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The Up-Scale Manufacture of Chondrocytes for Allogeneic Cartilage Therapies

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Key Words: Chondrocytes, hollow-fibre bioreactor, large-scale expansion, allogeneic cell therapy, cartilage cell repair, human platelet lysate, fetal bovine serum

33 **Abstract**

34 **Background:** Allogeneic chondrocyte therapies need to be developed to allow more
35 individuals to be treated with a cell therapy for cartilage repair and to reduce the burden
36 and cost of current two-stage autologous procedures. Up-scale manufacture of
37 chondrocytes using a bioreactor could help provide an off-the-shelf allogeneic chondrocyte
38 therapy with many doses being produced in a single manufacturing run. Here we assess a
39 Good Manufacturing Practice compliant hollow-fibre bioreactor (Quantum®) for adult
40 chondrocyte manufacture.

41 **Methods:** Chondrocytes were isolated from knee arthroplasty derived cartilage (n=5) and
42 expanded in media supplemented with 10% fetal bovine serum (FBS) or 5% human platelet
43 lysate (hPL) on tissue culture plastic (TCP) for a single passage. hPL supplemented cultures
44 were then expanded in the Quantum® bioreactor for a further passage. Matched, parallel
45 cultures in hPL or FBS were maintained on TCP. Chondrocytes from all culture conditions
46 were characterised in terms of growth kinetics, morphology, immunoprofile, chondrogenic
47 potential (chondrocyte pellet assays) and single telomere length analysis.

48 **Results:** Quantum® expansion of chondrocytes resulted in $86.4\pm38.5\times10^6$ cells in 8.4±1.5
49 days, following seeding of $10.2\pm3.6\ \times10^6$ cells. This related to 3.0±1.0 population doublings in
50 the Quantum® bioreactor, compared with 2.1±0.6 and 1.3±1.0 on TCP in hPL and FBS
51 supplemented media, respectively. Quantum® and TCP expanded cultures retained
52 equivalent chondropotency and mesenchymal stromal cell markers immunoprofiles, with
53 only integrin marker, CD49a, decreasing following Quantum® expansion. Quantum®
54 expanded chondrocytes demonstrated equivalent chondrogenic potential (as assessed by
55 ability to form and maintain chondrogenic pellets) with matched hPL TCP populations. hPL
56 manufacture however, led to reduced chondrogenic potential and increased cell surface
57 positivity of integrins CD49b, CD49c and CD51/61 compared with FBS cultures. Quantum®
58 expansion of chondrocytes did not result in shortened 17p telomere length when compared
59 with matched TCP cultures.

60 **Discussion:** This study demonstrates that large numbers of adult chondrocytes can be
61 manufactured in the Quantum® hollow-fibre bioreactor. This rapid, up-scale expansion, does
62 not alter chondrocyte phenotype when compared with matched TCP expansion. Therefore,
63 the Quantum® provides an attractive method of manufacturing chondrocytes for clinical
64 use. Media supplementation with hPL for chondrocyte expansion may, however, be
65 unfavourable in terms of retaining chondrogenic capacity.

Impact Statement

This is the first study, to our knowledge, to manufacture adult chondrocytes in a Good Manufacturing Practice (GMP) compliant hollow fibre bioreactor (Quantum®). We provide evidence that chondrocytes can be manufactured using this methodology whilst retaining comparable properties to chondrocytes expanded in matched tissue culture plastic conditions. Up-scale Quantum® expansion may provide an appropriate method for developing allogeneic chondrocyte therapies.

Introduction

The use of autologous chondrocyte implantation (ACI) to treat chondral/osteochondral defects has been applied clinically for nearly 30 years [1]. Despite this, advancement of such cell therapies is needed to reduce their cost and to improve their availability to a wider patient population. Currently, the production of autologous chondrocytes as Advanced Therapy Medicinal Products (ATMPs) is laborious and costly due to the need to produce a cell product for each patient. Furthermore, chondrocyte therapies are limited by the number of cells that can be expanded within a limited timeframe using traditional tissue culture plastic (TCP) methods. Currently, multiple (2-3) passages are required to achieve the numbers of cells used to treat small defects [2] and thus treatment of multiple or large chondral/osteochondral lesions is often not possible, restricting the option of this therapy for some patients.

The move from autologous to allogeneic chondrocyte therapy has the potential to produce large quantities of homogenous cells that can be cultured from 'optimal' donors, selected for their therapeutic potential, to produce an 'off-the-shelf' bank of multiple doses. Along with the beneficial cost implications, this would allow for a 'single-stage' surgical procedure as opposed to the current ACI treatment in which the patient undergoes two surgeries (the first to harvest a cartilage biopsy and the second to implant the patients' culture-expanded chondrocytes). In order for allogeneic chondrocyte therapy to become a reality, a good

92 manufacturing practice (GMP)-compliant process in which large numbers of high quality
93 chondrocytes can be cultured is needed.

94 We have previously demonstrated that use of the Quantum® bioreactor (manufactured by
95 TerumoBCT, Lakewood, USA) can generate large numbers of bone marrow- and umbilical
96 cord-derived mesenchymal stromal cells (MSCs), which maintain their phenotypic properties
97 following rapid expansion [3]. This bioreactor system has been widely used to expand human
98 MSCs from various tissue sources, including production of cells used clinically, as we have
99 reviewed and illustrated in Hulme and Mennan et al., 2023 [4]. We are, however, unaware of
100 any studies that have culture expanded chondrocytes using this platform. The Quantum™
101 cell expansion system is comprised of hollow fibres that provide a surface area of 2.1 m^2 for
102 cell adherence, equivalent to 120 T175 flasks used in standard tissue culture practise. The
103 Quantum™ is GMP-compliant, having been used in multiple clinical trials [4]. All the
104 constituent parts of the bioreactor are produced to a safety and quality standard, with
105 relevant documentation and traceability such that the European Medicine Agency approve
106 the use for GMP [5]. For expansion of adherent cells, the polysulphone fibres are lined with a
107 thin surface coating of a substrate to allow for the attachment of a monolayer of cells. Once
108 cells are adhered to the fibres, culture media is continuously perfused through the fibres
109 and the cell growth is assessed through monitoring of cellular metabolism by measuring the
110 secretion of lactate into and also the consumption of glucose from the conditioned media.
111 The flow rate at which media is perfused over the cells is altered as the cell numbers
112 increase to maximise cell expansion.

113 In the ACI procedure, chondrocytes are expanded in media supplemented with autologous
114 serum collected from patients by venepuncture at the time of initial surgery for cartilage
115 harvest [2,6]. For large-scale allogeneic cultures this would not represent a viable serum
116 source. Despite, clinical-grade foetal bovine serum (FBS) currently being used in the culture

117 of GMP-compliant ATMPs, identification of xeno-free alternatives to FBS are needed to
118 improve the safety profile of these products while adhering to regulatory policies [7]. The use
119 of pooled human platelet lysate (hPL) provides an attractive alternative which is
120 manufactured to GMP standards and without the risk of xenogeneic reaction or transmission
121 of bovine pathogens [8]. Akin to FBS, hPL is rich in vitamins, minerals, cytokines and growth
122 factors and has been demonstrated to support culture of numerous cell types, particularly
123 MSCs [9]. However, the culture of chondrocytes in FBS remains the 'gold standard' for
124 research purposes against which alternative serum sources should be compared [9].

