THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Platelet-Rich Plasma Attenuates 30-kDa Fibronectin Fragment-Induced Chemokine and Matrix Metalloproteinase Expression by Meniscocytes and Articular Chondrocytes

Citation for published version:

Wang, C-C, Lee, C-H, Peng, Y-J, Salter, D & Lee, H-S 2015, 'Platelet-Rich Plasma Attenuates 30-kDa Fibronectin Fragment-Induced Chemokine and Matrix Metalloproteinase Expression by Meniscocytes and Articular Chondrocytes', *American Journal of Sports Medicine*, vol. 43, no. 10, pp. 2481-2489.
<https://doi.org/10.1177/0363546515597489>

Digital Object Identifier (DOI):

[10.1177/0363546515597489](https://doi.org/10.1177/0363546515597489)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Peer reviewed version

Published In:

American Journal of Sports Medicine

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

- 1 Platelet-Rich Plasma Attenuates 30 kDa Fibronectin Fragment**
- 2 Induced Chemokine and MMP Expression by Meniscocytes and**
- 3 Articular Chondrocytes**

4 **Abstract**

5 **Background:** Proteolytic fragments of fibronectin have catabolic effects on cartilage
6 and menisci. Platelet-rich plasma (PRP) is increasingly being used in treatment of a
7 range of joint pathologies but it is unknown whether PRP influences fibronectin
8 fragment (FN-f) pro-catabolic activity.

9 **Hypotheses:** The pro-catabolic activity of FN-f on meniscocytes and articular
10 chondrocytes is attenuated by co-treatment with PRP.

11 **Study Design:** Controlled laboratory study.

12 **Methods:** Human meniscocytes were treated with FN-f (30 kDa) with/without PRP co-
13 incubation and gene expression analyzed by cDNA microarray analysis. Validation of
14 altered expression of known and novel chemokine and protease genes was undertaken
15 by real-time PCR in articular chondrocytes and meniscocytes. Chemokine release was
16 assayed by ELISA and intracellular pathway signaling was evaluated by western
17 immunoblotting.

18 **Results:** Microarray analysis and RT-PCR showed increased expression of MMP1,

19 MMP2, MMP3, MMP9, MMP13, IL-6 and IL-8 (CXCL8), CCL5, CCL20 and
20 CXCL10 chemokines in meniscocytes following treatment with FN-f. Upregulation of
21 these genes was significantly attenuated by PRP. Similar results were seen with
22 articular chondrocytes although no change in MMP2 or MMP9 levels were identified.
23 PRP induced suppression of gene expression was associated with activation of Akt and
24 p44/p42.

25 **Conclusions** : 30 kDa FN-f induced expression of a range of pro-inflammatory
26 chemokines and MMPs including IL-8, IL-6, CCL20, CCL5, CXCL10, MMP1, MMP3
27 and MMP13 by both meniscocytes and articular chondrocytes is attenuated by PRP
28 treatment.

29 **Clinical Relevance:** These observations provide support for the use and further trials
30 of PRP in management of cartilage and meniscal injuries.

31 **Keywords:** platelet-rich plasma; fibronectin fragment; chondrocyte; chemokine;
32 matrix metalloproteinase

33

34 **What is known about the subject:**

35 Proteolytic fragments of fibronectin are released from articular cartilage following
36 impact injury and are increased in synovial fluid of patients with osteoarthritis (OA).
37 These fibronectin fragments (FN-f) have catabolic activity inducing expression of a
38 range of inflammatory mediators and proteases that contribute to both cartilage and
39 meniscus degeneration. Removal of FN-f from OA synovial fluid decreases detrimental
40 indicating that targeting these molecules may be of benefit in attenuating the
41 development or progression of cartilage and meniscal pathology. There is increasing
42 interest and application of platelet-rich plasma (PRP) for the treatment of osteoarthritis
43 and a range of other joint and musculoskeletal conditions. PRP is a key source of
44 molecules involved in tissue repair and regeneration and can deliver a variety of
45 bioactive molecules that have the potential to suppress pro-inflammatory and
46 proteolytic pathways. It is however not known whether PRP acts to inhibit the broad
47 range of recognized and novel inflammatory and proteolytic pathways activated by
48 FN-f in human meniscocytes and articular chondrocytes.

49 **What this study adds to existing knowledge:**

50 In addition to confirming recent observations that FN-f increase expression of a range
51 of matrix metalloproteinases and chemokines in meniscocytes and articular
52 chondrocytes we have, for the first time, identified that FN-f also increase expression
53 of MMP9 and CCL5, CCL20 and CXCL10 chemokines in human meniscocytes. Co-
54 stimulation of both meniscocytes and articular chondrocytes with PRP significantly
55 attenuated the FN-f increased expression of chemokines and MMPs providing
56 mechanistic support for the use of intra-articular PRP injection for the treatment of
57 degenerative and traumatic joint conditions including OA.

58 **Introduction**

59 Fibronectin (FN) is a multidomain glycoprotein present in most extracellular matrices
60 (ECM), including cartilage⁸ and synovium²⁷, as a dimeric glycoprotein. The dimeric
61 glycoprotein is formed through a pair of anti-parallel disulfide bonds at the C terminus
62 linking single glycoproteins with a molecular weight of 230–270 kDa.⁴⁰ FN isoforms
63 in adult cartilage are significantly different from fibronectins in other tissues and
64 include predominantly the cartilage specific (V + C) isoform (50-80%) with smaller
65 amounts of the of ED-B (+) isoform.⁹ Intact FN has important roles in matrix assembly,
66 morphogenesis, cell migration and inflammation through the binding of multiple
67 domains to itself and a range of matrix proteins and cell surface receptors including
68 collagen, fibulin-1, syndecan and integrins.³⁵ $\alpha 5 \beta 1$ integrin is the major FN receptor
69 expressed by articular chondrocytes and has important roles in regulating chondrocyte
70 responses to mechanical loading.^{33, 45, 58}

71 FN levels are elevated in cartilage in osteoarthritis (OA) as a result of increased
72 production and retention.⁵⁹ This increase of FN in OA cartilage is associated with an

