Adenovirus BMP2-induced osteogenesis in combination with collagen carriers

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Abstract

Adenovirus BMP2 gene therapy has potential of a robust endogenous BMP2 production, while circumventing many of the problems currently associated with recombinant BMP2. The study objective was to determine and compare the ability of adenovirus BMP2 ex vivo gene therapy in combination with three types of collagen carriers to release BMP2 in vitro and to induce heterotopic bone formation in vivo. Human CD45-negative bone marrow cells were ex vivo transduced with a chimeric Ad5F35BMP2. The bioactivity of BMP2 produced by the transduced cells without a carrier, or in combination with three types of collagen carriers (injectable gel, microporous sponge, collagen–mineral composite) was measured and compared to rhBMP2. The heterotopic osteoinductivity assay was performed in immunocompromised NOD/SCID mice. A statistically significant decrease in the amount of rhBMP2 and adenoviral BMP2 released in vitro from the collagen–mineral composite carrier was noted (21% and 12%, respectively), whereas the amounts of rhBMP2 and adenoviral BMP2 released from the gel or sponge carriers were comparable. In vivo, 14 days post-implantation, no bone was formed consistently in groups with the empty Ad5F35HM4 control vector. New bone formation was evident radiographically and histologically in all groups with the Ad5F35BMP2-transduced cells irrespective of the presence or absence of a carrier. The presence of a carrier resulted in osteogenesis limited to the implantation site, and was most pronounced for solid (sponge, composite) carriers. The physical characteristics of the carrier determined the new bone spatial distribution at the site. Solid carriers reduced the clearance of Ad5F35-transduced cells by the host immune cells. Adenoviral ex vivo BMP2 gene therapy in combination with collagen carriers with distinct physical characteristics offers the prospects of adjusting this approach to optimally match the specific therapeutic requirements.

Keywords: Osteogenesis; Gene therapy; Bone morphogenetic protein; Adenovirus; Collagen carriers

1. Introduction

The application of recombinant human bone morphogenetic proteins (rhBMPs) has recently emerged as a novel treatment for the induction and enhancement of bone healing [1,2]. The efficient osteoinductive properties of rhBMP demonstrated in experimental models [3,4] and pre-clinical studies [5,6] supported the Food and Drug Administration (FDA) clearance of rhBMP2 and -7 (OP1) for clinical application. Although the initial clinical experience with exogenous rhBMP has been promising, routine rhBMP application has been hindered by the extremely high concentration of the active protein required to produce a clinical effect [6,7]. The compromised efficiency of the exogenous rhBMP likely results from the inability of currently available delivery systems to ensure BMP commensurate with the receptive potential of the target cells.

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Gene therapy approach to stimulate endogenous BMP production has recently emerged as an alternative to rhBMP [8–10]. Direct delivery of vectors carrying BMP gene or ex vivo transduced cells has resulted in the robust endogenous production of the protein [11,12]. The potential for BMP gene therapy to rapidly achieve high concentrations of endogenous BMP, while circumventing problems associated with current rhBMP systems, is of great advantage.

The ex vivo transduction of cells with adenovirus encoding BMP has become the most popular gene therapy for osteoinduction [13,14]. The replication-deficient recombinant adenovirus carrying the BMP gene results in a transient expression of active protein, and more efficient osteogenesis compared to rhBMP systems [8,15,16]. Furthermore, a recently described chimeric Ad5 fiber Ad35 (Ad5F35) adenovirus [17] demonstrated even greater endogenous BMP2 expression [12,18,19]. The high transduction efficiency of Ad5F35BMP2 enhanced production of BMP2 at a lower, safer viral multiplicity of infection [19]. Additionally, Ad5F35BMP2 demonstrated a unique ability to efficiently transduce cells of various types and stage of differentiation, irrespective of their osteogenic potential [18,20].

