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RESEARCH AND EDUCATION

The effect of embryonic origin on the osteoinductive potential of bone allografts

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ABSTRACT

Statement of problem. Allografts with osteoinduction potential are widely used to augment bone in surgical and prosthetic rehabilitations. However, osteoinduction potential varies among commercially available allografts. Donor bones are derived from different embryonic origins, either the neural crest or mesoderm. Whether the origin of the bones affects the osteoinductivity of allograftsis is unclear.

Purpose. The purpose of this ex vivo study was to investigate the osteoinduction potential of allografts derived from bones with distinct embryonic origins.

Material and methods. Allografts were obtained from human frontal and parietal bones at 2 different ages (fetal and adult). The specimens were divided into 4 groups: adult frontal (n=5), adult parietal (n=5), fetal frontal (n=10), and fetal parietal (n=10). Two investigations were conducted to assess the osteoinductive potential of these allografts. First, the osteogenesis of human osteoblasts exposed to these allografts were evaluated by analyzing the expression of runt-related transcription factor 2 (RUNX2), collagen type 1 alpha 2 chain (COL1A2), and bone gamma-carboxyglutamate protein (BGLAP) genes using quantitative real-time polymerase chain reaction (qRT-PCR). Second, the protein content of the adult frontal and parietal bone matrices was analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS). One-way ANOVA and the *t* test were used for statistical analyses of the gene and protein expression of the groups (α=.05).

Results. No difference was found in the gene expression of the cells exposed to frontal or parietal bones. However, all 3 genes were significantly overexpressed in cells treated with fetal bones compared with adult bones. No difference was found in protein expression between adult frontal and adult parietal bones.

Conclusions. No difference was found in the osteoinductive capacity of frontal and parietal bones used as allografts. However, the osteoinductivity of fetal bones can be higher than that of adult bones. Further microanalyses are needed to determine the protein content of fetal bones. (J Prosthet Dent 2019;121:651-8)

Resorption of the alveolar bone after tooth loss often contributes to remarkable alterations in face morphology and compromises the placement of dental implants.[1-3] Correcting these conditions often involves augmenting the affected bone with various types of graft materials.[4]

Allogeneic bone grafts represent an attractive bone graft material, as they lack the comorbidities and limitations associated with autografts, yet retain the osteoinductive potential necessary for bone regeneration.[4-6] Unfortunately, the regenerative potential of allogeneic graft materials is limited because of the high level of variability in their osteoinductive properties.[7-9] This affects clinical performance and reduces predictability.[6,10] Contributing to this variability

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Clinical Implications

The embryonic origin of donor bones does not contribute to the osteoinduction potential of allografts, but donor age does.

is that commercially available allografts are obtained from different types of donor bones at different ages.[10-15] For example, studies on mandibular and tibial bones demonstrate that genes such as vascular endothelial growth factor, transforming growth factor beta, collagen type 1, osteopontin, osteonectin, and β catenin are expressed differently in bones derived from the neural crest and mesoderm.[16,17]

Distinct ossification modes (intramembranous or endochondral),[18,19] distinct structural types (cortical or cancellous),[20] and donor age have been reported to affect the osteoinductive potential of allografts.[18-29] The embryonic origin of bones[17] could also contribute to variability in the osteoinductive capacity of allografts, but the authors are unaware that such a variable has been studied. Bones originate from the neural crest or mesoderm.[17] Frontal and parietal bones are among the few bones which share the same ossification mode and structural type but have different embryonic origins. Frontal bone is derived from the neural crest, while parietal bone is derived from the mesoderm.[17,30,31] These characteristics make them ideal to investigate the effect of different embryonic origins on the osteoinduction potential of allografts.

