Age and donor site do not affect cell growth and biologic activity in Autologous Tendon-cell Implantation (OrthoATI™) for treatment of patellar tendon and palmaris longus tendon

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Age and donor site do not affect cell growth and biologic activity in Autologous Tendon-cell Implantation (OrthoATI™) for treatment of patellar tendon and palmaris longus tendon

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*The work was selected as oral presentation at 14th Biennial ISAKOS Congress in Boston, USA*
1. **ABSTRACT**

**Objectives:** Autologous tendon cell implantation (OrthoATI™) therapy has demonstrated efficacy in treating patients with tendinopathy at various anatomical sites. This study evaluates the effect of patient age, gender and tendon biopsy site on morphology, growth and gene expression of autologous tendon cells used to treat chronic tendinopathy.

**Methods:** Patients undergoing OrthoATI™ for tendinopathies between 2020 and 2022 were initially treated by biopsies taken from patella tendon (PT) or palmaris longus tendon (PL). Autologous tenocytes were treated at a Good Manufacturing Practice (GMP) cell laboratory where they were isolated, cultured and expanded for four to six weeks. Cell morphology was assessed using phase contrast microscopy. Droplet digital PCR (ddPCR) was utilised for gene expression analysis. Dichotomous results were compared between groups using x² or Fisher’s exact tests with no adjustment for multiple comparisons. The non-parametric Mann–Whitney U and Kruskal–Wallis tests were utilised for the sex and age (<35y, 35-44y, 45-54y, >55y) analyses respectively. All analyses were performed using IBM SPSS v27, and a two-tailed P-value of <0.05 was considered statistically significant.

**Results:** 149 patients were included in the analysis. The PT was biopsied in 63 patients, and PL in 86 patients. There were no observer effects for age and gender between PT and PL groups. There was no statistical significance between the PT and PL tendons for cell morphology, average cell population doubling time (PDT) (PT 83.9 vs PL 82.7 hours, p=0.482), cellular yield (PT 16.2 vs PL 15.2×10^6, p=0.099), and cell viability (PT 98.7 vs PL 99.0%, p=0.277). Additionally, ddPCR analyses showed no statistical significance found in tenogenic gene expression including collagen type I (COL1, p=0.86), tenomodulin (TNMD, p=0.837) and scleraxis (SCX, p=0.331) between PT- and PL-derived tendon cells. An age stratification analysis found no effect on growth and gene expression. COL1 was
found to be higher in males when compared to females (P<0.001), but otherwise no difference was
seen in growth and gene expression in the gender analysis. No post-biopsy clinical complications were
reported for either group.

Conclusion: This study has shown that the growth and bioactivities of tendon cells from tendon
biopsies for OrthoATI™ are not affected by tendon donor site and age.

Level of evidence: IV

WHAT ARE THE NEW FINDINGS?

- Tendon tissue acquisition for autologous tendon cell implantation is not affected by
tendon donor site and gender.
- Patients from the older age groups (> 60 years) have equivalent cell culture times as
younger patients.
2. INTRODUCTION

Chronic degenerative tendinopathy is prevalent with socioeconomic impact in the athletic and working population, but also aging population [1; 2; 3; 4]. First line treatment for tendinopathy is conservative, including rest, non-steroidal anti-inflammatory drugs, bracing, eccentric strengthening, and other rehabilitation exercises [5; 6; 7]. Initial non-surgical treatment results in favourable outcomes in the majority of patients. However, for those patients with high functional demands or ongoing symptoms, further treatment, including surgical intervention, may be required.