Although efficient osteoinduction with adenovirus BMP has been achieved, the precise role and function of a carrier in this process remains unclear. Both in vivo and ex vivo methods of adenoviral BMP gene therapy have successfully induced bone in the absence or presence of a carrier [21–23]. Carriers are more germane for orthotopic BMP gene therapy compared to the heterotopic applications, although the evidence for either is currently lacking. Unlike exogenous BMP, in which carriers are crucial to ensure a sustained release the preadsorbed protein, continuous expression of endogenous BMP at the site may eliminate the need for a carrier. Furthermore, the carrier’s properties of binding and sequestering BMP, critical for exogenous rhBMP, have not been proven essential for endogenous systems. Although the theoretical functions of immobilizing the delivered and local host cells by the carrier can be recognized, its ability to affect osteogenesis by endogenous BMP is yet to be studied.

The objective of the study was to determine and compare the potential of adenoviral BMP2 ex vivo gene therapy in combination with three distinct collagen carriers to express and release BMP2 in vitro, and to induce heterotopic bone in vivo.

2. Materials and methods

2.1. Carriers

Three collagen carriers with dissimilar physical characteristics were used in the experiment, and they included injectable gel, microporous sponge, and a collagen–mineral composite. All three carriers are commercially available clinical products, and their handling and preparations followed the specific recommendations of the manufacturer.

An injectable carrier consisted of a high-viscosity gel (Zyderm® II; INAMED Aesthetics, Santa Barbara, CA) containing collagen (95% type I and 5% type III) extracted form bovine skin. The collagen was provided in a purified and sterile form at the concentration of 65 mg/ml suspended in phosphate-buffered saline (PBS) solution containing 0.3% lidocaine. This preparation was approved by the FDA in 1983, and is used clinically for soft tissue augmentation procedures [24]. A standard volume (50 µl) of gel was utilized in the experiment.

A microporous sponge carrier consisted of an atelopeptide collagen type I derived from bovine Achilles tendons, approved by the FDA in 2003 for spine fusion and in 2004 for complex lower extremity fractures as the carrier for an rhBMP2 (INFUSE®; Medtronic-Sofamor Danek, Memphis TN) [25]. In the dry state, the sponge exhibits porosity of 82–86%; pore size of 180–280 μm, and the pore wall thickness of 20–40 μm. The sponge was cut into cubes with the dimension of 5 × 5 × 5 mm3 prior to cell seeding.

The third carrier comprised a bone graft substitute consisting of an equal proportion of highly purified type I bovine collagen and mineral granules (Collagraft® Bone Graft Matrix Strip; Zimmer, Warsaw, IN). The hydroxyapatite (HA) and tricalcium phosphate (TCP) mineral granules were combined in a 3-to-2-volume ratio. In the dry state, Collagraft® exhibits 70% porosity and a pore size of 300–700 μm. Collagraft® structural integrity withstands compression loads even after hydration. Collagraft® was cleared by the FDA in 1993 as bone graft substitute for the treatment of long bone fractures and osseous defects [26,27]. The composite was cut into cubes with the dimension of 5 × 5 × 5 mm3 for this experiment.

2.2. Cells

The W20-17 mouse stromal cell line (a gift from Genetics Institute; Cambridge, MA) were propagated in DMEM (Life Technologies, Inc.; Gaithersburg, MD), 10% fetal bovine serum (FBS; HyClone; Logan, UT), 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B, and grown in a humidified chamber at 37 °C and 5% CO2 [28]. The cells were passaged at a subconfluent density. CD45-negative bone marrow cells (BMCs) were obtained from discarded marrow of healthy human donors in compliance with state and federal regulations following the Institutional Review Board approval. Mononuclear cells were isolated by centrifugation on Ficoll-Paque™ PLUS (Amersham Pharmacia Biotech; Piscataway, NJ) [12]. Cells were plated at a density of 5 × 10⁵ cells/cm² in DMEM with 10% FBS and antibiotics–antimycotics [29]. After 1 week of culture, dead cells and debris were removed, and the attached cells passaged prior to become confluent.

