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Growth Factor Delivery to a Cartilage-Cartilage Interface Using Platelet-Rich Concentrates on a Hyaluronic Acid Scaffold

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**1 Growth Factor Delivery to a Bovine Defect Using Leukocyte -Rich Platelet-Rich
2 Concentrates on a Hyaluronic Acid Scaffold**

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25 **Abstract:**

26 Purpose: To determine if (1) Human L-PRP or (2) L-PRF delivered on a HA scaffold at a bovine
27 chondral defect, a simulated cartilage tear interface, *in vitro* would improve tissue formation
28 based on biomechanical, histological, and biochemical measures.

29 Methods: L-PRF and L-PRP was prepared from 3 healthy volunteer donors which was delivered
30 in conjunction with Hyaluronic acid (HA scaffolds) to defects created in full thickness bovine
31 cartilage plugs harvested from bovine femoral condyle and trochlea. Specimens were cultured in
32 vitro for up to 42 days. Treatment groups included an HA scaffold alone and scaffolds containing
33 L-PRF or L-PRP. Cartilage repair was assessed using biomechanical testing, histology, DNA
34 quantification, and measurement of sulfated glycosaminoglycan (sGAG) and collagen content at
35 28 and 42 days.

36 Results: L-PRF elicited the greatest degree of defect filling and improvement in other
37 histological measures. L-PRF treated specimens also had the greatest cellularity when compared
38 to L-PRP and control at day 28 (560.4 vs. 191.4 vs. 124.2, $p=0.015$); at day 48 there remains a
39 difference though not significant between L-PRF vs L-PRP, (761.1 vs 589.3, $p=0.219$) . L-PRF
40 had greater collagen deposition when compared to L-PRP at day 42 (40.1 vs 16.3, $p< 0.0001$). L-
41 PRF had significantly higher maximum interfacial strength compared to the control at day 42
42 (10.92 N vs 0.66 N, $p=0.015$), but had no significant difference compared to L-PRP (10.92 N vs
43 6.58, $p=0.536$). L-PRP facilitated a greater amount of sGAG production at day 42 when
44 compared to L-PRF (15.9 vs. 4.3, $p=0.009$).

45 Conclusions: Delivery of leukocyte rich platelet concentrates in conjunction with a HA scaffold
46 may allow for improvements in cartilage healing through different pathways. L-PRF was not

47 superior to L-PRP in its biomechanical strength suggesting that both treatments may be effective
48 in improving biomechanical strength of healing cartilage through different pathways.

49

50 Statement of Clinical Relevance: The delivery of platelet-rich concentrates in conjunction HA
51 scaffolds may augment healing cartilaginous injuries.

52

53 **Keywords: cartilage repair, PRP, leukocyte-rich platelet-rich fibrin, leukocyte rich**
54 **platelet enriched concentrates**

55

56 **Introduction**

57 Focal cartilage defects may be caused by sports injury, trauma, and other activities of daily
58 living¹. As cartilage has limited capacity for intrinsic repair, such defects often further
59 degenerate, progressing into osteoarthritis with associated symptoms of pain, swelling, and
60 stiffness^{1,2}. There are few effective treatments for articular cartilage injuries. Previous efforts
61 have used cell-based therapies (autologous chondrocyte implantation, matrix-induced autologous
62 chondrocyte implantation, bone marrow and adipose-derived stem cells), microfracture, and
63 cytokines to promote cartilage repair; however, these attempts have not achieved ideal
64 osteochondral defect repair with regeneration of morphologically similar hyaline cartilage
65 tissue³. Autologous chondrocyte implantation as well as matrix-induced autologous chondrocyte
66 implementation has been shown improved clinical outcomes, however its cost is high, and the
67 procedure to obtain the cartilage pieces is invasive^{3,4}. These cells can produce hyaline cartilage,
68 however, this procedure is also associated with the formation of fibrocartilage, which typically
69 occurs secondary to de-differentiation of chondrocytes during cell expansion after harvest³.

70 Mesenchymal stem cells harvested from bone marrow or adipose tissue have been shown to give
71 rise to a mixture of hypertrophic, cartilaginous, and fibrous tissues and can lead to loss of tissue
72 in the long run^{3,5}. Microfracturing has been shown to significantly improve the symptoms of
73 patients and is thought to bring about the recruitment of mesenchymal stem cells, it is only
74 effective for small defects and has been shown to only have a short-term functional improvement
75 due to the formation of fibrocartilage rather than hyaline articular cartilage^{3,6}.

76

77 The use of scaffolds is promising as a viable surgical option in cartilage repair^{1,7}. Scaffolds can
78 augment cell migration from the surrounding tissue, stimulate cell proliferation, and aid in
79 maintenance of cell phenotype in order to facilitate regeneration of functional tissue^{7,8}.
80 Specifically, hyaluronic acid (HA) scaffolds may facilitate cellular migration by providing both
81 biochemical and biophysical cues improving articular cartilage repair^{9,10}. HA scaffolds have been
82 shown to increase early-stage gene expression of SOX-9 and collagen type II as well as cartilage
83 matrix production¹¹. Although HA has been found to be useful in reducing pain and symptoms
84 and recovering articular function, Kon et al. found that autologous platelet rich concentrates
85 showed more and longer efficacy than HA, suggesting that using HA scaffold alone may have
86 limited therapeutic effects¹².

87

88 Platelet-rich concentrates have been proposed to improve healing of different tissues due to their
89 abundance of growth factors and cytokines. Platelet-rich plasma (PRP) and platelet-rich fibrin
90 (PRF) have both been shown to promote the repair of articular cartilage defects^{4,13,14}. PRP is a
91 concentrate of platelet-rich plasma derived from whole blood, centrifuged to reduce the number

92 of red blood cells in a liquid form, whereas PRF is composed of autologous platelets which are
93 present in a complex stable fibrin matrix (gel-like consistency).

94

95 There is significant variability in the methods of preparation and composition of platelet-rich
96 concentrates¹⁵⁻¹⁷. Previous studies that have evaluated platelet-rich concentrates have used a
97 variety of protocols for preparation of platelet-rich concentrates with variation in initial blood
98 volume, velocity and time of centrifugation, number of centrifugation cycles, and inclusion or
99 exclusion of leukocytes, which can alter the effect on tissue repair by changing its composition¹⁸.
100 Specifically, platelet-rich concentrates with different compositions have different contents of
101 growth factors and numerous other plasma proteins, which may alter its efficacy^{19,20}. The
102 optimal preparation method of platelet-rich concentrates has not yet been identified, and will
103 likely differ for various tissues and pathologies. Leukocyte-platelet-rich concentrates have been
104 shown to contain higher levels of growth factors and cytokines, as well as inflammatory
105 cytokines that may have a catabolic effect, all of which is currently thought to be related to the
106 presence of leukocytes²¹. Number of centrifugations of the whole blood has been found to alter
107 the cytokine and growth factors release as well as the number of platelets, which has been found
108 to be higher in double spin protocols to prepare PRP²²⁻²⁴. Leukocyte-platelet-rich plasma (L-
109 PRP) have not been found to be effective in stimulating proliferation of new cartilage tissue^{25,26}.
110 It is not currently clear if L-PRF can consistently release growth factors given its
111 microstructure²². Current literature is inconclusive as to the ability of L-PRP and L-PRF to
112 improve cartilage integration²³.

