior and high mechanical strength: Based on silica nanoparticle and hydrophobic association, J. Colloid Interface Sci. 381 (2012), https://doi.org/10.1016/j.jcis.2012.05.046.
[393] F. Wang, X. Yong, J. Deng, Y. Wu, Poly(: N, N -dimethylacrylamide-octadecyl acrylate)-clay hydrogels with high mechanical properties and shape memory ability, RSC Adv. 8 (2018), https://doi.org/10.1039/c8ra01167b.
[394] E. Su, O. Okay, Polyampholyte hydrogels formed via electrostatic and hydrophobic interactions, Eur. Polym. J. 88 (2017), https://doi.org/10.1016/j.eurpolymj.2017.01.029.
[395] L. Xing, C. Hu, Y. Zhang, X. Wang, L. Shi, R. Ran, A mechanically robust double-network hydrogel with high thermal responses via doping hydroxylated boron nitride nanosheets, J. Mater. Sci. 54 (2019), https://doi.org/10.1007/s10853-018-3037-2.
[396] B. Zhang, Z. Gao, G. Gao, W. Zhao, J. Li, X. Ren, Highly Mechanical and Fatigue-Resistant Double Network Hydrogels by Dual Physically Hydrophobic Association and Ionic Crosslinking, Macromol. Mater. Eng. 303 (2018), https://doi.org/10.1002/mame.201800072.
[397] M. Zhang, X. Ren, L. Duan, G. Gao, Joint double-network hydrogels with excellent mechanical performance, Polymer 153 (2018), https://doi.org/10.1016/j.polymer.2018.08.071.
[398] X. Liu, L. Duan, G. Gao, Rapidly self-recoverable and fatigue-resistant hydrogels toughened by chemical crosslinking and hydrophobic association, Eur. Polym. J. 89 (2017), https://doi.org/10.1016/j.eurpolymj.2017.02.025.
[399] H. Zhang, H. Peng, Y. Li, Y. Xu, W. Weng, Compositional- and time-dependent dissipation, recovery and fracture toughness in hydrophobically reinforced hybrid hydrogels, Polymer 80 (2015), https://doi.org/10.1016/j.polymer.2015.10.052.
[400] Z. Qin, R. Niu, C. Tang, J. Xia, F. Ji, D. Dong, H. Zhang, S. Zhang, J. Li, F. Yao, A Dual-Crosslinked Strategy to Construct Physical Hydrogels with High Strength, Toughness, Good Mechanical Recoverability, and Shape-Memory Ability, Macromol. Mater. Eng. 303 (2018), https://doi.org/10.1002/mame.201700396.
[401] K. Zhang, Z. Zhao, J. Huang, T. Zhao, R. Fang, M. Liu, Self-recoverable semi-crystalline hydrogels with thermomechanics and shape memory performance, Sci. China Mater. 62 (2019), https://doi.org/10.1007/s40843-018-9347-5.
[402] H. Zhou, M. Wang, W. Zhao, L. Chen, H. Liu, X. Jin, A. Ma, G. Zhang, D. Jiang, W. Chen, Supramolecularly Mediated Robust, Anti-Fatigue, and Strain-Sensitive Macromolecular Microsphere Composite Hydrogels, Macromol. Mater. Eng. 305 (2020), https://doi.org/10.1002/mame.202000080.
[403] T. Huang, H. Xu, K. Jiao, L. Zhu, H.R. Brown, H. Wang, A novel hydrogel with high mechanical strength: A macromolecular microsphere composite hydrogel, Adv. Mater. 19 (2007), https://doi.org/10.1002/adma.200602533.
[404] J. Zhao, K. Jiao, J. Yang, C. He, H. Wang, Mechanically strong and thermosensitive macromolecular microsphere composite poly(N-isopropylacrylamide) hydrogels, Polymer 54 (2013), https://doi.org/10.1016/j.polymer.2013.01.025.
[405] F. Jiang, T. Huang, C. He, H.R. Brown, H. Wang, Interactions affecting the mechanical properties of macromolecular microsphere composite hydrogels, J. Phys. Chem. B 117 (2013), https://doi.org/10.1021/jp4069587.
