TISSUE ENGINEERING
Volume 8, Number 3, 2002

Bone Morphogenetic Protein-Transduced Human Fibroblasts Convert to Osteoblasts and Form Bone *in Vivo*

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ABSTRACT

Experimental cell or *ex vivo* gene therapy for localized bone formation typically uses osteoprogenitor cells propagated from periosteum or bone marrow. Both require bone or marrow biopsies to obtain cells. We have demonstrated that implantation of gingival or dermal fibroblasts transduced with BMP *ex vivo*, using a recombinant adenovirus (AdCMVBMP) attached to porous biodegradable scaffolds, form bone *in vivo*. Here we show that BMP-7-transduced fibroblasts suspended in injectable thermoset hydrogels form complete ossicles on subcutaneous injection and repair segmental defects in rat femurs. Bone formation was preceded by an intermediate cartilage stage. To determine the fate of the implanted transduced cells, thermoset hydrogel suspensions of *ex vivo* BMP-7-transduced or nontransduced fibroblasts were placed in diffusion chambers and implanted to allow development *in vivo* without direct contact with host cells. Only the BMP-transduced fibroblasts formed bone within the diffusion chambers *in vivo*, revealing that BMP transduction induces osteoblastic conversion of these cells.

INTRODUCTION

CELL THERAPY, one current approach to tissue engineering, may be utilized for the restoration of congenital or acquired loss of tissue structure and function. In a combination of cell and gene therapy, known as *ex vivo* gene therapy,[1] the cells to be implanted are removed from the patient and genetically altered before implantation *in vivo*. The genetically altered cells have typically been fibroblasts or myoblasts that are easily biopsied and propagated *in vitro*.[2] Genetic alteration may be accomplished with recombinant plasmid or viral vectors.

Osteoprogenitor cells such as those derived from bone marrow cultures or periosteum form bone on implantation *in vivo* without[3,4] or with *ex vivo* genetic modification.[5–13] However, this approach requires that bone or bone marrow be biopsied; a procedure often associated with substantial donor site morbidity. Obtaining cells for autologous grafts from easily biopsied, readily regenerating tissues such as skin or gingiva would likely be associated with less morbidity. Therefore we have utilized *ex vivo* gene therapy to test the idea that autologous cells for tissue regeneration need not be obtained from the tissue to be regenerated.[14]

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This approach was suggested by the well-documented capacity of recombinant human bone morphogenetic proteins (BMP) to induce endochondral-like bone formation on implantation into dermis, muscle,[15,16] or gingiva (R.B. Rutherford, unpublished observations) and the evidence that local cells initiate healing responses (reviewed by Prokop[17]). These observations suggest that several tissue types contain populations of cells responsive to the inductive effects of BMP. We have demonstrated that cultured nonimmortal strains of gingival or dermal fibroblasts, transduced *ex vivo* by the mouse BMP-7 full-length cDNA, secrete biologically active BMP-7 *in vitro*, and when absorbed to porous, preformed sponges and implanted *in vivo*, induce ectopic and orthotopic bone formation. Both the implanted donor and host cells participate in the bone formation, resulting in chimeric ossicles comprising both donor and host-derived extracellular matrix.[14] These data suggested that the BMP-transduced fibroblasts in contact with host tissue converted to osteoblasts *in vivo*.

The use of BMP-transduced fibroblasts that contribute to bony matrix production as well as induce host tissues to produce bone could be advantageous in clinical conditions characterized by relatively large acellular lesions such as those produced by surgical resection of tumors and radiation therapy. Our hypothesis is that BMP-responsive fibroblasts may be propagated from skin and gingiva *ex vivo* and stimulated to form predetermined amounts of bone on implantation *in vivo*. Here we report that BMP-transduced fibroblasts suspended in thermoset hydrogels from cartilage that converts to marrow-containing ectopic ossicles *in vivo*. The size of these ectopic ossicles varies with the number of cells injected. Such preparations absorbed to preformed porous sponges also induce repair of femoral segmental defects. In addition, BMP-transduced fibroblasts suspended in a hydrogel form bone *in vivo* in the absence of direct contact with host tissue. These data reveal that BMP-7 transduction induces an osteoblastic conversion of nonosteoblastic fibroblasts that is not dependent on direct interaction of the transduced cells with host tissue.

MATERIALS AND METHODS

Materials

All reagents were purchased from Sigma (St. Louis, MO) or GIBCO-BRL (Grand Island, NY) unless otherwise noted. Defined fetal bovine sera were obtained from HyClone (Provo, UT). Gelfoam was obtained from Upjohn (Kalamazoo, MI) and tissue culture ware was obtained from Corning (Corning, NY). NIH-bg-nu-xid br (NIH III) and C57BL/6 mice were purchased from Charles River (Wilmington, MA).

