Supplementary Information (SI) for Biomaterials Science.

Strategies to Engineer Articular Cartilage with Biomimetic Zonal Features: A Review

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Supporting Information

Table S1. Strategies exploring zonal chondrocyte and MSCs-based phenotypes.

Ref.	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
			Zonal chondrocytes				
65,256	Morphological and ECM synthesis differences between ZC	ZC	AGA	TIC	Chondrocytes (bovine)	12 days	–
259	Cytoskeletal organisation differences between ZC in AGA and TCPS	ZC	TCPS	–	Chondrocytes (bovine)	3 weeks	–
			AGA	TIC			
263	Quantification of SZP of ZC on monolayer, cartilage explants or decellularized cartilage substrate	ZC	TCPS	–	Chondrocytes (bovine)	9 days	–
			Cartilage explants				
			Decellularized cartilage substrate				
176,177	Culture environments that maintain ZCs phenotype	ZC	AGA	TIC	Chondrocytes (goat)	2 weeks	–
			COL II and AGC-coated surfaces	Solvent casting			
265	Fabrication of self-assembled constructs with different ZC	ZC	–	–	Chondrocytes (goat)	4 weeks in nonadherent AGA wells	–
267	Differences in ZCs isolated from immature and adult articular cartilage	ZC	–	–	Chondrocytes (bovine)	1 week in micromass	–
262	Effect of monolayer expansion dedifferentiation on the mechanical properties and gene expression of ZC	ZC	Micropatterned fibronectin-coated substrate	UV photocrosslinking	Chondrocytes (porcine)	1 week	–

Ref.	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
264	Redifferentiation of expanded ZC in ALG beads	ZC	ALG	Ionic crosslinking	Chondrocytes (equine)	4 weeks with bFGF	–
266	Clarification of ZC spheroids' characteristics	ZC spheroids	–	–	Chondrocytes (bovine)	3 weeks	–
268	Generation of stratified cartilage constructs	ZC	Pre incubation in ALG beads	Ionic crosslinking	Chondrocytes (porcine)	2 weeks	–
269						12 days	
270						4 weeks	1 week chondral (pig)
260	Fabrication of zonal constructs with photocrosslinked hydrogels	ZC	TCPS PEGDA	UV photocrosslinking	Chondrocytes (bovine)	3 weeks	–
272			PEODA			6 weeks	
261	Composition and organization evaluation of engineered cartilage and the effect chondrocyte dedifferentiation by monolayer expansion	ZC	COL II-coated filter inserts (monolayer expansion)	Solvent casting	Chondrocytes (bovine)	4 weeks in synthetic filter membranes with TGF-β2	–
273	Shaping scaffold-free zonal constructs	ZC	Pre incubation in ALG beads followed by seeding in AGA shaped-base	Ionic crosslinking and TIC	Chondrocytes (bovine)	29 days	–
271	Generation of stratified cartilage by incorporating ZC pellets in 3D printed scaffolds	ZC pellets	PEGT/PBT copolymer	FDM	Chondrocytes (equine)	31 days with TGF-β2	–
274	Fabrication of stratified constructs with ZC sheets obtained by monolayer expansion	ZC sheets	–	–	Chondrocytes (porcine)	3 weeks	12 weeks osteochondral (pig)

Ref.	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
275	Engineering zonal constructs with chondrocytes obtained by size-based inertial spiral microchannel separation	ZC	Fibrin	Thrombin-induced crosslinking	Chondrocytes (porcine)	3 weeks with TGF-β3	6 weeks osteochondral (rat)
276						16 days in dMC and 3 weeks with TGF-β3	–
277						16 days in dMC and 3 weeks with TGF-β3	6 months osteochondral (pig)
278	Friction improvement in self-assembled constructs	Different SZ:MZ chondrocytes ratios	–	–	Chondrocytes (bovine)	4 weeks in nonadherent AGA wells with TGF-β1	–
279		Treatment with extracts of SZ articular cartilage ECM					
			Co-culture of chondrocytes and mesenchymal stem cells				
284	Influence of BMMSCs co-culture with ZC on their biosynthetic activity	Co-culture with ZC	ALG	Ionic crosslinking	Chondrocytes and BMMSCs (ratio 1:2) (rabbit)	3 weeks with TGF-β3	8 weeks subcutaneous (mouse)
285	Fabrication of trilayered construct with zonal phenotype stratification combined with BMMSCs	ZC	HA-MA	UV photocrosslinking	Chondrocytes and BMMSCs (ratio 1:4) (bovine)	16 weeks with TGF-β3	–
286	Engineering zonal constructs using chondrocyte-laden hydrogel with a superficial self-assembled layer of MSCs	MSCs in UL and chondrocytes in BL	AGA	TIC	Chondrocytes and FPMSCs or BMMSCs (porcine)	5 weeks with TGF-β3	–