Studies on osteoblasts obtained from frontal and parietal bones show that genes encoding extracellular matrix (ECM) proteins such as collagen1, osteopontin, cadherin, and the fibroblast growth factor family and their receptors are expressed differently because of their distinct embryonic origins.[32-35] Moreover, neural crest and mesoderm osteoblasts exhibit different proliferation and osteogenesis potentials and utilize different molecular pathways for osteogenesis.[32,34,36,37]

Taken together, this synopsis of the literature suggests that ECM proteins, including growth factors in bones originating from the neural crest, may be different from the bones of mesodermal origin. Yet, to the authors' knowledge, no study has investigated ECM composition or the bone induction capacity of allografts prepared from bones of distinct embryonic origins. The purpose of this ex vivo study was to investigate the osteoinduction potential of allografts obtained from frontal and parietal bones at 2 different ages. To evaluate the osteoinductive capacity of these bones, the osteoblasts treated by them were studied. To evaluate the transcription, matrix formation, and mineralization stages, the expression of runt-related transcription factor 2 (RUNX2), collagen type 1 alpha 2 chain (COL1A2), and bone gamma-carboxyglutamate protein (BGLAP) genes were analyzed.[38] In addition, a large array of proteins in the 2 bone types was studied using proteomics. The null hypothesis was that gene expression in osteoblasts and protein expression in bones would not differ between the frontal and parietal groups, regardless of age.

Table 1. Baseline data

Specimen	n	Age (Mean)	Sex (M/F)
Adult	5	74.8 y	2/3
Fetal	10	17 wk	5/5

MATERIAL AND METHODS

Tissue specimens were obtained from the National Disease Research Interchange and University of Maryland Women's Center. This study was performed according to the University of Maryland Institutional Review Board approved protocol (protocol number: HP-00063249).

Frontal and parietal specimens were obtained from the crania of 10 normal aborted human fetuses, 15 to 19 weeks of age, and 5 deceased adults, 65 to 89 years of age (Table 1). Adult and fetal specimens were used for gene expression analysis. Only adult specimens were used for protein analysis.

For osteoblast gene expression, bone specimens were placed on ice, and soft tissues were dissected and removed. Bone marrow was flushed out with 1× phosphate-buffered saline (PBS). Bone specimens were stored in 70% ethanol until use. The specimens were transferred to 90% ethanol and allowed to dehydrate for 24 hours. They were then air-dried in a tissue culture hood overnight[39] and pulverized (Biopulverizer BioSpec 59012MS 316; Biospec). Bone particles were sieved to obtain bone powder with particles <60 μm.[39] The bone powder was transferred to a sterile centrifuge tube and stored at room temperature. Five milligrams of the bone particles were soaked in 1 mL Ham's Nutrient Mixture F12 Dulbecco's Modified Eagle's Medium (DMEM/Nutrient Mixture F12 Ham; Gibco-Life Technologies) supplemented by 1% amphotericin B, penicillin, and streptomycin solution (Antibiotic-Antimycotic; Gibco-Life Technologies) and 10% fetal bovine serum (Fetal Bovine Serum; Gibco-Life Technologies) and left at room temperature overnight before being added to the cell culture.

Human osteoblast cell line hFOB 1.19 was purchased from the American Type Culture Collection (ATCC CRL-11372). The cells were expanded in a 25 mL flask in DMEM/Nutrient Mixture F12 Ham media supplemented by 1% antibiotic-antimycotic solution and 10% fetal bovine serum. Once the cells reached 95% confluency, they were detached from the flasks using 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA) (Trypsin-EDTA; Gibco-Life Technologies). One million cells were plated in duplicates in 6-well plates. Prepared bone particles

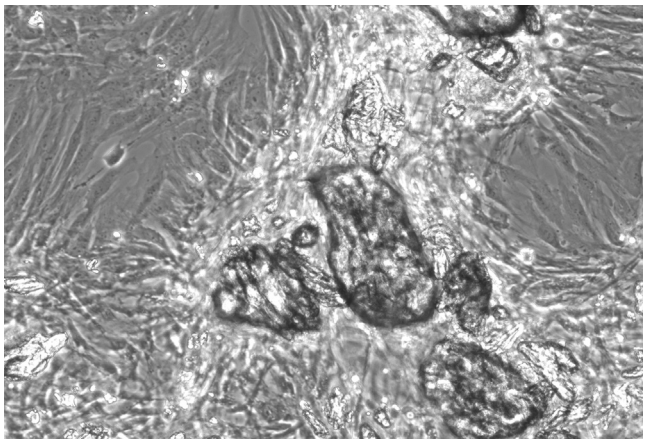

Figure 1. Day 6, culture of osteoblasts treated with bone particles (original magnification ×100).

(5 mg) were added to each well. The number of cells and the amount of bone were optimized in a prior series of experiments. Cells were incubated in a CO_2 incubator at 37°C. The media were changed every 3 days. After 6 days (Fig. 1), trypsin-EDTA was used to detach the cells before RNA isolation.