113

114 Both intraarticular injections of platelet rich concentrates and hyaluronic acid have been found
115 independently to improve knee functional status and symptoms over time, however evidence of
116 the superiority of one treatment over another remains mixed²⁷⁻³⁰. The combination of these two
117 therapies (platelet-rich concentrates and HA scaffolds) may allow for optimized cytokine delivery
118 of the platelet concentrates and promote tissue regeneration by allowing cells to migrate into the
119 scaffold, adhere, and produce new matrix to fill the defect. However, minimal research has been
120 performed to investigate the use of scaffold materials in combination with platelet-rich
121 concentrates to enhance cell migration. Therefore, the purpose of this study is to determine if (1)
122 Human L-PRP or (2) L-PRF delivered on a HA scaffold at a bovine chondral defect, a simulated
123 cartilage tear interface, *in vitro* would improve tissue formation based on biomechanical,
124 histological, and biochemical measures. We hypothesized that the use of L-PRP and L-PRF in
125 conjunction with an HA scaffold will improve biomechanical strength, improve cellular
126 migration, have increased sulfated glycosaminoglycan (sGAG), and have increased collagen
127 content when compared to an HA scaffold alone.

128

129

130 **Materials and Methods**

131 Preparation of the Cartilage Plugs:

132 The explant model used in this study has previously been used to evaluate cartilage repair,
133 scaffold integration, and cartilage interface development *in vitro*³¹. Three operators performed
134 the specimen preparations. 24 full-thickness osteochondral plugs were harvested from a bovine
135 femoral condyle and trochlea in a sterile manner using a 7mm diameter osteochondral graft
136 harvester (single use OATS set 7mm, Arthrex, Naples, FL, USA). The diameter of these plugs

137 were 7mm and thickness was 2.5mm. Osteochondral plugs were kept in Advanced DMEM
138 (ADMEM)/F12 with 100 nM dexamethasone, 50 µg/mL ascorbate-2-phosphate, and
139 penicillin/streptomycin (Life Technologies, Carlsbad, CA) within 5% CO₂ and air-humidified
140 incubator at 37 degrees Celsius for 6 hours. They were then washed briefly using phosphate-
141 buffered saline and antibiotics – (penicillin/streptomycin, Life Technologies, Carlsbad, CA). A
142 5.5mm diameter biopsy punch was used to create a circular defect through the center of the
143 explant (the defect had a diameter 5.5mm and a depth of 2.5mm) to allow for a press fit of a
144 6mm diameter scaffold with a thickness of 2.5mm placed within the core. **Figure 1, Figure 2A,**
145 **Figure 2B).**

146

147 Preparation of HA Scaffolds:

148 HA scaffolds (Hyalofast, USA) were used for this study. The scaffolds were punched out using
149 sterile 6 mm biopsy punches (McKesson, San Francisco, USA). The size of the scaffolds was 0.5
150 mm larger than the diameter of the cartilage defect to allow for a press-fit. The thickness of the
151 scaffold was the same as the plug, 2.5mm.

152

153 Groups and Timepoints:

154 Eight specimens were used at each time point in each group: Six for biomechanical testing of
155 interface strength and biochemical analysis of DNA, collagen, and sGAG content, and two
156 specimens used for histology (total number of specimens: 24). The specimen test and timepoint
157 allocation was determined at the time of preparation. The specimens used for biomechanical
158 testing were processed for biochemical evaluation after the biomechanical testing was complete.
159 Biochemical testing was done on specimens from days 28 and 42 as we expected the largest

160 differences between groups at these time points. The early (28 day) time-point was chosen based
161 on prior studies indicating that improvements in functional properties can be seen as early as 28
162 days²⁶. The later time point was selected as we expected there to be greater differences between
163 groups over time.

164

165 Blood Collection:

166 L-PRP and L-PRF was produced from blood samples from three fasting healthy male human
167 donors after they provided informed consent (average age: 34.6, range: 27-39). In each subject a
168 total of 37mL of peripheral venous blood was collected in order to prepare the two platelet
169 concentrates. From these 37mL of whole blood, 1 mL was used to measure platelet- and
170 leukocyte-counts with a cell counter Sysmex KX-21N (Sysmex-Suisse AG, Horgen,
171 Switzerland). Of the addition 36 mL, 27 mL of the blood was collected in a 30-mL syringe
172 containing 3 mL of ACD-A to prepare the L-PRP and 9 mL was collected in 10-mL glass-coated
173 to prepare the L-PRF. In the whole blood specimen the average number of platelets was $188.3 \times$
174 10^3 cells/ μ L ($\pm 19 \times 10^3$ cells/ μ L) ; and the average number of leukocytes was 4.9×10^3 cells/ μ L
175 ($\pm 0.5 \times 10^3$ cells/ μ L). This protocol was approved by the Institutional Review Board.

176

177 L-PRP Preparation:

178 Given that previous literature supports that double-centrifugation results in a greater number of
179 platelets and growth factor release we opted to use a previously described double-centrifugation
180 method^{20,22-24}. 27 mL of the blood was collected in a 30-mL syringe containing 3 mL of ACD-A.
181 The blood was separated into its different cell elements by centrifugation at 160 G for 20 minutes
182 at room temperature (Beckman J-6M Induction Drive Centrifuge, Beckman Instruments Inc.,

183 Palo Alto, CA, USA). This resulted into three basic components: an upper layer referred to as the
184 supernatant that contains most platelets, an intermediate thin layer that is known as the buffy coat
185 which is rich in white blood cells and some platelets, and a bottom layer that contains all the red
186 blood cells. A mark was made 6mm below the line that separated the red blood cell component
187 from the buffy coat and the serum component. All of the content above this mark was removed
188 in order to increase the total amount of platelets collected after the second centrifugation. The
189 sample was then centrifuged again at 400 G for 15 minutes resulting in two components: the
190 supernatant containing the serum, buffy coat containing the platelets and the leukocytes. The L-
191 PRP (approximately 2.7 ml) was separated out. Of this, 500 uL was taken to measure platelet-
192 and leukocyte-counts with a cell counter Sysmex KX-21N (Sysmex-Suisse AG, Horgen,
193 Switzerland).