Ref.	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
287	Control of spheroid growth and fusion to engineer native-like COL anisotropies in engineered cartilage cultured in printed microchambers	Posterior native-like COL fibre alignment	PCL, GEL-MA	FDM and extrusion and inkjet-based printing UV photocrosslinking	Chondrocytes and BMMSCs (ratio 1:3) (porcine)	10 weeks with TGF-β3, 3 % O_2 and dynamic stirring (6 mm amplitude at 0.5 mm/sec)	–
Co-culture of articular cartilage progenitor cells and mesenchymal stem cells							
227	Generation of stratified cartilage with ACPCs and BMMSCs -laden hydrogel layers	ACPCs on the UP and BMMSCs on the BL	GEL-MA	Extrusion-based printing and UV photocrosslinking	BMMSCs or ACPCs (equine)	8 weeks with TGF-β1	–
289			GEL-MA, GG, HA-MA			6 weeks with TGF-β1	
290	Engineering zonal constructs with ACPCs and BMMSCs -laden hydrogel layers	ACPCs on the UP and BMMSCs on the BL	AGA	TIC	BMMSCs or ACPCs (equine)	4 weeks with TGF-β1 and 2 and 21 % O_2	–
Mesenchymal stem cells							
291	Control of spheroid growth and fusion to engineer native-like COL anisotropies in engineered cartilage cultured in printed microchambers	Posterior native-like COL fibre alignment	PCL	MEW and inkjet-based printing	BMMSCs (porcine)	8 weeks with TGF-β3 and 5 % O_2	–
292				FDM and inkjet-based printing		8 weeks with TGF-β3, 5 % O_2 and dynamic stirring (5 mm at 0.048 Hz)	
293	Fabrication of stratified AC using differentiated ADSCs and their endogenous ECM in tissue strands	Cartilage tissue strands alignment	Sacrificial ALG	Extrusion-based printing	AMSCs (human)	5 weeks	–

EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; FDM, fused deposition modelling; TCPS, tissue culture polystyrene; TIC, thermally-induced crosslinking; UV, ultraviolet.

Table S2. Strategies exploring biological, chemical, mechanical and structural cues separately in zonal constructs and their influence on chondrocytes and MSCs behaviour.

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
			Decellularized cartilage explants				
294	Repopulation of a decellularized cartilage explant for the development of a specific COL architecture	Immature Benninghoff COL architecture	Decellularized cartilage explants	–	FPMSCs (human)	4 weeks with TGF-β3 and dynamic stirring	–
			Cell density and alignment				
295	Fabrication of zonal constructs with a biomimetic chondrocyte density gradient	Chondrocyte density gradient	COL II	Extrusion-based printing, TIC	Chondrocytes (rabbit)	3 weeks	–
296	Fabrication of zonal constructs with a biomimetic chondrocyte density gradient	Chondrocyte density gradient	PCL, ALG, methylcellulose	FDM, extrusion-based printing and ionic crosslinking	Chondrocytes (human)	25 days with TGF-β1 and TGF-β3	–
297	Engineering zonal cytoarchitecture using magnetically guided cell patterning	Zonal chondrocyte alignment	ALG	Ionic crosslinking	Chondrocytes (human)	*In vitro*: 2 weeks with TGF-β1; *Explant*: 1 week	4 weeks osteochondral (rabbit)
298	Engineering DZ-like cytoarchitecture with high-resolution acoustic cell patterning	DZ-like chondrocyte alignment	AGA	TIC	Chondrocytes (bovine)	5 weeks with TGF-β3	–

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
			Matrix molecules				
299	Influence of ECM molecules on chondrocyte phenotype and neocartilage formation on 2D and 3D environments	Biomimetic ECM molecules	Decorin, biglycan, tenascin, COL II, HA, osteopontin-coated surfaces; ALG	Solvent-casting	Chondrocytes (human)	2 weeks with TGF-β1	–
300	Incorporation of HA and CS onto ALG hydrogels to differentiate MSCs into ZC	ZC	ALG, HA, CS	Ionic crosslinking	Chondrocytes and BMMSCs (bovine)	3 weeks with TGF-β3	–
301	Biological response of ZC to biomimetic matrix molecules	Biomimetic matrix molecules	PEGDA, PEG-COL I, PEG-HA, PEG-CS	UV photocrosslinking	Chondrocytes (bovine)	3 weeks	–
302 303	Differentiation of BMMSCs into ZC on homogeneous or trilayered constructs with biomimetic matrix molecules	Biomimetic matrix molecules	PEGDA, PEG-CS-MMP-pep, PEG-CS, PEG-HA, CS, HA	UV photocrosslinking	BMMSCs (mouse)	6 weeks with TGF-β1	–
304	Differentiation of BMMSCs into ZC on trilayered construct with biomimetic matrix molecules	Biomimetic matrix molecules	PEGDA, MMP-pep, CS-MA, HA-MA	UV photocrosslinking	BMMSCs (human)	3 weeks with TGF-β3	–
305	Effect of matrix molecule gradient of trilayered constructs on chondrocyte fate	Matrix molecules gradient	GEL-MA, HA-MA	Stereolithography	Chondrocytes (porcine)	2 weeks with TGF-β3	–
307	Promoting endogenous AC regeneration with cECM-derived constructs and a bone-fixation device	SZ-like fibre alignment in the surface and cECM	cECM	Freeze-drying and annealing and dehydrothermal crosslinking	BMMSCs (human)	4 weeks with TGF-β3	6 months chondral (goat)