125 In this study we have assessed the potential of the Quantum® bioreactor as a platform for
126 the large-scale culture expansion of chondrocytes. This has been undertaken using a two-
127 step 'hybrid' process in which chondrocytes were isolated and expanded via standard
128 protocols using TCP [2,10] in culture media supplemented with either 10% FBS or 5% hPL
129 (Stemulate™, Cook Regentec, Indianapolis, IN, USA). Those chondrocytes culture expanded
130 in 5% hPL were either seeded in the Quantum® or onto TCP (again in 5% hPL) and 10% FBS
131 cultured cells were seeded onto TCP (in 10% FBS) for the second expansion phase. The need
132 for a hybrid process allows for the limited number of chondrocytes that can be isolated to be
133 culture expanded such that a sufficient number can be seeded into the large surface area of
134 the Quantum® bioreactor. We have carried out a comprehensive characterisation of the
135 Quantum® expanded chondrocytes and compared them to parallel cultures on TCP both
136 using hPL and FBS, determining whether these cells maintain their chondrogenic phenotype
137 via assessment of a panel of chondropotency markers and chondrogenesis assays, as well as
138 evaluating the effect of rapid expansion in relation to telomere length distributions.

139

140

141

142 ## Methods

143 **Patients**

144 Cartilage samples were collected with informed consent from five patients undergoing total
145 knee replacement (TKR) surgery for the treatment of end-stage osteoarthritis (OA). Ethical
146 approvals from the National Research Ethics Service- North West Committee (11/NW/0875)
147 was in place. The individual donors were aged 56-71 and there were four females and one
148 male (Table 1).

149 **Chondrocyte isolation and expansion**

150 Femoral condyles from patients undergoing TKR surgery were assessed for areas of
151 macroscopically normal articular cartilage. Maintaining sterile conditions, this full depth
152 cartilage was excised, weighed and minced into small pieces which were then digested for
153 16 hours at 37 °C using collagenase (250 IU/mg dry weight, Worthington, New Jersey, USA)
154 in serum free Dulbecco's Modified Eagle's Medium/F-12 (DMEM/F-12; Life Technologies,
155 Paisley, UK). Following digestion, the media/cell suspension was strained using a 40μm cell
156 strainer and then centrifuged at 350*g* for 10 minutes to produce a cell pellet which was
157 reconstituted in 1ml media. Following a cell count, approximately 75% of the cells were
158 seeded on TCP (Sarstedt, Leicester, UK) in DMEM-F12 with 1% (v/v) penicillin/streptomycin
159 (P/S; Life Technologies, Paisley, UK) and 5% human platelet lysate (hPL; Stemulate®, Cook
160 Regentec, Indianapolis US), hereafter referred to as hPL-medium. hPL was used from a
161 commercial source, Cook Regentec, which produced hPL to a GMP-compliant standard. In all
162 manufacturing runs the same batch was used to minimise the influence between donors.
163 The proportion of hPL in the media (5%) was used based on recommendations from the
164 supplier and as this is the most widely published concentration in studies looking to move
165 away from foetal bovine serum (FBS) [11]. Seeding of cells into the hPL was prioritised to
166 ensure there were sufficient cells at P0-1 to seed into the Quantum® bioreactor. The
167 remaining cells were seeded in DMEM-F12 with 1% P/S and 10% FBS (Life Technologies,

168 Paisley, UK), hereafter referred to as FBS-medium. The FBS-medium, represents the typical
169 chondrocyte culture medium, where 10% FBS is typically used as a 'gold standard'
170 comparator [6]. All donor samples were maintained separately (non-pooled) and expanded in
171 hPL or FBS (i.e. to compare matched donors expansion in the different supplements on TCP
172 or in the Quantum™). Chondrocytes were seeded at $5x10^3$ cells/cm^2 and were maintained in
173 a humidified atmosphere at 37°C with 21% O_2 and 5% CO_2, changing the media every 2-3
174 days. This culture expansion phase is deemed passage 0 (P0).
175 Once the chondrocytes cultured in hPL-medium reached 70-80% confluence, they were
176 trypsinised and seeded into the Quantum® or re-seeded onto TCP at 5 $x10^3$ cells/cm^2 in hPL-
177 medium (deemed passage 1 (P1)). The numbers of chondrocytes seeded into the Quantum®
178 or onto TCP are detailed in Table 2. Chondrocytes initially seeded onto TCP in FBS-medium
179 were trypsinised at 70-80% confluence and then re-seeded onto TCP at 5 $x10^3$ cells/cm^2 in
180 FBS-medium (P1).

181 **The Quantum® cell expansion system**

182 The Quantum® cell expansion system was prepared as described previously [3]. Briefly, the
183 system was pre-coated overnight with 100 ml of pooled human cryoprecipitate from five
184 donors (NHS Bloods and Transplant, Birmingham, UK) diluted 1:1 (v/v) with PBS. This coating
185 allows for the adherence of cells to the polysulphone hollow fibres. The system was
186 conditioned with hPL-media, after which 5-10 $x10^6$ chondrocytes were seeded into the
187 Quantum® cell expansion system and were left to adhere with uniform suspension for 24
188 hours. The Quantum® cell expansion system maintained perfusion of the hPL-medium over
189 the cells whilst removing an equal volume of conditioned medium. The concentration of
190 lactate and glucose within the conditioned medium were assessed daily using a Lactate Plus
191 meter (Nova Biomedical, Runcorn, UK) and a clinical blood glucose meter (Kinetik Wellbeing,
192 Redhill, UK), respectively. The lactate and glucose concentrations served as indicators of
193 cellular metabolism and consequently as a proxy of cell number. As the number of cells

194 within the system increased, the perfusion rate of fresh medium was increased from a
195 baseline rate of 0.1 ml/min to 1.6 ml/min. Once a flow rate of 1.6 ml/min was achieved,
196 chondrocytes were cultured for a further 12-24 hours before being harvested. Alternatively,
197 chondrocytes were harvested if their growth rate was deemed to plateau, as assessed via
198 conditioned medium lactate and/or glucose concentration. Chondrocytes were harvested
199 using *TrypLE™ (Gibco, New York, US),* as this product can easily be switched to a GMP-
200 compliant alternative should the process be adopted for ATMP manufacture. Briefly, the
201 pre-programmed harvest protocol from TerumoBCT was used in which the hollow fibers
202 containing the cells were fully washed with PBS; TrypLE loaded into the fibers and held at
203 37°C for 8 minutes and then a matched volume of culture medium added and flushed out of
204 the system into a cell harvest bag. This process was repeated twice to ensure all cells were
205 harvested. Harvested cells, in TrypLE and culture medium, were aliquoted into tubes prior to
206 centrifugation at 400*g* for 10 minutes to produce a cell pellet, which was reconstituted in
207 1ml media.

208 **Calculation of growth kinetics**

209 Doubling time (DT) was calculated using the formula $DT = (t2 - t1) \times (\ln(2)/\ln(\frac{n2}{n1})$

210 where $t1$ is the time at seeding, $t2$ is the time at harvesting, $n1$ is the cell number at seeding
211 and $n2$ is the cell number at harvest. To calculate the number of population doublings of
212 chondrocytes the following formula was used: $DT = 3.32 * (logN_2 - logN_1)$, where
213 N_1 is the cell number at seeding and N_2 is the cell number at harvest.

