Optimal Properties of the Scaffold

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Osteoarthritis leads to the progressive decay of articular cartilage. Due to its intrinsic avascular character, cartilage shows an inadequate capacity for regeneration. Cartilage loss may result in chronic pain, movement disorder and morbidity, which lack effective treatments except for joint replacement for late-stage osteoarthritis. To overcome this challenge, tissue engineering has emerged as a promising method. Scaffolds provide mechanical and biochemical support to stem cells that undergo differentiation and secrete a cartilage-specific matrix, and this strategy has been proven to have positive results. The ideal 3D scaffolds need to have versatile properties to mediate cell–cell signalling and cell–matrix interactions for controlling the cellular behaviour of MSCs, specifically: (1) sufficient mechanical strength; (2) biocompatibility; (3) suitable surface morphology for cell attachment; (4) appropriate porosity and pore size to allow the cells to infiltrate as well as nutrients and waste to diffuse; (5) promoting cell proliferation, differentiation and maintenance of a chondrogenic phenotype of seeded cells; (6) capability of integrating with native tissues; and (7) controlled degradation without toxic byproducts.

osteoarthritis cartilage repair biomaterial

1. Introduction

All of the criteria are for a successful scaffold that promotes sustained ECM deposition and integrates neocartilage into the surrounding native cartilage. Scientists have studied how to improve the chondrogenic properties of scaffolds by means of various methods and have obtained some encouraging results. However, the correlating mechanisms are not fully understood. Until now, the optimal material and technique have yet to emerge.

2. Composition of the Scaffold

The composition of the ECM that encloses stem cells illustrates the pivotal influence on directing cell differentiation. Matrices offer biochemical and physical cues to drive stem cells to a particular lineage [1]. For example, cells produced type II collagen on type II scaffolds and type I collagen on type I scaffolds [2]. The possible mechanism by which the composition modulates differentiation is cell–matrix interactions, including integrin expression and cytoskeleton organisation. First, different types of ECM stimulate cells to express different integrins on the cell membrane and transmit various chemical and mechanical signals into cells through integrins, which elicits a cascade of gene translational events, thus influencing cell adhesion, migration, proliferation and differentiation [3][4]. Second, cytoskeletal organisation regulates chondrogenesis via changes in microfilaments (consisting of actin) and microtubules (consisting of tubulin) [4]. Hence, the properties of scaffolds can benefit greatly from mimicking the natural composition of ECM. This strategy leads researchers to find a suitable material from or similar to the ECM.

Articular cartilage is predominantly composed of type II collagen and GAGs. Knowing this, many researchers have utilised these materials to fabricate scaffolds to direct MSC differentiation. Murphy et al. [5] investigated murine BMSC-seeded scaffolds made of collagen and two types of GAGs, either chondroitin sulfate or HA. The collagen-HA scaffolds promoted higher *SOX9* expression than collagen-chondroitin sulfate scaffolds; in contrast, collagen-chondroitin sulfate scaffolds expressed higher *RUNX2* expression than collagen-HA scaffolds, which indicated that HA had a chondrogenic influence, while chondroitin sulfate had an osteogenic influence on murine BMSCs. In some cases, the mixed composition did not increase ECM production; for example, incorporating HA into chitosan scaffolds displayed no significant impact on enhancing chondrogenesis. This phenomenon may come down to a low dose of HA (0.01%) added to the mixtures [6]. Nevertheless, materials with high water uptake and swelling ratios act as physical cues to promote chondrogenic differentiation [7].

