A Murine Calvarial Defect Model for the Investigation of the Osteogenic Potential of Newborn Umbilical Cord Mesenchymal Stem Cells in Bone Regeneration

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Short Running Head: Umbilical Cord Stem Cell Bone Formation

Abstract:

Background: The standard graft material for alveolar cleft repair (ACR) is autogenous iliac crest. However, a promising alternative potential graft adjunct - newborn human umbilical cord mesenchymal stem cells (h-UCMSC) - has yet to be explored *in vivo*. Their capacity for self-renewal, multipotent differentiation, and proliferation allows h-UCMSC to be harnessed for regenerative medicine. Our study seeks to evaluate the efficacy of using tissue-derived h-UCMSC and their osteogenic capabilities in a murine model to improve ACR.

Methods: *Foxn1* mice were separated into three groups with the following calvarial defects: (1) no-treatment (empty defect; n=6), (2) poly (D,L-lactide-*co*-glycolide) (PLGA) scaffold (n=6), and (3) h-UCMSC with PLGA (n=4). Bilateral 2-mm diameter parietal bone critical-sized defects were created using a dental drill. Micro-CT imaging occurred at 1, 2, 3, and 4 weeks postoperatively. The mice were euthanized 4 weeks postoperatively for RNAscope analysis, immunohistochemistry, and histology.

Results: No mice experienced complications during the follow-up period. Micro-CT and histology demonstrated that the no-treatment (1) and PLGA-only (2) defects remained patent without significant defect size differences across groups. In contrast, the h-UCMSC with PLGA group (3) had significantly greater bone fill on micro-CT and histology.

Conclusions: We demonstrate a successful calvarial defect model for the investigation of h-UCMSC-mediated osteogenesis and bone repair. Furthermore, evidence reveals that PLGA alone has neither short-term effects on bone formation nor any unwanted side effects, making it an

attractive scaffold. Further investigation using h-UCMSC with PLGA in larger animals is warranted to advance future translation to patients requiring ACR.

Clinical Relevance Statement: Our results demonstrate a successful murine calvarial defect model for the investigation of h-UCMSC-mediated osteogenesis and bone repair and provide preliminary evidence for the safe and efficacious use of this graft adjunct in alveolar cleft repair.

Introduction:

Cleft lip and/or palate (CLP) is the most common craniofacial birth defect, affecting an estimated 1/700 live births.[1,2] Most patients with complete CLP also have an alveolar cleft. The mainstay of alveolar cleft repair (ACR) at many institutions is secondary alveolar bone grafting (ABG), with autogenous iliac crest bone graft (ICBG) being the gold standard graft material. However, this procedure is associated with significant donor site morbidity, including hematoma, pain, and disfiguring scars.[3] More recently, studies have demonstrated the efficacy of recombinant human bone morphogenetic protein-2 (rh-BMP-2) as a substitute for autologous grafting in patients with CLP.[4,5] This treatment modality is also associated with complications including prolonged swelling and wound dehiscence.[4]

Improving alveolar cleft graft success and reducing morbidity are key goals in CLP research,[4,6–8] as patients with alveolar clefts are unable to generate sufficient native bone in the cleft site. Though orthodontic intervention can facilitate the narrowing of this cleft non-operatively, most medium-and-large-sized defects require surgical management in addition to bone grafting. While there have been pre-clinical and clinical studies using various bone adjunct materials in alveolar cleft repair, there is a paucity of data on the ability to harness h-UCMSC for this purpose.

Neonatal tissue-derived stem cells such as umbilical cord (UC) blood or UC tissue have recently been started to be investigated as a treatment adjunct in ABG.[6–8] Preliminary animal model investigation in rats has demonstrated that implantation of human Wharton's Jelly, which contains mesenchymal stem cells, can lead to bone regeneration in critical-sized alveolar bone defects[9]. Both UC blood and tissue contain human mesenchymal stem cells (h-UCMSC), which

