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Impact of Adipose-Derived Mesenchymal Stem Cell Isolation Parameters on Cell Yield, Growth Factor Secretion, and Clinical Outcomes in Knee Osteoarthritis: A Randomized Controlled Trial

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Abstract

Background: Intra-articular injection of adipose-derived mesenchymal stem cells (AD-MSCs) is increasingly offered for symptomatic knee osteoarthritis, but the laboratory step that converts lipoaspirate into an injectable stromal vascular fraction is not standardised. Centrifugation force and centrifugation duration vary several-fold between published protocols, and it is unknown how much of the variability in reported clinical results is attributable to the isolation procedure itself.

Objective: To determine, in a full 3 × 2 factorial randomised design, how centrifugation force and duration influence nucleated cell yield, cell viability, clonogenic capacity and growth factor content of the injected preparation, and whether those laboratory differences translate into differences in pain, function and structural outcome over 36 months.

Methods: Sixty patients (age 45–75 years) with symptomatic Kellgren–Lawrence grade II–III knee osteoarthritis were randomised 1:1:1:1:1:1 to six isolation protocols (n = 10 each) combining three centrifugation forces (300, 600 and 1200 ×g) with two durations (5 and 10 min). Lipoaspirate (148 ± 22 mL) was harvested from the lumbar region under tumescent local anaesthesia, digested, and centrifuged according to the allocated protocol. Every patient received a single ultrasound-guided intra-articular injection of 5×10^6 AD-MSCs in 3 mL of saline. Nucleated cell yield, trypan blue viability and colony-forming unit-fibroblast (CFU-F) frequency were measured in the final product, and TGF-β, VEGF and PDGF were quantified by ELISA. Visual analogue scale (VAS), WOMAC and KOOS were recorded at baseline and at 3, 6, 12, 18, 24, 30 and 36 months; 3 T MRI with T2 mapping and WORMS scoring was performed at baseline and every 6 months. Endpoints were analysed by one-way ANOVA with Tukey correction, 3 × 2 factorial ANOVA, repeated-measures ANOVA and Pearson correlation.

Results: Nucleated cell yield increased monotonically from $2.8 \pm 0.5 \times 10^6$ cells/mL at 300 ×g for 5 min to $7.2 \pm 0.8 \times 10^6$ cells/mL at 1200 ×g for 10 min ($F(5, 54) = 65.8$, $p < 0.001$), a 2.6-fold difference, while viability fell from 96.4% to 92.1% ($F(5, 54) = 10.2$, $p < 0.001$). CFU-F frequency and the concentrations of TGF-β, VEGF and PDGF all rose in parallel with yield (all $p < 0.001$). Factorial analysis attributed 78% of the variance in cell yield to centrifugation force and only 7.7% to duration, with no interaction ($p = 0.62$); the same pattern held for every laboratory endpoint. All six groups improved markedly from baseline (all within-group $p < 0.001$). At 36 months VAS fell by 42% in the 300 ×g / 5 min group versus 68% in the 1200 ×g / 10 min group (pairwise $p = 0.008$), WOMAC improvement was 19.2 versus 35.2 points (ANOVA $p < 0.001$), and cartilage thickness gain was 0.20 versus 0.40 mm (ANOVA $p < 0.001$). Cell yield correlated with 36-month WOMAC improvement ($r = 0.59$, $p < 0.001$) and with cartilage gain ($r = 0.61$, $p < 0.001$). Responder rates rose from 20% to 80% for a 50% WOMAC improvement ($\chi^2(5) = 11.4$, $p = 0.043$). No adjacent pair of protocols differed significantly on any clinical endpoint.

Conclusion: Higher centrifugation force yields more nucleated cells with greater clonogenic and secretory capacity, and this laboratory gradient is mirrored by a graded clinical and structural benefit sustained to 36 months. Force is roughly ten times more influential than duration, and the two factors act independently. Because the loss of viability at 1200 ×g is modest and does not offset the gain in cell number, protocols in the 600–1200 ×g range for 10 min are preferred over gentler protocols; the trial was not powered to separate adjacent protocols and the optimum within that range remains to be defined.

Keywords

Adipose-derived mesenchymal stem cells; Stromal vascular fraction; Knee osteoarthritis; Centrifugation; Cell yield; Growth factors; Regenerative medicine; Randomised controlled trial.

Abbreviations

AD-MSC, adipose-derived mesenchymal stem cell; CFU-F, colony-forming unit-fibroblast; ELISA, enzyme-linked immunosorbent assay; KL, Kellgren–Lawrence; KOOS, Knee injury and Osteoarthritis Outcome Score; MSC, mesenchymal stem cell; OA, osteoarthritis; PDGF, platelet-derived growth factor; SVF, stromal vascular fraction; TGF-β, transforming growth factor beta; VAS, visual analogue scale; VEGF, vascular endothelial growth factor; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

Introduction

Osteoarthritis of the knee is among the most common causes of chronic pain and physical disability worldwide, and its prevalence continues to rise with population ageing and increasing obesity [1,2]. Symptomatic knee osteoarthritis affects a substantial minority of adults over the age of 60 and imposes a considerable economic burden through direct medical costs, lost productivity and premature retirement [2,3]. Conventional management — physiotherapy, weight reduction, oral and topical analgesics, intra-articular corticosteroid and hyaluronic acid injections — relieves symptoms for limited periods and does not alter the underlying degenerative process [3]. Total knee arthroplasty is highly effective in advanced disease but is a major intervention with a finite implant lifespan, and it is poorly suited to younger, active patients with moderate radiographic change.

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The step that converts lipoaspirate into an injectable product is enzymatic or mechanical release of the stromal vascular fraction followed by centrifugation. It is precisely this step that is least standardised. Published protocols use relative centrifugal forces from below 300 ×g to above 1500 ×g and durations from 3 to 15 min, often without justification [8,19,20]. Centrifugation physics predicts a trade-off: higher force sediments a larger proportion of the nucleated cell population, including the small, low-density stromal cells that carry most of the regenerative activity, but also increases shear and compaction stress on the cell pellet. Whether the additional cells recovered at high force are worth the accompanying loss of membrane integrity has not been tested prospectively in patients.

Because centrifugation force and duration are usually varied together, their individual contributions cannot be separated in observational comparisons of published series. A factorial randomised design is required. We therefore conducted a single-centre randomised controlled trial in which patients with symptomatic Kellgren–Lawrence grade II–III knee osteoarthritis were allocated to one of six isolation protocols formed by crossing three centrifugation forces with two durations, holding every other element of the harvest, processing and injection procedure constant and delivering the same nominal cell dose to every patient. The primary aim was to quantify the effect of each isolation parameter on nucleated cell yield, viability, clonogenic capacity and growth factor content; the secondary aim was to determine whether those laboratory differences produce measurable differences in pain, function and joint structure over 36 months of follow-up.

