Human Periosteum Is a Source of Cells for Orthopaedic Tissue Engineering: A Pilot Study

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Published online: 6 May 2011
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Abstract

Background Periosteal cells are important in embryogenesis, fracture healing, and cartilage repair and could provide cells for osteochondral tissue engineering.

Questions/purpose We determined whether a population of cells isolated from human periosteal tissue contains cells with a mesenchymal stem cell (MSC) phenotype and whether these cells can be expanded in culture and used to form tissue in vitro.

Methods We obtained periosteal tissue from six patients. Initial expression of cell surface markers was assessed using flow cytometry. Cells were cultured over 10 generations

and changes in gene expression evaluated to assess phenotypic stability. Phenotype was confirmed using flow cytometry and colony-forming ability assays. Mineral formation was assessed by culturing Stro-1⁺ and unsorted cells with osteogenic supplements. Three cell culture samples were used for a reverse transcription–polymerase chain reaction, four for flow cytometry, three for colony-forming assay, and three for mineralization.

Results Primary cultures, containing large numbers of hematopoietic cells were replaced initially by Stro-1 and ALP-expressing immature osteoblastic cell types and later by ALP-expressing cells, which lacked Stro-1 and which became the predominant cell population during subculture. Approximately 10% of the total cell population continued to express markers for Stro1⁺/ALP⁺ cells throughout.

Conclusions These data suggest periosteum contains a large number of undifferentiated cells that can differentiate into neotissue and persist despite culture in noncell-specific media for over 10 passages.

Clinical Relevance Cultured periosteal cells may contribute to tissue formation and may be applicable for tissue engineering applications.

One or more of the authors (MMS) received funding from EPSRC (grant number EP/C520742/1) and the Leverhulme Trust. One of the authors (ICB) received funding from the Marshall Aid Commemoration Commission. One of the authors (AW) received funding from the British Association of the Knee. Each author certifies that his or her institution has approved the reporting of these cases, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participating in the study was obtained.

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Introduction

Periosteum is a connective tissue forming the outer lining of long bones and is involved in driving cell differentiation in bone development and repair [1, 25, 32]. Periosteum has two discrete layers: an outer fibrous layer and an inner juxtaosseous cambial layer [21]. The fibrous layer appears to be composed of fibroblastic cells in a collagen and elastin fiber matrix along with a nerve and microvascular network [9, 19]. The cambium layer is highly cellular and contains numerous cell types, including fibroblasts,

osteoblasts, and osteochondral precursor cells [47]. Mesenchymal precursor cells in the periosteum differentiate into neochondrocytes, producing cartilage tissue during embryogenesis and contributing to bone apposition during intramembranous ossification by differentiating into osteoblasts [40].

Studies have explored the use of periosteal explants [28, 29, 37] or isolated cells [6, 11, 22] to generate bone [16, 20] or cartilage [10, 23, 28, 37, 38]. Factors influencing cell phenotype include sex steroids [41, 43], endogenous growth factors [26, 45] and mechanical loading environment in vivo [27, 42], and isolation [30], culture methods [8, 13, 20, 31, 37] and the donor site [14] in vitro. Lim [24] identified potential precursor populations within periosteal cultures. Previous studies have identified stem cell markers in single isolates [24] and noted chondrogenesis in pellet cultures [14]. Further knowledge of the putative mesenchymal precursor cells in the periosteum could aid understanding of bone formation and fracture healing in vivo. For successful cell-based tissue engineering and regenerative medicine, changes in protein and gene expression during expansion must be well studied and controlled.

In this pilot study, we asked: (1) What is the initial expression within the periosteum by human periosteum-derived cells (HPDCs) of markers associated with mesenchymal stems cells? (2) How does this expression and functionality change when the periosteal cells are expanded in culture? (3) Are there cell phenotypes present in expanded HPDC cultures that warrant further exploration in bone tissue engineering applications as a result of their osteogenic potential?

Materials and Methods

Periosteal tissue was obtained from proximal tibias of six disease-free patients (mean age, 53 years; range, 25–75 years) providing informed consent when undergoing elective knee surgery (Fig. 1). The two females were aged 25 and 74 years; the four males were aged 38, 51, 57, and 61 years. Tissues were transported to the laboratory in Earl's balanced salt solution at 37°C. Gene expression (using real-time reverse transcription–polymerase chain reaction [RT-PCR]) and cell surface marker expression (flow cytometry) were used to assess initial population phenotype and stability over 10 generations (key research questions 1 and 2). Colony-forming unit (CFU) ability was used to explore the presence of progenitor cells and examine changes in cell functionality during culture (key research question 2). Osteogenicity was assessed by culturing sorted (Stro-1⁺) and unsorted cells with osteogenic supplements (key research question 3). Three samples were used for a RT-PCR, four for flow cytometry, three for colony-forming assay, and three for mineralization. This study was approved by the NHS ethical committee (project RREC2700).

