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Proliferative capacity and osteogenic potential of novel dura mater stem cells on poly-lactic-co-glycolic acid

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Abstract

The rational design of biomimetic structures for the regeneration of damaged or missing tissue is a fundamental principle of tissue engineering. Multiple variables must be optimized, ranging from the scaffold type to the selection and properties of implanted cell(s). In this study, the osteogenic potential of a novel stem cell was analyzed on biodegradable poly(lactic-co-glycolic acid) (PLGA) bio-materials as a step toward creating new cell-materials constructs for bony regeneration. Dura mater stem cells (DSCs), isolated from rat dura mater, were evaluated and compared to bone marrow stem cells (BMSCs) for proliferative and differentiative properties *in vitro*. Experiments were carried out on both tissue culture plastic (TCP) and 2D planar films of PLGA. Proliferation of DSCs on both TCP and PLGA films increased over 21 days. Positive fold inductions in all five bone marker genes were observed at days 7, 14, 21 in all experimental samples compared with day 0 controls. DSCs demonstrated greater cell coverage and enhanced matrix staining on 2D PLGA films when compared with BMSCs. These cells can be isolated and expanded in culture and can subsequently attach, proliferate, and differentiate on both TCP and PLGA films to a greater extent than BMSCs. This suggests that DSCs are promising for cell-based bone tissue engineering therapies, particularly those applications involving regeneration of cranial bones.

Keywords

biomimetic material; bone regeneration; bone tissue engineering; plasticity; dura mater

INTRODUCTION

Skeletal bone loss due to trauma, tumor resection, congenital disorders, infection, or other causes pose significant reconstructive challenges.¹ Primary reconstruction most frequently employs the use of autologous bone tissue, but limitations in supply and donor site morbidity often necessitate the need for alternative strategies. In recent years, tissue engineering strategies have evolved to provide alternative bone grafting materials. Commonly proposed strategies for tissue-engineered bone make use of biodegradable scaffolds, multipotent precursor cells, and growth factors, alone or in combination, to recreate a suitable environment for regeneration.^{2–4} Cells proposed for bone regeneration have been isolated from a range of different sources including bone, bone marrow, skin, adipose tissue, and dental pulp. To date, few studies have examined the respective

therapeutic potential of stem cells isolated from different tissue-specific sources, so it remains unclear which types of stem cells work well in specific applications.⁵⁻⁹ Moreover, new data continues to emerge which challenges the historically accepted lineage restrictions of adult stem cells, suggesting potentially new sources of multipotent cells for tissue engineering.

Our group is investigating the therapeutic potential of multipotent stem cells derived from dura mater, called dura mater stem cells (DSCs). The dura mater is a component of the meninges, a group of fibrous connective tissues which function to protect both the brain and spinal cord. Many studies have demonstrated that the initial patterning and subsequent regeneration of the cranial vault is regulated by secreted cytokines and donated precursor cells from the dura mater.¹⁰⁻¹³ Dura mater has also been demonstrated to be powerfully osteoinductive in several experimental animal models. Enhanced bone formation in mature animals was often observed when graft materials were implanted directly opposed to the dura.¹⁴ It is notable that this effect decreased with age of the animal correlating to a reduction in the number of dura cells present within the tissue.¹⁵ Physical blockades of the underlying dura mater also inhibited cranial re-ossification of surgical defects in both immature and mature rats, suggesting the importance of tissue-tissue interactions and/or progenitor cell migration.¹⁴ Significant reductions in new bone formation have also been reported when the dura was removed.¹⁶ Moreover, *in vitro* studies have shown that osteoblasts cocultivated in the presence of dura mater cells show enhanced proliferation and mineralization behavior that is attributed to autocrine and paracrine signaling behaviors of these cells.¹⁷