BMP transduction and preparation of cell–hydrogel suspensions

Human gingival (HGF), C57BL/6 mouse dermal (MDF), or Fischer rat (RDF) fibroblastic cell strains were derived from explants of tissue biopsies and cultured as described.[18] Cells from passage 3–12 were removed from liquid nitrogen storage and grown to near confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin, and streptomycin. HGF infection with AdRSVlacZ or with AdCMVBMP-2, -4, or -7[19] was at a multiplicity of infection (MOI) of 200 PFU/cell whereas MDF and RDF infection was at 1000 PFU/cell. Cells, confluent in 75-cm^2 flasks, were exposed to the appropriate dilution of virus in 8 ml of medium for 24 h at 37°C in a humidified atmosphere of 5% CO_2 before trypsinization, counting, and resuspension in the appropriate hydrogel at the desired concentration. Matrigel (growth factor reduced; Collaborative Biomedical Products, Bedford, MA) and acid-soluble rat tail collagen solution (final concentration, 2.5 mg/mL; Collaborative Biomedical Products) were prepared according to the manufacturer directions. Pluronic F127 (BASF, Mt. Olive, NJ) was mixed in sterile distilled water to a final concentration of 30%. All these materials are thermoset hydrogels at the concentrations used: liquids at 4°C that gel as the temperature approaches 37°C. The mass of ossicles was determined by wet weight and the data were analyzed by analysis of variance (ANOVA) and post-hoc analysis for significance with the Scheffe F test. The student *t* test for paired samples was also used where appropriate.

Implantation of transduced cells and tissue analysis

Transduced cells were transplanted via subcutaneous injection of 200 μL of the indicated number of cells in the backs of anesthetized NIH III mice for HGFs, or of C57BL/6 mice for MDFs. For the injections, a 26-

gauge hypodermic needle was used. The C57BL/6 cells and host animals are syngeneic, hence this system models autologous transplantation into an immunocompetent host. For diffusion chamber experiments, 30 μL containing a 10^5 BMP-7-transduced or nontransduced HGF–collagen suspension was injected into 4-mm-diameter chambers prepared in sterilized 14 $\times$ 2 mm custom plastic disks and sealed with nitrocellulose membranes (0.45 μm; Millipore, Bedford, MA). The sealed diffusion chambers were implanted subcutaneously.

For orthotopic assays of bone formation, Fisher rats were anesthetized with isoflurane and the hindlimbs were aseptically prepared with Betadine and alcohol. The femurs were surgically exposed and a custom drill template was used to drill and insert four fixation pins into the femur. An external fixator with a central hinge, locked in a neutral position, was secured on the pins. A transverse simple 2- to 3-mm osteotomy was created midway between the central two fixator pins with a micro-oscillating saw. The BMP-7-transduced RDF–collagen construct was inserted into the osteotomy site and the soft tissues were closed. BMP-7-transduced RDF–collagen construct comprised 10^4 cells suspended in type I collagen hydrogel (2.5 mg/mL, final concentration) and absorbed to moistened and flattened precut Gelfoam sponges. Control constructs contained 10^6 *lacZ*-transduced RDF.

After the indicated developmental interval *in vivo*, the tissues were harvested and fixed in fresh 4% paraformaldehyde, demineralized in 10% formic acid, and processed for histological or immunocytochemical analysis. Immunocytochemical analysis for BMP-7 expression was performed according to the Vectastain ABC kit protocol (Vector Laboratories, Burlingame, CA). BMP-7 ligand and antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). For calcium analysis, the ossicles were blotted dry after harvest, weighed, and incubated at 400°C for at least 8 h. The ash was weighed and analyzed for calcium content according to the manufacturers specifications (kit 587A; Sigma). The photomicrographs taken with a digital camera were altered for brightness and contrast only in Adobe Photoshop (version 5.0). All procedures using animals were performed under protocols approved by the University of Michigan Committee on the Use and Care of Animals.

RESULTS

Ectopic ossicle formation by injected BMP-transduced fibroblast–hydrogel suspensions

Human gingival fibroblasts (HGFs), transduced *ex vivo* with a recombinant adenovirus containing the full-length mouse BMP-7 cDNA,[19] formed marrow-containing ossicles *in vivo* when suspended in acid-soluble type I collagen and injected subcutaneously (Fig. 1). All ossicles produced by injecting BMP-2, -4, or -7-transduced HGFs revealed a similar histological structure (Fig. 1A,B). However, nontransduced HGFs, or HGFs transduced with bacterial *lacZ*, failed to form ossicles (Fig. 1C) and consistently produced identifiable tissue masses only after large numbers of cells were implanted.

The mass of the ossicles varied with the number of BMP-7-transduced HGFs injected when suspended in any of three distinct types of thermoset hydrogel (Table 1). Matrigel is a mixture of type IV collagen, laminin, and trace amounts of growth factors. The type I collagen is an acid-soluble fraction extracted from rat tails and Pluronic F127 is a surfactant with the common chemical name polyethylene-polyoxypropylene block copolymer previously demonstrated to be effective as a cell delivery vehicle.[20] Increasing the number of cells (10^3 to 2 $\times$ 10^6) injected in a constant volume of hydrogel (200 μL) yielded larger ossicles in both immunodeficient and immunocompetent hosts (Table 1). As few as 10^3 and 10^4 BMP-transduced cells suspended in Matrigel or collagen, respectively, formed complete ectopic ossicles (Fig. 1 and Table 1) on subcutaneous injection. The mass of the ossicles produced by equal numbers of cells was greatest in Matrigel (Table 1). All ossicles, regardless of hydrogel carrier or cell number injected, were composed of cortical and medullary bone and contained marrow (Fig. 1). The calcium content of the ossicles produced by BMP-7-transduced HGFs suspended in collagen increased with increasing numbers of implanted transduced cells and was significantly higher than tissue formed in *lacZ*-transduced cell implants (Fig. 2).