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
306	Fabrication of bilayered zonal constructs with enhanced lubrification and compressive properties	HA's top layer for improved lubrification and cECM	HA, cECM particles	Extrusion-based printing, TIC	Chondrocytes (bovine)	*In vitro:* 2 weeks; *Explant:* 1 week	–
308	Compositional and cellular gradients to regenerate full-thickness chondral defect using microfluidic	Cellular and matrix molecules gradient	ALG, GEL-MA, CS-MA, HA-MA, TCP	Co-axial extrusion-based printing, UV photocrosslinking	Chondrocytes and BMMSCs (human)	3 weeks with TGF-β3	12 weeks osteochondral (rat)
			Zone-specific growth factors				
320	Effect of growth factors on ZC	ZC	–	–	Chondrocytes (goat)	3 weeks with TGF-β1, IGF-I and bFGF	–
321	Effect of growth factors on ZC biomechanics and cytoskeleton	ZC	–	–		18 hours with TGF-β1 and IGF-I	–
322	Effect of growth factors on differentiation and maturation of encapsulated BMMSCs	Sequential addition of growth factors	Fetal or adult cECM-MA	UV photocrosslinking	BMMSCs (human)	3 weeks with TGF-β1, BMP-7, IHH and IGF-1	–
323	Induction of spatiotemporal gradients in self-assembled BMMSCs constructs	Spatiotemporal growth factors induction	COL I-coated filter inserts	Solvent-casting	BMMSCs (human)	10 weeks with TGF-β3, β-GP and thyroxine	4 weeks subcutaneous (mouse)
325	Fabrication of trilayered zonal constructs with encapsulated zonal-specific growth factors	Zonal-specific growth factors	PEGDA, PEO, mECM, TGF-β1, BMP-7, IGF-1	EHD printing and UV photocrosslinking	BMMSCs (rabbit)	3 weeks	24 weeks chondral (rabbit)

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
			Stiffness				
326	Effect of different stiffnesses of bilayered construct on encapsulated chondrocytes or ZC	Depth-dependent stiffness	AGA	TIC	Chondrocytes (bovine)	4 weeks	–
327						6 weeks with TGF-β3	
328	Fabrication of bilayered constructs mimicking zonal mechanical and lubrication properties	Depth-dependent stiffness and GO incorporation on SZ	PEGDA, GG, GO	UV photocrosslinking and ionic crosslinking	Osteoarthritic chondrocytes (human)	6 days treatment with hydrogels' degradation product	–
331	Fabrication of bi-layered hydrogel construct with stiffness gradient	Stiffness gradient	PEG, PEGDA, silk fibre	UV photocrosslinking	BMMSCs (human)	3 weeks with TGF-β3	–
332	Fabrication of 3D woven textiles with stiffness gradients infused with a hybrid hydrogel		PVDF, PAAm-ALG	Ionic and MBAA-induced crosslinking	–	–	–
334	Effect of gradient stiffness on encapsulated chondrocytes and BMMSCs behaviour		PEG, CS	UV photocrosslinking	Chondrocytes (bovine) and BMMSCs (human)	3 weeks with TGF-β3 and bFGF	–
335			PEG-SH, PEG-NB, CS-MA	UV photocrosslinking	Chondrocytes (bovine) and BMMSCs (human)	3 weeks with TGF-β3 and 20 % O_2	6 weeks subcutaneous (mouse)

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
333	Mimicking AC's mechanical behaviour	Posterior native-like DZ chondrocyte arrangement	Polyprotein	Chain entanglement and denatured crosslinking	MC3T3-E1 cells	10 days with β-glycerophosphate	12 weeks osteochondral (rabbit)
			Topographic cues				
338	Mimicking the SZ fibre alignment and nanotopography	SZ fibre alignment and nanotopography	PCL	Electrospinning	BMMSCs (human)	5 weeks with TGF-β1	–
339	Effect of fibre orientation on the friction and damage of SZ	SZ frictional and mechanical properties			Chondrocytes (bovine)	4 weeks with TGF-β3	–
340	Fabrication of SZ-like constructs with improved bio-inductivity and porosities	Biomimetic matrix molecule and SZ-like nanotopography	PCL, sacrificial PEG, GEL	Electrospinning	ACPCs (bovine)	2 weeks	–
341	Effect of zonal fibre size and orientation on the mechanical properties of the trilayered constructs and their ability to support *in vitro* cartilage formation	Zone-specific fibre size and orientation	PCL	Electrospinning	Chondrocytes (bovine)	5 weeks with TGF-β3	–
342	Fabrication of trilayered constructs with zone-specific topographic alignments	Zone-specific fibre and channel alignments	PLA, HA	Electrospinning and BDDE-induced crosslinking	Chondrocytes (bovine)	2 weeks	–
343	Fabrication of bi and trilayered constructs with zone-specific fibre alignments	Zone-specific fibre alignments	PCL	Electrospinning	–	–	–
345			PCL, sacrificial PEG		ACPCs (bovine)	3 weeks	
344			PCL, sacrificial PEG, GEL				