All explants were weighed, minced, and digested with 3 mg/mL Collagenase D (Roche Applied Science, West Sussex, UK), 3 mg/mL collagenase type II (Sigma-Aldrich, Poole, UK), and 5 mM CaCl₂ in high glucose Dulbecco's Modified Eagle Medium containing 10% v/v fetal bovine serum, 1% v/v L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (all from Invitrogen, Paisley, UK) at 37°C for 4.5 hours. The resulting suspension was washed, passed through a 70-µm cell strainer

Fig. 1 Diagram representing scope of study including samples used with replicants and statistical tests. qRT-PCR = quantitative reverse transcription–polymerase chain reaction; ANOVA = analysis of variance.

(Falcon, Becton Dickinson, Oxford, UK), and cells counted. A proportion of each sample was analyzed using flow cytometry and the remaining cells placed into culture and allowed to adhere for 4 days. Medium was changed to remove nonadherent cells while attached cells were cultured as passage 0 until confluence. Cells were trypsin-released (0.25% v/v trypsin, 1 mM ethylenediaminetetraacetic acid [EDTA; Invitrogen]), and replated at 5000 cells/cm².

To characterize initial and changing HDPC phenotypes, we stained cell isolates from six donors with cell-specific antibodies (Table 1) and measured cell types using flow cytometry. Antibodies were tested for specificity and titrated. Approximately 1 to 3 × 10⁵ HPDCs (passages 0 through 10) were incubated in 1 mL of blocking buffer (phosphate-buffered saline pH 7.4 0.01 M [PBS] containing 2% w/v bovine serum albumin [BSA], 0.1% v/v fetal bovine serum, and 0.1% v/v normal mouse serum; all Sigma-Aldrich) for 30 minutes at room temperature to limit nonspecific binding. Directly stained cells were incubated with conjugated mAbs for 45 minutes at 4°C, washed in PBS containing w/v 2% BSA + 0.1% w/v sodium azide (flow buffer), centrifuged, and resuspended in 300 µL flow buffer before analysis. Cells stained with Stro-1 Ab were incubated with primary mAb (45 minutes at 4°C), washed,

and incubated with a fluorescein isothiocyanate-conjugated fragment F(Ab')₂ goat antimouse secondary Ab (Jackson ImmunoResearch, Suffolk, UK) for 45 minutes at 4°C. Primary Abs were matched with a negative control isotype of the same concentration. Fluorescence was evaluated using a FACSCalibur (BD Biosciences, Oxford, UK) and data analyzed using FlowJo software (Tree Star Inc, Ashland, OR). At least 20,000 events were collected per sample and percentage positive expression for each mAb was defined as the fluorescence level greater than 99% of the isotype-matched control antibodies. Passage 0 HDPCs from the same donors were grown for 10 days in complete medium. Cells were fixed in 4% w/v paraformaldehyde for 20 minutes and stained with filtered Oil-Red O solution (6 parts 0.5% v/v Oil-Red O stock:4 parts dH₂O) for 1 hour, both at room temperature. Nuclei were counterstained with Harris hematoxylin (Sigma-Aldrich) for 1 minute and mounted with 10% v/v glycerol/PBS. Attempts to identify fibroblasts were made using antibodies D7-Fib and CD90 (Thy-1); both were broadly expressed on greater than 95% of HDPCs tested, making specific characterization of these cell types unfeasible.

RT-PCR was performed on HDPCs isolated from four different patients (females 25 and 74 years; males 38 and 62 years). Total RNA was extracted from monolayered

Table 1. Antibodies and TaqmanTM gene probes used in this study, with details of their sources and specificities.

