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# Efficient and stable gene transfer of growth factors into chondrogenic cells and primary articular chondrocytes using a VSV.G pseudotyped retroviral vector

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## 1. Introduction

Until now an optimal solution for the treatment of chondral and osteochondral lesions has not been found [1]. Transplantation of cultured chondrocytes into chondral lesions has shown promise as a new method [2], but leads histologically only to fibrocartilage or hyaline-like cartilage. In this regard

tissue engineering has attracted great attention because of its potential to promote healing of articular cartilage [3]. Type I collagen sponges, other scaffolds, fibrin-glue and gels have previously shown to be suitable carriers for chondrocytes [4–6]. Growth factors have been found to enhance the healing process in cartilage lesions [7]. Some of these cytokines stimulate cell proliferation, differentiation and matrix synthesis [8–12]. High dosages and repeated injections of these proteins are often required due to their relatively short biological half lives [13]. BMP2, a growth factor and member of the TGF $\beta$  superfamily, seems to be involved in the growth and

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differentiation of mesenchymal stem cells to chondroblasts and osteoblasts [14–16]. Sellers et al. [17] used recombinant human BMP2 for the treatment of full-thickness defects of articular cartilage in rabbits and found an accelerated formation of new subchondral bone with an improved histological appearance of the overlying articular surface. Others have shown that bone morphogenetic proteins stimulated the ingrowth of subchondral bone [15]. Van Beuningen et al. [18] observed that chondrocytes induced by BMP2 were found predominantly in the region where the growth plates meet the joint space. Stimulation of perichondrium-derived mesenchymal cells by transfer of BMP2 growth factor cDNA in a partial-thickness defect model allows for satisfactory cartilage restoration by a repair tissue comparable with hyaline articular cartilage [19].

The gene transfer of defined genes encoding therapeutic proteins represents a promising way to efficiently deliver suitable growth factors into injured tissue [20–23]. But the success of gene therapy is largely dependent on the development of vectors that can selectively and efficiently deliver genes to target cells with minimal toxicity [24]. Gene delivery systems generally fall into two categories: viral and non-viral vectors. Currently, viral vectors represent a more efficient method. The delivery of foreign genes to joints using adenoviral vectors [25,26], AAV vectors [27], lentiviral vectors [28] and retroviral vectors [4,14,29] has been explored as a strategy for the treatment of various joint disorders. But none of the used viral vectors achieved a transduction efficiency of more than 90% in primary articular chondrocytes without selecting cells.

ATDC5 cells, a chondrogenic cell line from AT805 mouse teratocarcinoma, were used for initial retroviral transduction experiments. Assuming a fibroblastic shape, ATDC5 cells rapidly proliferate to form a confluent monolayer until growth ceases as a result of contact inhibition. Supplementation of culture medium with insulin or growth factors, however, induces typical cellular condensation before overt chondrogenesis [30,31].

The aim of this study was to prove, if the VSV.G pseudotyped retroviral vector system is feasible for further *in vitro* and *in vivo* experiments, and is superior to other gene transduction systems in terms of transduction efficiency and long-term gene expression.

## 2. Materials and methods

### 2.1. Cell culture

Primary articular chondrocytes were obtained from knee joints of New Zealand white rabbits as previously described [4]. Isolated chondrocytes were seeded on 35 mm cell culture dishes, grown to a density of 80%, then seeded on 60 mm dishes and split in a ratio of 1:3 every fifth day. For further experiments articular chondrocytes were plated on 96- and 12-well plates or 60 mm dishes at an initial cell density of 1 and  $6 \times 10^4$  or  $1 \times 10^6$  cells/well, respectively. ATDC5 cells were cultured with DMEM/Ham's F12 hybrid medium (Biochrom, Berlin, Germany) containing 5% FBS (GIBCO/Invitrogen, Karlsruhe, Germany), 1% penicillin/streptomycin and 1% glutamine in a 5% CO<sub>2</sub>/95% air atmosphere on 35 and 60 mm dishes. Cells were split every third day at a ratio of 1:4. ATDC5 cells were plated for experiments on 96- and

12-well-plates or 60 mm dishes at an initial cell density of 1 and  $6 \times 10^4$  or  $1 \times 10^6$  cells/well, respectively. Cell viability and numbers were determined by trypan blue staining. Adherent cells were released from dishes by a 3-min exposure to 0.25% trypsin/EDTA 10 mM.