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
f346	Fabrication of a trilayered construct with zonal-fibre alignment	Zonal fibre alignment	PLLA, fibrinogen	Electrospinning, thrombin-induced crosslinking	C28/I2 immortalized chondrocyte line (human)	1 week	–
347	Fabrication of a woven porous scaffold from electrocompacted COL threads to accommodate cell-pellets	COL fibril alignment mimicked by weaving pattern	Col	Electrochemical compaction, thread weaving and genipin-induced crosslinking	BMMSCs (human)	3 days for pellet formation and 4 weeks with TGF-β3 and bFGF	–
349	Effect of extrusion-induced polymer molecule alignment on BMMSCs differentiation	Micro and nanoscaled polymer molecule alignment	PLGA	Extrusion-based printing	BMMSCs (human)	1 day	7 days subcutaneous (rat)
350	Effect of the extrusion-induced COL fibril alignment on BMMSCs migration from pellets	COL fibril extrusion-induced alignment	THA, COL I	Enzymatic cross-linking and extrusion-based printing	BMMSCs (human)	3 weeks with TGF-β1	–
351	Fabrication of trilayered constructs with zone-specific fibre alignments	Zone-specific fibre alignments	PEOT/PBT	EHD inkjet printing	BMMSCs (human)	3 weeks with TGF-β3	–
352	Fabrication of bilayered constructs with one layer magnetically aligned	COL fibril magnetic alignment	AGA, COL I, streptavidin-coated iron nanoparticles	Inkjet-based printing	Chondrocytes (human)	3 weeks with TGF-β1	–
353	COL I fibrils alignment gradient triggered by matrices' confined compression	Fibrils alignment gradient through densification	COL I	TIC	Chondrocytes (bovine)	1 week	–

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
355	Generation of 3D patterned constructs to control cellular orientation for SZ and MZ	Alignment patterns	PEGDMA, CH-g-PNIPAAm	Photolithography and TIC	BMMSCs (murine)	4 weeks with TGF-β1	–
356	Influence of SZ-based chondroinductive nanotopography on AMSCs	SZ nanotopographic cues	Silk, kartogenin	Solvent casting and colloidal lithography	AMSCs (human)	2 weeks with chondrogenic media	–
357	Effect of the surface micro and nanotopographic on chondrocyte behaviour	Zonal topographic cues	PMDS, CNTs	Curing in patterned molds and spraying	Chondrocytes (bovine)	2 weeks	–
	Pore size, alignment and/or porosity						
362	Effect of depth-dependent pore sizes on cell behaviour	Pore size gradient (200 to 1650 µm)	PEGT/PBT	FDM	Chondrocytes (bovine)	3 weeks in spinner flask	–
363		Pore size gradient (500 to 1100 µm)			BMMSCs (human)	5 weeks with TGF-β3	
364	Effect of pore size on chondrogenic differentiation of BMMSCs and underlying mechanisms	Pore size gradient (150 to 750 µm)	PCL, GEL, Fibrinogen, HA	Extrusion-based printing and thrombin-induced crosslinking	BMMSCs (rabbit)	6 weeks	24 weeks chondral (rabbit)
365	Effect of pore size and geometry of trilayered constructs on differentiated BMMSCs alignment and biosynthetic activity	Pore size (300 to 700 µm) and geometry (rectangular, triangular and rhombic) gradient	PCL, ALG-MA	FDM and blue light photocrosslinking	BMMSCs (rat)	3 weeks with TGF-β3	–
366	Design of computational model to predict the most biomechanically-suited soft network composite	Pore size gradient (800 to 200 µm)	PCL, HAp nanoparticles, GEL-MA	MEW, visible light photocrosslinking	–	–	–

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
367	Effect of GEL source and photoinitiator type on chondrocyte redifferentiation	Pore size gradient (800 to 200 µm)	PCL, GEL-MA	MEW and UV or visible light photocrosslinking	Chondrocytes (human)	4 weeks with TGF-β3	–
368	Fabrication of bilayered zonal constructs with SZ and DZ-like pore sizes	Depth-dependent pore sizes (50 and 800 µm)	PCL, GEL-MA	MEW and UV photocrosslinking	Chondrocytes (equine)	4 weeks with TGF-β1 or DC loading (0-20 % strain at 1 Hz)	–
369	Effect of vertical pore alignment on cell behaviour and zonal tissue formation	DZ vertical alignment	PLGA	TIPS and freeze-drying	Chondrocytes (porcine)	12 weeks	12 weeks subcutaneous (mouse)
370			PLGA, cECM	TIPS and freeze-drying	BMMSCs (rabbit)	1 week	–
371				TIPS, freeze-drying, EDC/NHS crosslinking	BMMSCs (rabbit)	3 days	24 weeks osteochondral (rabbit)
372			cECM	TIPS, freeze-drying, genipin-induced crosslinking	BMMSCs (rabbit)	13 days with TGF-β3	4 weeks subcutaneous (mouse)
373						1 week with TGF-β3	24 weeks chondral (rabbit)
374			PVA, carrageenan	TIPS, freeze-drying	ATDC5 cells (mouse)	4 weeks	4 weeks subcutaneous (rat)
375	Effect of aligned pore architecture on chondrogenic differentiation of FPMSCs	Aligned pore alignment (SZ and DZ)	GEL	Directional freeze-drying and EDC/NHS crosslinking	FPMSCs (goat)	4 weeks with TGF-β1	–