In recent years, there has been increasing interest in orthobiologic treatment options for tendinopathy. The use of platelet rich plasma (PRP) has been extensively studied in treating tendinopathy at various anatomic sites [8; 9; 10]. The release of growth factors from activated platelets at the site of tendon injury can enhance the tendon healing cascade and relieve tendonopathy symptoms to a variable degree. Bone marrow aspirate concentrate (BMAC) is an orthobiologic treatment with recent popularity in treating tendon, ligament and other soft tissue injuries. Mesenchymal stromal cells (MSC) can be concentrated from bone marrow aspirated from the anterior iliac crest or proximal tibia or calcaneus [11; 12; 13]. The mechanism of action for MSC is unclear, but there may be a positive effect on immune modulation and the cytokine pathways involved in tissue healing [14]. Early clinical studies using adipose-derived MSCs support a positive treatment effect in rotator cuff tendinopathy [15; 16].

OrthoATI™ is a cell therapy which has gained recognition over the past decade. OrthoATI™ addresses the underlying cause of severe chronic tendinopathy – the loss of functional tendon cells producing and remodelling COL1 [17; 18; 19; 20]. Pre-clinical studies have shown that implanted autologous tendon cells maintain their functional phenotype in vivo, producing COL1 and improving tendon structure [21; 22]. In the clinical setting, OrthoATI™ is a two-stage procedure that involves an initial
tendon biopsy under ultrasound guidance and local anaesthetic, followed by tendon cell harvest and
expansion in a GMP laboratory. Sonography guided injection of autologous tendon cells (2-5×10^6
cells/ml) at the site of tendinopathy is performed four to five weeks later [23]. Clinical trials of
OrthoATI™ show successful treatment of tendinopathy in the common extensor tendon origin at the
efficiency [23; 24], the supraspinatus tendon of the shoulder [25], and the gluteal tendon of the hip [26].
In a case series of chronic resistant lateral epicondylitis, Wang et al [24] reported positive and durable
clinical and radiological outcomes of OrthoATI extending to 4.5 years after treatment.

Currently the patella tendon (PT) and the palmaris longus tendon (PL) have been used as the preferred
tendon biopsy sites for OrthoATI™. Differences between the two donor sites in cell morphology, cell
growth characteristics, and tenogenic gene expression of the derived tendon cells remain unclear. In
addition, the influence of patient demographics has not been evaluated. As the use of OrthoATI™ for
treatment of tendinopathy becomes more widespread, factors which may influence the utility and
efficacy of autologous tendon cells require further investigation. The primary objective of this study
was to evaluate the effect of patient factors (age and gender) on the morphology, growth characteristics
and tenogenic gene expression (quality parameters) of tendon cells derived from the patella and
palmaris longus tendons.
3. METHODS

3.1 Patient selection and age stratification analysis

Patients included in this study had undergone OrthoATI™ for the clinical treatment of a range of tendinopathies including lateral epicondylitis, gluteal tendinopathy, injury of the rotator cuff, achilles and hamstring tendinopathy between the year 2020 and 2022. This project has gained exemption from Human Research Ethics Review by the University of Western Australia (Ref # 2023/ET001074). An age stratification analysis was conducted for the age ranges < 35 (N = 12), 35 - 44 (N = 41), 45 - 60 (N = 57), and > 60-years-old (N = 39).

3.2 Cell harvesting and preparation

Of the treated patients, a needle biopsy of the healthy PT or surgical biopsy of the PL was performed. The PT biopsy was performed under local anaesthesia in an outpatient setting with a Temno Evolution needle biopsy device (14 gauge × 11 cm; CareFusion, Seven Hills, NSW, Australia). Typically a 3×1 mm sample of tendon was harvested from the superficial aspect of either the PT or PL tendon. As part of the Code of Good Manufacturing Practice (GMP): Human Blood and Tissues protocol, a 75-mL sample of venous blood was taken for infection and viral screening. The tendon cells were extracted and expanded at a GMP-accredited facility as governed by the Therapeutic Goods Administration’s (TGA) Code of GMP: Human Blood and Tissues. Cultivation of tendon cells followed the standard manufacture protocol established by Orthocell Ltd (Murdoch, Australia). Tendon tissue biopsies were dissected, digested with type II collagenase and suspended to remove debris. Tendon tissue was pelleted by centrifugation, re-suspended and cultured in fresh growth medium (Dulbecco's Modified Eagle Medium with Fetal Bovine Serum, ThermoFisher, USA) until 80-90% cell confluence. Once achieved, cells were enzymatically detached, counted and passaged or cryopreserved for analysis. General cell morphology were monitored by phase contrast microscopy from passage 1 to 5.
3.3 Measurement of population doubling time and morphological assessment