2.3. Vector construction and transduction

A replication-deficient human chimeric E1–E3-deleted adenovirus type 5 with a fiber gene substituted from adenovirus type 35 (Ad5F35) was constructed to contain cDNA for human BMP2 in the E1 region (Ad5F35BMP2) [30]. The virus was propagated and purified [11,19]. The control vector included the same adenovirus but lacking a transgene cassette (Ad5F35HM4). Viral particle-to-plaque-forming unit ratios were 1:77 for Ad5F35BMP2 and 1:111 for Ad5F35HM4. The absence of contamination with a wild-type replication-competent adenovirus was confirmed [30].

Human BMCs were transduced with Ad5F35BMP2 or Ad5F35HM4 in DMEM supplemented with 2% FBS at a consistent concentration of 2500 viral particles per cell [19]. Adenovirus was allowed to adsorb overnight (ca. 10 h) in a incubator (37 °C, humidified atmosphere; 5% CO2). The transduction efficiency was validated with Western blot [31]. An anti-BMP2 monoclonal antibody (h3b2/17.8.1; Genetics Institute; Cambridge, MA) was used for BMP2 detection in Western blot [12,19].

2.4. In vitro quantification and bioactivity of BMP2

W20-17 cells were assessed at 4 days of culture for the inherent alkaline phosphatase activity (background) and under stimulation with rhBMP2
directly or with supernatant rhBMP2 released from the carriers [11]. The supernatant BMP2 produced by the adenovirally transduced cells alone or in combination with a carrier was used to stimulate W20-17 cells, similarly to rhBMP2. Cellular alkaline phosphatase was extracted by three freeze-thaw cycles in 100 μl of 25 mM Tris–HCl, pH 8.0 and 0.5% Triton X-100 and the activity measured by adding CSPD® ready-to-use with Sapphire II enhancer (Tropix; Bedford, MA) to the samples. The light output from each sample was integrated for 10s after a 2s delay by the luminometer (TD-20/20; Turner Designs; Sunnyvale, CA). The alkaline phosphatase activity was recorded in relative luminescence units (RLU) and normalized to protein content with the bovine serum albumin to derive a standard curve.

2.5. In vivo heterotopic bone formation assay

2.5.1. Implantation

Animal experiments were performed in compliance with the regulations of the Department of Comparative Medicine (Baylor College of Medicine; Houston, TX), following approval by the Animal Use and Care Committee. The experimental groups included the implantation of human BMCs transduced with Ad5F35BMP2 without a carrier (Group 0); BMCs suspended in the collagen gel (Group I); BMCs seeded on absorbable collagen sponge (Group II), or on the collagen-mineral composite (Group III). All experimental groups had matched controls that consisted of the same number of BMCs transduced with the empty Ad5F35HM4. Non-obese Diabetic Severe Combined Immunodeficiency (NOD/SCID) mice were implanted bilaterally in the hind extremities under inhaled anesthesia. For Groups 0 and I, the transduced cell suspensions were injected transcutaneously into the lateral middle region of the quadriceps muscle using an 18-gauge needle and 1-ml syringe. For Groups II and III, a small 10-mm-long stab incision was performed on the lateral aspect of the thigh to implant the transduced cells seeded on the structural carriers into the lateral middle region of the quadriceps. The animals were allowed food and activity ad libitum. Fourteen days post-implantation, animals were euthanized, and the limb specimens placed in a neutral fixative 3% formaldehyde solution.

2.5.2. Radiographic evaluation

The harvested limbs were radiographically analyzed using Faxitron (Faxitron MX2D X-ray Corp., Wheeling IL) in biplanar (anteroposterior

![Fig. 1. The alkaline phosphatase-determined in vitro bioactivity of rhBMP2 (A) and adenoviral BMP2 (B) released from various carriers at 4 days of W20-17 cells stimulation.](image-url)
and lateral) projections. The exposure time (83 s) and acceleration voltage (31 kV) was consistent for all specimens. The distance of the specimen from the radiation source resulted in four-fold magnification.