RNA was isolated from the cells using an RNA isolation kit (High Pure miRNA Isolation Kit; Roche) according to the manufacturer's protocol. The quality and quantity of the RNA specimens were evaluated using a spectrophotometer (NanoDrop 2000/2000c spectrophotometer; Thermo Fisher Scientific). Complementary DNA (cDNA) was made using a cDNA synthesis kit (RevertAid First Strand cDNA Synthesis Kit; Bioline) according to the manufacturer's protocol. RUNX2, COL1A2, and BGLAP genes were selected for quantitative real-time polymerase chain reaction (PCR) analysis, performed in a real-time PCR machine (7900HT Fast Real-Time PCR System; Applied Biosystems) using primers (RT^2 qPCR Primer Assay; Qiagen) and SYBR Green mastermix (RT^2 SYBR Green ROX qPCR Mastermix; Qiagen). The thermocycling condition was set according to Qiagen recommendations.

Raw data were analyzed with software (SDS software; Applied Biosystems). Expression of housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. The fold change of the expression of each gene relative to GAPDH was calculated using the $2^{-\Delta Ct}$ method (ΔCt is Ct of target gene minus Ct of GAPDH). Similarly, the fold change of gene expression in a group relative to another group was calculated using the $2^{-\Delta\Delta Ct}$ method ($\Delta\Delta Ct$ is ΔCt of target gene in one group minus ΔCt of the same gene in another group)[40] Statistical comparison of the groups was carried out using 1-way ANOVA (α=.05). Three comparisons were made for each gene which include comparison of fetal frontal versus fetal parietal groups, adult frontal versus adult parietal groups, and fetal versus adult groups.

For bone protein expression, bone specimens were placed on ice, and soft tissues were dissected and removed. Bone fragments were agitated in 1× PBS to flush out the bone marrow. The fragments were pulverized (BioPulverizer BioSpec 59012MS 316; BioSpec) in liquid nitrogen. Protein extraction from bone specimens was performed following a modified method from the study by Yang et al and Jiang et al.[41,42] Briefly, bone protein specimens were incubated at 4°C overnight in 1× PBS pH 7.4 containing a protease inhibitor cocktail. After an overnight wash in PBS, bone specimens were demineralized overnight in 1.2 M hydrogen chloride at 4°C. The specimens were centrifuged, and the collected supernatant-1 was stored at −80°C. The pellet was washed with 1× PBS pH 7.4 and incubated in 100 mM Tris, 6 M guanidine-hydrogen chloride (pH 7.4) with a protease inhibitor cocktail overnight. The following morning, the specimens were centrifuged, and the supernatant-2 was stored at −80°C; the pellet was further extracted in radioimmunoprecipitation assay (RIPA) buffer (RIPA Buffer; Thermo Fisher Scientific) for 72 hours at 4°C, after which the supernatant-3 was collected. Finally, the pellet was incubated in 1× PBS pH 7.4 overnight at 4°C. After incubation, the supernatant-4 was collected and pooled with the supernatant fractions 1 to 3. The supernatant specimens were precipitated in trichloroacetic acid for an hour at 4°C, and the precipitated proteins were pelleted by centrifugation and redissolved in RIPA buffer. All centrifugation steps were performed at 12 000 rpm for 20 minutes at 4°C. The specimens were quantified for the protein concentration using the bicinchoninic acid (BCA) assay (BCA Assay; Pierce Biotechnology).

After the BCA analysis of the specimens, trypsin digestion was performed on 200 μg protein of each specimen using the filter-aided sample preparation.[43] Protein specimens were mixed with 250 μL of reduction buffer comprising 7.5 mM Tris-(2-carboxyethyl)-phosphine hydrochloride, 8 M urea, and 100 mM ammonium bicarbonate pH 8.0. Afterward, the specimens were loaded into a 3kDa molecular-weight cutoff filter (Pierce Protein Concentrator PES; Thermo Fisher Scientific) and incubated in a dry bath at 37°C for 1 hour for protein reduction. Specimens were then centrifuged at 14 000 rpm for 15 minutes and supplemented with 250 μL of alkylation buffer (8M urea, 100 mM ammonium bicarbonate, and 50 mM iodoacetamide). The specimens were incubated at room temperature in the dark for 1 hour. They were then centrifuged to remove the alkylation buffer, supplemented with 25 μL of 500 mM DL-dithiothreitol to deactivate residual iodoacetamide and recentrifuged. They were washed 4 times with 300 μL washing buffer

