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Characterization of Scaffold Carriers for BMP9-Transduced Osteoblastic Progenitor Cells in Bone Regeneration

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Abstract

Successful bone tissue engineering at least requires sufficient osteoblast progenitors, efficient osteoinductive factors, and biocompatible scaffolding materials. We have demonstrated that BMP9 is one of the most potent factors in inducing osteogenic differentiation of mesenchymal progenitors. To facilitate the potential use of cell-based BMP9 gene therapy for bone regeneration, we characterize the in vivo osteoconductive activities and bone regeneration potential of three clinically-used scaffold materials, type I collagen sponge, hydroxyapatite-tricalcium phosphate (HA-TCP) and demineralized bone matrix (DBM), using BMP9-expressing C2C12 osteoblastic progenitor cells. We find that recombinant adenovirus-mediated BMP9 expression effectively induces osteogenic differentiation in C2C12 cells. Although direct subcutaneous injection of BMP9-transduced C2C12 cells forms ectopic bony masses, subcutaneous implantation of BMP9-expressing C2C12 cells with collagen sponge or HA-TCP scaffold yields the most robust and mature cancellous bone formation, whereas the DBM carrier group forms no or minimal bone

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CONFLICT OF INTEREST

The authors state that they have no conflicts of interest.

masses. Our results suggest that collagen sponge and HA-TCP scaffold carriers may provide more cell-friendly environment to support the survival, propagation, and ultimately differentiation of BMP9-expressing progenitor cells. This line of investigation should provide important experimental evidence for further pre-clinical studies in BMP9-mediated cell based approach to bone tissue engineering.

Keywords

BMP9; bone regeneration; scaffold carriers; cell-based therapy; bone tissue engineering; bone formation

INTRODUCTION

Effective bone regeneration holds promise as an improved method of bone and skeletal reconstruction^{1–6}. Successful bone regeneration may require at least three important components: osteogenic progenitor cells that can undergo effective osteogenic differentiation and produce extracellular matrix, biological factors that are osteoinductive and able to induce or enhance new bone ingrowth, and scaffolding materials that are biocompatible and osteoconductive^{4–6}. Osteogenic differentiation is a sequential cascade that recapitulates most of the molecular events occurring during skeletal development⁷. Mesenchymal stem cells (MSCs) are commonly used osteoblast progenitor cells for bone formation⁸. MSCs are multipotent progenitors which can undergo self-renewal and differentiate into multi-lineages, including osteogenic, chondrogenic, and adipogenic lineages^{9–11}. MSCs have been isolated from numerous tissues, and one of the major sources in adults is the bone marrow stromal cells. For cell-based bone tissue engineering, ideal scaffolding materials should provide certain initial biomechanical support at the injury site; allow for the attachment of osteoblast progenitor cells; offer a cell-friendly environment enabling progenitors to survive, propagate, and ultimately differentiate; enable the ingrowth of vascular tissue to ensure the survival of the transplanted cells; and undergo biodegradable process with a controllable rate of degradation into molecules that easily can be metabolized or excreted¹².

Bone morphogenetic proteins (BMPs) play an important role during development^{11,13,14} and regulate stem cell proliferation and osteogenic differentiation^{15,16}. BMPs belong to the TGF β superfamily; and there are at least 14 BMPs in humans and rodents^{11,13,14,17}. We previously found that BMP9 is one of the most potent BMPs among the 14 types of BMPs in inducing osteogenic differentiation^{2,3,14,18–24}. BMP9 (also known as growth differentiation factor 2, or GDF-2) was identified in the developing mouse liver²⁵. We have demonstrated that BMP9 effectively induces osteoblast differentiation by regulating several important downstream targets during BMP9-induced^{2,14,18–23,26}, as well as cross-talking with other important signaling pathways^{2,24,27–30}. Our recent findings suggest that BMP9 is resistant to noggin inhibition, which may partially contribute to BMP9's potent osteogenic activity³¹. Thus, it is conceivable that cell-mediated gene therapy using BMP9-expressing progenitor cells may hold promise to promote bone regeneration in large bony defects and/or fracture nonunion in clinical settings^{1–3}.