We used population doubling time (PDT) [27] to measure the growth rate of cells from tissues obtained from different anatomical sites. In brief, we calculate the baseline cell count at the first passage of culture. Population doubling (PD) were calculated as the log\(_{10}\) of the ratio of the final cell count (N) over the initial seeding baseline count (N\(_0\)), divided by the log\(_{10}\) of 2.

\[
P_D = \log\left(\frac{N}{N_0}\right) \div \log 2, \text{ as } \log_{10} \text{ function}
\]

To calculate the population doubling time (PDT), the time period in which the cells are growing (i.e. cell culture time in hours) was divided by the PD value that is gained in the same time period:

\[
PDT \ (hours) = \frac{Time \ Period \ (hours)}{PD}
\]

We have developed a matrix system for morphological assessment to validate the healthy appearance of tendon cell culture. In brief, When cultures have reached confluence, phase-contrast images from 10 random fields were captured at 10× magnification. Visual inspection by trained operators form the assessment of healthy tendon cell culture to ensure no visual evidence of cellular transformation including (i) no focal expansion of irregular shaped fusiform cells combined with persistently low (< 60) or high (> 200) PDT, (ii) no formation of multilayered dense foci aggregates distinct from background monolayered cells, and (iii) no decrease in cell-cell and cell-substrate adhesion.

3.4 Measurement of total cellular yield

Total cellular yield was obtained from manual counting performed by using a hemocytometer. Cells were stained with trypan blue for 2 minutes prior to completing the manual cell count. To calculate total cellular yield the following calculation was used.
Viability was obtained by counting cells which had absorbed the trypan blue. Percentage was determined using the following calculation:

\[
\text{Viability} = \left( \frac{\text{Number of viable cells}}{\text{Total number of cells (viable and non-viable)}} \right) \times 100
\]

3.6 Gene expression analysis by droplet digital PCR (ddPCR)

Total RNA was extracted from cultured cells using PureLink™ RNA Mini Kit (Invitrogen, ThermoFisher Scientific, MA, USA) in accordance with manufacturer’s standard protocol. First-strand cDNA was synthesized from 2μg extracted total RNA using 200 U of M-MLV reverse transcriptase, 20 U of RNasin ribonuclease inhibitor, 0.5 mM of deoxynucleotide triphosphates (dNTPs) and 300 um of oligo-dT (a short sequence of deoxythymine nucleotides) (Promega, Madison, WI, USA) in a total volume of 20 uL for each sample. cDNA samples were purified using the Roche HighPure Kit as per manufacturer’s instruction, purified cDNA were stored at −30°C until further use. The concentration of purified cDNA were quantified using a Nanodrop Lite spectrophotometer (ThermoFisher).

For analyzing gene expression, ddPCR was performed. Tenogenic genes associated with tendon matrix including COL1 and markers that are tendon cell specific such as SCX and TNMD were assessed. SCX and TNMD specifically marks the tendon cell lineage, whereas Collagen 1 (COL1) is the major extracellular matrix in tendon which marks the tendon cell differentiation, GAPDH was used as internal housekeeping control. All applied probes and primers were designed and purchased from Integrated DNA Technologies (IDT, IA, USA). For the quantitative ddPCR either ddPCR
Supermix for Probes or ddPCR™ EvaGreen Supermix (BioRad, CA, USA) was used in accordance
with manufacturer’s protocol. From the PCR reaction mixes, 20 µL was used in droplet generation
using DG8 Cartridge and the QX200 Droplet Generator™ (BioRad). Amplification was carried out
with the following cycling conditions: 1 cycle of 95°C for 5 min, 40 cycles of 95°C for 30 secs,
53/58°C for 1 min, and 72°C for 30 secs, 1 cycle of 4°C for 5 min and 90°C for 5 min with ramp rate
at 2°C/sec in all steps. Droplet reading and analysis of data conducted on the QX200 Droplet Reader
and QuantaSoft™ analysis software (BioRad). Gene expression was determined in absolute number
of mRNA copies/µg cDNA. Primers were listed in supplementary figure 1.