73 increase in FN levels in synovial fluid.⁴⁸ As well as being increased in amount, the FN
74 in OA cartilage and synovium is fragmented, comprising FN-fragments (FN-f) of 30–
75 200 kDa.²² Unlike intact FN these FN-f have catabolic activity.²¹ FN-f bind to and
76 penetrate cartilage tissue resulting in proteinase expression and cartilage damage.⁶⁰
77 FN-f show potent catabolic activity increasing expression of Toll-like receptors and
78 inflammatory cytokines including TNF- α , IL-1 β , and IL-1 α , elevating production of
79 matrix metalloproteinase(MMP) proteases, and suppressing proteoglycan synthesis.^{13,}
80 ^{19, 20, 49, 51} FN-f are also released from articular cartilage following impact injury⁷, and
81 have been shown to increase production of MMPs by normal and OA meniscocytes in
82 vitro⁵⁰, indicating that a general increase in levels of these biologically active
83 fragments in synovial fluid in OA or other joint pathologies is also detrimental to
84 meniscus structure and function.
85 Removal of FN-f from OA synovial fluid has been shown to diminish detrimental
86 effects on cartilage²² indicating that targeting these molecules may be of benefit in
87 attenuating the development or progression of cartilage and meniscal pathology. A

88 specific pharmacological agent that inhibits the catabolic effects of FN-f has not yet
89 been developed but there is increasing interest in the use of biological therapies such as
90 hyaluronan (HA) and platelet-rich plasma (PRP) for the treatment of osteoarthritis and
91 a range of other joint and musculoskeletal conditions.^{15, 28, 29, 31, 38}

92 PRP is a key source of molecules involved in tissue repair and regeneration and can
93 deliver a variety of bioactive molecules. These include a number of growth factors
94 recognized to be important in regulation of chondrocyte proliferation and anabolic
95 function including platelet-derived growth factor, transforming growth factor beta,
96 fibroblast growth factor and insulin-like growth factor 1.^{5, 56} Delivery of a cocktail of
97 agents, as is present in PRP, would be expected to be beneficial for repair of cartilage
98 and meniscus injury and enhance tissue repair by stimulation of anabolic activity
99 whilst proinflammatory and catabolic pathways would be inhibited.^{2, 26, 53}

100 The aim of this study was to assess whether PRP has inhibitory effects on expression of
101 pro-inflammatory and proteolytic molecules induced by FN-f in human meniscocytes
102 and articular chondrocytes.

103 **Materials and Methods**

104 **Human articular chondrocyte and meniscocyte isolation and culture**

105 Human cartilage and meniscus samples were obtained from surgical discard tissue,
106 with consent (TMU-JIRB No.201305003), at knee joint arthroplasty from patients with
107 OA (n = 43, mean age 72.86 years, range 56-84 years). Residual OA cartilage with
108 predominantly grade II and III lesions (Collins/McElligot system) from each joint was
109 removed and pooled. Cartilage and meniscus tissue were cut into small fragments,
110 incubated with antimicrobial solution, containing 500 IU/mL penicillin (Gibco,
111 Invitrogen, Burlington, Ontario, Canada), 500 mg/mL streptomycin (Gibco) and 2.5
112 µg/mL Fungizone (Sigma, St Louis, MO, USA) for 4 h, and then washed with sterile
113 phosphate-buffered saline (PBS) before digestion. Cells were extracted by sequential
114 enzymatic digestion with 0.25% trypsin (Gibco) and collagenase type H (Sigma).
115 Extracted cells were re-suspended in 10 mL Dulbecco's modified Eagle's
116 medium/Nutrient Mixture F-12 HAM medium (Gibco) supplemented with 10% FBS
117 (Gibco), 100 I.U./mL penicillin and 100 mg/mL streptomycin; seeded in complete

118 medium at a density of 5×10^5 cell/mL in 60 mm Petri dishes (TPP, Trasadingen,
119 Switzerland); and cultured in a humidified 5% CO₂ incubator at 37°C for further
120 experimental procedures. Cells between passages 3 and 5 were used.

121 **Experimental protocol**

122 Human articular chondrocytes and meniscocytes were seeded at 5×10^5 cells/dish and
123 grown as a monolayer for 5 days in 60 mm tissue culture Petri dishes. Cells were
124 washed with sterile PBS twice, placed in serum-free media for 2 hours, and then co-
125 incubated with fibronectin proteolytic fragments 30 kDa at a concentration of 0.5
126 µg/mL for 24 hours. Freeze-dried powder of PRP was prepared by Regen Lab and
127 Regenkit (Regen Lab, Lausanne, Switzerland) by centrifugation of peripheral blood,
128 using a thixotropic gel for cell separation and citrate as anticoagulant. The obtained
129 PRP was approximately 3.3-fold platelet increase above baseline. Each ampoule of
130 PRP was dissolved with 1 mL of distilled water. The concentrations of two major
131 growth factors were - transforming growth factor-beta1 (TGF- β1) 125.9 pg/mL and
132 platelet-derived growth factor (PDGF) 40.8 pg/mL. 80 µL of PRP solution was added

133 to cells with 2 mL medium to yield a final concentration of TGF- β 1 5.036 pg/mL and
134 PDGF 1.632 pg/mL.

135

136 **Extraction of RNA and Real-Time Polymerase Chain Reaction**

137 Total RNA was extracted using TRIzol® RNA Isolation Reagents (Invitrogen, NY,
138 USA). For first-strand cDNA synthesis, 2 μ g total RNA was used in a single-round
139 reverse-transcription reaction by High-Capacity cDNA Reverse Transcription Kit
140 (Applied Biosystems, Foster City, CA, USA). qPCR reactions were carried out in a
141 final volume of 20 μ L containing 1 μ L of 20X TaqMan® Gene Expression Assay probe
142 (Applied Biosystems), 10 μ L of 2X TaqMan® Gene Expression Master Mix, 5 μ L of
143 RNase-free water, and 4 μ L of cDNA. Complement DNAs were amplified with the
144 following condition: 50°C for 2 minutes, 95°C for 10 minutes, 40 cycles of 95°C for
145 15 seconds and 60°C for 60 seconds, using a ViiA7 real-time PCR system (Applied
146 Biosystems). Resultant cycle threshold (Ct) values were normalized to the endogenous
147 control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and analyzed using the

148 $2^{-\Delta\Delta C_t}$ method. The Taqman Probes used for gene expression studies are listed in Table

149 1.