can be harnessed in regenerative medicine due to their capacity for self-renewal, multipotent differentiation, and enhanced proliferative capacity.[6] Using newborn tissue as a stem cell source is advantageous because UC blood and tissue are usually disposed of as medical waste following the delivery of the neonate, which makes collection straightforward and non-invasive. Given the potential therapeutic value, ease of collection, and reduced risk of exposure to viruses and environmental toxins when compared to harvesting stem cells from adult tissues, newborn UC stems cells present distinct advantages over other stem cell sources.[10,11] UC tissue can be cryopreserved as a composite material for future isolation of the mesenchymal stem cell population[12]. Further, as CLP can be diagnosed early in pregnancy (~11-13 weeks) with ultrasonography, UC collection can be planned long before birth.[6,13,14] Albeit, while prenatal diagnosis has improved with ultrasonography, absolute confirmation of the neonate's final anomaly cannot be determined with 100% certainty. The existing literature reports that only 14-25% of isolated cleft lip cases are detected antenatally, and 12% of these presumed isolated clefts are found to be a feature of a more pervasive malformation or syndrome. Regardless, tissue collection can still occur in preparation for the possibility of needing the UC stem cells in a safe and proactive manner [15].

At our institution, under an Institutional Review Board (IRB)-approved study (CHLA-21-00298), parents of patients with a prenatal diagnosis of CLP are referred to the Cord Blood Registry (CBR) and are presented with the option to store UC blood and tissue at no cost as part of a medical needs-based program for qualifying families who may benefit from using their newborn stem cells in various experimental settings, such as in ACR repair. Our study seeks to evaluate the efficacy of utilizing the osteogenic and regenerative capacity of human UC tissue-

derived mesenchymal stem cells in a mouse model. In so doing, we hope for future clinical translation of h-UCMSC to ACR and subsequent improvement in outcomes for patients with CLP.

Methods:

Animal Information:

All animal studies were conducted with approval by the Institutional Animal Care and Use Committee (IACUC #21329) at the University of Southern California (USC) and in accordance with federal regulations. Homozygous *Foxn1* (JAX#007850) mice obtained from Jackson Laboratory were used. At the time of surgery, the mice were 6 months old. Half of the mice were female, and half were male. Before surgery, males and females (maximum of 3 mice/cage) were housed together. However, because of their inherent immunosuppression, they were isolated after surgery to minimize the risk of postoperative complications. Mice were monitored for complications daily by the primary surgeon (ES) and the Department of Animal Resources at USC.

Umbilical Cord Tissue Collection and Cryopreservation, Mesenchymal Stem Cell Isolation & Culture

Umbilical cord tissue was collected in a noninvasive procedure following the neonate's birth and was then transported in a collection kit at ambient temperature to CBR's processing laboratory in Tucson, Arizona. All cord tissues were collected at birth from full-term, healthy donor neonates (two male donors; one female donor). No complications or donor syndromes

were reported. Upon receipt, the tissue was subjected to a series of rinses consisting of buffered saline and an antiseptic wash. The tissue was segmented into small sections, submerged in a dimethyl sulfoxide (DMSO)-based clinical-grade cryoprotective medium, and maintained in the vapor phase of liquid nitrogen at -196°C. After being stored in the vapor phase of liquid nitrogen for ≥6 months, the composite tissue was transferred to an ISO-7-certified clean room where it was thawed rapidly and smaller pieces of the composite tissue were explanted into cell cultureware. MSCs were harvested after 2 weeks in MSC-supportive media. They were then washed in DPBS and resuspended in a DMSO-based clinical-grade cryoprotective medium. A representative vial was subsequently thawed and the identity of the cells confirmed by flow cytometric analysis, with lines exhibiting greater than 95% positive expression of MSC markers CD73, CD90, and CD105 and less than 1% of cells expressing hematopoietic markers CD34/45 (Figure 1). As previously described, the explant method provides reliable isolation of an MSC population from cord tissue previously cryopreserved as a composite material, as confirmed by plastic adherence, flow cytometry, and osteo-, adipo-, and chondrogenic differentiation potential.[16]

Three distinct mesenchymal stem cell lines isolated from research-donated umbilical cord tissue units were shipped overnight on dry ice from CBR. Upon arrival, all cells were stored in liquid nitrogen until expansion. The cells were dethawed according to the following steps adapted from ThermoFisher: 1) the cryovial with frozen cells was removed from the liquid nitrogen storage and immediately placed into a 37°C water bath; 2) the cells were rapidly thawed in the 37°C water bath until there was a small (~1-3mm^3) piece of cells remaining in the vial; 3) 10mL of MesenCult-ACF Plus medium was added dropwise to the cells, which was followed by

centrifugation at 300g for 10 minutes; 4) the supernatant was removed, and additional media was added; and 5) the cells were plated in three pre-coated 75mL cultureware plates.