Table 2. Comparison of RUNX2, COL1A2, and BGLAP expression in osteoblasts treated with different allografts

	RUNX2			COL1A2			BGLAP		
Comparison	**Fold Change**	***F* Value**	***P***	**Fold Change**	***F* Value**	***P***	**Fold Change**	***F* Value**	***P***
Fetal frontal versus fetal parietal	1.076	0.012	.915	1.115	2.076	.167	1.103	0.004	.951
Adult frontal versus adult parietal	1.062	0.001	.975	1.095	0.681	.433	1.119	0.019	.894
Fetal versus adult	1.336	7.048	.013	1.237	9.898	.004	1.362	4.762	.038

BGLAP, bone gamma-carboxyglutamic acid-containing protein; COLIA2, collagen type 1 alpha 2 chain; RUNX2, runt-related transcription factor 2.

(50 mM ammonium bicarbonate pH 7.4) and digested using trypsin (Trypsin Gold; Promega) overnight at 37°C. Digested specimens were dried in a SpeedVac concentrator and resuspended to a concentration of 0.5 μg/μL protein in 94.9% water/5.0% acetonitrile/0.1% formic acid. The specimens were subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

Peptides were analyzed by LC-MS/MS using a rapid separation nano-LC (Dionex UltiMate 3000 HPLC and UHPLC Systems; Thermo Fisher Scientific) coupled to a linear ion trap mass spectrometer (MS) (LTQ Velos Orbitrap; Thermo Fisher Scientific). Peptide specimens were loaded onto a 150 μm×3 cm trap column packed with 3-μm beads (ReproSil-Pur Basic; Dr. Maisch GmbH HPLC). A loading buffer of 94.9% water/5% acetonitrile/0.1% formic acid was used at a flow rate of 5 μL/minute for 5 minutes. The analytical column was a 75 μm×10.5 cm column (PicoChip System, New Objectives) packed with 1.9-μm beads (ReproSil-Pur; New Objectives). The flow rate was kept at 300 nL/minute. For the mobile phase, solvent A was 0.1% formic acid in water, and solvent B was 0.1% formic acid in acetonitrile. Peptides were separated and eluted using an acetonitrile gradient of 5% to 40%. The source voltage was 2.40 kV, and the capillary temperature was 275°C. MS scans were acquired from 400 to 2000m/z at 60 000 resolving power and automatic gain control set to 1×10^6. The MS was operated in a data-dependent mode.

The 10 most abundant precursor ions in each MS scan were selected for fragmentation. Precursors were selected with an isolation width of 1 Da. They were fragmented by collision-induced dissociation (CID) at 35% normalized collision energy in the ion trap. Previously selected ions were dynamically excluded from reselection for 60 seconds. The MS automatic gain control was set to 3×10^5. The MS/MS data were analyzed for protein identification using software (MaxQuant v1.5; Max Planck Institute of Biochemistry) and queried against the UniprotKB Homo sapiens database. Two missed cleavage sites were allowed for the peptide search. Peptide precursor and fragment ions mass tolerance was set at 20 ppm. Carbamidomethyl on cysteine was taken as fixed modification, whereas oxidized methionine and protein N-terminal acetylation were considered as variable modifications. The "second peptides" option was also enabled with peptide-spectrum

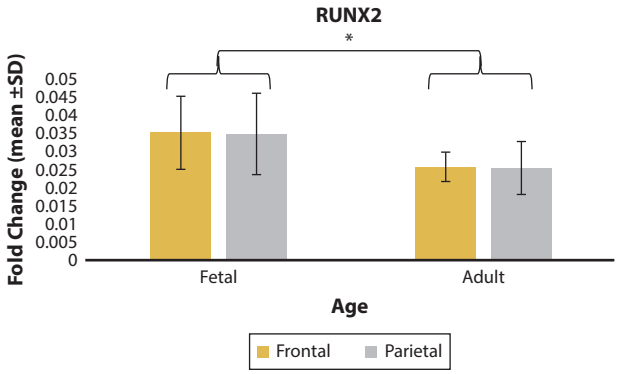

Figure 2. qRT-PCR detection of runt-related transcription factor 2 (RUNX2) in osteoblasts treated with frontal and parietal bone particles in fetal and adult age groups (Fold change determined relative to glyceraldehyde 3-phosphate dehydrogenase). SD, standard deviation; qRT-PCR, quantitative real-time polymerase chain reaction. *Significant difference.