Dura mater is believed to arise from neural crest which is created during neural tube closure by delamination from the ectodermal epithelial sheet. Neural crest cells are known to be highly migratory and plastic cells that ultimately give rise to many diverse tissues in the body including peripheral nerve, melanocytes, bone, cartilage, muscle, heart, and adrenal glands.^{18,19} Indeed, the neural crest origin of the dura mater tissue and the reported role of this tissue in skull morphogenesis fueled our search for multipotential stem cells residing in the dura mater that are capable of differentiating into osteoblasts, neurons, and other tissues of neural crest origin. Recently, Tholpady and Ogle have reported that stem cells isolated from the dura mater differentiate toward an osteogenic phenotype along a glucocorticoid independent pathway.²⁰ This pathway is unique compared with other stem cells of mesenchymal origin, which often require steroid supplementation for long-term expansion and osteogenic commitment²¹; a characteristic that could prove especially advantageous in cell-material strategies proposed for bone repair.

While tissue interactions are important for cranial development, a key signaling molecule for osteogenic mineralization, FGF-2, is secreted at high levels by dura.^{22,23} This growth factor is thought to be one of the primary regulators of cranial bone formation,¹² controlling proliferation and subsequent mineralization at localized osteogenic fronts.²⁴ In this study, we hypothesize that intrinsic autocrine signaling of DSC populations permit robust expansion and osteogenic differentiation in the absence of both glucocorticoid treatment and exogenous FGF-2 stimulation. The *in vitro* osteogenic differentiation potential of DSCs was compared with the well-studied bone mesenchymal stem cell type, BMSC, as a control. This was performed through longitudinal studies analyzing the expression of bone-specific RNAs as well as histochemical detection of bone-specific proteins. Finally, the ability of DSCs and BMSCs to attach, grow, and differentiate on biodegradable poly(lactic-co-glycolic acid) (PLGA) planar films was determined.

MATERIALS AND METHODS

Cell harvest and culture

Dura mater beneath the frontal and parietal bones were removed aseptically from rat fetuses at 19 days according to an approved ACUC animal protocol. The tissue was washed in a physiologic buffer and plated in tissue culture dishes with basal media to allow outgrowth of DSCs from these explants (Fig. 1). BMSCs were isolated from the hind leg of juvenile rats according to an approved ACUC animal protocol. Cells were flushed from the excised femur with media and immediately plated onto tissue culture dishes. Both primary cell types were maintained at sub-confluent levels and cultured at 37°C with 5% CO₂ in a base media of DMEM supplemented with 10% FBS, 1% L-glutamine, and 0.5% gentamycin. Only cells that were passage number 5 or less were used for these experiments.

Differentiation of DSCs to bony and to neural phenotype

Isolated DSCs were differentiated to a neural phenotype, as previously described.²⁰ Briefly, neural differentiation was induced using a traditional osteogenic cocktail with the addition of 1 mM dexamethasone. DSCs began to exhibit neuronal morphology following 6 h incubation with dense cell bodies and multiple extending processes [Fig. 2(A)]. To induce an osteogenic phenotype in isolated DSCs, basal media was supplemented with a mix of organic/inorganic phosphates along with 50 μM ascorbic acid. No dexamethasone was added to DSC osteogenic media. Osteogenic differentiation was carried out to 21 days [Fig. 2(B)].

Differentiation of BMSCs to bony phenotype

Isolated BMSC were differentiated to a bony phenotype by adding 10 mM β-glycerophosphate, 50 μM ascorbic acid, and 10 μM dexamethasone to the basal media [Fig. 2(C)]. To determine the necessity of dexamethasone for osteogenic differentiation of BMSCs, these cells were also cultured in basal media without dexamethasone, but including 10 mM β-glycerophosphate and 50 μM ascorbic acid [Fig. 2(D)]. BMSC osteogenic differentiation was carried out to 21 days.

