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REVIEW ARTICLE

Hybrid and Composite Scaffolds Based on Extracellular Matrices for Cartilage Tissue Engineering

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Cartilage consists of chondrocytes and a special extracellular matrix (ECM) having unique biochemical, biophysical, and biomechanical properties that play a critical role in the proliferation and differentiation of cells inherent to cartilage functions. Cartilage tissue engineering (CTE) requires recreating these microenvironmental physicochemical conditions to lead to chondrocyte differentiation from stem cells. ECM-derived hybrid scaffolds based on chondroitin sulfate, hyaluronic acid, collagen, and cartilage ECM analogs provide environments conducive to stem cell proliferation. In this review, we describe hybrid scaffolds based on these four cartilage ECM derivatives; we also categorize these scaffolds based on the methods used for their preparation. The use of hybrid scaffolds is increasing in CTE to address the complexity of cartilage tissue. Thus, a comprehensive review on the topic should be a useful guide for future research.

Keywords: cartilage, tissue engineering, hybrid scaffolds, extracellular matrix (ECM), ECM derivatives

Impact Statement

Scaffolds fabricated from extracellular matrix (ECM) derivatives are composed of conducive structures for cell attachment, proliferation, and differentiation, but generally do not have proper mechanical properties and load-bearing capacity. In contrast, scaffolds based on synthetic biomaterials demonstrate appropriate mechanical strength, but the absence of desirable biological properties is one of their main disadvantages. To integrate mechanical strength and biological cues, these ECM derivatives can be conjugated with synthetic biomaterials. Hence, hybrid scaffolds comprising both advantages of synthetic polymers and ECM derivatives can be considered a robust vehicle for tissue engineering applications.

Introduction

Articular cartilage (AC) is a connective tissue comprising also the main component of joints' surface, which covers and protects bones. Cartilage is composed of specialized cells and extracellular matrix (ECM).[1] Three major types of cartilaginous tissues are distinguished: hyaline cartilage, fibrocartilage, and elastic cartilage. The chemistry and the supramolecular structure of the matrices determine the biomechanical and functional properties of these three cartilage types. Hyaline cartilage is the most abundant and well characterized, found as AC on the surfaces of bones.[2,3] AC can bear loads of up to around 20 MPa during normal joint movements.[1] The major macromolecular components of hyaline cartilage include collagen (COL) type II and X, cartilage oligomeric matrix protein, and proteoglycans,

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among which aggrecan (ACAN) is the major component. Collagens and proteoglycans play critical roles in maintaining cartilage ECM structure. The mechanical strength of the different zones of AC is defined by changes in chemistry and biomechanical cues of the macromolecular components.[3–5] Mechanical characteristics and load-bearing ability of AC are related to and optimized by the arrangement of COL fiber organization, proteoglycan content, and chondrocyte shape.[6] The ECM also forms a niche for stem cells (SCs) and provides biochemical and physical signals to control their proliferation.[7] Recent studies demonstrated that SCs necessitate a particular tissue niche for proliferation and differentiation.[8] In addition to the biochemical environment, mechanical cues of the ECM, such as stiffness, also impact SC lineage differentiation.[9] Soft, medium, and hard matrices can induce mesenchymal stromal cells (MSCs) to differentiate into neurons, myocytes, and osteoblasts, respectively.[10] MSCs are site regulated; they secrete different factors to modulate tissue protection and regeneration by interacting with their niche microenvironment mediated by ECM components.[11]

Recent studies demonstrate that chondrocytes sense physical cues such as receptor/ligand density, rigidity/softness, and shape (dimensionality) of the ECM surface by mechanoreceptors. In response to these cues, the cells modulate the organization of the cytoskeleton, extracellular adhesion sites, and cell shape.[12,13] Studies performed on artificial scaffolds, such as hydrogels, porous/fibrous scaffolds, and ECM-based constructs, demonstrated that SCs expand and differentiate, faster on the softer materials such as COL than stiffer substrates such as chitosan (CHSN).[14,15] One approach to create three-dimensional (3D) structures that mimic the cartilage natural niche is to utilize scaffolds based on cartilage ECM, but generally these scaffolds have lower mechanical strength and suffer from lot-to-lot variations.[16] Synthetic and polymeric materials, including poly(DL-lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), poly(D,L-lactic acid) (PLA) and their copolymers, poly vinyl alcohol (PVA) and also methacrylamide (MA) modification, have been used to fabricate scaffolds for AC replacement and to serve as cell transplantation vehicle for tissue engineering (TE).[17–20] Synthetic polymeric scaffolds allow for good control of mechanical properties, but suffer from a lack of biological properties. These biomaterials are hydrophobic, lack functional adhesion sites and cell recognition signals, and therefore must be modified before cell seeding.[21] In contrast, scaffolds manufactured from ECM components contain surface structures conducive to cell growth.[22] Hence, hybrid scaffolds comprising both advantages of synthetic polymers and ECM derivatives can be considered a strong vehicle for TE applications.[23]

Although other reviews related to cartilage ECM derivatives have been published already, this review focuses on cartilage ECM-derived hybrid and composite scaffolds, prepared and used specifically for cartilage tissue engineering (CTE). Four sections describe (1) COL and COL-based scaffolds, (2) hyaluronic acid (HA) and HA-based scaffolds, (3) chondroitin sulfate (CS) and CS-based scaffolds, and (4) cartilage ECM analogs (cECMa) and cECMa-based scaffolds. The fifth section reviews the different hybrid scaffold processing approaches, and in the sixth section, results from ECM-based scaffolds are discussed in detail.

Hybrid and Composite Scaffolds for CTE

COL-based scaffolds

Collagens can be considered the most important proteins of connective tissue in mammals and also the most abundant proteins in their body.[24] About 30 different forms of COL have been defined according to the amino acid sequences and the structures that they are arranged into. The three most common types of collagens are type II, type I, and type X, in hyaline cartilage, fibrocartilage, and hypertrophic cartilage, respectively.[25] As abundant and biocompatible natural products, collagens have been thoroughly investigated and are widely used as TE scaffolds.[26] Important drawbacks of collagens are, however, inferior mechanical strength and quick degradation, compromising their use for CTE applications. These disadvantages can be partially rectified by crosslinking[27] collagens with natural and synthetic polymers to afford blends and composite materials of superior properties.[28] For instance, blend scaffolds composed of COL crosslinked with CHSN have improved mechanical strength than unmodified COL.[29] A mixture of COL and CHSN exhibits higher mechanical strength because of their miscibility, hydrogen bonding, and electrostatic interactions that reinforce the composite.[30] Partial hydrolysis of COL leads to gelatin (GEL) production. Despite its similarity to chemical COL composition, it lacks antigenicity and immunogenicity. GEL has been used for cartilage, bone, and nerve TE.[31] Hybrid scaffolds composed of COL and synthetic materials, such as PLA, PLGA, PVA, PCL, MA, poly(ethylene glycol) (PEG), or natural biomaterials, including CHSN, agarose (AGR), alginate (ALG), elastin, silk fibroin (SF), and others that have been used for CTE, are listed in Table 1.

HA-based scaffolds

Hyaluronic acid (HA) is a key component in the ECM of cartilage. It is an anionic glycosaminoglycan (GAG) of up to 4000 kDa with hydrogel-like properties.[63,77–80] Elasticity and viscosity of the synovial fluid and shock absorbance capacity in articular joints are due to HA. HA is the primary ligand of the CD44 receptor, and several functions of the CD44 receptor are mediated through interaction with HA.[81,82] Crosslinking is the most common modification of HA, to form hydrogels for TE applications. The methacrylation is one of the frequently used technique for modification of HA hydrogels. Changing the methacrylation percentage directly effects on the crosslinking density and stiffness of the hydrogel matrix.[83] HA has also been chemically functionalized for the attachment of reporter groups for drug delivery systems. Composite materials of HA with natural and synthetic polymers were developed into biomimetic scaffolds with ability of enhancing wound healing and angiogenesis.[84] Hybrid and composite scaffolds composed of HA and synthetic materials (PLGA, PVA, PEG, and MA) and with natural biomaterials (CHSN, fibrin, AGR, ALG, and dextran) have been used for CTE (Table 2).

CS-based scaffolds

CS is a GAG, a long unbranched and polar polysaccharide consisting of amino sugars and sugar acids. CS plays a critical role as a main component of the ECM in cartilage functions.[125] The ECM regulates metabolism as well as

Table 1. Collagen-Based Hybrid Scaffolds for Cartilage Tissue Engineering

Author	*Scaffold composition*	*Biological assessment*	*Fabrication process*	*Advantages*	*Ref.*
Zhu *et al.*	GEL-MA/PEG diacrylate/(TGF-β1)-embedded nanospheres	hBMSCs *in vitro*	Tabletop stereolithography-based 3D bioprinter	Cell-laden bioprinted construct, a promising strategy for CTE	32
Cheng *et al.*	COL–CHSN/GO-np	Hydrogels transplanted in the rats	Composite 3D printing	The hydrogel/GO-np protected the CT by the signal pathway of Rank/Rankl/OPG	33
He *et al.*	PLCL/COL I	Rabbit articular chondrocytes *in vitro*	3D printing technology, LDM	PLCL scaffolds coated with COL I show a great potential application in TE	34
Kaczmarek *et al.*	CHSN/COL/GAGs crosslinked by sodium ALG	Chondrocytes *in vitro*	FD	Modified biomechanical properties, open-pore structures after 3 days in a perfusion bioreactor	35
Yang *et al.*	COL1/ALG bioink	Rat articular chondrocytes *in vitro*	3D cell printing	Bioprinted ALG/COL with favorable mechanical strength and biological functionality	36
Gao *et al.*	COL I/activated CS (with NHS)	Rabbit articular chondrocytes *in vitro*	Hydrogels	Injectable and self-crosslinkable hydrogels	37
Chen *et al.*	PEG/COL	MC3T3-E1 cells	Double network hydrogel	Potential as a load-bearing tissue repair material	38
Mekhileri *et al.*	Thermoplastic (PEGT/PBT) cell-laden GEL-based hydrogel microspheres	Human chondrocytes *in vitro*	3D plotting, high-throughput fabrication techniques	Automated and scalable pathway for bioassembly of both simple and complex 3D tissue constructs	39
Bas *et al.*	MA-GEL/PCL fiber	—	MEW	A strategy based on a numerical model was applied to accelerate the design of specific scaffolds for TE	40
Saghebasl *et al.*	(PNIPAAm-PCL-PEG-PCL-PNIPAAm)/GEL	Human chondrocytes *in vitro*	Thermosensitive hydrogel	Useful hydrophilic properties for growth and cell embedding and secretion of ECM	41
Song *et al.*	Blends of duck's feet COL/PLGA	Rabbit costal chondrocytes *in vitro*	SC/SL	Scaffolds with pore size 250 to 425 μm, highly suitable constructs for enhanced cartilage repair	42
Clearfield *et al.*	COL-HA/COL-hydroxyapatite	—	Unidirectional freeze casting/lyophilization bonding process	Combination of biomimetic compositional and architectural multidirectional scaffolds	43
Huang *et al.*	Sodium cellulose sulfate/GEL	hMSCs *in vitro*	Electrospinning	Use as a scaffolding material for CTE	44
D 'Amora *et al.*	PCL/COL	—	3D printing	Mimicking tissue gradients for interface TE	45
Jeon *et al.*	O A/GEL-MA IPN-structured hybrid hydrogels	hMSCs *in vitro*	Hydrogel	Biocompatible, biodegradable, and tough elastomeric hydrogels	46
Agheb *et al.*	GEL-tyrosine	Chondrocyte	Electrospinning	Excellent matrices in cell adhesion and proliferation	47
Kalaithong *et al.*	PLCL/GEL	L929 mouse fibroblast cells	Electrospinning and wet spinning	Wet-spun scaffold gave the best combination of properties	48
Shi *et al.*	SF/GEL	BMSCs *in vivo*	3D printing	Promising biomaterial for knee cartilage repair	49
Almeida *et al.*	ALG functionalized with COL I or II	Human IFP-derived stem cells	FD	Shape-memory ALG scaffold	50
Levato *et al.*	GEL methacryloyl bioinks	BMSCs and chondrocytes	Bioprinting	Biofabrication of 3D constructs with multiple cell types	51

(*continued*)

Table 1. (Continued)

Author	*Scaffold composition*	*Biological assessment*	*Fabrication process*	*Advantages*	*Ref.*
Wang *et al.*	COL/SF/PLGA	BMSCs *in vitro* and fully thick AC defects in rabbits	COL/SF incorporated with PLGA microsphere	Enhance AC regeneration, good integration between the scaffold and the surrounding tissue	52
Naseri *et al.*	Cellulose nanofibers/a genipin-crosslinked GEL/CHSN	Chondrocytes	FD	Good mechanical properties	53
Studer *et al.*	ALG/COL	ECPs *in vitro* and *in vivo*	—	Introducing a highly chondrogenic and off-the-shelf cell type	54
Bas *et al.*	PVA/heparin/GEL	—	UV photopolymerized hydrogels	Promoting cellular adhesion and sequestering growth factors	55
He *et al.*	GEL/PCL membranes	BMSC/chondrocyte cocultures, subcutaneously in nude mice	Electrospinning	May provide a potentially clinically feasible approach for cartilage repairs	56
Liu *et al.*	(PLA-co-PCL)/COL I	hMSCs in rabbit model	Dynamic liquid electrospinning	Improved repair scores and compressive modulus	57
Yin *et al.*	TGF-β1-loaded GEL microspheres into PLGA framework	ADMSCs *in vitro*	—	Enhances quality of tissue-engineered cartilage	58
Haaparanta *et al.*	COL/CHSN/PLA	Adult bovine chondrocytes	FD	COL/PLA hybrids promising scaffolds for CTE	59
Lomas *et al.*	PHBHHx/COL	hESCs and hMSCs	SL	Successfully used to culture hMSCs and hESCs	60
Dai *et al.*	COL/PLGA	Bovine chondrocytes subcutaneous in nude mice	PLGA mesh	New approach consists of designed shapes for regeneration of CT	61
Xu *et al.*	PCL fiber/fibrin–COL hydrogel	Chondrocytes	Fiber-hydrogel	Printed hybrid scaffolds with good mechanical properties	62
Kim *et al.*	HA/COL	*In vitro* cell adhesion, proliferation/chondrocyte implantation in rabbit ears	FD	Easily processed	63
Bhat *et al.*	CHSN–AGR–GEL	Goat chondrocytes	Cryogels	Designed 3D scaffold for CTE	64
Chen *et al.*	Genipin-crosslinked COL II scaffolds	MSCs for cartilage repair in osteochondral defect	—	New cartilage formation with natural cartilage structure	65
Hwang *et al.*	PEG-based hydrogels/type II COL, or HA	MSCs *in vitro*	Hydrogel	Differentiation of MSCs to chondrocytes marginally enhanced in hydrogel constructs	23
Abedi *et al.*	COL/PVA	Seeded with autologous MSCs for osteochondral defect in rabbit joints	Nanofibers	Controlling cell differentiation to chondrocytes	66
Bi *et al.*	Genipin-crosslinked CHSN/COL	(BDCs) and (ADCs) used to observe biocompatibility of scaffolds	FD	Optimized scaffold in genipin concentration of 1.0% and temperature of 20°C exhibited good biological properties	67
Qu *et al.*	PVA/GEL/nanohydroxyapatite/ polyamide-6	Culturing neonatal rabbit MSCs	FD	High mechanical strength composite with promotion of cell adhesion and proliferation ability	68
Zhang *et al.*	HA/COL/CS	In rabbit models	FD	Crosslinking in mild conditions	69