194

195 L-PRF Preparation:

196 To prepare L-PRF, 9 mL of blood was collected in 10-mL glass-coated, plastic tubes
197 (Vacutainer; BD Biosciences, Allschwil, Switzerland). Using a similar protocol performed by
198 Schär et al., immediately after collection the blood was centrifuged at 400 x g for 12 minutes at
199 room temperature using a table-top centrifuge specifically designed for this application (EBA 20;
200 Andreas Hettich GmbH & Co KG, Tuttlingen, Germany)²³. The L-PRF was then allowed to
201 congeal on a sterilized custom draining system and was cored using a 6 mm biopsy punch,
202 ensuring a consistent volume for each specimen (**Figure 2C**).

203

204 Preparation of Test Groups:

205 The following groups were tested: (1) control group where only a HA scaffold was applied
206 (**Figure 2D**), (2) L-PRP with HA scaffold (**Figure 2E**), (3) L-PRF with HA scaffold (**Figure**
207 **2F**). In the L-PRP groups, the scaffold was soaked for 5 minutes, a time used by previous studies
208 to ensure that PRP had been completely soaked up by the scaffold, in 100uL of undiluted PRP at
209 day 0 prior to being placed into the defect in the culture plates^{32,33} (**Figure 2E**). In the L-PRF
210 groups, a 6mm biopsy punch of the L-PRF was carefully layered around the scaffold and then
211 placed within a bovine cartilage defect in Corning plates taking care to ensure that the scaffold
212 now layered with L-PRF had the same thickness of the defect of 2.5mm(Corning Inc, Corning,
213 NY). There were 8 specimens per plate with a total of 3 plates per timepoint; specimens cultured
214 on the same plate were in the same treatment group. Each dish was then filled with 30ml of
215 media. Therefore, the initial PRP concentration was 3% (amount of PRP/total media volume in
216 the dish). In the control group, only a HA scaffold was placed in the defect. Scaffold–cartilage
217 constructs were cultured in individually wells in culture media the consisted of 30 mL Advanced
218 DMEM (ADMEM)/F12 with 100 nM dexamethasone, 50 µg/mL ascorbate-2-phosphate, and
219 penicillin/streptomycin (Life Technologies, Carlsbad, CA) within 5% CO₂ and air-humidified
220 incubator at 37 degrees Celsius. Media was changed every three days by replacing half of the
221 media in the culture dishes for 28 days and 42 days, respectively. The primary outcome measure
222 was interface strength assessed by a pushout test. The secondary outcome measurements
223 included scaffold DNA content (picogreen assay, Invitrogen, Carlsbad, CA), scaffold sulfated-
224 glycosaminoglycan (sGAG) content (Dimethylmethylene Blue Assay described by Farndale et
225 al.)³⁴, collagen content (hydroxyproline assay described by Stegmann et al.)³⁵, and histology. All
226 biochemical tests were performed on the tissue formed at the scaffold-explant interface.

227

228 Biomechanical Testing

229 The cartilage interface strength was determined using a push-out test. A 2.75-mm stainless steel
230 indenter applied load to the scaffold by advancing at 10 $\mu\text{m/s}$ using a custom built compression
231 testing system previously described by Ng et al.²⁵. The test was considered completed when there
232 was a consistent drop in force. The maximum load was recorded and normalized to the
233 interfacial surface area for each sample to determine the maximum interfacial strength.

234

235 Biochemical Analysis

236 After biomechanical testing samples were digested in papain for analysis of DNA, sGAG, and
237 collagen content. DNA content per specimen was determined using the dsDNA Picogreen Assay
238 (Molecular Probes, Eugene, OR). sGAG content was determined using the 1,9-
239 dimethylmethylene blue dye binding assay with chondroitin-6 sulfate as a standard²⁰. Digested
240 aliquots were also hydrolyzed for 16 h in 12 N HCl at 110°C, and the hydroxyproline (HP)
241 content was quantified via colorimetric reaction with chloramine T (Sigma-Aldrich, St Louis,
242 MO) and 4-Dimethylamino benzaldehyde (Sigma-Aldrich, St Louis, MO), against an HP
243 standard curve³⁵.

244

245 Histological Analysis

246 Histological analysis was undertaken to evaluate the scaffold–cartilage interface. The explants
247 were fixed in neutral buffered formalin with 0.5% cetylpyridinium chloride for 4 hours at room
248 temperature, washed briefly in phosphate-buffered saline (PBS) to remove residue formalin,
249 cryoprotected in a 30% sucrose solution at 4°C overnight, incubated for 2 hours in 5% gelatin +
250 5% sucrose embedding medium, and then embedded in the gelatin–sucrose medium. Blocks

251 were cut on a cryotome for histological analysis of interfacial sGAG deposition and cellular
252 infiltration using Safranin-O/Fast Green histological stain. Slides were reviewed at a
253 magnification of 10x. Consensus grading was done by two evaluators in a blinded fashion, with
254 grading of surface regularity, cellularity, stain uptake, and adherence/bonding of the scaffold to
255 adjacent cartilage.

256

257 Statistical Analysis

258 Two-way ANOVA was performed using Graphpad PRISM 6 software (GraphPad Software, Inc,
259 San Diego, CA, USA, 2015) to compare different conditions, with Tukey's post-hoc testing to
260 identify differences between groups. Significance was set at $p < 0.05$.

261

262 **Results:**

263 **Blood Collection:**

264 The number of platelets within the L-PRP was 188.4×10^4 cells/ μ L, representing a 10-fold
265 increase from the original blood specimens collected. The average number of white blood cells
266 was 15.5×10^3 cells/ μ L, which was a 3.2 fold increase from the original blood specimens
267 collected. The hematocrit value was 36, which was a 0.12 fold decrease from the original blood
268 specimen collected. We did not evaluate changes in cellular concentrations in L-PRF given its
269 gelatinous form.

270

271 Mechanical Strength of the Interface:

272 Following 42 days in culture, L-PRF demonstrated a maximum interfacial strength was
273 significantly greater than the control ($10.92N \pm 9.25$ vs. $0.66N \pm 0.35$, $p=0.015$, **Figure 3, Table 1**).

274 L-PRF though did had higher mean interfacial strength compared to that of L-PRP it was not
275 significantly different ($10.92N \pm 9.25$ vs. $6.58N \pm 10.87$, $p=0.536$). There were no significant
276 differences seen at day 28 between L-PRF, L-PRP, and the control ($3.08N \pm 1.23$, $1.74N \pm 1.07$,
277 $0.63N \pm 0.29$ $p = 0.055$) In all groups, the maximum interfacial strength increased at each time
278 point, though not significantly.