Fabrication of PLGA films

PLGA (85:15) with a molecular weight of 110,000 Da was purchased from Alkermes (Canton, MA) and was dissolved into 20 mL methylene chloride in a ratio of 1:4 (w/v). Films were poured into petri dishes lined with Bytac paper. Films were allowed to dry for 3 days at -80°C, followed by 3 days at room temperature. After complete evaporation of the methylene chloride, films were stored in a vacuum desiccator. In preparation for cell seeding, films were bored to a 1.5 cm diameter, treated with UV irradiation for 15 min, soaked in ethanol for 10 min, and washed in sterile water 5 × 10 min. Following washes, films were placed into the bottom of sterile 24-well plates in preparation for cell seeding.

Cell proliferation

Changes in cell growth were analyzed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma) mitochondrial reduction kit. This assay is based on the ability of live cells to reduce the tetrazolium-based compound, MTT. Following 7, 14, or 21 days in culture, existing media was removed and replaced with fresh media and MTT solution (2:10 ratio). This solution was incubated with the cell monolayer for 2 h at 37°C. Sodium dodecyl sulfate (SDS) was added to stop the reaction and absorbance of the supernatant was read with a spectrophotometer at 570 nm. A standard curve to relate cell number to absorbance was established to determine cell numbers in the unknown samples.

Alkaline phosphatase activity

Following 7, 14, or 21 days in culture, existing media was removed and 1% Triton was added to each sample for 15 min to permeabilize the cell monolayer. *p*-Nitrophenyl phosphate and alkaline phosphatase were then added to the Triton solution and incubated at 37°C for 15 min. The amount of *p*-nitrophenol produced was determined by measuring the absorbance of the solution at 415 nm. This measurement was then related to alkaline phosphatase activity of the cell monolayer. Cell monolayers were also stained for alkaline phosphatase following 7, 14, and 21 days in culture. At each time point, existing media was removed and replaced with a staining solution (50 mM Tris, naphthol, *N,N*-dimethyl formamide, fast red). Cell monolayers were incubated for 30 min in the staining solution and subsequently fixed with 2% paraformaldehyde.

Alizarin red

Mineralized matrix deposition was analyzed using an alizarin red staining method specific for calcium deposition. This assay uses a colorimetric analysis based on solubilizing the red matrix precipitate with cetylpyridinium chloride to yield a purple solution. Cells were fixed with 70% ethanol at 4°C for 1 h and then stained with 10% alizarin red solution for 10 min. After images of the stained monolayers were taken, cells were washed five times with distilled water, the red matrix precipitate was solubilized in 10% cetylpyridinium chloride, and the absorbance was read at 562 nm with a spectrophotometer. The mineralized matrix deposition was expressed as a function of absorbance.

RT-PCR

DSCs were plated on both tissue culture plastic (TCP) and PLGA substrates in triplicate at 25,000 cells/cm² and cultured under both basal and osteogenic media conditions. Following 7, 14, and 21 days in culture, total RNA was isolated from each group (OM-TCP: DSC culture on TCP in osteogenic media; OM-PLGA: DSCs cultured on PLGA in osteogenic media; TCP: DSCs cultured in basal media on TCP) using a Qiagen mini-kit following the manufacturer's instructions. Reverse transcription of the total RNA was performed using a Biorad iCycler (Biorad, Hercules, CA). Each 50 µL reaction mixture contained iQ SYBR Green Supermix (Biorad, Hercules, CA), both forward and reverse primers for each gene listed in Table I (XXIDT, Coralville, IA), and the cDNA. Manufacturer's suggested melting temperatures were used for each primer. 18s rRNA was used as the housekeeping gene. All fold changes in expression were calculated from the CT numbers of each experimental group and compared with RNA isolated from the day 0 control (DSCs seeded at 25,000 cell/cm² on TCP after 1 day culture in basal media).