### 2.2. Construction of the retroviral transfer vector

The *lacZ* gene fused to the SV40 virus nuclear leading sequence (*nlslacZ*), the green fluorescent protein gene (*eGFP*) and the human bone morphogenetic protein-2 gene (*hBMP2*) were cloned into the NcoI site of the retroviral vector pBullet [32] after blunt-ending all cloning sites with Klenow enzyme. The resulting vectors were designated Bullet-*nlslacZ*, Bullet-*eGFP* and Bullet-*hBMP2*.

### 2.3. Retroviral vector production and target cell transduction

VSV.G pseudotyped retroviral vectors were produced by transient transfection of 293 T cells as previously described [33]. Viral supernatants were collected and stored at  $-80^\circ\text{C}$  for further use. The day prior to retroviral transduction, cells were seeded on 60 mm dishes and retroviral transduction was performed using 750  $\mu\text{l}$  retroviral supernatant in the presence of 8  $\mu\text{g}/\text{ml}$  polybrene (final concentration). After 2 h of incubation 4 ml fresh medium was added. In the integrated provirus, gene expression is driven by the retroviral MoMLV-LTR promoter (Fig. 1). Transduction efficiency of Bullet-*nlslacZ* transduced primary articular chondrocytes was determined by using the fluorogenic *lacZ*-substrate fluorescein- $\beta$ -D-galactopyranosid (FDG; FluoroReporter *lacZ* Flow Cytometry Kit, Molecular Probes, Leiden, Netherlands) followed by Fluorescence-Activated-Cell-Sorter (FACS; Vantage, Beckton Dickinson, San Jose, CA, USA) analysis. The transduction efficiency of Bullet-*eGFP* in ATDC5 cells and primary articular chondrocytes was directly measured by detecting eGFP positive cells and FACS analysis.

### 2.4. Analysis of long-term expression of *lacZ* and *eGFP*

The stability of the described retroviral transduction was analysed for ATDC5 cells and primary articular chondrocytes. After transduction of ATDC5 cells by Bullet-*eGFP*, cells were cultured for several months and analysed by FACS. GFP positive cells were detected at different time-points (up to one year). Bullet-*nlslacZ* transduced articular chondrocytes were FACS-analysed regarding the *nlslacZ* expression at different time-points described above (up to 15 weeks; afterwards, only greatly reduced cell divisions were observed). *LacZ* staining was performed as described previously [34].

### 2.5. BMP2 quantification

BMP2 production in cell culture supernatants of ATDC5 cells and articular chondrocytes cultured on 12-well plates was measured by BMP2 immunoassay (Quantikine<sup>®</sup>, R&D systems, Wiesbaden, Germany) according to the manufacturer's protocol. Supernatants were frozen at  $-80^\circ\text{C}$  in 1 ml aliquots after centrifugation for clearance from cells and debris until time of analysis.

### 2.6. MTT-assay

Cell growth rates were determined by MTT-assay according to manufacturer's protocol (Vybrant<sup>®</sup>, Molecular Probes, Leiden, Netherlands). Cells

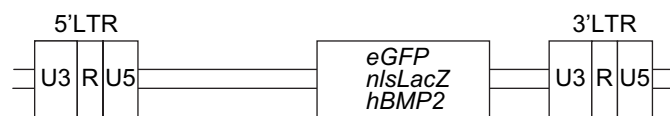


Fig. 1. Structure of the integrated provirus expressing *eGFP*, *nlsLacZ* or *hBMP2*, respectively. Transgene expression is controlled by the retroviral promoter of the MoMLV 5'LTR. LTR: long terminal repeat; U3, U5: unique regions; R: redundant region.

( $1 \times 10^4$ ) were plated on 96-micro-plates. Absorbance was read at 570 nm in triplicates.

### 2.7. Measurement of cartilage-specific proteoglycans

ATDC5 cells and primary articular chondrocytes (wild-type, Bullet-*eGFP* and Bullet-*hBMP2* transduced,  $6 \times 10^4$  cells) were plated and cultured on 12-well plates. Additionally, non-transduced cells were treated with insulin/transferrin/sodium selenite (10  $\mu\text{g}/\text{ml}$ , 10  $\mu\text{g}/\text{ml}$ ,  $3 \times 10^{-8}$  M, see Fig 5a caption) (ITS; Sigma-Aldrich, Taufkirchen, Germany) as a control. The medium was replaced every day (ATDC5) or every third day (articular chondrocytes) and culture was continued up to 28 days. Cartilaginous nodule formation was analysed under a phase-contrast microscope. At the indicated time-points, cells were rinsed twice with ice-cold phosphate-buffered-saline (PBS), fixed with methanol (2 min at  $-20^\circ\text{C}$ ), rinsed once with distilled water, and then stained overnight at room temperature with 0.1% alcian blue (Alcian Blue 8 GX; Sigma) in 0.1 N HCl. Cells were rinsed three times with distilled water, and the amount of cell-associated dye was measured at 620 nm after extraction with 6 M guanidine-HCl (200  $\mu\text{l}/\text{well}$ ). Results were normalized to total-DNA levels of cells. DNA was determined by DNeasy Blood & Tissue Kit according to the manufacturer's protocol at OD 260 nm (Qiagen, Germany).