214 **Flow cytometry immunoprofiling of chondrocytes**

215 Chondrocytes were harvested by trypsinisation at P0 and P1 from TCP and/or Quantum®
216 systems. Cells were centrifuged, counted and prepared at 20,000 cells per tube. The cells
217 were blocked using 10% human IgG in 2% bovine serum albumin (BSA) and then
218 resuspended in 2% BSA for flow cytometry. The following fluorochrome-conjugated

219 antibodies were used to assess chondrogenic potency markers [10,12–16]: CD166-Brilliant Violet
220 421 (BV421) (clone 3A6), CD39- Allophycocyanin (APC) (clone TU66), CD44- Peridinin-
221 chlorophyll proteins-Cyanine 5.5 (PerCP-Cy5.5) (clone G44-26)(all from Becton Dickinson and
222 Company, Oxford, UK) and CD151-PE (clone14A2.H1) (R&D Systems, Abingdon, UK). Markers
223 to indicate MSC profiles [17] were assessed using antibodies: CD105-APC (clone 266), CD73-
224 BV421 (clone AD2), CD90- Phycoerythrin (PE) (clone 5E10), CD19-BV421 (clone HIB19),
225 CD45-PE (clone HI30), CD34-APC (clone 581) and CD14-PerCP-Cy5.5 (clone MϕP9) (all
226 antibodies Becton Dickinson and Company, Oxford, UK). Antibodies used to assess integrin
227 immunoprofiles were CD29-APC (MAR4), CD49a-PE (clone SR84), CD49b-BV421 (clone 12F1),
228 CD49c-PE (clone C3 II.1) and CD151/61-PE (clone 23C6) (also all from Becton Dickinson and
229 Company, Oxford, UK).

230 Chondrogenic potency was also assessed using an antibody to intracellular SOX-9 (clone 3C
231 10; Abcam, Cambridge, UK). Briefly, cells were fixed in 80% (v/v) methanol, permeabilised in
232 0.1% (v/v) tween-20 in PBS, blocked in 0.1% (v/v) tween-20 in PBS with 10% human IgG,
233 then resuspended in 0.1% (v/v) tween-20 in PBS for flow cytometry.

234 Isotype-matched IgG controls were used in the gating strategy for all antibodies. Flow
235 cytometry analysis was performed using a FACSCanto II flow cytometer using Diva 7
236 software (Becton Dickinson & Company, Oxford, UK).

237 **<u>Chondrogenic Differentiation assays</u>**

238 Chondrogenic pellet cultures were established following expansion in the Quantum® or on
239 matched TCP conditions. In brief, $2x10^5$ chondrocytes per pellet were centrifuged at 500*g* for
240 8 minutes in chondrogenic differentiation media. This comprised of 1% Insulin Transferrin
241 Selenium (ITS-G; Gibco™, FisherScientific, UK), 10 $ngml^{-1}$ Transforming Growth Factor- β
242 (TGF-β; PeproTech, USA), 1 mM ascorbic acid-2-phosphate, 10 µM dexamethasone, 20 µM
243 linoleic acid, 1 mM sodium pyruvate (all Sigma Aldrich, UK) and 1% P/S (Life Technologies,

244 Paisley, UK) made up in DMEM-F12 (Life Technologies, Paisley, UK). Following three days in
245 static culture, the chondrogenic pellets were dislodged from the Eppindorf tube. Media was
246 changed on the pellets every 2-3 days and the pellets were maintained in culture for 28
247 days, then washed with PBS (Life Technologies, Paisley, UK) and snap frozen in liquid
248 nitrogen. Frozen chondrogenic pellets were stored at -80°C until subsequent analysis.

249 **Histological Analysis of Chondrogenic Pellets**

250 Chondrogenic pellets were cryosectioned (7 μm) using a cryostat (Bright Instrument Co Ltd,
251 Huntingdon, UK) onto poly-L-lysine coated slides. Slides were stained for glycosaminoglycans
252 (GAGs) using the metachromatic stain, 1% aqueous toluidine blue (BDH) covering the slides
253 for 30 seconds and then washing in tap water. Following air drying, slides were mounted in
254 Pertex (Cell Path Ltd, Newtown, UK).

255 **Glycosaminoglycan (GAG)/DNA Analysis of Chondrogenic Pellets**

256 Chondrogenic pellets were digested in 125 μg/ml papain, made up in a buffer of 5 mM
257 EDTA, 5 mM cysteine hydrochloride and 0.1 M sodium phosphate (all Sigma Aldrich, UK)
258 and adjusted to pH 6.5, for 3 hours at 60°C. The pellets in buffered papain were vortexed
259 every 30 minutes throughout the 3 hour digest, to release GAGs and DNA. These samples
260 were then centrifuged at 1000*g* for five minutes and stored at -20°C for subsequent analysis.

261 Quantitative assessment of GAG concentration was performed to indicate the capacity of
262 the chondrocytes to form extracellular matrix when driven towards chondrogenesis, in
263 pellet assays. This method is widely used to provide an indication of the chondrocyte's
264 cartilage forming potential and hence their likely capacity to repair damaged cartilage [10,18–20].
265 GAGs were quantitated using the dimethyl blue (DMMB) assay [21,22]. Bovine trachea derived
266 chondroitin sulphate (Sigma Aldrich, UK) was used to prepare standards in PBS, with serial
267 dilutions from 0 to 20 μg/ml. Fifty microlitres of sample or standard and 200 μl of 4x DMMB

staining solution was combined per well of a 96 well plate. The assay absorbance was immediately read at 530 nm. The total GAG content for each sample was calculated using the equation of the linear portion of the standard curve.

A picogreen assay (Invitrogen, Massachusetts, US) was used to quantitate the amount of double stranded DNA in the papain digested pellet solution. The assay was performed according to manufacturer's instructions. Assay fluorescence was measured on a plate reader (Omega FLUROStar, BMG Labtech, Ortenberg, Germany) with excitation at 480 nm and emission at 520 nm.

The GAG content of each chondrogenic pellet was normalised to its DNA content, calculated by dividing the total GAG content by the DNA content of the same pellet.

DNA Extraction and Single Telomere Length Analysis

DNA was isolated from $3x10^5$ chondrocytes immediately after harvest from culture on TCP or in the Quantum® in hPL-medium (P1). DNA was extracted using the using the High Pure PCR Template Preparation Kit (Roche, Sussex, UK). Extracted DNA was then stored at -80°C until the time of analysis. The DNA from matched donor chondrocytes expanded in the Quantum® or on TCP were subjected to single length telomere analysis (STELA) at the 17p telomeres, as described previously [23,24].

Statistical Analysis

Statistical analysis was performed using Prism software version 9.0 (GraphPad Software, CA, USA). The normality of the data was assessed using a Shapiro-Wilk test, which was used to inform whether parametric or non-parametric statistical tests were appropriate. Unpaired data were analysed using an unpaired Student t test or Mann-Whitney U test, where appropriate based on the normality of the dataset. Paired data were analysed using a paired t-test or a Wilcoxon-matched pairs signed rank test, where appropriate. For multiple comparisons, analysis of variance (ANOVA) or Kruskal Wallis were used, with either a Holm-

293 Sidaks or Dunn's multiple comparisons post-hoc test, respectively, where appropriate. P
294 values ≤ 0.05 were considered significant.