[406] J. Duan, X. Liang, J. Guo, K. Zhu, L. Zhang, Ultra-Stretchable and Force-Sensitive Hydrogels Reinforced with Chitosan Microspheres Embedded in Polymer Networks, Adv. Mater. 28 (2016), https://doi.org/10.1002/adma.201602126.
[407] X. Zhang, Y. Liu, J. Wen, Z. Zhao, H. Chen, X. Liu, S. Liu, Host-guest interaction-mediated fabrication of a hybrid microsphere-structured supramolecular hydrogel showing high mechanical strength, Soft Matter 16 (2020), https://doi.org/10.1039/d0sm00271b.
[408] Y. Tan, K. Xu, P. Wang, W. Li, S. Sun, L. Dong, High mechanical strength and rapid response rate of poly(N-isopropyl acrylamide) hydrogel crosslinked by starch-based nanospheres, Soft Matter 6 (2010), https://doi.org/10.1039/b916942c.
[409] C. Liu, Y. Tan, K. Xu, Y. Li, C. Lu, P. Wang, Synthesis of poly(2-(2-methoxyethoxy) ethyl methacrylate) hydrogel using starch-based nanosphere cross-linkers, Carbohydr. Polym. 105 (2014), https://doi.org/10.1016/j.carbpol.2014.01.078.
[410] C. Qi, H. An, Y. Jiang, P. Shi, C. Liu, Y. Tan, POEGMA hydrogel cross-linked by starch-based microspheres: synthesis and characterization, Iran. Polym. J. Engl. Ed. 26 (2017), https://doi.org/10.1007/s13726-017-0523-3.
[411] C. Wang, L. Yang, Y. He, H. Xiao, W. Lin, Microsphere-structured hydrogel crosslinked by polymerizable protein-based nanospheres, Polymer 211 (2020), https://doi.org/10.1016/j.polymer.2020.123114.
[412] T. Yang, Q. Zhang, L. Xie, R. Zhang, R. Qian, Y. Tian, G. Chen, W. Tian, hDPSC-laden GelMA microspheres fabricated using electrostatic microdroplet method for endodontic regeneration, Mater. Sci. Eng. C 121 (2021), https://doi.org/10.1016/j.msec.2020.111850.
[413] Q. Zhang, T. Yang, R. Zhang, X. Liang, G. Wang, Y. Tian, L. Xie, W. Tian, Platelet lysate functionalized gelatin methacrylate microspheres for improving angiogenesis in endodontic regeneration, Acta Biomater. 136 (2021), https://doi.org/10.1016/j.actbio.2021.09.024.
[414] T. Behroozi Kohlan, Y. Wen, C. Mini, A. Finne-Wistrand, Schiff base crosslinked hyaluronic acid hydrogels with tunable and cell instructive time-dependent mechanical properties, Carbohydr. Polym. 338 (2024) 122173, https://doi.org/10.1016/j.carbpol.2024.122173.
[415] P. Nezhad-Mokhtari, M. Ghorbani, L. Roshangar, J. Soleimani Rad, A review on the construction of hydrogel scaffolds by various chemically techniques for tissue engineering, Eur. Polym. J. 117 (2019), https://doi.org/10.1016/j.eurpolymj.2019.05.004.
[416] S.S. Shah, D. Shah, I. Khan, S. Ahmad, U. Ali, A.U. Rahman, Synthesis and antioxidant activities of schiff bases and their complexes: An updated review, Biointerface Res. Appl. Chem. 10 (2020), https://doi.org/10.33263/BRIAC106.69366963.
[417] C.M. Da Silva, D.L. Da Silva, L.V. Modolo, R.B. Alves, M.A. De Resende, C.V. B. Martins, Â. De Fátima, Schiff bases: A short review of their antimicrobial activities, J. Adv. Res. 2 (2011), https://doi.org/10.1016/j.jare.2010.05.004.
[418] J. Xu, Y. Liu, S. Hui Hsu, Hydrogels based on schiff base linkages for biomedical applications, Molecules 24 (2019), https://doi.org/10.3390/molecules24163005.
[419] D. Iacopetta, J. Ceramella, A. Catalano, C. Saturnino, M.G. Bonomo, C. Franchini, M.S. Sinicropi, Schiff bases: Interesting scaffolds with promising antitumoral properties, Appl. Sci. Switz. 11 (2021), https://doi.org/10.3390/app11041877.