The cells were expanded using the MesenCult-ACF Plus Umbilical Cord Culture Kit (StemCell Technology, Vancouver, Canada, Cat#100-0234) according to the manufacturer's protocol. After reaching the cell number needed for operations (6-8 x10^5 cells/defect), the cells were extracted, resuspended in MesenCult-ACF Plus medium, and mixed with PLGA powder (Acros Organics, Cat#436200050).

Calvarial Bone Injury Assays

To investigate the bone regeneration capabilities of h-UCMSC, we implemented a calvarial defect model that has been well-established in the study of bone repair[17,18] and has been used in previous animal model studies of alveolar cleft repair[19]. Further, the calvarial defect size is larger than a mouse alveolar cleft, and thus the bony gap more closely represents the size of human alveolar cleft defects.

The calvarial defect surgeries involved a sagittal skin incision with subcutaneous tissue and periosteum dissection to expose the calvarial bone. Creation of bilateral 2 mm-diameter parietal bone full-thickness critical-sized calvarial defects using a 1.8 mm dental burr followed.[20] A representation of these defects can be found in Figure 2. The skin was sutured with 4-0 absorbable sutures. *Foxn1* mice 6-8 weeks of age were included in the study and were separated into three experimental groups with calvarial defects treated as follows: (1) no-treatment (n=6 mice), (2) defect filled with poly(D,L-lactide-*co*-glycolide) (PLGA) (n=6 mice), and (3) defect filled with h-UCMSC mixed with PLGA (n=4 mice). *Foxn1* mice were used as they have been

used in prior calvarial defect surgeries at our institution during the transplantation of xenografts. PLGA was chosen as a vehicle for the h-UCMSC due to its established ability to facilitate bone mineralization and osteogenesis, its optimal rapid degradation *in vivo*, and its mechanical properties similar to those found in human cancellous bone[21–23]. In group (3), each calvarial defect was treated with 6-8 x 10^5 cells suspended in a mixture of complete MesenCult-ACF Plus medium and PLGA powder. A schematic of the experimental design, from h-UCMSC harvest through implantation into the calvarial defects can be found in Figure 3. The dependent variables of interest were bone fill, represented by micro-CT analysis and histology. Additional variables included level of *Runx2* expression and anti-human IgG expression.

Live micro-CT Analysis

At 1, 2, 3, and 4 weeks postoperatively, mice underwent live micro-CT imaging of the calvaria. The images were processed and analyzed using Amira 3D software for Life Sciences (ThermoFisher Scientific, Waltham, Massachusettes). To quantify the differences in bone fill among the groups, we used a previously-described healing ratio in calvarial defects, defined as the (initial diameter of the injury site) - (the final diameter of the injury site)/(initial diameter of the injury site)[20].

Histology

Calvarial bone samples including the original defect area were dissected and fixed overnight in 4% paraformaldehyde (PFA) at room temperature. Calvarial samples were decalcified while stationary in 10% ethylenediaminetetraacetic acid (EDTA) for 14 days and then dehydrated with graded sucrose solutions: 15% sucrose in 1x phosphate-buffered saline (PBS) overnight followed by 30% sucrose with 50% optimal cutting temperature (OCT) solution

(Sakura Finetek, Torrance, California) overnight at 4℃. After embedding in OCT compound, frozen sections of 8.0μm thickness were cut using a cryostat (CM1850; Leica Biosystems Inc., Buffalo Grove, IL) and stained with hematoxylin and eosin (H&E). Standard protocols were followed for H&E staining.

RNAscope Analysis

Four weeks after the operation, mice were euthanized for histological examination, immunohistochemistry, and RNAscope analysis. The expression of *Runx2* (Advanced Cell Diagnostics, Cat#712231) was assayed 4 weeks after the operation using an RNAscope 2.5 HD Chromogenic Assay (Single-plex, Advanced Cell Diagnostics, Cat#323110) and was analyzed qualitatively. Sections were acquired as described above through embedding in an OCT compound. As with the H&E sections, only bone was harvested, while the remaining surrounding subcutaneous and periosteal tissues were removed. The frozen sections of bone were sliced into 10μm segments that included the defect site and ~3mm of surrounding calvarial bone from each side. After the sections were generated, *in situ* hybridization was conducted according to the manufacturer's instructions.