(*continued*)

Table 1. (Continued)

Author	*Scaffold composition*	*Biological assessment*	*Fabrication process*	*Advantages*	*Ref.*
Ho *et al.*	PCL–COL	MSCs	Electrospun meshes	Inhibition of hypertrophic response from cells	70
Dai *et al.*	PLGA/COL	Bovine chondrocytes, subcutaneously in nude mice	COL microsponge formed in PLGA mesh	PLGA mesh aided as a mechanically strong frame while COL microsponges enhanced cell seeding and tissue formation	71
He *et al.*	PLA–COL	Chondrocytes	Hybrid sponge	New technique for the preparation of functional porous scaffolds	21
Guo *et al.*	(PEG fumarate)/GMP loaded with (TGF-β1)	Rabbit BMSCs	Crosslinked hydrogel	Composite hydrogels for localized delivery of SCs and bioactive molecules	72
Kathuria *et al.*	CHSN–GEL	Fibroblasts	Cryogels	Enhanced cell adhesion, proliferation, and ECM secretion	73
Ko *et al.*	COL II/CS/HA crosslinked by genipin	Cartilage repair *in vivo*	FD	Mimics the natural ECM of AC	74
Buttafocoa *et al.*	COL/elastin crosslinking with (EDC/NHS)	Smooth muscle cells	Electrospinning from aqueous solutions	Suitable scaffold for TE applications	75
Tan *et al.*	CHSN–COL	K562 cell line	Nanostructured gel	Hybrid scaffold with biological and mechanical potential benefits for use in CTE	76

3D, three-dimensional; AC, articular cartilage; ADCs, adipose tissue-derived cells; ADMSCs, adipose tissue-derived mesenchymal stem cells; AGR, agarose; ALG, alginate; BDCs, bone marrow-derived cells; BMSCs, bone marrow-derived mesenchymal stem cells; COL, collagen; CS, chondroitin sulfate; CT, cartilage tissue; CTE, cartilage tissue engineering; ECM, extracellular matrix; ECPs, epiphyseal chondroprogenitor cells; EDC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride; FD, freeze-drying; GAGs, glycosaminoglycans; GEL, gelatin; GEL-MA, gelatin-methacrylamide; GMP, GEL microparticles; GO-np, graphene oxide nanoparticles; HA, hyaluronic acid; hBMSCs, human bone marrow-derived mesenchymal stem cells; hESCs, human embryonic stem cells; hMSCs, human mesenchymal stromal cells; IFP, infrapatellar fat pad; IPN, interpenetrating polymer network; LDM, low-temperature deposition manufacturing; MA, methacrylamide; MEW, melt electrospinning writing; MSCs, mesenchymal stromal cells; NHS, N-hydroxysuccinimide; PBT, poly(butylene terephthalate); PCL, poly(ε-caprolactone); PEG, poly(ethylene glycol); PEOT, poly(ethylene oxide terephthalate); PLCL, poly(L-lactide-co-??-caprolactone); PLGA, poly(DL-lactic-co-glycolic acid); PLA, poly(D,L-lactic acid); PHBHHx, poly(3-hydroxybutyrate-co-3-hydroxyhexanoate); PNIPAAm, poly(N-isopropyl acrylamide); PVA, poly vinyl alcohol; SC, solvent casting; SF, silk fibroin; SL, salt leaching; TGF-β1, transforming growth factor-beta 1; UV, ultra violet.

Table 2. Hyaluronan-Based Hybrid Scaffolds for Cartilage Tissue Engineering

Author	*Scaffold composition*	*Biological assessment*	*Fabrication process*	*Advantages*	*Ref.*
Jung *et al.*	Hybrid (MCG-HG-PLGA-PD-B) composite system	ATDC5 prechondrocyte cell line	PLGA microspheres coated by HG	Scaffold can be a promising candidate for CTE	85
Hsieh *et al.*	PCL/hydroxyapatite/glycidyl-MA-HA	The knees of Lanyu miniature pigs for a period of 12 months	FDM	A new clinical option to be considered alongside current treatments of cartilage injury	86
Zhu *et al.*	HA/S-ALG	Fibroblasts and keratinocytes *in vitro*	Hydrogels were prepared crosslinking via epoxy groups on HA and ionic crosslinking on S-ALG	Cell carrier role	87
Karabiyik Acar *et al.*	Coacervate-based HA/CHSN	MSCs *in vitro*	Coacervate preparation	Proper cell viability, well-spread morphology	88
Schiavi *et al.*	ALG/HA	hMSCs *in vitro*	Hydrogel	Capable *in vivo* to mimic all depths of chondral defects	89
Teong *et al.*	HA-MA	hADMSCs *in vitro*	Hydrogel	Degree of methacrylation can modulate matrix stiffness of a hydrogel, thus affecting chondrogenesis	83
Han *et al.*	DBCO/HA/PEG	Subcutaneously injected into Balb-c mice	Injectable hydrogel	Hydrogel supported cell survival, regeneration of CT	90
Wang *et al.*	Hydrazine-modified elastin-like protein/aldehyde-modified HA	hMSCs *in vitro*	Injectable hydrogel	Significant mechanical protection to encapsulated hMSCs	91
Zhu *et al.*	Hydrazine-modified elastin-like protein/aldehyde-modified HA	Chondrocyte *in vitro*	Injectable hydrogel	3D scaffolds with decoupled niche properties	92
Shie *et al.*	Light-cured polyurethane/HA	Wharton's jelly MSCs	3D printing	Mimics the mechanical properties of AC	93
Raia *et al.*	SF/HA	hBMSCs *in vitro*	Hydrogel	Biologically relevant system with controllable temporal stiffening and elasticity	94
Lin *et al.*	PGA-coated HA	*In vivo* subcutaneous implantation in rabbit	Electrospinning	HA coating can significantly enhance biocompatibility	95
Chen *et al.*	HA/RGD-functionalized pectin hydrogel	Mouse subcutaneous implantation	Injectable hydrogel	Acceptable tissue compatibility	96
Kim *et al.*	Oxidized HA/glycol CHSN	ATDC5 cells *in vitro*	Injectable hydrogel	Respectable biocompatibility and stability under physiological conditions	97
Park *et al.*	ALG/HA	ATDC5 cells *in vitro* derived from mouse chondrogenic cells	Hydrogel	Well-characterized composition and mechanical properties	98
Lynch *et al.*	PVCL/HA-MA	Fetal bovine calf leg chondrocyte	Thermosensitive hydrogel	ECM production *in vitro* showed 10-fold increase compared with HA-MA controls	99
Dai *et al.*	PLGA/HA-MA	BMSCs *in vitro* and cell-free scaffolds in rabbit knees	Directional cooling of HA-MA solution	Anti-inflammatory properties, bioactivity, and good repair of full-thickness cartilage defect *in vivo*	100
Shim *et al.*	ateloCOLagen/HA	*In vivo* repair in knee joints of rabbits	Hydrogel	3D printing-based platform technology for regeneration	101

(*continued*)

TABLE 2. (CONTINUED)

Author	*Scaffold composition*	*Biological assessment*	*Fabrication process*	*Advantages*	*Ref.*
Tavakoli *et al.*	PLGA/HA/fibrin/bioactive glass	—	SC and PL	Microstructural and mechanical properties for CTE	102
Bas *et al.*	GEL-MA/HA-MA hydrogels/ highly oriented PCL fibers	hMSCs *in vitro*	MEW hydrogels/highly oriented fibers	Improvement in mechanical properties	55
Snyder *et al.*	Fibrin/HA-MA	BMSCs *in vitro*	Hydrogel	Gene expression of COL-1 was decreased and increased in SOX9 in the presence of a platelet lysate, early chondrogenesis was observed	103
Mintz *et al.*	HA-based hydrogel/PCL	Dedifferentiated chondrocytes	PL	Mechanical properties same as human cartilage	104
Levett *et al.*	GEL-MA/HA-MA/CS-MA	Human chondrocytes	Photocrosslinkable hydrogel	Enhanced chondrogenesis and mechanical properties	105
Wang *et al.*	CS/HA/heparin sulfate	hADSCs	3D hydrogel	Biochemical and biomechanical cues interact with hADSCs in a 3D environment to regulate chondrogenesis	106
Schuurman *et al.*	GEL-MA/HA	—	Hydrogel	GEL-MA for using in biofabrication processes	107
Murphy *et al.*	COL-GAG	Wistar rat BMSCs	—	Lowest stiffness showed upregulation of SOX9 expression; highest stiffness showed upregulation of RUNX2 expression	108
Correia *et al.*	HA/CHSN	*In vitro*	FD	Noncytotoxic, promotes cell adhesion	109
Coburn *et al.*	PVA-MA and CS-MA	Cultured with MSCs for 6 weeks in both chondrogenic induction medium and *in vivo* osteochondral defect of rat model	Electrospinning	Enhancing type II COL synthesis and mechanical strength of tissues	110
Lee *et al.*	HA/CHSN	Chondrocytes in full-thickness cartilage defects on patellar grove of rabbit knee	—	GAG and COL II gene expression and presence of lacunae were exhibited	111
Jin *et al.*	HA/PEG	Chondrocytes	Injectable hydrogels	Injectable hydrogels with potential for CTE	112
Jin *et al.*	HA grafted with dextran–TA	Bovine chondrocytes	Injectable hydrogels via enzymatic crosslinking of the TA residues	Enhanced chondrocyte proliferation and matrix production	113
Fan *et al.*	PLGA–GEL/CS/HA (GCH)	MSCs *in vitro* and in full-thickness cartilage defect in rabbits	Incorporating GCH microsponges into PLGA framework and then crosslinked with TGF-β3	The group treated by the scaffold exhibited better chondrocyte morphology, integration with surrounding tissues, continuous subchondral bone	114
Im *et al.*	HA-atelocollagen/ hydroxyapatite-tricalcium phosphate	Seeded chondrocytes, repair of osteochondral defects in minipigs	—	Showed significantly performance for repair	115
Tan *et al.*	HA/CHSN	Bovine articular chondrocytes	FD	Nontoxic reagents, injectable	116

(*continued*)

Table 2. (Continued)

Author	*Scaffold composition*	*Biological assessment*	*Fabrication process*	*Advantages*	*Ref.*
Tan *et al.*	PLGA/GEL-CHSN-HA	Chondrocytes	FD	Increasing compressive modulus	117
Chen *et al.*	HA-g-CHSN-PNIPAM	Articular chondrocytes and meniscal cells of rabbit *in vitro*	Hydrogel	Enhanced ECM secretion and mechanical properties	118
Erggelet *et al.*	Cell-free polyester/HA scaffold	Full-thickness AC defects in sheep	—	Enhancements in histological structure, and COL type-II expression	119
Pereira *et al.*	Carrageenan/fibrin/HA	Human articular chondrocytes *in vitro* and *in vivo*	Injectable hydrogel	Novel cell carrier for CTE	120
Kasahara *et al.*	HA/CHSN	Osteochondral defects in the patellar groove of rabbits	—	Integration with surrounding natural cartilage and normal reconstruction of subchondral bone	121
Kim *et al.*	HA/COL	Bovine chondrocytes	Electrospinning process combined with SL	Cellular adhesion and proliferation enhanced, chondrocytes maintained chondroblastic morphology	122
Solchaga *et al.*	HA- and polyester (HYAFF-11)-based scaffolds	Polyester-based sponge in rabbit osteochondral defects	SC/PL	Slow degradation of HYAFF-11, delayed cartilage formation and endochondral bone construction	123
Yamane *et al.*	CHSN-based HA	Rabbit chondrocytes	Fibers	Fabrication of scaffolds, which can control porous structure	124

DBCO, dibenzocyclooctyl; FDM, fused deposition modeling; GCH, GEL/CS/HA; hADMSCs, human adipose tissue-derived mesenchymal stem cells; hADSCs, human adipose-derived stem cells; HA-MA, hyaluronic acid-methacrylamide; MCG-HG-PLGA-PD-B, multichannel biphasic calcium phosphate granule–hyaluronic acid-gelatin–poly(lactic-co-glycolic acid)–polydopamine–BMP-7; PL, particulate leaching; PVCL, poly(N-vinylcaprolactam); RGD, Arg-Gly-Asp; RUNX2, runt-related transcription factor 2; SOX9, Sry-type high-mobility group box-9; TA, tyramine.

gene expression and stimulates proliferation and differentiation of chondrocytes.[126] The biological activities of CS make it an ideal biomaterial for CTE. The chondroprotective action of CS can be explained by the stimulation of the anabolic progression of cartilage metabolism, and its anti-inflammatory action, delaying numerous inflammation-induced catabolic processes in the tissue.[127,128] The inclusion of CS in composite scaffolds improves their mechanical properties and compressive strength by interacting with cell surface receptors to regulate chondrocyte behavior.[129] Hybrid scaffolds containing CS and synthetic as well as natural polymers have been used for CTE and are listed in Table 3.

ECM analog-based scaffolds

The use of decellularized ECM scaffolds and tissues is speedily expanding in TE.[146] There are many reasons for utilization of ECM-based materials in TE applications, including promotion of SC recruitment, cell infiltration, and differentiation without the need of additional biological factors. Cartilaginous ECM may be innovative in orthopedic medicine because of its chondroinductive potential, particularly in hydrogel-based systems.[147] The potential of ECM-based scaffolds to retain growth factors such as transforming growth factor-beta 1 (TGF-β1), fibroblast growth factor, and insulin-like growth factor is one of the major advantages of utilizing these materials as a scaffold.[148] However, the inferior mechanical properties of scaffolds composed completely of natural materials compared with synthetic materials are a major disadvantage for load-bearing tissue applications. Therefore, combinations of these ECM-based materials with suitable synthetic materials can be of advantage for CTE.[147] Hybrid scaffolds composed of ECM analogs and synthetic materials, as well as natural biomaterials, have been used for CTE and are listed in Table 4.

Hybrid Scaffold Processing Approaches

A multitude of porous ECM-derived hybrid scaffolds and scaffold processing approaches have been utilized in CTE. The most frequently used types are discussed below.