3. Stiffness of the Scaffold

When MSCs anchor onto the substrate surface through integrin-mediated adhesion, the substrate stiffness reorganises ligands and modulates integrin binding. Meanwhile, cells reshape cytoskeletal organisation by sensing substrate stiffness and transferring mechanical signals into cells via nonmuscle myosin II [8][9]. A study reported that MSCs exhibited different morphologies and behaviours on gels with different stiffnesses; MSCs aggregated into clusters in round shapes on soft gels, spread out in elongated shapes, proliferated rapidly on stiff gels, and partially aggregated on medium-stiff gels [9]. The condensation and spherical shape are highly relevant to chondrogenesis [10]. Similarly, Wang et al. [11] reported that chondrogenesis relied on the interaction of matrix stiffness and biochemical cues. Wu et al. [12] also indicated that chondrogenesis and ECM accumulation depended on matrix stiffness, and soft scaffolds promoted better chondrogenesis in a dose-dependent manner, and vice versa. These findings illustrate that MSCs are extremely sensitive to stiffness, which could significantly affect stem cell fate [2][8], especially for the first 1–2 weeks of the early stage of chondrogenic differentiation. As mentioned above, the compression modulus of cartilage is ~1 MPa, with dynamic compressive stiffness at ~10 MPa. Hence, a soft scaffold with a similar compressive stiffness would promote chondrogenesis, and the mismatch of scaffold stiffness and adjacent native tissues may result in unexpected differentiation and the failure of the long-term integration of implants. For instance, the literature revealed that MSCs differentiated into osteoblasts when cultured on a matrix that stiffened at later time points [13].

Regarding some natural polymers that have insufficient stiffness, adding some synthetic polymers can improve mechanical properties. For example, the incorporation of HAp into Alg improved the integral stiffness of the scaffold [14]. In another aspect, a study illustrated that scaffold stiffness is integrally influenced not only by the robust elastic modulus of the composition but also by the porosity and topology (interconnection and shape of pores) [15]. Another study reported that not only would the cross-link density increase stiffness and result in the formation of fibrocartilage [16], but also that newly deposited cartilage matrices would gradually heighten the stiffness [17]. When researchers are designing scaffolds, they should consider these parameters and situations.

Unlike differentiation, whether stiffness impacts cell proliferation remains controversial. Wu et al. [12] found that the proliferation rate remained similar across PCL, PLA and PGA scaffolds with different stiffnesses. In contrast, some

studies showed a higher proliferation rate and larger spreading area on stiffer substrates [18][19].

4. Porosity, Pore Size and Pore Shape of the Scaffold

Porosity has also been known as a regulator of cellular behaviours. It is an essential parameter because it guarantees the viability of the cells on the scaffold before expansion and differentiation. High porosity and interconnected inner structure ensure that nutrients and gases diffuse inward into the deep zone of the scaffold and remove metabolic wastes inside-out [2], allowing the cells to migrate deep into the scaffold. Porosity was found to decrease with increased cross-link density, but the pore size was not affected [20]. It has been reported that a minimum of 50% porosity is adequate for the attachment, migration and proliferation of cells on scaffolds [21][22], but a more significant porosity (more than 90%) is more favourable [23].

The pore size affects initial cell adhesion and subsequent events, including proliferation, migration and differentiation. If the pore size far exceeds the dimensions of MSCs, it will influence MSC migration ability and speed. On the flip side, the pore size should not be too small; otherwise, it would be easily blocked by expanded cells, leading to a limitation of cell infiltration and apoptosis [24]. For cartilage regeneration, an article pointed out that collagen scaffolds with pore sizes of 50–300 μm are generally favourable to stimulate cartilaginous tissue formation [25], consistent with another study indicating that collagen-HA scaffolds with pore sizes of 90–300 μm promoted chondrogenesis. In addition, among them, the largest mean pore size (300 μm) displayed significantly higher cell proliferation, cartilage-specific gene expression, cartilage-like matrix deposition, and compressive modulus compared to other smaller sizes [26]. The reported maximum pore diameter was approximately 500–550 μm in SF scaffolds, which showed not only the best cell adhesion and cell proliferation but also facilitated chondrogenic differentiation [27][28]. Hereto, the literature showed that the pore size suitable for chondrogenic differentiation is not limited to a specific figure, but rather a wide range from 50 μm to 550 μm is acceptable. It is presumed that the optimal pore size may change from material to material, and the possible mechanism for this may be that porosity and pore size simultaneously affect both substance exchange and mechanical properties [29] to different extents in different materials, finally showing an overall effect on cell behaviour. Collectively, a pore size between 200 and 500 μm for chondrogenesis is recommended. For microparticles, the literature usually recommends a bead diameter in the range of 100–500 μm [13][30], which ensures that the maximum substance diffusion distance is within the range of metabolically active tissues [15].