Antibodies				
Antigen	Concentration (µg/10 ⁶ cells)	Catalog number	Manufacturer	Cell specificity
Stro-1	2.5	MAB1038	R&D Systems	Progenitor cells
Alkaline phosphatase (ALP)	2.5	FAB1448A	R&D Systems	Osteoblastic cells
CD45	1.0	MCA87APC	Abd Serotec	Leukocytes
CD34	1.0	MCA1578PE	Abd Serotec	Hematopoietic stem cells
CD31 (PECAM-1)	1.0	MCA1738F	Abd Serotec	Endothelial cells
SSEA-1 (stage-specific antigen-1)	5.0	FAB2155P	R&D Systems	Primitive stem cells
α-smooth muscle actin	10.0	MAB1420	R&D Systems	Smooth muscle cells
Taqman TM gene probes (Applied Biosystems)				
Gene name	Symbol	Catalog number	Accession number	Cell specificity
Alkaline phosphatase	ALP	Hs00758162	NM000478	Osteoblast
SRY (sex determining region-y)-box 9	Sox9	Hs00165814	NM000346	Chondrocyte
Collagen Type 1	Col1A1	Hs00164004	NM000088	Osteoblast, fibroblast
Runt-related transcription factor 2	Runx2	Hs00298328	NM001015051	Osteoblast
Bone gamma-carboxyglutamate protein	Osteocalcin	Hs01587813	NM199173	Osteoblast
Platelet/endothelial cell adhesion molecule	CD31	Hs00354641	NM000442	Platelet/endothelial cell adhesion molecule
Eukaryotic 18S rRNA	18s	Hs99999901	X03205	All cells
Collagen Type 2, alpha 1	COL2A1	Hs00156568	NM001844.4	chondrocyte

HPDCs by the addition of 10 μL β -mercaptoethanol in 1 mL RLT buffer (QIAGEN, West Sussex, UK), isolated using the RNeasy mini-kit, treated with RNase-free DNase 1 (both QIAGEN) according to manufacturer protocol, and quantified using the ND-1000 UV-Vis Spectrophotometer (Nanodrop, Wilmington, DE). A total of 1000 ng of total RNA was combined with a master mix for each reaction (10 μL TaqMan universal master mix [Applied Biosystems, Foster City, CA], 7 μL 0.1% v/v diethylpyrocarbonate water [Invitrogen], 2 μL extracted cDNA, and 1 μL TaqMan® probe [Applied Biosystems]). TaqMan® Gene Expression assays are detailed in Table 1. 18S was used as a control gene and each reaction carried out in triplicate. PCR was initiated by 2-minute 50°C and 10-minute 95°C steps to optimize thermal cycling conditions for the ABI Prism 7700 sequence detection system (Applied Biosystems). Amplifications were performed for 40 cycles in a Corbett Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia) at 95°C for 15 seconds and 60°C for 1 minute. Target signal was plotted against the number of cycles and threshold set at 0.05. Data comparisons were made at the intercept, where sample reactions crossed this phase of amplification. Results were correlated using the comparative C_T method [44].

To assess changes in CFU ability over time, HPDCs from three different donors (38 and 51 years male; 25 years female) were seeded at 10,000 cells/dish and cultured in growth medium for 12 days. Cells were stained after 12 days with 0.5% crystal violet in methanol for 5 minutes, rinsed twice in distilled (d)H₂O, and colonies per dish counted by two independent observers. Colonies less than 1 mm diameter (approximately 50 cells) and those faintly stained were discounted [12]. Mean values for each passage per patient were derived from three dishes using cells between passages 1 and 17. Interobserver variability was factored into standard deviations represented on graphs.

In vitro mineralization capacity was assessed in cells derived from one donor (38-year-old man). Stro-1⁺ cells were selected from passage 10 HPDCs using a cell-sorting kit containing streptavidin-coated magnetic beads (Biotin binder kit; Invitrogen) according to the manufacturer's instructions. 10⁷ HPDCs were incubated with primary mAb (antihuman Stro-1; R&D Systems) at 2.5 $\mu\text{g}/10^6$ cells for 1 hour at 4°C. After washing in 2% w/v BSA buffer, cells were incubated with biotin-SP-conjugated fragment F(ab')₂ goat antimouse secondary antibody (Jackson ImmunoResearch, Suffolk, UK) at 1.5 $\mu\text{g}/10^6$ cells for 45 minutes at 4°C. After washing, biotin-conjugated magnetic beads were added to the suspension, incubated for 20 minutes, then Stro-1⁺ cells separated using a magnetic field. Stro-1⁺ populations were counted using a hemocytometer and viability determined using trypan blue dye exclusion assay.

