

# Chondrobags: A high throughput alginate-fibronectin micromass platform for *in vitro* human cartilage formation

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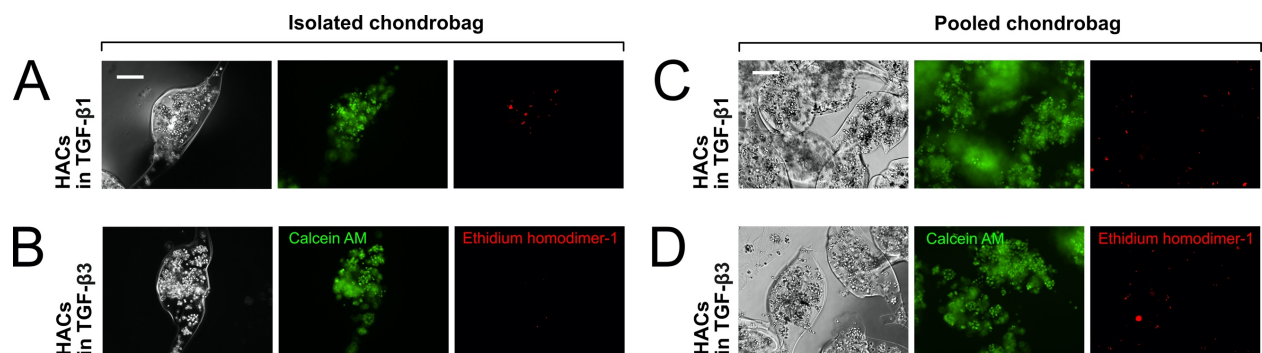
## Supplementary data

**Table S1.** Primer sequences for genes examined and the corresponding amplicon size.

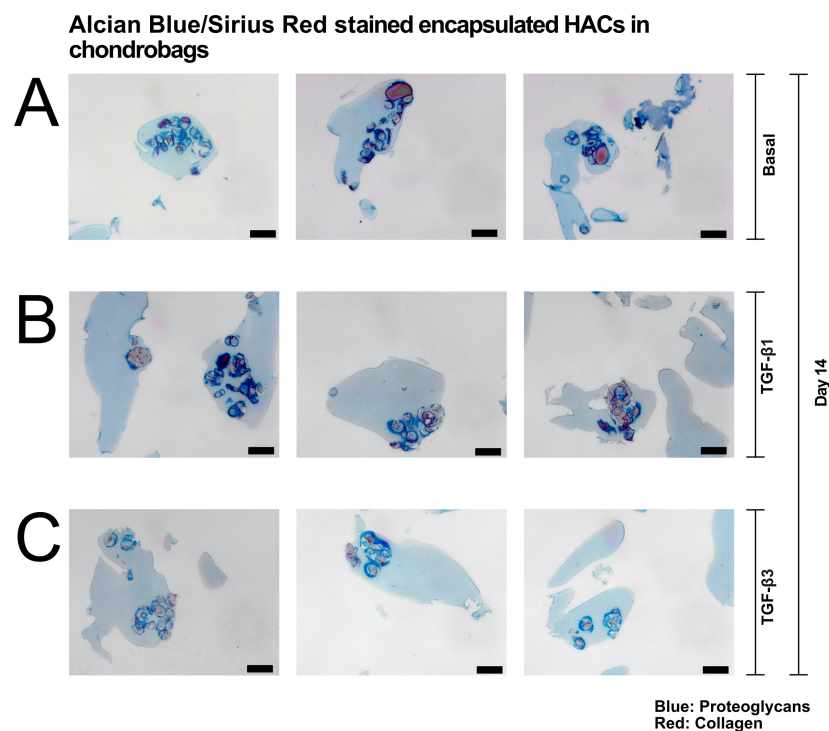
	Gene	Primers sequences	Amplicon size
<b>Housekeeping</b>	Human <i>ACTB</i>	F: 5' ggcatcctcacctgaagta 3'	82 bp
		R: 5' aggtgtggtgccagatttc 3'	
	Human <i>GAPDH</i>	F: 5' ccagggtggtctcctctgacttc 3'	108 bp
		F: 5' tcataccaggaaatgagcttgaca 3'	
<b>Chondrogenic</b>	Human <i>SOX9</i>	F: 5' ccctcaacctccacacta 3'	74 bp
		R: 5' tgggtggtcggtgtagtcgta 3'	
	Human <i>COL2A1</i>	F: 5' cctgggtcccctggtcttg 3'	58 bp
		R: 5' catcaaatcctccagccatc 3'	
	Human <i>ACAN</i>	F: 5' gacggcttcaccagtgt 3'	90 bp
		R: 5' gtctccatagcagccttc 3'	
<b>Hypertrophy</b>	Human <i>COL10A1</i>	F: 5' cccactaccaacaccaaga 3'	95 bp
		R: 5' gtggaccaggagtacctgc 3'	
	Human <i>MMP13</i>	F: 5' ttaaggagcatggcgacttct 3'	71 bp
		R: 5' cccaggaggaaaagcatgag 3'	
<b>Proliferation</b>	Human <i>PCNA</i>	F: 5' aggtgttgaggcactcaag 3'	69 bp
		R: 5' gggttacaccgctggagc 3'	
	Human <i>MKI67</i>	F: 5' ggtctgttattgatgacctgta 3'	11bp
		R: 5' cagttgacttccttcattctg 3'	
<b>Cell cycle</b>	human <i>CCND1</i>	F: 5' ctaccgcctcacacgctt 3'	130 bp
		R: 5' cttgggtccatgttctgc 3'	
	Human <i>CDK6</i>	F: 5' tttcgtggaagtcagatgttg 3'	85 bp
		R: 5' catctctaggccagtcttctct 3'	
	Human <i>CDKN2A</i>	F: 5' gtggacctggctgaggag 3'	132 bp
		R: 3' cttcaatcggggatgtctg 3'	