RESULTS

Role of steroid treatment in BMSC osteogenic differentiation on TCP

BMSCs were cultured in osteogenic media with and without the inclusion of dexamethasone to assess its role in BMSC osteogenic differentiation. Both quantitative and qualitative results (Fig. 2) confirmed that *in vitro* osteogenic differentiation of BMSCs was dependent upon dexamethasone supplementation. After 21 days, BMSCs cultured with dexamethasone exhibited robust matrix staining [Fig. 2(C)], whereas those cultured without steroid treatment showed no staining [Fig. 2(D)]. Quantitative differences in staining were statistically different at days 14 and 21. These results highlight BMSCs dependence on dexamethasone for successful osteogenic differentiation under *in vitro* culture conditions.

Differentiation of DSCs to neural and osteogenic phenotypes

From the previous set of experiments, it was noted that the osteogenic media used to induce bony differentiation of BMSCs instead caused neural differentiation of DSCs [Fig. 2(A)]. Although DSCs are capable of differentiating to nerve under similar neurogenic media conditions, as described for BMSCs,²⁰ they are uniquely sensitive to steroid treatment. Steroid treatment created cells that were multipolar, with extensive branching outward from the cell body within 6 h of media induction. Thus, differentiation conditions were modified to eliminate steroid supplements from the osteogenic media. This steroid removal from the media created conditions permissible for osteogenic differentiation of DSCs, as demonstrated by positive staining of both mineralized nodules [Fig. 2(B)] and alkaline phosphatase (Fig. 3). Dexamethasone-free osteogenic media was used for the remainder of DSC experiments in this study.

Quantitative comparisons of proliferation, alkaline phosphatase expression, and alizarin red staining between DSCs and BMSCs were assessed over a 21-day time course (Figs. 2 and 4). Proliferation of both cell types followed similar trends of increasing growth. During osteoblast maturation, alkaline phosphatase is typically reported to peak at day 7 and subsequently decrease.²⁵ As expected, BMSCs mimicked this expression pattern. However, in the case of DSCs, alkaline phosphatase expression peaked at day 14 and remained high at day 21. Similar trends were observed in DSC alkaline phosphatase staining (Fig. 3). Alizarin red results demonstrated statistically higher mineralization at earlier time points (day 7, day 14) in DSCs compared with BMSCs. However, by day 21, BMSCs exhibited statistically higher amounts of mineralization (Fig. 2).

DSC culture on PLGA Films

Given the robust osteogenic differentiation of DSCs on TCP, characterization of DSCs behavior on PLGA was also investigated. Initially, DSCs seeded on PLGA remained viable over 24 h as assessed by MTS and adherent cells exhibited similar morphology to those grown on TCP (data not shown). Subsequent experiments were developed to characterize osteogenic differentiation potential of DSCs on PLGA films. Figure 5(C) shows robust cell growth on films maintained in basal media, and also strong positive staining for alizarin red and alkaline phosphatase, Figure 5(A) and (B), respectively.

DSC versus BMSC osteogenic differentiation on PLGA

Like DSCs, BMSCs have the ability to differentiate into a bony phenotype given the appropriate osteogenic media. Successful osteogenic differentiation on PLGA polymers using BMSCs has been reported previously.^{26–28} Here, we compare proliferation and osteogenic differentiation behavior of DSCs to BMSCs grown on PLGA films. Comparison of histochemical staining, both qualitative and quantitative results show significantly greater cell growth and overall mineralized matrix production by DSCs when compared with BMSCs at days 7 through 28 (Fig. 5). This difference in total mineralized matrix quantity is likely attributable to the significantly greater mineralizing osteogenic cells among cultured DSCs.