3.7 Gene expression analysis by quantitative qPC (qPCR)
For analysis of gene expression by qPCR, SYBR Green qPCR Master Mix were added to the cDNA
samples of three tendon cells from patients who received ATI and adipocyte cDNA (ScienCell™
Research Laboratories, California, USA) according to the manufacturer’s standard protocol. Primers
Primers were listed in supplementary figure 1.. The samples were run on Biorad Cfx 384 machine.
Data was exported and analyzed by t test.

3.8 Statistical analysis
Baseline characteristics and outcome data for each procedure group were described using mean
(standard deviation), median (Interquartile Range- IQR) or frequencies/proportions (%), depending on
the distribution. Dichotomous results were compared between groups using χ² or Fisher’s exact tests
with no adjustment for multiple comparisons. The nonparametric Mann–Whitney U and Kruskal–
Wallis tests analysed outcomes for continuous unpaired variables. All analyses were performed using
IBM SPSS v27, and a two-tailed P-value of <0.05 was considered statistically significant.
4. RESULTS

4.1 Patient Cohort and Tissue Biopsy

A total of 149 patients underwent a biopsy of either their patella tendon (N = 63) or palmaris longus (N = 86) (Table 1). The mean patient age was 48.9y (range of 22 - 75y) and 47.1y (range of 31 – 64y) for the PT and PL groups respectively. In terms of gender, 44.4% and 32.6% of the patient population were female for the PT and PL groups respectively. No statistical significance was observed in donor age and gender between the PT and PL groups (p = 0.085 and p = 0.139 respectively). Due to the nature of tissue harvest, the tendon biopsy obtained from the PL via surgical resection was in general larger than tendon tissues obtained by needle biopsy of the PT (p < 0.001).

4.2 Morphology

All tendon cells were successfully expanded in culture and morphological appearance were monitored at every passage. Confluent tendon cells from both PT and PL exhibited spindle-shaped and bipolar morphology with long processes and round to oval nuclei (Figure 1). Morphological appearance was maintained up to passage 5 (Figure 1). Tendon cells supplied for ATI is validated to meet strict quality attributes for up to 6 passages. Cells at passage 3 were most commonly supplied to patients. No major differences in morphological structures were observed between cells derived from PT and PL tendons. No unintended morphological features suggestive of alternative cell types such as adipocytes (fat cells), chondrocytes (cartilage cells), and osteoblasts (bone cells) were observed.

4.3 Growth Characteristics

Growth characteristics of cultured tendon cells such as population doubling time, cellular yields, and cell viability during manufacture are routinely evaluated to confirm the potency of the final OrthoATI product. The population doubling time (PDT) number is the approximate number of doublings (in hours) that the cell population has undergone since isolation. The mean population doubling time was
similar between tendon cells derived from PT and PL, 83.9 and 82.7 hours respectively (p = 0.482), were within specifications and consistent with tenogenic cell populations (Figure 2). Total cellular yields and cell viability were comparable with no statistically significant difference in growth characteristics between tendon cells derived from PT and PL (Figure 2). Mean cellular yield was 16.2 and 15.2×10^6 cells (p = 0.099) with mean cell viability of 98.6% and 99.0% (p = 0.277) for the PT and PL groups respectively (Figure 2).