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
376	Effect of pore orientation on *in vivo* tissue formation	Constructs with zonal-like pore alignments	Silk fibroin	Directional freeze-drying and methanol-induced crosslinking	BMMSCs (human)	1 week	12 weeks osteochondral (rabbit)
377	Fabrication of TGF-β3-loaded constructs with aligned pore microstructure reinforced with PLCL framework	Aligned pores	PLCL, ALG, ALG sulphate, TGF-β3	Extrusion-induced printing hydrazide or ionic crosslinking and directional freeze-drying	BMMSCs (porcine)	2 weeks with TGF-β3 and 5 % O_2, followed by 10 days with continuous stirring	–
378	Influence of pore orientation on the mechanical properties, cell distribution of zonal constructs and zonal tissue formation	Pore alignment mimicking COL organization	CH, GEL	TIPS, freeze-drying and genipin-induced crosslinking	Chondrocytes (goat)	2 weeks	–
379					BMSCs (rabbit)	4 weeks	4 months osteochondral (rabbit)
380			COL, HA-MA	Glutaraldehyde-induced crosslinking, freezing and photocrosslinking	Chondrocytes (human)	1 day	–
381	Fabrication of trilayered constructs with zonal pore alignment	Pore alignment mimicking COL organization	PLGA, cECM	LT-FDM, TIPS, freeze-drying and genipin-induced crosslinking	–	–	–

MMP-pep, matrix metalloproteinase-sensitive peptides; mECM, meniscus-derived extracellular matrix; PET, polyethylene terephthalate; PAAm-ALG, polyacrylamide-ALG hybrid; PEG-SH, PEG-dithiol; PEG-NB, PEG-norbornene; PEOT/PBT, poly(ethylene oxide terephthalate)/poly-(butylene terephthalate); BDDE, 1,4-butanediol diglycidyl ether; EDAC/NHS, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/ N-hydroxysuccinimide; LT-FDM, low temperature fused deposition modelling; PVA-BA, poly(vinyl alcohol)-boric acid; MBAA, N'-methylene bis-acrylamide.

Table S3. Strategies exploring the combination of multiple cues in zonal constructs and their influence on chondrocytes and MSCs behaviour.

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
			Cell density and zone-specific factors and stiffness and/or topographic cues				
382	Effect of biomimetic cell densities, growth factors culture, stiffnesses and nanofiber alignment on chondrogenic BMMSCs behaviour	Zonal cell densities (60 to 15×10^6), growth factors, stiffnesses and nanofiber topography and alignments	SPELA, Ac-GRGD, HAp, PLA nanofibres	UV photocrosslinking and electrospinning	BMMSCs (human)	3 weeks with TGF-β1 and BMP-7 for SZ, TGF-β1 and IGF-1 for MZ, TGF- β1 and β-GP for CCZ	–
383							–
			Matrix molecules and stiffness				
384	Combinatorial effect of ECM matrix molecules and stiffnesses on chondrogenic AMSCs behaviour	Biomimetic matrix molecules and stiffnesses	PEG-DMA, CS-MA, HA-MA, HS-MA, GEL-MA	UV photocrosslinking	AMSCs (human)	3 weeks with TGF-β3 and bFGF	–
385	Fabrication of dual-gradient zonal constructs	Biochemical and stiffness gradients	PEG, CS	UV photocrosslinking	Chondrocytes (bovine)	3 weeks	–
			Matrix molecules and topographic cues				
386	Influence of mAGC on the constructs' proteolytic degradation and chondrocytes' gene expression modulation	Biomimetic matrix molecule and COL fibrils magnetic alignment	COL I, CS, HA	TIC	Chondrocytes (bovine)	8 days with IL-1β	–
387	Fabrication of trilayered constructs with biomimetic chemical and topographic alignment	Biomimetic matrix molecules and μRB topographic alignment	GEL-MA, CS-MA	μRB wet-spinning, glutaraldehyde and dithiothreitol crosslinking, UV photocrosslinking	BMMSCS (human)	3 weeks with TGF-β3	–