BMP-transduced fibroblasts induce cartilage and bone

Implantation of exogenous BMP induces an endochondral-like bone formation process.[21] To determine whether ossicles produced by BMP-7-transduced fibroblasts follow a similar developmental pathway, in-

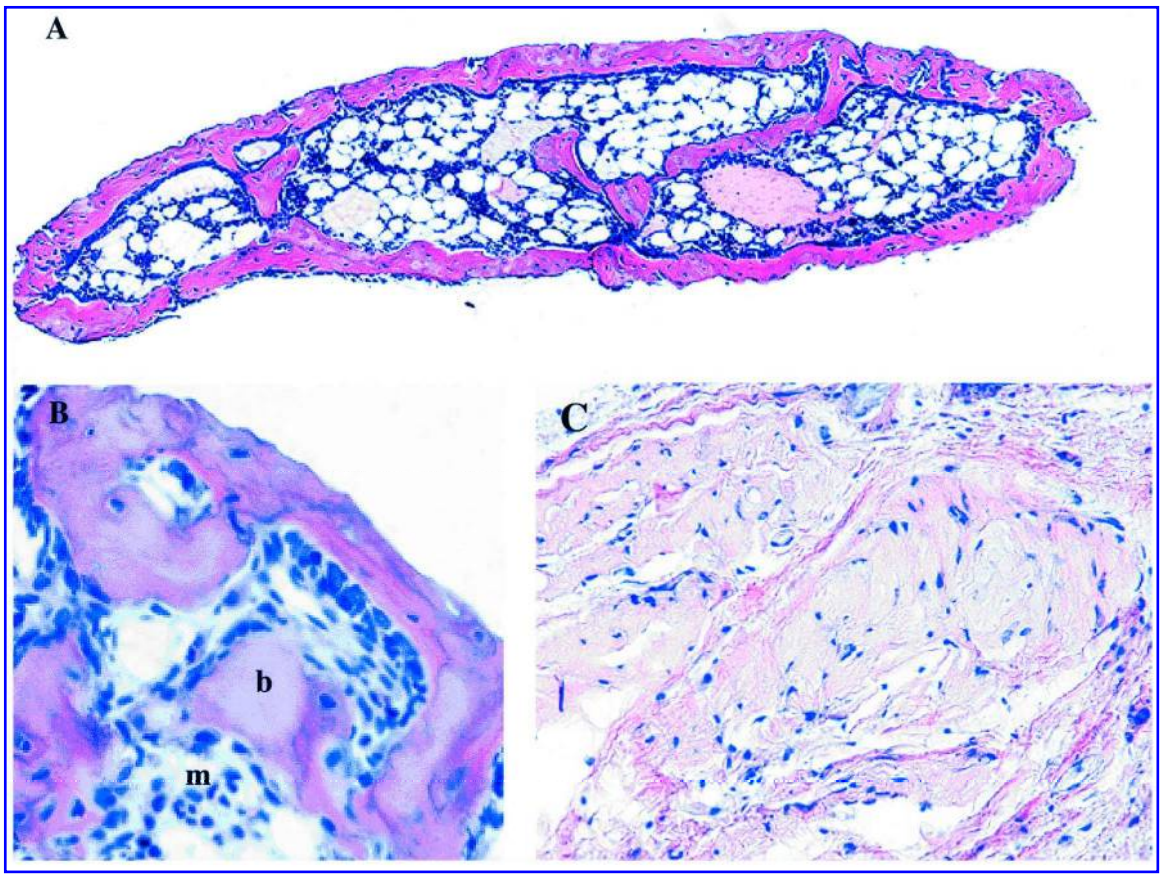

FIG. 1. BMP-transduced fibroblasts suspended in a collagen thermoset hydrogel form ectopic marrow-containing ossicles *in vivo.* BMP-7-transduced (**A, B**) or nontransduced (**C**) HGFs were suspended in soluble thermoset collagen hydrogel (2.5 mg/ml, final concentration), injected subcutaneously into NIH III mice, harvested after 3 weeks, and analyzed histologically. (**A**) Histomicrograph of a typical ossicle formed with BMP-7-transduced HGFs suspended at 10^4 cells/200 μL of collagen; original magnification, ×10. (**B**) Higher power magnification of sample in (**A**), demonstrating bony trabeculae (b) and marrow (m); original magnification, ×50; (**C**) histomicrograph of tissue produced by injection of *lacZ*-transduced, collagen hydrogel-suspended HGFs original magnification, ×50. All sections were stained with H&E.

duced tissues were harvested at various intervals and histological specimens were prepared. Histochemical analyses of tissues produced by subcutaneous injection of 5×10^5 C57BL/6 fibroblasts into syngeneic hosts reveal that cartilage precedes bone ($n = 4/4$; Fig. 3). After 3 days, a condensation of cells is present (Fig. 3A) that matures to cartilage by 5–7 days (Fig. 3B) and to bone by 14 days (Fig. 3C). The cartilage phenotype is confirmed by the presence of cartilage extracellular matrix proteoglycans as detected by Safranin O staining after 6 days *in vivo* (Fig. 3B). By 14 days developing marrow is evident (Fig. 3C, arrow).