279

280 Biochemical Analysis:

281 The number of cells and thus DNA content of the constructs varied between groups and with
282 culture time (**Figure 4**). The L-PRF group had a significantly greater number of cells compared
283 to the L-PRP and control groups at both 28 ($560.5\mu\text{g} \pm 142.6$, $124.2\mu\text{g} \pm 65.8$, $191.4\mu\text{g} \pm 44.6$
284 $p < 0.001$, **Table 1, Figure 4**) and 42 days ($761.1\mu\text{g} \pm 270.7$, $589.3\mu\text{g} \pm 224.2$, $296.4\mu\text{g} \pm 101.8$, $p <$
285 0.01 , **Figure 4, Table 1**). Across all groups the DNA content increased between days 28 and 42,
286 however only the L-PRP group had a significant increase ($124.2\mu\text{g} \pm 65.8\mu\text{g}$ vs.
287 $589.3\mu\text{g} \pm 224.2\mu\text{g}$, $p=0.0004$, **Figure 4, Table 1**).

288

289 Across groups, the total sGAG production increased from day 28 to day 42 (**Figure 5, Table 1**).
290 L-PRP had larger total sGAG production compared to L-PRF at 28 and 42 days, but only had a
291 significantly greater amount at day 42 ($4.3\mu\text{g} \pm 11.5$ vs $11.6\mu\text{g} \pm 7.2$, $p=0.0095$, **Figure 5, Table**
292 **1**).

293

294 Hydroxyproline assay demonstrated significantly greater collagen content in the L-PRF group
295 compared to the control group on day 28 ($31.2\mu\text{g} \pm 5.3$ vs. $17.6\mu\text{g} \pm 7.5$, $p=0.017$, **Figure 6, Table**
296 **1**), but not to the L-PRP treatment group on day 28 ($31.2\mu\text{g} \pm 5.3$ vs. $21.1\mu\text{g} \pm 10.2$, $p=0.092$,

297 **Figure 6, Table 1).** On day 42 there was a significant difference between L-PRF and the control
298 group ($40.1\mu\text{g}\pm 9.7$ vs. $16.3\mu\text{g}\pm \mu\text{g}$, $20.4\mu\text{g}\pm 6.8$ vs. $p=0.0006$, **Figure 6, Table 1).** There was no
299 significant difference observed between L-PRP and the control group at either timepoint
300 ($21.1\mu\text{g}\pm 10.2$ vs. $17.6\mu\text{g}\pm 7.5\mu\text{g}$, $p=0.723$, $16.3\mu\text{g}\pm 7.6$ vs. $20.4\mu\text{g}\pm 6.8$, $p=0.659$, **Figure 6, Table**
301 **1).**

302

303 Histological evaluation:

304 Most specimens had at least some positive staining for proteoglycans. In all groups the Safranin-
305 O staining increased from day 28 to day 42, suggesting increased cellular infiltration and
306 increased proteoglycan synthesis as demonstrated by the intensity of the staining (**Figure 7).** All
307 the specimens started to form disorganized cartilage-like tissue at the edge with ECM deposition
308 with more apparent cellular integration and extracellular matrix deposition at the edges of the
309 construct in the L-PRP and L-PRF groups. At the interface of the L-PRP group, lacuna
310 structures, typical of cartilage, are observed at the interface. There was more intense Safranin-O
311 staining in the L-PRF and L-PRP groups compared to control on day 42. The L-PRP group had
312 the most intense staining of Safranin-O on day 42.

313

314 **Discussion:**

315 The two principal findings of our study are that L-PRF elicited the greatest degree of cellularity,
316 collagen production, while L-PRP facilitated the greatest amount of sGAG production. There
317 was no superiority observed in biomechanical strength between the platelet concentrates utilized
318 in this study.

319

320 An important finding was that L-PRF had the greatest number of cells. The significant increase
321 in the L-PRF group may be artificially elevated because of the presence of leukocytes in the
322 preparation compared to the control at both day 28 and 42. However, there were quantitatively
323 more cells in the cartilage interface for L-PRF compared to L- PRP (**Figure 4**), which suggests
324 that it may promote cell migration and/or proliferation to a greater extent than L-PRP. The
325 gelatinous nature of L-PRF may allow for sustained release of growth factors over a greater
326 period of time when compared to L-PRP and the control. PRF has been previously been shown
327 to more gradually release growths factors especially in comparison to PRP^{20,36}. Schär et al.
328 specifically found that more TGF- β 1 and VEGF was released from L-PRF compared with L-
329 PRP. The release of TGF- β 1 was bimodal in nature and peaked at 8 hours and 7 days, while L-
330 PRF had a single higher peak at 7 days. VEGF release from L-PRP peaked at 8 hours and 3 days
331 in contrast with L-PRF in which it peaked at 7 days²³. This sustained release may also offset the
332 known catabolic response cells have in response to leukocyte-rich platelet concentrates²¹ as there
333 is clearly an increase in cell number in the L-PRF group. This is supported by the fact that IL-1B,
334 which is known to play a role in inflammation and/or matrix degradation, has been shown to
335 have a more sustained concentration in L-PRP when compared to L-PRF²³. Schär et al. also
336 found better migration *in vitro* for MSC and endothelial cells in L-PRF compared to L-PRP²³.
337 Further, even the preparation of PRP can alter the extent and duration of cytokine release²⁰ and
338 as a result, great care must be taken in ensuring the proper protocol is used for L-PRP given the
339 significant variability in its preparation in the clinical setting.

340

341 Interestingly, our findings demonstrated that L-PRP elicited the greatest amount of sGAG
342 production. Kazemi et al. found that L-PRF and L-PRP demonstrated similar macroscopic and

343 microscopic improvements in articular cartilage defects in a dog model³⁷. However, it should be
344 noted that both centrifuge spin velocity and time differed between our study and theirs making it
345 difficult to truly make comparisons.

346

347 It is important to highlight that although L-PRF seemed to have increased biomechanical
348 strength and collagen content, such improvements were not seen in sGAG accumulation. Instead,
349 L-PRF had the lowest amount of sGAG accumulation compared to the control and L-PRP. This
350 may be secondary to the fact that L-PRP and L-PRF release cytokines and growth factors at
351 different rates and amounts^{20,23,36}. Histological analysis demonstrated that L-PRP-treated
352 specimens had more intense Safranin-O staining at day 42 compared to the other two groups.
353 This finding was expected, as the production of sGAG helps stimulate the production of
354 proteoglycans³⁸. L-PRP appeared to successfully incorporate newly-synthesized sGAG into the
355 matrix in the L-PRP group, resulting in increased staining compared to the HA only group.
356 Further study is required to identify how these platelet concentrates affect production and
357 incorporation of a proteoglycan-rich matrix.

358

359 The mechanism for the differences between groups is not known. We hypothesize that the
360 differences relate to the kinetics of cytokine production and release. The fibrin matrix in the L-
361 PRF may entrap platelets and lead to differences in the kinetics of cytokine release. Schär et al.
362 have shown that growth factor release is different between L-PRF and L-PRP²³. There may also
363 be differences in the platelet number and platelet activation status between the different
364 preparations. There are also differences regarding the presence or absence of leukocytes³⁹.
365 Additionally, given that PRP has a liquid consistency, with media changes there is more likely to

366 be an incremental decrease in residual PRP concentration in the media. Further studies are
367 required to identify the underlying biological mechanisms for the observed differences.