5. Surface Properties of the Scaffold

MSC attachment to the surface of the scaffold is the first step prior to subsequent cellular activities. Apart from the surface roughness facilitating cell adhesion, nonreceptor mediation (weak chemical bonding), such as electrostatic, hydrogen or ionic bonding, also achieves adhesion. However, this type of adhesion lacks cell–matrix signal transmission, which is vital for the viability of cells and ECM secretion. In contrast, receptor-mediated adhesion via ECM molecules, including FN or collagen, allows cells to receive physiological signals [15]. These specific adhesion motifs on ECM molecules contain at least three amino acids symbolised by Arg-Gly-Asp (RGD) [31]. RGD is

commonly used to assist cells in adhering to scaffolds without intrinsic binding sites. When RGD is integrated onto the surface of the scaffold, it can work as a ligand and specifically bind with integrin of receptor cells. In this way, cells can anchor to the surface of the scaffold and sense cell–matrix signal transmission. Scaffolds made from natural materials (e.g., collagen, AG, and fibrin) naturally possess RGD sequences, but synthetic polymers (e.g., PCL, PLA, and PLGA) may require deliberately incorporating RGD through protein adsorption or other methods [15]. The literature reported that hPDC-embedded PEG hydrogels combined with RGD promoted higher GAG deposition and chondrogenic gene expression than RGD-free PEG hydrogels [32]. Another study indicated that the E7 peptide (an RGD sequence) could significantly enhance murine BMSC aggregation, viability and chondrogenic differentiation [33]. RGD density is another crucial indicator that influences MSC focal adhesion, spreading and proliferation. Lower RGD density has been shown to enhance chondrogenesis of hMSCs on electrospun methacrylated HA scaffolds [34].

Apart from RGD, selective specific chemical groups can be an alternative. Commonly, plasma surface modification is used to introduce chemical groups onto scaffolds. Some chemical groups, such as the carboxyl (−COOH) group and −OH, present on the scaffold surface have been shown to upregulate chondrogenic marker gene expression in MSCs in the absence of GFs [35][36], while $–NH_2$ facilitates osteogenesis [35]. For instance, cellulose comprising three −OH groups per repeat could facilitate chondrogenic differentiation [37]. PLGA scaffolds that originally had poor bioactivities were activated by introducing –COOH groups and heparin onto the surface. This method finally formed –CONH– and showed binding affinity to MSCs and TGF-β1. As a result, modified PLGA scaffolds with GFs sharply increased the expression of cartilage-specific markers, along with type II collagen production [38]. The plasma surface treatment of scaffolds with N_2, O_2 and NH_3 endowed the construct surface with more hydrophilicity and more bioadhesion [39].

The abovementioned RGD or chemical groups belong to surface chemistry. On the other hand, surface physics and the nanoscale topography, including patterns of the surface, influence chondrogenesis. They affect cellular processes through changes in focal adhesion and the actin cytoskeleton. Currently, nanoscale surface modification is attracting increasing interest in tissue engineering. Specifically, a nanopillar surface facilitated hyaline-like cartilage, while a nanograting surface tended to induce fibro/superficial zone-like cartilage [12]. BMP-2-coated TiO_2 nanotubes 100 nm in size strongly supported chondrogenic differentiation, while 15 nm nanotubes greatly facilitated osteogenic differentiation, which showed fewer focal contacts and stress fibres but allowed cell aggregation to facilitate chondrogenesis [40]. Nevertheless, matrix stiffness still showed a far more dominant effect than surface patterns on differentiation [12][41].