150 **Microarray assay and data analysis**

151 RNA was extracted from control and cells incubated with 30 kDa FN-f. 0.2 µg of total

152 RNA was amplified by a Low Input Quick-Amp Labeling kit (Agilent Technologies,

153 Palo Alto, CA, USA) and labeled with Cy3 (Agilent Technologies). 0.6 µg of Cy3-

154 labeled cRNA was fragmented to an average size of 50-100 nucleotides by incubation

155 with fragmentation buffer at 60°C for 30 minutes. Correspondingly fragmented labeled

156 cRNA is then pooled and hybridized to Agilent SurePrint G3 Human V2 GE 8×60K

157 Microarray (Agilent Technologies) at 65°C for 17 h. After washing and drying by

158 nitrogen gun blowing, microarrays were scanned with an Agilent microarray scanner at

159 535 nm. Scanned images are analyzed by Feature extraction10.5.1.1 software (Agilent

160 Technologies). The microarray data comply with MIAME (Minimum Information

161 About a Microarray Experiment) guidelines, and the raw data have been deposited in a

162 MIAME-compliant database.

163 **Protein Extraction and Western blotting**

164 Following stimulation cells were immediately washed with ice-cold PBS and protein
165 extracted with standard lysis buffer at 4°C for 15 min. Whole-cell lysates were
166 collected after centrifugation at 13,000 rpm for 10 min. Equal amounts of protein were
167 loaded onto 10% SDS-polyacrylamide gel and following electrophoresis were
168 transferred to polyvinylidene fluoride (PVDF) membranes (Millipore). Membranes
169 were blocked overnight at 4°C with 2% BSA in TBST (12.5 mM Tris/HCl, pH 7.6, 137
170 mM NaCl, 0.1% Tween 20). After washing with TBST, blots were incubated at 4°C
171 overnight with primary antibodies (PathScan® Multiplex Western Cocktail I 1/1000;
172 p44/42 MAPK 1/1000; AKT 1/1000) (all from Cell Signaling Technology, MA, USA)
173 diluted in TBST respectively, washed 6 times before incubation with HRP-labeled
174 secondary antibody 1/5000 (DakoCytomation, Copenhagen, Denmark) for 1 h at room
175 temperature. Membranes were rewashed extensively and antibody binding was
176 visualized with Immobilon™ Western HRP Substrate (Millipore). Immunoblots were
177 scanned by a UVP BioSpectrum AC image system (UVP, Upland, CA, USA) and

178 quantitated using VisionWork LS software (UVP). Anti-alpha-tubulin (1/5000; Abcam,
179 Cambridge, UK) acted as internal control.

180 **ELISA**

181 Quantification of IL-8 (CXCL8) in supernatants of cultured medium was carried out
182 using Quantikine ELISA kits (R&D Systems, Minneapolis, USA).

183 **Statistical analysis**

184 The values were expressed as fold of band intensity of the target gene or protein to the
185 internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene or alpha-
186 tubulin. The results are expressed as the mean $\pm$ SD. Data are analyzed using SPSS
187 (Statistical Package for Social Sciences) statistical software 18.0; all statistical tests use
188 Student's t-test, and the p value < 0.05 is considered statistically significant.

189 **Results**

190 **PRP attenuates FN-f induced chemokine gene expression in meniscocytes and** 191 **articular chondrocytes**

192 *Microarray analysis*

193 To investigate the global gene change in meniscocytes as a result of stimulation with
194 0.5 µg/mL 30 kDa FN-f an initial microarray assay was carried out. Analysis using the
195 GeneSpring GX software showed that expression of 258 genes was significantly
196 altered. Novel findings were the upregulation of several chemokine genes. The top 5
197 upregulated genes were IL-8 (CXCL8), CCL20, IL-6, CXCL10, and CCL5,
198 respectively (Table 2). To assess whether PRP had any effect on the 30 kDa FN-f
199 induced changes in gene expression meniscocytes were co-incubated with PRP and 30
200 kDa FN-f. With co-incubation there was a significant downregulation of expression of
201 each of these top 5 upregulated genes rather than the increased expression seen with
202 FN-f treatment alone (Table 2).

203 *Quantitative gene expression*

204 To confirm these results and to establish whether similar effects were seen in articular
 205 chondrocytes experiments were undertaken in which changes in gene expression were
 206 assessed by Q-PCR. Following 24 hours incubation with 30 kDa FN-f expression of
 207 IL-8 (410.1 ± 310.9 fold), CCL20 (287.8 ± 50.3 fold), IL-6 (281.0 ± 199.4 fold),
 208 CXCL10 (859.9 ± 665.2 fold), and CCL5 (167.7 ± 90.4 fold) was significantly
 209 increased in meniscocytes. These changes were attenuated by co-treatment with PRP
 210 for each of the 5 genes: IL-8 (18.1 ± 13.7 fold, $p = 0.042$); CCL20 (14.5 ± 11.5 fold, p
 211 $= 0.0008$); IL-6 (12.7 ± 7.8 fold, $p = 0.0362$); CXCL10 (6.2 ± 5.6 fold, $p = 0.0448$);
 212 CCL5 (2.9 ± 2.1 fold, $p = 0.011$) (Figure 1A). 30 kDa FN-f also significantly
 213 upregulated expression of IL-8, CCL20, IL-6, CXCL10, and CCL5 in articular
 214 chondrocytes (164.4 ± 78.2 fold, $p = 0.0095$; 122.6 ± 76.34 fold, $p = 0.0014$; $43.8 \pm$
 215 22.9 fold, $p = 0.033$; 186.7 ± 140.4 fold, $p = 0.042$; 14.29 ± 11.27 fold, $p = 0.003$,
 216 respectively). These changes were significantly attenuated by co-treatment with PRP
 217 (IL-8 - 25.3 ± 22.1 fold, $p = 0.005$; CCL20 - 18.7 ± 9.5 fold, $p = 0.0009$; IL-6 - $10.7 \pm$
 218 7.9 fold, $p = 0.034$; CXCL10 - 8.6 ± 4.9 fold, $p = 0.044$, and CCL5 - 2.4 ± 1.9 fold, $p =$

219 0.003, respectively) (Figure 1B). With PRP treatment alone there was no significant
220 change in expression of IL-8, CCL20, IL-6, CXCL10, and CCL5 genes in either
221 meniscocytes or articular chondrocytes over the time period tested.