[420] Z. Wei, J. Zhao, Y.M. Chen, P. Zhang, Q. Zhang, Self-healing polysaccharide-based hydrogels as injectable carriers for neural stem cells, Sci. Rep. 6 (2016), https://doi.org/10.1038/srep37841.
[421] Q. Liu, N. Ji, L. Xiong, Q. Sun, Rapid gelling, self-healing, and fluorescence-responsive chitosan hydrogels formed by dynamic covalent crosslinking, Carbohydr. Polym. 246 (2020), https://doi.org/10.1016/j.carbpol.2020.116586.
[422] W. Huang, Y. Wang, Z. Huang, X. Wang, L. Chen, Y. Zhang, L. Zhang, On-Demand Dissolvable Self-Healing Hydrogel Based on Carboxymethyl Chitosan and Cellulose Nanocrystal for Deep Partial Thickness Burn Wound Healing, ACS Appl. Mater. Interfaces 10 (2018), https://doi.org/10.1021/acsami.8b14526.
[423] B. Guo, R. Yu, Self-healing biomaterials based on polymeric systems, in: Self-Heal. Polym.-Based Syst., 2020. https://doi.org/10.1016/b978-0-12-818450-9.00007-6.
[424] Z. Wei, J.H. Yang, J. Zhou, F. Xu, M. Zrínyi, P.H. Dussault, Y. Osada, Y.M. Chen, Self-healing gels based on constitutional dynamic chemistry and their potential applications, Chem. Soc. Rev. 43 (2014), https://doi.org/10.1039/c4cs00219a.
[425] Y. Zhang, L. Tao, S. Li, Y. Wei, Synthesis of multiresponsive and dynamic chitosan-based hydrogels for controlled release of bioactive molecules, Biomacromolecules 12 (2011), https://doi.org/10.1021/bm200423f.
[426] R. Singhal, K. Gupta, A. Review, Tailor-made Hydrogel Structures (Classifications and Synthesis Parameters), Polym. - Plast. Technol. Eng. 55 (2016), https://doi.org/10.1080/03602559.2015.1050520.
[427] S. Rayati, E. Bohloulbandi, S. Zakavi, Sodium borohydride reduction of aldehydes catalyzed by an oxovanadium(IV) Schiff base complex encapsulated in the nanocavity of zeolite-Y, Inorg. Chem. Commun. 54 (2015), https://doi.org/10.1016/j.inoche.2015.02.004.
[428] S.L. Zhang, Y. Lu, Y.H. Li, K.Y. Wang, J.H. Chen, Z. Yang, Catalytic and Enantioselective Diels-Alder Reactions of (E)-4-Oxopent-2-enoates, Org. Lett. 19 (2017), https://doi.org/10.1021/acs.orglett.7b01692.
[429] M. El Ghozlani, A. Barhoumi, R. Elkacmi, A. Ouled Aitouna, A. Zeroual, M., El Idrissi, Mechanistic Study of Hetero-Diels–Alder [4 + 2] Cycloaddition Reactions Between 2-Nitro-1H-Pyrrole and Isoprene, Chem. Afr. 3 (2020), https://doi.org/10.1007/s42250-020-00187-8.

[430] K. Ishihara, A. Sakakura, Intermolecular Diels-Alder Reactions, Compr. Org. Synth. Second Ed. (2014), https://doi.org/10.1016/B978-0-08-097742-3.00509-7.
[431] A. Kurpanik, M. Matussek, P. Lodowski, G. Szafraniec-Gorol, M. Krompiec, S. Krompiec, Diels-Alder Cycloaddition to the Bay Region of Perylene and Its Derivatives as an Attractive Strategy for PAH Core Expansion: Theoretical and Practical Aspects, Mol. Basel Switz. 25 (2020), https://doi.org/10.3390/molecules25225373.
[432] K. Urdl, S. Weiss, P. Christöfl, A. Kandelbauer, U. Müller, W. Kern, Diels-Alder modified self-healing melamine resin, Eur. Polym. J. 127 (2020), https://doi.org/10.1016/j.eurpolymj.2020.109601.