Fibrous scaffolds

Electrospinning of natural and synthetic biomaterials is a promising technique to produce fibrous scaffolds for TE applications.[102] The combination of synthetic polymeric materials and natural components such as COL could increase cell attachment while presenting ideal mechanical properties for TE applications.[56] PCL–COL electrospun meshes were used in autologous chondrocyte implantation as an innovative substitute to conventional grafts, which was the first try to design a mechanically enhanced cartilage resurfacing membrane composed of strong PCL mesh with bioactive COL. MSCs adhered on the surface of the mesh after seeding. More importantly, the mesh induced MSC differentiation into chondrocytes and inhibited a cellular hypertrophic response. This study showed the impact of the use of PCL–COL hybrid mesh as a cartilage patch and showed the importance of incorporation of the ECM-derived component COL into the synthetic PCL.[163] In a similar study, oriented PLA-co-PCL/COL I nanofiber yarn meshes were fabricated by dynamic liquid electrospinning and aided as a skeleton for a freeze-dried (FD) COL I/HA chondral phase to improve the mechanical properties of the scaffolds. *In vitro* and *in vivo* results demonstrated that the hybrid constructs allowed cell infiltration similar to sponge scaffolds, and repaired the rabbit model osteochondral defects with improved mechanical properties of the newly engineered cartilage.[57] In another approach, COL/PVA nanofiber scaffolds were prepared and seeded with autologous MSCs to repair osteochondral defects of rabbit joints. The hybrid scaffolds induced higher chondrocyte morphology and new cartilage formation compared with the control defect without any treatment. The results showed that the nanofibrous COL/PVA scaffolds provide a supportive environment for cartilage tissue (CT) regeneration over 12 weeks. The histological results demonstrated that the COL/PVA group had better cartilage repair, more new matrix formation, and continuous subchondral bone compared with the control group.[66] In a further approach, oriented PCL fibers were fabricated by melt electrospinning writing (MEW) and combined with gelatin-methacrylamide (GEL-MA) and GEL-MA/HA-MA hydrogels to produce fiber-reinforced GEL-MA/HA-MA composites with enhanced mechanical properties. The results demonstrate that reinforcement of hydrogels with fibers leads to increases in the mechanical properties of the hybrid construct.[55] *In vitro* and *in vivo* studies on a sandwich model of electrospun GEL/PCL membranes, seeded with MSC/chondrocyte cocultures, were performed. To engineer the sandwich model, a GEL/PCL mesh was placed at the bottom of a well and seeded with cell suspension. A second mesh was then stacked on top of the first sheet, followed by cell seeding. The stacking was repeated until ten sheets. After implantation of the sandwich constructs 12 weeks subcutaneously into nude mice, histological analysis, GAG assay, and mechanical property measurement confirmed the formation of mature cartilage-like tissue. The strategy indicated that designed constructs were suitable for SC-based CTE. The constructs showed white appearance, flexibility, and a well-distributed synthesized neomatrix typical of cartilage.[56]

Yamane *et al.*[164] developed hybrid fibers based on CHSN and CHSN-HA by wet spinning. Articular chondrocytes from rabbits were cultured in the sheets of CHSN- and HA-based hybrid fibers. The fibers exhibited potential as an appropriate biomaterial for cartilaginous tissue scaffolds.[124] In a similar study, 3D scaffolds based on novel CHSN-based HA hybrid polymer fibers, which could control porous structure, were fabricated by wet spinning. The results showed that cell adhesion, proliferation, and synthesis of ACAN were higher in the CHSN-HA hybrid fiber than in the CHSN-only group.[164]

Chondroinductive nanofiber composites of PVA-MA and chondroitin sulfate-methacrylamide (CS-MA) were synthesized and used for AC repair. MSCs cultured on these scaffolds were shown to support an increase in cell proliferation, ECM production, and cartilage-specific gene expression (Fig. 1) *in vitro* as well as *in vivo*.[165]

ECM-coated scaffolds display the advantages of both natural and synthetic components. Polymeric scaffolds provide the strength and robustness to support tissue growth, while the ECM coating acts in bioactive signal providing needed cues for differentiation.[160] Electrospun PCL microfibers

Table 3. Chondroitin Sulfate-Based Hybrid Scaffolds for Cartilage Tissue Engineering

Author	*Scaffold composition*	*Biological assessment*	*Fabrication process*	*Advantages*	*Ref.*
Agrawal *et al.*	SF/CHSN/CS	hMSCs–spinner flask bioreactor	FD	Potential of SF/CHSN/CS scaffolds for hMSC recruitment and directing CT regeneration	130
Bang *et al.*	CS–A/GEL–TCEP	Fibroblast *in vitro*	*In situ* hydrogel	Excellent biocompatibility, mimicking ECM components	131
Li *et al.*	CS/pullulan	Rabbit articular chondrocytes	Injectable hydrogel	Hydrogel could conserve chondrocyte phenotype and increase chondrogenesis	132
Piai *et al.*	CS immobilized PCL	Human articular chondrocytes *in vitro*	Electrospun nanofiber meshes	Effective method for surface functionalization	133
Fan *et al.*	CHSN/CS	Bovine articular chondrocytes *in vitro*	Injectable hydrogel	Decreases in swelling ratio and degradation rate	134
Vishwanath *et al.*	Glucosamine/SF/CHSN	Umbilical cord blood MSCs	FD	Increased cell supportive properties	135
Zhou *et al.*	SF/CS	Human articular chondrocytes *in vitro*	Salt-leaching, FD	Anti-inflammatory activities	136
Shahali *et al.*	Poly-3-hydroxybutyrate scaffolds loaded with glucosamine sulfate	Human chondrocytes *in vitro*	Electrospining	Excellent cell viability, cell adhesion, and cell penetration	137
Chen *et al.*	CMP-TA and CS-TA	Porcine auricular chondrocytes, mouse subcutaneous implantation	Enzymatically crosslinked injectable and biodegradable hydrogel	Acceptable tissue compatibility	138
Costantini *et al.*	GEL-MA/CS amino ethyl MA/ HA-MA	MSCs	Hydrogel	Promoted viability and chondrogenic differentiation of MSCs	139
Liao *et al.*	CS-MA/PECA/GO	CT repair of rabbit	Porous scaffold	Scaffold is applicable in articular CTE	140
Sawatjui *et al.*	SF/GEL/CS/HA	hMSCs or chondrocytes evaluated their performance with dynamic compression *in vitro*	Hydrogel	The microenvironment provided by the scaffolds and dynamic compression enhanced tissue regeneration	141
Naeimi *et al.*	SF-CS-S-ALG porous scaffold containing CHSN NPs	ADSCs	Porous scaffold	Incorporation of NPs into the scaffold improved compressive modulus	142
Nanda *et al.*	PVA/CS	Cartilage repair *in vivo*	Hydrogel	Fill of cartilage defects without inflammation, integration with surrounding tissues	126
Silva *et al.*	CHSN/CS	hMSCs *in vitro*	Layer-by-layer technology	Chondrogenic differentiation of hMSCs	143
Chen *et al.*	CHSN/CS/dermatan sulfate	Chondrocyte	—	Stimulating ECM production and cartilage regeneration	144
Lee *et al.*	PVA-CS	Growth of BHK cells	Hydrogel crosslinking with glutaraldehyde	Advantages of both PVA and CS	145

BHK, baby hamster kidney fibroblasts; CHSN, chitosan; CMP-TA, carboxymethyl pullulan-tyramine; GO, graphene oxide; MPEG-PCL-AC, poly(ethylene glycol) methyl ether-ε-caprolactone-acryloyl chloride (PECA was used as abbreviation for MPEG-PCL-AC); NPs, nanoparticles; TCEP, tris(carboxyethyl)phosphine.

Table 4. Hybrid Scaffolds Based on Extracellular Matrix Analogs for Cartilage Tissue Engineering

Author	*Scaffold composition*	*Biological assessment*	*Fabrication process*	*Advantages*	*Ref.*
Kim *et al.*	MPC/PLGA	NIH/3 T3, KCLB216480, and (RAW 264.7, KCLB40071) cells *in vitro*, implanted subcutaneously in rats	Solvent-casting/salt-leaching	Cell adhesion and proliferation increased, inflammatory cytokines and cellular ROS reduced, interaction between tissue and scaffolds *in vivo*	149
Ghosh *et al.*	DCM encapsulated in PLA microspheres	hMSCs *in vitro*	Filament	Filaments containing chondroinductive microspheres	150
Jung *et al.*	CAM-silk bioink	Rabbit MSCs *in vitro*	3D printing	3D printing of cartilage-shaped scaffolds	151
Ghassemi *et al.*	Single-wall CNTs/ decellularized bovine cartilage	hADSCs	FD	Increased dECM mechanical strength	152
Masaeli *et al.*	Polyhydroxyalkanoate/dECM	hADMSCs, human primary chondrocytes *in vitro*	Nanofibrous scaffold	Mimicked the natural motifs of cartilage ECM	153
Rothrauff *et al.*	MA-ECM	hMSCs	Photocrosslinked hydrogels	Thermoresponsive, photocrosslinkable hydrogels	154
Levingstone *et al.*	Multilayered biomimetic COL-based scaffolds	Femoral condyle (MC) defects in the caprine stifle joint	FD-EDC/NHS cross-linked	Recruitment of host cells	155
Nogami *et al.*	ECM-PLGA	Rat MSCs *in vitro*, implanted into osteochondral defect in rat knees	ECM-coated PLGA	Cell-free scaffolds providing an environment for growth of MSCs and facilitating cartilage repair	156
Beck *et al.*	MeSDCC gels	Rat MSCs	Photocrosslinked hydrogel	MeSDCC hydrogels may be promising materials for CTE	147
Almeida *et al.*	Fibrin/ECM	Infrapatellar fat pad-derived stem cells	Injectable hydrogel	Invasive single-stage, cell-based therapies for joint regeneration	157
Sutherland *et al.*	PLGA surface coated with decellularized cartilage	Rat MSCs *in vitro*	Microsphere	Bioactive approach to cartilage regeneration with microsphere-based scaffolds	158
Garrigues *et al.*	PCL/CDM	Human ADSCs	Electrospinning	Role of lower elastic modulus, particularly from CDM, in promoting chondrogenesis	159
Levorson *et al.*	PCL/ECM	Chondrocytes and MSC coculture	Microfiber scaffolds coated with ECM	PCL/ECM supporting chondrogenic differentiation of MSCs	160
Liao *et al.*	PCL/ECM	MSCs *in vitro*	Microfiber scaffolds coated with cartilaginous ECM	Method for fabrication of polymer/ECM composite scaffolds	161
Wang *et al.*	Demineralized BMG/fibrin	Rabbit chondrocytes *in vitro*	BMG scaffolds soaked in a chondrocyte–fibrin suspension	Potentially cell carrier vehicle and a structural basis for CTE	162

BMG, bone matrix-GEL; CAM, cartilage acellular matrix; CDM, cartilage-derived matrix; CNTs, carbon nanotubes; DCM, decellularized cartilage matrix; dECM, decellularized ECM; MeSDCC, MA-solubilized decellularized cartilage; MC, Femoral condyle; MPC, micronized porcine cartilage; NIH/3 T3 cells, mouse embryo fibroblasts, KCLB216480; RAW 264.7, KCLB40071, mouse leukemic monocyte macrophage cell line; ROS, reactive oxygen species.

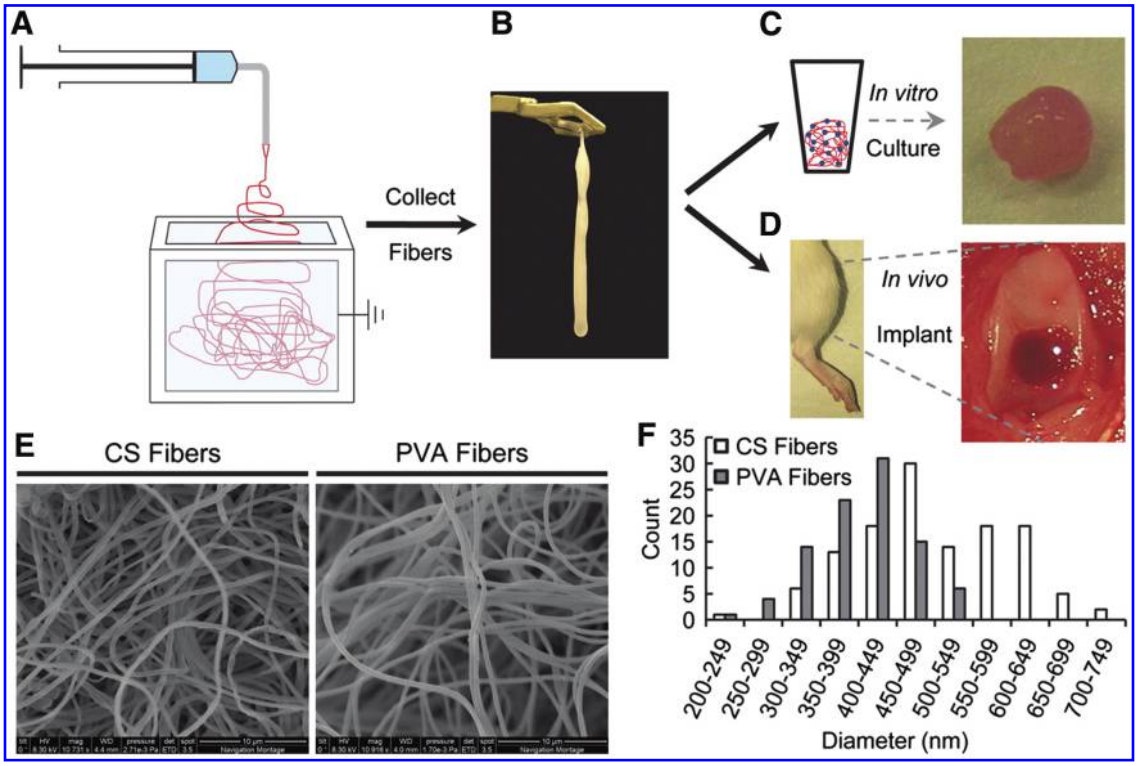

FIG. 1. **(A, B)** Nanofiber composites of PVA-MA and CS-MA were synthesized using electrospinning system in which the nanofibers were collected into an ethanol bath. **(C)** *In vitro* chondrogenesis of MSCs was performed over 42 days. **(D)** Nanofibers were implanted into osteochondral defects in the rat hind limbs for 6 weeks. **(E)** Scanning electron microscopy imaging showed the morphology and **(F)** size distribution of the fibers.[165] CS, chondroitin sulfate; MA, methacrylamide; MSCs, mesenchymal stromal cells; PVA, poly vinyl alcohol. Reprinted with permission from Coburn *et al.*[165] Color images are available online.

were coated with cartilaginous ECM to fabricate PCL/ECM composite scaffolds. Briefly, chondrocytes were cultured in a flow perfusion bioreactor, and then, the cellular constructs were decellularized. The composite scaffolds in the presence of TGF-β1 exposure showed upregulation of ACAN and COL II gene expression.[161] In a similar approach, coculture of chondrocytes and MSCs on electrospun fibrous scaffolds was performed to produce polymer/ECM hybrid constructs. The results indicated the capacity of cocultures to deposit cartilaginous matrix within a polymeric scaffold. Thus, cocultures of MSCs and chondrocytes can be used to reduce the needed chondrocytes to fabricate polymer/ECM hybrid scaffolds.[160] An innovative technique was performed to fabricate PCL and PCL/cartilage-derived matrix scaffolds by the serial collection of 60 electrospun single-layer scaffolds, which were then seeded with human adipose-derived stem cells (hADSCs). The results indicated that multilayer hybrid constructs improved homogeneous cell seeding and showed chondrogenesis-related bioactivity.[159] Recent studies demonstrated that electrospinning can produce fibrous scaffolds with a high surface/volume ratio, resembling the natural ECM pore structure, thus useful in terms of cell adhesion, proliferation, and differentiation. To improve cell–scaffold interactions, a range of natural and synthetic biomaterials can be blended into the fibrous scaffolds. In view of the fact that the biodegradation rate of scaffolds can influence cell behavior and the following tissue regeneration, a proper biodegradation rate of the hybrid meshes can also be produced to match and control the rate of tissue regeneration.