Skeletal defect repair

To determine whether BMP-7-transduced HGFs suspended in collagen hydrogels induce local skeletal regeneration, surgical osteotomies were prepared in rat femurs and implanted with BMP-7- or *lacZ*-transduced syngeneic rat dermal fibroblasts (RDFs). In these experiments, 10^4 BMP-7- or 10^6 *lacZ*-transduced RDFs suspended in a collagen hydrogel and absorbed into gelatin sponges were delivered to the respective sites. *lacZ*-transduced RDFs failed to form bone bridging the defect ($n = 3/3$; Fig. 4A,C,E). In contrast, BMP-7-transduced RDFs induced cartilage and bone formation within the surgical defect within 6 weeks ($n = 3/3$; Fig. 4B,D,F). The regenerating bone fused with the existing fragments so that the cut ends are not evident

Table 1. Relationship of Mass of Ossicle to Number of Cells Injected[a]

	Cell number							
	0	*10^2*	*10^3*	*10^4*	*10^5*	*5×10^5*	*10^6*	*2×10^6*
Matrigel	0.02 (0.001)	0.03 (0.002)	0.05 (0.003)	0.03[b] (0.003)	0.08[b] (0.004)	0.15[b] (0.007)	0.2[b] (0.01)	0.45[b] (0.04)
Collagen[c]	n/t	n/t	n/t	0.015[d] (0.004)	n/a	n/t	0.065[d] (0.003)	0.125 (0.01)
Collagen[e]	n/t	n/t	n/t	0.012[d] (0.005)	0.04[d] (0.002)	0.07[d] (0.004)	0.05[d] (0.002)	0.09 (0.008)
Pluronic F127	n/t	n/t	n/t	0.015[d] (0.007)	n/a	0.06[d] (0.005)	0.06[d] (0.004)	0.08 (0.007)
No carrier	n/a	n/a	n/t	n/t	n/t	n/a	n/t	n/t

Abbreviations: n/t; No identifiable transplanted tissue at time of biopsy (3 weeks); n/a, not attempted.

[a]Equal numbers of cells were transduced with BMP-7 and suspended in 200 μl of hydrogel and injected subcutaneously. Values represent wet weight of tissue in grams (standard error), $n = 3$.

[b]Significantly different than ossicles produced in thermoset collagen or Pluronic hydrogels ($p < 0.05$). The mass of all ossicles was significantly different than that produced by fewer or more cells ($p < 0.05$). No ossicles were produced by fewer than 10^3 cells suspended in Matrigel.

[c]HGFs suspended in collagen and injected into nude mice.

[d]Significantly different ($p < 0.05$) than ossicles produced by injection of more cells.

[e]Syngeneic mouse dermal fibroblasts suspended in collagen and injected into C57Bl/6 mice.

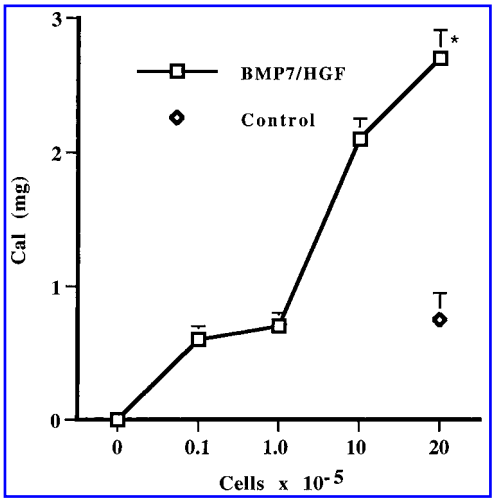

FIG. 2. Calcium content of collagen-suspended, BMP-7-transduced HGF-induced ossicles increases with increasing numbers of implanted cells. No identifiable implanted tissues were recovered from control sites containing fewer than 2×10^6 cells. Calcium content was measured from ashed samples by colorimetric assay according to the manufacturer instructions (Sigma). Data represent means ± standard deviation ($n = 3$, representative of triplicate experiments). Calcium content of samples from 2×10^6 cells is significantly different from control ($^*p < 0.01$, Student t test for paired samples).

in the histological specimen (Fig. 4B,D). In the *lacZ*-transduced RDF control experiments, the defect contained fibrous connective tissue with bone formation limited to remodeling the free ends of the bony fragments.

Osteoblastic transformation of transduced fibroblasts

Our previous data demonstrated that the BMP-transduced fibroblasts as well as the host fibroblasts contributed to the bony matrix,[14] suggesting that the BMP-7-transduced fibroblasts converted to osteoblasts *in vivo*. The ossicles formed by transduced fibroblasts developed through a cartilage intermediate that was usually replaced with bone and marrow by 2–3 weeks (Fig. 3). To demonstrate that implanted cells directly produce cartilage matrix, HGFs cotransduced with *lacZ* and BMP-7 were injected subcutaneously and the