Sorted population viability and purity were greater than 98%. Total (undepleted) and Stro-1⁺ cell populations were seeded at 10,000 cells/cm² in 48-well plates (n = 6) and mineralization induced by addition of osteogenic supplements (10 mM β -glycerophosphate, 50 mg/L L-ascorbate, 10 nM dexamethasone [all Sigma-Aldrich]). Twenty-eight-day cultures were rinsed with PBS, fixed in 4% formaldehyde in PBS for 15 minutes at room temperature, rinsed with dH₂O, then stained with 1 mL 40 mM alizarin red (pH 4.1) per well for 30 minutes at room temperature with gentle shaking. Unincorporated dye was aspirated, wells washed five times with dH₂O, and rinsed in a water bath. Images were immediately taken using phase contrast and fluorescence microscopy at 10 $\times$ magnification (DP-70 Olympus Microscope, Watford, UK) and fluorescent nodules per well counted. Mineralized matrix was quantified using a modified dye extraction protocol [15]. A total of 800 μL 10% v/v acetic acid was added to each well and the plate incubated at room temperature for 30 minutes on a plate shaker. The monolayer was scraped from the well and transferred to a 1.5-mL microcentrifuge tube. After vortexing for 30 seconds, the slurry was overlaid with 500 μL mineral oil (Sigma-Aldrich), heated to 85°C for 10 minutes, and transferred to ice for 5 minutes, centrifuged at 20,000 g for 15 minutes, and 500 μL of the supernatant removed to a new 1.5-mL microcentrifuge tube. Addition of 200 μL of 10% v/v ammonium hydroxide neutralized the acid. Aliquots of 150 μL supernatant were read in triplicate at 405 nm in a 96-well plate using opaque-walled, transparent-bottomed plates (Fisher Lifesciences, Loughborough, UK). Mineralization data were normalized against total DNA, determined in three sorted and three unsorted HPDC populations from the same donor (38-year-old man) using Hoechst 33258 (Sigma-Aldrich) dye. Cells were lysed by one freeze/thaw cycle (30 minutes at -80°C and 20 minutes at 37°C). Hoechst 33258 was added to the lysate and reacted for 5 minutes in the dark. Fluorescence was measured using a fluorescent plate reader at 360 nm excitation wavelength, 460 nm emission wavelength, and read against a standard curve (calf thymus DNA [Sigma-Aldrich] in TNE buffer [10 mM Tris, 1 mM EDTA, 2 M NaCl, pH 7.4]).

To determine changes in phenotype over time, differences in passages between different cell markers in flow cytometry were measured using a two-way analysis of variance (ANOVA) with a Tukey post hoc test. Data were normally distributed considering passage number and cell population as factors. To determine changes in colony-forming ability versus passages, a one-way ANOVA was applied to CFU measurements from each donor. To identify statistical differences in mineralization data, the nonparametric Mann-Whitney U test was performed. For data analysis, the SPSS statistical package was used.

Results

Antibodies expressed and cell types present in initial HPDC cultures (Table 2) were representative of all donors. Cells expressing ALP and CD34 represented large populations within passage 0 cells, constituting $30\% \pm 5.9\%$ and $32\% \pm 6.9\%$ (mean $\pm$ SE) of total cell population, respectively (Fig. 2).

We observed no changes in the population of Stro-1⁺ cells across 10 passages ($p = 0.069$ between p1 and p8). Stro-1⁺/ALP⁻ cell populations comprised $6\% \pm 1.7\%$ of the cell population (all donors). ALP⁺/Stro-1⁻ populations peaked at passages 1 and 2 and declined subsequently with the lowest frequency of ALP⁺ cells occurring at passage 10

Table 2. Summary of expression of cell markers with passage

Antibody	Passage 0	Expression at passage 10
Stro-1	+	+
CD34	+	+
ALP	+	+
SSEA-1	—	—
Cell type	Passage 0	Expression at passage 10
Smooth muscle cell	+	Lost by passage 2
Endothelial cell (CD31)	+	Lost by passage 10
Leukocytes (CD45)	+	Lost by passage 10
Adipocyte-like cells	+	Lost by passage 2

Fig. 2 Bar chart of flow cytometry data showing cell surface marker expression as a function of passage number. Mean change in expression of cell surface proteins on periosteal cells isolated from three patients. Values are shown as percentage positive expression of total cells analyzed using flow cytometry (greater than 20,000 events). Positivity for each antibody was defined as level fluorescence greater than 99% of the isotype-matched control antibodies. CD45⁺ and CD31⁺ expression are displayed as the same data set, because those antibodies were expressed by periosteal cells in almost identical numbers and temporal profiles.