Materials and Methods

Study design and ethical approval

This was a single-centre, prospective, parallel-group randomised controlled trial with a full 3 × 2 factorial allocation to isolation protocol. Patients were enrolled between January 2019 and January 2023, and every participant completed a minimum of 36 months of follow-up. The protocol was approved by the institutional review board (protocol number to be inserted) and conducted in accordance with the Declaration of Helsinki and applicable national regulations governing the clinical use of autologous cell preparations. The trial was registered prospectively (registry identifier to be inserted). All participants gave written informed consent, including specific consent for lipoaspiration, cell processing, intra-articular injection and repeated magnetic resonance imaging.

Participants

Eligible patients were aged 45 to 75 years, had knee pain on most days for at least six months, a visual analogue scale pain score of 4 or more out of 10, and Kellgren–Lawrence grade II or III osteoarthritis on weight-bearing radiographs [9]. Patients were required to have failed at least three months of conservative management including physiotherapy and analgesia. Exclusion criteria were Kellgren–Lawrence grade IV disease, inflammatory or crystal arthropathy, previous knee arthroplasty or realignment osteotomy, intra-articular injection of any kind within the preceding three months, body mass index above 35 kg/m², active malignancy, uncontrolled diabetes mellitus, coagulopathy or anticoagulant therapy that could not be interrupted, active local or systemic infection, pregnancy, and any contraindication to magnetic resonance imaging. Seventy-eight patients were screened, 18 were excluded, and 60 were randomised (**Figure 1**).

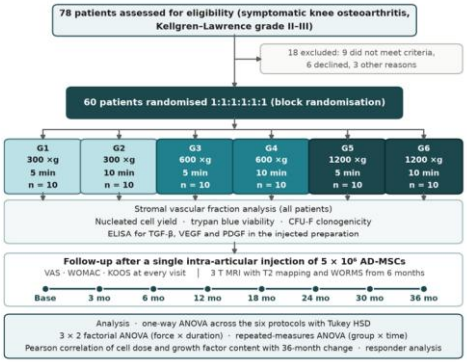

Figure 1: Trial profile, isolation protocols and analysis plan. Sixty of 78 screened patients were randomised 1:1:1:1:1:1 to six isolation protocols formed by crossing three centrifugation forces (300, 600 and 1200 ×g) with two durations (5 and 10 min). All patients received a single ultrasound-guided intra-articular injection of 5×10^6 AD-MSCs and completed 36 months of clinical and imaging follow-up.

Randomisation and blinding

Randomisation used a computer-generated sequence in permuted blocks of six, prepared by an independent statistician and concealed in sequentially numbered, opaque, sealed envelopes opened only after the lipoaspirate had been harvested. Patients remained blinded to their allocated isolation protocol for the whole study. The outcome assessors who administered the questionnaires, the musculoskeletal radiologists who read the magnetic resonance images and the statistician were all blinded to allocation.

The laboratory personnel performing the isolation could not be blinded, and the treating physician was aware of allocation at the time of processing but took no part in outcome assessment.

Lipoaspiration and stromal vascular fraction isolation

Adipose tissue was harvested from the lumbar region under tumescent local anaesthesia using a 3 mm blunt cannula and manual syringe aspiration at low negative pressure, which preserves cell viability better than machine-assisted suction [19]. A mean of 148 ± 22 mL of lipoaspirate was obtained per patient. The aspirate was washed with an equal volume of sterile phosphate-buffered saline until the infranatant ran clear, then digested with 0.075% collagenase type I at 37 °C for 30 min with continuous agitation. Digestion was neutralised with an equal volume of medium containing autologous serum, and the digest was filtered sequentially through 500 µm and 100 µm mesh.

The filtrate was then centrifuged according to the allocated protocol: 300, 600 or 1200 ×g for either 5 or 10 min at room temperature in a fixed-angle rotor (**Table 2**). All other steps — harvest technique, aspirate volume, wash protocol, collagenase concentration, digestion time and temperature, filtration, erythrocyte lysis and final resuspension — were identical across groups, so that the only systematic difference between arms was the centrifugation step. The pellet was resuspended, erythrocytes were lysed with ammonium chloride buffer, and the stromal vascular fraction was washed twice and resuspended in sterile saline.

Laboratory characterisation of the injected product

Nucleated cell yield was determined on an automated cell counter and confirmed manually on a haemocytometer, and is reported per millilitre of processed lipoaspirate. Viability was assessed by trypan blue exclusion on at least 200 cells counted in duplicate. Clonogenic capacity was measured as the colony-forming unit-fibroblast frequency: 1×10^5 nucleated cells were plated in 60 mm dishes in α-MEM with 10% autologous serum, cultured for 14 days, fixed and stained with crystal violet, and aggregates of at least 50 cells were counted as a colony. Immunophenotype was confirmed on a subset by flow cytometry for CD73, CD90 and CD105 positivity and CD34 and CD45 negativity, in accordance with the minimal criteria for mesenchymal stromal cells [10]. Concentrations of TGF-β, VEGF and PDGF in the final injectate were measured in duplicate by sandwich enzyme-linked immunosorbent assay according to the manufacturers' instructions, with the mean of the duplicates used for analysis.

Intra-articular injection and rehabilitation

Every patient received a single intra-articular injection of 5×10^6 AD-MSCs suspended in 3 mL of sterile saline, delivered under ultrasound guidance through a superolateral approach with the knee in slight flexion. Standardising the delivered cell dose across all groups is a deliberate feature of the design: it means that any clinical difference between arms reflects the biological quality of the cells rather than the number injected. Where the isolation protocol produced more cells than required, the surplus was not administered. Patients rested the limb for 24 hours, avoided anti-inflammatory medication for two weeks, and followed an identical graded rehabilitation programme of range of motion and quadriceps strengthening exercises supervised for the first six weeks.

Outcome measures

Clinical outcomes were the 100 mm visual analogue scale for pain, the Western Ontario and McMaster Universities Osteoarthritis Index total score (0–96, higher scores indicating worse status) [11] and the Knee injury and Osteoarthritis Outcome Score (0–100, higher scores indicating better status) [13], all recorded at baseline and at 3, 6, 12, 18, 24, 30 and 36 months. Structural outcomes were assessed on a 3 T scanner at baseline and every 6 months using a standardised protocol including sagittal and coronal proton density-weighted sequences and a multi-echo spin-echo sequence for T2 mapping. Mean cartilage thickness of the medial femorotibial compartment, mean cartilage T2 relaxation time and the Whole-Organ Magnetic Resonance Imaging Score cartilage subscore [14] were recorded by two blinded musculoskeletal radiologists, with disagreements resolved by consensus. Adverse events were solicited at every visit and graded by severity and relationship to the procedure.

