

## Electronic Supplementary Information

### **Combining biomimetic collagen/hyaluronan hydrogels with discogenic growth factors promotes mesenchymal stroma cell differentiation into Nucleus Pulposus like cells**

Prince David Okoro<sup>†a</sup>, Antoine Frayssinet<sup>†a</sup>, Stéphanie De Oliveira<sup>a</sup>, Léa Rouquier, Gregor Milkosic, Matteo D'Este, Esther Potier, Christophe Héлары\*

<sup>a</sup> Laboratoire de Chimie de la Matière Condensée de Paris, Sorbonne Université, CNRS, UMR 7574, F-75005, Paris, France.

<sup>c</sup> Université Paris Cité, CNRS, INSERM, B3OA, Paris, France.

<sup>b</sup> AO Research Institute Davos (ARI), Clavadelerstrasse 8, 7270 Davos, Switzerland

\* Corresponding authors: Christophe Héлары

Email address : [christophe.helary@sorbonne-universite.fr](mailto:christophe.helary@sorbonne-universite.fr)

Telephone: +33144276539

Postal address: Sorbonne University - Campus Pierre et Marie Curie, 4 place Jussieu, 75252 Paris Cedex 05, France

<sup>†</sup> These authors have contributed equally to this work

Gel formulation/tube (i.e. 6 replicates)

Collagen	PBS 10x [600ul]	NaOH [240ul]	PBS 1x [2416.8]	✘	✘	Coll. [2743.2] 0.4%	✘
1:2	PBS 10x [600ul]	NaOH [240ul]	PBS 1x [1347]	HRP [30ul]	THA [799.8] 0.8%	Coll. [2743.2] 0.4%	H <sub>2</sub> O <sub>2</sub> [240u]
1:5	PBS 10x [600ul]	NaOH [240ul]	PBS 1x [147]	HRP [30ul]	THA [1999.8] 2%	Coll. [2743.2] 0.4%	H <sub>2</sub> O <sub>2</sub> [240u]
HA	✘	✘	PBS 1x [3730.2]	HRP [30u]	THA [1999.8] 2%	✘	H <sub>2</sub> O <sub>2</sub> [240u]

X 4 time-points!

- 600ul cell suspension/tube at 500k/gel
- 6 replicates/condition in 15ml tubes

ACAN	Forward	TCGAGGACAGCGAGGCC
	Reverse	TCGAGGGTGTAGCGTAGAGA
COL2A1	Forward	CAGACGCTGGTGCTGCT
	Reverse	TCCTGGTTGCCGGACAT
COL1A1	Forward	CCAGTCAGAGTGGCACATCTTGA
	Reverse	GCTCACGATGGTGCCGCTACTA
SOX 9	Forward	AGGTGCTCAAAGGCTACGAC
	Reverse	GTAATCCGGGTGGTCCTTCT
COL 10	Forward	GTGGACCAGGAGTACCTTGC
	Reverse	TGCTGCCACAAATACCCTTT
IBSP	Forward	AACGAACAAGGCATAAACGGCACCA
	Reverse	CTTGCCCTGCCTTCCGGTCT
KRT18	Forward	AAAGGCCTACAAGCCCAGAT
	Reverse	CACTGTGGTGCTCTCCTCAA
GAPDH	Forward	TTGATTTTGGAGGGATCTCG
	Reverse	GAGTCAACGGATTGGTCGT