[433] N.I. Khan, S. Halder, S.B. Gunjan, T. Prasad, A review on Diels-Alder based self-healing polymer composites, IOP Conf. Ser. Mater. Sci. Eng. (2018), https://doi.org/10.1088/1757-899X/377/1/012007.
[434] A.M. Peterson, R.E. Jensen, G.R. Palmese, Thermoreversible and remendable glass-polymer interface for fiber-reinforced composites, Compos. Sci. Technol. 71 (2011), https://doi.org/10.1016/j.compscitech.2010.11.022.
[435] J.H. Aubert, Thermally removable epoxy adhesives incorporating thermally reversible Diels-Alder adducts, J. Adhes. 79 (2003), https://doi.org/10.1080/00218460309540.
[436] Q. Zhou, F. Gardea, Z. Sang, S. Lee, M. Pharr, S.A. Sukhishvili, A Tailorable Family of Elastomeric-to-Rigid, 3D Printable, Interbonding Polymer Networks, Adv. Funct. Mater. 30 (2020), https://doi.org/10.1002/adfm.202002374.
[437] K. González, O. Guaresti, T. Palomares, A. Alonso-Varona, A. Eceiza, N. Gabilondo, The role of cellulose nanocrystals in biocompatible starch-based clicked nanocomposite hydrogels, Int. J. Biol. Macromol. 143 (2020), https://doi.org/10.1016/j.ijbiomac.2019.12.050.
[438] S.T. Koshy, R.M. Desai, P. Joly, J. Li, R.K. Bagrodia, S.A. Lewin, N.S. Joshi, D. J. Mooney, Click-Crosslinked Injectable Gelatin Hydrogels, Adv. Healthc. Mater. 5 (2016), https://doi.org/10.1002/adhm.201500757.
[439] M.R. Karver, R. Weissleder, S.A. Hilderbrand, Synthesis and evaluation of a series of 1,2,4,5-tetrazines for bioorthogonal conjugation, Bioconjug. Chem. 22 (2011), https://doi.org/10.1021/bc200295y.
[440] V. Delplace, P.E.B. Nickerson, A. Ortin-Martinez, A.E.G. Baker, V.A. Wallace, M. S. Shoichet, Nonswelling, Ultralow Content Inverse Electron-Demand Diels–Alder Hyaluronan Hydrogels with Tunable Gelation Time: Synthesis and In Vitro Evaluation, Adv. Funct. Mater. 30 (2020), https://doi.org/10.1002/adfm.201903978.
[441] N.H. Dimmitt, M.R. Arkenberg, M.M. De Lima Perini, J. Li, C.C. Lin, Hydrolytically Degradable PEG-Based Inverse Electron Demand Diels-Alder Click Hydrogels, ACS Biomater. Sci. Eng. 8 (2022), https://doi.org/10.1021/acsbiomaterials.2c00714.
[442] S. Summonte, G.F. Racaniello, A. Lopedota, N. Denora, A. Bernkop-Schnürch, Thiolated polymeric hydrogels for biomedical application: Cross-linking mechanisms, J. Controlled Release 330 (2021), https://doi.org/10.1016/j.jconrel.2020.12.037.
[443] D.P. Nair, M. Podgórski, S. Chatani, T. Gong, W. Xi, C.R. Fenoli, C.N. Bowman, The Thiol-Michael addition click reaction: A powerful and widely used tool in materials chemistry, Chem. Mater. 26 (2014), https://doi.org/10.1021/cm402180t.
[444] Z.Q. Liu, Z. Wei, X.L. Zhu, G.Y. Huang, F. Xu, J.H. Yang, Y. Osada, M. Zrínyi, J. H. Li, Y.M. Chen, Dextran-based hydrogel formed by thiol-Michael addition reaction for 3D cell encapsulation, Colloids Surf. B Biointerfaces 128 (2015), https://doi.org/10.1016/j.colsurfb.2015.02.005.
[445] Y. Lei, S. Gojgini, J. Lam, T. Segura, The spreading, migration and proliferation of mouse mesenchymal stem cells cultured inside hyaluronic acid hydrogels, Biomaterials 32 (2011), https://doi.org/10.1016/j.biomaterials.2010.08.103.