295

296 **Results**

297 **Chondrocyte Growth and Cell Morphology**

298 Chondrocytes could be maintained and expanded in the Quantum® bioreactor. At the end of
299 passage 2 a mean cell harvest of 86.4±38.5 x10^6 (mean±SD) chondrocytes was generated
300 following Quantum® expansion for 8.4±1.5 days, after seeding the bioreactor with 10.2±3.6
301 x10^6 cells (Table 2). Significantly fewer chondrocytes (5.9±4.3 x10^6) were expanded from
302 1.4±0.7 x10^6 in 7±2.7 days on TCP with hPL media (p=0.009; paired t-test; Figure 1). In
303 comparison, 1.0± 1.1 x10^6 chondrocytes seeded on TCP in FBS media yielded 2.3±0.8 x10^6
304 cells after 17.5±21.8 days (Table 2). The number of cells harvested from TCP was not
305 significantly different between chondrocytes cultured in either hPL or FBS at both passage 1
306 and 2 (Figure 1 A&B). When considering the differing starting numbers of chondrocytes on
307 TCP (0.4-2.1x10^6) compared with Quantum® (5.0-15.0x10^6), the % cellular increase was not
308 significantly different between the TCP and Quantum® hPL expansion (p=0.13; paired t-test;
309 Figure 1).

310 Chondrocytes maintained comparative doubling times in the Quantum® compared to
311 matched hPL TCP sister populations (Figure 2D; p=0.30; paired t-test). The total population
312 doublings was higher but not significantly increased following Quantum® expansion when
313 compared with TCP (Figure 2C; p=0.06; paired t-test). Moreover, the number of population
314 doublings and the doubling times were not significantly higher when comparing matched
315 FBS and hPL cultures on TCP (Figure 2 C&D; population doublings: p= 0.26; doubling time:
316 p=0.35; paired t-test). Culture in hPL resulted in a different cell morphology compared to

standard culture in FBS, with cells demonstrating more clustered, fibroblast-like growth formations (Figure 2 A & B).

Immunoprofiling

Chondrocytes cultured in hPL on TCP or in the Quantum® or in FBS adhered to the International Stem Cell Therapy (ISCT) minimal reporting criteria [17] for being >95% positive for CD105, CD73 and CD90 and negative (<2%) for CD34, CD45 and CD19. However, chondrocytes were immunopositive for CD14 regardless of the expansion method used (TCP hPL: 17.2±14.6; TCP FBS: 25.8±24.4; Quantum hPL: 25.1±29.3; mean±SD).

Quantum® expansion did not result in altered expression of chondropotency indicators (CD markers 166, 39, 44, 151, SOX9) or MSC profile indicators (CD105, CD73, CD90, CD19, CD45, CD34, CD14) (Figure 3; $p>0.05$; paired t-test). One of the integrin markers, CD49a (integrin alpha-1) was found to be significantly lower in Quantum® expanded chondrocytes compared to matched cells grown on TCP in hPL (Quantum®=95.8±2.9%, TCP= 99.6±0.4%; (mean±SD; $p=0.04$; paired t-test).

The culture of chondrocytes in hPL demonstrated equivalent immunopositivity for the panel of chondropotency markers and MSC markers tested, compared to matched chondrocytes cultured in FBS (Figure 3; $p<0.05$; paired t-test). However, the immunopositivity of a number of integrin markers was lower following expansion in FBS compared to hPL (Figure 3). These include CD49b (integrin alpha 2) which was 99.0±0.8% positive on chondrocytes cultured in hPL and was 47.5±24.1% positive following FBS expansion ($p=0.02$; paired t-test). Further, CD49c demonstrated 96.2±2.6% immunopositivity following hPL expansion cf. 71.2±15.1% FBS expansion ($p=0.02$; paired t-test). CD151/61 positivity was decreased following FBS expansion compared with hPL (FBS= 69.9±27.6%; hPL=31.9±25.8%; $p=0.02$; paired t-test).

Chrondrogenesis

341 Following TCP expansion in hPL or FBS and Quantum® bioreactor expansion in hPL,
342 chondrogenic pellets were established and maintained in chondrogenic media for 28 days.
343 There were two donors in which chondrocytes were set for pellet expansion in hPL but a
344 chondrogenic pellet did not form; rather a cell pellet collected within the Eppendorf tube
345 following centrifugation, but the pellet did not form a sphere over the first few days and
346 once the pellet was dislodged after 3 days, the cells dispersed throughout the media (Suppl.
347 File 1). Furthermore, there were two donors for which there were insufficient cells
348 harvested at the end of passage 1 to set chondrogenic pellets for analysis, once cells had
349 been utilised for DNA and flow cytometry analysis. The conditions for which pellets were
350 formed and maintained to 28 days is demonstrated in Figure 4b.

351 The concentration of chondrogenic pellet GAG content was calculated and normalised to
352 DNA content. Chondrogenic pellets that were formed from Quantum® expanded
353 chondrocytes retained consistent GAG/DNA concentrations when compared to matched
354 sister populations of chondrocytes cultured in hPL on TCP ($p>0.05$; Kruskal-Wallis; Figure 5).
355 However, Figure 5 demonstrates that when comparing the matched TCP cultures, expansion
356 in FBS resulted in significantly increased GAG/DNA content in comparison with hPL
357 expansion ($p>0.05$; Kruskal-Wallis).

358 **<u>Analysis of telomere length</u>**

359 Single telomere length analysis (STELA) of 17p telomeres demonstrated that there was a bi-
360 modal distribution for many of the chondrocyte samples. In general, this distribution was
361 maintained whether expanded on TCP or in the Quantum® bioreactor. For three of the
362 donors, there was no difference in telomere lengths between up-scale and TCP expanded
363 chondrocytes ($p>0.05$; Mann-Whitney). However, for two donors, there was a difference in
364 telomere length when expanded in the Quantum® bioreactor in comparison with matched
365 TCP (Figure 6B). The direction of change, however was inconsistent for these two donors

366 with donor 3 demonstrating decreased and donor 5 displaying increased 17p telomere
367 length following Quantum® expansion cf. matched hPL expansion on TCP (Figure 6B)
368 ($p<0.05$; Mann Whitney).

369 **Discussion**

370 The development of allogeneic chondrocyte therapies is a rapidly advancing field [25,26].
371 However, it is recognised that the translation of these allogeneic therapies into clinical
372 practice has been limited, likely due to difficulties in sourcing appropriate adult articular
373 cartilage, growing sufficient numbers of cells under GMP conditions and difficulties with
374 preserving cells appropriately [27]. As part of developing allogeneic chondrocyte therapies,
375 there is a need to optimise existing autologous chondrocyte manufacturing processes,
376 particularly to grow large numbers of cells from a cartilage harvest biopsy, with standard ACI
377 chondrocyte expansion on TCP for 2-3 weeks [6,28] unlikely to be capable of producing
378 sufficient cell yields to treat more than a single patient.