4.4 Tenogenic Gene Expression

The evaluation of tenogenic gene expression in cultured tendon cells within defined specifications is a key readout for quality attributes (purity, potency and identity) of tendon cells making up the final ATI cellular product. Potency of cultured tendon cells is verified through the expression of the key tendon matrix gene, COL1. COL1 is the major component of tendon extracellular matrix [28]. Purity of cultured tendon cells is verified through the expression of SCX, a tenogenic transcription factor required for tendon cell differentiation [29]. SCX induces the downstream expression of COL1 and TNMD [30]. Identity of cultured tendon cells is verified through the co-expression of tendon specific matrix genes, TNMD and COL1. By qPCR, TMND expression was not detectable in the three samples of human tendon cells and adipocytes, whereas the expression of COL1 and SCX was significantly higher (tendon cells vs non-tendon cells COL1 p<0.05) in all samples of human tendon cells than in non tendon cells (Adipocytes) except one sample with (p=0.073 close to 0.05 when compared on COL1 expression ), showing that these genes are relatively tendon-specific. (Supplementary Figure 1). By ddPCR, the expression of COL1, TNMD, and SCX were comparable with no statistically significant difference (p = 0.860, p = 0.837, and p = 0.331 respectively) between tendon cells derived from PT and PL tendons (Figure 3). Consistent to qPCR, TNMD expression was extremely low in all samples by ddPCR.
4.5. Impact of Demographics on Growth Characteristics and Quality Attributes

An age stratification analysis was conducted for the age ranges < 35 (N = 12), 35 - 44 (N = 41), 45 - 60 (N = 57), and > 60-years-old (N = 39). Using Kruskall-Wallis (comparison of > 2 groups), no statistical significance was demonstrated between age groups for cellular growth characteristics and gene expression quality attributes of PT- and PL-derived tendon cells (Table 2). Similarly, using the Mann-Whitney U test, gender did not influence growth parameters from both tendon sources (Table 3). However, expression of COL1 was statistically significantly greater in male than female patients (P<0.001).
5. DISCUSSION

Many cell therapies including BMAC, adipose derived MSCs, skin cells, and tendon cells have been used for treatment of tendinopathy. This study reports that patient age does not affect the growth characteristics or biologic potency of the harvested and cultured tendon cells used in ATI cell therapy. Patients from the older age groups (45-60 years and greater than 60 years) who are especially prone to degenerative tendinopathy, have equivalent cell culture times as younger patients. Similarly, morphological characteristics, markers for tendon cell activity and potency in older patients are comparable to younger patients. The data presented in this study indicates that older patients are also able to provide similar tendon cells for OrthoATIM as the younger patients.

Several studies have evaluated the effect of age on the number of bone marrow stromal cells. Previous studies on the utility of BMAC in treating older patients have suggested that increasing patient age correlates with decreased concentration of bone marrow stromal cells [31; 32; 33]. In contrast, Huddlestone [14] in a study of 80 patients undergoing BMAC treatment for various soft tissue injuries, reported that patient age and gender are not factors associated with the concentration of MSCs. However, the oldest patient cohort evaluated in that study was the rotator cuff repair group with a mean age of 55.8 ± 8.3 years. This is younger than the oldest age cohort evaluated in the current study.

In a review of aging and tendinopathy, Korcari [20] noted a marked decrease in cell density and metabolic activity and an increase in senescent cells in aged tendon with an impact on development of degenerative tendinopathy. However, it was noted that the age categories of “young”, “mid-aged” and “old” were not chronologically well defined. In addition the impact of age on tendinopathy likely varies between different anatomic sites and individuals with different activity levels or sporting participation [20].
As tendon from different anatomical locations experience different levels of mechanical load and stress, it is probable that tendon cells populations residing in these different tendons possess differences in growth characteristics and/or biological activity (such as gene expression) when in culture. In the current study, we showed that the palmaris longus (PL) tendon in the distal forearm is equivalent to the patella (PT) tendon as a source for autologous tendon cells. This study is limited by only including two harvest donor sites. Other potential harvest sites could be plantaris and tensor fasciae lata [34]. The selection of tendon for tissue biopsy is dependent on the clinician’s preference and consideration of the patients’ individual circumstances and needs. For example, the clinician may choose the PL biopsy of the non-dominant upper extremity over a PT biopsy in an athlete engaging in running or jumping sports. Nonetheless, PT- and PL-derived tendon cells expanded in culture through several passages were comparable in growth characteristics such as population doubling time, cellular yield, and viability at time of implantation. In addition, no major differences were observed in the gene expression of COL1, the major component of the tendon ECM, or markers that characterises the tendon phenotype including the transcription factor SCX, and pro-proliferative glycoprotein tenomodulin. The expression of COL1, SCX, and TNMD comprises the key readout parameters for quality attributes of purity, potency and identity (PPI) of tendon cells making up the final OrthoATI cellular product.