368

369 Currently, while a number of strategies have been suggested, there has been no consistent
370 method for augmenting biological integration of scaffold materials with host cartilage tissue. In
371 addition to stem cells and/or growth factors used experimentally and clinically, several blood-
372 derived products are currently used in clinical practice for the treatment of articular cartilage
373 defects. The most well-known and commonly used are the platelet-rich concentrates. They are
374 prepared by differential centrifugation of autologous whole blood and contain a higher
375 concentration of platelets compared with whole blood. There is an established minimum number
376 of required platelets (250,000) to ensure efficacy of PRP by the FDA⁴⁰, however, the remains a
377 wide variety of protocols currently used to create platelet-rich concentrates suggests that the
378 optimal protocol to promote repair and regeneration of cartilage and other tissues is not yet fully
379 known.

380

381 **Limitations:**

382 This study has a several limitations. We used *in vitro* analysis only for this study. In cell culture
383 cells are grown in artificial non-physiological conditions without mechanical stimulation,
384 limiting the *in vivo* relevance. Additionally, each group was co-cultured within the same dish,
385 which may have allowed for additive effects of circulating growth factors or for individual
386 specimens to influence each other. We did not include gross analysis regarding the extent to
387 which the defect was filled in our study in that all of the scaffolds remained fully present in the
388 defects as it takes at least 10 weeks for the scaffolds to dissolve in culture⁴¹. In regards to our

389 mechanical testing we only measured interfacial shear strength, and we did not evaluate
390 additional mechanical properties of the new tissue (i.e. compressive modulus). When evaluating
391 cellularity via the picogreen assay, we did not specifically investigate chondrocyte proliferation
392 and migration, both of which are important in evaluating healing cartilaginous tissue. We did not
393 determine the platelet or leukocyte count for L-PRF. Prior studies have indirectly assessed the
394 PRF clot platelet count by subtracting the platelet count in the residual serum⁴². Additionally, the
395 choice of using a double centrifugation technique for preparing L-PRP can result in an extended
396 force on the platelets which could possibly alter the microstructure of the platelets²⁴. Finally, this
397 results of this study may be dampened by use of both bovine and human derived factors and its
398 applicability, however, this methodology has been previously used^{43,44} and our study has
399 produced data consistent regarding the ability of L-PRP and L-PRF to facilitate cartilage healing
400 via a scaffold.

401

402 **Conclusions:**

403 Delivery of leukocyte rich platelet concentrates in conjunction with a HA scaffold may allow for
404 improvements in cartilage healing through different pathways. L-PRF was not superior to L-PRP
405 in its biomechanical strength suggesting that both treatments may be effective in improving
406 biomechanical strength of healing cartilage through different pathways.

407

408

409

410 The authors declare no conflicts of interest

411

412

413

414 **Table and Figure Legends:**

415 **Table 1:** Analysis of Biomechanical and Biochemical Data for Day 28 and Day 42; NS = not
416 significant

417 **Figure 1:** *In vitro* model of cartilage plug with scaffold +/- L-PRP/L-PRF

418 **Figure 2:** Photographs of the experimental specimens including: A) osteochondral with punch
419 biopsy defect, B) hyaluronic acid scaffold punchouts, C) L-PRF after preparation prior to punch
420 biopsy, D) hyaluronic acid scaffold plug within the defect of the osteochondral specimen, E)
421 hyaluronic acid scaffold plug saturated with PRP within the defect of the osteochondral
422 specimen F) placement of a hyaluronic acid scaffold plug with PRF layered on it within the
423 defect of the osteochondral specimen

424 **Figure 3:** Treatment with L-PRF and L-PRP led to improved maximum interfacial strength
425 compared to the control; At day 42 L-PRF demonstrated a maximum interfacial strength was
426 significantly greater than the control ($P = 0.015$); (* $P < 0.05$); Footnote: Two-way ANOVA was
427 performed with a significance set to $P < 0.05$

428 **Figure 4:** DNA content increased between days 28 and 42 in all groups, with L-PRF having a
429 significantly larger increase at day 28 ($P < 0.001$) and day 42 ($P < 0.01$); (* $P < 0.05$; ** $P < 0.01$;
430 *** $P < .0001$); Footnote: Two-way ANOVA was performed with a significance set to $P < 0.05$

431 **Figure 5:** Total sGAG production increased from day 28 to day 42, with L-PRP demonstrating
432 the greatest production, which a significant increased compared to L-PRF at day 42 ($p = 0.0095$);
433 (** $P < 0.01$); Footnote: Two-way ANOVA was performed with a significance set to $P < 0.05$

434 **Figure 6:** Collagen content was significantly higher in the L-PRF group compared to both the
435 control and L-PRP groups on day 28 and day 42 (* $P < 0.05$; ** $P < 0.01$); Footnote: Two-way
436 ANOVA was performed with a significance set to $P < 0.05$

437 **Figure 7:** Day 42 Safranin-O histological specimens at 10x original magnification from Left to
 438 Right: L-PRP, L-PRF, Control; All the specimens started to form disorganized cartilage-like
 439 tissue at the edge with ECM deposition with more apparent cellular integration and extracellular
 440 matrix deposition at the edges of the construct in the L-PRP and L-PRF groups. L-PRP group
 441 had the greatest amount of ECM deposition and cellular integration compared to L-PRF and the
 442 control group.