222

223 **PRP reduces FN-f induced IL-8 secretion by meniscocytes and articular**
224 **chondrocytes**

225 IL-8 secretion by primary meniscocytes was significantly increased following 30 kDa
226 FN-f stimulation for 24 hours (603.59 ± 594.81 vs 112.8 ± 32.3 pg/mL, $p = 0.0037$)
227 (Figure 2A). This enhanced production was significantly suppressed by PRP co-
228 incubation (266.38 ± 214.09 pg/mL, $p = 0.0087$) (Figure 2A). Treatment with PRP
229 alone had no significant effect on basal IL-8 secretion levels (128.37 ± 51.29 vs 112.8
230 ± 32.3 pg/mL, $p = 0.0583$). 30 kDa FN-f had similar but more pronounced effects on
231 articular chondrocytes showing a significant increase in IL-8 secretion following
232 stimulation for 24 hours (5672.86 ± 5266.53 vs 155.15 ± 52.37 pg/mL, $p = 0.0026$).
233 The increased secretion of IL-8 induced by 30 kDa FN-f was significant suppressed by

234 PRP but remained above basal levels (1049.26 ± 866.66 pg/mL, $p = 0.0024$) (Figure
235 2B). There was a small, but non-significant increase in IL-8 levels when articular
236 chondrocytes were incubated with PRP alone (237.14 ± 97.45 vs 155.15 ± 52.37
237 pg/mL, $p = 0.0523$).

238 **FN-f induced MMP expression by meniscocytes and articular chondrocytes is**
239 **inhibited by PRP**

240 Following incubation of meniscocytes with 30 kDa FN-f for 24 hours gene expression
241 of MMP1, MMP2, MMP3, MMP9, and MMP13 was significantly increased ($112.7 \pm$
242 58.0 fold; 1.9 ± 0.4 fold; 41.7 ± 22.0 fold; 1.7 ± 0.3 fold; 15.6 ± 2.2 fold, respectively).
243 Co-incubation with PRP significantly attenuated the effect of 30 kDa FN-f on MMP1,
244 MMP2, MMP3 and MMP13 gene expression (6.6 ± 4.2 fold; 1.0 ± 0.2 fold; 3.4 ± 2.0
245 fold; 2.3 ± 1.3 fold, respectively). MMP9 gene expression was also decreased by co-
246 incubation with PRP but the results did not reach statistical significance. There was no
247 change in gene expression of MMP1, MMP2, MMP3, MMP9, and MMP13 in
248 meniscocytes cultured for 24 hours in PRP alone (Figure 3A).

249 Articular chondrocyte expression of MMP1, MMP3 and MMP13 genes was
250 significantly upregulated by incubation with 30 kDa FN-f (17.6 ± 11.3 fold; $29.2 \pm$
251 18.3 folds; 6.3 ± 5.7 fold, respectively). This effect was significantly attenuated by co-
252 treatment with PRP (3.4 ± 1.9 fold; 4.3 ± 3.1 fold; 2.4 ± 0.6 fold, respectively). In
253 contrast to meniscocytes, MMP2 and MMP9 genes expression was not upregulated by
254 30 kDa FN-f stimulation. MMP1, 2, 3, 9, and 13 showed no significant gene regulation
255 following PRP treatment alone (Figure 3B).

256 **Akt and p44/42 MAP kinase phosphorylation induced by PRP**

257 To investigate potential mechanisms by which PRP may be influencing chemokine and
258 MMP gene expression we looked at the activation of Akt and p44/42 MAP kinase, both
259 molecules being recognized as important regulatory intracellular signaling pathways in
260 chondrocytes and meniscocytes. Following stimulation with PRP Akt phosphorylation
261 was rapidly increased in meniscocytes and maintained for up to 3 hours (16.63 ± 5.28
262 fold, $p = 0.0096$ at 0.5 h; 10.47 ± 1.94 fold, $p = 0.0023$ at 1 h; 4.74 ± 2.42 fold, $p =$
263 0.0533 at 3 h; compared to baseline). p44/42 phosphorylation was similarly increased

264 over the same time course (5.24 ± 1.23 fold, $p = 0.0015$ at 0.5 h; 3.66 ± 0.95 fold, $p =$
265 0.0033 at 1 h; 4.54 ± 2.29 fold, $p = 0.0258$ at 3 h, compared to baseline). Under
266 identical conditions similar results were seen when articular chondrocytes were
267 incubated with PRP (Figure 4). Phosphorylation of Akt was increased at each time
268 point (0.5 h = 19.01 ± 5.30 fold, $p = 0.0016$; 1 h = 21.26 ± 4.45 fold, $p = 0.0005$; 3 h =
269 12.47 ± 5.91 fold, $p = 0.0122$, compared to baseline). Phosphorylation of p44/42 MAP
270 kinase followed an analogous pattern (0.5 h = 3.58 ± 2.06 fold, $p = 0.0487$, 1 h = 3.33
271 ± 2.62 fold, $p = 0.1175$, 3 h = 2.67 ± 2.47 fold, $p = 0.204$, compared to baseline).

272 **Discussion**

273 In this study we aimed to assess the influence of PRP on FN-f induced pro-
274 inflammatory and proteolytic activity of human meniscocytes and articular
275 chondrocytes. Using a gene microarray we have identified that 30-kDa FN-f induces
276 increased gene expression of several chemokines in meniscocytes and chondrocytes. In
277 addition to confirming observations that FN-f increases expression of MMP1, MMP2,
278 MMP3, MMP13, IL-6 and IL-8 in meniscocytes⁵⁰ we have, for the first time, identified
279 that FN-f also increases expression of MMP9 and CCL5, CCL20 and CXCL10
280 chemokines in human meniscocytes. Similar effects are evident in articular
281 chondrocytes although we found no change in MMP2 or MMP9 expression in these
282 cells consistent with findings by others.⁴⁹ Importantly, co-stimulation of both
283 meniscocytes and articular chondrocytes with PRP significantly attenuated the FN-f
284 increased expression of chemokines and MMPs.