Gene expression profiles of DSCs

DSC osteogenic potential was further characterized by investigating expression profiles of five important bone markers using real time RT-PCR (Table I, Fig. 6). All five genes (bone sialoprotein, cbfa-1, alkaline phosphatase, collagen I, osteopontin) were quantitatively upregulated in all experimental samples compared with day 0 controls. Alkaline phosphatase expression was significantly higher in the TCP sample compared with both OM samples for all days. Collagen I expression peaked in the OM-TCP sample at day 14, whereas the

expression levels of the other samples remained lower across all time points. Osteopontin expression was significantly higher at each time point in the OM-TCP sample compared with the others. Osteopontin expression in both OM-TCP and OM-PLGA samples peaked at day 21. Cbfa 1 expression remained higher in the TCP sample compared with the OM samples across 21 days. Both OM-TCP and OM-PLGA showed significantly higher expression of BSP at day 7, and this higher expression continued to increase in the OM-TCP sample at days 14 and 21.

DISCUSSION

Current strategies employed to address the need for bone replacement therapies utilize a combination of appropriately tailored biomaterials and multipotent cell populations to recreate a suitable tissue environment. Several anatomic sites have been reported to house resident stem cell populations that can be isolated, expanded, and used for tissue-engineering applications.⁵⁻⁹ However, few studies have explored differences between these resident stem cell niches, and specifically, characteristics related to bio-material compatibility and differentiation potential between individual populations.^{29,30} In the present study, the *in vitro* proliferative capacity, osteogenic potential, and biocompatibility of a new progenitor population was evaluated and compared to that of a common osteoprogenitor, the BMSC. These studies demonstrate the feasibility of isolating a new population of multipotent progenitor cell from dura mater, growing and expanding these cells in culture, and cultivating robust matrix production on both TCP and 2D films of PLGA.

While Sieber-Blum et al. have recently reported that neural crest derived stem cells from hair follicles can be used for tissue regenerative applications,¹⁹ DSCs are a unique osteoprogenitor cell type. They are derived from neural crest in contrast to other common osteoprogenitor cells, which are derived from mesoderm. While the heterogeneity of this cell population is currently unknown, it is totally derived from neural crest.³¹ Given the uniformity of cellular response to induction, this population of progenitor cells most likely contains less heterogeneity than marrow stromal cells, which are known to have a significant hematopoietic population in addition to the adherent mesenchymal fraction. However, proliferation and differentiation responses of DSCs were consistently uniform across populations and experiments, a characteristic unlike other popular multipotent sources, such as adipose-derived stem cells (ADSCs).^{32,33}

Cranial neural crest derivatives are known to differentiate into a variety of tissue types including bone and peripheral nerve.¹⁸ However, the specific differentiation pathway of DSCs towards these phenotypes was unanticipated. Our data demonstrates that these cells differentiate towards an osteoblastic phenotype along a unique pathway that is glucocorticoid independent. The presence of dexamethasone, a potent synthetic glucocorticoid, produces DSCs that are highly dendritic with dense cell bodies and complex processes reminiscent of neural cells from the CNS. Therefore, it was determined that the omission of dexamethasone from the osteogenic media would be required for successful osteogenic differentiation. In contrast, BMSCs are known to be target cells for steroid treatment,³⁴ as these hormones act to enhance proliferation, alkaline phosphatase expression, and osteoblastic differentiation.³⁵ Our results confirm that BMSCs, in the absence of other pro-osteogenic stimuli (i.e. flow, mechanical loading), require dexamethasone supplementation to acquire an osteoblastic phenotype. These results were similar to others previously reported in literature using both rat and human-derived stromal mesenchymal stem cells.³⁶⁻³⁸ Therefore, in subsequent comparison studies between these two cell populations, BMSC osteogenic media included dexamethasone, while DSC

osteogenic media did not. Unique responses to steroid treatment and differences in cell origin clearly distinguish DSCs and BMSCs as two distinct progenitor populations.

It must be acknowledged however, that, there may also exist potential differences between juvenile BMSCs and fetal DSCs and the significance of this difference remains uncertain. Some recent evidence suggests no significant difference in mesenchymal cells' matrix production between different age groups.^{39,40} Others have reported age-related differences, noting that self-renewal, and not matrix production, was affected with age.^{41,42} However, addressing uncertainties in the timeframe of rodent BMSC niche development⁴³ and difficulties in separating committed osteoprogenitors from BMSCs in the marrow at this stage of development are beyond the scope of this current study. Future studies will address these concerns and compare fetal DSCs directly to fetal BMSCs.