379 In this study we have presented, what we believe to be the first attempt to manufacture
380 chondrocytes in the GMP compliant Quantum® bioreactor. Although FBS is used in current
381 ATMP manufacture [6,29], there is an aim to minimise the use of xeno-products in ATMP
382 manufacture, in accordance with the Note for Guidance on Minimizing the Risk of
383 Transmitting Animal Spongiform Encephalopathy (TSE) Agents via Human and Veterinary
384 Medicinal Products (EMA/410/01 rev 3) [30]. Therefore, we have aimed to 'future-proof' this
385 study by investigating pooled human platelet lysate as a GMP-compliant, xeno-free
386 alternative [31] to FBS and testing its potential for use in up-scale bioreactor manufacturing.
387 Currently, autologous serum is used for autologous chondrocyte manufacture; this would
388 not be available from an allogeneic donor, particularly in the volumes required for
389 bioreactor expansion. Furthermore, the Quantum® expansion products and TCP 'sister'
390 populations have been extensively characterised.

391 Our findings demonstrate that chondrocytes can be successfully maintained and expanded
392 using the Quantum® bioreactor system. Manufacture in this advanced culture platform
393 should be easily transferrable to a GMP facility and so significantly reduce the need for
394 'open-handling processes', in which there is increased potential for culture contamination [32].
395 Moreover, we have highlighted that large quantities of chondrocytes (mean- 86M) can be
396 manufactured in a single passage in this system so as to maximise the cell yields from donor
397 tissues. Currently patients treated with ACI in our centre have 1-16M cells delivered into a
398 defect with a diameter of 20.7±7.5mm (mean±SD; n=306; unpublished data from our onsite
399 GMP facility). With this in mind, our data indicate that an average Quantum® yield could
400 potentially produce up to 84 batches of chondrocytes from a single expansion phase. In
401 developing cell-based therapies, staffing and consumables are the two biggest associated
402 costs [33]. Quantum® bioreactor expansion of chondrocytes has the potential to significantly
403 reduce costs in-terms of expert staffing, with 5 staff having been proposed to be required to
404 harvest the number of cells that one user could harvest from the Quantum® [32]. However, the
405 consumables required for Quantum® manufacture are expensive and would need to be
406 considered before an accurate cost/benefit could be determined, as has been performed for
407 other cell types [32,34].

408 Importantly, chondrocytes manufactured in the Quantum® retained many of the
409 characteristics of matched cultures grown on TCP. This included immunopositivity of
410 chondropotency and MSC markers post-expansion and capacity for producing chondrogenic
411 pellets. Importantly, chondrocytes retained high levels of CD44 and CD166. Surface
412 expression of CD44 on chondrocytes used for ACI is associated with improved clinical
413 outcomes (International Knee Documentation Committee (IKDC) score[35] and Lysholm score
414 at 24 months[15]). Higher CD44 chondrocyte expression has also been demonstrated to
415 correlate with chondrocyte capacity to form GAGs[16]. Further, increased expression of CD166
416 on chondrocytes has been shown in groups with enhanced clinical success following ACI[15], as

417 well as, having increased expression during chondrogenic re-differentiation [36].
418 Immunoprofiling of chondrocyte products from each culture condition, indicates that these
419 cells will have capacity to repair cartilage, however, future work would further benefit from
420 analysis of additional chondrocyte markers such as collagen type II[35] and aggrecan, as
421 alternative markers of chondrocyte potency. On the whole the integrin profiles were
422 comparable between Quantum® and hPL TCP expanded chondrocytes, with the exception of
423 CD49a (integrin α1) which was reduced post-Quantum® expansion. The alpha 1 subunit
424 makes up half of the α1β1 integrin, which is expressed on normal chondrocytes and binds to
425 collagen types VI and II and also to matrilin-1 [37]. Djouard et al., demonstrated that gene
426 expression of integrin α1 increases over time as MSCs undergo chondrogenesis [38]. This may
427 perhaps indicate that the chondrocytes are starting to de-differentiate following Quantum®
428 expansion, but this would require confirmation at the gene expression level.

429 Telomere lengths were not altered following up-scale expansion of the chondrocytes,
430 indicating cellular aging was not induced as a response to rapid expansion [39]. These findings
431 suggest that Quantum® bioreactor expansion could provide a safe method of producing
432 large numbers of chondrocytes without detrimentally ageing the chondrocytes. It will be
433 important however, to determine whether the capacity of these chondrocytes for repairing
434 cartilage is maintained in *in vivo* models of pre/early-osteoarthritis, particularly, as studies
435 have indicated that potency of chondrocytes as assessed via immunoprofile and gene
436 expression relationship to chondrogenic pellet assays does not always relate with clinical
437 outcomes [15].

438 Interestingly, chondrocytes manufactured in hPL resulted in greater cells yields, as
439 normalised to seeding density, with a much quicker doubling time than those cultured in
440 FBS. Sykes et al. (2018) also demonstrated that chondrocytes manufactured in hPL had an
441 increased rate of proliferation when compared with manufacture in FBS. Akin with our

442 findings, chondrogenic pellets derived from hPL manufactured chondrocytes demonstrated
443 reduced chondrogenicity (GAG content normalised to cell number) and formed less stable
444 pellets [40]. Chondroprogenitors, a subpopulation of fibronectin adherent proliferative
445 chondrocytes, expanded in hPL had decreased gene expression of chondrogenic markers
446 (aggrecan and collagen II) [41]. Conversely, Rikkers et al. (2020) demonstrated increased GAG
447 content in 28 day chondrogenic pellets established from chondrocytes expanded in hPL
448 compared with those grown in FBS [42]. However, when re-differentiated in the presence of
449 hPL in the chondrogenic differentiation media, matrix production and chondrogenic gene
450 expression were negatively influenced [42]. When seeded in fibrin scaffolds, however,
451 chondrocytes differentiated using media supplemented with hPL produced higher GAG
452 content compared to standard chondrogenic differentiation media and demonstrated a
453 trend towards increased gene expression of chondrogenic markers (aggrecan, collagen type
454 II alpha 1 chain and cartilage oligomeric protein) [43]. All these data suggest that hPL results in
455 greater numbers of cells in a given time period. How hPL influences their capacity to form
456 cartilage extracellular matrix, however, remains less conclusive with inconsistent findings
457 across studies. Future studies, are required to assess how different hPL concentrations and
458 from different suppliers influence chondrocytes potency to determine if suboptimal
459 chondrogenic differentiation is a direct influence of the quality of the hPL being used for
460 their expansion.

461 It is important to note that for many current clinical manufacturing processes, human
462 autologous serum is used for chondrocyte expansion [2,6,28]. A recent study has compared
463 chondrocyte expansion in autologous serum and hPL [44]. Philippe et al. (2021) demonstrated
464 that human articular chondrocytes maintained similar morphology and growth kinetics
465 when cultured in hPL compared with autologous serum and indicated that when these hPL
466 grown chondrocytes were cultured to form chondrogenic pellets, they accumulated GAG
467 and demonstrated increased chondrogenic gene expression increasingly with time

468 throughout their culture. A limitation of this study was that we unfortunately did not have
469 sufficient cells to derive enough chondrogenic pellets such as to harvest them for analysis at
470 timepoints throughout the 28 day culture period, although it would certainly have been
471 interesting to determine how the chondrogenic potential compared longitudinally between
472 chondrocytes manufactured in hPL and FBS. Furthermore, the variability between batches of
473 hPL, even from commercial, GMP-compliant sources is more recently becoming
474 acknowledged [45]. In an attempt to limit this effect across the different donor sources, the
475 same batched of hPL was used in all experiments. In future work, however, it would be
476 important to ensure that the hPL source adheres with the recommendations of the Working
477 Party for Cellular Therapies of the International Society of Blood Transfusion [45].