6. Hydrophilicity and Electric Charge of the Scaffold

The moderate hydrophilicity and positive charge of the scaffold are considered to represent the optimal adhesive properties for cells [42]. The ability to retain water and a high swelling rate have been shown to promote cell infiltration, proliferation and differentiation [43]. The underlying mechanism is that adhesion molecules are adsorbed in a favourable geometry in this situation, making it easier for ligands to bind with cell receptors [44]. SF is quite hydrophobic in the dry state, but it becomes hydrophilic when wetted with a water contact angle of 0°, which

successfully induces chondrogenesis [45]. Synthetic polymers usually exhibit intrinsic hydrophobicity that goes against cell attachment [46]. For instance, the hydrophobicity of PLGA limits the adhesion and proliferation of osteoblasts, chondrocytes and MSCs [47].

The cell membrane has a negative charge that results in a difficulty in attaching to negatively charged materials but an affinity to positively charged surfaces [48]. CS showed an intrinsic high positive charge density in acidic solution due to primary amine groups. Thus, CS can easily facilitate cell adhesion and consequent chondrogenic differentiation [49]. To enhance the affinity of intrinsically negatively charged materials such as PLGA, it needs to integrate another cationic or absorb some specific proteins onto the scaffold surface, to which the cells attach via integrin receptors [50]. For example, after combining P188, PLGA-P188-PLGA microbeads showed a positive charge, which facilitated the adhesion of cells [30][51].

7. Anisotropic Structure of the Scaffold

Anisotropic structures of collagen in cartilage have remarkable effects on the mechanical properties of the cartilage [52] and the differential fate of MSCs, similar to scaffolds. For instance, adding aligned nanofibers parallel to the direction of the hydrogel surface would significantly enhance the superficial zone, similar to the differentiation of hBMSCs [53]. Chou et al. [54] indicated that a bovine type I collagen scaffold containing parallel micrometre-wide channels displayed enhanced compressive properties (elasticity modulus ranged from 1.2 to 2.1 MPa) compared to control constructs without these channels in mechanical testing, along with extensive GAG and type II collagen deposition. These channels were in favour of cell localisation, aggregation and rounding, facilitating aligned neo-cartilage formed perpendicularly along the length of guidance channels, similar to the deep zone of native articular cartilage. Scaffolds fabricated using natural materials through gelatinisation and other methods typically form unoriented and disarrayed matrices. On the other hand, synthetic materials are more accessible to form oriented structures via precisely controlled 3D printing or woven methods, which endows an advantage to synthetic materials for customisation.

Abbreviations

Abbr.	Full Name
GFs	growth factors
ACI	autologous chondrocyte implantation
MSCs	mesenchymal stem cells
hBMSCs	human bone marrow-derived MSCs
2D	two-dimensional
3D	three-dimensional

3D-TIPS	3D printing-guided thermally induced phase separation
GAGs	glycosaminoglycans
SF	silk fibroin
HA	hyaluronic acid
Alg	alginate
HAp	hydroxyapatite
CS	chitosan
PLA	poly(lactic acid)
PGA	poly(glycolic acid)
PLGA	poly(lactic-co-glycolic acid)
PUU	poly(urea-urethane)
POSS	polyhedral oligomeric silsesquioxane
PEG	polyethene glycol
AG	agarose
MOCART	magnetic resonance observation of cartilage repair tissue
MNPs	magnetic nanoparticles
GO	graphene oxide
TGF	transforming growth factor
BMP	bone morphogenetic protein
IGF	insulin-like growth factor
−OH	hydroxyl group
−COOH	carboxyl group
HP	hydrostatic pressure

References

1. Tamaddon, M.; Burrows, M.; Ferreira, S.A.; Dazzi, F.; Apperley, J.F.; Bradshaw, A.; Brand, D.D.; Czernuszka, J.; Gentleman, E. Monomeric, porous type II collagen scaffolds promote chondrogenic differentiation of human bone marrow mesenchymal stem cells in vitro. Sci. Rep. 2017, 7, 43519.