**Table S2.** TaqMan® MiRNA Assays used for miRNA expression analysis from Applied Biosystems, Life Technologies.

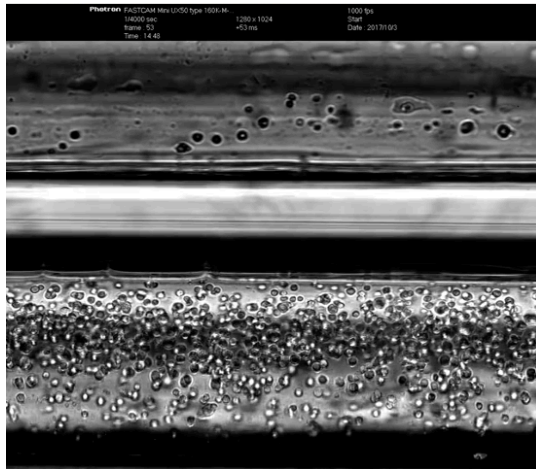
Small Nucleolar RNA, C/D Box44	RNU44	001094
miR-138-5p	Has-miR-138-5p	002284
miR-140-3p	has-miR-140-3p	002234
miR-146b-5p	has-miR-146b-5p	001097



**Figure S1. Isolated and pooled chondrobags.** Microscopy images of isolated alginate-fibronectin chondrobags containing human articular chondrocytes (HACs) cultured in the presence of TGF-β3 (A) and TGF-β1 (B) for up to 28 days. Scale bar: 100 μm. Microscopy images of pooled alginate-fibronectin chondrobags containing Stro-1 enriched skeletal stem cells (SSCs) cultured in the presence of TGF-β3 (C) or TGF-β1 (D) for up to 28 days. The hydrogel was stained with Viability/Cytotoxicity Kit with viable cells in green (Calcein AM) and non-viable cells in red (Ethidium homodimer-1). See figure 2 and 3 for more images.



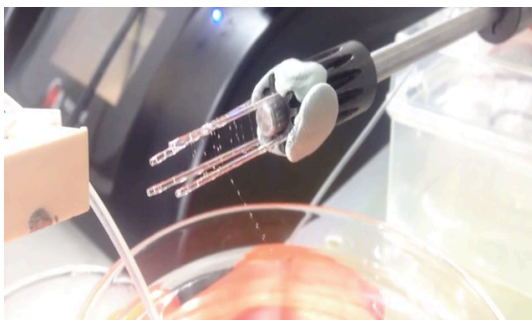
**Figure S2. Stained encapsulated human articular chondrocytes (HACs) in alginate-fibronectin chondrobags.** Alcian Blue/Sirius Red histological sections of chondrocytes-laden chondrobags (5 μm sections). Histological appearance of chondrobags cultured for 14 days in basal media (A), in the presence of TGF-β1 (B) and in the presence of TGF-β3 (C). Scale bar: 50 μm. The cell donor for this set of histological analysis is different from those of Figure 7.



Still from SV1 - HACs encapsulation. Video is submitted



Still from SV2 - HACs encapsulation. Video is submitted



Still from SV3 - Pearl-on-thread printing. Video is submitted

## The table of contents entry

A microfluidic-based development of alginate-fibronectin based microgels that support human skeletal stem cell (SSCs) chondrogenic differentiation and human articular chondrocyte (HACs) phenotype preservation – *chondrobags*. Chondrobags are introduced, *in vitro*, as quantitative and high-throughput 3D culture systems to replace current undefined and low-yield practices of generating micromass, which are currently in use for cartilage studies.

### Microgel

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