Qualitative and quantitative radiographic analyses were performed using computed microtomography (Skyscan 1072; MicroPhotonics, Inc., Allentown, PA). Two-dimensional Micro-CT with a spatial resolution of 5 μm was carried out to assess the mineral component thickness and mineral component separation (porosity) for each specimen. Three-dimensional reconstructions were performed to visualize the pattern, spatial distribution, and extent of osteogenesis.

2.5.3. Histological evaluation

The fixed specimens were decalcified in EDTA, embedded in paraffin, and sectioned at a thickness of 5 μm. Select sections were stained with hematoxylin–eosin; others were used for immunohistochemical analysis following de-paraffinizing using EZ-DeWax™ (InnoGenex, San Ramon, CA). The sections were washed with PBS with 0.05% Tween-20, and incubated for 5 min in 0.3% Triton-X-100. Multiple incubations prior to antibody staining were done to block endogenous hydrogen peroxidase, avidin, and biotin. The incubation with a 1:500 dilution of a mouse monoclonal antibody (Ms X Hu Mitochondrial Mab 1237; Chemicon; Temecula, CA) against a human mitochondrial protein not cross-reacting with mouse [32,33] was done using a mouse-on-mouse kit (Vectorlabs; Burlingame, CA) and Vector™ Nova Red™ reagent (Vectorlabs) for color. The dehydrated sections were cleared using NeoClear (EM Science; Gibbstown, NJ). Select sections were counterstained with hematoxylin. All specimens were observed using a Zeiss Axiophot light microscope equipped with a Spot RT Camera (Diagnostic Instruments; Sterling Heights, MI).

2.6. Statistical analysis

All values obtained from the in vitro and in vivo experiments are expressed as means ± standard deviations (n = 6). The parametric data in the study was reduced within each experimental group to its matched control and subsequently cross-comparisons between the experimental groups were done. Analysis of variance (ANOVA) with a post-hoc Bonferroni test was carried out to detect significant (p < 0.05) differences between the specimens (Statview 5; SAS Institute; Cary, NC).

3. Results

3.1. In vitro bioactivity of rhBMP2 and Ad5F35BMP2 released from the carriers

The alkaline phosphatase activity in W20-17 cells under rhBMP2 stimulation at the 4 day post-stimulation is illustrated in Fig. 1A. Statistically significant differences in the alkaline phosphatase activity were observed between the stimulated and non-stimulated cells in Group 0 (p = 0.0005), Group I (p = 0.002) and Group II (p = 0.001). Conversely, Group III (collagen–mineral carrier) demonstrated no significant difference (p = 0.11). The bioactivities of rhBMP2 released from a carrier at 4 days of stimulation were 61.8% (p = 0.002) for Group I, 78% (p = 0.03) for Group II, and 21.1% (p = 0.0007) for Group III, compared to total rhBMP preadsorbed on the carrier (100%).

The bioactivity of BMP2 produced by Ad5F35BMP2-transduced cells and released from the carriers at the 4 day is depicted in Fig. 1B. Statistically significant differences in the bioactivity of supernatant BMP2 between the Ad5F35BMP2- and Ad5F35HM4-transduced cells were noted in Group 0 (p = 0.006), Group I (p = 0.001) and Group II (p = 0.007). Conversely, Group III demonstrated no significant (p = 0.08) difference. The average bioactivity of supernatant BMP2 of Ad5F35BMP2-transduced cells seeded on the carriers were 79.8% (p = 0.1) for Group I, 110.9% (p = 0.2) for Group II, and 11.9% (p = 0.007) for Group III, compared to the transduced cells without a carrier (100%).

3.2. In vivo heterotopic bone induction

3.2.1. Radiographic evaluation

All animals completed the 14-day experiment without systemic or local complications, and they maintained an unrestricted level of activity throughout the entire study period. Biplanar radiography demonstrated heterotopic
bone formation in all animals implanted with the BMCs transduced with Ad5F35BMP2, irrespective of the presence or absence of a carrier (Fig. 2). The new bone demonstrated a typical heterotopic ossicle in Group 0 (no carrier) (Fig. 2B) and Group I (gel) (Fig. 2D). The radiographic opacity of new bone in these groups was uniform on both anteroposterior and lateral projections, whereas in Group II (sponge) new bone demonstrated radiographic lucency in the center (Fig. 2F). The presence of a mineralized component in Group III (collagen-mineral) prevented the radiographic delineation between mineralized new bone and the mineral granules. New bone could be only detected in voids between mineral granules and at the carrier-adjacent muscle interface. No new bone formation was observed in all control animals implanted with the BMCs transduced with Ad5F35HM4 in the absence or presence of a carrier (Fig. 2A,C,E,G).