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
388	Engineering fibre-reinforced gradient hydrogels to facilitate integration with bone	Matrix molecules and fibrous topographic cues	PCL, CS, bioactive glass, AGA, GEL	Sol-gel, electrospinning and TIC	Chondrocytes (goat)	3 weeks	–
389	Biological effect of the CS-functionalized aligned fibrous network on chondrocyte and BMMSCs behaviour *in vitro* and *in vivo*	Biomimetic matrix molecule and SZ-like fibre alignment	PLLA, CS	Electrospinning and PDA-induced coating	Chondrocytes and BMMSCs (rabbit)	7 days with TGF-β3	16 weeks chondral (rabbit)
Matrix molecules and pore size or alignment							
390	Fabrication of multilayered zonal constructs with biochemical and pore sizes gradients	Matrix molecules and pore size gradients	CH, PCL, COL II	TIPS, freeze-drying, photothermal heating and EDAC/NHS crosslinking	Chondrocytes (rabbit)	3 weeks	–
361			CH, PCL, COL II, CS				
391	Fabrication of trilayered constructs with zone-specific biochemical composition and pore orientations	Matrix molecules ratio gradient and pore alignments	COL I, HA, HAp	TIPs, freeze-drying and EDAC/NHS crosslinking	–	–	–
392					Chondrocytes and BMMSCS (mouse)	3 weeks with TGF-β1 and 5 % O_2	8 weeks subcutaneous (mouse)
393	Fabrication of trilayered constructs with biomimetic chemical properties and pore alignments	Matrix molecules gradient and pore alignments	PLA, sCNCs, pCNCs, HAp	TIPS	Fetal chondrocytes (human)	4 weeks	–
Matrix molecules and zone-specific growth factors and pore size or alignment							
394	Fabrication of bilayer biomimetic construct with depth-dependent biochemical and morphological properties	Matrix molecules, growth factors and pore size gradient	pCOL I, CS, SF, HA, PLGA microspheres with kartogenin, TPHNs with TGF-β1	TIPS, freeze-drying	BMMSCS (rat)	1 week	16 weeks osteochondral OA model (rabbit)

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
	Zone-specific growth factors and pore size or alignment						
395	Fabrication of dual-factor releasing and gradient-structured BMMSCS-laden multilayered constructs	Zone-specific growth factors and pore size gradients	PCL, GEL, Fibrinogen, HA, PLGA microspheres with TGF-β3 and BMP-4	Extrusion-based printing and thrombin-induced crosslinking	BMMSCS (rabbit)	12 weeks	12 weeks subcutaneous (mouse) and 24 weeks osteochondral (rabbit)
396	Effect of zone-specific growth factors and pore size and geometry of trilayered constructs on the differentiation and tissue formation of encapsulated BMMSCS	Zone-specific growth factors and pore size and geometry gradients	PCEC, GEL-MA and PLGA microspheres with TGF-β3, BMP-7 and BMP-2	MEW, FDM, inkjet-based printing and UV photocrosslinking		2 weeks	24 weeks osteochondral (rabbit)
397	Effect of zone-specific growth factors and pore size gradient of trilayered constructs on the differentiation and tissue formation of BMMSCS	Zone-specific growth factors and pore size gradients	PCL, HAp, PLGA microspheres with TGF-β3, BMP-7 and IGF-1	MEW and inkjet-based printing		3 weeks	12 weeks osteochondral (rabbit)
	Stiffness and topographic cues						
398	Effect of the substate stiffness and topography on the chondrogenic differentiation of BMMSCs	Stiffness and topography	PCL, PLA, PGA	Nanoimprint lithography	BMMSCs (human)	6 weeks with TGF-β3	–
399	Effect of genipin concentration on the mechanical properties of zonal electrospun constructs	Topographic cues and zonal stiffness	CH, PEO	Electrospinning and genipin-induced crosslinking	Chondrocytes (human)	3 weeks with TGF-β3	–
400	Fabrication of bio-inspired cellulose reinforced anisotropic composite hydrogel with zonal properties	Topographic cues and zonal stiffness	PEGDA, cellulose fabric, nanofibers and wood	UV photocrosslinking	BMMSCs (–)	2 weeks with TGF-β3	–

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*In vitro*	*In vivo*
			Topographic cues and pore size or alignment				
401	Fabrication of bilayered zonal constructs by electrospinning of aligned polymeric layer onto particulate-templated scaffolds	Zone-specific fibre alignment and pore size	PCL	Electrospinning and solvent-casting and particulate leaching	Chondrocyte (bovine)	5 weeks with TGF-β3	
402	Fabrication of five-layered zonal constructs with combinatorial morphologic cues	Zone-specific fibre alignment and pore orientation and stiffness gradient	PCL and sacrificial GEL microspheres	Electrospinning, porogen leaching, TIPS and freeze-drying	Chondrocytes (bovine and porcine)	4 weeks with TGF-β3	6 months osteochondral (pig)
403	Fabrication of trilayered zonal constructs with zone-specific fibre alignment and pore orientation by combining cryoprinting with electrospinning	Zone-specific fibre alignment and pore orientation	PCL	Extrusion-based printing, TIPS, freeze-drying and electrospinning	BMMSCs (rat)	4 weeks with TGF-β3	–
404					Chondrocytes (human)	5 weeks with TGF-β3	–
405	Fabrication of four-layered zonal constructs by combining electrospinning with freeze-drying	Zone-specific fibre alignment and pore size and orientation	GEL, bMP, cMP	Electrospinning, TIC, freeze-drying and glutaraldehyde crosslinking	BMMSCs (human)	10 days	–

Ac-GRGD, acrylamide-terminated glycine-arginine-glycine aspartic acid peptide; IL-1β, interleukin 1 beta; bMP, bone microparticles; cMP, cartilage micropraticles.