In vitro analysis, via mitochondrial assays and histochemical staining, revealed statistically similar trends in proliferative capacity and different trends in osteogenic potential between groups when cultured on TCP. Both groups, grown in their respective osteogenic medias, exhibited increasing growth with no significant differences in proliferative capacity by day 21. BMSCs exhibited the classic day 7 peak in alkaline phosphatase enzymatic expression and showed significantly greater matrix production at day 21. In contrast, DSCs demonstrated a delayed peak in alkaline phosphatase expression with significantly elevated levels at day 14 which remained elevated thru day 21. Also of note, DSCs exhibited significantly greater matrix production at earlier time points (day 7, day 14) when compared with BMSCs. Although robust staining in DSCs was observed at day 21, it was significantly less than BMSC staining.

With culture on 2D PLGA films, DSCs demonstrated superior cell coverage and significantly greater overall matrix staining over a 28-day time course (Fig. 6). DSCs, which are known to express high levels of FGF-2,⁴⁴ are most likely self-stimulating their proliferation and differentiation on PLGA. FGF-2, often added as a supplement in osteogenic media, has been shown to enhance proliferation and differentiation of BMSCs.^{45,46} Others have reported using this factor in basal media for expansion purposes.⁴⁷ Our studies did not include FGF-2, which may provide explanation for why DSCs performed significantly better than BMSCs on 2D PLGA films. Previous studies have reported BMSCs interactions with PLGA 2D films,²⁷ but none have provided quantitative data regarding long-term proliferative and differentiation potential. Previous studies comparing the biocompatibility of certain cell types with defined biomaterials^{48,49} have elucidated the importance of optimizing the biomaterial-cell-type match, given the variability in differentiation response. Using DSCs instead of BMSCs on PLGA constructs may prove advantageous for bone tissue-engineered applications, given how rapidly these cells attach, proliferate, and cover this substrate.

Further characterization of DSC osteogenic potential on both TCP and 2D PLGA films included gene expression measurements of five important bone markers. For each gene (alkaline phosphatase, collagen I, bone sialoprotein, osteocalcin, osteopontin), positive fold induction was observed in all samples compared with day 0 controls. Expression levels of alkaline phosphatase remained high in the TCP sample compared with the OM samples across 3 weeks. Although early increases in alkaline phosphatase protein expression can be indicative of osteogenic differentiation, this marker is also known to be highly expressed in many progenitor cells. Therefore, consistently higher levels of alkaline phosphatase gene expression in the TCP case may suggest maintenance of an undifferentiated phenotype. Type I collagen protein expression is known to increase early during osteoblast maturation.²⁵ Therefore, significant increases in collagen I gene expression at day 14 (OM-TCP) are consistent with an advance towards osteogenic maturation. Osteopontin, a late

bone-specific protein marker, shows increasing expression through day 21 in both differentiated samples. Another bone-specific extracellular protein, BSP, exhibited significantly higher gene expression in the OM-TCP sample across all time points compared with TCP, lending support to the notion that these cells are undergoing osteoblastic maturation. Given the robust staining on PLGA films, which was comparable to that on TCP, the overall lower BSP expression profiles on film were unanticipated. Additionally, Cbfa1, an essential transcription factor for bone formation, was more highly expressed in the TCP sample compared with the OM samples. This result was also unexpected. Recent reports questioning the importance of cbfa1 in osteogenesis⁵⁰ and/or post-transcriptional modifications may support these unanticipated results.⁵¹