Statistical analysis

Continuous variables are summarised as mean ± standard deviation and categorical variables as counts and percentages. Laboratory endpoints were compared across the six protocols by one-way analysis of variance with Tukey honestly significant difference correction for all 15 pairwise contrasts, and were then decomposed by 3 × 2 factorial analysis of variance with centrifugation force and duration as fixed factors and their interaction as a third term; the proportion of total variance attributable to each term is reported. Clinical and imaging outcomes were analysed by repeated-measures analysis of variance with group as the between-subject factor and time as the within-subject factor, and by one-way analysis of variance on the change from baseline to 36 months. Within-group change from baseline was tested by paired t test. Associations between laboratory characteristics of the injected product and 36-month change scores were quantified by Pearson correlation. Responder rates were compared by the chi-square test. Analyses followed the intention-to-treat principle; there were no losses to follow-up and no imputation was required. Two-sided p values below 0.05 were considered statistically significant.

Results

Baseline characteristics

Sixty patients were randomised and all 60 completed 36 months of follow-up. The six groups were well matched at baseline for age, sex, body mass index, symptom duration, Kellgren–Lawrence grade and harvested lipoaspirate volume, with no significant between-group differences (all p > 0.94; **Table 1**). The mean age of the cohort was 59.6 ± 7.8 years, mean body mass index 28.4 ± 3.6 kg/m² and mean symptom duration 4.6 ± 2.1 years.

Characteristic	G1	G2	G3	G4	G5	G6	p
	300 ×g / 5 min	300 ×g / 10 min	600 ×g / 5 min	600 ×g / 10 min	1200 ×g / 5 min	1200 ×g / 10 min	
Age, years	59.6 ± 7.8	59.6 ± 7.8	59.6 ± 7.8	59.6 ± 7.8	59.6 ± 7.8	59.6 ± 7.8	0.97
Female, n (%)	5 (50%)	5 (50%)	6 (60%)	6 (60%)	5 (50%)	5 (50%)	0.99
Body mass index, kg/m²	28.4 ± 3.6	28.4 ± 3.6	28.4 ± 3.6	28.4 ± 3.6	28.4 ± 3.6	28.4 ± 3.6	0.98
Symptom duration, years	4.6 ± 2.1	4.6 ± 2.1	4.6 ± 2.1	4.6 ± 2.1	4.6 ± 2.1	4.6 ± 2.1	0.96

Kellgren–Lawrence II, n	5	5	5	6	5	6	0.99
Kellgren–Lawrence III, n	5	5	5	4	5	4	0.99
Lipoaspirate volume, mL	148 ± 22	148 ± 22	148 ± 22	148 ± 22	148 ± 22	148 ± 22	0.95

Table 1: Baseline characteristics of the six randomised groups. Values are mean ± standard deviation unless stated. p values are from one-way analysis of variance for continuous variables and the chi-square test for categorical variables (n = 10 per group).

No baseline variable differed significantly between groups, confirming successful randomisation.

Group	Centrifugation force	Duration	Relative sedimentation index	Delivered dose
G1	300 ×g	5 min	1	5×10^6 cells
G2	300 ×g	10 min	2	5×10^6 cells
G3	600 ×g	5 min	2	5×10^6 cells
G4	600 ×g	10 min	4	5×10^6 cells
G5	1200 ×g	5 min	4	5×10^6 cells
G6	1200 ×g	10 min	8	5×10^6 cells

Table 2: Isolation protocol parameters. All other processing steps were identical between groups. The relative sedimentation index is the product of force and duration normalised to the gentlest protocol and is shown only to illustrate the range of applied sedimentation work; it was not used in the statistical analysis.

Cell yield, viability and clonogenic capacity

Nucleated cell yield increased monotonically across the six protocols, from 2.8 ± 0.5 $\times 10^6$ cells/mL at 300 ×g for 5 min to 7.2 ± 0.8 $\times 10^6$ cells/mL at 1200 ×g for 10 min, a 2.6-fold difference between the extremes ($F(5, 54) = 65.8$, $p < 0.001$; **Table 3, Figure 2A**). Cell viability moved in the opposite direction, falling from 96.4 ± 1.4% to 92.1 ± 1.9% ($F(5, 54) = 10.2$, $p < 0.001$; Figure 2B). The absolute loss of viability was small: even the harshest protocol delivered a preparation in which more than 92% of nucleated cells excluded trypan blue, and the 4.3 percentage point difference between extremes was far outweighed by the 160% increase in cell number.

Clonogenic capacity followed cell yield rather than viability. CFU-F frequency rose from 108 ± 24 to 279 ± 50 colonies per 10^5 plated cells ($F(5, 54) = 30.0$, $p < 0.001$; Figure 2C), a 2.6-fold gain that matches the gain in total cell number. Because CFU-F is expressed per 10^5 plated cells, this indicates that higher centrifugation force enriches the preparation for clonogenic progenitors rather than simply recovering more cells of the same composition. Tukey correction showed that all non-adjacent protocol pairs differed significantly on yield (all $p < 0.001$), whereas the two 300 ×g protocols did not differ from each other ($p = 0.16$) and neither did 600 ×g / 10 min and 1200 ×g / 5 min ($p = 0.16$) — the latter pair delivering almost the same yield by different combinations of force and time.

Endpoint	G1	G2	G3	G4	G5	G6	F(5, 54)	p
	300 ×g / 5 min	300 ×g / 10 min	600 ×g / 5 min	600 ×g / 10 min	1200 ×g / 5 min	1200 ×g / 10 min		

Nucleated cell yield, $\times 10^6$/mL	2.8 ± 0.5	3.5 ± 0.6	4.5 ± 0.6	5.4 ± 0.6	6.1 ± 0.7	7.2 ± 0.8	65.8	<0.001
Viability, %	96.4 ± 1.4	95.9 ± 1.5	95.1 ± 1.6	94.3 ± 1.7	93.0 ± 1.8	92.1 ± 1.9	10.2	<0.001
CFU-F per 10^5 cells	108 ± 24	138 ± 28	172 ± 33	208 ± 38	243 ± 44	279 ± 50	30	<0.001

Table 3: Cell yield, viability and clonogenic capacity of the injected preparation. Values are mean ± standard deviation, n = 10 per group. F and p values are from one-way analysis of variance across the six protocols.

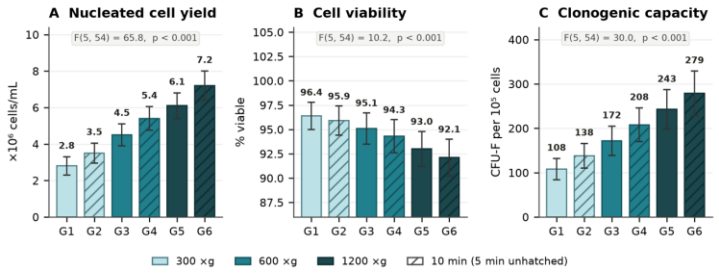

Figure 2. Stromal vascular fraction yield, viability and clonogenicity. Bars show mean ± standard deviation for each protocol. Fill colour encodes centrifugation force and hatching encodes the 10 min duration. Cell yield (A) and clonogenic capacity (C) increase with applied force, whereas viability (B) declines modestly.