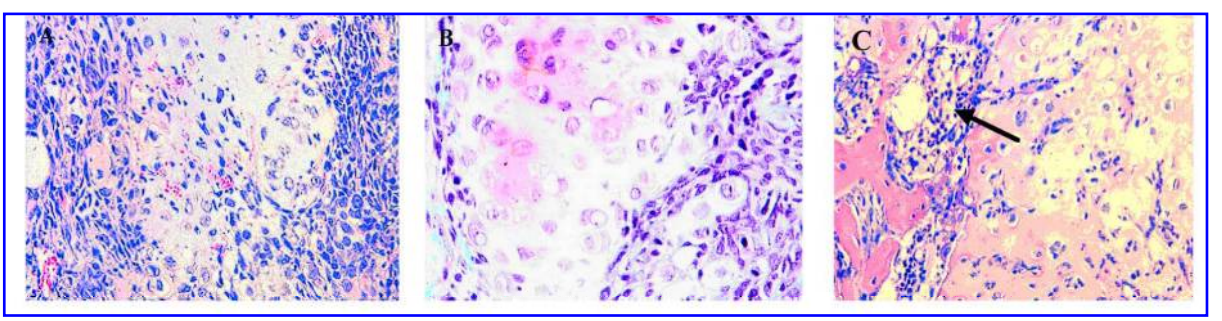

FIG. 3. BMP-7-transduced fibroblasts induce bone via an endochondral-like process *in vivo*. BMP-7-transduced C57BL/6 dermal fibroblasts (5×10^5) suspended in type I collagen thermoset hydrogel (2.5 mg/mL, final concentration) were injected subcutaneously into C57BL/6 mice and the resulting tissue was harvested after 3 days (**A**), 6 days (**B**), and 14 days (**C**). (**A, C**) Stained with H&E; (**B**) stained with Safranin O, which stains cartilage proteoglycans red. Developing marrow is evident by 14 days (arrow, **C**), concomitant with the conversion of the cartilage to a bony matrix. Original magnification, ×100.

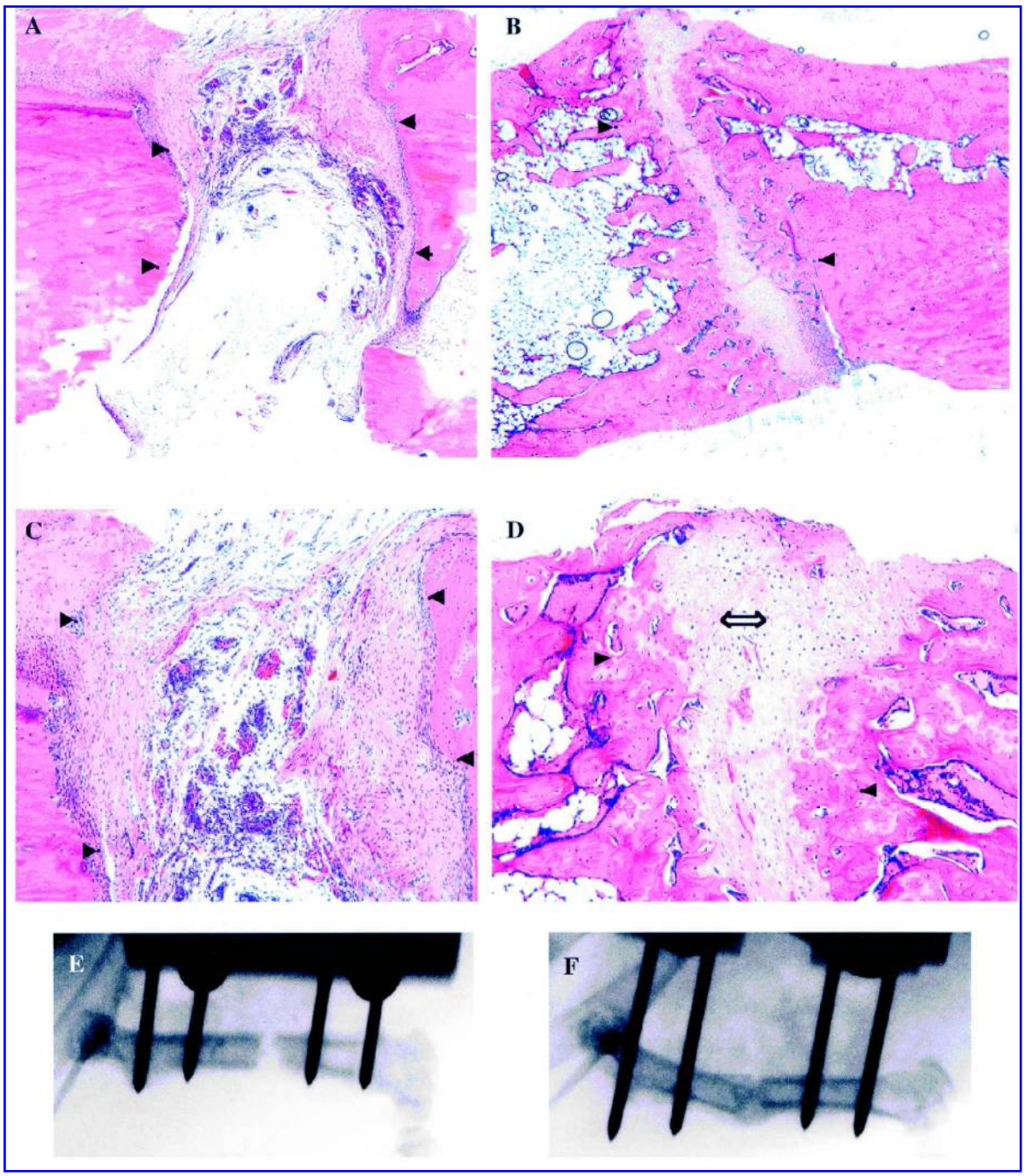

FIG. 4. BMP-7-transduced syngeneic dermal fibroblasts induce skeletal regeneration. Fischer rat dermal fibroblasts were transduced with *lacZ* (**A, B**; $n = 3$) or BMP-7 (**C, D**; $n = 3$) and suspended at 10^6 or 10^4 cells/30 μL of collagen hydrogel, respectively, absorbed to 5 × 5 × 5 mm pieces of gelatin sponge, and implanted into 3-mm surgical osteotomies in Fischer rat femurs for 6 weeks. Histologic analyses revealed only fibrous connective tissue forming in osteotomies implanted with *lacZ*-transduced RDFs (**A, C**; arrowheads mark bony margins); whereas bone (arrowheads) and cartilage (double arrow) formed in lesions implanted with BMP-7-transduced RDFs (**B, D**). Radiographs of *lacZ* control (**E**) and BMP-7-transduced (**F**) treated bones were taken after 6 weeks, just before sacrifice. Original magnification: (**A, B**) ×10; (**C, D**) ×50.