Hydrogel scaffolds

Hydrogels are composed of hydrophilic polymer chains with natural or synthetic origin. They offer many advantages, including biodegradability, easy processing, minimally invasive delivery manner, and modulating ability by regulation of crosslink density. For these reasons, hydrogels have been widely used in TE application and especially in CTE.[79] COL is one of the most common ECM derivatives used for culturing cells *in vitro*. COL can be reconstructed to form hydrogel to mimic the connective tissue *in vitro*. Numerous ECM-like composites that combine 3D COL hydrogels with synthetic and natural polymers have been examined.[76] Hybrid gel matrices composed of COL and CHSN had been examined for their capacity to regulate cellular activity. The K562 (a human hematopoietic cell line) cells were cultured in 3D gels to examine cell proliferation and differentiation.[76] In completion of the previous studies, elastic cryogels composed of CHSN–GEL were prepared via crosslinking with glutaraldehyde. Hybrid cryogels exhibited efficient cell adherence, proliferation, and ECM deposition by culturing a fibroblast cell line (Cos-7). The results showed the potential of the hybrid hydrogel for TE applications.[73] To achieve proper biomechanical and biological properties, researchers tried to fabricate scaffolds based on an optimized ratio of polymer solutions (CHSN,

Aga, and GEL) and glutaraldehyde as the crosslinker, via cryogelation technology with incubation at subzero temperature. The fabricated scaffolds showed proper biodegradation, mechanical, and biological properties for effective cell interaction in subsequent tissue development. *In vivo* biocompatibility examination of the scaffolds suggested the potential of these cryogels as a 3D scaffold for CTE.[166] Human chondrocytes were encapsulated in GEL-MA-based hydrogels, and combined with small amounts of photocrosslinkable CS-MA and HA-MA. According to the results, the incorporation of HA-MA to GEL-MA resulted in further round cell morphologies, improved chondrogenesis, and increased synthesized neomatrix throughout the hydrogel. As a result, the compressive modulus of the hydrogel containing HA-MA and CS-MA increased to 114 kPa compared with the control GEL-MA (26 kPa) after 8 weeks of culture.[105]

Entrapment of ECM molecules within a synthetic hydrogel is ultimately the most comprehensive method to create hydrogels containing biological signals. The ultra violet (UV) photopolymerized PVA hydrogels were prepared via methacrylated functionalization, and the effects of incorporation of heparin and GEL within a PVA hydrogel were examined.[167] Chen *et al.* advanced the novel double network PEG/COL, whose mechanical strength could be modified by changing the rigidity, molecular weight, and crosslinking density of the two components. Results indicated that the incorporation of COL significantly enhanced the strength and toughness of hydrogels. The resulting hydrogels could offer a proper environment for cell attachment and proliferation.[38]

Injectable hydrogels can be presented by minimally invasive procedures and activated by environmental conditions, including pH, temperature, ultrasound, ionic strength, or electric fields, to undertake a shape compliant to the surrounding defect site. Moreover, biological cues such as cells and growth factors could be coinjected with the hydrogels.[80] Injectable synthetic/natural hydrogel composites containing oligo(PEG)-fumarate (OPF), and GEL microparticles (GMP), were synthesized. OPF/GMP TGF-β1-loaded composites, hydrogel encapsulating rabbit MSCs, supported osteochondral tissue generation in rabbit osteochondral defects at 12 weeks.[72] Novel biodegradable, biocompatible, and tough elastomeric hybrid hydrogels based on photocrosslinkable GEL and ionically crosslinkable ALG were engineered. These hydrogels offer an exciting venue to investigate the effect of mechanical stimulation on SC proliferation and differentiation.[46] *In situ* CS/GEL hydrogels were achieved by simple mixing of aqueous solutions of both GEL–tris(carboxyethyl)phosphine and CS–acrylate via click chemistry strategy. *In vitro* studies showed excellent biocompatibility and potential of the hydrogel in various biomedical applications, including TE and drug delivery.[131]

Various ECM derivative molecules, such as CS and HA, have been used as the 3D hydrogels to support SC chondrogenesis. However, because of the lack of proper mechanical properties and matrix stiffness, it is difficult to explain the relative contribution of matrix stiffness on SC fate using ECM derivative hydrogels. Improvement of mechanical properties of these hydrogels can occur by incorporation of polymeric biomaterials.[106] Three major types of cartilage ECM derivatives, HA, CS, and heparan sulfate, were incorporated to fabricate biomimetic hybrid scaffolds. The degree of methacrylation of these ECM molecules was a key factor to produce ECM-based hydrogel scaffolds. To investigate the effects of mechanical and biochemical cues on chondrogenesis, ADSCs were encapsulated in different hydrogel compositions with various ECM derivative concentrations [0.5%, 1.25%, 2.5%, and 5% (w/v)] and different matrix stiffnesses (3, 30, and 90 kPa). The results indicated that the influence of matrix stiffness on chondrogenesis is dependent on the composition of hydrogels in a nonlinear manner.[106] A chondrogenic hydrogel composed of fibrin/HA-MA seeded with MSCs was further evaluated. The results indicated that the hydrogel could be considered a proper vehicle for MSC delivery and chondrogenesis induction.[103]

Regarding the disadvantage of using crosslinking agents in biological systems, a novel biocompatible hybrid hydrogel was prepared by an oxidized HA/CHSN solution in the absence of a crosslinker. This composite hydrogel supported the survival of encapsulated bovine articular chondrocytes, which retained a chondrocytic morphology.[116] Novel injectable mixtures were synthesized under physiological conditions with PEG vinylsulfone macromers and thiol functionalized HA, which were crosslinked via Michael addition. On mixing with chondrocytes, these hydrogels afforded a homogeneous distribution of cells.[113] Another injectable hydrogel, using blending of thermoresponsive engineered proteins and a dynamic covalent crosslinking system, was fabricated. By mixing aldehyde-modified HA and hydrazine-modified elastin-like protein via dynamic covalent hydrazone bonds, hydrogel formation occurred. This hydrogel represented proper mechanical support to encapsulated human mesenchymal stromal cells (hMSCs) during injection.[91] In a recently published study by Raia *et al.*,[94] HA and SF were enzymatically crosslinked to fabricate biocompatible hydrogels with mechanical strength comparable with the native tissues. The SF proteins crosslinked via horseradish peroxidase (HRP) by formation of dityrosine bonds, and tyramine (TA)-substituted HA was synthesized by the same reactions. Consequently, HA was covalently crosslinked with silk to form a composite hydrogel (Fig. 2). The results show that synthesized hydrogel can provide a proper biologically system with controllable mechanical properties for TE applications.[94] According to previous works, HA-MA with different degrees of methacrylation were synthesized. The degree of methacrylation modulated matrix stiffness of the hydrogels, therefore affecting the ability of hADSC chondrogenesis.[83]

PVA is one of the most studied polymers in biomedical application, because of its great biocompatibility in combination with a variety of appropriate biomechanical properties, swelling capacity, and crosslinking opportunities. Nanda *et al.*[126] crosslinked the PVA-CS hydrogels with glutaraldehyde and used them as a scaffold in TE.[144] Also, hydrogel scaffolds of PVA/CS were fabricated to imitate the ECM to provide an environment for improved AC cartilage repair *in vivo*.[126] Recently, based on CS, enzymatically crosslinkable, injectable, minimally invasive, and biodegradable hydrogels have been described under physiological conditions. The hydrogels consisted of carboxymethyl pullulan-TA (CMP-TA) and CS-TA conjugates, which were enzymatically crosslinked by horseradish peroxidase and hydrogen peroxide. According to the results, hydrogels were cytocompatible. The CMP-TA/CS-TA composite hydrogels

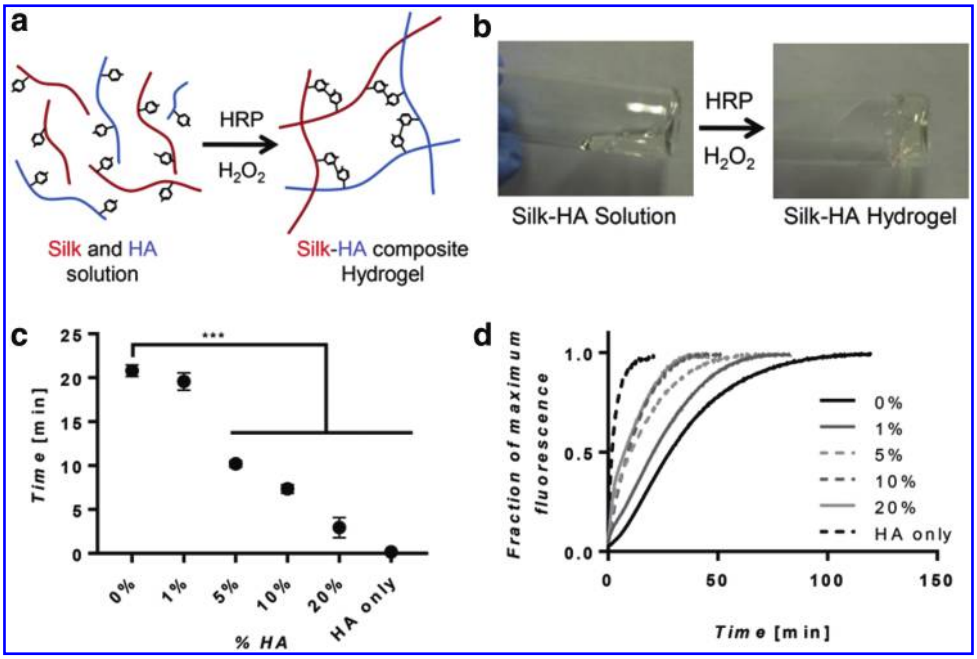

FIG. 2. **(a)** The silk-HA gel formation by covalent crosslinking of tyramine side chains on HA and tyrosine residues on silk. **(b)** The images of silk-HA gelation through a vial inversion test. **(c)** Vial inversion test results to evaluate gelation times. **(d)** The increasing of HA concentration affected crosslinking kinetics ($n=7$).[94] ($n=4$, ***$p<0.001$). Reprinted from Biomaterials, Raia *et al.*,[131] Enzymatically crosslinked silk-hyaluronic acid hydrogels, pp, 58–67, 2017, with permission from Elsevier. HA, hyaluronic acid; H_2O_2, hydrogen peroxide. Color images are available online.

enhanced cartilaginous ECM synthesis and cell chondrogenesis.[138] Injectable and self-crosslinkable hydrogels were fabricated based on COL I and activated CS with N-hydroxysuccinimide by chemical and physical crosslinking without catalysts. The results suggest that these hydrogels were suitable candidates for applications in the fields of cell delivery and TE.[37]

Synthetic and natural compounds can be used to produce hydrogels taking advantage of their complementary and sometimes synergistic properties. Hydrogels consisting of ECM analogs can be similar to the natural cartilaginous ECM. Through the elastic network, they facilitate transport of nutrients and cellular metabolites, and they can be applied by simple, minimally invasive procedures to fill large and irregular complex defects.[138,147] Similarly, injectable hydrogel based on methacrylated solubilized decellularized porcine cartilage was prepared by methacrylation and UV photocrosslinking modifications. *In vitro* studies showed that these hydrogels induced chondrogenic gene expression and new ECM cartilage formation with mechanical characteristics of native cartilage.[147] In conclusion, hydrogels can be prepared from both synthetic and natural biomaterials. Synthetic biomaterials have the ability to control the chemical composition, mechanical properties, and biodegradation rate of the composite hydrogels. In contrast, hydrogels composed of natural biomaterials have the ability of providing biological cues and signals to enhance cell adhesion, proliferation, and differentiation. However, hybrid hydrogels composed of both synthetic/natural biomaterials can be an effective tool to achieve optimal cartilage regeneration.

3D printing and biofabrication

Additive manufacturing tools are based on the development of computer science and manufacturing technologies. The potential to fabricate highly complex constructs such as whole organs directly from a computer model is one of the main advantages of these approaches.[168] Additive manufacturing offers a different potential to understand the structural constraints for TE scaffolds. On the contrary, it is also critical to develop the methods allowing efficient scaffold cellularization independent of shape and porosity.[169] Frequently due to the absence of biological cues and hydrophobicity of the synthetic biomaterials used in scaffold fabrication by additive manufacturing, these scaffolds generally offer a limited environment for cell attachment and growth. Conversely, in those biofabrication techniques using living cells and biological materials, tissues are directly produced with ECM derivatives by controlling their 3D structures. However, inferior mechanical properties of such biofabricated constructs are considered a main limitation of these approaches.[170] Recently, to overcome these restrictions, multihead deposition systems with the ability of bioprinting different row constituents consisting of synthetic and natural biomaterials, proteins, and cells have been developed.[171] Xu *et al.*[62] tried to use inkjet-based cell printing in conjunction with electrospinning to fabricate constructs with improved mechanical properties. After spinning of a PCL layer, a rabbit chondrocyte/fibrinogen/COL solution was deposited onto the electrospun PCL fibrous layer. After gelation of the cell-printed solution, PCL was spun another time and followed by inkjet cell printing again. A final construct consisting of five layers of 1 mm thickness was fabricated. Cells showed >80% viability a week after culture.[62] Before using GEL-MA as a biomaterial for biofabrication purposes, several crosslinking parameters consisting of UV exposure time, polymer concentration, and thermal gelation before UV exposure were investigated to control the swelling and mechanical properties of the hydrogel. The opportunity to control mechanical properties, swelling behavior, and high cell compatibility and the ability to synthesize cartilaginous matrix make GEL-MA a suitable material for CTE. Results showed that when GEL-MA is combined with HA as a viscosity-enhancing additive, it could be printed into layered hydrogel structures. The results