### 2.8. Alkaline phosphatase measurement

Alkaline phosphatase (AP) activity was measured as a marker of BMP2 effect. Alkaline phosphatase activity in cell culture supernatants of ATDC5 and articular chondrocytes seeded on 12-well plates was determined according to the manufacturer's protocol (Ecoline 25, Alkaline Phosphatase; Merck, Darmstadt, Germany). Briefly, 20  $\mu\text{l}$  of supernatants were incubated with 1000  $\mu\text{l}$  substrate solution composed of 0.1 M diethanolamine HCl buffer, pH 9.8, 1 mM  $\text{MgCl}_2$ , and 10 mg/ml p-nitrophenyl phosphate for 1 min and increase in absorbance was measured at 405 nm every minute for 3 min. Enzyme activity was defined as  $[U/l] = (\Delta A/\text{min}) \times 2754$  ( $\Delta A/\text{min}$  = differences in absorbance per minute).

## 3. Results

### 3.1. Retroviral transduction of ATDC5 cells and rabbit articular chondrocytes

Transduction efficiency of ATDC5 cells and articular chondrocytes was determined using VSV.G pseudotyped retroviral vectors coding for reporter genes *eGFP* and *nlsLacZ*, respectively. Stability of gene expression was observed over time.

ATDC5 cells ( $98.3 \pm 0.6\%$  SD) were transduced by retroviral vector Bullet-*eGFP* (mean fluorescence intensity (MFI)  $1712.1 \pm 60.8$  SD). After 52 weeks  $94.7\%$  (SD  $\pm 0.6\%$ ) of these cells were eGFP positive in FACS analyses (Fig. 2A) (MFI  $1622.2 \pm 33.3$  SD). In articular chondrocytes retroviral transduction efficiency for reporter gene *nlsLacZ* exceeded  $92.3\%$  (SD  $\pm 6.1\%$ ) (MFI  $122.2 \pm 3.6$  SD) and expression remained high after 15 weeks ( $75.7 \pm 14.2\%$  SD) (MFI  $154.3 \pm 23.0$  SD) in FACS analysis with lacZ-substrate FDG (Fig. 2B).

### 3.2. BMP2 production

To analyse the stability of *BMP2* expression after Bullet-*hBMP2* transduction in ATDC5 cells and articular chondrocytes, the *BMP2* concentration in cell culture supernatants was measured by *BMP2* immunoassay at different time-points. *BMP2* secreted into cell culture supernatants (accumulated within 24 h) of Bullet-*hBMP2* transduced ATDC5 cells increased within the first week and remained nearly constant after full confluence of cells. *BMP2* concentration (accumulated within 72 h) in cell culture supernatants of articular chondrocytes increased continuously by a factor of three over the observation period of three weeks. The highest increase was observed during the initial 14 days, followed by a slower increase due to confluence of cells. Concentration of *BMP2* in supernatants of control cells (Bullet-*eGFP* transduced) was below detection limit (Fig. 3A, B). In general *hBMP2* production was 5–10-fold higher in ATDC5 cells as in articular chondrocytes.

### 3.3. Effects of Bullet-*hBMP2* on chondrogenesis in ATDC5 cells

Clonal mouse teratocarcinoma cells, ATDC5, display a number of characteristics as committed to chondroprogenitors [30,31]. When cultured in the presence of insulin, ATDC5 cells form cartilaginous nodules (chondrogenesis), and enter into sequential chondrogenic maturation processes. In the