Supplementary information N°2: List of primers for Q-PCR

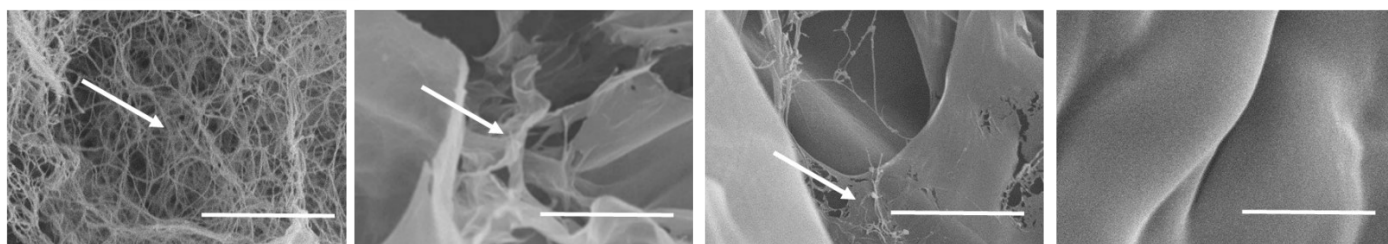
Pure Collagen

Collagen/THA  
1:2 Ratio

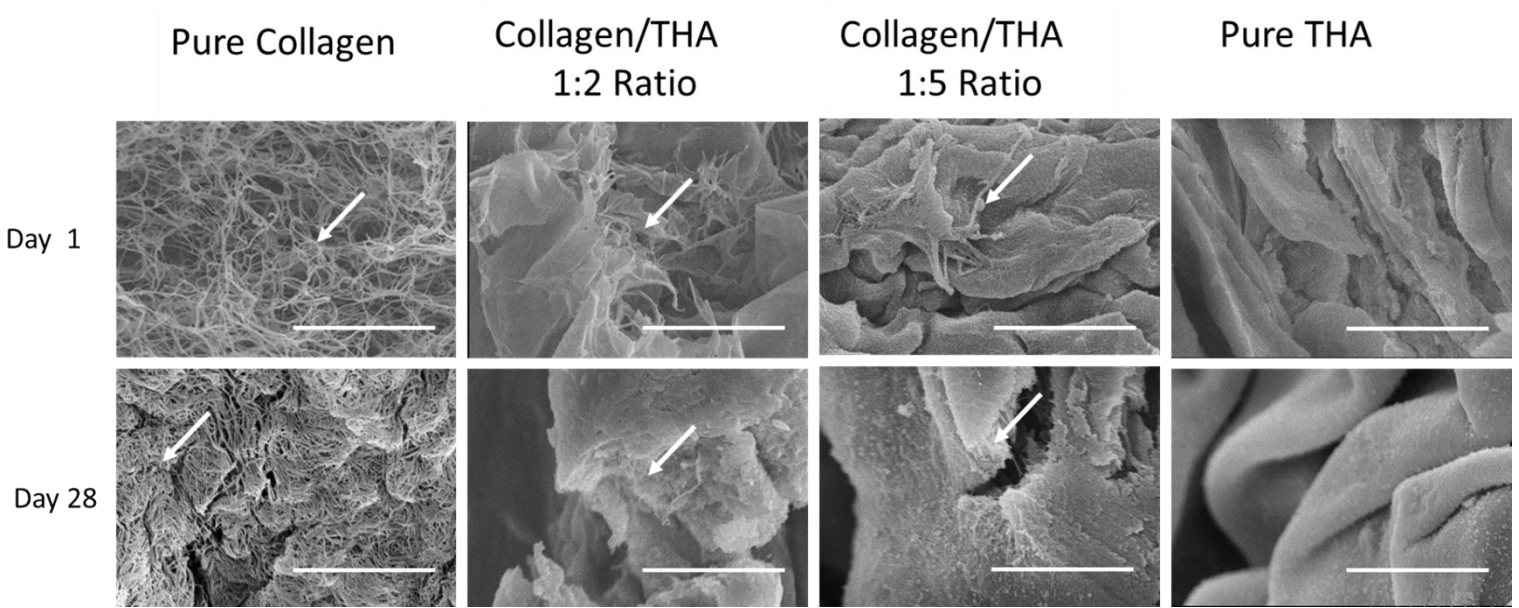
Collagen/THA  
1:5 Ratio

Pure THA

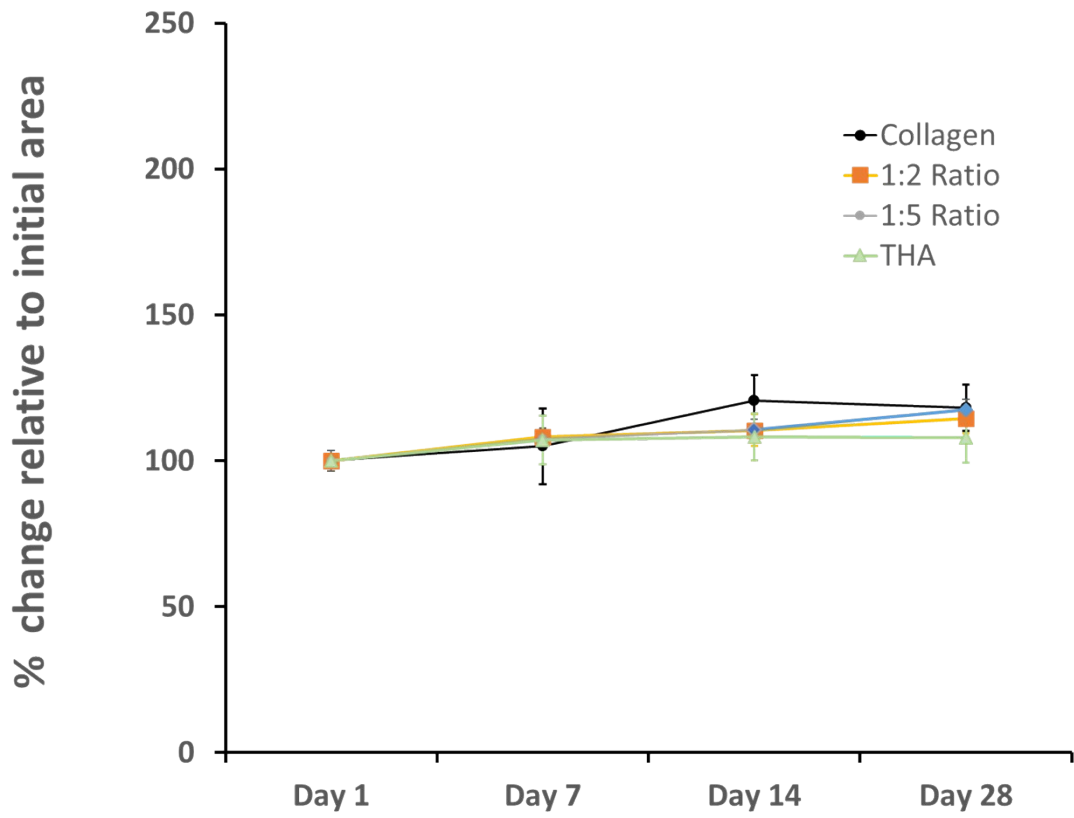
Day 28



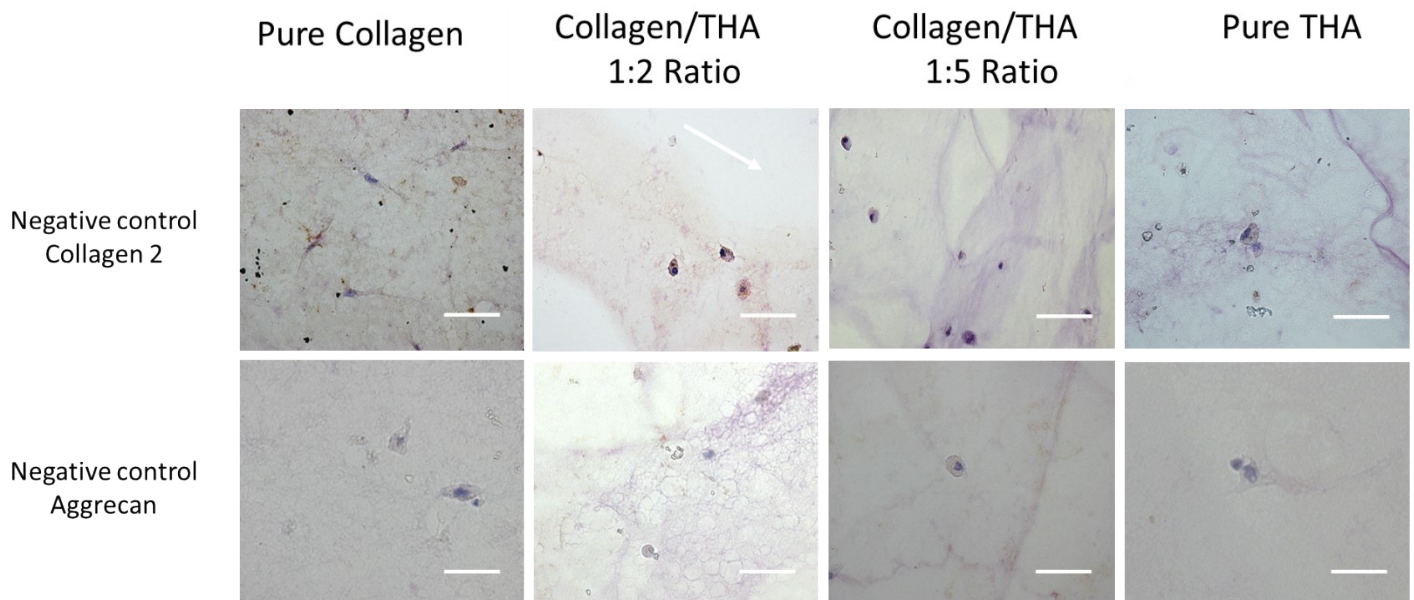
Supplementary Information N°3: Ultrastructure of acellular collagen/THA composite hydrogels observed by scanning electron microscopy after 1 28 days of culture in differentiation medium. Hydrogel surface observed after hydrogel fracture. White arrows show collagen fibrils. Scale Bar: 5  $\mu$ m.



Supplementary Information N°4: Ultrastructure of cellularized collagen/THA composite hydrogels observed by scanning electron microscopy after 1 day (top) or 28 days (bottom) of culture in differentiation medium. Hydrogel surface observed after hydrogel fracture. White arrows show collagen fibrils. Scale Bar: 5  $\mu$ m.



Supplementary Information N°5: Changes in surface area of acellular composite hydrogels cultured in differentiation medium over 28 days. Results expressed as a percentage of the initial area +/- SD (n=6).



Supplementary Information N°6: Negative controls carried out on histological sections by omitting the primary antibody (anti collagen 2 or anti-aggrecan) to show the antibody specificity. Sections were counterstained with Mayer's Hematoxyline to visualize BM-MSCs. Negative controls performed on cellularized hydrogels after 14 days in culture in differentiation medium.