confirmed that the engineered constructs allow matching the natural functional variations in cartilage biomechanical properties.[107] In a more recent approach, Visser *et al.*[172] reinforced soft GEL-MA hydrogels with high porosity and a highly organized 3D-printed PCL network that was fabricated via the MEW technique. The mechanical properties of the gel/scaffold composites improved compared with microfiber scaffolds or hydrogels alone. Chondrocytes embedded in the hybrid construct were viable, and retained their round morphology and physiological behavior *in vitro*.[172] A novel hybrid scaffold based on PLA/photopolymerizable cell-laden hydrogels has been established. A hydrogel precursor was prepared from a solution of MA-modified GEL and the photoinitiator Li-TPO-L in cell culture medium. The fabricated TE constructs merged both advantages, synthetic additive manufactured constructs and a natural hydrogel matrix.[169] Recently, a bioink consisting of GEL-MA, CS-amino ethyl-MA, and HA-MA was loaded with MSCs to fabricate 3D biomimetic hydrogel scaffolds for CTE. Two coaxial needles were used to establish a proper system to bioprint hybrid constructs with high cell viability, high cell density, and high bioprinting resolution. The results confirmed that this method is a valuable candidate for advanced CTE.[139] By combining SF and GEL with MSCs and a specific-affinity peptide E7, a functionally and structurally improved scaffold was designed via an indirect 3D printing method. Briefly, The SF-GEL mixture solution was dispensed to a 3D computer-aided design mold. After decreasing the temperature to form the gel, the mold was dissolved, and the scaffold then crosslinked with genipin. The scaffold showed efficient recruiting capacity for MSCs and provided a mechanical support and proper microenvironment for proliferation, differentiation, and neocartilage tissue production.[49] Fabrication of gradient structures has been made by using the layer-by-layer inherent fabrication of additive manufacturing technologies. Gradient functionalization with controlled geometry and porosity on the surface of an additive manufactured PCL scaffold was investigated. First, the surface of PCL scaffold was aminolysed using a continuous gradient of amine concentration; second, by using EDC reaction, a COL gradient was formed via protein grafting. The results showed that for the construction of 3D scaffolds with chemical gradients and controlled structural properties, a combination of surface modification and additive manufacturing is an appealing strategy.[45] A tabletop stereolithography-based bioprinter has been used for a new cell-laden CT construct fabrication. The bioink was composed of GEL-MA, various concentrations of PEG diacrylate, 2-hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone as a photoinitiator, and TGF-β1-embedded nanospheres (Fig. 3). Cell growth, viability, and chondrogenesis were explored to develop an optimized 3D-bioprinted construct for CTE. A significant increase in chondrocyte-specific gene expression on printed constructs containing TGF-β1 nanospheres over 3 weeks showed that cell-laden bioprinting is a promising strategy for CTE.[32] A bioprintable natural bioink based on cartilage acellular matrix (CAM) for bioprinting of irregular shape tissues has been developed. As a support of the CAM powder, SF was used because of its physical crosslinking ability and controllable viscosity. Bioprinting of a cartilage-shaped scaffold using this CAM-SF bioink has been done successfully.[151] The results of *in vitro* culture showed that a printed CAM-SF construct provided better cell morphology and neomatrix synthesis from rabbit BMSCs compared with a printed PCL construct.

According to recently published articles, bioprinting approaches have shown great potential in CTE applications. However, several limitations are still remaining in achieving

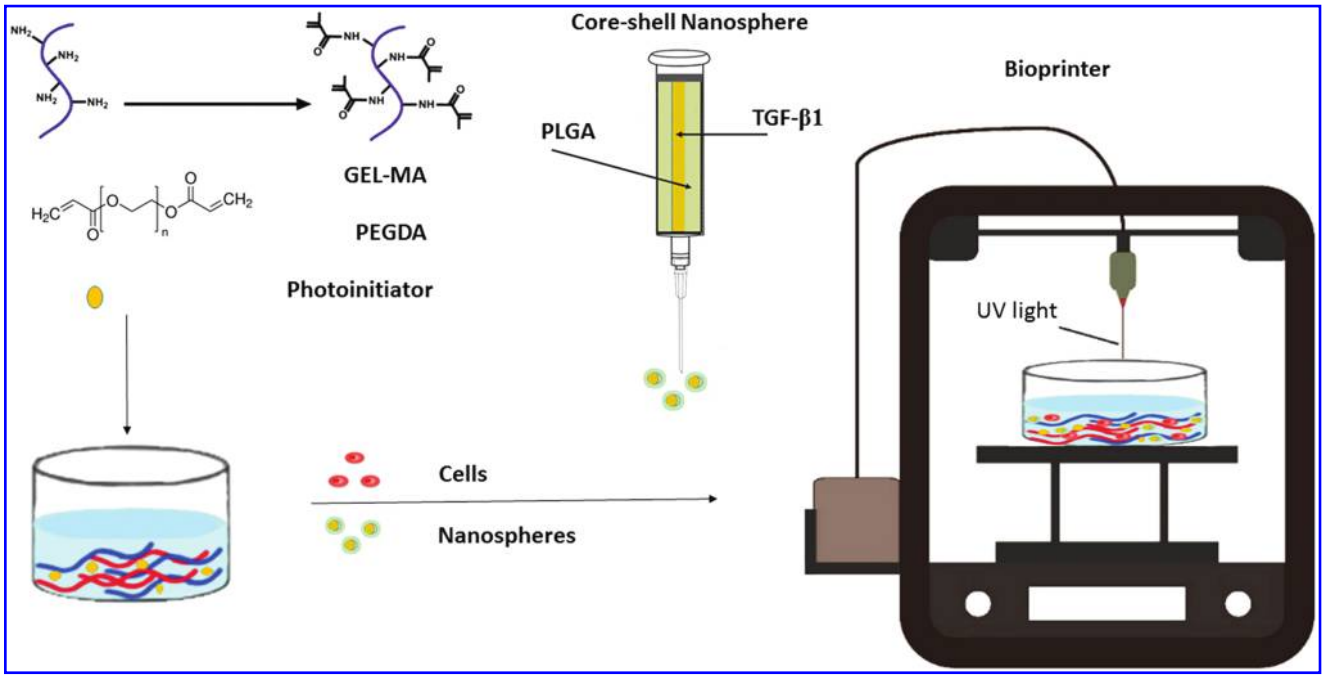

FIG. 3. Schematic design of cartilage construct printing via stereolithography-based bioprinter and synthesized bioink.[32] GEL-MA, gelatin-methacrylamide; PEGDA, poly ethylene glycol diacrylate; PLGA, poly(DL-lactic-co-glycolic acid); TGF-β1, transforming growth factor-beta 1; UV, ultra violet. Color images are available online.

clinical applicability of these methods. The most challenging limitations are technical improvement and standardization, bioink formulation, and more closely mimicking the native cartilage mechanical properties.[151] However, when different synthetic and natural biomaterials are associated within bioinks, they appear to mimic the cartilage microenvironment and to enhance cell viability and chondrogenic ability.

Freeze-drying

In FD, the material is frozen in a hydrogel shape and the water forms pockets of ice throughout the matrix. Consequently, these ice pockets are sublimated and removed under vacuum to produce the porous network. The size of pores can be controlled by the freezing temperature, thus producing porous structures with varying pore sizes and interconnectivities, due to varying heat transfer coefficients. Interconnected network composite scaffolds based on incorporating type II COL with CS and HA were fabricated utilizing chemical crosslinking and FD procedures, to mimic the native ECM of AC and upregulate cartilage ECM biosynthetic activities.[74] The 3D porous hybrid scaffolds were prepared with incorporation of PLGA microspheres into GEL/CHSN/HA scaffolds and crosslinking with EDC, using the simple FD method. Cell culture confirmed that chondrocytes could secrete ECM and proliferate similar to the control (GEL/CHSN/HA scaffolds).[117] The FD method was also used to prepare interconnected PVA/GEL/nanohydroxyapatite/polyamide-6 bilayered hybrid scaffolds for *in situ* osteochondral defect repair.[68] In another study, COL/PLA, CHSN/PLA, and COL/CHSN/PLA hybrid scaffolds were fabricated via the combination of FD of the natural biomaterials COL and CHSN and PLA meshes. The 3D PLA meshes provided mechanical properties and the natural biomaterials mimicked the natural niche of chondrocytes.[59] GEL scaffolds with interconnected pore structures and with good mechanical strength were fabricated using ice particulates and FD. Bovine articular chondrocytes were cultured on these scaffolds, for CT formation *in vitro*.[173] The 3D porous scaffold-based cellulose nanofibers, stabilized using a genipin crosslinked matrix of Gel and CHSN, were prepared using FD. The results showed that the scaffolds have interconnected and homogenous pores, which supported chondrogenesis, thus highlighting the impact and efficiency of FD in the fabrication of hybrid scaffolds for CTE application of CHSN.[53] Open and interconnected pore structures are considered the most important characteristic of the TE scaffolds.[35] Uniform multidirectional COL-based scaffolds were fabricated by unidirectional freeze casting of COL/HA and COL/hydroxyapatite suspensions. The scaffolds were joined by a lyophilization bonding process. With the arrangement of these compositional and architectural biomimetic cues, the scaffolds hold great capacity for zonal CTE.[43] A novel class of hybrid scaffolds based on CHSN as a structural material and low portion of HA to mimic cartilage ECM were fabricated using FD. The results showed that incorporation of HA to the CHSN scaffolds enhanced the biological properties of the scaffolds, which had a superior porous structure network and exhibited higher cartilage ECM deposition.[109] To fabricate porous hybrid constructs, CS was blended with SF to fabricate SF/CS scaffolds via FD. The scaffolds showed a pore size of 37–212 μm, contact angle 46.2–50.38, biodegradation, and controlled swelling. Biocompatibility was confirmed by implantation of scaffolds subcutaneously in a mouse. The results indicated that incorporation of CS to the scaffolds promoted proliferation, cell attachment, and metabolic activity of hMSCs *in vitro*.[130] Despite the great advantage of using the FD technique in fabrication of hybrid constructs in CTE, a small and nonhomogeneous pore size can be considered the main limitation of this method for several materials.

Solvent casting and particulate leaching techniques

In particulate leaching (PL) techniques, pore size and porosity of the scaffolds can be controlled by a selection of appropriate porogen materials. Using this method, numerous porous structures have been fabricated from varied synthetic and natural biomaterials for TE applications.[79] Electrospinning combined with salt leaching (SL) has also been used to fabricate macroporous and nanofibrous HA scaffolds. HA and COL were electrospun into a nanofiber mesh by the deposition of salt particles during electrospinning and following crosslinking and SL. Cytocompatibility of the scaffold was evaluated by culturing bovine chondrocytes on the HA/COL scaffolds. The results demonstrated that cell proliferation was improved and COL content enhanced.[122] Gas foaming/SL was used for the fabrication of PLGA/HA hybrid scaffolds. The PLGA scaffolds were fabricated by blending PLGA with varying amounts of amine-terminated PLGA-PEG diblock copolymers. Gene expression results, biochemical assays, and histological analysis showed that HA-modified scaffolds supported higher cartilage ECM formation, COL II gene expression, and morphological characteristics.[174] Porous scaffolds based on poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) tubes were fabricated using a dipping method followed by SL. Culture of hMSCs on hybrid scaffolds showed early chondrogenic differentiation by expression of SOX9 in the presence of a proper induction medium.[60] Nanocomposite scaffolds composed of PLGA/HA/fibrin/bioglass were prepared using solvent casting and PL (SC-PL) techniques. The scaffolds showed proper porosity percentage ($87.01 \pm 3\%$) with interconnected pore morphologies and pore size between 100 and 200 μm. The nanocomposite scaffold was cytocompatible, and the human adipose tissue-derived mesenchymal stem cells attached and proliferated on the scaffold. These scaffolds can be used for CTE applications.[102] To overcome the possible disadvantages of PLGA, such as its hydrophobicity, limited support of nutrient exchange, and induction of inflammatory responses, micronized porcine cartilage (MPC) was added to the scaffolds to reduce the inflammatory effects and improve cell attachment and proliferation. PLGA and MPC/PLGA scaffolds were fabricated by the SC-PL technique. The results showed that incorporation of bioactive materials of MPC had constructive effects for enhancing PLGA scaffold biocompatibility.[149]

Future Outlook

About 132 articles published between 2000 and 2018, describing ECM-based hybrid and composite scaffolds used in CTE, were reviewed in the present study (Tables 1–4). A statistical analysis demonstrates which materials and methods are the most frequently used for ECM-based

hybrid scaffold fabrication. To evaluate frequency of the different materials and methods, the number of published articles for each material or method was divided by total number of published articles during these years [frequency percentage = (number of published articles for each materials or methods/total number of published articles) ×100]. The results indicated that between cartilage ECM derivatives, the most frequently used materials were COL (40%), HA (34%), CS (14%), and cECMa (12%). According to the results, the most used synthetic materials to design hybrid and composite scaffolds in CTE are PLGA (18%), MA (16%), PCL (15%), PEG (9%), PVA (9%), and PLA (8%), and other polymeric materials consisting of poly-N-(vinylcaprolactam), other polyesters, polyurethanes, and PGA (25% totally). Among natural biomaterials, CHSN (41%), SF (21%), ALG (14%), fibrin (11%), and AGR (5%), respectively, were the most frequently used in CTE. Finally, among scaffolding methods for fabrication of hybrid scaffolds at CTE applications, hydrogels (38%), fibrous scaffolds (22%), FD (15%), 3D printing (10%), SCPL (6%), and other methods consisting of the microsphere, mesh-microsponge, fiber–hydrogel (9% totally) were more familiar, respectively. COL and HA have been mostly used in the fabrication of hybrid scaffolds in comparison with other ECM derivatives. These two polymers have been thoroughly investigated because of their biocompatibility, biomimetic properties, and abundance. As shown in the beginning, synthetic polymers, PLGA, PCL, and MA, were more frequently used hybrid scaffolds than PVA, PLA, and PEG polymers. PLGA and PCL are biodegradable, biocompatible, and their rate of biodegradation can be controlled by the degree of hybridization with other synthetic and natural biomaterials. Results demonstrated that CHSN has been used the most frequently as a natural biomaterial in the fabrication of hybrid scaffolds. CHSN biostability is due to its large number of reactive amino groups that play a useful role as sites for specific crosslinking. CHSN is miscible at the molecular level and it exhibits hydrogen bonding or electrostatic interactions that contribute to mechanical stability. Furthermore, adding CHSN to ECM derivatives increases the number of crosslinking sites. When crosslinked, these hybrid scaffolds prevent access to hydrolytic enzymes to the sensitive cleavage sites of ECM derivatives. Biostability on the one hand and degradation rates on the other hand can be controlled by the extent and type of crosslinking. Finally, among the fabrication methods, hydrogels and fibrous approaches were the most common in CTE application.

As the results show, collagens and HA were used most frequently in the fabrication of hybrid scaffolds. However, according to articles published in recent years, the use of ECM analogs in the preparation of hybrid scaffolds for CTE applications has been expanding due to the potential to induce SC differentiation.

Among natural biomaterials, CHSN has been widely used due to its miscibility with ECM derivatives, and the presence of functional groups for crosslinking with ECM derivatives, all important features for cartilage tissue repair. With regard to fabrication, hydrogels and fibers are used most frequently in hybrid scaffold fabrication due to their similarity to natural cartilage structure. There is also a growing trend with the use of injectable hydrogels and biofabrication methods for CTE.