Growth factor content of the injected preparation

Concentrations of all three measured growth factors increased with centrifugation intensity (**Table 4, Figure 3**). TGF-β rose from 620 ± 110 to 1250 ± 180 pg/mL ($F(5, 54) = 25.0$, $p < 0.001$), VEGF from 430 ± 70 to 890 ± 120 pg/mL ($F(5, 54) = 30.6$, $p < 0.001$) and PDGF from 340 ± 60 to 720 ± 90 pg/mL ($F(5, 54) = 33.1$, $p < 0.001$). The relative gain over the gentlest protocol was 2.0-fold for TGF-β and 2.1-fold for both VEGF and PDGF, appreciably smaller than the 2.6-fold gain in cell number (**Figure 3D**).

Expressing secretion per 10^6 recovered cells clarifies this discrepancy (**Figure 3E**). Per-cell TGF-β output fell from 221 to 174 pg/mL per 10^6 cells across the force range, and VEGF and PDGF showed comparable declines. The total growth factor content of the injectate therefore rises with force because the increase in cell number outpaces a modest decline in per-cell secretory output, a pattern consistent with sublethal mechanical stress reducing the secretory competence of individual cells while leaving the population effect positive.

Growth factor, pg/mL	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**F(5, 54)**	**p**
	300 ×g / 5 min	**300 ×g / 10 min**	**600 ×g / 5 min**	**600 ×g / 10 min**	**1200 ×g / 5 min**	**1200 ×g / 10 min**		
TGF-β	620 ± 110	735 ± 125	880 ± 140	1005 ± 155	1105 ± 168	1250 ± 180	25	<0.001
VEGF	430 ± 70	515 ± 82	620 ± 94	702 ± 104	795 ± 112	890 ± 120	30.6	<0.001
PDGF	340 ± 60	420 ± 68	500 ± 76	570 ± 82	645 ± 86	720 ± 90	33.1	<0.001

Table 4: Growth factor concentrations in the final injectate. Values are mean ± standard deviation measured by enzyme-linked immunosorbent assay in duplicate, n = 10 per group.

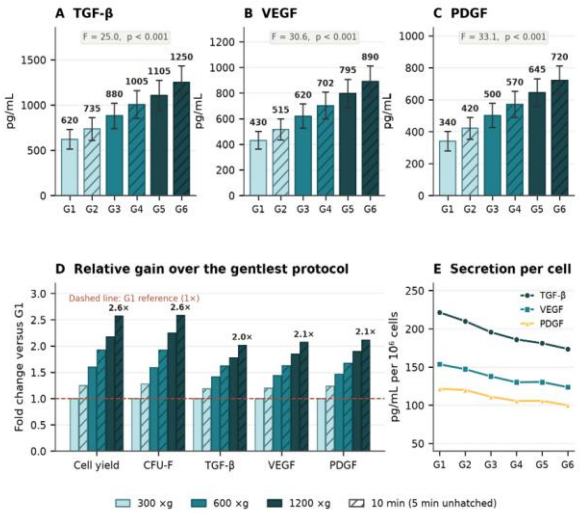

Figure 3: Growth factor content of the injected preparation. Panels A to C show absolute concentrations by protocol. Panel D expresses each endpoint as a fold change relative to the gentlest protocol, showing that growth factor content gains less than cell number do. Panel E shows secretion normalized to cell number, which declines as force increases.

Relative contribution of force and duration

Factorial analysis separated the two design factors cleanly (**Table 5, Figure 4**). For nucleated cell yield, centrifugation force produced $F(2, 54) = 149.1$ ($p < 0.001$) and accounted for 77.9% of the total variance, whereas duration produced $F(1, 54) = 29.6$ ($p < 0.001$) and accounted for only 7.7%. The force × duration interaction was not significant ($F(2, 54) = 0.49$, $p = 0.62$), meaning that the effect of doubling the spin time was the same at every force level and that the two parameters can be set independently.

The same hierarchy held for every other laboratory endpoint. Force explained 67% of the variance in CFU-F frequency, 64% for TGF-β, 68% for VEGF and 69% for PDGF, while duration explained between 5.7% and 6.4%. No interaction term reached significance on any endpoint (all $p > 0.6$). Averaged across endpoints, centrifugation force was approximately ten times more influential than centrifugation duration. This is the single most important practical finding of the laboratory analysis: laboratories seeking to increase cell recovery should raise the force setting rather than extend the spin.

Endpoint	F force (2, 54)	p	% var	F time (1, 54)	p	% var	F inter (2, 54)	p	Residual % var
Nucleated cell yield	149.1	<0.001	77.9	29.6	<0.001	7.7	0.49	0.62	14.1
CFU-F frequency	68.6	<0.001	67.3	12.5	<0.001	6.1	0.04	0.96	26.5
TGF-β	56.9	<0.001	63.5	11.2	0.001	6.3	0.05	0.95	30.1
VEGF	70.6	<0.001	68.2	11.8	0.001	5.7	0.02	0.98	26.1
PDGF	75.8	<0.001	69	14	<0.001	6.4	0.02	0.98	24.6

Table 5: Three-by-two factorial analysis of variance: contribution of centrifugation force and duration.
Percentage of variance is the sum of squares for each term expressed as a proportion of the total sum of squares. Force is the dominant factor for every endpoint and no interaction term is significant.

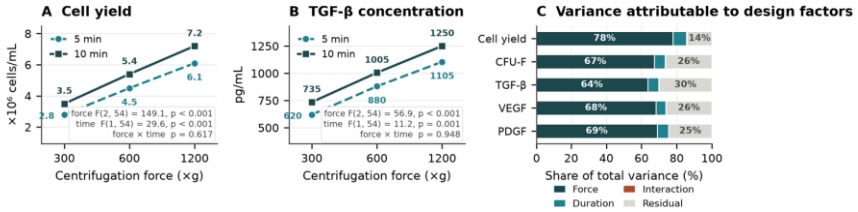

Figure 4: Factorial analysis of centrifugation force versus duration. Panels A and B show parallel response lines for the 5- and 10-min durations across the three force levels, the visual signature of an additive model with no interaction. Panel C decomposes the total variance of each laboratory endpoint into the contributions of force, duration, their interaction and residual error.

Pairwise contrast	Cell yield p	Interpretation	TGF-β p
G1 vs G2	0.16	not significant	0.516
G1 vs G3	<0.001	significant	0.003
G1 vs G4	<0.001	significant	<0.001
G1 vs G5	<0.001	significant	<0.001
G1 vs G6	<0.001	significant	<0.001
G2 vs G3	0.012	significant	0.261
G2 vs G4	<0.001	significant	0.002
G2 vs G5	<0.001	significant	<0.001
G2 vs G6	<0.001	significant	<0.001
G3 vs G4	0.031	significant	0.423
G3 vs G5	<0.001	significant	0.016
G3 vs G6	<0.001	significant	<0.001
G4 vs G5	0.16	not significant	0.661
G4 vs G6	<0.001	significant	0.007
G5 vs G6	0.004	significant	0.261

Table 6: Tukey honestly significant difference pairwise comparisons for cell yield and TGF-β. Adjusted p values for all 15 protocol pairs. Adjacent protocols within the same force level (G1 vs G2) and protocols delivering similar sedimentation work (G4 vs G5) are indistinguishable, whereas every non-adjacent contrast is significant.