Given the observed differences in cell origin, differentiation pathway, and biomaterial compatibility, future studies will evaluate how the osteogenic potential of DSCs compared with BMSCs may translate to an *in vivo* environment. Previously, Cowan et al. compared healing of critical-sized cranial defects in mice using 3D PLGA scaffolds seeded with BMSCs, ADSCs, and DSCs.⁵² Results showed “minimal bony islands that did not span the area of the defect at 12 weeks,” using scaffolds seeded with DSCs, whereas complete healing was shown using BMSCs and ADSCs. These evidences suggest little therapeutic *in vivo* potential of resident DSCs in cranial bone healing. However, Cowan et al. and others^{10,53} have noted that the first bone layers within a cranial defect form directly opposed to the host dura tissue and have often alluded to the possible signaling role of the host dura mater in the wound healing process. Therefore, further studies will need to be completed to address biocompatibility, optimal preculture environments, and synergistic activity with the host dura, before the healing potential of this cell source is fully understood.

CONCLUSIONS

These studies show that DSCs isolated and expanded in culture can subsequently attach, proliferate, and differentiate to degradable PLGA films without addition of glucocorticoid and growth factor supplements. This initial phase of our studies establishes that DSCs are a promising candidate for cell-based bone tissue engineering therapies and merit further investigation.

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Figure 1.
DSCs outgrowth from explanted dura mater tissue following 2 days of culture in basal media on TCP. Montage made from individual images taken at 10 $\times$.

Figure 2.

Cell monolayers of DSCs (A, B) and BMSCs (C, D) cultured in osteogenic media *with* and *without* dexamethasone supplementation stained for alizarin red following 21 days in culture. A, C: Dexamethasone was included in the osteogenic media. B, D: Dexamethasone was omitted from the osteogenic media. E: Quantitative results of solubilized alizarin red staining over 21 days in culture. BMSC cultured in osteogenic media *without* dexamethasone (BMSC OM – Dex), BMSC cultured in osteogenic media *with* dexamethasone (BMSC OM + Dex), DSC cultured in osteogenic media *without* dexamethasone (DSC OM – Dex). Legend bars = 0.2 mm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 3.

Qualitative images of alkaline phosphatase staining following 3, 7, and 14 days (A, B, C) in osteogenic culture media. DSCs were plated at an initial seed density of 25,000 cells/cm². Legend bars = 0.05 mm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 4. Quantitative results of DSCs and BMSCs osteogenic differentiation over 21-day period. Top graph: Cell Proliferation. Bottom graph: Alkaline phosphatase expression.

Figure 5. Comparison of DSCs and BMSCs osteogenic differentiation on 2D planar PLGA films. Shown is staining following 28 days culture in osteogenic media. A, B, C: DSCs. D, E, F: BMSCs. A, D: Alizarin red staining. B, E: Alkaline phosphatase staining. C, F: Alizarin red stained undifferentiated control. G: Cell proliferation. H: Quantitative alizarin red staining. Legend bars = 1 mm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Figure 6.

Real time RT-PCR results of DSCs over 21 days on both TCP and PLGA films. RNA was extracted at days 7, 14, and 21 from DSCs cultured in osteogenic media on TCP (white), from DSCs cultured in osteogenic media on PLGA films (gray), and from DSCs cultured in basal media on TCP (black).

TABLE I

Tissue-Specific Rat Primers Used to Amplify Genes of Interest

Gene	Primer Sequence
Alkaline phosphatase	5' AACCTCGAGCAGGAACACAAG 3' CCTAAGGACGACGGCAAC
Collagen I	5' ATGGTGCTCCTGGTGCCA 3' GTTCTGTCAGTAGCTTATGT
Bone sialoprotein	5' CTGACGCTGGAAAGTTGGAGT 3' CTCTACGTCTCCCGTTCCG
Cbfa 1	5' CACTCACTACCACACCTACC 3' CCACAACGCGACTACCT
Osteopontin	5' GTAAGTCCAACGAAAGCC 3' CCACTATCACACCAAATACC