2. He, J.; Guo, J.; Jiang, B.; Yao, R.; Wu, Y.; Wu, F. Directing the osteoblastic and chondrocytic differentiations of mesenchymal stem cells: Matrix vs. induction media. Regen. Biomater. 2017, 4, 269–279.
3. Li, Y.Y.; Choy, T.H.; Ho, F.C.; Chan, P.B. Scaffold composition affects cytoskeleton organization, cell-matrix interaction and the cellular fate of human mesenchymal stem cells upon chondrogenic differentiation. Biomaterials 2015, 52, 208–220.
4. Murphy, C.M.; Matsiko, A.; Haugh, M.G.; Gleeson, J.P.; O'Brien, F.J. Mesenchymal stem cell fate is regulated by the composition and mechanical properties of collagen-glycosaminoglycan scaffolds. J. Mech. Behav. Biomed. Mater. 2012, 11, 53–62.
5. Schwartz, Z.; Griffon, D.J.; Fredericks, L.P.; Lee, H.B.; Weng, H.Y. Hyaluronic acid and chondrogenesis of murine bone marrow mesenchymal stem cells in chitosan sponges. Am. J. Vet. Res. 2011, 72, 42–50.
6. Jaipaew, J.; Wangkulangkul, P.; Meesane, J.; Raungrut, P.; Puttawibul, P. Mimicked cartilage scaffolds of silk fibroin/hyaluronic acid with stem cells for osteoarthritis surgery: Morphological, mechanical, and physical clues. Mater. Sci. Eng. C Mater. Biol. Appl. 2016, 64, 173–182.
7. Engler, A.J.; Sen, S.; Sweeney, H.L.; Discher, D.E. Matrix elasticity directs stem cell lineage specification. Cell 2006, 126, 677–689.
8. Kwon, H.J.; Yasuda, K. Chondrogenesis on sulfonate-coated hydrogels is regulated by their mechanical properties. J. Mech. Behav. Biomed. Mater. 2013, 17, 337–346.
9. McBride, S.H.; Falls, T.; Tate, M.L.K. Modulation of stem cell shape and fate B: Mechanical modulation of cell shape and gene expression. Tissue Eng. Part A 2008, 14, 1573–1580.
10. Wang, T.; Lai, J.H.; Han, L.H.; Tong, X.; Yang, F. Chondrogenic differentiation of adipose-derived stromal cells in combinatorial hydrogels containing cartilage matrix proteins with decoupled mechanical stiffness. Tissue Eng. Part A 2014, 20, 2131–2139.
11. Wu, Y.; Yang, Z.; Law, J.B.; He, A.Y.; Abbas, A.A.; Denslin, V.; Kamarul, T.; Hui, J.H.; Lee, E.H. The Combined Effect of Substrate Stiffness and Surface Topography on Chondrogenic Differentiation of Mesenchymal Stem Cells. Tissue Eng. Part A 2017, 23, 43–54.
12. Guvendiren, M.; Burdick, J.A. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. Nat. Commun. 2012, 3, 792.
13. Yu, H.; Cauchois, G.; Louvet, N.; Chen, Y.; Rahouadj, R.; Huselstein, C. Comparison of MSC properties in two different hydrogels. Impact of mechanical properties. Biomed. Mater. Eng. 2017, 28, S193–S200.
14. Tamaddon, M.; Liu, C. Enhancing Biological and Biomechanical Fixation of Osteochondral Scaffold: A Grand Challenge. Adv. Exp. Med. Biol. 2018, 1059, 255–298.