285 Currently 44 human chemokine ligands 21 chemokine receptors have been described.

286 Chemokine receptors contain 7 transmembrane domains and are G protein-coupled.⁴²

287 Whilst chemokines act as critical extracellular mediators of cell migration, particularly
288 in the immune system³⁴ there is increasing interest in potential roles as inflammatory
289 mediators in joint tissues. Chondrocytes and meniscocytes express numerous CC and
290 CXC chemokines, including CCL2 (MCP-1), CCL3 (MIP-1 α), CCL11 (eotaxin-1),
291 CXCL1, CXCL2, CXCL3, IL-8 (CXCL8), in addition to a number of chemokine
292 receptors which potentially play important roles in activating catabolic pathways.^{23, 47,}
293 ⁵⁰ The pattern of chemokine and chemokine receptor expression in normal and OA
294 chondrocytes suggests that chemokines have an impact in cartilage homeostasis and
295 release of matrix-degrading enzymes in normal cartilage remodeling and cartilage
296 breakdown.¹¹ Chemokine levels are increased in synovial fluid in patients with
297 osteoarthritis.¹⁸ Chemokine and chemokine receptor expression is also elevated in
298 damaged menisci⁶ indicating that chemokine production in cartilage or meniscus due to
299 biomechanical injury is important in the development of degenerative joint disease.⁷
300 Our observation that meniscocytes, in addition to chondrocytes, express CCL5, CCL20
301 and CXCL10 chemokines and that expression of these cytokines is increased in

302 articular chondrocytes and meniscocytes on exposure to the proinflammatory 30 kDa
303 FN-f is novel. The effect of increased expression of these chemokines in cartilage and
304 menisci is not yet clear. Whilst IL-1 β and high-mobility group protein 1 (HMGB1)
305 increase expression of CCL5, CCL20 and CXCL10 chemokines in human
306 chondrocytes, expression of CCL5 is decreased by the chondroprotective cytokine IL-4
307 indicating likely roles as pro-inflammatory mediators in the OA.^{1, 3, 39}
308 Cartilage breakdown results in production of metabolically active breakdown products
309 such as FN-f. It is likely, although not yet confirmed that FN-f can also be produced in
310 menisci following acute or chronic degeneration and have effects on all intra-articular
311 tissues. Meniscocytes express a range of MMPs including MMP1, MMP2, MMP3,
312 MMP8, MMP9 and MMP13.³² Levels of MMPs in synovial fluid are elevated rapidly
313 in patients with a meniscal injury and remain elevated for at least 20 years post
314 injury.³⁰ Targeting MMP activity within the joint may prevent long-term damage. Pro-
315 catabolic FN-fs appear to act predominantly through the $\alpha 5 \beta 1$ integrin receptor.⁵⁷ Thus,
316 it would be possible to attempt to influence FN-f activity through targeting this

317 receptor. However, as single agent therapies are increasingly found to be ineffective
318 there is interest in more broad based biological approaches to treating joint injury and
319 osteoarthritis, especially in its early phase. Targeting pathways activated by FN-f may
320 be an effective means of inhibiting production of multiple mediators of cartilage
321 destruction.⁴³

322 Autologous PRP injections were first used in 1987 in open heart surgery.¹⁴ As a low
323 cost and minimally invasive way to obtain a natural concentration of autologous
324 growth factors, PRP is being studied in different fields of medicine for its ability to aid
325 tissue regeneration.^{12,16} PRP has been shown to enhance the healing of meniscal defects
326 in a rabbit model.²⁴ Preclinical studies showing that PRP enhances chondrocyte
327 viability, proliferation and matrix production provide mechanistic support for the use of
328 intra-articular PRP injection.^{25, 36}

The optimal concentration, composition and relative
importance of each of the components of PRP for clinical use remain unclear. Platelet
concentrations of 2.5-3 fold above baseline are considered to be ideal, with higher
concentrations potentially inhibiting tissue healing.^{17, 46, 55} Furthermore the “ideal”

platelet concentration may depend on the target parameter (e.g., direct promotion of tissue healing or stem cell recruitment), the tissue being treated (e.g., bone, cartilage, or tendon), and stage of disease or wound healing. Consequently, the “ideal” platelet concentration for various clinical scenarios remains unknown. PRP concentration can vary considerably depending on an individual’s blood platelet levels from day to day, diet, general health, medication and exercise. The method of PRP preparation also influences platelet concentration and levels of growth factors. PRP used in treatment of osteoarthritis usually contains between 2-6 fold normal platelet concentrations⁴¹ although the optimal protocol for PRP injection in knee OA has not been defined. PRP, approximately 6.8 fold above baseline, inhibits the inflammatory processes in human osteoarthritic chondrocytes⁵³ whilst a double-blinded, randomized controlled trial using PRP with a platelet concentration of approximately 3 fold above baseline showed benefit over placebo.³⁷ Other randomized controlled trials have also shown that PRP injection, although with variable preparation formulae, provides symptomatic relief in early knee OA³⁷, a significantly better clinical outcome compared with HA treatment

347 in grade III gonarthrosis¹⁰ and efficacy in management of pain and inflammation in
348 OA.^{2, 26} The commercially available PRP preparation we used had a platelet
349 concentration of 3.3 fold and is within the 'therapeutic range'. The preparation
350 contained 125.9 and 40.8 pg/mL of TGF- β 1 and PDGF respectively which, at the final
351 concentration used in our experimental model system, are at a level at which they
352 would be expected to be biologically active.⁴⁴ . In the current study the levels of Akt
353 and p44/42 MAP kinase phosphorylation rapidly increase in both meniscocytes and
354 articular chondrocytes upon incubation with PRP. This is in line with the published
355 literature on Akt and p44/42 MAP kinase phosphorylation following incubation of
356 chondrocytes with a range of growth factors and cytokines.⁴

357 We have shown that PRP can attenuate the effects of FN-f on both chondrocyte and
358 meniscocyte production of pro-catabolic chemokines and MMPs. The mechanisms by
359 which these effects are produced are not clear but are likely to be multifactorial as PRP
360 can potentially affect numerous overlapping pathways simultaneously due to the
361 presence of a number of anti-inflammatory cytokines and anabolic growth factors.²

362 Interestingly PRP has been shown to stimulate endogenous HA production and show
363 similar effects to HA in the suppression of inflammatory gene and protein expression
364 in synoviocytes and cartilage.⁵² High-molecular-weight (800 kDa) HA is believed to be
365 effective *in vitro* and *in vivo* in blocking the catabolic action of FN-f by preventing
366 entry of the fragments into the cartilage.⁵⁴ A recent *in vitro* study comparing the
367 activity of HA and PRP on the expression of anabolic and catabolic genes and
368 inflammatory mediators from human OA cartilage and synoviocytes indicated that
369 whilst both agents decreased catabolic activity, PRP treatment also caused a significant
370 reduction of MMP13, an increase in HAS-2 expression in synoviocytes and an increase
371 in cartilage synthetic activity compared with HA.⁵²

372 **Conclusion**

373 In the current study, 30 kDa FN-f induced production of a range of chemokines and
374 MMPs including IL-8, IL-6, CCL20, CCL5, CXCL10, MMP1, MMP3 and MMP13
375 by both meniscocytes and articular chondrocytes was attenuated by PRP treatment.