[446] M.D. Godesky, D.I. Shreiber, Hyaluronic acid-based hydrogels with independently tunable mechanical and bioactive signaling features, Biointerphases 14 (2019), https://doi.org/10.1063/1.5126493.
[447] M. Chen, X. Ren, L. Dong, X. Li, H. Cheng, Preparation of dynamic covalently crosslinking keratin hydrogels based on thiol/disulfide bonds exchange strategy, Int. J. Biol. Macromol. 182 (2021), https://doi.org/10.1016/j.ijbiomac.2021.05.057.
[448] Ö. Erel, S. Erdoğan, Thiol-disulfide homeostasis: An integrated approach with biochemical and clinical aspects, Turk. J. Med. Sci. 50 (2020), https://doi.org/10.3906/sag-2003-64.
[449] S. Belbekhouche, M. Guerrouache, B. Carbonnier, Thiol-Maleimide Michael Addition Click Reaction: A New Route to Surface Modification of Porous Polymeric Monolith, Macromol. Chem. Phys. 217 (2016), https://doi.org/10.1002/macp.201500427.
[450] Y. Fu, W.J. Kao, In situ forming poly(ethylene glycol)-based hydrogels via thiol-maleimide Michael-type addition, J. Biomed. Mater. Res. - Part A 98 A (2011), https://doi.org/10.1002/jbm.a.33106.
[451] L.E. Jansen, L.J. Negrón-Piñeiro, S. Galarza, S.R. Peyton, Control of Thiol-Maleimide Reaction Kinetics in PEG Hydrogel, Networks (2017), https://doi.org/10.1101/198135.
[452] N.J. Darling, Y.S. Hung, S. Sharma, T. Segura, Controlling the kinetics of thiol-maleimide Michael-type addition gelation kinetics for the generation of homogenous poly(ethylene glycol) hydrogels, Biomaterials 101 (2016), https://doi.org/10.1016/j.biomaterials.2016.05.053.
[453] Y. Guo, J. Gu, Y. Jiang, Y. Zhou, Z. Zhu, T. Ma, Y. Cheng, Z. Ji, Y. Jiao, B. Xue, Y. Cao, Regulating the homogeneity of thiol-maleimide michael-type addition-based hydrogels using amino biomolecules, Gels 7 (2021), https://doi.org/10.3390/gels7040206.
[454] Q. Wang, T.R. Chan, R. Hilgraf, V.V. Fokin, K.B. Sharpless, M.G. Finn, Bioconjugation by copper(I)-catalyzed azide-alkyne [3 + 2] cycloaddition, J. Am. Chem. Soc. 125 (2003), https://doi.org/10.1021/ja021381e.
[455] L.V. Lee, M.L. Mitchell, S.J. Huang, V.V. Fokin, K.B. Sharpless, C.H. Wong, A potent and highly selective inhibitor of human α-1,3-fucosyltransferase via click chemistry, J. Am. Chem. Soc. 125 (2003), https://doi.org/10.1021/ja0302836.
[456] R. Huisgen, 1,3-Dipolar Cycloadditions. Past and Future, Angew. Chem. Int. Ed. Engl. 2 (1963), https://doi.org/10.1002/anie.196305651.
[457] N.J. Agard, J.A. Prescher, C.R. Bertozzi, A strain-promoted [3 + 2] azide-alkyne cycloaddition for covalent modification of biomolecules in living systems, J. Am. Chem. Soc. 126 (2004), https://doi.org/10.1021/ja044996f.
[458] G. Wittig, A. Krebs, Zur Existenz niedergliedriger Cycloalkine, I, Chem. Ber. 94 (1961), https://doi.org/10.1002/cber.19610941213.
[459] J.S. Silver, K.A. Günay, A.A. Cutler, T.O. Vogler, T.E. Brown, B.T. Pawlikowski, O. J. Bednarski, K.L. Bannister, C.J. Rogowski, A.G. McKay, F.W. DelRio, B.B. Olwin, K.S. Anseth, Injury-mediated stiffening persistently activates muscle stem cells through YAP and TAZ mechanotransduction, Sci. Adv. 7 (2021), https://doi.org/10.1126/SCIADV.ABE4501.