Table S4. Exploring mechanical and biochemical stimuli and their influence on chondrocytes and BMMSCs behaviour

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*Stimulus*	*In vitro*
				Mechanical stimulus			
410	Influence of compressive strain on the response of ZC	ZC and DC stimulus	AGA	TIC	Chondrocytes (bovine)	DC	3 days with DC loading (15 % strain at 0.3, 1, 3 Hz)
411	Influence of short- and long-term DC on zonal osteoarthritic chondrocytes	ZC and DC stimulus	ALG	Ionic crosslinking	Osteoarthritic chondrocytes (human)	DC	2 weeks pre-culture and 2 weeks with TGF- β1 and DC loading (5, 15 and 50 % strain at 1 Hz) during 1, 3 or 12 hours per day
412	Influence of pre-culture and DC on zonal osteoarthritic chondrocytes expression						2 weeks pre-culture and 2 weeks with TGF- β1 and DC loading (50 % strain at 1 Hz) during 3 hours per day
413	Analysis of compressive strain fields of cell-laden hydrogel constructs subjected to horizontal DC loading	Zone-specific chondrocyte deformation patterns	COL I, AGA	TIC	Chondrocytes (mouse)	DC	1 week after which DC was applied (5 and 15 % strain at 20 µm/sec)
414	Effect of dynamic loading on chondrocyte-laden zonal constructs with stiffness gradient	Stiffness gradient and DC stimulus	AGA	TIC	Chondrocytes (bovine)	DC	4 weeks with DC loading (40 µm amplitude at 1 Hz) for 3 hours per day and 5 days per week
415	Computational model to predict collagen network architecture upon exposure to sliding indentation with lateral compression	Depth-dependent COL fibril alignment	–	–	–	Sliding indentation	–

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*Stimulus*	*In vitro*
416	Evaluation of sliding indentation effect on the depth-dependent ECM deposition in engineered constructs	Biomimetic depth-dependent mechanical stimulus	AGA	TIC	Chondrocytes (bovine)	Sliding indentation	4 weeks with TGF-β3, 20 % O_2 and sliding indentation (10 % strain at 1 Hz) 4 hours per day and 5 days per week
417	Triggering SZ-like layer formation with moving point contact stimulation	SZ-like layer through moving point contact stimulus					8 days with 5 days pre-culture and 3 days with dynamic moving point contact stimulus (3 mm amplitude at 0.5, 1 and 2 Hz) between 5 and 60 minutes
419	Effect of DC with or without articular surface motion on ZCs' gene expression	SZ-like surface motion and DC	PU, fibrin	TIPS, freeze-drying and thrombin-induced crosslinking	Chondrocytes (bovine)	DC and DS	10 days with 5 days of DC (10-20 % strain at 0.1 Hz) and ball oscillation (± 30 ° at 0.1 Hz) for 1 hour, twice a day
420	Effect of surface motion on the response of ZC						8 days with DC and shear loading (10 % strain at 0.1 Hz and ball oscillation of 25 ° at 0.1 Hz) twice a day
421	Modulation of chondrocyte-laden constructs' stiffness and friction using sliding surface motion						4 weeks with 1 week pre-culture and 3 weeks DC (10-20 % strain at 1 Hz) and ball oscillation (± 25 ° at 1 Hz) 1 hour per day, 6 days per week
422	Effect of joint-mimicking loading conditions on zonal ECM synthesis and cartilage explant maturation	Joint-mimicking mechanical stimulus	Cartilage explants	–	Chondrocytes (porcine)	DC and DS	4 weeks with DC and shear loading (0.25 to 0.3 MPa) for 1 hour daily

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*Stimulus*	*In vitro*
423	Influence of surface layer's friction on the chondrocyte phenotypic expression upon dynamic shear loading	Bilayered construct with cell-free top layer with SZ-like shear loading	PEGDA, ALG-MA	UV photocrosslinking	Chondrocytes (bovine)	Intermittent DC and DS	32 days with TGF-β3 and 3 weeks pre-culture followed by 11 days of intermittent dynamic shear loading (1 mm amplitude at 1 Hz with an offset compression of 15 %) for 1 hour daily
424	Cartilage-on-chip system to assess the effect of different mechanical stimuli on chondrocyte phenotype and ECM production	Zonal cell deformation and ECM synthesis induced by DC and multi-directional mechanical stimuli	AGA	TIC	Chondrocytes (human)	DC and DS	15 days with TGF-β3 and DC loading (300 mbar at 1 Hz) and dynamic shear loading (−350 mbar at 0.33 Hz) 1 hour daily after day 1
426	Effect of hydrodynamic stimulation in rotating bioreactor system on the evolution of surface zone features in engineered constructs	Biomimetic oscillatory shear stress on the surface of the construct	AGA	TIC	Chondrocytes (porcine)	Fluid-induced DS	2 weeks with 5 % O_2 and periodical agitation in a half-square wave (0–25 rpm) for 40 min (1 min on, 1 min off), followed by steady rotational speed (25 rpm) for the rest of the day, daily
427	Effect of perfused conditions on chondrocyte-laden hydrogels	SZ-like shear stress by perfusion	ALG	Ionic crosslinking	Chondrocytes (bovine)	Fluid-induced DS	2 weeks with perfusion (3 mL
344	Effect of depth-dependent fibre alignment of zonal constructs and DC or perfusion stimuli on ACPCs behaviour	Depth-dependent fibre alignment and DC stimulus and perfusion	PCL, GEL, COL I, GO	Electrospinning and freeze-drying	ACPCs (bovine)	DC or fluid-induced DS	3 weeks with 5 days pre-culture and 16 days with DC loading (10 % strain at 0.5 Hz) for 2 hours daily or continuous perfusion (0.75 mL/min per scaffold)