376 These observations suggest the mechanism by which PRP might help osteoarthritis

377 and suggests a rationale for continued limited clinical trials. Variations in the

378 composition of PRP from patient to patient are however recognized and composition

379 may also vary depending on the device and protocols used for preparation, methods

380 and time of storage. Due to these limitations and questions remaining on potential

381 interactions with other biologics or materials the current use of PRP in orthopedics

382 needs to be further established.

383 **References**

- 384 1. Amin AR, Islam AB. Genomic analysis and differential expression of HMG and
385 S100A family in human arthritis: upregulated expression of chemokines, IL-8
386 and nitric oxide by HMGB1. *DNA Cell Biol.* 2014;33(8):550-565.
- 387 2. Andia I, Maffulli N. Platelet-rich plasma for managing pain and inflammation
388 in osteoarthritis. *Nat Rev Rheumatol.* 2013;9(12):721-730.
- 389 3. Assirelli E, Pulsatelli L, Dolzani P, et al. Human osteoarthritic cartilage shows
390 reduced in vivo expression of IL-4, a chondroprotective cytokine that
391 differentially modulates IL-1beta-stimulated production of chemokines and
392 matrix-degrading enzymes in vitro. *PLoS One.* 2014;9(5):e96925.
- 393 4. Beier F, Loeser RF. Biology and pathology of Rho GTPase, PI-3 kinase-Akt, and
394 MAP kinase signaling pathways in chondrocytes. *J Cell Biochem.*
395 2010;110(3):573-580.
- 396 5. Borriore P, Gianfrancesco AD, Pereira MT, Pigozzi F. Platelet-rich plasma in
397 muscle healing. *Am J Phys Med Rehabil.* 2010;89(10):854-861.
- 398 6. Brophy RH, Rai MF, Zhang Z, Torgomyan A, Sandell LJ. Molecular analysis of
399 age and sex-related gene expression in meniscal tears with and without a
400 concomitant anterior cruciate ligament tear. *J Bone Joint Surg Am.*
401 2012;94(5):385-393.
- 402 7. Buckwalter JA, Anderson DD, Brown TD, Tochigi Y, Martin JA. The Roles of
403 Mechanical Stresses in the Pathogenesis of Osteoarthritis: Implications for
404 Treatment of Joint Injuries. *Cartilage.* 2013;4(4):286-294.
- 405 8. Burton-Wurster N, Butler M, Harter S, et al. Presence of fibronectin in
406 articular cartilage in two animal models of osteoarthritis. *J Rheumatol.*
407 1986;13(1):175-182.

- 408 9. Burton-Wurster N, Lust G, Macleod JN. Cartilage fibronectin isoforms: in
409 search of functions for a special population of matrix glycoproteins. *Matrix*
410 *Biol.* 1997;15(7):441-454.
- 411 10. Cerza F, Carni S, Carcangiu A, et al. Comparison between hyaluronic acid and
412 platelet-rich plasma, intra-articular infiltration in the treatment of
413 gonarthrosis. *Am J Sports Med.* 2012;40(12):2822-2827.
- 414 11. Cristino S, Piacentini A, Manferdini C, et al. Expression of CXC chemokines
415 and their receptors is modulated during chondrogenic differentiation of
416 human mesenchymal stem cells grown in three-dimensional scaffold:
417 evidence in native cartilage. *Tissue Eng Part A.* 2008;14(1):97-105.
- 418 12. Daher RJ, Chahine NO, Greenberg AS, Sgaglione NA, Grande DA. New
419 methods to diagnose and treat cartilage degeneration. *Nat Rev Rheumatol.*
420 2009;5(11):599-607.
- 421 13. Dang Y, Cole AA, Homandberg GA. Comparison of the catabolic effects of
422 fibronectin fragments in human knee and ankle cartilages. *Osteoarthritis*
423 *Cartilage.* 2003;11(7):538-547.
- 424 14. Ferrari M, Zia S, Valbonesi M, et al. A new technique for hemodilution,
425 preparation of autologous platelet-rich plasma and intraoperative blood
426 salvage in cardiac surgery. *Int J Artif Organs.* 1987;10(1):47-50.
- 427 15. Firestein GS, Zvaifler NJ. Anticytokine therapy in rheumatoid arthritis. *N Engl*
428 *J Med.* 1997;337(3):195-197.
- 429 16. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-Rich
430 Plasma: From Basic Science to Clinical Applications. *The American Journal of*
431 *Sports Medicine.* 2009;37(11):2259-2272.
- 432 17. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro
433 effect of different PRP concentrations on osteoblasts and fibroblasts. *Clin*
434 *Oral Implants Res.* 2006;17(2):212-219.

- 435 18. Hampel U, Sesselmann S, Iserovich P, Sel S, Paulsen F, Sack R. Chemokine and
436 cytokine levels in osteoarthritis and rheumatoid arthritis synovial fluid. *J*
437 *Immunol Methods*. 2013;396(1-2):134-139.
- 438 19. Homandberg GA, Hui F. Association of proteoglycan degradation with
439 catabolic cytokine and stromelysin release from cartilage cultured with
440 fibronectin fragments. *Arch Biochem Biophys*. 1996;334(2):325-331.
- 441 20. Homandberg GA, Meyers R, Williams JM. Intraarticular injection of
442 fibronectin fragments causes severe depletion of cartilage proteoglycans in
443 vivo. *J Rheumatol*. 1993;20(8):1378-1382.
- 444 21. Homandberg GA, Meyers R, Xie DL. Fibronectin fragments cause chondrolysis
445 of bovine articular cartilage slices in culture. *Journal of Biological Chemistry*.
446 1992;267(6):3597-3604.
- 447 22. Homandberg GA, Wen C, Hui F. Cartilage damaging activities of fibronectin
448 fragments derived from cartilage and synovial fluid. *Osteoarthritis Cartilage*.
449 1998;6(4):231-244.
- 450 23. Houard X, Goldring MB, Berenbaum F. Homeostatic mechanisms in articular
451 cartilage and role of inflammation in osteoarthritis. *Curr Rheumatol Rep*.
452 2013;15(11):375.
- 453 24. Ishida K, Kuroda R, Miwa M, et al. The regenerative effects of platelet-rich
454 plasma on meniscal cells in vitro and its in vivo application with
455 biodegradable gelatin hydrogel. *Tissue Eng*. 2007;13(5):1103-1112.
- 456 25. Kruger JP, Hondke S, Endres M, Pruss A, Siclari A, Kaps C. Human platelet-rich
457 plasma stimulates migration and chondrogenic differentiation of human
458 subchondral progenitor cells. *J Orthop Res*. 2012;30(6):845-852.
- 459 26. Laudy AB, Bakker EW, Rekers M, Moen MH. Efficacy of platelet-rich plasma
460 injections in osteoarthritis of the knee: a systematic review and meta-
461 analysis. *Br J Sports Med*. 2015;49(10):657-672.