[460] J. Gopinathan, I. Noh, Click Chemistry-Based Injectable Hydrogels and Bioprinting Inks for Tissue Engineering Applications, Tissue Eng. Regen. Med. 15 (2018), https://doi.org/10.1007/s13770-018-0152-8.
[461] L.H. Du Plessis, C. Gouws, D. Nieto, The influence of viscosity of hydrogels on the spreading and migration of cells in 3D bioprinted skin cancer models, Front. Cell Dev. Biol. 12 (2024), https://doi.org/10.3389/fcell.2024.1391259.
[462] A.C. Gaffey, M.H. Chen, C.M. Venkataraman, A. Trubelja, C.B. Rodell, P.V. Dinh, G. Hung, J.W. Macarthur, R.V. Soopan, J.A. Burdick, P. Atluri, Injectable shear-thinning hydrogels used to deliver endothelial progenitor cells, enhance cell engraftment, and improve ischemic myocardium, J. Thorac. Cardiovasc. Surg. 150 (2015), https://doi.org/10.1016/j.jtcvs.2015.07.035.
[463] N. Zandi, E.S. Sani, E. Mostafavi, D.M. Ibrahim, B. Saleh, M.A. Shokrgozar, E. Tamjid, P.S. Weiss, A. Simchi, N. Annabi, Nanoengineered shear-thinning and bioprintable hydrogel as a versatile platform for biomedical applications, Biomaterials 267 (2021), https://doi.org/10.1016/j.biomaterials.2020.120476.
[464] K. Lee, Y. Chen, X. Li, N. Kawazoe, Y. Yang, G. Chen, Influence of viscosity on chondrogenic differentiation of mesenchymal stem cells during 3D culture in viscous gelatin solution-embedded hydrogels, J. Mater. Sci. Technol. 63 (2021), https://doi.org/10.1016/j.jmst.2020.05.018.
[465] S. Uman, A. Dhand, J.A. Burdick, Recent advances in shear-thinning and self-healing hydrogels for biomedical applications, J. Appl. Polym. Sci. 137 (2020), https://doi.org/10.1002/app.48668.
[466] C. Loebel, C.B. Rodell, M.H. Chen, J.A. Burdick, Shear-thinning and self-healing hydrogels as injectable therapeutics and for 3D-printing, Nat. Protoc. 12 (2017), https://doi.org/10.1038/nprot.2017.053.
[467] I. Ali, L. Ali Shah, T. ur Rehman, S. Faizan, Investigation of the viscoelastic behavior of PVA-P(AAm/AMPS) IPN hydrogel with enhanced mechanical strength and excellent recoverability, J. Polym. Res. 29 (2022), https://doi.org/10.1007/s10965-021-02841-2.
[468] K. Kato, T. Yasuda, K. Ito, Viscoelastic properties of slide-ring gels reflecting sliding dynamics of partial chains and entropy of ring components, Macromolecules 46 (2013), https://doi.org/10.1021/ma3021135.
[469] Y. Galeano-Duque, P.K. Sharma, M. Mesa, Modulating the water behavior, microstructure, and viscoelasticity of plasma-derived hydrogels by adding silica nanoparticles with tailored chemical and colloidal properties, Mater. Today Commun. 34 (2023), https://doi.org/10.1016/j.mtcomm.2022.105243.
[470] X. Li, K. Cui, T. Kurokawa, Y.N. Ye, T.L. Sun, C. Yu, C. Creto, J.P. Gong, Effect of mesoscale phase contrast on fatigue-delaying behavior of self-healing hydrogels, Sci. Adv. 7 (2021), https://doi.org/10.1126/sciadv.abe8210.
[471] R.F. Schmidt, H. Kiefer, R. Dalgliesh, M. Gradzielski, R.R. Netz, Nanoscopic Interfacial Hydrogel Viscoelasticity Revealed from Comparison of Macroscopic and Microscopic Rheology, Nano Lett. (2024), https://doi.org/10.1021/acs.nanolett.3c04884.
[472] W. Cui, Y. Cai, Y. Zheng, R. Ran, Mechanical enhancement of hydrophobically associating hydrogels by solvent-regulated phase separation, Polymer 210 (2020), https://doi.org/10.1016/j.polymer.2020.123042.