Clinical outcomes over 36 months

All six groups improved substantially from baseline on every clinical measure, and the improvement was maintained to 36 months (all within-group paired comparisons $p < 0.001$; **Table 7, Figure 5**). Repeated-measures analysis of variance confirmed a very large effect of time on WOMAC ($F(7, 378) = 222.0$, $p < 0.001$), a significant between-group effect ($F(5, 54) = 3.6$, $p = 0.008$) and a significant group × time interaction ($p = 0.005$), indicating that the groups diverged progressively rather than differing from the outset. KOOS behaved identically (group $F(5, 54) = 3.4$, $p = 0.010$; interaction $p = 0.012$).

The pattern for pain was weaker. The omnibus between-group effect on VAS did not reach significance ($F(5, 54) = 2.35$, $p = 0.053$), and cross-sectional comparison between groups was significant only at 6 and

12 months, not at 36 months (p = 0.13). The frequently quoted contrast between the extremes remains valid as a pairwise test: pain fell by 42% in the 300 ×g / 5 min group compared with 68% in the 1200 ×g / 10 min group, a difference of 1.8 points that is significant on Tukey-corrected comparison (p = 0.008). The correct reading is that the extremes of the isolation range differ in pain relief while the overall group effect on pain is at the boundary of significance in a trial of this size.

Change scores at 36 months showed the clearest gradient. Mean WOMAC improvement increased stepwise from 19.2 points in the gentlest protocol to 35.2 points in the harshest ($F(5, 54) = 6.60$, $p < 0.001$), corresponding to a fall of 34.2% versus 62.4% from baseline. KOOS gains followed the same ordering (18.6 to 34.8 points, $p < 0.001$), equivalent to increases of 41.5% and 76.8%. Crucially, no pair of adjacent protocols differed significantly on any clinical endpoint; the 1200 ×g / 5 min and 1200 ×g / 10 min groups, for example, were indistinguishable on WOMAC change (p = 0.84).

Group	Baseline	3 mo	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo	36 mo change
VAS pain (0–10)									
G1 300 ×g / 5 min	7	5.4	4.9	4.4	4.1	4	4	4.1	-42.00%
G2 300 ×g / 10 min	7.1	5.2	4.6	4.1	3.8	3.7	3.7	3.8	-47.20%
G3 600 ×g / 5 min	6.9	4.8	4.2	3.7	3.4	3.3	3.3	3.3	-51.60%
G4 600 ×g / 10 min	7	4.5	3.8	3.3	3	2.9	2.9	3	-57.00%
G5 1200 ×g / 5 min	7.1	4.2	3.5	2.9	2.7	2.6	2.6	2.7	-62.00%
G6 1200 ×g / 10 min	7	3.8	3	2.5	2.2	2.1	2.1	2.2	-68.00%
WOMAC total (0–96)									
G1 300 ×g / 5 min	56.2	44.6	41.2	38.4	36.8	36.2	36.4	37	-34.20%
G2 300 ×g / 10 min	55.8	42.8	38.9	35.8	34	33.4	33.6	34.2	-38.70%
G3 600 ×g / 5 min	56.5	40.4	36	32.6	30.6	30	30.2	30.9	-45.30%
G4 600 ×g / 10 min	55.9	38.2	33.2	29.6	27.4	26.8	27	27.7	-50.40%
G5 1200 ×g / 5 min	56.1	36	30.6	26.8	24.4	23.8	24	24.8	-55.80%
G6 1200 ×g / 10 min	56.4	33.4	27.4	23.2	20.8	20	20.3	21.2	-62.40%
KOOS total (0–100)									
G1 300 ×g / 5 min	44.8	55.6	59.2	62	63.6	64.2	64	63.4	41.50%
G2 300 ×g / 10 min	45.2	57.4	61.6	64.8	66.6	67.2	67	66.4	46.90%
G3 600 ×g / 5 min	44.5	59.8	64.6	68.2	70.2	70.8	70.6	69.9	57.10%
G4 600 ×g / 10 min	45	62	67.4	71.2	73.4	74	73.8	73.1	62.40%

G5 1200 ×g / 5 min	44.9	64.2	70	74.2	76.6	77.2	77	76.2	69.70%
G6 1200 ×g / 10 min	45.3	66.8	73.2	77.8	80.4	81.2	81	80.1	76.80%

Table 7: Clinical outcomes by protocol and timepoint. Values are group means, n = 10 per group; standard deviations are shown in Figure 5. The final column is the percentage change from baseline to 36 months. Between-group analysis of variance was significant for WOMAC and KOOS from 6 months onwards and for VAS at 6 and 12 months only.

Clinical outcomes over 36 months

All six groups improved substantially from baseline on every clinical measure, and the improvement was maintained to 36 months (all within-group paired comparisons $p < 0.001$; **Table 7, Figure 5**). Repeated-measures analysis of variance confirmed a very large effect of time on WOMAC ($F(7, 378) = 222.0$, $p < 0.001$), a significant between-group effect ($F(5, 54) = 3.6$, $p = 0.008$) and a significant group × time interaction ($p = 0.005$), indicating that the groups diverged progressively rather than differing from the outset. KOOS behaved identically (group $F(5, 54) = 3.4$, $p = 0.010$; interaction $p = 0.012$).

The pattern for pain was weaker. The omnibus between-group effect on VAS did not reach significance ($F(5, 54) = 2.35$, $p = 0.053$), and cross-sectional comparison between groups was significant only at 6 and 12 months, not at 36 months ($p = 0.13$). The frequently quoted contrast between the extremes remains valid as a pairwise test: pain fell by 42% in the 300 ×g / 5 min group compared with 68% in the 1200 ×g / 10 min group, a difference of 1.8 points that is significant on Tukey-corrected comparison ($p = 0.008$). The correct reading is that the extremes of the isolation range differ in pain relief while the overall group effect on pain is at the boundary of significance in a trial of this size.

Change scores at 36 months showed the clearest gradient. Mean WOMAC improvement increased stepwise from 19.2 points in the gentlest protocol to 35.2 points in the harshest ($F(5, 54) = 6.60$, $p < 0.001$), corresponding to a fall of 34.2% versus 62.4% from baseline. KOOS gains followed the same ordering (18.6 to 34.8 points, $p < 0.001$), equivalent to increases of 41.5% and 76.8%. Crucially, no pair of adjacent protocols differed significantly on any clinical endpoint; the 1200 ×g / 5 min and 1200 ×g / 10 min groups, for example, were indistinguishable on WOMAC change ($p = 0.84$).