Immunohistochemistry

Sections were obtained as described above and then were stained with recombinant rabbit monoclonal [EPR4421] anti-human immunoglobulin G (IgG) (Abcam, Cat #ab109489) to confirm the source of the cells found in the defect site after healing. The staining protocol followed the manufacturer's instructions.

Data/Statistical Analyses

In all of the aforementioned analyses, a minimum of three samples were analyzed for each group to allow for statistical analysis. One author (ES) derived the data, which was then reviewed by additional authors. Analyses were not blinded, and one author (ES) evaluated slides/data.

Data were analyzed with SPSS software Version 28.0 (SPSS Inc., Chicago, IL). Tests of statistical significance were performed, including ANOVA with Bonferroni post hoc correction. Prior to ANOVA, skewness and kurtosis tests were performed to confirm the normality of the data. Statistical significance was indicated by $p<0.05$.

Results:

h-UCMSC promote bone healing in calvarial bone critical defects as evidenced by microCT analysis

To test whether h-UCMSC can promote bone healing and regeneration, we conducted critical-sized 2mm calvarial defect surgeries in the parietal bone. All 16 mice underwent calvarial defect operations without postoperative complications or infections. All mice were ambulatory and healthy with no signs of neurologic deficits postoperatively. In humans, following alveolar cleft repair, patients are evaluated with CT scans to investigate bone fill. Likewise, we conducted micro-CT analysis to evaluate the bone fill. As evidenced by micro-CT imaging, at 1, 2, 3, and 4 weeks following the operation, all calvarial defects in the no-treatment (1) and PLGA-only groups (2) remained patent without significant differences in defect sizes between the two groups. In contrast, the h-UCMSC group had significantly greater bone fill,

demonstrated by micro-CT at each postoperative time point ($p<0.001$) (Figure 4). Summary statistics for the various groups are demonstrated in Table 1.

Histologic analysis of the h-UCMSC-treated group demonstrated bone fill of the defects

To confirm the evidence of bone fill seen on our micro-CT results, we conducted histological analysis. Morphologically, neither the defects in the no-treatment group (1) nor the PLGA-only group (2) demonstrated patency without appreciable size differences from each other at 4-weeks postoperatively. The defects were filled with soft tissue rather than bone. In contrast, the h-UCMSC + PLGA group (3) exhibited bone fill across the entire defect site by 4-weeks (Figure 5).

The h-UCMSC-treated group demonstrated increased Runx2 *expression in comparison to the two control groups (no-treatment and PLGA-only)*

To test relative levels of osteoblastic activity across the groups, we performed RNA in-situ hybridization of *Runx2,* a gene known to induce differentiation of multipotent mesenchymal stem cells into osteoblasts. *Runx2* expression was increased in the PLGA-only group and h-UCMSC + PLGA group compared to the no-treatment group (1). The h-UCMSC + PLGA group (3) had more *Runx2* expression at 4 weeks following the operation than the other groups, providing evidence for the differentiation and proliferation of osteoblastic activity and bone formation (Figure 6).

The bone filling the defect in the h-UCMSC group stained positively for human IgG

To confirm that the new bone formation was due to the h-UCMSC, we conducted antibody staining for human IgG. Anti-human IgG staining was positive in the h-UCMSC + PLGA group at 4 weeks following the operation, demonstrating that the bone fill of the defect

was generated by the progeny of the transplanted h-UCMSCs (Figure 7). These results provide evidence that the regenerated bone was human-derived and thus resulted from the h-UCMSC implantation.

Discussion:

In the growing field of regenerative medicine, h-UCMSC present an exciting opportunity to improve cleft care. The advantages of h-UCMSC are numerous, including their capacity for multipotential differentiation and the fact that they can be harvested from umbilical cord tissue, a noncontroversial, nearly inexhaustible source material. We used undifferentiated h-UCMSC in our study because prior evidence suggested that mesenchymal stem cells like these help suppress the immune response, thus enabling improved healing of the defect postoperatively without complications of immuno-rejection[24]. Further, differentiation of MSCs has been reported to lead to increased expression of MHC proteins[25], providing additional rationale that an autologous cell source, such as tissue-derived h-UCMSCs, may be particularly useful in this clinical application. This approach demonstrated safety and efficacy: no mice experienced any adverse outcomes following surgery, and h-UCMSC demonstrated the capacity to regenerate bone in a critical-sized, calvarial defect. This study thus provides *in vivo* evidence that h-UCMSC could serve as an alternative to autologous bone grafts or to growth factors delivered on resorbable scaffolds and hold the potential for a new treatment paradigm in cleft care; additional investigation comparing h-UCMSC to these existing modalities is warranted.