Microtomographic analysis permitted quantitative, qualitative, and spatial distribution assessment of the heterotopically formed new bone. Within a standard volume of interest (2.9 mm³) randomly measured within the sample, the average new bone volume for Group 0, I, and II was 9.9%, 10.1%, and 7.8%, respectively. These differences were not statistically significant. The presence of mineral granules in Group III obscured the quantitation of new bone volume. The qualitative assessment of bone formation included the distribution of the mineral component thickness and the mineral component separation (porosity) (Fig. 3). The distribution of mineral component thickness for Groups 0, I and II were comparable, with the most prevalent values in the range of 21–75 μm (Fig. 3A). The presence of mineral granules in Group III produced a significant shift of the mineral component thickness distribution extending up to 162 μm. In this group, new bone was formed in the voids between mineral granules (Fig. 4B) and resulted in a shift to the right in thickness distribution compared to the matched control (Fig. 4C). The mineral component separation (porosity) demonstrated a wide variation between experimental groups (Fig. 3B). A narrow range (i.e. homogenous bone porosity) was observed only in specimens from Group 0, whereas Group I demonstrated bimodal distribution with half of the pores size in the range 350–700 μm. Significant gaps within new bone were present in Group II, in which voids

![Distribution of Mineral Component Thickness](image)

**Fig. 3.** Qualitative computed microtomography analysis of the new bone mineral content across three types of carriers at 14 days post-implantation: (A) distribution of mineral component thickness and (B) distribution of mineral component separation (porosity).
of a size exceeding 691 μm constituted about 25% of the entire carrier volume. New bone in Group III demonstrated pores of all sizes prevailing in the range of 300–400 μm. The specific comparison of specimens in this group with the matched control (empty vector) demonstrated a reduction of pores sizes (shift to the left), which can be attributed to new bone formation in-between mineral granules (Fig. 4D).

Three-dimensional microtomographic reconstructions permitted visualization of the spatial distribution of new bone at the site and its relationship to the carrier (Fig. 5). New bone formed without a carrier (Group 0) was uniformly distributed at the injection site, whereas the presence of the collagen gel carrier (Group I) resulted in bone formation, which was more pronounced at the periphery of the carrier. This observation was most evident for the collagen sponge carrier (Group II), in which the new bone was formed almost exclusively at the periphery of the sponge. Finally, the new bone formation with the collagen-mineral composite carrier (Group III) could be visualized both at the periphery and throughout the carrier.

3.2.2. Histological evaluation

The histological evaluation confirmed the radiographic results. No new bone formation occurred in groups with the Ad5F35HM4-transduced cells, irrespective of the absence or presence of a carrier. Ad5F35BMP2-transduced BMCs without a carrier (Group 0) elicited significant bone formation within 2 weeks (Fig. 6A and B). The new bone was formed in a process of endochondral ossification, demonstrated uniform density, well-developed vessels, and a tentative marrow features. The new bone formation permeated beyond the injection site by splitting the distant muscle fibers. The Ad5F35-BMP2-transduced cells suspended in the collagen gel carrier (Group I) resulted in a more localized osteogenesis; the new bone formation within the muscle fibers was observed only in select locations and limited to the immediate interface between the carrier and the muscle (Fig. 6C and D). This group demonstrated denser bone at the carrier’s periphery. The application of the Ad5F35BMP2-transduced cells seeded on the collagen sponge carrier (Group II) resulted in osteogenesis that was confined to the periphery of the sponge (Fig. 6E). The new bone was formed within
the pores along the sponge surface (Fig. 6F), and furthermore invaded the cracks that developed within the structure of the carrier (Fig. 6E). The new bone did not form in the sponge center, which consisted of compacted collagen. The osteogenesis was restricted to the sponge boundaries. The application of Ad5F35BMP2-transduced cells seeded on the collagen–mineral composite carrier (Group III) resulted in bone formation upon and between the mineral granules. This process was contiguous with the mineral portion of the carrier with appositional bone formation within the boundaries of the carrier (Fig. 6G and H).