Together, our data suggests that healthy tendon from any anatomical location would be suitable donor tissue for the extraction and culture of autologous tendon cells without compromising the quality and biological potency of final implanted OrthoATI products.

Less than 10% of the cohort was over the age of 60. It is known that senescent cells in tendon increase with age [20], however it is unclear from this data whether patients in the upper age limit are likely to attain a successful outcome with OrthoATI.

6. CONCLUSION
This study has shown that age and donor site for tendon tissue biopsies do not affect the growth characteristics or quality attributes of extracted and in vitro expanded autologous tendon cells.

7. DECLARATION OF COMPETING INTEREST

MHZ & AW hold shares of Orthocell Ltd
8. REFERENCES


Table 1. Demographics of PT and PL groups.

<table>
<thead>
<tr>
<th></th>
<th>PT (N = 63)</th>
<th>PL (N = 86)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gender</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>28 (44.4%)</td>
<td>28 (32.6%)</td>
<td>p = 0.139</td>
</tr>
<tr>
<td>Male</td>
<td>35 (56.6%)</td>
<td>58 (67.4%)</td>
<td></td>
</tr>
<tr>
<td>§ Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>48.89 ± 11.13</td>
<td>47.12 ± 8.39</td>
<td>p = 0.085</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>50.0 (11)</td>
<td>47.5 (13)</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>22 – 75</td>
<td>31 – 64</td>
<td></td>
</tr>
<tr>
<td>§ Biopsy weight (mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>61.62 ±37.03</td>
<td>119.07 ± 76.24</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>60.0 (40)</td>
<td>100.0 (60)</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2 – 230</td>
<td>20 – 520</td>
<td></td>
</tr>
</tbody>
</table>

* Independent samples proportion test
§ Two-sided Student’s T-test (parametric) or Mann-Whitney U (non-parametric)
Patella tendon (PT), palmaris longus (PL), Standard Deviation (SD), Interquartile Range (IQR)

Table 2. Age stratification analysis evaluating growth and quality parameters. PT and PL observed as a single cohort.

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt;35 (N = 12)</th>
<th>35-44 (N = 41)</th>
<th>45-54 (N = 57)</th>
<th>&gt;55 (N = 39)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth parameters (Mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average PDT (hours)</td>
<td>84.54 ± 39.25</td>
<td>80.62 ± 27.71</td>
<td>82.56 ± 35.12</td>
<td>83.24 ± 33.30</td>
<td>0.879</td>
</tr>
<tr>
<td>Total Cell Yield (×10⁶)</td>
<td>14.83 ± 5.20</td>
<td>15.95 ± 3.97</td>
<td>15.82 ± 4.62</td>
<td>15.29 ± 4.30</td>
<td>0.814</td>
</tr>
<tr>
<td>Cell Viability (%)</td>
<td>98.97 ± 1.05</td>
<td>98.99 ± 0.10</td>
<td>98.92 ± 1.04</td>
<td>98.47 ± 2.31</td>
<td>0.388</td>
</tr>
<tr>
<td>Gene Expression (copies/μg cDNA; Mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COL1 (×10⁸)</td>
<td>1.99 ± 1.02</td>
<td>1.93 ± 1.19</td>
<td>1.48 ± 0.80</td>
<td>1.63 ± 1.11</td>
<td>0.125</td>
</tr>
<tr>
<td>TNMD* (×10³)</td>
<td>1.36 ± 1.14</td>
<td>0.74 ± 0.61</td>
<td>0.67 ± 0.67</td>
<td>0.78 ± 1.16</td>
<td>0.419</td>
</tr>
<tr>
<td>SCX (×10³)</td>
<td>1.36 ± 1.09</td>
<td>5.21 ± 1.47</td>
<td>4.28 ± 1.12</td>
<td>3.78 ± 1.04</td>
<td>0.505</td>
</tr>
</tbody>
</table>