In humans, alveolar clefts are often surgically repaired with bone grafts/substitutes. Historically, when repairing an alveolar cleft, ICBG has been utilized to fill the defect and create a favorable environment for bone growth. However, ICBG carries significant morbidity at the

bone harvest site, with the reported rates of debilitating postoperative pain rising as high as 70%.[26] The need to decrease donor site morbidity has led to the utilization of scaffold-delivered rh-BMP-2 for the stimulation of bone growth at the cleft site. A recently-published, large-scale clinical study at our institution comparing rh-BMP-2 to ICBG has demonstrated comparable results, thus providing an alternative to ICBG and the associated morbidity from bone harvest technique.[4] However, the critical-sized defect at which rh-BMP-2 fails to generate sufficient bone stock remains unknown. Both ICBG and rh-BMP-2 were previously thought to have a bone fill of 60-80% on 2-Dimensional panorex X-ray; however, when critically evaluated by a cone-beam computed tomography scan, they demonstrate bone fill rates of merely 32%.[27] These data provide evidence that our understanding of bone fill is not truly complete, which has significant future clinical implications to orthodontics and orthognathic surgery. Success is delineated on three entities: dental eruption, the stability of the lower lateral nasal cartilage, and the continuity of the maxilla. While good orthodontic work can camouflage incomplete bone fill, improved bone stock provides stability for plate fixation in orthognathic surgery and better supports dental implants as children reach maturity. Further, enhanced stability of the lower lateral nasal cartilage allows for improved aesthetic outcomes. Thus, suboptimal bone fill and its clinical significance necessitate continual evaluation and technical advancements to augment bone stock fill in ACR.

Stem cell-based strategies for bone regeneration have been employed both in humans and animal models, typically by means of a resorbable scaffold seeded with various types of autologous stem cells[28–32]. However, issues such as the low rate of cell survival[29], invasive cell harvesting procedures[30,33], and low bone fill[32] are commonly reported. In contrast, umbilical cord

tissue is easily collected, rich in mesenchymal stem cells, and can be cryopreserved at a newborn stem cell bank for future use. The cell survival and resultant bone fill in our study provide evidence for their ability to serve as a regenerative therapy for craniofacial bony defects. Further investigation is necessary to safely translate these findings to clinical practice. To date, no large-animal studies have been conducted on the ability of h-UCMSC to regenerate bone. Important questions remain regarding the number of cells required for regeneration as well as whether h-UCMSC can regenerate bone in larger defects that may be encountered clinically.

A small number of pioneering studies using UC blood for augmenting ACR have recently been published outside of the United States.[7,8] One case-control found that the use of h-UCMSC from umbilical blood and placental blood in CLP repair decreased inflammation and scar formation compared to traditional CLP repair without h-UCMSC.[7] Another case report showed that the implantation of h-UCMSC during primary gingivoperiosteoplasty mitigated the future need for SABG.[8] However, isolation of mesenchymal stem cells from UC blood can be both time- and volume-dependent. In contrast, mesenchymal stem cells can reliably and consistently be isolated from cryopreserved umbilical cord tissue[12]. Considerable future research is necessary to validate the ability of h-UCMSC to promote bone regeneration in humans.

This study was limited by a relatively small sample size. The number of mice included in the study was restricted by the finite amount of donated umbilical cord stem cells donated for research purposes. Consequently, this study serves as a pilot and proof of concept on which further evidenced-based research can be subsequently conducted. In addition, while this model does not accurately represent cleft defects, we implemented a calvarial defect approach due to its

larger size and thus better approximation of human alveolar clefts. In future studies, both investigations with a physiologic model using alveolar bone as well as a larger and more robust animal model would provide additional/improved data to support our initial findings. Furthermore, the selection of osteogenic markers can be expanded upon in future experiments in order to better understand the bone dynamics affected by the h-UCMSCs. For example, while *Runx2*, an early marker of osteogenesis, was the focus of the manuscript, investigation of late markers (i.e. osteocalcin/osteopontin) can bolster our analyses. Finally, the histologic analysis included in this study can be improved upon, as herein bone formation was determined qualitatively, without staining for cartilage formation or evaluation of the type of bone generated (i.e. woven vs. cancellous). We plan to address these aforementioned limitations in our ongoing experiments and future studies.