3.2.3. Immunohistochemistry

The donor (human) or host (murine) origin of the cells present within the carrier and/or heterotopic bone was determined by identification of human mitochondrial antigens. In the absence of a carrier, sections reveal positive human markers up to 3 days post-injection (Fig. 7A), whereas subsequent 7-, 10-, 14-day sections did not (Fig. 7B). Similar was observed for the transduced cells implanted with the collagen gel carrier (Fig. 7C and D). However, human antigens were apparent within the collagen sponge carrier for 14 days post-implantation (Fig. 7E and F). Also, the presence of cells exhibiting human markers was evident within collagen–mineral composite up to 14 days post-implantation (Fig. 7F and H).

4. Discussion

Determining the potential benefits, functions, and optimal characteristics of a carrier for ex vivo BMP2 gene therapy is essential in the development of safe and efficacious systems for osteoinduction. The carriers for exogenous rhBMP have been studied extensively [34–36]; however, there is a paucity of data on the use of carriers for endogenous BMP systems. Among the variety of carriers [25,36,37] used experimentally, an absorbable bovine collagen type I sponge is the only rhBMP carrier currently approved for clinical use. Collagen’s ability to bind and sequester rhBMP [39] potentiates the osteoinductive activity of the protein [38,39], and induces bone formation at lower BMP concentrations than its free-diffusible form [39,40]. The merits of using collagen carriers for adenoviral BMP2 can also be supported. In a recent study, Xu et al. [41] identified collagen gel as a suitable carrier for the Ad5BMP2-transduced human marrow cells to efficiently induce heterotopic osteogenesis. Also the structural integrity of a carrier exogenous rhBMP2 has potential to affect in vivo osteogenesis. Minamide et al. [42] demonstrated that the augmenting a collagen rhBMP2 carrier with TCP resulted in more efficient bone formation in a rabbit spine fusion model compared with a carrier without the mineral. Also, Akamaru et al. [43] consistently achieved a solid posterolateral spine fusion in monkeys by combining rhBMP2 with a collagen carrier containing biphasic mineral granules (15% HA and 85% TCP). Both these studies concluded that the mineral component augmented the structural integrity of the carrier, maintained its porosity, and thereby, enhanced rhBMP2-induced osteogenesis.

In the present study, three distinct forms of collagen carriers were used owing to their specific structural advantages: collagen gel–cell suspension favors atraumatic percutaneous application; a solid absorbable collagen...
sponge provides an excellent matrix for cells to attach; whereas a collagen–mineral composite possesses a structural integrity which allows load transfer and favors adjacent bone ingrowth. No significant differences were observed in the extent of bone formation induced with Ad5F35BMP2-transduced BMCs in the absence or presence of collagen carriers. The fact that a similar atelopeptide clinical-purity bovine collagen type I was the entire or main carrier component may account for this lack of difference. Interestingly, the spatial distribution of osteogenesis at the site was dissimilar and correlated with the physical properties of the carrier used. Implantation of the cells without a carrier resulted in homogenous bone formation, and, as previously reported [29], exhibited a pattern of an invasive osteogenesis with splitting the muscle fibers and extending beyond the injection site. Collagen gel demonstrated new bone formation restricted to the carrier boundaries with significantly less osteogenesis occurring.