resultant tissue was analyzed after 7 days. BMP-7- and LacZ-producing cells are directly associated with and surrounded by the developing matrix (Fig. 5A,B) during the cartilagenous phase. These observations reinforce our previously reported data,[14] that *ex vivo* BMP-transduced fibroblasts directly contribute to extracellular matrix production. As a definitive test to demonstrate the osteoblastic conversion of fibroblasts by ruling out a contribution by host cells, BMP-7-transduced HGF–collagen suspensions were sealed within diffusion chambers and implanted subcutaneously. A mineralized matrix formed within sealed diffusion chambers containing BMP-7-transduced HGF–collagen composites but not nontransduced fibroblast–collagen composites within 6 weeks ($n = 9$, six positive; Fig. 6A–C). Similar tissues were obtained from chambers containing control nontransduced neonatal mouse calvarial-derived periosteal cells (Fig. 6D). The host dermis was in close contact with the diffusion chamber at harvest, with little to no inflammation evident. Extensive histologic analysis of the tissue formed within the chambers (Fig. 5A,C) failed to detect any evidence of host tissue contamination. In striking contrast to results obtained with injection of HGF–hydrogel composites (Figs. 2 and 4), no evidence of angiogenesis or marrow formation was evident in the tissues harvested from the diffusion chambers.

DISCUSSION

Among the pleiotropic effects of BMPs is their remarkable capacity to induce endochondral-like bone formation in nonosteogenic tissues such as gingiva (R.B. Rutherford, unpublished observations), skin, or muscle (reviewed by Kingsley[15] and Reddi[21,22]). Our data reveal that osteogenesis induced by *ex vivo* BMP-transduced fibroblasts follows a similar endochondral-like process (Figs. 3–5). The cartilage phase was completely converted to bone even though the rate of conversion appeared to vary with the number of cells implanted and the site of injection (i.e., ectopic vs. orthotopic) (Figs. 3 and 4). However, an organized cartilage–bone interface resembling a growth plate was not detected in any of the several hundred sections examined. These observations reveal that secreted and exogenous recombinant BMP-7 induces bone formation by similar pathways.

One of the major challenges for the tissue engineering of bone is to regulate the amount and form of the new tissue. In our earlier studies, using BMP-transduced fibroblasts and preformed scaffolds, the volume of the ectopic ossicles was largely limited to the volume of the scaffold implanted, and did not vary substantially with the number of attached cells.[14] Hence the resulting ossicle resembled the size and shape of, and replaced, the implanted cell-laden scaffold. Although robust in producing bone that mimicks a preformed shape, this approach is limited with regard to its ability to deliver osteogenic cells to lesions with complicated anatomy. In the current study, we demonstrate that BMP-7-transduced fibroblasts suspended in thermoset hydrogels from bone *in vivo*. In this ectopic bone induction assay, the size of the ossicle varied with the number of BMP-7-transduced human cells injected subcutaneously and conformed to the shape

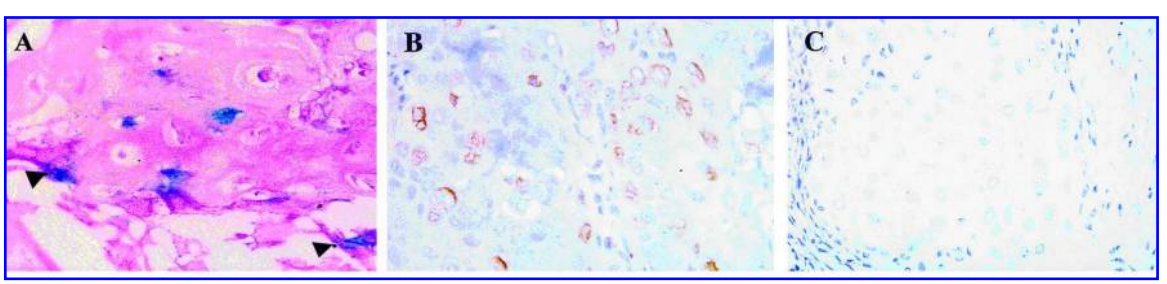

FIG. 5. *Ex vivo*-transduced fibroblasts are associated with cartilage-like matrix. BMP-7- and *lacZ*-cotransduced HGFs were suspended in a soluble collagen hydrogel (2.5 mg/mL, final concentration), injected subcutaneously into NIH III mice, harvested after 7 days, and processed for histochemical and immunocytochemical analyses. (**A**) LacZ expression by chondroblast-like cells; arrowheads indicate LacZ-producing cells. (**B**) BMP-7 expression by chondroblast-like cells. (**C**) Specificity control section [for (**B**)] developed with anti-BMP-7 antiserum preincubated with BMP-7 ligand. Original magnification: ×100.