Conclusions

In hybrid scaffold fabrication, ECM-derived biomolecules are combined with natural or synthetic biomaterials. Because of their specific biophysical and biochemical properties, hybrid scaffolds provide superior interactions with cells and better control of cell adhesion, spreading, proliferation, and differentiation. ECM-derived hybrid scaffolds are promising materials for cartilage and other TE needs. Hybrid scaffolds consisting of cell-derived ECM and synthetic materials have superior mechanical properties compared with acellular tissues and pure ECM scaffolds. Based on the reviewed publications, it is possible to conclude that COL and HA are the most frequently used in hybrid scaffolds for CTE, and that from the perspective of fabrication or process or material format, fibrous and hydrogel scaffolds are the most popular.

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References

1. Tuan, R.S., and Chen, F.H. Cartilage. In: Battler, A., and Leor, J., eds. Stem Cell and Gene-Based Therapy. London: Springer, 2006, pp. 179–193.
2. Mankin, H.J., Mow, V.C., Buckwalter, J.A., Iannotti, J.P., and Ratcliffe, A. Articular cartilage structure, composition, and function. Orthop Basic Sci **2,** 443, 2000.
3. Mow, V.C., Ratcliffe, A., and Poole, A.R. Cartilage and diarthrodial joints as paradigms for hierarchical materials and structures. Biomaterials **13,** 67, 1992.
4. Eggli, P.S., Hunzinker, E.B., and Schenk, R.K. Quantitation of structural features characterizing weight- and less-weight-bearing regions in articular cartilage: a stereological analysis of medical femoral condyles in young adult rabbits. Anat Rec **222,** 217, 1988.
5. Wong, M., Wuethrich, P., Eggli, P., and Hunziker, E. Zone-specific cell biosynthetic activity in mature bovine articular cartilage: a new method using confocal microscopic stereology and quantitative autoradiography. J Orthop Res **14,** 424, 1996.
6. Wu, J.Z., and Herzog, W. Elastic anisotropy of articular cartilage is associated with the microstructures of collagen fibers and chondrocytes. J Biomech **35,** 931, 2002.
7. Scadden, D.T. The stem-cell niche as an entity of action. Nature **441,** 1075, 2006.
8. Weissman, I.L. Translating stem and progenitor cell biology to the clinic: barriers and opportunities. Science **287,** 1442, 2000.
9. Mousavi, S.J., and Hamdy Doweidar, M. Role of mechanical cues in cell differentiation and proliferation: A 3D numerical model. PLoS One **10,** e0124529, 2015.
10. Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. Matrix elasticity directs stem cell lineage specification. Cell **126,** 677, 2006.
11. Becerra, J., Santos-Ruiz, L., Andrades, J.A., and Marí-Beffa, M. The stem cell niche should be a key issue for

cell therapy in regenerative medicine. Stem Cell Rev **7,** 248, 2011.
12. Geiger, B., Spatz, J.P., and Bershadsky, A.D. Environmental sensing through focal adhesions. Nat Rev Mol Cell Biol **10,** 21, 2009.
13. Holle, A.W., and Engler, A.J. More than a feeling: discovering, understanding, and influencing mechanosensing pathways. Curr Opin Biotechnol **22,** 648, 2011.
14. Sekiya, I., Larson, B.L., Smith, J.R., Pochampally, R., Cui, J.G., and Prockop, D.J. Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. Stem Cells **20,** 530, 2002.
15. Bertolo, A., Mehr, M., Aebli, N., Baur, M., Ferguson, S.J., and Stoyanov, J.V. Influence of different commercial scaffolds on the in vitro differentiation of human mesenchymal stem cells to nucleus pulposus-like cells. Eur Spine J **21,** S826, 2012.
16. Farrell, E., O'Brien, F.J., Doyle, P., *et al.* A collagen-glycosaminoglycan scaffold supports adult rat mesenchymal stem cell differentiation along osteogenic and chondrogenic routes. Tissue Eng **12,** 459, 2006.
17. Charlton, D.C., Peterson, M.G., Spiller, K., Lowman, A., Torzilli, P.A., and Maher, S.A. Semi-degradable scaffold for articular cartilage replacement. Tissue Eng Part A **14,** 207, 2008.
18. Kang, S.W., Seo, S.W., Choi, C.Y., and Kim, B.S. Porous poly(lactic-co-glycolic acid) microsphere as cell culture substrate and cell transplantation vehicle for adipose tissue engineering. Tissue Eng Part C Methods **14,** 25, 2008.
19. Young, R.C., Schumann, R., and Zhang, P. Three-dimensional culture of human uterine smooth muscle myocytes on a resorbable scaffolding. Tissue Eng **9,** 451, 2003.
20. Heo, S.-J., Kim, S.-E., Wei, J., *et al.* In vitro and animal study of novel nano-hydroxyapatite/poly(ε-caprolactone) composite scaffolds fabricated by layer manufacturing process. Tissue Eng Part A **15,** 977, 2009.
21. He, X., Lu, H., Kawazoe, N., Tateishi, T., and Chen, G. A novel cylinder-type poly(L-lactic acid)–collagen hybrid sponge for cartilage tissue engineering. Tissue Eng Part C Methods **16,** 329, 2010.
22. Varghese, S., and Elisseeff, J.H. Hydrogels for musculoskeletal tissue engineering. In: Werner, C., ed. Polymers for Regenerative Medicine. Berlin: Springer, 2006, pp. 95–144.
23. Hwang, N.S., Varghese, S., Li, H., and Elisseeff, J. Regulation of osteogenic and chondrogenic differentiation of mesenchymal stem cells in PEG-ECM hydrogels. Cell Tissue Res **344,** 499, 2011.
24. Van der Rest, M., and Garrone, R. Collagen family of proteins. FASEB J **5,** 2814, 1991.
25. Fratzl, P. Collagen: Structure and Mechanics. Boston, MA: Springer Science & Business Media, 2008, pp. 1–13.
26. Glowacki, J., and Mizuno, S. Collagen scaffolds for tissue engineering. Biopolymers **89,** 338, 2008.
27. Doulabi, A.H., Mequanint, K., and Mohammadi, H. Blends and nanocomposite biomaterials for articular cartilage tissue engineering. Materials **7,** 5327, 2014.
28. Yang, X., Guo, L., Fan, Y., and Zhang, X. Preparation and characterization of macromolecule cross-linked collagen hydrogels for chondrocyte delivery. Int J Biol Macromol **61,** 487, 2013.
29. Yan, L.P., Wang, Y.J., Ren, L., *et al.* Genipin-cross-linked collagen/chitosan biomimetic scaffolds for articular cartilage tissue engineering applications. J Biomed Mater Res Part A **95,** 465, 2010.
30. Sionkowska, A., Wisniewski, M., Skopinska, J., Kennedy, C.J., and Wess, T.J. Molecular interactions in collagen and chitosan blends. Biomaterials **25,** 795, 2004.
31. Liu, X., Won, Y., and Ma, P.X. Porogen-induced surface modification of nano-fibrous poly(L-lactic acid) scaffolds for tissue engineering. Biomaterials **27,** 3980, 2006.
32. Zhu, W., Cui, H., Boualam, B., *et al.* 3D bioprinting mesenchymal stem cell-laden construct with core-shell nanospheres for cartilage tissue engineering. Nanotechnology **29,** 185101, 2018.
33. Cheng, Z., Landish, B., Chi, Z., *et al.* 3D printing hydrogel with graphene oxide is functional in cartilage protection by influencing the signal pathway of Rank/Rankl/OPG. Mater Sci Eng C **82,** 244, 2018.
34. He, Y., Liu, W., Guan, L., *et al.* A 3D-printed PLCL scaffold coated with collagen type I and its biocompatibility. BioMed Res Int **2018,** 5147156, 2018.
35. Kaczmarek, B., Sionkowska, A., and Stojkovska, J. Characterization of scaffolds based on chitosan and collagen with glycosaminoglycans and sodium alginate addition. Polym Test **68,** 229, 2018.
36. Yang, X., Lu, Z., Wu, H., Li, W., Zheng, L., and Zhao, J. Collagen-alginate as bioink for three-dimensional (3D) cell printing based cartilage tissue engineering. Mater Sci Eng C **83,** 195, 2018.
37. Gao, Y., Kong, W., Li, B., *et al.* Fabrication and characterization of collagen-based injectable and self-crosslinkable hydrogels for cell encapsulation. Colloids Surf B Biointerfaces **167,** 448, 2018.
38. Chen, J.X., Yuan, J., Wu, Y.L., *et al.* Fabrication of tough poly(ethylene glycol)/collagen double network hydrogels for tissue engineering. J Biomed Mater Res A **106,** 192, 2018.
39. Mekhileri, N.V., Lim, K.S., Brown, G.C.J., *et al.* Automated 3D bioassembly of micro-tissues for biofabrication of hybrid tissue engineered constructs. Biofabrication **10,** 024103, 2018.
40. Bas, O., Lucarotti, S., Angella, D.D., *et al.* Rational design and fabrication of multiphasic soft network composites for tissue engineering articular cartilage: a numerical model-based approach. Chem Eng J **340,** 15, 2018.
41. Saghebasl, S., Davaran, S., Rahbarghazi, R., Montaseri, A., Salehi, R., and Ramazani, A. Synthesis and in vitro evaluation of thermosensitive hydrogel scaffolds based on (PNIPAAm-PCL-PEG-PCL-PNIPAAm)/Gelatin and (PCL-PEG-PCL)/Gelatin for use in cartilage tissue engineering. J Biomater Sci Polym Ed **29,** 1185, 2018.
42. Song, J.E., Tripathy, N., Cha, S.R., *et al.* Three-dimensional duck's feet collagen/PLGA scaffold for chondrification: role of pore size and porosity. J Biomater Sci Polym Ed **29,** 932, 2018.
43. Clearfield, D., Nguyen, A., and Wei, M. Biomimetic multidirectional scaffolds for zonal osteochondral tissue engineering via a lyophilization bonding approach. J Biomed Mater Res A **106,** 948, 2018.
44. Huang, G.P., Molina, A., Tran, N., Collins, G., and Arinzeh, T.L. Investigating cellulose derived glycosaminoglycan mimetic scaffolds for cartilage tissue engineering applications. J Tissue Eng Regen Med **12,** e592, 2018.

45. D'Amora, U., D'Este, M., Eglin, D., *et al.* Collagen density gradient on three-dimensional printed poly(ε-caprolactone) scaffolds for interface tissue engineering. J Tissue Eng Regen Med **12,** 321, 2018.
46. Jeon, O., Shin, J.-Y., Marks, R., *et al.* Highly elastic and tough interpenetrating polymer network-structured hybrid hydrogels for cyclic mechanical loading-enhanced tissue engineering. Chem Mater **29,** 8425, 2017.
47. Agheb, M., Dinari, M., Rafienia, M., and Salehi, H. Novel electrospun nanofibers of modified gelatin-tyrosine in cartilage tissue engineering. Mater Sci Eng C **71,** 240, 2017.
48. Kalaithong, W., Molloy, R., Theerathanagorn, T., and Janvikul, W. Novel poly(L-lactide-co-caprolactone)/gelatin porous scaffolds for use in articular cartilage tissue engineering: comparison of electrospinning and wet spinning processing methods. Polym Eng Sci **57,** 875, 2017.
49. Shi, W., Sun, M., Hu, X., *et al.* Structurally and functionally optimized silk-fibroin-gelatin scaffold using 3D printing to repair cartilage injury in vitro and in vivo. Adv Mater **29,** 1701089, 2017.
50. Almeida, H.V., Sathy, B.N., Dudurych, I., Buckley, C.T., O'Brien, F.J., and Kelly, D.J. Anisotropic shape-memory alginate scaffolds functionalized with either type I or type II collagen for cartilage tissue engineering. Tissue Eng Part A **23,** 55, 2017.
51. Levato, R., Webb, W.R., Otto, I.A., *et al.* The bio in the ink: cartilage regeneration with bioprintable hydrogels and articular cartilage-derived progenitor cells. Acta Biomater **61,** 41, 2017.
52. Wang, J., Yang, Q., Cheng, N., *et al.* Collagen/silk fibroin composite scaffold incorporated with PLGA microsphere for cartilage repair. Mater Sci Eng C **61,** 705, 2016.
53. Naseri, N., Poirier, J.-M., Girandon, L., Fröhlich, M., Oksman, K., and Mathew, A.P. 3-Dimensional porous nanocomposite scaffolds based on cellulose nanofibers for cartilage tissue engineering: tailoring of porosity and mechanical performance. Rsc Adv **6,** 5999, 2016.
54. Studer, D., Cavalli, E., Formica, F.A., *et al.* Human chondroprogenitors in alginate–collagen hybrid scaffolds produce stable cartilage in vivo. J Tissue Eng Regen Med **11,** 3014, 2017.
55. Bas, O., De-Juan-Pardo, E.M., Chhaya, M.P., *et al.* Enhancing structural integrity of hydrogels by using highly organised melt electrospun fibre constructs. Eur Polym J **72,** 451, 2015.
56. He, X., Feng, B., Huang, C., *et al.* Electrospun gelatin/polycaprolactone nanofibrous membranes combined with a coculture of bone marrow stromal cells and chondrocytes for cartilage engineering. Int J Nanomater **10,** 2089, 2015.
57. Liu, S., Wu, J., Liu, X., *et al.* Osteochondral regeneration using an oriented nanofiber yarn-collagen type I/hyaluronate hybrid/TCP biphasic scaffold. J Biomed Mater Res A **103,** 581, 2015.
58. Yin, F., Cai, J., Zen, W., *et al.* Cartilage regeneration of adipose-derived stem cells in the TGF-β1-immobilized PLGA-gelatin scaffold. Stem Cell Rev **11,** 453, 2015.
59. Haaparanta, A.M., Järvinen, E., Cengiz, I.F., *et al.* Preparation and characterization of collagen/PLA, chitosan/PLA, and collagen/chitosan/PLA hybrid scaffolds for cartilage tissue engineering. J Mater Sci Mater Med **25,** 1129, 2014.
60. Lomas, A.J., Webb, W.R., Han, J., *et al.* Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate)/collagen hybrid scaffolds for tissue engineering applications. Tissue Eng Part C Methods **19,** 577, 2013.
61. Dai, W., Yao, Z., Dong, J., Kawazoe, N., Zhang, C., and Chen, G. Cartilage tissue engineering with controllable shape using a poly(lactic-co-glycolic acid)/collagen hybrid scaffold. J Bioact Compat Polym **28,** 247, 2013.
62. Xu, T., Binder, K.W., Albanna, M.Z., *et al.* Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. Biofabrication **5,** 015001, 2013.
63. Kim, H.J., Kim, K.K., Park, I.K., Choi, B.S., Kim, J.H., and Kim, M.S. Hybrid scaffolds composed of hyaluronic acid and collagen for cartilage regeneration. Tissue Eng Regen Med **9,** 57, 2012.
64. Bhat, S., Lidgren, L., Kumar, A. In vitro neo-cartilage formation on a three-dimensional composite polymeric cryogel matrix. Macromol Biosci **13,** 827, 2013.
65. Chen, W.C., Yao, C.L., Wei, Y.H., and Chu, I.M. Evaluating osteochondral defect repair potential of autologous rabbit bone marrow cells on type II collagen scaffold. Cytotechnology **63,** 13, 2011.
66. Abedi, G., Sotoudeh, A., Soleymani, M., Shafiee, A., Mortazavi, P., and Aflatoonian, M.R. A collagen-poly(vinyl alcohol) nanofiber scaffold for cartilage repair. J Biomater Sci Polym Ed **22,** 2445, 2011.
67. Bi, L., Cao, Z., Hu, Y., *et al.* Effects of different cross-linking conditions on the properties of genipin-cross-linked chitosan/collagen scaffolds for cartilage tissue engineering. J Mater Sci Mater Med **22,** 51, 2011.
68. Qu, D., Li, J., Li, Y., *et al.* Ectopic osteochondral formation of biomimetic porous PVA-n-HA/PA6 bilayered scaffold and BMSCs construct in rabbit. J Biomed Mater Res B Appl Biomater **96B,** 9, 2011.
69. Zhang, L., Li, K., Xiao, W., *et al.* Preparation of collagen–chondroitin sulfate–hyaluronic acid hybrid hydrogel scaffolds and cell compatibility in vitro. Carbohydr Polym **84,** 118, 2011.
70. Ho, S.T., Cool, S.M., Hui, J.H., and Hutmacher, D.W. The influence of fibrin based hydrogels on the chondrogenic differentiation of human bone marrow stromal cells. Biomaterials **31,** 38, 2010.
71. Dai, W., Kawazoe, N., Lin, X., Dong, J., and Chen, G. The influence of structural design of PLGA/collagen hybrid scaffolds in cartilage tissue engineering. Biomaterials **31,** 2141, 2010.
72. Guo, X., Park, H., Young, S., *et al.* Repair of osteochondral defects with biodegradable hydrogel composites encapsulating marrow mesenchymal stem cells in a rabbit model. Acta Biomater **6,** 39, 2010.
73. Kathuria, N., Tripathi, A., Kar, K.K., and Kumar, A. Synthesis and characterization of elastic and macroporous chitosan-gelatin cryogels for tissue engineering. Acta Biomater **5,** 406, 2009.
74. Ko, C.S., Huang, J.P., Huang, C.W., and Chu, I.M. Type II collagen–chondroitin sulfate–hyaluronan scaffold cross-linked by genipin for cartilage tissue engineering. J Biosci Bioeng **107,** 177, 2009.
75. Buttafoco, L., Kolkman, N.G., Engbers-Buijtenhuijs, P., *et al.* Electrospinning of collagen and elastin for tissue engineering applications. Biomaterials **27,** 724, 2006.
76. Tan, W., Krishnaraj, R., and Desai, T.A. Evaluation of nanostructured composite collagen–chitosan matrices for tissue engineering. Tissue Eng **7,** 203, 2001.