443

444 **References:**

- 445 1. Nehrer S, Domayer S, Dorotka R, Schatz K, Bindreiter U, Kotz R. Three-year clinical
 446 outcome after chondrocyte transplantation using a hyaluronan matrix for cartilage repair.
 447 *Eur J Radiol.* 2006;57(1):3-8.
- 448 2. Park IS, Jin RL, Oh HJ, et al. Sizable Scaffold-Free Tissue-Engineered Articular
 449 Cartilage Construct for Cartilage Defect Repair. *Artif Organs.* 2019;43(3):278-287.
- 450 3. Medvedeva EV, Grebenik EA, Gornostaeva SN, et al. Repair of Damaged Articular
 451 Cartilage: Current Approaches and Future Directions. In: *Int J Mol Sci.* Vol 19.2018.
- 452 4. Sermer C, Devitt B, Chahal J, Kandel R, Theodoropoulos J. The Addition of Platelet-
 453 Rich Plasma to Scaffolds Used for Cartilage Repair: A Review of Human and Animal
 454 Studies. *Arthroscopy.* 2015;31(8):1607-1625.
- 455 5. Ogata Y, Mabuchi Y, Yoshida M, et al. Purified Human Synovium Mesenchymal Stem
 456 Cells as a Good Resource for Cartilage Regeneration. In: *PLoS One.* Vol 10.2015.
- 457 6. Gobbi A, Karnatzikos G, Kumar A. Long-term results after microfracture treatment for
 458 full-thickness knee chondral lesions in athletes. *Knee Surg Sports Traumatol Arthrosc.*
 459 2014;22(9):1986-1996.
- 460 7. Park SH, Park SR, Chung SI, Pai KS, Min BH. Tissue-engineered cartilage using
 461 fibrin/hyaluronan composite gel and its in vivo implantation. *Artif Organs.*
 462 2005;29(10):838-845.
- 463 8. Tognana E, Borriero A, De Luca C, Pavesio A. Hyalograft C: hyaluronan-based
 464 scaffolds in tissue-engineered cartilage. *Cells Tissues Organs.* 2007;186(2):97-103.
- 465 9. Rhodes NP. Inflammatory signals in the development of tissue-engineered soft tissue.
 466 *Biomaterials.* 2007;28(34):5131-5136.
- 467 10. Facchini A, Lisignoli G, Cristino S, et al. Human chondrocytes and mesenchymal stem
 468 cells grown onto engineered scaffold. *Biorheology.* 2006;43(3,4):471-480.
- 469 11. Matsiko A, Levingstone TJ, O'Brien FJ, Gleeson JP. Addition of hyaluronic acid
 470 improves cellular infiltration and promotes early-stage chondrogenesis in a collagen-
 471 based scaffold for cartilage tissue engineering. *J Mech Behav Biomed Mater.* 2012;11:41-
 472 52.
- 473 12. Kon E, Mandelbaum B, Buda R, et al. Platelet-rich plasma intra-articular injection versus
 474 hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early
 475 degeneration to osteoarthritis. *Arthroscopy.* 2011;27(11):1490-1501.

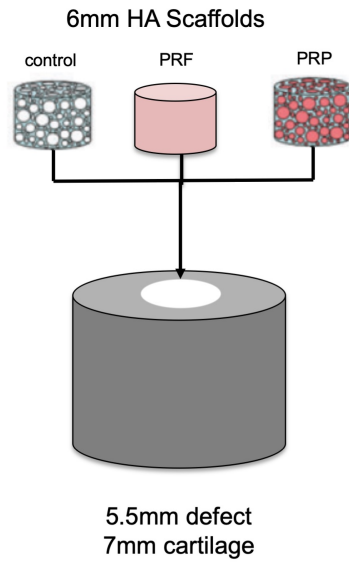
- 476 13. Haleem AM, Singergy AA, Sabry D, et al. The Clinical Use of Human Culture-Expanded
477 Autologous Bone Marrow Mesenchymal Stem Cells Transplanted on Platelet-Rich Fibrin
478 Glue in the Treatment of Articular Cartilage Defects: A Pilot Study and Preliminary
479 Results. *Cartilage*. 2010;1(4):253-261.
- 480 14. Xie X, Wang Y, Zhao C, et al. Comparative evaluation of MSCs from bone marrow and
481 adipose tissue seeded in PRP-derived scaffold for cartilage regeneration. *Biomaterials*.
482 2012;33(29):7008-7018.
- 483 15. Chahla J, Cinque ME, Piuzzi NS, et al. A Call for Standardization in Platelet-Rich
484 Plasma Preparation Protocols and Composition Reporting: A Systematic Review of the
485 Clinical Orthopaedic Literature. *The Journal of bone and joint surgery American volume*.
486 2017;99(20):1769-1779.
- 487 16. Grecu AF, Reclaru L, Ardelean LC, Nica O, Ciucă EM, Ciurea ME. Platelet-Rich Fibrin
488 and Its Emerging Therapeutic Benefits for Musculoskeletal Injury Treatment. In:
489 *Medicina (Kaunas)*. Vol 55.2019.
- 490 17. Oudelaar BW, Peerbooms JC, Huis In 't Veld R, Vocteloo AJH. Concentrations of
491 Blood Components in Commercial Platelet-Rich Plasma Separation Systems: A Review
492 of the Literature. *The American journal of sports medicine*. 2019;47(2):479-487.
- 493 18. Everts PA, Brown Mahoney C, Hoffmann JJ, et al. Platelet-rich plasma preparation using
494 three devices: implications for platelet activation and platelet growth factor release.
495 *Growth Factors*. 2006;24(3):165-171.
- 496 19. Assirelli E, Filardo G, Mariani E, et al. Effect of two different preparations of platelet-
497 rich plasma on synoviocytes. *Knee Surg Sports Traumatol Arthrosc*. 2015;23(9):2690-
498 2703.
- 499 20. Roh YH, Kim W, Park KU, Oh JH. Cytokine-release kinetics of platelet-rich plasma
500 according to various activation protocols. In: *Bone Joint Res*. Vol 5.2016:37-45.
- 501 21. Cavallo C, Filardo G, Mariani E, et al. Comparison of platelet-rich plasma formulations
502 for cartilage healing: an in vitro study. *The Journal of bone and joint surgery American*
503 *volume*. 2014;96(5):423-429.
- 504 22. Nagata MJH, Messori MR, Furlaneto FAC, et al. Effectiveness of Two Methods for
505 Preparation of Autologous Platelet-Rich Plasma: An Experimental Study in Rabbits. In:
506 *Eur J Dent*. Vol 4.2010:395-402.
- 507 23. Schär MO, Diaz-Romero J, Kohl S, Zumstein MA, Nestic D. Platelet-rich Concentrates
508 Differentially Release Growth Factors and Induce Cell Migration In Vitro. In: *Clin*
509 *Orthop Relat Res*. Vol 473.2015:1635-1643.
- 510 24. Croise B, Pare A, Joly A, Louisy A, Laure B, Goga D. Optimized centrifugation
511 preparation of the platelet rich plasma: Literature review. *J Stomatol Oral Maxillofac*
512 *Surg*. 2019.
- 513 25. Ng KW, Wanivenhaus F, Chen T, et al. A Novel Macroporous Polyvinyl Alcohol
514 Scaffold Promotes Chondrocyte Migration and Interface Formation in an In Vitro
515 Cartilage Defect Model. In: *Tissue Eng Part A*. Vol 18.2012:1273-1281.
- 516 26. Madry H, Kaul G, Cucchiari M, et al. Enhanced repair of articular cartilage defects in
517 vivo by transplanted chondrocytes overexpressing insulin-like growth factor I (IGF-I).
518 *Gene Ther*. 2005;12(15):1171-1179.
- 519 27. Di Martino A, Di Matteo B, Papio T, et al. Platelet-Rich Plasma Versus Hyaluronic Acid
520 Injections for the Treatment of Knee Osteoarthritis: Results at 5 Years of a Double-Blind,