478 To date, several studies have manufactured a plethora of cell types in the Quantum®, using
479 hPL (5-10%) as a growth supplement. This has included numerous studies which have up-
480 scaled mesenchymal stromal cells (MSCs) from various sources including adipose tissue [46–48]
481 and bone-marrow [5,32,34,49–51]. However, only Haack-Sorensen (2018) have directly compared
482 Quantum® manufacture in 5% hPL or 10% FBS. This study highlighted that hPL manufacture
483 resulted in an average of 546 million adipose derived-MSCs in 9 days compared with 111
484 million cells in 17 days following FBS manufacture [46]. Moreover, these cells maintained
485 equivalent quality in terms of adherence with the ISCT criteria and genomic stability [46]. As
486 our study results also indicate slower chondrocyte growth in FBS cf. hPL on TCP, a study of
487 Quantum® chondrocyte expansion in FBS to assess yields, doubling times and chondrogenic
488 potential is warrented.

489 Although our data indicate that hPL may be suboptimal for chondrocyte manufacture in
490 terms of chondrogenic function, there is a GMP requirement to move away from the use of
491 xeno-sera. Therefore, further investigation of the potential of GMP-compliant defined serum
492 free media for manufacture of chondrocytes is required. There are several such products

493 commercially available, but the majority have been manufactured for the growth of MSCs
494 and their utility for chondrocyte expansion without encouraging de-differentiation needs to
495 be comprehensively evaluated. Moreover, the paracrine and immunomodulatory function
496 of these Quantum expanded chondrocytes has not been assessed. Perhaps this activity
497 could prove to influence the repair or regenerative properties of chondrocytes in much the
498 same way as appears to be the case for MSCs [52,53].

499 Alongside optimisation of manufacturing procedures for allogeneic chondrocyte therapies,
500 there is a need to identify optimal donor sources of cartilage. This study highlights that
501 chondrocytes can be up-scale expanded within the Quantum® bioreactor system. However
502 the use of chondrocytes which have been derived from joint arthroplasty tissue, is unlikely
503 to be the best source of cartilage for clinical use, as the extracted chondrocytes may have
504 been detrimentally preconditioned within the osteoarthritic joint. Therefore, identification
505 of optimal 'healthy' donors is required, perhaps from cadaveric adult cartilage donors, akin
506 to clinical allograft sources. Alternatively, more proliferative chondrocyte populations, such
507 as those of chondroprogenitors which can be isolated via selective adhesion to fibronectin
508 [54,55] or from juvenile tissue sources, such as excised polydactyl digits [56,57] may be superior.
509 Moreover, before chondrocytes from any of these donors could be used in clinical practice,
510 a concerted effort is required to ensure all the manufacturing steps comply with GMP
511 regulations and that rigorous safety testing of the cell products e.g. donor serological testing
512 and product microbiology, endotoxin and mycoplasma tests are performed. Further, to
513 confirm minimum batch to batch functionality is achieved a potency release test would need
514 to be established. Regardless, a method of assessing and scoring the quality of the donor
515 cartilage tissue and extracted chondrocytes will be a requisite before costly up-scale
516 bioreactor expansion is performed.

517 Cartilage repair therapy has been at the forefront in introducing advanced therapies into the
518 clinic, with ACI having been applied for over 30 years [1,58]. Further, ACI was one of the
519 pioneering cell therapy techniques to be recommended by the UK National Institute of
520 Clinical Excellence (NICE) [59]. As cell therapy for cartilage repair continues to evolve, there is a
521 need to manufacture large numbers of chondrocytes for 'off-the-shelf' delivery. The findings
522 of this study indicate that there is potential to up-scale expand chondrocytes using the
523 Quantum® bioreactor. With refinement, bioreactor expansion of allogeneic chondrocytes
524 could result in a relatively cheap and consistent ATMP with the potential to repair cartilage
525 and prevent osteoarthritis in many patients.

526 **References**

527 1. Davies RL, Kuiper NJ. Regenerative Medicine: A Review of the Evolution of
528 Autologous Chondrocyte Implantation (ACI) Therapy. Bioengineering
529 2019;6(1):1–16; doi: 10.3390/bioengineering6010022.

530 2. Harrison PE, Ashton IK, Johnson WEB, et al. The in vitro growth of human
531 chondrocytes. Cell Tissue Bank 2000;1(4):255–260; doi:
532 10.1023/A:1010131729208.

533 3. Mennan C, Garcia J, Roberts S, et al. A comprehensive characterisation of
534 large- scale expanded human bone marrow and umbilical cord mesenchymal
535 stem cells. Stem Cell Res Ther 2019;4:1–15.

536 4. Hulme CH, Mennan C, H.S. M, et al. A Comprehensive Review of Quantum
537 Bioreactor Cell Manufacture: Research and Clinical Applications. Cytotherapy
538 2023;In Submiss.

539 5. Rojewski MT, Fekete N, Baila S, et al. GMP-Compliant Isolation and Expansion
540 of Bone Marrow-Derived MSCs in the Closed , Automated Device Quantum
541 Cell Expansion System. 2013;22:1981–2000; doi:
542 10.3727/096368912X657990.

543 6. Harrison P, Hopkins T, Hulme CH, et al. Chondrocyte Isolation and Expansion.
544 In: Methods in Molecular Biology- "Cartilage Tissue Engineering." (Springer.
545 ed) 2022.

546 7. Giancola R, Bonfini T, Iacone A. Cell therapy: cGMP facilities and
547 manufacturing. Muscles Ligaments Tendons J 2012;2(3):243–247.

548 8. Shih DTB, Burnouf T. Preparation, quality criteria, and properties of human
549 blood platelet lysate supplements for ex vivo stem cell expansion. N

550 Biotechnol 2015;32(1):199–211; doi: 10.1016/j.nbt.2014.06.001.

551 9. Hemeda H, Giebel B, Wagner W. Evaluation of human platelet lysate versus
552 fetal bovine serum for culture of mesenchymal stromal cells. Cytotherapy
553 2014;16(2):170–180; doi: 10.1016/j.jcyt.2013.11.004.

554 10. Garcia J, Mennan C, McCarthy HS, et al. Chondrogenic Potency Analyses of
555 Donor-Matched Chondrocytes and Mesenchymal Stem Cells Derived from
556 Bone Marrow, Infrapatellar Fat Pad, and Subcutaneous Fat. Stem Cells Int
557 2016;2016(October); doi: 10.1155/2016/6969726.

558 11. Guiotto M, Raffoul W, Hart AM, et al. Human platelet lysate to substitute fetal
559 bovine serum in hMSC expansion for translational applications: A systematic
560 review. J Transl Med 2020;18(1):1–14; doi: 10.1186/s12967-020-02489-4.