77. Hillel, A., Shah, P., and Elisseeff, J.H. Hydrogels in cell encapsulation and tissue engineering. In: Biodmedical Polymers. Woodhead Publishing, 2007. pp. 57–82.
78. Kim, D.-D., Kim, D.-H., and Son, Y.-J. Three-dimensional porous scaffold of hyaluronic acid for cartilage tissue engineering. In: Zilberman, M., ed. Active Implants and Scaffolds for Tissue Regeneration (Studies in Mechanobiology, Tissue Engineering and Biomaterials). Berlin: Springer, 2010, pp. 329–349.
79. Collins, M.N., and Birkinshaw, C. Hyaluronic acid based scaffolds for tissue engineering—a review. Carbohydr Polym **92,** 1262, 2013.
80. Muzzarelli, R.A., Greco, F., Busilacchi, A., Sollazzo, V., and Gigante, A. Chitosan, hyaluronan and chondroitin sulfate in tissue engineering for cartilage regeneration: a review. Carbohydr Polym **89,** 723, 2012.
81. Widner, B., Behr, R., Von Dollen, S., *et al.* Hyaluronic acid production in Bacillus subtilis. Appl Environ Microbiol **71,** 3747, 2005.
82. Mattheolabakis, G., Milane, L., Singh, A., and Amiji, M.M. Hyaluronic acid targeting of CD44 for cancer therapy: from receptor biology to nanomedicine. J Drug Target **23,** 605, 2015.
83. Teong, B., Wu, S.C., Chang, C.M., *et al.* The stiffness of a crosslinked hyaluronan hydrogel affects its chondro-induction activity on hADSCs. J Biomed Mater Res B Appl Biomater **106,** 808, 2018.
84. Seidlits, S.K., Drinnan, C.T., Petersen, R.R., Shear, J.B., Suggs, L.J., and Schmidt, C.E. Fibronectin-hyaluronic acid composite hydrogels for three-dimensional endothelial cell culture. Acta Biomater **7,** 2401, 2011.
85. Jung, A., Makkar, P., Amirian, J., and Lee, B.T. A novel hybrid multichannel biphasic calcium phosphate granule-based composite scaffold for cartilage tissue regeneration. J Biomater Appl **32,** 775, 2018.
86. Hsieh, Y.-H., Shen, B.-Y., Wang, Y.-H., Lin, B., Lee, H.-M., and Hsieh, M.-F. Healing of osteochondral defects implanted with biomimetic scaffolds of poly(ε-caprolactone)/hydroxyapatite and glycidyl-methacrylate-modified hyaluronic acid in a minipig. Int J Mol Sci **19,** 1125, 2018.
87. Zhu, C., Yang, R., Hua, X., *et al.* Highly stretchable HA/SA hydrogels for tissue engineering. J Biomater Sci Polym Ed **29,** 543, 2018.
88. Karabiyik Acar, O., Kayitmazer, A.B., and Torun Kose, G. Hyaluronic acid/chitosan coacervate-based scaffolds. Biomacromolecules **19,** 1198, 2018.
89. Schiavi, J., Reppel, L., Charif, N., *et al.* Mechanical stimulations on human bone marrow mesenchymal stem cells enhance cells differentiation in a three-dimensional layered scaffold. J Tissue Eng Regen Med **12,** 360, 2018.
90. Han, S.-S., Yoon, H.Y., Yhee, J.Y., *et al.* In situ cross-linkable hyaluronic acid hydrogels using copper free click chemistry for cartilage tissue engineering. Polym Chem **9,** 20, 2018.
91. Wang, H., Zhu, D., Paul, A., *et al.* Covalently adaptable elastin-like protein–hyaluronic acid (ELP–HA) hybrid hydrogels with secondary thermoresponsive crosslinking for injectable stem cell delivery. Adv Funct Mater **27,** 2017, 1605609.
92. Zhu, D., Wang, H., Trinh, P., Heilshorn, S.C., and Yang, F. Elastin-like protein–hyaluronic acid (ELP–HA) hydrogels with decoupled mechanical and biochemical cues for cartilage regeneration. Biomaterials **127,** 132, 2017.
93. Shie, M.Y., Chang, W.C., Wei, L.J., *et al.* 3D printing of cytocompatible water-based light-cured polyurethane with hyaluronic acid for cartilage tissue engineering applications. Materials **10,** 136, 2017.
94. Raia, N.R., Partlow, B.P., McGill, M., Kimmerling, E.P., Ghezzi, C.E., and Kaplan, D.L. Enzymatically crosslinked silk-hyaluronic acid hydrogels. Biomaterials **131,** 58, 2017.
95. Lin, X., Wang, W., Zhang, W., *et al.* Hyaluronic acid coating enhances biocompatibility of nonwoven PGA scaffold and cartilage formation. Tissue Eng Part C Methods **23,** 86, 2017.
96. Chen, F., Ni, Y., Liu, B., *et al.* Self-crosslinking and injectable hyaluronic acid/RGD-functionalized pectin hydrogel for cartilage tissue engineering. Carbohydr Polym **166,** 31, 2017.
97. Kim, D.Y., Park, H., Kim, S.W., Lee, J.W., and Lee, K.Y. Injectable hydrogels prepared from partially oxidized hyaluronate and glycol chitosan for chondrocyte encapsulation. Carbohydr Polym **157,** 1281, 2017.
98. Park, H., Lee, H.J., An, H., and Lee, K.Y. Alginate hydrogels modified with low molecular weight hyaluronate for cartilage regeneration. Carbohydr Polym **162,** 100, 2017.
99. Lynch, B., Crawford, K., Baruti, O., *et al.* The effect of hypoxia on thermosensitive poly(N-vinylcaprolactam) hydrogels with tunable mechanical integrity for cartilage tissue engineering. J Biomed Mater Res **105,** 1863, 2017.
100. Dai, Y., Gao, Z., Ma, L., Wang, D., and Gao, C. Cell-free HA-MA/PLGA scaffolds with radially oriented pores for in situ inductive regeneration of full thickness cartilage defects. Macromol Biosci **16,** 1632, 2016.
101. Shim, J.H., Jang, K.M., Hahn, S.K., *et al.* Three-dimensional bioprinting of multilayered constructs containing human mesenchymal stromal cells for osteochondral tissue regeneration in the rabbit knee joint. Biofabrication **8,** 014102, 2016.
102. Tavakoli, E., Mehdikhani-Nahrkhalaji, M., Hashemi-Beni, B., Zargar-Kharazi, A., and Kharaziha, M. Preparation, characterization and mechanical assessment of poly(lactide-co-glycolide)/hyaluronic acid/fibrin/bioactive glass nano-composite scaffolds for cartilage tissue engineering applications. Procedia Mater Sci **11,** 124, 2015.
103. Snyder, T.N., Madhavan, K., Intrator, M., Dregalla, R.C., and Park, D. A fibrin/hyaluronic acid hydrogel for the delivery of mesenchymal stem cells and potential for articular cartilage repair. J Biol Eng **8,** 10, 2014.
104. Mintz, B.R., and Cooper, J.A. Hybrid hyaluronic acid hydrogel/poly(ε-caprolactone) scaffold provides mechanically favorable platform for cartilage tissue engineering studies. J Biomed Mater Res **102,** 2918, 2014.
105. Levett, P.A., Melchels, F.P., Schrobback, K., Hutmacher, D.W., Malda, J., and Klein, T.J. A biomimetic extracellular matrix for cartilage tissue engineering centered on photocurable gelatin, hyaluronic acid and chondroitin sulfate. Acta Biomater **10,** 214, 2014.
106. Wang, T., Lai, J.H., Han, L.H., Tong, X., and Yang, F. Chondrogenic differentiation of adipose-derived stromal cells in combinatorial hydrogels containing cartilage matrix proteins with decoupled mechanical stiffness. Tissue Eng Part A **20,** 2131, 2014.
107. Schuurman, W., Levett, P.A., Pot, M.W., *et al.* Gelatin-methacrylamide hydrogels as potential biomaterials for

fabrication of tissue-engineered cartilage constructs. Macromol Biosci **13,** 551, 2013.
108. Murphy, C.M., Matsiko, A., Haugh, M.G., Gleeson, J.P., and 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 **11,** 53, 2012.
109. Correia, C.R., Moreira-Teixeira, L.S., Moroni, L., *et al.* Chitosan scaffolds containing hyaluronic acid for cartilage tissue engineering. Tissue Eng Part C Methods **17,** 717, 2011.
110. Coburn, J., Gibson, M., Bandalini, P.A., *et al.* Biomimetics of the extracellular matrix: an integrated three-dimensional fiber-hydrogel composite for cartilage tissue engineering. Smart Struct Syst **7,** 213, 2011.
111. Lee, J.S., and Lee, E.K. FGF-2-expanded costal chondrocytes regenerate hyaline cartilage in rabbit osteochondral defects. Tissue Eng Regen Med **8,** 200, 2011.
112. Jin, R., Moreira Teixeira, L.S., Krouwels, A., *et al.* Synthesis and characterization of hyaluronic acid–poly(ethylene glycol) hydrogels via Michael addition: an injectable biomaterial for cartilage repair. Acta Biomater **6,** 1968, 2010.
113. Jin, R., Teixeira, L.S., Dijkstra, P.J., Van Blitterswijk, C.A., Karperien, M., and Feijen, J. Enzymatically-crosslinked injectable hydrogels based on biomimetic dextran-hyaluronic acid conjugates for cartilage tissue engineering. Biomaterials **31,** 3103, 2010.
114. Fan, H., Tao, H., Wu, Y., Hu, Y., Yan, Y., and Luo, Z. TGF-β3 immobilized PLGA-gelatin/chondroitin sulfate/hyaluronic acid hybrid scaffold for cartilage regeneration. J Biomed Mater Res A **95,** 982, 2010.
115. Im, G.I., Ahn, J.H., Kim, S.Y., Choi, B.S., and Lee, S.W. A hyaluronate-atelocollagen/beta-tricalcium phosphate-hydroxyapatite biphasic scaffold for the repair of osteochondral defects: a porcine study. Tissue Eng Part A **16,** 1189, 2010.
116. Tan, H., Chu, C.R., Payne, K.A., and Marra, K.G. Injectable in situ forming biodegradable chitosan-hyaluronic acid based hydrogels for cartilage tissue engineering. Biomaterials **30,** 2499, 2009.
117. Tan, H., Wu, J., Lao, L., and Gao, C. Gelatin/chitosan/hyaluronan scaffold integrated with PLGA microspheres for cartilage tissue engineering. Acta Biomater **5,** 328, 2009.
118. Chen, J.-P., and Cheng, T.-H. Preparation and evaluation of thermo-reversible copolymer hydrogels containing chitosan and hyaluronic acid as injectable cell carriers. Polymer **50,** 107, 2009.
119. Erggelet, C., Endres, M., Neumann, K., *et al.* Formation of cartilage repair tissue in articular cartilage defects pretreated with microfracture and covered with cell-free polymer-based implants. J Orthop Res **27,** 1353, 2009.
120. Pereira, R.C., Scaranari, M., Castagnola, P., *et al.* Novel injectable gel (system) as a vehicle for human articular chondrocytes in cartilage tissue regeneration. J Tissue Eng Regen Med **3,** 97, 2009.
121. Kasahara, Y., Iwasaki, N., Yamane, S., *et al.* Development of mature cartilage constructs using novel three-dimensional porous scaffolds for enhanced repair of osteochondral defects. J Biomed Mater Res A **86,** 127, 2008.
122. Kim, T.G., Chung, H.J., and Park, T.G. Macroporous and nanofibrous hyaluronic acid/collagen hybrid scaffold fabricated by concurrent electrospinning and deposition/leaching of salt particles. Acta Biomater **4,** 1611, 2008.
123. Solchaga, L.A., Temenoff, J.S., Gao, J., Mikos, A.G., Caplan, A.I., and Goldberg, V.M. Repair of osteochondral defects with hyaluronan- and polyester-based scaffolds. Osteoarthritis Cartilage **13,** 297, 2005.
124. Yamane, S., Iwasaki, N., Majima, T., *et al.* Feasibility of chitosan-based hyaluronic acid hybrid biomaterial for a novel scaffold in cartilage tissue engineering. Biomaterials **26,** 611, 2005.
125. Athansou, N.A., Puddle, B., and Sallie, B. Highly sulphated glycosaminoglycans in articular cartilage and other tissues containing β2 microglobulin dialysis amyloid deposits. Nephrol Dial Transplant **10,** 1672, 1995.
126. Nanda, S., Sood, N., Reddy, B.V.K., and Markandeywar, T.S. Preparation and characterization of poly(vinyl alcohol)-chondroitin sulphate hydrogel as scaffolds for articular cartilage regeneration. Indian J Mater Sci **2013,** 1, 2013.
127. Li, Q., Williams, C.G., Sun, D.D., Wang, J., Leong, K., and Elisseeff, J.H. Photocrosslinkable polysaccharides based on chondroitin sulfate. J Biomed Mater Res A **68,** 28, 2004.
128. Ronca, F., Palmieri, L., Panicucci, P., and Ronca, G. Anti-inflammatory activity of chondroitin sulfate. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society **6**(Suppl. A), 14, 1998.
129. Yang, Y.L., Sun, C., Wilhelm, M.E., Fox, L.J., Zhu, J., and Kaufman, L.J. Influence of chondroitin sulfate and hyaluronic acid on structure, mechanical properties, and glioma invasion of collagen I gels. Biomaterials **32,** 7932, 2011.
130. Agrawal, P., Pramanik, K., Vishwanath, V., Biswas, A., Bissoyi, A., and Patra, P.K. Enhanced chondrogenesis of mesenchymal stem cells over silk fibroin/chitosan–chondroitin sulfate three dimensional scaffold in dynamic culture condition. J Biomed Mater Res B Appl Biomater **106,** 2576, 2018.
131. Bang, S., Jung, U.W., and Noh, I. Synthesis and biocompatibility characterizations of in situ chondroitin sulfate–gelatin hydrogel for tissue engineering. Tissue Eng Regen Med **15,** 25, 2018.
132. Li, T., Song, X., Weng, C., *et al.* Self-crosslinking and injectable chondroitin sulfate/pullulan hydrogel for cartilage tissue engineering. Appl Mater Today **10,** 173, 2018.
133. Piai, J.F., da Silva, M.A., Martins, A., *et al.* Chondroitin sulfate immobilization at the surface of electrospun nanofiber meshes for cartilage tissue regeneration approaches. Appl Surf Sci **403,** 112, 2017.
134. Fan, M., Ma, Y., Tan, H., *et al.* Covalent and injectable chitosan–chondroitin sulfate hydrogels embedded with chitosan microspheres for drug delivery and tissue engineering. Mater Sci Eng C **71,** 67, 2017.
135. Vishwanath, V., Pramanik, K., and Biswas, A. Development of a novel glucosamine/silk fibroin–chitosan blend porous scaffold for cartilage tissue engineering applications. Iran Polym J **26,** 11, 2017.
136. Zhou, F., Zhang, X., Cai, D., *et al.* Silk fibroin–chondroitin sulfate scaffold with immuno-inhibition property for articular cartilage repair. Acta Biomater **63,** 64, 2017.
137. Shahali, Z., Karbasi, S., Avadi, M.R., Semnani, D., Naghash Zargar, E., and HashemiBeni, B. Evaluation of