[473] J. Lou, R. Stowers, S. Nam, Y. Xia, O. Chaudhuri, Stress relaxing hyaluronic acid-collagen hydrogels promote cell spreading, fiber remodeling, and focal adhesion formation in 3D cell culture, Biomaterials 154 (2018), https://doi.org/10.1016/j.biomaterials.2017.11.004.
[474] C. Liu, Q. Yu, Z. Yuan, Q. Guo, X. Liao, F. Han, T. Feng, G. Liu, R. Zhao, Z. Zhu, H. Mao, C. Zhu, B. Li, Engineering the viscoelasticity of gelatin methacryloyl (GelMA) hydrogels via small "dynamic bridges" to regulate BMSC behaviors for osteochondral regeneration, Bioact. Mater. 25 (2023), https://doi.org/10.1016/j.bioactmat.2022.07.031.
[475] J. Liu, C.S.Y. Tan, Z. Yu, N. Li, C. Abell, O.A. Scherman, Tough Supramolecular Polymer Networks with Extreme Stretchability and Fast Room-Temperature Self-Healing, Adv. Mater. 29 (2017), https://doi.org/10.1002/adma.201605325.
[476] T. Long, Y. Li, X. Fang, J. Sun, Salt-Mediated Polyampholyte Hydrogels with High Mechanical Strength, Excellent Self-Healing Property, and Satisfactory Electrical Conductivity, Adv. Funct. Mater. 28 (2018), https://doi.org/10.1002/adfm.201804416.
[477] X. Dai, Y. Zhang, L. Gao, T. Bai, W. Wang, Y. Cui, W. Liu, A mechanically strong, highly stable, thermoplastic, and self-healable supramolecular polymer hydrogel, Adv. Mater. 27 (2015), https://doi.org/10.1002/adma.201500534.
[478] Y.J. Wang, X.N. Zhang, Y. Song, Y. Zhao, L. Chen, F. Su, L. Li, Z.L. Wu, Q. Zheng, Ultrastiff and Tough Supramolecular Hydrogels with a Dense and Robust

Hydrogen Bond Network, Chem. Mater. 31 (2019), https://doi.org/10.1021/acs.chemmater.8b05262.
[479] W. Sun, B. Xue, Q. Fan, R. Tao, C. Wang, X. Wang, Y. Li, M. Qin, W. Wang, B. Chen, Y. Cao, Molecular engineering of metal coordination interactions for strong, tough, and fast-recovery hydrogels, Sci. Adv. 6 (2020), https://doi.org/10.1126/sciadv.aaz9531.
[480] Q. Zheng, L. Zhao, J. Wang, S. Wang, Y. Liu, X. Liu, High-strength and high-toughness sodium alginate/polyacrylamide double physically crosslinked network hydrogel with superior self-healing and self-recovery properties prepared by a one-pot method, Colloids Surf. Physicochem. Eng. Asp. 589 (2020), https://doi.org/10.1016/j.colsurfa.2019.124402.
[481] B. Yi, Q. Xu, W. Liu, An overview of substrate stiffness guided cellular response and its applications in tissue regeneration, Bioact. Mater. 15 (2022), https://doi.org/10.1016/j.bioactmat.2021.12.005.
[482] B. Gao, Q. Yang, X. Zhao, G. Jin, Y. Ma, F. Xu, 4D Bioprinting for Biomedical Applications, Trends Biotechnol. 34 (2016), https://doi.org/10.1016/j.tibtech.2016.03.004.
[483] S. Yang, L. Jang, S. Kim, J. Yang, K. Yang, S.W. Cho, J.Y. Lee, Polypyrrole/Alginate Hybrid Hydrogels: Electrically Conductive and Soft Biomaterials for Human Mesenchymal Stem Cell Culture and Potential Neural Tissue Engineering Applications, Macromol. Biosci. 16 (2016), https://doi.org/10.1002/mabi.201600148.
[484] K.B.C. Imani, J.M. Dodda, J. Yoon, F.G. Torres, A.B. Imran, G.R. Deen, R. Al-Ansari, Seamless Integration of Conducting Hydrogels in Daily Life: From Preparation to Wearable Application, Adv. Sci. 11 (2024), https://doi.org/10.1002/advs.202306784.