We hypothesize that a cell-friendly scaffold environment is a key to effective bone formation for BMP9-expressing cell-based approach to bone tissue engineering as scaffold materials can serve as a template for cell interactions and form osteoid extracellular matrix to provide structural support for the newly formed osseous tissue. Our objective is to characterize the in vivo osteoconductive activities and bone regeneration potential of three clinically used scaffold materials, type I collagen sponge, hydroxyapatite-tricalcium phosphate (HA-TCP), and demineralized bone matrix (DBM), using BMP9-expressing osteoblastic progenitor C2C12 cells. We find that although direct subcutaneous injection of the BMP9-transduced C2C12 cells forms ectopic bony masses, subcutaneous implantation of the BMP9-expressing C2C12 cells with type I collagen sponge or HA-TCP scaffold yields the most robust and mature cancellous bone formation, while the DBM carrier group forms minimal bony masses. Thus, our results suggest that collagen sponge and HA-TCP scaffold carriers may provide a more cell-friendly environment to support the survival, propagation, and ultimately differentiation of BMP9-expressing osteoblastic progenitor cells.

MATERIALS AND METHODS

Cell culture and chemicals

Human HEK-293 and mouse C2C12 pre-osteoblastic lines were from ATCC (Manassas, VA) and maintained in complete Dulbecco modified Eagle medium (DMEM) with 10% fetal calf serum (FBS, Mediatech, Herndon, VA), 100 units/ml penicillin, and 100µg/ml streptomycin at 37°C in 5% CO₂, as described^{18,20,32}. Unless indicated otherwise, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or Fischer Scientific (Pittsburgh, PA).

Scaffold carriers

Three commonly-used scaffold materials were used in the study, including type I collagen sponge, hydroxyapatite-tricalcium phosphate (HA-TCP), and demineralized bone matrix (DBM) were kindly provided by Medtronic Sofamor-Danek (Minneapolis, MN), Biomet (Warsaw, IN), and Wright Medical (Arlington, TN), respectively.

Construction of recombinant adenovirus expressing BMP9 or GFP

Recombinant adenovirus was generated using AdEasy technology as described^{18,19,33,34}. The coding region of human BMP9 was PCR amplified and cloned into an adenoviral shuttle vector and subsequently used to generate recombinant adenovirus in HEK293 cells. The resulting adenovirus was designated as AdBMP9, which also expresses GFP as a marker for monitoring infection efficiency. Analogous adenovirus expressing only GFP (AdGFP) was used as a control^{22–24,35,36}.

Alkaline phosphatase (ALP) assay

ALP activity was assessed by a modified Great Escape SEAP Chemiluminescence assay (BD Clontech, Mountain View, CA) and/or histochemical staining assay (using a mixture of 0.1 mg/ml naphthol AS-MX phosphate and 0.6 mg/ml Fast Blue BB salt) as described^{18,23,27,32,37,38}. For the chemiluminescence assays, each assay condition was

performed in triplicate and the results were repeated in at least three independent experiments. ALP activity was normalized by total cellular protein concentrations among the samples.

Alizarin Red S staining

C2C12 cells were seeded in 24-well cell culture plates and infected with AdBMP9, or AdGFP. Infected cells were cultured in the presence of ascorbic acid (50 μ g/mL) and β -glycerophosphate (10mM). At 10 days post infection, mineralized matrix nodules were stained for calcium precipitation by means of Alizarin Red S staining, as described previously^{18,19,32}. Cells were fixed with 0.05% (v/v) glutaraldehyde and stained with 0.4% Alizarin Red S (Sigma-Aldrich). The staining of calcium mineral deposits was recorded under bright field microscopy.

Immunohistochemical (IHC) staining

IHC staining was carried out as previously described^{38–42}. Cultured cells were infected with adenoviruses. At 12 days post infection, cells were fixed with 10% formalin and washed with PBS. The fixed cells were permeabilized with 1% NP-40 and blocked with 10% goat serum, followed by incubation with an anti-osteocalcin (OCN) antibody (Santa Cruz Biotechnology) for 1h. After being washed, cells were incubated with biotin-labeled secondary antibody for 30 min, followed by incubating cells with streptavidin-HRP conjugate for 20 min at room temperature. The presence of OCN was visualized by DAB staining and examined under a microscope. Stains with control IgG were used as negative controls.

Subcutaneous implantation of carriers seeded with AdBMP9-transduced cells in nude mice and radiographic imaging

The use and care of animals followed the guidelines approved by the Institutional Animal Care and Use Committee. Young adult athymic nude mice (male, 4–6 week old, Harlan Laboratories, Indianapolis, IN) were used in this study. Each experimental group had ten animals, including a group without carrier (cells only). For the preparation of the adenovirus-transduced C2C12 cells for carrier implantation, subconfluent C2C12 cells were infected with AdBMPs or AdGFP at a pre-optimized titer (or multiplicity of infection, MOI = 10) for 15h. The infected cells were collected and resuspended in PBS at a density of 10⁸ cells/ml and 50 μ l of the cell suspension was used for each implantation site.