561 12. Pretzel D, Linss S, Rochler S, et al. Relative percentage and zonal distribution
562 of mesenchymal progenitor cells in human osteoarthritic and normal cartilage.
563 Arthritis Res Ther 2011;13(2):1–15; doi: 10.1186/ar3320.

564 13. Vinod E, Padmaja K, Livingston A, et al. Prospective Isolation and
565 Characterization of Chondroprogenitors from Human Chondrocytes Based on
566 CD166/CD34/CD146 Surface Markers. Cartilage 2021;13(2_suppl):808S-817S;
567 doi: 10.1177/19476035211042412.

568 14. Hamada T, Sakai T, Hiraiwa H, et al. Surface markers and gene expression to
569 characterize the differentiation of monolayer expanded human articular
570 chondrocytes. Nagoya J Med Sci 2014;76(1–2):101–111.

571 15. Islam A, Fossum V, Hansen AK, et al. In vitro chondrogenic potency of surplus
572 chondrocytes from autologous transplantation procedures does not predict
573 short-term clinical outcomes. BMC Musculoskelet Disord 2019;20(1):1–15;
574 doi: 10.1186/s12891-018-2380-4.

575 16. Grogan SP, Barbero A, Diaz-Romero J, et al. Identification of markers to
576 characterize and sort human articular chondrocytes with enhanced in vitro
577 chondrogenic capacity. Arthritis Rheum 2007;56(2):586–595; doi:
578 https://doi.org/10.1002/art.22408.

579 17. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining
580 multipotent mesenchymal stromal cells. The International Society for Cellular
581 Therapy position statement. Cytotherapy 2006;8(4):315–317; doi:
582 10.1080/14653240600855905.

583 18. Padmaja K, Amirtham SM, Rebekah G, et al. Supplementation of articular
584 cartilage-derived chondroprogenitors with bone morphogenic protein-9
585 enhances chondrogenesis without affecting hypertrophy. Biotechnol Lett
586 2022;44(9):1037–1049; doi: 10.1007/s10529-022-03280-9.

587 19. Bekkers JEJ, Saris DBF, Tsuchida AI, et al. Chondrogenic Potential of Articular
588 Chondrocytes Depends on Their Original Location. Tissue Eng Part A
589 2013;20(3–4):663–671; doi: 10.1089/ten.tea.2012.0673.

590 20. Rikkers M, Korpershoek J V., Levato R, et al. Progenitor Cells in Healthy and
591 Osteoarthritic Human Cartilage Have Extensive Culture Expansion Capacity
592 while Retaining Chondrogenic Properties. Cartilage 2021;13(2_suppl):129S-
593 142S; doi: 10.1177/19476035211059600.

594 21. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination
595 of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim
596 Biophys Acta - Gen Subj 1986;883(2):173–177.

597 22. Farndale RW, Sayers CA, Barrett AJ. A Direct Spectrophotometric Microassay
598 for Sulfated Glycosaminoglycans in Cartilage Cultures. Connect Tissue Res
599 1982;9(4):247–248; doi: 10.3109/03008208209160269.

600 23. Capper R, Britt-Compton B, Tankimanova M, et al. The nature of telomere
601 fusion and a definition of the critical telomere length in human cells. Genes
602 Dev 2007;21(19):2495–2508; doi: 10.1101/gad.439107.

603 24. Fellows CR, Williams R, Davies IR, et al. Characterisation of a divergent
604 progenitor cell sub-populations in human osteoarthritic cartilage: The role of
605 telomere erosion and replicative senescence. Sci Rep 2017;7(February):1–11;
606 doi: 10.1038/srep41421.

607 25. Hulme CH, Perry J, McCarthy HS, et al. Cell therapy for cartilage repair. Emerg
608 Top Life Sci 2021;0(July):1–15; doi: 10.1042/etls20210015.

609 26. Vonk LA, de Windt TS, Slaper-Cortenbach ICM, et al. Autologous, allogeneic,
610 induced pluripotent stem cell or a combination stem cell therapy? Where are
611 we headed in cartilage repair and why: a concise review. Stem Cell Res Ther
612 2015;6(1):94; doi: 10.1186/s13287-015-0086-1.

613 27. Ibrahim S, Nagesh HY, Pandey V. Allogeneic chondrocyte implantation: What
614 is stopping it from being a standard of care? J Arthrosc Surg Sport Med
615 2021;3(1):34–39; doi: 10.25259/jassm_8_2021.

616 28. Brittberg M. Autologous Chondrocyte Implantation- Technique and long-term
617 follow-up. Inj Int J Care Inj 2008;39(1):40–49.

618 29. Lechanteur C, Briquet A, Bettonville V, et al. Msc manufacturing for academic
619 clinical trials: From a clinical-grade to a full gmp-compliant process. Cells
620 2021;10(6):1–19; doi: 10.3390/cells10061320.

621 30. European Medicines Agency. Note for guidance on minimising the risk of
622 transmitting animal spongiform encephalopathy agents via human and
623 veterinary medicinal products (EMA/410/01 rev.3). Off J Eur Union
624 2011;C(73):1–18; doi: 2011/C 73/01.

625 31. Oeller M, Laner-plamberger S, Krisch L, et al. Human platelet lysate for good
626 manufacturing practice-compliant cell production. Int J Mol Sci 2021;22(10).

627 32. Hanley PJ, Mei Z, Durett AG, et al. Ef fi cient manufacturing of therapeutic
628 mesenchymal stromal cells with the use of the Quantum Cell Expansion

629 System. J Cytotherapy 2014;16(8):1048–1058; doi: 10.1016/j.jcyt.2014.01.417.

630 33. ten Ham RMT, Nievaart JC, Hoekman J, et al. Estimation of manufacturing
631 development costs of cell-based therapies: a feasibility study. Cytotherapy
632 2021;23(8):730–739; doi: 10.1016/j.jcyt.2020.12.014.

633 34. Russell AL, Lefavor RC, Zubair AC. Characterization and cost–benefit analysis
634 of automated bioreactor- expanded mesenchymal stem cells for clinical
635 applications. Transfusion 2018;1–9; doi: 10.1111/trf.14805.

636 35. Niemeyer P, Pestka JM, Salzmann GM, et al. Influence of Cell Quality on
637 Clinical Outcome After Autologous Chondrocyte Implantation. Am J Sports
638 Med 2012;40(3):556–561.

639 36. Krüger M, Krüger JP, Kinne RW, et al. Are surface antigens suited to verify the
640 redifferentiation potential and culture purity of human chondrocytes in cell-
641 based implants. Tissue Cell 2015;47(5):489–497; doi:
642 10.1016/j.tice.2015.07.004.

643 37. Loeser RF, Hill C, States U. Integrins and chondrocyte–matrix interactions in
644 articular cartilage. Matrix Biol 2014;39:11–16; doi:
645 10.1016/j.matbio.2014.08.007.Integrins.

646 38. Djouad F, Delorme B, Maurice M, et al. Microenvironmental changes during
647 differentiation of mesenchymal stem cells towards chondrocytes. Arthritis Res
648 Ther 2007;9(2):1–12; doi: 10.1186/ar2153.