15. Toh, W.S.; Lim, T.C.; Kurisawa, M.; Spector, M. Modulation of mesenchymal stem cell chondrogenesis in a tunable hyaluronic acid hydrogel microenvironment. Biomaterials 2012, 33, 3835–3845.
16. Li, C.H.; Chik, T.K.; Ngan, A.H.; Chan, S.C.; Shum, D.K.; Chan, B.P. Correlation between compositional and mechanical properties of human mesenchymal stem cell-collagen microspheres during chondrogenic differentiation. Tissue Eng, Part A 2011, 17, 777–788.
17. Wang, L.S.; Boulaire, J.; Chan, P.P.; Chung, J.E.; Kurisawa, M. The role of stiffness of gelatin-hydroxyphenylpropionic acid hydrogels formed by enzyme-mediated crosslinking on the differentiation of human mesenchymal stem cell. Biomaterials 2010, 31, 8608–8616.
18. Evans, N.D.; Minelli, C.; Gentleman, E.; LaPointe, V.; Patankar, S.N.; Kallivretaki, M.; Chen, X.; Roberts, C.J.; Stevens, M.M. Substrate stiffness affects early differentiation events in embryonic stem cells. Eur. Cells Mater. 2009, 18, 1–13.
19. Yang, C.; Frei, H.; Rossi, F.M.; Burt, H.M. The differential in vitro and in vivo responses of bone marrow stromal cells on novel porous gelatin-alginate scaffolds. J. Tissue Eng. Regen. Med. 2009, 3, 601–614.
20. Wu, L.; Virdee, J.; Maughan, E.; Darbyshire, A.; Jell, G.; Loizidou, M.; Emberton, M.; Butler, P.; Howkins, A.; Reynolds, A.; et al. Stiffness memory nanohybrid scaffolds generated by indirect 3D printing for biologically responsive soft implants. Acta Biomater. 2018, 80, 188–202.
21. Kokorev, O.V.; Hodorenko, V.N.; Chekalkin, T.L.; Kim, J.S.; Kang, S.B.; Dambaev, G.; Gunther, V.E. In vitro and in vivo evaluation of porous TiNi-based alloy as a scaffold for cell tissue engineering. Artif. Cells Nanomed. Biotechnol. 2016, 44, 704–709.
22. Huang, Z.; Nooeaid, P.; Kohl, B.; Roether, J.A.; Schubert, D.W.; Meier, C.; Boccaccini, A.R.; Godkin, O.; Ertel, W.; Arens, S.; et al. Chondrogenesis of human bone marrow mesenchymal stromal cells in highly porous alginate-foams supplemented with chondroitin sulfate. Mater. Sci. Eng. C Mater. Biol. Appl. 2015, 50, 160–172.
23. Murphy, C.M.; O'Brien, F.J.; Little, D.G.; Schindeler, A. Cell-scaffold interactions in the bone tissue engineering triad. Eur. Cells Mater. 2013, 26, 120–132.
24. Nehrer, S.; Breinan, H.A.; Ramappa, A.; Young, G.; Shortkroff, S.; Louie, L.K.; Sledge, C.B.; Yannas, I.V.; Spector, M. Matrix collagen type and pore size influence behaviour of seeded canine chondrocytes. Biomaterials 1997, 18, 769–776.
25. Matsiko, A.; Gleeson, J.P.; O'Brien, F.J. Scaffold mean pore size influences mesenchymal stem cell chondrogenic differentiation and matrix deposition. Tissue Eng. Part A 2015, 21, 486–497.
26. Wang, Y.; Kim, U.J.; Blasioli, D.J.; Kim, H.J.; Kaplan, D.L. In vitro cartilage tissue engineering with 3D porous aqueous-derived silk scaffolds and mesenchymal stem cells. Biomaterials 2005, 26, 7082–7094.