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*Stimulus*	*In vitro*
428	Influence of oscillatory tensile strain on the response of ZC	ZC and dynamic tensile loading	Fibrin	Thrombin-induced crosslinking	Chondrocytes (rabbit)	Intermittent DT	15 days, 3 of those with oscillatory tensile loading (10 % strain at 1 Hz) for 12 hours
429	Effect of DT loading on the expression of SZP	Tensile stimulus for SZ chondrocyte phenotype	Laminin-coated surfaces		Chondrocytes (porcine)	DT	48 hours with DT loading (7 and 21 % strain at 0.5 Hz) for 1 second, with another one of rest
430	Effect of excessive tensile load on gene expression and protein synthesis of ZC	ZC	COL II-coated surface	Solvent casting	Chondrocytes (rabbit)	DT	24 hours with DT loading (17 kPa at 30 cycles per minute) for 1 second, with another one of rest
431	Sensitivity of ZC to pure hydrostatic pressure	ZC	COL I-coated surface		Chondrocytes (bovine)	HP	5 days of constant hydrostatic pressure (0.5 MPa)
				Biochemical stimulus			
434	Reproducing native physiological O_2 environment to reduce culture time of chondrocyte sheets	Physiological O_2 concentrations	–	–	Chondrocytes and synoviocytes (human)	O_2	3 weeks with TGF-β1 and 2, 5 or 21 % O_2
435	Effect of O_2 tension on ZC	ZC and physiological O_2 concentrations	–	–	Chondrocytes (human)	O_2	4 weeks with TGF-β3 and 5 or 20 % O_2
436	Evaluation of SPZ expression under different O_2 tensions	ZC and physiological O_2 concentrations	Cartilage explants	–	ATDC5 cells (mouse) and chondrocytes (bovine)	O_2	4 days with TGF-β1 and 1, 5 or 21 % O_2

Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*Stimulus*	***In vitro***
Mimicking the nutrients gradients for the generation of zonal constructs	Glucose concentration gradient	AGA	TIC	Chondrocytes (bovine)	Glucose	1 week with 2.5 and 20 % O_2, in a bioreactor designed to create glucose gradients (0, 5 and 20 mM)
Prediction of ZCs' O_2 consumption based on changes in the nutritional environment	ZC and O_2 consumption behaviour	–	–	Chondrocytes (bovine)	Glucose and O_2	12 hours with 0-22 mM glucose
Measurement of zonal OP in cartilage explants	ZC and physiological OP	Cartilage explants	–	Chondrocytes (bovine)	OP	30 min with low and high Osm (200 to 500 mOsm)
Effect of OP on ZCs' ECM synthesis	ZC and physiological OP	–	–	Chondrocytes (bovine)	OP	10 days with low and high Osm (310 and 450 ± 10 mOsm)
			Combination of mechanical and biochemical stimuli			
Assessment of chondrocyte response to both DC loading and gradient nutrient supply	DC stimulus and glucose concentration gradient	Cartilage explants	–	Chondrocytes (bovine)	DC and glucose	2 weeks with 0-25 mM glucose and DC loading (2.5 % strain at 0.33 Hz) for 1 hour three times a day
Effect of biomimetic depth-dependent mechanical stimulus and oxygen gradient on the depth-dependent ECM deposition in cell-laden constructs	DC stimulus and O_2 concentration gradient	AGA	TIC	BMMSCs (porcine)	DC and O_2	6 weeks with TGF-β3 and DC loading (10 % strain at 1 Hz) 4 hours per day and 5 days per week
Effect of biomimetic depth-dependent mechanical stimulus and oxygen gradient on the depth-dependent ECM				FPMSCs (porcine)		6 weeks with TGF-β3 and DC (10 % strain at 1 Hz) 2 hours per day and 5 days per week

	Study goal	Zonal features	Matrix	Fabrication	Cells (origin)	*Stimulus*	*In vitro*
	deposition in cell-laden constructs						
447	Effect of serial passaging on chondrocytes’ PRG4 and COL 2 expression under different O_2 tensions	ZC and physiological O_2 concentrations	ALG beads	Ionic crosslinking	Chondrocytes (bovine)	DT and O_2	4 days with TGF-β1 and 1 or 21 % O_2 and DT loading (5 % strain at 1 Hz) for 2 hours per day
			COL 1-coated PDMS	Solvent casting			

Osm, osmolarity; PVA-MA, poly(vinyl alcohol) methacryloyl.