649 39. Shammas MA. Telomeres, lifestyle, cancer, and aging. Curr Opin Clin Nutr
650 Metab Care 2011;14(1):28–34; doi: 10.1097/MCO.0b013e32834121b1.

651 40. Sykes JG, Kuiper JH, Richardson JB, et al. Impact of human platelet lysate on
652 the expansion and chondrogenic capacity of cultured human chondrocytes for
653 cartilage cell therapy. Eur Cells Mater 2018;35(2014):255–267.

654 41. Kachroo U, Zachariah SM, Thambaiah A, et al. Comparison of Human Platelet
655 Lysate versus Fetal Bovine Serum for Expansion of Human Articular Cartilage–
656 Derived Chondroprogenitors. Cartilage 2021;13(2_suppl):107S-116S; doi:
657 10.1177/1947603520918635.

658 42. Rikkers M, Levato R, Malda J, et al. Importance of Timing of Platelet Lysate-
659 Supplementation in Expanding or Redifferentiating Human Chondrocytes for
660 Chondrogenesis. Front Bioeng Biotechnol 2020;8(July):1–13; doi:
661 10.3389/fbioe.2020.00804.

662 43. Pötter N, Westbrock F, Grad S, et al. Evaluation of the influence of platelet-
663 rich plasma (PRP), platelet lysate (PL) and mechanical loading on
664 chondrogenesis in vitro. Sci Rep 2021;11(1):1–11; doi: 10.1038/s41598-021-
665 99614-0.

666 44. Philippe V, Laurent A, Abdel-Sayed P, et al. Human Platelet Lysate as an
667 Alternative to Autologous Serum for Human Chondrocyte Clinical Use.

668 Cartilage 2021;13(1_suppl):509S-518S; doi: 10.1177/19476035211035433.

669 45. Schallmoser K, Henschler R, Gabriel C, et al. Production and Quality
670 Requirements of Human Platelet Lysate: A Position Statement from the
671 Working Party on Cellular Therapies of the International Society of Blood
672 Transfusion. Trends Biotechnol 2020;38(1):13–23; doi:
673 10.1016/j.tibtech.2019.06.002.

674 46. Haack-sørensen M, Juhl M, Follin B, et al. Development of large-scale
675 manufacturing of adipose-derived stromal cells for clinical applications using
676 bioreactors and human platelet lysate. Scand J Clin Lab Invest 2018;0(0):1–8;
677 doi: 10.1080/00365513.2018.1462082.

678 47. Pirrone C, Gobbetti A, Caprara C, et al. Chondrogenic potential of hASCs
679 expanded in flask or in a hollow-fiber bioreactor. 2017;2(2):1–10; doi:
680 10.15761/JSCRM.1000123.

681 48. Coccè V, La Monica S, Bonelli M, et al. Inhibition of human malignant pleural
682 mesothelioma growth by mesenchymal stromal cells. Cells 2021;10(6); doi:
683 10.3390/cells10061427.

684 49. Nold P, Brendel C, Neubauer A, et al. Biochemical and Biophysical Research
685 Communications Good manufacturing practice-compliant animal-free
686 expansion of human bone marrow derived mesenchymal stroma cells in a
687 closed hollow-fiber-based bioreactor. Biochem Biophys Res Commun
688 2013;430(1):325–330.

689 50. Savelli S, Trombi L, Alessandro DD, et al. Pooled human serum : A new culture
690 supplement for bioreactor-based cell therapies . Preliminary results.
691 Cytotherapy 2018;20(4):556–563; doi: 10.1016/j.jcyt.2017.12.013.

692 51. Maeda T, Briggs CM, Datar A, et al. Influence of administration of
693 mesenchymal stromal cell on pediatric oxygenator performance and
694 inflammatory response. JTCVS Open 2021;5(C):99–107; doi:
695 10.1016/j.xjon.2021.02.003.

696 52. Lohan P, Treacy O, Lynch K, et al. Culture expanded primary chondrocytes
697 have potent immunomodulatory properties and do not induce an allogeneic
698 immune response. Osteoarthr Cartil 2016;24(3):521–533; doi:
699 10.1016/j.joca.2015.10.005.

700 53. Osiecka-Iwan A, Hyc A, Radomska-Lesniewska DM, et al. Antigenic and
701 immunogenic properties of chondrocytes. Implications for chondrocyte
702 therapeutic transplantation and pathogenesis of inflammatory and
703 degenerative joint diseases. Cent Eur J Immunol 2018;43(2):209–219; doi:
704 10.5114/ceji.2018.77392.

705 54. Dowthwaite GP, Bishop JC, Redman SN, et al. The surface of articular cartilage
706 contains a progenitor cell populations. J Cell Sci 2004;117(6):889–897; doi:
707 10.1242/jcs.00912.

708 55. Williams R, Khan IM, Richardson K, et al. Identification and clonal
709 characterisation of a progenitor cell sub-population in normal human articular
710 cartilage. PLoS One 2010;5(10); doi: 10.1371/journal.pone.0013246.

711 56. Maehara M, Sato M, Toyoda E, et al. Characterization of polydactyly-derived
712 chondrocyte sheets versus adult chondrocyte sheets for articular cartilage
713 repair. Inflam Regen 2017;37:1–10; doi: 10.1186/s41232-017-0053-6.

714 57. Cavalli E, Levinson C, Hertl M, et al. Characterization of polydactyly
715 chondrocytes and their use in cartilage engineering. Sci Rep 2019;9(1); doi:
716 10.1038/s41598-019-40575-w.

717 58. Brittberg M, Lindahl A, Nilsson A, et al. Treatment of Deep Cartilage Defects in
718 the Knee with Autologous Chondrocyte Transplantation. N Engl J Med
719 1994;331(14):889–895.

720 59. National Institute for Health and Care Excellence (NICE). Autologous
721 Chondrocyte Implantation for Treating Symptomatic Articular Cartilage
722 Defects of the Knee. Technology Appraisal Guidance [TA477]. 2017.; doi:
723 https://www.nice.org.uk/guidance/ta477/resources/autologous-chondrocyte-
724 implantation-for-treating-symptomatic-articular-cartilage-defects-of-the-
725 knee-pdf-82604971061701.

726

727 ***<u>Declarations</u>***

728 <u>Ethics approval and consent to participate</u>

729 Patient samples were collected under the ethical approvals: 'Investigating the potential for
730 cells and molecules isolated from orthopaedic patients for modelling and understanding
731 pathogenic conditions and developing diagnostic markers and therapies for musculoskeletal
732 disorders and spinal cord injury' (11/NW/0875) which was approved by the NRES committee
733 North West- Liverpool East. All patients gave valid informed consent prior to samples being
734 collected.

735 <u>Competing interests</u>

736 The authors declare that they have no competing interests.

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742 Authors' contributions

743 CHH, JKG KTW & CM came up with conception and design of the study. JKG, CHH, CM, DB,
744 LR & JP collected data which was then analysed and interpreted by JKG, CHH, CM & KTW.
745 JKG, CHH, CM, SR, RB, CM, DB, LR & KTW drafted the manuscript, critically revised and
746 approved the final article. RB & CM provided patients' cartilage samples. Funding for the
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748 manuscript.

749

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