Despite these limitations, this study provides important preliminary evidence of the promise for h-UCMSC in ACR. Investigation to tailor h-UCMSC implantation for patients with ACR is warranted; such research should include optimizing the delivery scaffold for these cells and identifying the appropriate cell-seeding concentration. While additional details need to be solidified prior to human application, the preliminary data presented in this study support the benefits of h-UCMSC, and their potential application in ACR demonstrates an exciting opportunity to improve current CLP treatment paradigms.

Conclusions:

These results demonstrate a successful murine calvarial defect model for the investigation of h-UCMSC-mediated osteogenesis and bone repair. Our use of this novel bone graft source is

an ideal preliminary model for clinical translation of h-UCMSC in bone regeneration applications such as ACR. These findings also provide evidence that PLGA alone has no short-term effects on bone formation or adverse side effects, making it an attractive vehicle for graft substitutes. Further investigation using this h-UCMSC and PLGA scaffold combination in a larger-animal model is warranted in the hopes of translating it to ACR in patients with CLP.

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Table 1. Summary Statistics of Healing Ratios Across Experimental Groups

	Healing Ratios	
	Median	IQR
No Treatment	0.20	0.25-0.41
PLGA Only	0.20	0.20-0.41
h-UCMSC + PLGA	0.79	0.79-0.94

IQR, 25-75 percentile interquartile range

Figure Legends

Figure 1. Flow cytometry results from a representative sample (W241721000276). Top left: 99.64% of cells were negative for CD34/CD45. Top right: 99.94% of CD34-/CD45- cells were positive for CD73. Bottom left: 98.24% of CD34-/CD45- cells were positive for CD90. Bottom right: 97.72% of CD34-/CD45- cells were positive for CD105.

Figure 2. Superior view of the 2 mm calvarial defects bilaterally in the parietal bone prior to any treatment.

Figure 3. Schematic representation of the experimental design, including: A) UC tissue B) UC tissue collection and processing, C) UC tissue cryopreservation, D) UC tissue explantation and h-UCMSC isolation, E) h-UCMSC culture and expansion and F) h-UCMSC implantation into mouse calvarial defects.

Figure 4. Micro-CT analysis of calvarial defects at 2 and 4 weeks postoperatively. Neither the no treatment group (1) (left) nor the PLGA-only group (2) (middle) had bone fill at 2 or 4 weeks postoperatively. In contrast, group (3) demonstrated nearly complete bone fill of the defect at 4 weeks postoperatively (right). The left hole in the h-UCMSC group served as a control with nothing inserted; there was migration of UCMSC initially to the contralateral side.

Figure 5. Histological analysis of calvarial defects at 4 weeks postoperatively at 4x magnification (top row), 10x magnification (middle row), and 20x magnification (bottom row). Black dotted lines outline native bone with the defect between. Neither the no treatment group (1) nor the PLGA-only group (2) demonstrated bone fill of the defect; instead, fibrous tissue was

present. In contrast, group (3) exhibited a complete bony bridge across the defect at 4 weeks postoperatively.

Figure 6. *Runx2* expression in cross-sections of calvarial defect sites at 4 weeks postoperatively at 4x magnification (top) and 10x magnification (bottom). The blue stain demonstrates expression of cell nuclei using 4′,6-diamidino-2-phenylindole (DAPI). Pink staining represents expression of *Runx2*. There was almost no *Runx2* expression in the no treatment group (1), while there was some expression in the PLGA-only group (2). The h-UCMSC + PLGA group demonstrated the greatest *Runx2* expression at 4 weeks postoperatively.

Figure 7. Anti-human immunoglobulin G (IgG) staining of the h-UCMSC + PLGA group at 4 weeks postoperatively at 4x magnification (left) and 10x magnification (right). The defect stained positively for anti-human IgG, providing evidence that the regenerated bone was a result of h-UCMSC implantation.

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