matches (PSM). The protein identifications were filtered at a false discovery rate (FDR) of 0.01. The data were mined using the Andromeda search engine.[44-46] For further analysis, a label-free quantitation (LFQ) minimum ratio count of 2 was taken into consideration. The proteins with 2 or more peptides were analyzed for relative protein expression using software (Perseus v1.5.0.9; Max Planck Institute of Biochemistry).[47] To compare the protein expression of the groups, proteins expressed in at least 3 specimens of each group were analyzed using the Student *t* test (α=.05). The Perseus v1.5.09 tool was used for the statistical analyses.

RESULTS

To compare the osteogenesis activity of the osteoblasts treated by bone particles, the expression of RUNX2, COL1A2, and BGAL genes were evaluated using quantitative real-time PCR. Comparisons were performed based on bone source and age. The analysis showed that expression levels of RUNX2, COL1A2, and BGLAP in the cells treated with the fetal frontal bone were not significantly different from those treated with the fetal parietal bone (P>.05). Similar results were observed with adult bones (P>.05) (Table 2, Figs. 2-4). However, the expression of these genes was significantly higher in osteoblasts exposed to allografts prepared from fetal subjects compared with adults (P<.05) (Table 2, Figs. 2-4). To test if similar expression in frontal and parietal groups was due to similar

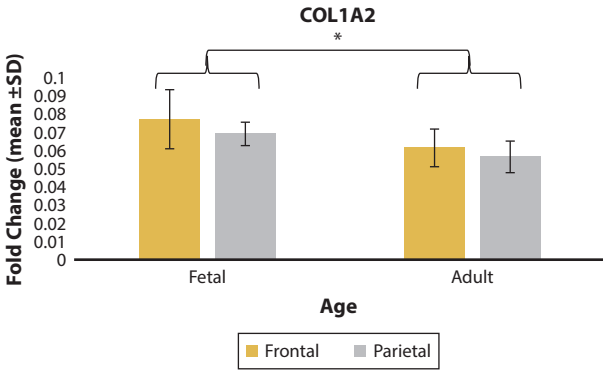

Figure 3. qRT-PCR detection of collagen type 1 alpha 2 chain (COL1A2) in osteoblasts treated with frontal and parietal bone particles in fetal and adult age groups (Fold change determined relative to glyceraldehyde 3-phosphate dehydrogenase). SD, standard deviation; qRT-PCR, quantitative real-time polymerase chain reaction. *Significant difference.

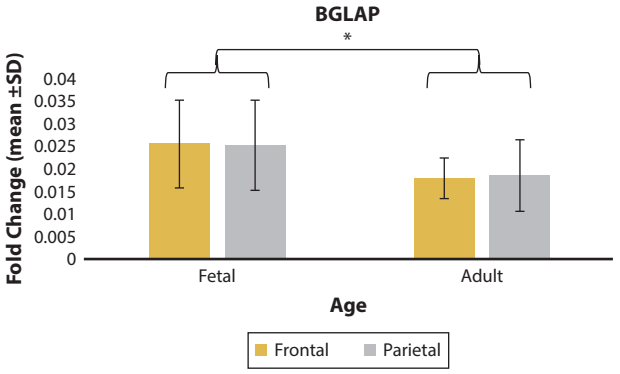

Figure 4. qRT-PCR detection of bone gamma-carboxyglutamate protein (BGLAP) in osteoblasts treated with frontal and parietal bone particles in fetal and adult age groups (Fold change determined relative to glyceraldehyde 3-phosphate dehydrogenase). SD, standard deviation; qRT-PCR, quantitative real-time polymerase chain reaction. *Significant difference.

bone protein content or experiment conditions, a protein analysis was performed on adult demineralized bones.

A total of 128 proteins were identified. Seventeen of the 128 proteins were only expressed in allografts prepared from frontal bones. Twenty-four of the 128 proteins were only expressed in parietal allografts. A list of the proteins unique to each bone group is presented in Tables 3 and 4. Thirty-six of the proteins were common to at least 3 specimens in each group. These proteins were considered for quantitative analysis, which showed no significant difference in the expression of proteins in frontal bones compared with parietal specimens ($P<.05$). A summary of this comparison is presented in Table 5.