- 462 27. Lavietes BB, Carsons S, Diamond HS, Laskin RS. Synthesis, secretion, and
463 deposition of fibronectin in cultured human synovium. *Arthritis Rheum.*
464 1985;28(9):1016-1026.
- 465 28. Lee AY, Korner H. CCR6 and CCL20: emerging players in the pathogenesis of
466 rheumatoid arthritis. *Immunol Cell Biol.* 2014;92(4):354-358.
- 467 29. Lisignoli G, Piacentini A, Cristino S, et al. CCL20 chemokine induces both
468 osteoblast proliferation and osteoclast differentiation: Increased levels of
469 CCL20 are expressed in subchondral bone tissue of rheumatoid arthritis
470 patients. *J Cell Physiol.* 2007;210(3):798-806.
- 471 30. Lohmander LS, Roos H, Dahlberg L, Hoerrner LA, Lark MW. Temporal patterns
472 of stromelysin-1, tissue inhibitor, and proteoglycan fragments in human knee
473 joint fluid after injury to the cruciate ligament or meniscus. *J Orthop Res.*
474 1994;12(1):21-28.
- 475 31. Mazzocca AD, McCarthy MBR, Chowaniec DM, et al. The Positive Effects of
476 Different Platelet-Rich Plasma Methods on Human Muscle, Bone, and Tendon
477 Cells. *The American Journal of Sports Medicine.* 2012;40(8):1742-1749.
- 478 32. McNulty AL, Weinberg JB, Guilak F. Inhibition of matrix metalloproteinases
479 enhances in vitro repair of the meniscus. *Clin Orthop Relat Res.*
480 2009;467(6):1557-1567.
- 481 33. Millward-Sadler SJ, Wright MO, Lee H-S, et al. Integrin-regulated Secretion of
482 Interleukin 4: A Novel Pathway of Mechanotransduction in Human Articular
483 Chondrocytes. *The Journal of Cell Biology.* 1999;145(1):183-189.
- 484 34. Olson TS, Ley K. Chemokines and chemokine receptors in leukocyte
485 trafficking. *Am J Physiol Regul Integr Comp Physiol.* 2002;283(1):R7-28.
- 486 35. Pankov R, Yamada KM. Fibronectin at a glance. *J Cell Sci.* 2002;115(Pt
487 20):3861-3863.

- 488 36. Park SI, Lee HR, Kim S, Ahn MW, Do SH. Time-sequential modulation in
489 expression of growth factors from platelet-rich plasma (PRP) on the
490 chondrocyte cultures. *Mol Cell Biochem.* 2012;361(1-2):9-17.
- 491 37. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-
492 rich plasma is more effective than placebo for knee osteoarthritis: a
493 prospective, double-blind, randomized trial. *Am J Sports Med.*
494 2013;41(2):356-364.
- 495 38. Pietrzak WS, Eppley BL. Platelet rich plasma: biology and new technology. *J*
496 *Craniofac Surg.* 2005;16(6):1043-1054.
- 497 39. Platas J, Guillen MI, del Caz MD, Gomar F, Mirabet V, Alcaraz MJ. Conditioned
498 media from adipose-tissue-derived mesenchymal stem cells downregulate
499 degradative mediators induced by interleukin-1beta in osteoarthritic
500 chondrocytes. *Mediators Inflamm.* 2013;2013:357014.
- 501 40. Potts JR, Campbell ID. Fibronectin structure and assembly. *Curr Opin Cell Biol.*
502 1994;6(5):648-655.
- 503 41. Pourcho AM, Smith J, Wisniewski SJ, Sellon JL. Intraarticular platelet-rich
504 plasma injection in the treatment of knee osteoarthritis: review and
505 recommendations. *Am J Phys Med Rehabil.* 2014;93(11 Suppl 3):S108-121.
- 506 42. Premack BA, Schall TJ. Chemokine receptors: gateways to inflammation and
507 infection. *Nat Med.* 1996;2(11):1174-1178.
- 508 43. Pulai JJ, Chen H, Im H-J, et al. NF- κ B Mediates the Stimulation of Cytokine
509 and Chemokine Expression by Human Articular Chondrocytes in Response to
510 Fibronectin Fragments. *The Journal of Immunology.* 2005;174(9):5781-5788.
- 511 44. Qureshi HY, Ahmad R, Sylvester J, Zafarullah M. Requirement of
512 phosphatidylinositol 3-kinase/Akt signaling pathway for regulation of tissue
513 inhibitor of metalloproteinases-3 gene expression by TGF-beta in human
514 chondrocytes. *Cell Signal.* 2007;19(8):1643-1651.