For the carrier implantation, mice were anesthetized using intraperitoneal injections of ketamine and xylazine. One centimeter incision was made over each flank approximately 1-cm from the midline of the mouse. The tested carriers included type I collagen sponge (Medtronic Sofamor-Danek, Minneapolis, MN), hydroxyapatite-tri-calcium phosphate (HA-TCP) (Biomet, Warsaw, IN), and demineralized bone matrix (DBM) (Wright Medical, Arlington, TN). The carriers were kept in sterile condition and opened in the biosafety cabinet within the animal care facilities. A subcutaneous pocket was then created using blunt dissection. Approximately 2mm³ of carrier substances was placed into each pocket. BMP9 or GFP-transduced cells (5 $\times$ 10⁶ cells per implantation site) were directly applied onto the implanted carriers. The operated animals resumed activities immediately without any

restraints on food and drink. At 1, 2, or 4 weeks, animals were sacrificed and subjected to X-ray radiography.

Histological evaluation

After radiographic analysis, implant sites were retrieved, decalcified, and paraffin-embedded. The samples were sectioned and stained with hematoxylin and eosin out as previously described^{23,27,32,37}. The slides were evaluated by three individuals, including musculoskeletal pathologists. For calculating the percentage of cancellous bone area over total area for each treatment group, at least 20 random high-power-fields (HPFs, 200x) for each treatment group were analyzed using the NIH ImageJ software. The average cancellous bone area/high-power-field (HPF) per treatment group was calculated.

Statistical analysis

All quantitative experiments were performed in triplicate and/or repeated three times. Data were expressed as mean±S.D. Statistical significances between different treatments were determined by one-way analysis of variance and the Student's *t* test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

BMP9 effectively induces osteogenic differentiation of pre-osteoblast progenitor cells in vitro

As C2C12 cells were used as seeding cells for the cell-based bone regeneration study, we first confirmed the osteogenic activity of BMP9 in C2C12 cells. When C2C12 cells were transduced with AdBMP9, early osteogenic marker ALP activity was significantly induced qualitatively (Fig. 1A, panel a) and quantitatively (Fig. 1A, panel b) compared with the GFP control treatment ($p < 0.001$). Furthermore, BMP9 was shown to effectively up-regulate late osteogenic marker OCN when compared with that of the GFP treatment (Fig. 1B). Lastly, we assessed the ability of BMP9 to induce matrix mineralization in C2C12 cells. As shown in Fig. 1C, mineralized nodules were readily formed in BMP9-transduced C2C12 culture compared with that of the GFP control treatment. These results indicate that BMP9 can effectively induce osteogenic differentiation in C2C12 cells and that C2C12 cells may be used as a reliable seeding cell source for the carrier studies.

BMP9 can induce robust ectopic bone formation in 4 weeks

We next determined the optimal timeline for BMP9-transduced C2C12 to form robust ectopic bone utilizing the commonly-used type I collagen sponge. We chose to use an ectopic bone formation animal model as this model would allow us to test if a scaffold carrier provides a cell friendly environment and subsequently supports bone formation. We transduced subconfluent C2C12 cells with an optimal titer of AdBMP9 or AdGFP and found the cells were effectively transduced (Fig. 2A) and effectively induced ALP activity (Fig. 2B). The cells were collected for seeding with the type I collagen carriers in the subcutaneous implantation of athymic nude mice. The animals were anesthetized and X-ray imaged at weeks 1, 2, and 4 post implantation (Fig. 2C). Opaque images at the implantation sites were observed in BMP9 treatment group as early as 2 weeks (Fig. 2C, panel a)

although more mature and mineralized masses were observed at week 4 (Fig. 2C, panel c). No significant opaque masses were observed in the GFP control group at all three time points (Fig. 2C). Histological evaluation further confirmed that robust bone formation was readily observed in the samples retrieved from the BMP9 treatment group, while the GFP control group only contained proliferative and undifferentiated cells without detectable bone formation (Fig. 2D). These results indicate that BMP9-transduced C2C12 cells can induce effective and robust bone formation in collagen sponge carriers in 4 weeks using the athymic nude mouse model.