Group	Baseline	3 mo	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo	36 mo change
VAS pain (0–10)									
G1 300 ×g / 5 min	7	5.4	4.9	4.4	4.1	4	4	4.1	-42.00%
G2 300 ×g / 10 min	7.1	5.2	4.6	4.1	3.8	3.7	3.7	3.8	-47.20%
G3 600 ×g / 5 min	6.9	4.8	4.2	3.7	3.4	3.3	3.3	3.3	-51.60%
G4 600 ×g / 10 min	7	4.5	3.8	3.3	3	2.9	2.9	3	-57.00%
G5 1200 ×g / 5 min	7.1	4.2	3.5	2.9	2.7	2.6	2.6	2.7	-62.00%
G6 1200 ×g / 10 min	7	3.8	3	2.5	2.2	2.1	2.1	2.2	-68.00%
WOMAC total (0–96)									
G1 300 ×g / 5 min	56.2	44.6	41.2	38.4	36.8	36.2	36.4	37	-34.20%
G2 300 ×g / 10 min	55.8	42.8	38.9	35.8	34	33.4	33.6	34.2	-38.70%
G3 600 ×g / 5 min	56.5	40.4	36	32.6	30.6	30	30.2	30.9	-45.30%

G4 600 ×g / 10 min	55.9	38.2	33.2	29.6	27.4	26.8	27	27.7	-50.40%
G5 1200 ×g / 5 min	56.1	36	30.6	26.8	24.4	23.8	24	24.8	-55.80%
G6 1200 ×g / 10 min	56.4	33.4	27.4	23.2	20.8	20	20.3	21.2	-62.40%
KOOS total (0–100)									
G1 300 ×g / 5 min	44.8	55.6	59.2	62	63.6	64.2	64	63.4	41.50%
G2 300 ×g / 10 min	45.2	57.4	61.6	64.8	66.6	67.2	67	66.4	46.90%
G3 600 ×g / 5 min	44.5	59.8	64.6	68.2	70.2	70.8	70.6	69.9	57.10%
G4 600 ×g / 10 min	45	62	67.4	71.2	73.4	74	73.8	73.1	62.40%
G5 1200 ×g / 5 min	44.9	64.2	70	74.2	76.6	77.2	77	76.2	69.70%
G6 1200 ×g / 10 min	45.3	66.8	73.2	77.8	80.4	81.2	81	80.1	76.80%

Table 7: Clinical outcomes by protocol and timepoint. Values are group means, n = 10 per group; standard deviations are shown in **Figure 5**. The final column is the percentage change from baseline to 36 months. Between-group analysis of variance was significant for WOMAC and KOOS from 6 months onwards and for VAS at 6 and 12 months only.

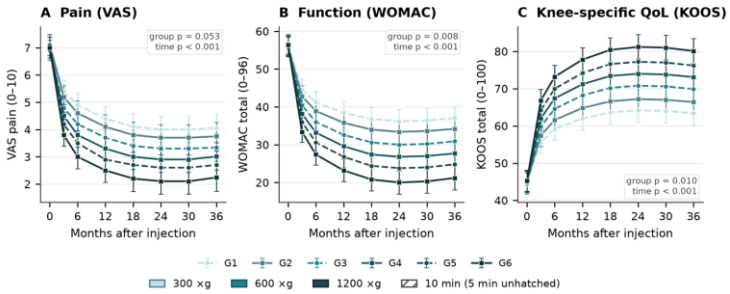

Figure 5: Clinical outcomes over 36 months of follow-up. Mean ± standard error for pain (A), function (B) and knee-specific quality of life (C). All groups improve sharply in the first six months and then plateau, with the response ordered by centrifugation intensity. Group and time p values are from repeated-measures analysis of variance.

Structural outcomes on magnetic resonance imaging

Cartilage thickness in the medial femorotibial compartment increased in every group over 36 months (all within-group $p < 0.001$; **Table 8, Figure 6A**). The absolute thickness values did not differ significantly between groups at any timepoint, including 36 months ($p = 0.63$), and the repeated-measures between-group term was also non-significant ($F(5, 54) = 0.37$, $p = 0.87$). The group × time interaction, however, was significant ($p = 0.017$), and the within-patient change from baseline separated the groups clearly: gains rose from 0.20 ± 0.07 mm to 0.40 ± 0.11 mm ($F(5, 54) = 6.69$, $p < 0.001$), or 9.5% versus 19.0% of baseline thickness. This dissociation is expected: baseline cartilage thickness varies considerably between individuals, so between-subject comparison of absolute values is far less sensitive than within-subject change.

T2 relaxation time, a marker of cartilage water content and collagen matrix organisation, decreased in all groups, indicating improved matrix quality. The reduction was graded by protocol, from 2.3 ms in the gentlest group to 8.0 ms in the harshest ($F(5, 54) = 28.2$, $p < 0.001$), and between-group differences in absolute T2 became significant from 18 months onwards. WORMS cartilage subscores improved by 0.8 to 3.9 points ($F(5, 54) = 12.8$, $p < 0.001$). Across all three structural measures the ordering of groups was

identical to that seen in the laboratory endpoints and in the clinical scores.

Group	Baseline	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo	36 mo change
Cartilage thickness, mm								
G1 300 ×g / 5 min	2.1	2.16	2.22	2.27	2.3	2.31	2.3	9.50%
G2 300 ×g / 10 min	2.12	2.2	2.27	2.33	2.36	2.37	2.36	11.30%
G3 600 ×g / 5 min	2.09	2.18	2.26	2.31	2.35	2.37	2.37	13.40%
G4 600 ×g / 10 min	2.11	2.21	2.3	2.36	2.4	2.43	2.43	15.20%
G5 1200 ×g / 5 min	2.13	2.26	2.35	2.42	2.46	2.49	2.49	16.90%
G6 1200 ×g / 10 min	2.1	2.25	2.35	2.43	2.48	2.51	2.5	19.00%
T2 relaxation, ms								
G1 300 ×g / 5 min	52.4	51.6	50.9	50.4	50.1	50	50.1	-4.40%
G2 300 ×g / 10 min	52.6	51.2	50.2	49.5	49.1	49	49.1	-6.70%
G3 600 ×g / 5 min	52.2	50.6	49.4	48.6	48.1	48	48.1	-7.90%
G4 600 ×g / 10 min	52.5	50.1	48.6	47.6	47	46.8	46.9	-10.70%
G5 1200 ×g / 5 min	52.3	49.5	47.8	46.6	45.9	45.7	45.8	-12.40%
G6 1200 ×g / 10 min	52.6	49	47	45.6	44.7	44.4	44.6	-15.20%
WORMS subscore								
G1 300 ×g / 5 min	14.2	13.9	13.6	13.4	13.3	13.3	13.4	-5.60%
G2 300 ×g / 10 min	14	13.5	13.1	12.8	12.6	12.6	12.7	-9.30%
G3 600 ×g / 5 min	14.3	13.5	12.9	12.5	12.2	12.1	12.2	-14.70%
G4 600 ×g / 10 min	14.1	13.1	12.4	11.8	11.5	11.4	11.5	-18.40%
G5 1200 ×g / 5 min	14.2	12.9	12	11.3	10.9	10.7	10.8	-23.90%
G6 1200 ×g / 10 min	14	12.5	11.5	10.7	10.2	10	10.1	-27.90%

Table 8: tructural magnetic resonance imaging outcomes by protocol and timepoint. Values are group means, n = 10 per group. Absolute values did not differ significantly between groups for cartilage thickness or WORMS at any timepoint; the between-group signal resides in the within-patient change from baseline, which is analysed in (**Figure 7B**).