27. Barlian, A.; Judawisastra, H.; Alfarafisa, N.M.; Wibowo, U.A.; Rosadi, I. Chondrogenic differentiation of adipose-derived mesenchymal stem cells induced by L-ascorbic acid and platelet rich plasma on silk fibroin scaffold. PeerJ 2018, 6, e5809.
28. Karageorgiou, V.; Kaplan, D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005, 26, 5474–5491.
29. Morille, M.; Toupet, K.; Montero-Menei, C.N.; Jorgensen, C.; Noel, D. PLGA-based microcarriers induce mesenchymal stem cell chondrogenesis and stimulate cartilage repair in osteoarthritis. Biomaterials 2016, 88, 60–69.
30. Ruoslahti, E.; Pierschbacher, M.D. New perspectives in cell adhesion: RGD and integrins. Science 1987, 238, 491–497.
31. Kudva, A.K.; Luyten, F.P.; Patterson, J. RGD-functionalized polyethylene glycol hydrogels support proliferation and in vitro chondrogenesis of human periosteum-derived cells. J. Biomed. Mater. Res. A 2018, 106, 33–42.
32. Meng, Q.; Man, Z.; Dai, L.; Huang, H.; Zhang, X.; Hu, X.; Shao, Z.; Zhu, J.; Zhang, J.; Fu, X.; et al. A composite scaffold of MSC affinity peptide-modified demineralized bone matrix particles and chitosan hydrogel for cartilage regeneration. Sci. Rep. 2015, 5, 17802.
33. Kim, I.L.; Khetan, S.; Baker, B.M.; Chen, C.S.; Burdick, J.A. Fibrous hyaluronic acid hydrogels that direct MSC chondrogenesis through mechanical and adhesive cues. Biomaterials 2013, 34, 5571–5580.
34. Curran, J.M.; Fawcett, S.; Hamilton, L.; Rhodes, N.P.; Rahman, C.V.; Alexander, M.; Shakesheff, K.; Hunt, J.A. The osteogenic response of mesenchymal stem cells to an injectable PLGA bone regeneration system. Biomaterials 2013, 34, 9352–9364.
35. Curran, J.M.; Chen, R.; Hunt, J.A. Controlling the phenotype and function of mesenchymal stem cells in vitro by adhesion to silane-modified clean glass surfaces. Biomaterials 2005, 26, 7057–7067.
36. Singh, N.; Rahatekar, S.S.; Koziol, K.K.; Ng, T.S.; Patil, A.J.; Mann, S.; Hollander, A.P.; Kafienah, W. Directing chondrogenesis of stem cells with specific blends of cellulose and silk. Biomacromolecules 2013, 14, 1287–1298.
37. Park, K.; Cho, K.J.; Kim, J.J.; Kim, I.H.; Han, D.K. Functional PLGA scaffolds for chondrogenesis of bone-marrow-derived mesenchymal stem cells. Macromol. Biosci. 2009, 9, 221–229.
38. Mahboudi, H.; Kazemi, B.; Soleimani, M.; Hanaee-Ahvaz, H.; Ghanbarian, H.; Bandehpour, M.; Enderami, S.E.; Kehtari, M.; Barati, G. Enhanced chondrogenesis of human bone marrow mesenchymal Stem Cell (BMSC) on nanofiber-based polyethersulfone (PES) scaffold. Gene 2018, 643, 98–106.