Fig. 6. Histological sections depicting new bone formation at 14 days post-implantation of the Ad5F35BMP2-transduced cells alone (A,B), the cells implanted in combination with the collagen gel carrier (C,D), the collagen sponge carrier (E,F), or the collagen–mineral composite carrier (G,H). New bone was formed in the process endochondral ossification (B). In no carrier or the gel carrier, the new bone formation permeated the soft tissue beyond the injection site by splitting the muscle fibers (D arrows). The bone was formed at the periphery of the sponge carrier invading the port at its surface or cracks (E,F arrows). More uniform bone formation was observed throughout the collagen–mineral composite carrier [H&E; inset magnification × 40].
remotely between muscle fibers. The absorbable collagen sponge, compressed in situ by the surrounding muscles, thereby displacing the seeded cells and BMP2, formed bone at the carrier’s periphery. In selected regions, cracks and voids permitted bone formation within the inner portions of the compacted collagen. In contrast, the collagen–mineral composite retained its integrity in vivo, and new bone was formed within and upon the carrier. The formation of new bone within the carrier decreased its inherent porosity while adding to its mineral content. In both solid carriers (sponge, collagen–mineral), bone formation was confined to the carrier boundaries, and did not involve the adjacent muscle fibers.

The in vitro results of present study suggest a significant role of carrier’s mineral component in determining BMP2 release. No significant differences in the in vitro release of both rhBMP2 and adenoviral BMP2 from the gel and sponge collagen carriers were detected, whereas the
presence of a mineral component (HA-TCP) resulted a considerable reduction of both rhBMP2 and adenoviral BMP2 release from the carrier (21.1% and 11.9%, respectively). However, these apparent BMP2 binding properties of HA-TCP did not affect in vivo osteogenesis suggesting that the free-release of BMP2 may not be essential for its osteoinductivity.

The newly formed heterotopic bone was entirely of host origin, although a few human donor cells could be detected within the sponge and collagen–mineral carriers at 14 days post-implantation. The Ad5F35-transduced cells injected alone or within collagen gel were cleared by the host within 7 days. The solid carriers (sponge, collagen–mineral) demonstrated the extended presence of the donor cells likely acting as an immunological shield protecting the human, xenogeneic cells in the murine host. Similar masking effects of solid carriers for adenovirus has recently been reported by Sonobe et al. [44], who demonstrated decreased Ad5BMP2 immunogenicity compared to a no carrier setting.

The present study attempts to determine the functions and benefits of carriers for ex vivo adenovirus-induced heterotopic osteogenesis. Previous studies focused solely on the merits of using carriers in orthotopic locations. The heterotopic osteoinductivity assay of the study permitted accurate determination of the extent and spatial distribution of new bone at the site of implantation. Unlike orthotopic setting, it excluded the adjacent bone ingrowth and exposure to osteogenic bone cells and/or mediators, thereby emphasizing the interactions between the carrier, adenoviral BMP2, and cells delivered and/or attracted in the host upon BMP2 activity.

5. Conclusions

The present study demonstrates the in vitro and in vivo abilities of adenovirally transduced cells to express BMP2 in the absence and presence of three collagen carriers with distinct physical characteristics. Although, the extent of BMP2 expression among the studied carriers was comparable, the nature of BMP2-induced osteogenesis differed and strongly correlated with the carrier’s structural integrity. The presence of mineral (HA-TCP) component within the carrier efficiently bound both rh- and adenoviral-BMP2, thereby preventing free diffusion of the protein from the carrier. The comparable extent of osteogenesis in the presence of the mineral-containing carrier suggests that BMP2 free release is not essential for its in vivo osteoinductivity. The structural integrity of a collagen carrier appears crucial in determining the spatial distribution of the new bone formed at the site. Moreover, solid collagen carriers (sponge, collagen–mineral composite) are able to confine the osteogenesis to the site, and further, are capable of masking the host immune response to the donor cells. In consequence, the clearance of the delivered Ad5F35BMP2-transduced cells is delayed, and the BMP2 expression at the site is extended. Applying collagen carriers with distinct physical characteristics for adenoviral ex vivo BMP2 gene therapy offers the prospect of adjusting this approach to optimally match specific therapeutic requirements.

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References