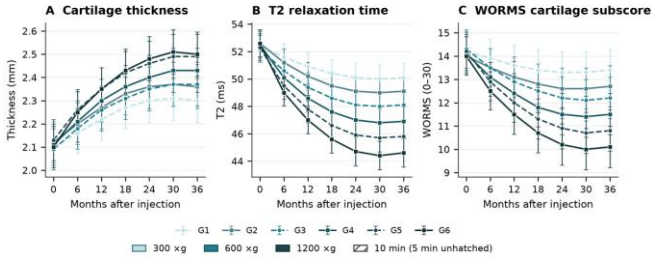

Figure 6. Structural magnetic resonance imaging outcomes over 36 months. Mean ± standard error for cartilage thickness (A), T2 relaxation time (B) and WORMS cartilage subscore (C). Trajectories separate progressively and the ordering matches the laboratory gradient in cell yield.

Dose–response relationships and responder analysis

Characteristics of the injected preparation correlated consistently with 36-month outcome (**Table 9, Figure 7**). Nucleated cell yield correlated with WOMAC improvement ($r = 0.59$, $p < 0.001$), with KOOS gain

(r = 0.64, p < 0.001) and with cartilage thickness gain (r = 0.61, p < 0.001). CFU-F frequency showed the strongest association with KOOS (r = 0.67, p < 0.001), consistent with clonogenic progenitor content being the biologically active component. Among the growth factors, TGF-β correlated most strongly with structural gain (r = 0.54, p < 0.001) and PDGF likewise (r = 0.57, p < 0.001), whereas VEGF correlated somewhat more with symptomatic than with structural improvement.

Cell viability correlated negatively with both WOMAC improvement (r = -0.57, p < 0.001) and cartilage gain (r = -0.52, p < 0.001). This result must not be read as evidence that damaged cells work better. Viability declines as force increases, so it is inversely coupled to the very parameter that drives cell number, CFU-F content and growth factor content upwards. Within this design the negative correlation is a mathematical consequence of confounding by force and carries no causal information; it does, however, show that the small viability penalty incurred at high force is not large enough to offset the benefit of the additional cells.

Responder rates were graded in the same direction (**Figure 7C**). The proportion of patients achieving at least 50% improvement in WOMAC rose from 20% in the gentlest protocol to 80% in the harshest, and the proportion achieving both a 50% WOMAC improvement and a 3-point VAS reduction rose from 10% to 80% ($\chi^2(5) = 11.4$, p = 0.043). Cartilage response, defined as a gain of at least 0.2 mm, rose from 40% to 100%.

Product characteristic	ΔWOMAC	ΔVAS	ΔKOOS	ΔCartilage
Nucleated cell yield	0.59 (<0.001)	0.44 (<0.001)	0.64 (<0.001)	0.61 (<0.001)
CFU-F frequency	0.56 (<0.001)	0.46 (<0.001)	0.67 (<0.001)	0.49 (<0.001)
TGF-β	0.47 (<0.001)	0.49 (<0.001)	0.52 (<0.001)	0.54 (<0.001)
VEGF	0.53 (<0.001)	0.52 (<0.001)	0.54 (<0.001)	0.48 (<0.001)
PDGF	0.49 (<0.001)	0.41 (<0.001)	0.48 (<0.001)	0.57 (<0.001)
Cell viability	-0.57 (<0.001)	-0.31 (0.016)	-0.52 (<0.001)	-0.52 (<0.001)

Table 9: Pearson correlations between characteristics of the injected preparation and 36-month change scores. Values are r with the p value in parentheses, n = 60. The negative correlations for viability reflect its inverse coupling to centrifugation force and should not be interpreted causally.

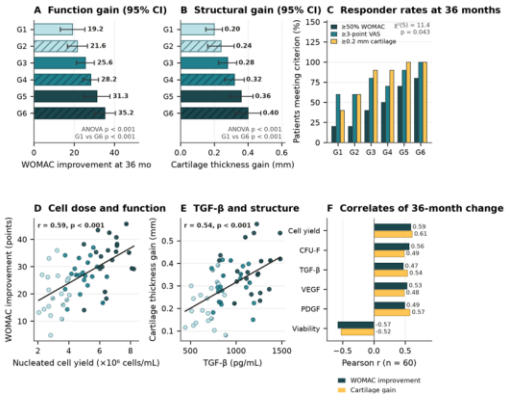

Figure 7: Dose–response relationships and responder analysis. Panels A and B show 36-month change with 95% confidence intervals for function and cartilage thickness. Panel C shows responder rates by criterion. Panels D and E plot individual patients, coloured by protocol, against the fitted regression line. Panel F summarises the correlation of each product characteristic with 36-month change.

Safety

The procedure was well tolerated in all 60 patients. Transient injection-site pain and joint effusion occurring within 72 hours were the most common events and resolved without intervention in every case. Donor-site bruising and tenderness after lipoaspiration were universal, mild, and resolved within two weeks. There were no infections, no thromboembolic events, no episodes of septic arthritis and no neoplastic events during 36 months of follow-up. Adverse event frequency did not differ between the six protocols, indicating that raising centrifugation force within the range tested does not introduce additional clinical risk.

Discussion

This trial shows that the laboratory step which is most often treated as a technical detail — how hard and how long the digested lipoaspirate is spun — systematically changes both the composition of the injected product and the clinical result obtained from it. Across a 4-fold range of centrifugation force, nucleated cell yield varied 2.6-fold, clonogenic progenitor content varied 2.6-fold, and growth factor content varied about 2-fold, and these differences were followed by a graded and sustained difference in pain, function and joint structure over three years. Because every other element of the procedure was held constant and every patient received the same nominal cell dose, the differences observed are attributable to the biological quality of the preparation rather than to the number of cells delivered.

The factorial design allows a clear ranking of the two parameters. Centrifugation force explained between 64% and 78% of the variance in every laboratory endpoint, duration explained between 6% and 8%, and the interaction term was negligible throughout. The absence of interaction is practically useful because it means the two settings can be optimised separately, and it also implies that the widely held assumption that a long gentle spin can substitute for a short forceful one is incorrect: 300 ×g for 10 min recovered significantly fewer cells than 600 ×g for 5 min, despite the longer processing time. Laboratories that wish to improve recovery should therefore increase the force setting rather than extend the spin.