structural, mechanical, and cellular behavior of electrospun poly-3-hydroxybutyrate scaffolds loaded with glucosamine sulfate to develop cartilage tissue engineering. Int J Polym Mater Polym Biomater **66,** 589, 2017.

138. Chen, F., Yu, S., Liu, B., *et al.* An injectable enzymatically crosslinked carboxymethylated pullulan/chondroitin sulfate hydrogel for cartilage tissue engineering. Sci Rep **6,** 20014, 2016.
139. Costantini, M., Idaszek, J., Szöke, K., *et al.* 3D bioprinting of BM-MSCs-loaded ECM biomimetic hydrogels for in vitro neocartilage formation. Biofabrication **8,** 035002, 2016.
140. Liao, J., Qu, Y., Chu, B., Zhang, X., and Qian, Z. Biodegradable CSMA/PECA/graphene porous hybrid scaffold for cartilage tissue engineering. Sci Rep **5,** 9879, 2015.
141. Sawatjui, N., Damrongrungruang, T., Leeanansaksiri, W., Jearanaikoon, P., Hongeng, S., and Limpaiboon, T. Silk fibroin/gelatin–chondroitin sulfate–hyaluronic acid effectively enhances in vitro chondrogenesis of bone marrow mesenchymal stem cells. Mater Sci Eng C **52,** 90, 2015.
142. Naeimi, M., Fathi, M., Rafienia, M., and Bonakdar, S. Silk fibroin–chondroitin sulfate–alginate porous scaffolds: structural properties and in vitro studies. J Appl Polym Sci, **131,** 41048, 2014.
143. Silva, J.M., Georgi, N., Costa, R., *et al.* Nanostructured 3D constructs based on chitosan and chondroitin sulphate multilayers for cartilage tissue engineering. PLoS One **8,** e55451, 2013.
144. Chen, Y.L., Lee, H.P., Chan, H.Y., Sung, L.Y., Chen, H.C., and Hu, Y.C. Composite chondroitin-6-sulfate/dermatan sulfate/chitosan scaffolds for cartilage tissue engineering. Biomaterials **28,** 2294, 2007.
145. Lee, C.-T., Kung, P.-H., and Lee, Y.-D. Preparation of poly(vinyl alcohol)–chondroitin sulfate hydrogel as matrices in tissue engineering. Carbohydr Polym **61,** 348, 2005.
146. Benders, K.E., van Weeren, P.R., Badylak, S.F., Saris, D.B., Dhert, W.J., and Malda, J. Extracellular matrix scaffolds for cartilage and bone regeneration. Trends Biotechnol **31,** 169, 2013.
147. Beck, E.C., Barragan, M., Tadros, M.H., Gehrke, S.H., and Detamore, M.S. Approaching the compressive modulus of articular cartilage with a decellularized cartilage-based hydrogel. Acta Biomater **38,** 94, 2016.
148. Vinatier, C., Mrugala, D., Jorgensen, C., Guicheux, J., and Noël, D. Cartilage engineering: a crucial combination of cells, biomaterials and biofactors. Trends Biotechnol **27,** 307, 2009.
149. Kim, S., Jang, J.E., Lee, J.H., and Khang, G. Composite scaffold of micronized porcine cartilage/poly(lactic-co-glycolic acid) enhances anti-inflammatory effect. Mater Sci Eng C **88,** 46, 2018.
150. Ghosh, P., Gruber, S.M.S., Lin, C.Y., and Whitlock, P.W. Microspheres containing decellularized cartilage induce chondrogenesis in vitro and remain functional after incorporation within a poly(caprolactone) filament useful for fabricating a 3D scaffold. Biofabrication **10,** 025007, 2018.
151. Jung, C.S., Kim, B.K., Lee, J., Min, B.H., and Park, S.H. Development of printable natural cartilage matrix bioink for 3D printing of irregular tissue shape. Tissue Eng Regen Med **15,** 155, 2018.
152. Ghassemi, T., Saghatolslami, N., Matin, M.M., Gheshlaghi, R., and Moradi, A. CNT-decellularized cartilage hybrids for tissue engineering applications. Biomed Mater **12,** 065008, 2017.
153. Masaeli, E., Karamali, F., Loghmani, S., Eslaminejad, M.B., and Nasr-Esfahani, M.H. Bio-engineered electrospun nanofibrous membranes using cartilage extracellular matrix particles. J Mater Chem B **5,** 765, 2017.
154. Rothrauff, B.B., Coluccino, L., Gottardi, R., *et al.* Efficacy of thermoresponsive, photocrosslinkable hydrogels derived from decellularized tendon and cartilage extracellular matrix for cartilage tissue engineering. J Tissue Eng Regen Med **12,** e159, 2018.
155. Levingstone, T.J., Ramesh, A., Brady, R.T., *et al.* Cell-free multi-layered collagen-based scaffolds demonstrate layer specific regeneration of functional osteochondral tissue in caprine joints. Biomaterials **87,** 69, 2016.
156. Nogami, M., Kimura, T., Seki, S., *et al.* A human amnion-derived extracellular matrix-coated cell-free scaffold for cartilage repair: in vitro and in vivo studies. Tissue Eng Part A **22,** 680, 2016.
157. Almeida, H.V., Eswaramoorthy, R., Cunniffe, G.M., Buckley, C.T., O'Brien, F.J., and Kelly, D.J. Fibrin hydrogels functionalized with cartilage extracellular matrix and incorporating freshly isolated stromal cells as an injectable for cartilage regeneration. Acta Biomater **36,** 55, 2016.
158. Sutherland, A.J., and Detamore, M.S. Bioactive microsphere-based scaffolds containing decellularized cartilage. Macromol Biosci **15,** 979, 2015.
159. Garrigues, N.W., Little, D., Sanchez-Adams, J., Ruch, D.S., and Guilak, F. Electrospun cartilage-derived matrix scaffolds for cartilage tissue engineering. J Biomed Mater Res A **102,** 3998, 2014.
160. Levorson, E.J., Hu, O., Mountziaris, P.M., Kasper, F.K., and Mikos, A.G. Cell-derived polymer/extracellular matrix composite scaffolds for cartilage regeneration, Part 2: construct devitalization and determination of chondroinductive capacity. Tissue Eng Part C Methods **20,** 358, 2014.
161. Liao, J., Guo, X., Grande-Allen, K.J., Kasper, F.K., and Mikos, A.G. Bioactive polymer/extracellular matrix scaffolds fabricated with a flow perfusion bioreactor for cartilage tissue engineering. Biomaterials **31,** 8911, 2010.
162. Wang, Z.H., He, X.J., Yang, Z.Q., and Tu, J.B. Cartilage tissue engineering with demineralized bone matrix gelatin and fibrin glue hybrid scaffold: an in vitro study. Artif Organs **34,** 161, 2010.
163. Ho, S.T.B., Ekaputra, A.K., Hui, J.H., and Hutmacher, D.W. An electrospun polycaprolactone–collagen membrane for the resurfacing of cartilage defects. Polym Int **59,** 808, 2010.
164. Yamane, S., Iwasaki, N., Kasahara, Y., *et al.* Effect of pore size on in vitro cartilage formation using chitosan-based hyaluronic acid hybrid polymer fibers. J Biomed Mater Res A **81,** 586, 2007.
165. Coburn, J.M., Gibson, M., Monagle, S., Patterson, Z., and Elisseeff, J.H. Bioinspired nanofibers support chondrogenesis for articular cartilage repair. Proc Natl Acad Sci U S A **109,** 10012, 2012.
166. Bhat, S., Tripathi, A., and Kumar, A. Supermacroprous chitosan-agarose-gelatin cryogels: in vitro characterization and in vivo assessment for cartilage tissue engineering. J R Soc Interface **8,** 540, 2011.
167. Nafea, E.H., Poole-Warren, L.A., and Martens, P.J. Bioactivity of permselective PVA hydrogels with

mixed ECM analogues. J Biomed Mater Res A **103,** 3727, 2015.
168. Chua, C.K., and Leong, K.F. Rapid Prototyping: Principles and Applications. Singapore: World Scientific, 2003.
169. Markovic, M., Van Hoorick, J., Hölzl, K., *et al.* Hybrid tissue engineering scaffolds by combination of three-dimensional printing and cell photoencapsulation. J Nanotechnol Eng Med **6,** 0210011, 2015,
170. Hofmann, S., and Garcia-Fuentes, M. Bioactive scaffolds for the controlled formation of complex skeletal tissues. In: Regenerative Medicine and Tissue Engineering-Cells and Biomaterials. IntechOpen, 2011, pp. 393–432.
171. Mironov, V., Visconti, R.P., Kasyanov, V., Forgacs, G., Drake, C.J., and Markwald, R.R. Organ printing: tissue spheroids as building blocks. Biomaterials **30,** 2164, 2009.
172. Visser, J., Melchels, F.P., Jeon, J.E., *et al.* Reinforcement of hydrogels using three-dimensionally printed microfibres. Nat commun **6,** 6933, 2015.
173. Chen, S., Zhang, Q., Nakamoto, T., Kawazoe, N., and Chen, G. Gelatin scaffolds with controlled pore structure and mechanical property for cartilage tissue engineering. Tissue Eng Part C Methods **22,** 189, 2016.
174. Yoo, H.S., Lee, E.A., Yoon, J.J., and Park, T.G. Hyaluronic acid modified biodegradable scaffolds for cartilage tissue engineering. Biomaterials **26,** 1925, 2005.

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2. Flavia Pedrini, Moema Hausen, Rodrigo Gomes, Eliana Duek. 2020. Enhancement of cartilage extracellular matrix synthesis in Poly(PCL–TMC)urethane scaffolds: a study of oriented dynamic flow in bioreactor. *Biotechnology Letters* **5**. . [Crossref]
3. Paul D. Dalton, Tim B. F. Woodfield, Vladimir Mironov, Jürgen Groll. 2020. Advances in Hybrid Fabrication toward Hierarchical Tissue Constructs. *Advanced Science* **7**:11, 1902953. [Crossref]
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5. Sadaf Saeedi Garakani, Mehdi Khanmohammadi, Zhaleh Atoufi, Seyed Kamran Kamrava, Mohsen Setayeshmehr, Rafieh Alizadeh, Faezeh Faghihi, Zohreh Bagher, Seyed Mohammad Davachi, Alireza Abbaspourrad. 2020. Fabrication of chitosan/agarose scaffolds containing extracellular matrix for tissue engineering applications. *International Journal of Biological Macromolecules* **143**, 533-545. [Crossref]
6. Anying Wang, Naixia Hu, Yefeng Zhang, Yuanzhen Chen, Changhui Su, Yao Lv, Yong Shen. 2019. MEG3 promotes proliferation and inhibits apoptosis in osteoarthritis chondrocytes by miR-361-5p/FOXO1 axis. *BMC Medical Genomics* **12**:1. . [Crossref]
7. Zhaleh Atoufi, Seyed Kamran Kamrava, Seyed Mohammad Davachi, Majid Hassanabadi, Sadaf Saeedi Garakani, Rafieh Alizadeh, Mohammad Farhadi, Shima Tavakol, Zohreh Bagher, Ghodratollah Hashemi Motlagh. 2019. Injectable PNIPAM/Hyaluronic acid hydrogels containing multipurpose modified particles for cartilage tissue engineering: Synthesis, characterization, drug release and cell culture study. *International Journal of Biological Macromolecules* **139**, 1168-1181. [Crossref]
8. Moira Loepfe, Anja Duss, Katerina-Alexandra Zafeiropoulou, Oddny Björgvinsdóttir, Matteo D'Este, David Eglin, Giuseppino Fortunato, Juergen Klasen, Stephen J. Ferguson, Karin Wuertz-Kozak, Olga Krupkova. 2019. Electrospray-Based Microencapsulation of Epigallocatechin 3-Gallate for Local Delivery into the Intervertebral Disc. *Pharmaceutics* **11**:9, 435. [Crossref]
9. Biao Duan, Yan Liu, He Hu, Fu-Guo Shi, Ya-Long Liu, Hao Xue, Xin-Yu Yun, Ming-Yu Yan, Xi-Rui Han, An-Fu Chen, Yong Wang, Zhe-Hai Li. 2019. Notch1-ADAM8 positive feed-back loop regulates the degradation of chondrogenic extracellular matrix and osteoarthritis progression. *Cell Communication and Signaling* **17**:1. . [Crossref]