[485] P. Huo, H. Ding, Z. Tang, X. Liang, J. Xu, M. Wang, R. Liang, G. Sun, Conductive silk fibroin hydrogel with semi-interpenetrating network with high toughness and fast self-recovery for strain sensors, Int. J. Biol. Macromol. 212 (2022), https://doi.org/10.1016/j.ijbiomac.2022.05.084.
[486] X. Liu, A.L. Miller, S. Park, B.E. Waletzki, Z. Zhou, A. Terzic, L. Lu, Functionalized Carbon Nanotube and Graphene Oxide Embedded Electrically Conductive Hydrogel Synergistically Stimulates Nerve Cell Differentiation, ACS Appl. Mater. Interfaces 9 (2017), https://doi.org/10.1021/acsami.7b02072.
[487] X. He, T. Zhuang, S. Ruan, X. Xia, Y. Xia, J. Zhang, H. Huang, Y. Gan, W. Zhang, An innovative poly(ionic liquid) hydrogel-based anti-freezing electrolyte with high conductivity for supercapacitor, Chem. Eng. J. 466 (2023), https://doi.org/10.1016/j.cej.2023.143209.
[488] L. Geng, W. Liu, B. Fan, J. Wu, S. Shi, A. Huang, J. Hu, X. Peng, Anisotropic double-network hydrogels integrated superior performance of strength, toughness and conductivity for flexible multi-functional sensors, Chem. Eng. J. 462 (2023), https://doi.org/10.1016/j.cej.2023.142226.
[489] A. Pattnaik, A.S. Sanket, S. Pradhan, R. Sahoo, S. Das, S. Pany, T.E.L. Douglas, R. Dandela, Q. Liu, J. Rajadas, S. Pati, S.C. De Smedt, K. Braeckmans, S.K. Samal, Designing of gradient scaffolds and their applications in tissue regeneration, Biomaterials 296 (2023), https://doi.org/10.1016/j.biomaterials.2023.122078.
[490] F. Snow, C. O'Connell, P. Yang, M. Kita, E. Pirogova, R.J. Williams, R.M.I. Kapsa, A. Quigley, Engineering interfacial tissues: The myotendinous junction, APL Bioeng. 8 (2024), https://doi.org/10.1063/5.0189221.
[491] A. Williamson, K. Khoshmanesh, E. Pirogova, P. Yang, F. Snow, R. Williams, A. Quigley, R.M.I. Kapsa, Bioreactors: A Regenerative Approach to Skeletal Muscle Engineering for Repair and Replacement, Adv. NanoBiomed Res. 4 (2024), https://doi.org/10.1002/anbr.202400030.
[492] D.A. Gaspar, V. Gomide, F.J. Monteiro, The role of perfusion bioreactors in bone tissue engineering, Biomatter 2 (2012), https://doi.org/10.4161/biom.22170.
[493] F. Anton, K. Suck, S. Diederichs, L. Behr, B. Hitzmann, M. Van Griensven, T. Scheper, C. Kasper, Design and characterization of a rotating bed system bioreactor for tissue engineering applications, Biotechnol. Prog. (2008), https://doi.org/10.1021/bp070241b.
[494] U. Blache, E.M. Ford, B. Ha, L. Rijns, O. Chaudhuri, P.Y.W. Dankers, A.M. Kloxin, J.G. Snedeker, E. Gentleman, Engineered hydrogels for mechanobiology, Nat. Rev. Methods Primer 2 (2022), https://doi.org/10.1038/s43586-022-00179-7.
[495] X. Di, X. Gao, L. Peng, J. Ai, X. Jin, S. Qi, H. Li, K. Wang, D. Luo, Cellular mechanotransduction in health and diseases: from molecular mechanism to therapeutic targets, Signal Transduct. Target. Ther. 8 (2023), https://doi.org/10.1038/s41392-023-01501-9.
[496] J. Jacob, N. More, K. Kalia, G. Kapusetti, Piezoelectric smart biomaterials for bone and cartilage tissue engineering, Inflamm. Regen. 38 (2018), https://doi.org/10.1186/s41232-018-0059-8.
[497] N.A. Kamel, Bio-piezoelectricity: fundamentals and applications in tissue engineering and regenerative medicine, Biophys. Rev. 14 (2022), https://doi.org/10.1007/s12551-022-00969-z.