DISCUSSION

Commercially available bone grafts have been prepared using various methods. The main common steps are

Table 3. Proteins only expressed in frontal demineralized bones

Protein Names	Protein IDs
Cathepsin B	CTSB
Ig gamma-3 chain C region	IGHG3
Ig gamma-4 chain C region	IGHG4
Myosin-9	MYH9
Pigment epithelium-derived factor	SERPINF1
Platelet glycoprotein 4	CD36
Protein disulfide-isomerase	P4HB
Protein S100-A11	S100A11
Rho GDP-dissociation inhibitor 2	ARHGDIB
SH3 domain-binding glutamic acid-rich-like protein	SH3BGRL
Tripeptidyl-peptidase 1	TPP1
ATP synthase subunit e, mitochondrial	ATP5I
Clusterin	CLU
Eosinophil cationic protein	RNASE3
Ferritin light chain	FTL
14-3-3 protein gamma	YWHAG
Actin	ACTA1; ACTC1; ACTG2; ACTA2

ATP, adenosine triphosphate; GDP, guanosine diphosphate.

rinsing, disinfecting, and defatting with solutions such as ethanol, freeze-drying to reduce antigenicity, pulverizing, and sterilizing with irradiation or ethylene oxide. These steps can reduce the protein content of the graft, but in clinical settings, they are necessary to control contamination and antigenicity.[48,49] In this ex vivo study, the steps such as freeze-drying and sterilizing were eliminated to preserve the protein content of the bones and reduce the procedural biases.

As an osteoinductive graft stimulates osteoblast differentiation and bone formation,[50-53] this study focused on investigating the osteoinductive capacity of allografts by assessing the molecular changes of the osteoblasts treated by the grafts. The study showed no difference in the expression of RUNX2, COL1A2, and BGLAP genes between the frontal and parietal groups in either age group. However, only 3 genes were analyzed in this study. The possibility that other osteogenesis-regulating genes were affected cannot be excluded.

As commercially available grafts are obtained from adult cadavers, the second part of this study focused on adult bones. The investigation did not find any differences in the protein composition of adult frontal and parietal demineralized bones. There were some proteins expressed in a few specimens that were not considered for quantitative comparison because of the stringent cutoff implemented in this study. However, the function of these proteins was evaluated, and it is not clear whether these proteins can affect the osteoinductivity of the specimens.

The influence of different factors such as ossification mode and the structure of donor bones on the induction capacity of allografts has been studied.[18-24] In some of the studies on the ossification mode of allografts, the

Table 4. Proteins only expressed in parietal demineralized allografts

Protein Names	Protein IDs
Fibronectin	FN1
Ig kappa chain V-III region WOL	
Complement C4-A	C4A; C4B
Complement C3	C3
Fibrinogen alpha chain	FGA
Alpha-1B-glycoprotein	A1BG
Ig mu chain C region	IGHM
Actin beta	ACTB
Immunoglobulin lambda-like polypeptide 5	IGLL5
Lactotransferrin	LTF
Azurocidin	AZU1
Annexin A3	ANXA3
Histone H2B type 1-C/E/F/G/I	HIST1H2BC
Voltage-dependent anion-selective channel protein 1	VDAC1
Pyruvate kinase PKM	PKM
Keratin, type I cytoskeletal 18	KRT18
Tubulin alpha-1	TUBA1
Phosphoglycerate kinase	PGK1; PGK2
60 kDa heat shock protein, mitochondrial	HSPD1
Heat shock protein HSP 90-alpha	HSP90A
Adenylyl cyclase–associated protein 1	CAP1
Lamin-B2	LMNB2
Tropomyosin alpha-4 chain	TPM4
Lamin-B1	LMNB1

PKM, pyruvate kinase M; WOL, wooden leg.