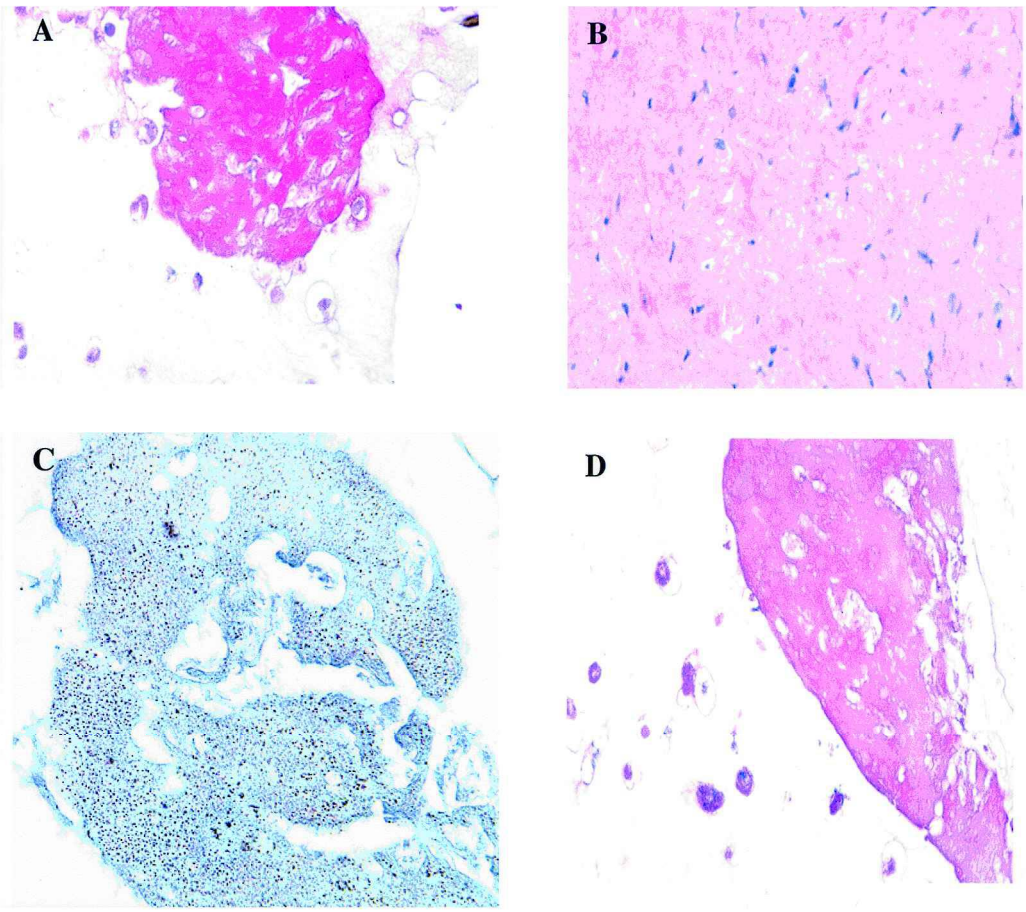

FIG. 6. BMP-7-transduced HGF–collagen gel composites from a cartilage–bone matrix on implantation in immunocompetent mice within diffusion chambers to isolate transduced from host cells. Cells (10^5) in 30 μL of collagen solution (3 mg/mL) were injected into diffusion chambers, gelled, and sealed with nitrocellulose filter paper. The diffusion chambers were implanted subcutaneously in C57BL/6 mice for 6 weeks. Tissues were harvested and processed for histochemical analyses. (**A**) BMP-7-transduced HGF implant, (**B**) nontransduced HGF implant, (**C**) nontransduced neonatal mouse calvarial cell implant, (**D**) BMP-7-transduced HGF implant stained with von Kossa. Original magnification: ×100.

of the tissue pouch created by injection (data not shown). The size was also dependent on the carrier used but not the immunological competence of the host (Table 1). Hence the type of hydrogel carrier is likely an important factor, because equal numbers of transduced HGFs in equal volumes of hydrogel produce different-sized ossicles depending on the hydrogel used (Table 1). The basis for this differential response has not been studied. The materials tested vary widely in composition and have diverse uses. Matrigel is produced by tumor cells *in vitro* and contains several extracellular matrix molecules including nonfibrillar type IV collagen and laminin.[23] The type I collagen contains several cell-binding domains whereas Pluronic, a synthetic surfactant, likely does not. Integrin-mediated cell–extracellular collagen interactions are important in osteoblast differentiation.[24–26] It is clear that, with fewer than 2 million cells, a carrier is necessary for bone formation (Fig. 2 and Table 1). However, when more than 2 million cells suspended in culture medium are injected, small ossicles develop (n = 4/4; data not shown). The biologic basis for this matrix effect is under investigation.

The use of either porous solid scaffolds or hydrogels alone as carriers for cell implants is not readily adaptable to all potential clinical applications. For example, cells suspended in culture medium are readily