*One-way ANOVA
*TNMD, N = 64 (Male, N = 39; Female, N = 25)
Patella tendon (PT), palmaris longus (PL), Collagen 1 (COL1), Tenomodulin (TMND), Scleraxis (SCX), Standard Deviation (SD).

Table 3. Gender analysis evaluating growth and quality parameters. PT and PL observed as a single cohort.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male (N = 93)</th>
<th>Female (N = 56)</th>
<th>p-value≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth parameters (Median, [Range])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average PDT (hours)</td>
<td>71.71, [29.74 - 153.69]</td>
<td>77.76, [32.99 - 152.41]</td>
<td>0.510</td>
</tr>
<tr>
<td>Total Cell Yield (×10⁶)</td>
<td>15.00, [7.14 - 25.95]</td>
<td>15.00, [2.85 - 21.00]</td>
<td>0.835</td>
</tr>
<tr>
<td>Cell Viability (%)</td>
<td>99.1, [97.6 - 100.0]</td>
<td>99.2, [97.9 - 100]</td>
<td>0.866</td>
</tr>
<tr>
<td>Quality parameters (copies/μg cDNA; Median, [Range])</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COL1</td>
<td>1.75×10⁸, [6.15×10⁷ - 4.02×10⁸]</td>
<td>1.31×10⁹, [2.74×10⁸ - 2.44×10⁹]</td>
<td>&lt; 0.001</td>
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<tr>
<td>TNMD*</td>
<td>8.29×10⁷, [0.00 - 1.95×10⁷]</td>
<td>1.95×10⁸, [34.8 - 2.09×10⁸]</td>
<td>0.180</td>
</tr>
<tr>
<td>SCX</td>
<td>1.83×10⁵, [1.54×10⁵ - 5.81×10⁵]</td>
<td>1.76×10⁵, [1.52×10⁵ - 7.30×10⁵]</td>
<td>0.959</td>
</tr>
</tbody>
</table>

≤Mann-Whitney Test
*TNMD N=64 (Male N=39; Female N=25)
Patella tendon (PT), palmaris longus (PL), population doubling time (PDT), Collagen 1 (COL1), Tenomodulin (TMND), Scleraxis (SCX).
Figure Legend

**Figure 1. Cell morphology of patella tendon (PT) and palmaris longus (PL) derived tendon cells.** Confluent tendon cells in monolayer culture (passage 1 – 5) with spindle shape and round nuclei (200× magnification, phase contrast microscopy). Images are representative of 10 fields observed for each cell culture at each passage derived from PT and PL biopsy samples.

**Figure 2. Growth characteristics of cultured patella tendon (PT) and palmaris longus (PL) derived tendon cells including population doubling time (PDT), total cellular yield and cell viability.** No statistical significance was observed in the PDT (p=0.482), total cellular yield (p=0.099) and cell viability (p=0.277) between PT (n=63) and PL samples (n=86).

**Figure 3. Gene expression analysis of collagen 1 (COL1), tenomodulin (TNMD) and scleraxis (SCX) in patella tendon (PT) and palmaris longus (PL) derived tendon cells.** No statistical significance was observed in the expression of COL1 (p=0.86), TNMD (p=0.837) and SCX (p=0.331) between PT (n=63) and PL samples (n=86).
Figure 2
Figure 3
DECLARATION OF COMPETING INTEREST

MHZ & AW hold shares of Orthocell Ltd