Three types of scaffold carriers exhibit distinct capability for supporting ectopic bone formation of BMP9-transduced C2C12 cells

Using the experimental conditions established in Fig. 2, we analyzed the effect of different scaffolds on the ectopic bone formation ability of BMP9-transduced pre-osteoblastic progenitors. When BMP9-transduced C2C12 cells were seeded with three types of scaffold carriers, type I collagen sponge, HA-TCP, and DBM, or cells only (without any carriers), robust ectopic bone formation was radiographically detected in collagen sponge, HA-TCP, and cells only groups (Fig. 3A–C), whereas no apparent bone formation was detected in DBM group (Fig. 3D). Furthermore, under the same conditions, no apparent bone formation was detected radiographically in the contralateral GFP control groups in all three carrier groups or cells only group although HA-TCP carrier had high background opaque imaging when X-ray imaging was performed (Fig. 3C). These radiographic results suggest that collagen sponge and HA-TCP scaffold carriers may provide a friendly environment for BMP9-transduced preosteoblast progenitors to survive, propagate, and eventually undergo osteogenic differentiation.

Type I collagen and HA-TCP carriers provide a more cell-friendly scaffolding environment for BMP9-induced cancellous bone formation

The retrieved implant samples were further analyzed histologically (Supplementary Fig. 1). Consistent with our earlier studies^{2,19,37,43,44}, a direct subcutaneous injection of BMP9-transduced C2C12 cells resulted in the presence of some immature, wormian bone and hyper-cellularity, while no evidence of bone formation was observed in GFP control group (Fig. 4A). Implantation of collagen sponge carriers containing BMP9-transduced C2C12 induced lamellar structure of bone formation although a hypercellular distribution of osteoblasts was noted, whereas GFP control group did not form any bone-like structure under in the same condition (Fig. 4B). In the HA-TCP carrier groups, histologic evaluation revealed rather mature trabecular bone architecture with some lamellar bone structure, as well with a hypercellular pattern of osteoblasts, and the presence of some carrier materials (Fig. 4C). The GFP control sites exhibited no bone formation except the presence of carrier materials (Fig. 4C). Lastly, in the DBM carrier group implanted with BMP9-transduced C2C12 cells, histologic evaluation revealed continued presence of the DBM carrier materials at the sites with minimal bone formation surrounding the DBM carriers (Fig. 4D), resembling immature, wormian bone without trabecular and lamellar pattern. Interestingly, there were limited but noticeable bone-lining cells on the surface of the DBM carriers on both BMP9 and GFP groups, suggesting that the DBM carrier may release certain osteoinductive factors to induce osteogenic differentiation.

We further analyzed the histologic features of the retrieved implant sites containing BMP9-transduced progenitor cells. Although direct cell injection led to effective bone formation, there were fewer and thinner trabecular bone structures lacking evident lamellar bone, and there was abundant presence of undifferentiated progenitor cells (Fig. 5A). On the other hand, both type I collagen sponge and HA-TCP scaffolds provided a cell-friendly environment and yielded effective formation of rather mature and lamellar bone structures (Fig. 5A). However, there was very limited ectopic bone formation observed in DBM carrier implant sites although there were osteoblast-like cells lined on the surface of DBM carriers in both BMP9 and GFP implantation groups (Fig. 5A). Nonetheless, it seems that the DBM carriers may lack of porous structure and thus limit the survival and growth of osteoblast progenitors inside the scaffolds. Quantitative analysis of the average area of cancellous bone formation from each implant group using high-power microscope images further confirms that the type I collagen sponge and HA-TCP scaffold groups formed the highest percentage of cancellous bone ($p < 0.01$), while the DBM group formed the lowest ($p < 0.05$), when compared with direct cell injection group (Fig. 5B). Taken together, our results strongly suggest that collagen sponge and HA-TCP scaffolds may provide a more cell-friendly environment for BMP9-transduced progenitor cell-based bone regeneration.