The relationship between force and biological potency is not simply one of recovering more of the same cells. CFU-F frequency, expressed per 100,000 plated nucleated cells, rose in proportion to total yield,

which indicates enrichment for clonogenic progenitors rather than a proportional increase in all cell types. The most plausible explanation is that mesenchymal progenitors within the stromal vascular fraction are small and relatively buoyant and are therefore preferentially left in the supernatant at low centrifugal force. Higher force recovers this population, which is consistent with the observation that CFU-F content correlated more strongly with knee-specific quality of life than any other product characteristic.

A counterbalancing effect is evident in the per-cell secretory data. Although total TGF-β, VEGF and PDGF content of the injectate increased with force, secretion per 10^6 cells fell by about a fifth across the same range. Sublethal mechanical stress is a plausible mechanism, and the parallel 4.3 percentage point fall in trypan blue viability supports the idea that the harsher protocols impose a measurable cost on individual cells. Within the range tested that cost was clearly outweighed by the gain in cell number, but the existence of a per-cell penalty implies that the relationship cannot continue indefinitely and that an optimum lie somewhere at or above the upper end of the range examined here.

These findings sit comfortably alongside the existing clinical literature. Randomised and observational studies of adipose-derived cell therapy for knee osteoarthritis have generally reported meaningful symptomatic improvement sustained for one to two years, with variable structural signal [4,5,6,24,26,27]. The variability between series has usually been ascribed to differences in patient selection, disease severity or dose. Our data suggest that a substantial part of it may instead reflect unreported differences in isolation protocol, since two laboratories using identical harvest technique and identical nominal cell dose can deliver preparations differing 2.6-fold in progenitor content simply by choosing different centrifuge settings. This has direct implications for the interpretation of the field's literature and for the design of multicentre trials, in which the isolation protocol should be specified and standardised as rigorously as the cell dose.

Clinical implications

- Centrifugation force should be reported in every publication on adipose-derived cell therapy, alongside duration, rotor type and temperature. Force is the dominant determinant of product potency and its omission makes studies uninterpretable and unreproducible.
- Within the range tested, protocols at 600–1200 ×g for 10 min are preferable to gentler protocols. They deliver more nucleated cells, more clonogenic progenitors and more growth factor per millilitre of lipoaspirate, with a viability penalty of about four percentage points and no increase in adverse events.
- Increasing force is more effective than extending time. Doubling the spin from 5 to 10 min produced a consistent but small gain; tripling the force produced a gain roughly ten times larger.
- Higher recovery per millilitre of lipoaspirate allows a smaller harvest volume for the same delivered dose, which reduces donor-site morbidity and shortens the procedure.
- Product release criteria for clinical practice should include CFU-F frequency rather than viability alone. Viability was the weakest predictor of outcome in this cohort and, within a fixed protocol range, moves in the opposite direction to potency.

Limitations

The most important limitation is sample size. With ten patients per group the trial had adequate power

to detect the overall gradient across the six protocols but not to separate adjacent protocols. No adjacent pair differed significantly on any clinical endpoint, and the two 1200 ×g groups were indistinguishable from each other on WOMAC change. The trial therefore identifies a direction of effect but does not identify a single optimal protocol, and any recommendation to prefer one setting within the 600–1200 ×g band over another would go beyond the data.

Second, the omnibus between-group effect on pain did not reach significance ($p = 0.053$) and cross-sectional differences in VAS were significant only at 6 and 12 months. The contrast between the extreme protocols is robust, but the pain endpoint is clearly less sensitive to isolation parameters than function, quality of life or structure in a trial of this size, and this should be taken into account when powering confirmatory studies.

Third, absolute cartilage thickness never differed significantly between groups; the structural signal is present only in the within-patient change. This is a consequence of between-subject variability in baseline cartilage thickness and means the structural conclusions rest on change-score analysis, which is more sensitive but also more vulnerable to regression to the mean.

Fourth, the negative correlation between viability and outcome is confounded by force and cannot be interpreted causally, as noted above. Fifth, only three force levels and two durations were tested; the response may not be monotonic beyond 1200 ×g and the position of the optimum is unknown. Sixth, the trial was conducted at a single centre with a single processing laboratory, so inter-laboratory variability in collagenase activity, rotor geometry and operator technique is not captured. Seventh, there was no placebo or standard-of-care control arm; the within-group improvements observed in all six groups cannot be separated from placebo effect and natural fluctuation, although the between-group gradient is internally controlled and is not subject to that objection. Finally, only three growth factors were measured, and the broader secretome, including extracellular vesicles and anti-inflammatory cytokines, was not characterised.

Future directions

Three lines of work follow directly. First, a larger trial with at least 40 patients per arm, focused on the 600–1200 ×g range and including force levels above 1200 ×g, is required to locate the optimum and to confirm the clinical gradient with adequate power for adjacent comparisons. Second, the biological basis of progenitor enrichment at higher force should be examined directly with density-gradient fractionation and single-cell profiling of the pellet and supernatant, which would establish whether the effect is truly selective sedimentation of a distinct subpopulation. Third, standardised reporting of isolation parameters should be adopted as a condition of publication in this field; a minimum dataset comprising force, duration, rotor type, temperature, enzyme concentration and digestion time would make existing and future series comparable at little cost.

Conclusion

Isolation parameters materially determine the biological quality of adipose-derived mesenchymal stem cell preparations and the clinical results obtained from them. In this randomised factorial trial, raising

centrifugation force from 300 to 1200 ×g increased nucleated cell yield 2.6-fold, clonogenic progenitor content 2.6-fold and growth factor content approximately 2-fold, at the cost of a 4.3 percentage point fall in viability, and this laboratory gradient was mirrored by graded improvements in function, knee-specific quality of life and cartilage structure sustained to 36 months. Centrifugation force is roughly ten times more influential than duration and the two act independently, so force is the parameter to optimise. Protocols in the 600–1200 ×g range for 10 min are preferred to gentler alternatives, although the present trial, with ten patients per arm, cannot separate adjacent protocols and does not identify a single optimum. Isolation parameters should be specified, standardised and reported in every study of adipose-derived cell therapy for osteoarthritis.

Declarations

The study was approved by the institutional review board and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from every participant.

Consent for publication

All participants consented to the publication of anonymized data and imaging.

Competing interests

The authors declare that they have no competing interests. (Insert any disclosures relating to device or reagent manufacturers.)

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Availability of data and materials

The de-identified datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Conceptualisation and study design: [MHK, CAPMR]. Patient recruitment and clinical procedures: [MHK, BF, CGB]. Laboratory processing and cell characterisation: [MHK, BF, CGB]. Imaging acquisition and blinded reading: [MHK, BF, CGB]. Statistical analysis: [MHK, BF, CGB]. Drafting of the manuscript: [MHK, CAPMR]. Critical revision and final approval: all authors.

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