

## Supplemental Materials and Methods

### *Safranin O*

Samples were incubated with haematoxylin for 8 min, and washed with running tap water for 20 min. Next, they were incubated with 0.05% Fast Green solution for 5 min and rinsed with 1% acetic acid solution for 10 seconds. Thereafter, the samples were incubated in 0.1% safranin O solution (Merck, Overijse, Belgium) for 5 min.

### *Alcian blue*

Samples were incubated with alcian blue solution (pH = 2.5) for 30 min at room temperature (RT). Subsequently, samples were washed with running tap water for 10 min and submerged in distilled water for 1 min. Next, nuclear fast red solution was applied for 10 min and samples were dipped for 1 second in distilled water.

### *Toluidine blue*

Samples were incubated with 1% toluidine blue solution for 20 min at RT. Subsequently, samples were washed with distilled water for 30 seconds.

### *Masson's trichrome*

After incubation with haematoxylin and running tap water, samples were incubated in Ponceau/Fuchsin solution for 5 min. Next, samples were incubated in 1% phosphomolybdic acid and Aniline blue solution for 5 min each. After incubation in 1% phosphomolybdic acid for 5 min, samples were placed in acetic acid for 2 min. Between each incubation, samples were washed with distilled water.

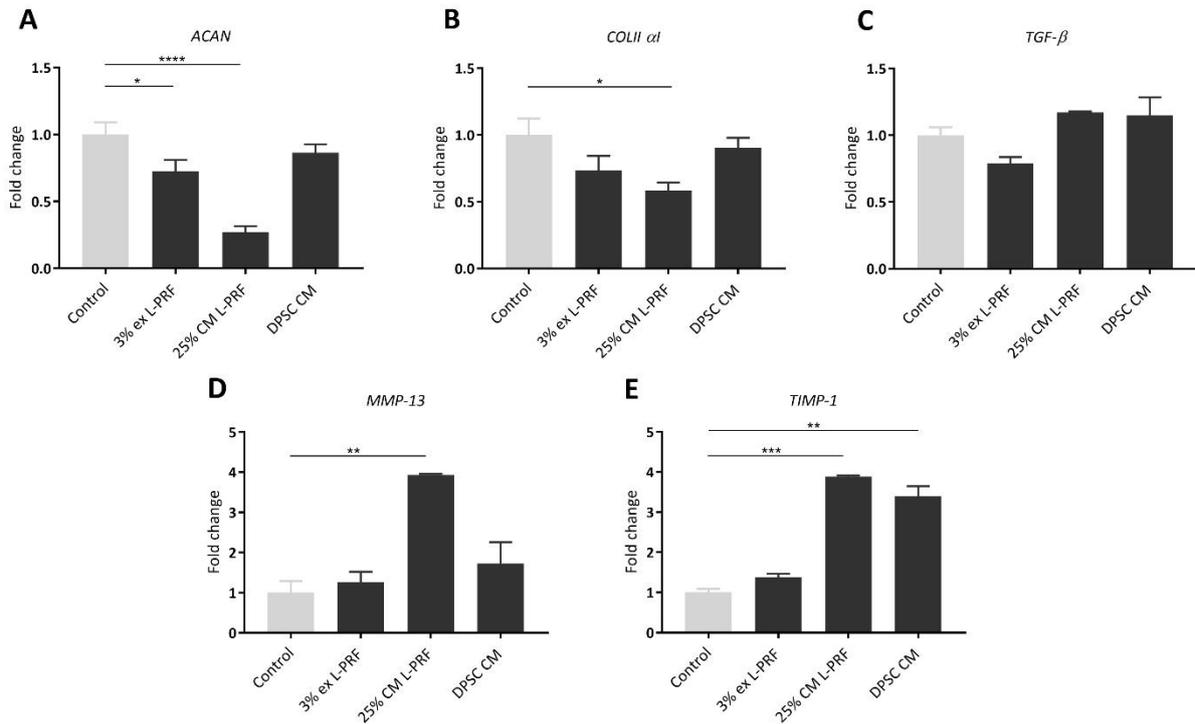
### *Primer sequences used for RT-qPCR*

**Table S1.** The Primers used for RT-qPCR analysis.

Gene	Forward primer 5'-3'	Reverse primer 5'-3'
<i>ADAM-17</i>	AGAGAGCCATCTGAAGAGTTTGT	CTTCTCCACGGCCCATGTAT
<i>ACAN</i>	GTCGCTCCCCAACTATCCAG	AAAGTCCAGGGTGTAGCGTG
<i>COL II<math>\alpha</math>1</i>	GAAGGATGGCTGCACGAAAC	AATAATGGGAAGGCCGGGAGG
<i>CYP A</i>	GCGTCTCCTTCGAGCTGTT	AAGTACCACCCTGGCA
<i>HMBS</i>	GATGGGCAACTGTACCTGACTG	CTGGGCTCCTCTTGAATG
<i>HPRT</i>	CTCATGGACTGATTATGGACAGGAC	GCAGGTCAGCAAAGAACTTATAGCC
<i>IL-6</i>	TACCACTTCAACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
<i>iNOS</i>	CCCTTCAATGGTTGGTACATGG	ACATTGATCTCCGTGACAGCC
<i>MMP-13</i>	TCGCCCTTTTGAGACCACTC	AGCACCAAGTGTTACTCGCT
<i>TGF-<math>\beta</math></i>	GGGCTACCATGCCAACTTCTG	GAGGGCAAGGACCTTGCTGTA
<i>TIMP-1</i>	TCCTAGAGACACACCAGAGCA	AGCAACAAGAGGATGCCAGA
<i>TNF-<math>\alpha</math></i>	GTCCCCAAAGGGATGAGAAGT	TTTGCTACGACGTGGGCTAC
<i>YWHAZ</i>	GCAACGATGTACTGTCTCTTTTGG	GTCCACAATTCCTTTCTGTGCATC

## Supplemental Figures

Figure S1.



**Figure S1.** The effect of L-PRF ex, L-PRF CM and DPSC CM on chondrogenic genes of iMACs. Gene expression levels of chondrogenic markers were determined by RT-qPCR of unstimulated iMACs exposed to 3% L-PRF ex, 25% L-PRF CM and DPSC CM. (A-B) 25% L-PRF CM significantly decreased expression levels of *collagen type II  $\alpha$  1* and *aggrecan*, while 3% L-PRF exudate only downregulated *aggrecan* expression levels. (C) *TGF- $\beta$*  mRNA levels were not significantly altered by L-PRF ex, L-PRF CM and DPSC CM. (D) *MMP-13* was significantly upregulated in iMACs cultured with 25% L-PRF CM compared to the control. (E) *TIMP-1* mRNA expression levels were significantly upregulated by the supplementation of 25% L-PRF CM and DPSC CM. Data correspond to n = 6 for L-PRF ex and L-PRF CM and n = 7 for DPSC CM. Data are represented as mean  $\pm$  S.E.M. \*, p  $\leq$  0.05. \*\*, p  $\leq$  0.01. \*\*\*, p  $\leq$  0.001. \*\*\*\*, p  $\leq$  0.0001.