DISCUSSION

Efficacious cell-mediated bone tissue engineering requires at least three essential components; osteoblastic progenitors, potent osteogenic factors, and cell-friendly scaffold materials. We have identified a potent osteogenic factor BMP9 and characterized several mesenchymal progenitor cells, including C2C12^{18,19,37,44}. In this study, we conduct a comparative analysis of the osteoconductive activities and bone regeneration potential of three commonly-used scaffold materials, type I collagen sponge, HA-TCP, and DBM, using BMP9-expressing C2C12 cells in vivo. We find that while direct subcutaneous injection of the BMP9-transduced C2C12 cells forms ectopic bony masses, subcutaneous implantation of the BMP9-expressing C2C12 cells with type I collagen sponge or HA-TCP scaffold yields robust and mature cancellous bone formation. The DBM carrier group forms minimal bony masses. These results suggest that collagen sponge and HA-TCP scaffold carriers may provide a more cell-friendly environment to support the survival, propagation, and ultimately differentiation of BMP9-expressing osteoblastic progenitor cells. It is conceivable that the use of primary bone marrow stromal cells may be more clinically relevant because these cells can be harvested from the affected individuals and transduced ex vivo with BMP9 to achieve effective cell-based therapies.

A number of carrier materials for recombinant BMP therapy were reported, including inorganic materials such as hydroxyapatite (HA) and tricalcium phosphate (TCP), natural polymers such as collagen and hyaluronans, synthetic polymers such as polylactide and composites of various groups⁴⁵. Ceramics have been shown to have a higher rate of fusion than type I collagen sponge in a rabbit fusion model using recombinant BMP2⁴⁶. A high spine fusion rate was reported using a compression resistant ceramic/collagen type I composite matrix in both rabbits and non-human primates⁴⁷. A PLGA scaffold has been proposed and tested, but no comparison of carriers was performed⁴⁸. Interestingly, high spine fusion rate was reported in studies using collagen sponge and DBM in rats using

BMP2 gene therapy⁴⁹. Additionally, animal spinal fusion models in rabbits and canines have shown successful fusion using collagen, demineralized bone matrix (DBM), hyaluronate, and HA-TCP^{50,51}. Although carriers have been compared using recombinant BMP, few carriers have emerged as an ideal vessel for cell-based gene therapy. No studies have been performed to compare carriers for cell-based BMP9 gene therapy.

In this study, we find that collagen sponge and HA-TCP carriers, when seeded with BMP9-expressing progenitors, produce more mature and a larger amount of bone compared to the GFP control. This is in contrast to the DBM groups which show minimal bone formation in both the experimental BMP9 group and the GFP control group. Our results indicate that the DBM may be least cell-friendly and affect cell survival and proliferation at the site of implantation. The low cellularity observed in the DBM samples may be due to the DBM itself as it contains glycerol as a bonding agent. Glycerol has been implicated in local cell death at high doses including that of muscle and may have been toxic enough to affect the cells post-implantation⁵². Nonetheless, our results indicate that collagen and HA-TCP scaffolds function as more effective carriers for cell-based BMP9 gene therapy.

While the reported study focused on the scaffold materials that are currently used in clinical settings, increasing efforts have been focused on the development of new biomaterials for bone tissue engineering⁵³. Potential bone scaffold materials include inorganic ceramics (e.g., hydroxyapatite, coralline-derived hydroxyapatite, tricalcium phosphate, calcium sulphates, glass ceramics, calcium phosphate-based cements, and bioglass), metals, and synthetic biodegradable polymer composites. Ideal biomaterials should easily integrate with the adjacent bone, favor new tissue ingrowth (osteoconduction) and are biodegradable, which can provide the initial structure and stability for tissue formation but degrade as tissue forms^{12,54,55}. Due to the physiochemical properties, biocompatibility, and controllable biodegradability, polylactic acid (PLA) and polyglycolic acid (PGA) polymers have emerged as a frequently investigated material in bone tissue engineering⁵³. More recently, there is a growing interest in developing artificial bone-mimetic nanomaterials with controllable mineral content, nanostructure, chemistry for bone tissue engineering and substitutes^{56,57}. It is conceivable that these efforts should ultimately lead to the development of biocompatible scaffold materials for progenitor cell based BMP9 gene therapy for bone regeneration in clinical setting.