Table 5. Comparison of protein expression between adult frontal and parietal subgroups (intensity difference=intensity in frontal subgroup−intensity in parietal subgroup)

Protein Names	Protein IDs	Intensity Difference	*P*
Histone H1.0	P07305	1.105	.300
Talin-1	Q9Y490	0.960	.261
Plastin-2	P13796	0.851	.095
Biglycan	A6NLG9	0.673	.509
Histone H1.3	P16402	0.570	.372
Histone H4	P62805	0.557	.226
Hemoglobin subunit alpha	P69905	0.456	.341
Serum albumin	P02768	0.325	.639
Myeloperoxidase	P05164-2	0.321	.560
Leukocyte elastase inhibitor	P30740	0.320	.713
Glyceraldehyde-3-phosphate dehydrogenase	P04406	0.284	.759
Protein S100-A8	P05109	0.256	.771
Ig gamma-1 chain C region	P01857	0.246	.838
Lysozyme C	P61626	0.214	.753
Hemoglobin subunit beta	P68871	0.191	.689
Neutrophil elastase	P08246	0.181	.856
Annexin A1	P04083	0.091	.789
Moesin	P26038	0.067	.883
Tropomyosin alpha-3 chain	D6R904	0.063	.922
Collagen alpha-1(I) chain	P02452	0.027	.965
Histone H2B type 1-H	Q93079	0.015	.984
Perilipin-1	O60240	0.012	.985
Histone H1.2	P16403	−0.054	.961
Acyl-CoA-binding protein	P07108	−0.064	.926
Collagen alpha-2(I) chain	P08123	−0.157	.878
Carbonic anhydrase 1	E5RH81	−0.178	.803
Hemoglobin subunit delta	P02042	−0.363	.682
Protein S100-A9	P06702	−0.498	.490
Alpha-1-antichymotrypsin	P01011	−0.510	.658
Filamin-A	P21333-2	−0.558	.408
Vinculin	P18206-2	−0.608	.122
Vimentin	P08670	−0.679	.305
Histone H1.5	P16401	−0.682	.609
Actin	P60709	−0.688	.249
Histone H1.4	P10412	−0.934	.478
Ig alpha-1 chain C region	P01876	−1.070	.319
Alpha-1-antitrypsin	P01009	−1.936	.173
Apolipoprotein A-I	P02647	−1.962	.471

terms “intramembranous bones” and “bones with neural crest origin” have been used interchangeably.[18,19] However, the Jiang et al[30] reported on calvarial bones which are intramembranous bones from different embryonic origins. The authors are unaware of any previous study investigating the relationship between embryonic origins and the osteoinductivity of allografts.

Previous studies have evaluated the effect of donor age on the osteoinduction potential of demineralized allografts using histological methods or protein analysis.[25-29] However, the authors are unaware of a previous study that has evaluated the effect of age on allografts using “osteoblast gene expression.” Syftestad et al[25] and Reddi[26] used demineralized bone matrix of different ages in animal models and histologically studied ectopic bone formation. They showed that the osteoinduction potential of allografts reduced with the age of the donor because of progressive reduction in the morphogenetic activity of the bone matrix.[25,26] Pinholt et al[27] and Zhang et al[11] used the same method. Their study showed that osteoinduction capacity increased with age in early adulthood and decreased later. The increase was due to bone maturation and a rise in bone protein concentration. The decrease was because of osteoporotic changes in the bones. Their study showed that the age range with the highest osteoinduction potential in humans is between 30 and 50 years.[11,27] The outcome of the present study was consistent with previous findings in that the bones of elderly donors were less osteoinductive relative to those of younger donors. However, it was not in concert with the studies of Nyssen-Behets et al[28] and Pietrzak et al,[29] who did not report any differences in osteoinduction potential between young and old allografts. The study of Pietrzak et al[29] did not include specimens from individuals older than 65 years. None of the previous studies investigated fetal bones.

Overdemineralization and underdemineralization may also affect the availability of ECM proteins. For maximum osteoinductivity, the demineralization protocol should be optimized for bones with different amounts of

minerals.[11,54] In our study, the 2 age groups did not have similar mineralization levels. Unlike adult bones, fetal bones were not fully mineralized. Since this study was primarily designed to investigate the difference between frontal and parietal bones, for the sake of consistency, neither age group was demineralized for gene expression analysis. Further proteomic studies on fetal bones are needed.

CONCLUSIONS

Based on the findings of this ex vivo study, the following conclusions were drawn:

1. No difference was found in the osteoinduction capacity of frontal and parietal bones.
2. Donor age is one of the contributory factors in the osteoinduction potential of allografts.

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