39. Park, J.; Bauer, S.; Pittrof, A.; Killian, M.S.; Schmuki, P.; von der Mark, K. Synergistic control of mesenchymal stem cell differentiation by nanoscale surface geometry and immobilized growth factors on TiO2 nanotubes. Small 2012, 8, 98–107.
40. Li, Z.; Gong, Y.; Sun, S.; Du, Y.; Lu, D.; Liu, X.; Long, M. Differential regulation of stiffness, topography, and dimension of substrates in rat mesenchymal stem cells. Biomaterials 2013, 34, 7616–7625.
41. Amani, H.; Arzaghi, H.; Bayandori, M.; Dezfuli, A.S.; Pazoki-Toroudi, H.; Shafiee, A.; Moradi, L. Controlling cell behavior through the design of biomaterial surfaces: A focus on surface modification techniques. Adv. Mater. Interfaces 2019, 6, 1900572.
42. Raftery, R.M.; Woods, B.; Marques, A.L.P.; Moreira-Silva, J.; Silva, T.H.; Cryan, S.A.; Reis, R.L.; O'Brien, F.J. Multifunctional biomaterials from the sea: Assessing the effects of chitosan incorporation into collagen scaffolds on mechanical and biological functionality. Acta Biomater. 2016, 43, 160–169.
43. Bacakova, L.; Filova, E.; Parizek, M.; Ruml, T.; Svorcik, V. Modulation of cell adhesion, proliferation and differentiation on materials designed for body implants. Biotechnol. Adv. 2011, 29, 739–767.
44. Voga, M.; Drnovsek, N.; Novak, S.; Majdic, G. Silk fibroin induces chondrogenic differentiation of canine adipose-derived multipotent mesenchymal stromal cells/mesenchymal stem cells. J. Tissue Eng. 2019, 10, 2041731419835056.
45. Chen, M.; Le, D.Q.; Baatrup, A.; Nygaard, J.V.; Hein, S.; Bjerre, L.; Kassem, M.; Zou, X.; Bunger, C. Self-assembled composite matrix in a hierarchical 3-D scaffold for bone tissue engineering. Acta Biomater. 2011, 7, 2244–2255.
46. Rai, V.; Dilisio, M.F.; Dietz, N.E.; Agrawal, D.K. Recent strategies in cartilage repair: A systemic review of the scaffold development and tissue engineering. J. Biomed. Mater. Res. Part A 2017, 105, 2343–2354.
47. Verma, A.; Stellacci, F. Effect of surface properties on nanoparticle-cell interactions. Small 2010, 6, 12–21.
48. Cao, X.; Deng, W.; Wei, Y.; Yang, Y.; Su, W.; Wei, Y.; Xu, X.; Yu, J. Incorporating pTGF-beta1/calcium phosphate nanoparticles with fibronectin into 3-dimensional collagen/chitosan scaffolds: Efficient, sustained gene delivery to stem cells for chondrogenic differentiation. Eur. Cells Mater. 2012, 23, 81–93.
49. Go, G.; Han, J.; Zhen, J.; Zheng, S.; Yoo, A.; Jeon, M.J.; Park, J.O.; Park, S. A Magnetically Actuated Microscaffold Containing Mesenchymal Stem Cells for Articular Cartilage Repair. Adv. Healthc. Mater. 2017, 6, 1601378.

50. Morille, M.; Van-Thanh, T.; Garric, X.; Cayon, J.; Coudane, J.; Noel, D.; Venier-Julienne, M.C.; Montero-Menei, C.N. New PLGA-P188-PLGA matrix enhances TGF-beta3 release from pharmacologically active microcarriers and promotes chondrogenesis of mesenchymal stem cells. J. Control. Release 2013, 170, 99–110.
51. Wilson, W.; Huyghe, J.M.; van Donkelaar, C.C. Depth-dependent compressive equilibrium properties of articular cartilage explained by its composition. Biomech. Model. Mechanobiol. 2007, 6, 43–53.
52. Moeinzadeh, S.; Shariati, S.R.P.; Jabbari, E. Comparative effect of physicomechanical and biomolecular cues on zone-specific chondrogenic differentiation of mesenchymal stem cells. Biomaterials 2016, 92, 57–70.
53. Chou, C.L.; Rivera, A.L.; Williams, V.; Welter, J.F.; Mansour, J.M.; Drazba, J.A.; Sakai, T.; Baskaran, H. Micrometer scale guidance of mesenchymal stem cells to form structurally oriented large-scale tissue engineered cartilage. Acta Biomater. 2017, 60, 210–219.