- 521 Randomized Controlled Trial. *The American journal of sports medicine*. 2019;47(2):347-
522 354.
- 523 28. Duymus TM, Mutlu S, Dernek B, Komur B, Aydogmus S, Kesiktas FN. Choice of intra-
524 articular injection in treatment of knee osteoarthritis: platelet-rich plasma, hyaluronic acid
525 or ozone options. *Knee Surg Sports Traumatol Arthrosc*. 2017;25(2):485-492.
- 526 29. Filardo G, Di Matteo B, Di Martino A, et al. Platelet-Rich Plasma Intra-articular Knee
527 Injections Show No Superiority Versus Viscosupplementation: A Randomized Controlled
528 Trial. *The American journal of sports medicine*. 2015;43(7):1575-1582.
- 529 30. Meheux CJ, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of Intra-
530 articular Platelet-Rich Plasma Injections in Knee Osteoarthritis: A Systematic Review.
531 *Arthroscopy*. 2016;32(3):495-505.
- 532 31. Xie X, Zhang C, Tuan RS. Biology of platelet-rich plasma and its clinical application in
533 cartilage repair. *Arthritis Res Ther*. 2014;16(1):204.
- 534 32. Betsch M, Schnependahl J, Thuns S, et al. Bone Marrow Aspiration Concentrate and
535 Platelet Rich Plasma for Osteochondral Repair in a Porcine Osteochondral Defect Model.
536 In: *PLoS One*. Vol 8.2013.
- 537 33. Kon E, Filardo G, Delcogliano M, et al. Platelet autologous growth factors decrease the
538 osteochondral regeneration capability of a collagen-hydroxyapatite scaffold in a sheep
539 model. In: *BMC Musculoskelet Disord*. Vol 11.2010:220.
- 540 34. Farndale RW, Sayers CA, Barrett AJ. A direct spectrophotometric microassay for
541 sulfated glycosaminoglycans in cartilage cultures. *Connect Tissue Res*. 1982;9(4):247-
542 248.
- 543 35. Stegemann H, Stalder K. Determination of hydroxyproline. *Clin Chim Acta*.
544 1967;18(2):267-273.
- 545 36. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma:
546 from basic science to clinical applications. *The American journal of sports medicine*.
547 2009;37(11):2259-2272.
- 548 37. Kazemi D, Fakhrjou A. Leukocyte and Platelet Rich Plasma (L-PRP) Versus Leukocyte
549 and Platelet Rich Fibrin (L-PRF) For Articular Cartilage Repair of the Knee: A
550 Comparative Evaluation in an Animal Model. *Iran Red Crescent Med J*.
551 2015;17(10):e19594.
- 552 38. Bassleer C, Rovati L, Franchimont P. Stimulation of proteoglycan production by
553 glucosamine sulfate in chondrocytes isolated from human osteoarthritic articular cartilage
554 in vitro. *Osteoarthritis Cartilage*. 1998;6(6):427-434.
- 555 39. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second-generation
556 platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med*
557 *Oral Pathol Oral Radiol Endod*. 2006;101(3):e37-44.
- 558 40. Jones IA, Togashi RC, Thomas Vangsness C. The Economics and Regulation of PRP in
559 the Evolving Field of Orthopedic Biologics. In: *Curr Rev Musculoskelet Med*. Vol
560 11.2018:558-565.
- 561 41. Solchaga LA, Temenoff JS, Gao J, Mikos AG, Caplan AI, Goldberg VM. Repair of
562 osteochondral defects with hyaluronan- and polyester-based scaffolds. *Osteoarthritis*
563 *Cartilage*. 2005;13(4):297-309.
- 564 42. Karde PA, Sethi KS, Mahale SA, Khedkar SU, Patil AG, Joshi CP. Comparative
565 evaluation of platelet count and antimicrobial efficacy of injectable platelet-rich fibrin

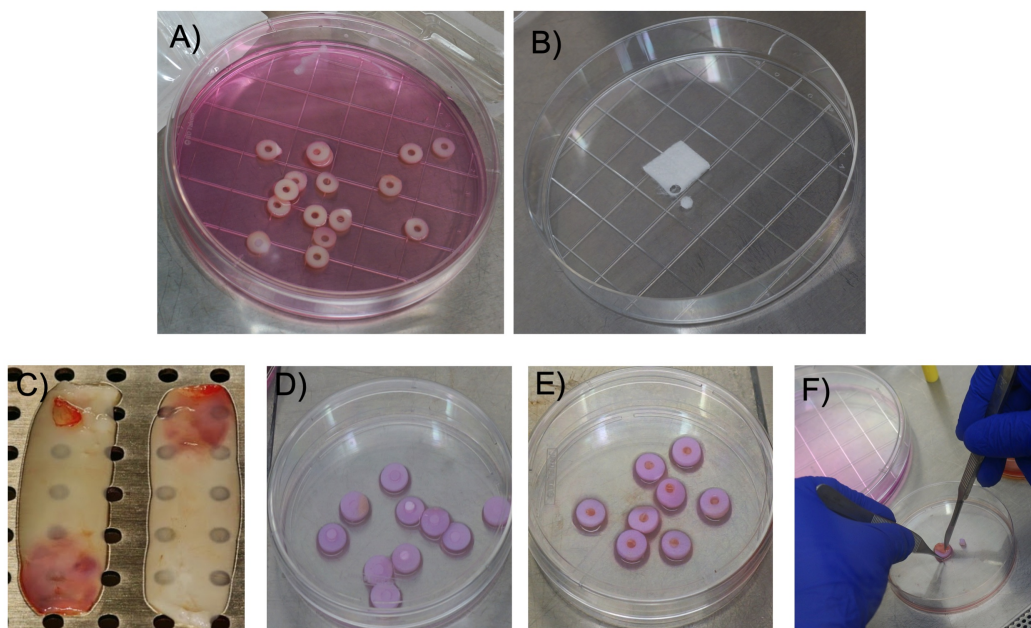
- 566 with other platelet concentrates: An in vitro study. *J Indian Soc Periodontol.*
567 2017;21(2):97-101.
- 568 43. Matuska A, O'Shaughnessey K, King W, Woodell-May J. Autologous solution protects
569 bovine cartilage explants from IL-1alpha- and TNFalpha-induced cartilage degradation.
570 *Journal of orthopaedic research : official publication of the Orthopaedic Research*
571 *Society.* 2013;31(12):1929-1935.
- 572 44. Zhang Y, Morgan BJ, Smith R, et al. Platelet-rich plasma induces post-natal maturation
573 of immature articular cartilage and correlates with LOXL1 activation. *Scientific reports.*
574 2017;7(1):3699.
- 575