- 515 45. Salter DM, Hughes DE, Simpson R, Gardner DL. Integrin expression by human
516 articular chondrocytes. *Br J Rheumatol*. 1992;31(4):231-234.
- 517 46. Sampson S, Gerhardt M, Mandelbaum B. Platelet rich plasma injection grafts
518 for musculoskeletal injuries: a review. *Curr Rev Musculoskelet Med*. 2008;1(3-
519 4):165-174.
- 520 47. Sandell LJ, Xing X, Franz C, Davies S, Chang LW, Patra D. Exuberant expression
521 of chemokine genes by adult human articular chondrocytes in response to IL-
522 1beta. *Osteoarthritis Cartilage*. 2008;16(12):1560-1571.
- 523 48. Scott DL, Wainwright AC, Walton KW, Williamson N. Significance of
524 fibronectin in rheumatoid arthritis and osteoarthrosis. *Annals of the*
525 *Rheumatic Diseases*. 1981;40(2):142-153.
- 526 49. Stanton H, Ung L, Fosang AJ. The 45 kDa collagen-binding fragment of
527 fibronectin induces matrix metalloproteinase-13 synthesis by chondrocytes
528 and aggrecan degradation by aggrecanases. *Biochem J*. 2002;364(Pt 1):181-
529 190.
- 530 50. Stone AV, Loeser RF, Vanderman KS, Long DL, Clark SC, Ferguson CM. Pro-
531 inflammatory stimulation of meniscus cells increases production of matrix
532 metalloproteinases and additional catabolic factors involved in osteoarthritis
533 pathogenesis. *Osteoarthritis Cartilage*. 2014;22(2):264-274.
- 534 51. Su SL, Tsai CD, Lee CH, Salter DM, Lee HS. Expression and regulation of Toll-
535 like receptor 2 by IL-1beta and fibronectin fragments in human articular
536 chondrocytes. *Osteoarthritis Cartilage*. 2005;13(10):879-886.
- 537 52. Sundman EA, Cole BJ, Karas V, et al. The Anti-inflammatory and Matrix
538 Restorative Mechanisms of Platelet-Rich Plasma in Osteoarthritis. *The*
539 *American Journal of Sports Medicine*. 2014;42(1):35-41.
- 540 53. van Buul GM, Koevoet WL, Kops N, et al. Platelet-rich plasma releasate
541 inhibits inflammatory processes in osteoarthritic chondrocytes. *Am J Sports*
542 *Med*. 2011;39(11):2362-2370.

- 543 54. Wang Y, Hall S, Hanna F, et al. Effects of Hylan G-F 20 supplementation on
544 cartilage preservation detected by magnetic resonance imaging in
545 osteoarthritis of the knee: a two-year single-blind clinical trial. *BMC*
546 *Musculoskelet Disord.* 2011;12:195.
- 547 55. Weibrich G, Hansen T, Kleis W, Buch R, Hitzler WE. Effect of platelet
548 concentration in platelet-rich plasma on peri-implant bone regeneration.
549 *Bone.* 2004;34(4):665-671.
- 550 56. Weibrich G, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in platelet-
551 rich plasma and correlations with donor age, sex, and platelet count. *J*
552 *Craniomaxillofac Surg.* 2002;30(2):97-102.
- 553 57. Werb Z, Tremble PM, Behrendtsen O, Crowley E, Damsky CH. Signal
554 transduction through the fibronectin receptor induces collagenase and
555 stromelysin gene expression. *The Journal of Cell Biology.* 1989;109(2):877-
556 889.
- 557 58. Wright MO, Nishida K, Bavington C, et al. Hyperpolarisation of cultured
558 human chondrocytes following cyclical pressure-induced strain: evidence of a
559 role for alpha 5 beta 1 integrin as a chondrocyte mechanoreceptor. *J Orthop*
560 *Res.* 1997;15(5):742-747.
- 561 59. Wurster NB, Lust G. Synthesis of fibronectin in normal and osteoarthritic
562 articular cartilage. *Biochim Biophys Acta.* 1984;800(1):52-58.
- 563 60. Xie D, Homandberg GA. Fibronectin fragments bind to and penetrate
564 cartilage tissue resulting in proteinase expression and cartilage damage.
565 *Biochim Biophys Acta.* 1993;1182(2):189-196.

566

567

568

569 **Table 1.** Taqman Probes used for Gene Expression Studies.

GeneSymbol	Accession No.	Applied Biosystems Order No.
IL-6	NM_000600	Hs00174131_m1
IL-8	NM_000584	Hs00174103_m1
CXCL10	NM_001565	Hs00171042_m1
CCL5	NM_002985	Hs00174575_m1
CCL20	NM_004591	Hs00355476_m1
MMP1	NM_002421	Hs00233958_m1
MMP2	NM_004530	Hs01548727_m1
MMP3	NM_002422	Hs00968305_m1
MMP9	NM_004994	Hs00234579_m1
MMP13	NM_002427	Hs00233992_m1
GAPDH	NM_002046	Hs02758991_g1

570

571 **Table 2.** Top of 5 upregulated genes (ranking 1-5) by 30 kDa FN-f stimulation and
572 downregulated chemokine genes by PRP treatment in meniscocytes.

Upregulated genes			Downregulated genes		
Ranking	GeneSymbol	Log Ratio (2)	Ranking	GeneSymbol	Log Ratio (2)
1	IL-8	9.358	1	CXCL10	-7.523
2	CCL20	8.750	4	CCL5	-5.121
3	IL-6	7.563	8	CCL20	-4.464
4	CXCL10	7.412	16	IL-8	-3.951
5	CCL5	6.545	17	IL-6	-3.830

573

574 **Figure legends**

575 **Figure 1.** Validation of microarray analysis in both (A) meniscocytes and (B) articular
576 chondrocytes. Top 5 upregulated genes including IL-8, CCL20, IL-6, CXCL10, and
577 CCL5 induced by 30 kDa FN-f all showed significant downregulation by the treatment
578 of PRP. (* $p < 0.05$, compared with control; * * $p < 0.01$, compared with control;
579 [#] $p < 0.05$, 30 kDa+PRP vs 30 kDa).

580

581 **Figure 2.** Effects of PRP on 30 kDa FN-f induced IL-8 release. (A) IL-8 release
582 induced by 30 kDa FN-f was suppressed by PRP treatment in meniscocytes. (B)
583 Similar effect was seen in articular chondrocytes. (* * $p < 0.01$, compared with control;
584 [#] $p < 0.05$, 30 kDa+PRP vs 30 kDa).

585

586 **Figure 3.** Effects of PRP on 30 kDa FN-f induced MMP expression in both (A)
587 meniscocytes and (B) articular chondrocytes. Upregulation of MMPs induced by 30
588 kDa FN-f was suppressed by the treatment of PRP in meniscocytes. In articular
589 chondrocytes MMP1, MMP3, and MMP13 gene expression showed the similar pattern.
590 There was no significant change in MMP2 and MMP9 gene expression in articular
591 chondrocytes. (* $p < 0.05$, compared with control; [#] $p < 0.05$, 30 kDa+PRP vs 30 kDa).

592

593 **Figure 4.** Protein phosphorylation of Akt and p44/42 by PRP treatment in human
594 meniscocytes (A) and articular chondrocytes (B). Left panel - representative blots ;
595 right panel - semiquantitative data. Rapid protein phosphorylation of Akt and p44/42
596 was recognized in both cell types. (* $p < 0.05$, compared with control; * * $p < 0.01$,
597 compared with control; * * * $p < 0.001$, compared with control).

A

B

A **B**

A

B

A **B**