($16\% \pm 6.5\%$; Fig. 2). Over 10 passages ALP⁺/Stro-1⁻ was the largest cell population ($p = 0.004$) compared with all other cell types. A small population of cells coexpressing ALP and Stro-1 (Stro-1⁺/ALP⁺) was maintained throughout ($4\% \pm 0.7\%$). Real-time RT-PCR confirmed increased expression of ALP and collagen I (Col I) in HPDCs cultured for up to 10 passages (Fig. 3). At passage 10, we observed a 4.6- and 7.6- fold increase in ALP and Col I expression, respectively, with a smaller ($p = 0.005$ versus ALP) 1.1-fold increase in Runx2 expression. In contrast, relative expression of Sox9 decreased steadily from passage 2 to passage 10 ($p = 0.002$ versus ALP). No mRNA expression of osteocalcin, collagen II, or CD31 was detected.

The highest numbers of colonies in cells from each donor were formed at passage 1 (mean for three donors $\pm$ SD: 44 ± 7.3 ; Fig. 4) after which a relative decline (all samples $p < 0.001$) in the number of colonies counted (per 10,000 plated) was noted with subsequent passages. In addition, no correlation ($R = 0.26$, $p = 0.229$) between colony-forming ability and the percentage of Stro-1⁺ cells in culture was identified.

Nodule counting and intensity measurement of alizarin red extracted from stained cultures showed that less ($p = 0.01$) mineralized tissue was formed by cultures depleted of Stro-1⁺ cells (Fig. 5).

Discussion

Mesenchymal precursor cells in the periosteum differentiate into neochondrocytes to produce cartilage tissue during embryogenesis and contribute to osteochondral and

Fig. 3 Real-time reverse transcriptase–polymerase chain reaction data showing mRNA expression profiles of human periosteum-derived cells (HPDCs) cultured for 10 passages. Expression levels of selected osteogenic and chondrogenic transcription factors and proteins were normalized to the 18 s housekeeping gene and compared with passage 2 expression levels. Average values $\pm$ SD of repeated measurements ($n = 3$) are presented of cultured periosteal cells isolated from a 25-year-old woman and are representative of data generated from all individuals.

intramembranous ossification by differentiating into osteoblasts [40]. Periosteum serves as a reservoir of osteochondral precursor cells [46] and represents a new

Fig. 4 Colony-forming units (CFU) assay of the number of colonies (mean $\pm$ SD) formed per 10,000 cells seeded into a 78-cm² Petri dish ($n = 3$). Colony scoring was performed by two independent observers and was based on colony size being greater than 1 mm in diameter and not inhibited by contact with other colonies. Results are displayed to show the change in colony-forming ability over passaging for three different patients.

Fig. 5A–D Graphs of alizarin red dye desorbed from wells, normalized to total DNA content (A) and the numbers of fluorescent nodules counted per well (B). The lower micrographs show representative images of unsorted (C) and sorted (Stro-1 depleted) (D) cell populations grown for 28 days in the presence of osteogenic media scale bar on images = 1.0 mm (Stain, Alizarin red dye; original magnification, $\times 25$).

candidate for cell-based therapies. To exploit this potential, studies must concentrate on exploring the link between in vitro periosteal phenotype and in vivo functionality. As a preliminary step in exploring this link, we sought to examine (1) the initial phenotypes of HPDCs; (2) the impact of culture expansion on cell expression patterns and functionality; and (3) the relationship between the HDPC Stro-1⁺ phenotype and bone mineralization in vitro.

Before discussing results, a number of experimental limitations need to be recognized. First, we analyzed a limited number of samples. A larger number of samples would allow for identification of age- and gender-related phenotypic trends in addition to providing greater statistical reliability for comparison. However, given the range, agreement, and expansion on existing literature, our data are reliable. Second, tissue formation in vivo has not been demonstrated. The “gold standard” for testing osteogenicity has been the formation of mineralised tissue in immunocompromised animals. These studies would be necessary to confirm our initial hypotheses. Third, we were not able to examine the expression of relevant markers within tissue sections as a result of limitations of sample numbers and available antibodies. Finally, we did not

perform clonal analyses to absolutely confirm that the cells we were examining were stem cells. As a result of the markers expressed, it is likely that the cells identified are progenitor cells.