absorbed to solid porous scaffolds. However, the most gentle manipulation may result in loss of cells from the scaffold. Therefore it may be useful to retain cells within porous, presized scaffolds by first resuspending the cells in an appropriate hydrogel for subsequent loading of the scaffold. Such a method could serve to better retain the cells within the scaffold than if the cells are suspended in a nongelling liquid. Therefore, in these studies, we have tested two strategies for implanting genetically modified fibroblasts. *Ex vivo*-transduced cells suspended in the thermoset hydrogel polymers were directly injected *in vivo* (Fig. 1) or the hydrogel–cell suspensions were absorbed to preformed porous sponges before surgical implantation (Fig. 4). This approach was utilized in the segmental defect assay, as we anticipated that the hydrogel would not gel quickly enough to form a gelled mass within the segmental defect. Bone formed in both cases, suggesting these delivery modes for BMP-transduced fibroblasts could be useful for localized skeletal regeneration. In the latter case sponges were easily manipulated and placed into the femoral segmental defect without losing absorbed cells. This finding suggests that hydrogel-suspended BMP-transduced fibroblasts could be loaded into porous, custom-designed solid scaffolds before implantation into specific bony sites.[27] It may not be necessary for the transduced fibroblasts to attach directly to the solid scaffold, providing more latitude in the choice of materials for scaffold construction. However, we have tested only a single solid scaffold (gelatin sponge)–hydrogel (collagen) combination to date. Cells suspended in thermoset hydrogels could be useful in spinal fusion, the restoration of digits, or complex craniofacial structures that may pose anatomical and structural limitations on the implantation of preformed materials.

In our current study, substantial skeletal repair was produced by only 10^4 BMP-7-transduced fibroblasts. Femoral defects contained bone and cartilage that filled the gap after 6 weeks (Fig. 4). Given that more cells produced larger ossicles in the ectopic assay (Table 1), it is likely that more cells would similarly produce a more robust orthotopic response. These data reveal the strong potential for bone regeneration with as few as 10^4 *ex vivo* BMP-7-transduced, readily available autologous fibroblasts. On the basis of our data (Table 1), it is unlikely that fewer cells will be effective in segmental defects. Other limiting factors affecting the amount of orthotopic bone formed by implantation of transduced fibroblasts have not been further studied.

Most current evidence suggests that reparative cells are recruited from the local tissue (reviewed by Prokop[17]). It is therefore likely that cells capable of responding to BMP to initiate bone formation are local. Our hypothesis is that BMP-responsive cells may be propagated from skin and gingiva *ex vivo* and stimulated to form predetermined amounts of bone on implantation *in vivo*. In support of this hypothesis we have demonstrated that low-passage human fibroblast strains (from different individual donors) express BMP receptor transcripts *in vitro*.[28] HGFs transduced with BMP-7 or cotransduced with BMP-7 and *lacZ* are found within the developing cartilaginous matrix (Fig. 5), suggesting that the *ex vivo* BMP-7-transduced cells directly produce bone via an endochondral-like developmental process and that BMP-7 secretion plays a role in cartilage development. Furthermore, BMP-7-transduced HGF–collagen composites, gelled in diffusion chambers and implanted in isolation from direct contact with host tissue, also produce a mineralizing bonelike matrix *in vivo* (Fig. 6). Such diffusion chamber experiments have been used extensively to determine or confirm the osteoblastic nature of cultured cells.[29–31] Others have demonstrated bone formation by BMP-transduced fibroblasts.[32] In addition, the BMP secreted by the implanted transduced cells induces adjacent host cells to form bone.[14] Taken together, our data reveal that autocrine/intracrine as well as the well-established paracrine modes of signaling operate to form bone on implantation of *ex vivo* BMP-transduced fibroblasts *in vivo*. These data support the findings of others, who report an autocrine mode of action for BMP[33] but do not distinguish between autocrine or intracrine modes.

Our experiments (Figs. 5 and 6) also demonstrate that BMP-7 transduction of fibroblasts alters the phenotype from fibroblastic to osteoblastic. Consistent with our results, fibroblastic cell lines in culture are known to differentiate to osteoblasts after treatment with exogenous recombinant BMP.[34] Furthermore, bone marrow and other adult mesenchymally derived tissues possess multipotential cells capable of differentiating along several different pathways, resulting in distinct mesenchymal tissues.[35–37] Moreover, several studies indicate that adult tissues contain cells capable of differentiating into cells typical of mature tissues derived from a different cell lineage, for example, bone marrow (mesoderm) to neurons (ectoderm).[38,39] We have no evidence suggesting that the populations of fibroblasts used in these experiments contain stem cells. It is possible that the fibroblastic cells propagated *ex vivo* and utilized in these experiments are descendents

of the cells that respond to exogenous BMP signals *in vivo*. However, the cells responsive to these exogenous BMP signals have not been identified. It is likely that transduction with BMP-7 alters the transcriptional program and hence the functional phenotype of the transduced fibroblasts.

Ex vivo gene therapy for localized skeletal regeneration, in which the induction of bone formation is by donor cells as well as host cells, could represent a distinct clinical advantage over recombinant protein[21] or *in vivo* gene therapy.[19,40] Both of these approaches require the migration of sufficient numbers of responsive host cells into the lesion. This lengthy process may not readily occur in relatively acellular lesions. In addition, the use of cells, such as dermal or gingival fibroblasts, derived from readily accessible, quickly healing tissues, would be associated with less donor-site morbidity than autologous bony transplants or bone marrow-derived cell therapy. However, a variety of clinical conditions requiring bone regeneration or autologous grafting exist. Further experiments are needed to determine the appropriate and most robust mode of therapy for each.

ACKNOWLEDGMENTS

We thank Tracy Matson, Rochelle Taylor, and Pat Racenis for excellent technical assistance and Bonnie Knowland and Kathy Sweet for outstanding animal care. We also thank Amy Koh and Laurie McCauley for kindly supplying the mouse osteoblasts. This work was supported by grants from the NIH: DE 12466 (R.B.R.), DE 13386 (R.T.F.), DE 00426 (P.H.K.), AG 00713 (M.M.), and AR 20557 (R.T.).

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