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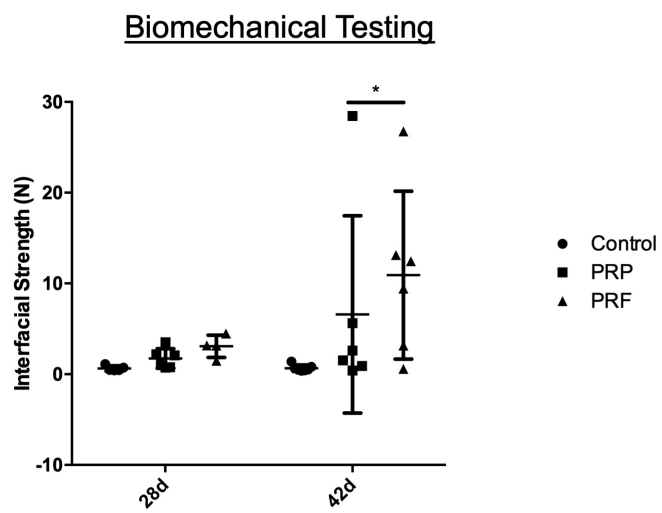
Comparison Group 1 / Comparison Group 2	Day 28		Day 42	
	Group 1 Mean \pm SD / Group 2 Mean \pm SD	P-Value	Group 1 Mean \pm SD / Group 2 Mean \pm SD	P-Value
Interfacial Strength (N)				
L-PRP/L-PRF	1.74 \pm 1.1/3.08 \pm 1.2	NS	6.58 \pm 10.9/10.92 \pm 9.3	NS
L-PRP/HA Only	1.74 \pm 1.1/0.63 \pm 0.3	NS	6.58 \pm 10.9/0.66 \pm 0.4	0.015
L-PRF/HA Only	3.08 \pm 1.2/0.63 \pm 0.3	NS	10.92 \pm 9.3/0.66 \pm 0.4	NS
DNA Content (μg)				
L-PRP/L-PRF	124.2 \pm 65.8/560.5 \pm 142.6	0.0002	589.3 \pm 224.2/761.1 \pm 270.7	NS
L-PRP/HA Only	124.2 \pm 65.8/191.4 \pm 44.6	NS	589.3 \pm 224.2/296.4 \pm 101.8	0.0125
L-PRF/HA Only	560.5 \pm 142.6/191.4 \pm 44.6	0.0015	761.1 \pm 270.7/296.4 \pm 101.8	<0.0001
sGAG (μg)				
L-PRP/L-PRF	0.5 \pm 1.1/0.0 \pm 0.0	NS	16.0 \pm 11.5/4.3 \pm 7.2	NS
L-PRP/HA Only	0.5 \pm 1.1/2.1 \pm 2.5	NS	16.0 \pm 11.5/9.7 \pm 7.1	NS
L-PRF/HA Only	0.0 \pm 0.0/2.1 \pm 2.5	NS	4.3 \pm 7.2/9.7 \pm 7.1	0.0095
Collagen Content (μg)				
L-PRP/L-PRF	21.1 \pm 10.2/31.2 \pm 5.3	NS	16.3 \pm 7/40.1 \pm 9.7	<0.0001
L-PRP/HA Only	21.1 \pm 10.2/17.6 \pm 7.5	NS	16.3 \pm 7/20.4 \pm 6.8	NS
L-PRF/HA Only	31.2 \pm 5.3/17.6 \pm 7.5	NS	40.1 \pm 9.7/20.4 \pm 6.8	0.0006

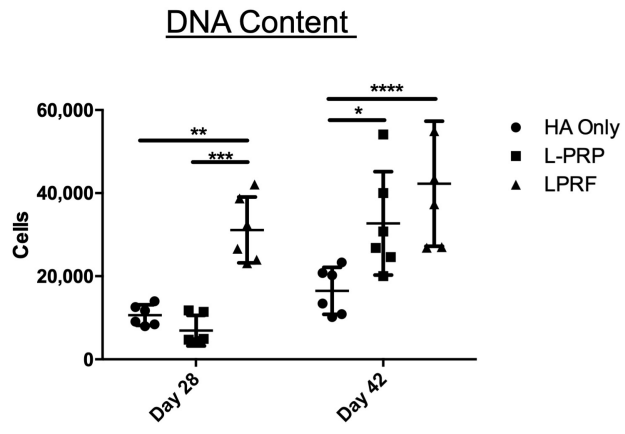


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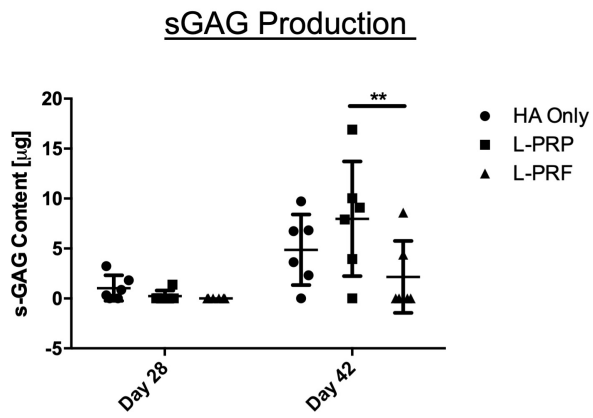


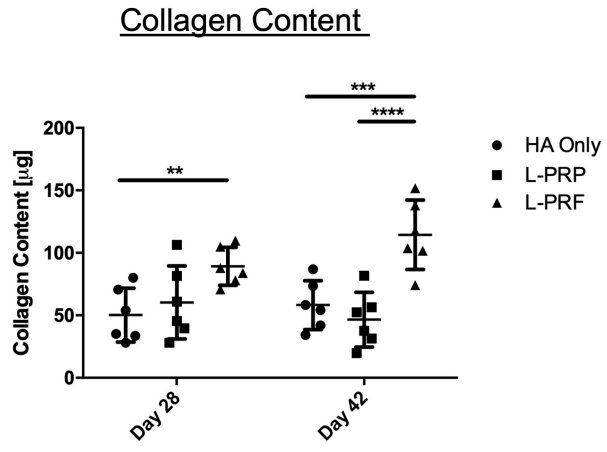
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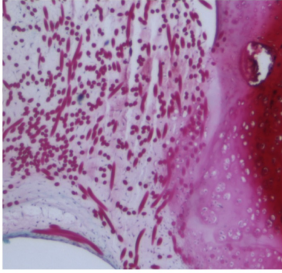
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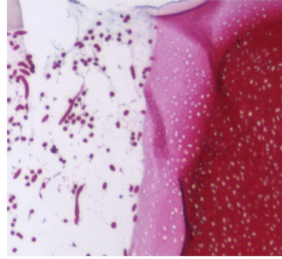
L-PRP



Scaffold

Plug

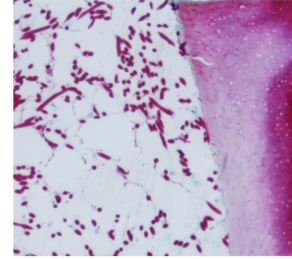
L-PRF



Scaffold

Plug

HA Only



Scaffold

Plug

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