As an important first step, initial phenotypes of uncultured human periosteal cell populations were examined across multiple donors. Initial cell isolates consistently contained large populations of CD34⁺ hematopoietic cells and ALP⁺ osteogenic cell types, small numbers of smooth muscle, endothelial and adipocytic cells, and small subpopulations of Stro-1⁺ cell types. Based on the study of developmental phenotypes of stromal cell cultures, it has been suggested that Stro-1⁺/ALP⁻ cells represent a less differentiated osteoblastic phenotype, Stro-1⁺/ALP⁺ cells an intermediate stage, and Stro-1⁻/ALP⁺ cells the most mature [17]. These findings build on work by Lim [24], who identified potential precursor populations within periosteal cultures. Attempts to characterize and localize these cell types in tissue sections were made; however, practical considerations associated with availability of progenitor surface markers for immunohistochemistry and small numbers of positively stained cells made such analysis impractical.

Next, we examined the impact of expansive cell culture on HPDC phenotypes. In unspecialized cell media, the shift of periosteal cell populations to a more osteogenic phenotype was seen through a large reduction in the population of hematopoietic cells (from 32% $\pm$ 6.9% to 3.5% $\pm$ 1.6% of the total population); disappearance of adipose, endothelial, leukocyte, and smooth muscle cells; and changes in bone and cartilage related RNA expression profiles. In culture, cells expressing Stro-1⁻/ALP⁺ accounted for approximately 40% of the population and Stro-1⁺/ALP⁺ a further 10%. These values are consistent with early passage cultured cells derived from known MSC sources: trabecular bone explants [17], bone marrow, synovial lining, adipose tissue, muscle, and a single passage of periosteum [35]. More surprising than the osteogenic phenotype of cultured HPDCs in this study was the presence of a consistent and stable Stro-1⁺ population. Stro-1 expression had been reported as varying between 2% and 80% in 28-day bone marrow stromal cultures [39] and noted to decrease, to 15% of their maximum frequency, after 6 weeks of culture [36]. These phenotypic expression profile differences noted in our study may reflect differences in culture techniques and cell types examined (eg, stromal versus periosteal cell isolates). To further examine changes in the presence, number, and phenotypes of MSCs and precursor cells in our HPDC cultures, the CFU assay was used. Each adherent colony, considered to be derived from the rapid proliferation of a single nonhematopoietic stem cell, is often referred to as MSCs or mesenchymal progenitor cells [5, 12, 33]. Interestingly, we found no correlation between

CFU number and the presence of Stro-1⁺ progenitor populations. This finding, combined with the pattern of osteogenic marker expression and mineralization seen in this study, is consistent with the observation of Bruder et al. suggesting the multipotentiality of human bone marrow-derived CFUs was progressively lost during subculture with progenitor cells only retaining their osteogenicity at late passage [4]. The progressive shift from multilineage potential to osteoblastic phenotype was associated with a progressive change in cell morphology from small, rapidly dividing spindle-shaped cells to large, flat, slowly proliferating cells with long cell projections, also seen in bone marrow-derived MSCs [7]. It is interesting to note that CFU efficiency is highest in the early passage cells, which contain large proportions of hematopoietic nonmesenchymal cell types. Although this needs to be confirmed in future studies, it may be an example of the role that “community effects” or cell-cell interactions/secreted factors have on the maintenance and control of periosteal progenitor cell populations [2, 3]. As well as the colony-forming capacity of Stro-1⁺ cells, they have been linked with enhanced osteogenicity in vitro [16, 48] and cementogenesis in teeth [34]. These observations are confirmed here by a decrease in overall culture when Stro-1⁺ cells were removed from the general cell population.

In terms of HPDCs' chondrogenic potential, gene expression of Sox-9 decreased over time and collagen II, a marker of mature chondrocytes, was not found at all. Low initial chondrogenicity of isolated periosteal cells is confirmed in the literature [18] and indicates that the chondrogenic cells in nongrowth factor-supplemented media are derived from precursor cells, not mature cells in the periosteum. Other findings show HPDCs in chondrogenic media retain their cartilage-forming capacity over a number of generations [8], indicating specialized media would be required to culture cells with chondrogenic capacity.

Our data suggest MSCs, at different stages of differentiation, are present within initial mixed cell populations of human periosteal isolates. We could initially isolate a population of Stro-1⁺ progenitor cells from human periosteal explants and cultured for up to 10 passages without loss in population numbers or ability to form mineralized tissue. Late passage cultures were osteogenic and had lost chondrogenic markers. Building on this new information, culture conditions and factors can be tailored to exploit the full potential of specific HPDC types for both bone and cartilage tissue engineering applications.

Acknowledgments We thank Natasa Devic, MB BS, for experimental and analytical support on many aspects of this study. In addition, we thank Maria Azevedo and Steve Mwenifumbo for their help counting and scoring CFU dishes.

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