CONCLUSIONS

To establish the cell-friendly scaffolding environment for cell-based BMP9 gene therapy approach for bone tissue engineering, we analyzed the *in vivo* osteoconductive activities and bone regeneration potential of three commonly-used scaffold materials, type I collagen sponge, HA-TCP and DBM, using BMP9-expressing C2C12 progenitor cells. We found that, while direct subcutaneous injection of the BMP9-transduced C2C12 cells formed ectopic bony masses, subcutaneous implantation of the BMP9-expressing C2C12 cells with type I collagen sponge or HA-TCP scaffold yielded the most robust and mature cancellous bone formation. The DBM carrier groups formed minimal bony masses. These results suggest that collagen sponge and HA-TCP scaffold carriers may provide a more cell-friendly environment to support the survival, propagation, and ultimately differentiation of

BMP9-expressing osteoblastic progenitor cells. This line of investigation should provide important experimental evidence for further pre-clinical studies in BMP9-mediated cell based approach to bone tissue engineering.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

BMP9 effectively induces osteogenic differentiation of mesenchymal stem cells in vitro. **(A)** BMP9-induced early osteogenic marker alkaline phosphatase (ALP) activity. Subconfluent C2C12 cells were infected with AdGFP or AdBMP9 (MOI=10). ALP activity was qualitatively assessed by histochemical staining at day 5 (*a*) or quantitatively determined at days 3, 5, 7, and 10 (*b*). Each assay was done in triplicate. Representative staining is shown in (*a*). **(B)** BMP9-induced late osteogenic marker osteocalcin (OCN) expression. Subconfluent C2C12 cells were infected with AdGFP or AdBMP9 for 12 days. Cells were fixed and subjected to immunohistochemical staining using an OCN antibody (Santa Cruz Biotechnology). Isotype IgG and minus primary antibody were used as negative controls (data not shown). Representative images are shown. **(C)** BMP9-induced matrix mineralization. Subconfluent C2C12 cells were infected with AdGFP or AdBMP9 and cultured in mineralization medium. Alizarin red staining was carried out on day 10 and

documented grossly (*a*) or under a microscope (*b*). Assays were done in duplicate and representative images are shown.

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FIGURE 2.

Determination of optimal timeline for BMP9-induced ectopic bone formation *in vivo*. (A) Verification of efficient gene transfer mediated by AdBMP9 and AdGFP in Subconfluent C2C12 cells. (B) Demonstration of BMP9-induced ALP activity. Subconfluent C2C12 cells were transduced with AdBMP9 or AdGFP for 4 days. The cells were subjected to histochemical staining of ALP activity. Representative results are shown. (C) Radiographs of ectopic bone formation. BMP9-transduced C2C12 cells with type I collagen carrier were implanted subcutaneously in the flanks of athymic nude mice. The animals were radiographically imaged at 2, 3, and 4 weeks after implantation. Implantation sites are indicated by arrows. Masses formed in the GFP group are circled with dotted lines. Representative results are shown. (D) Histologic evaluation of the retrieved samples from BMP9 or GFP-treated samples. Animals were sacrificed at the endpoint (4wk), masses were retrieved from the implant sites, fixed, decalcified, paraffin-embedded, and subjected to H & E staining. Representative results are shown. ‘b’, osteoid matrix; ‘c’, injected and undifferentiated cells; magnification, 200x

FIGURE 3.

Ectopic bone formation by BMP9-transduced C2C12 cells in three different scaffold carriers. AdBMP9 or AdGFP-infected C2C12 cells were prepared in a similar fashion as described in Fig. 2. The cells were seeded with either cells only (**A**) or onto three different scaffold carriers, type I collagen sponge (**B**), HA-TCP (**C**) and DBM (**D**), which were implanted subcutaneously in the flanks of athymic nude mice. Animals were sacrificed at 4 weeks after implantation and subjected to radiographic imaging. Implantation sites are indicated with arrows. Masses formed in the GFP group are circled with dotted lines. Representative images are shown.

FIGURE 4.

Histologic analysis of BMP9-induced ectopic bone formation with different scaffold carriers. Masses retrieved from implant sites were fixed, decalcified and subjected to paraffin-embedded sectioning. Sectioned samples from samples retrieved from cells only (A), type I collagen (B), HA-TCP (C), and DBM (D) groups were H & E stained. Representative results are shown. “b”, osteoid matrix; “c”, injected/undifferentiated cells; magnification, 200x

FIGURE 5.

Quantitative analysis of BMP9-induced cancellous bone formation under different carrier conditions. (A) High-power histologic images of BMP9-induced bone formation under four conditions, cells only (a), type I collagen (b), HA-TCP (c) and DBM (d). Osteocytes are indicated with arrows. Representative results are shown. “b”, osteoid matrix; “c”, injected/undifferentiated cells. (B) Higher cancellous bone formation in the collagen and HA-TCP carriers. Percentage of cancellous bone area over total area for each treatment group was calculated by measuring the average cancellous bone area/high-power-field (HPF) for at least 20 random HPFs for each sample analyzed with the NIH ImageJ software.