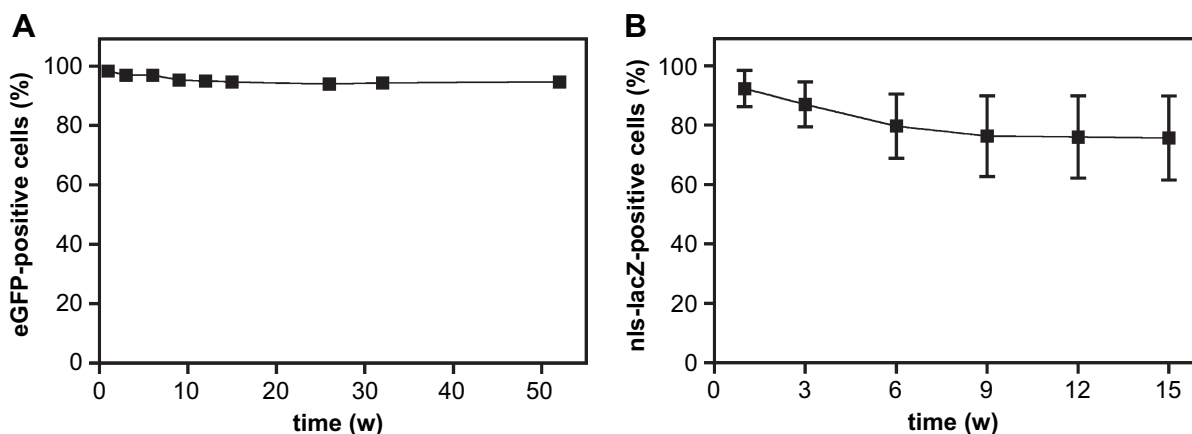


Fig. 2. Initial transduction rate and long-term stability of retroviral vector gene transfer in ATDC5 cells (Bullet-*eGFP*) (A) and primary chondrocytes (Bullet-*nlsLacZ*) (B). After retroviral vector transduction, cells were incubated over time and FACS-analysed at the indicated time-points (w = weeks) (means  $\pm$  SD,  $n = 3$ ).

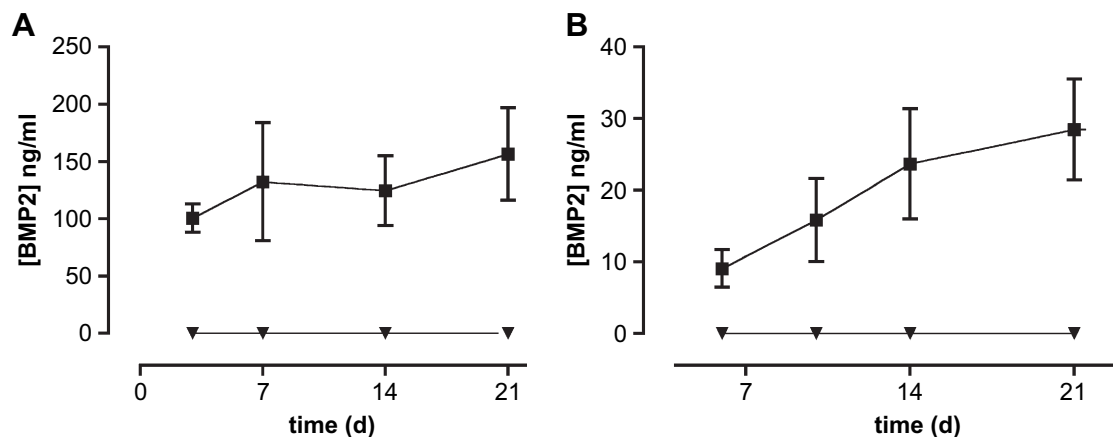


Fig. 3. BMP2 determination by ELISA in cell culture supernatants of ATDC5 cells (A) and primary chondrocytes (B). After retroviral vector transduction (Bullet-hBMP2, ■ and Bullet-eGFP, ▼), cells were incubated for the indicated times and BMP2 concentration in supernatants was determined by ELISA at different time-points. For ATDC5 cells medium was changed every day, for primary chondrocytes every third day. The BMP2 concentration was measured directly before medium change (means  $\pm$  SD,  $n = 3$ ).

present study, therefore, the effects of Bullet-hBMP2 on chondrogenesis in ATDC5 cells were also determined.

### 3.3.1. Cartilaginous nodule formation

Bullet-hBMP2 transduced ATDC5 cells showed typical cartilaginous nodule formation starting at day seven of cell culture. This nodule formation was strongly increased in comparison to insulin/transferrin/sodium selenite treated cells. In control ATDC5 cells (wild-type and Bullet-eGFP transduced cells) no nodule formation was observed (data not shown).

### 3.3.2. MTT-assay

The MTT-assay was used to analyse if Bullet-hBMP2 transduction influences the proliferation rate of ATDC5 cells. This transduction initially increased the proliferation rate until day five in comparison to control cells (Bullet-eGFP transduced ATDC5 cells). After one week there was only a slight increase and after two weeks, when cells were nearly confluent, the proliferation rate was similar to control cells (Fig. 4A).

### 3.3.3. Proteoglycan production

To investigate if Bullet-hBMP2 leads to an increase in proteoglycan production, Bullet-hBMP2 transduced, insulin/transferrin/sodium selenite treated and control ATDC5 cells (wild-type and Bullet-eGFP transduced cells) were seeded on 12-well plates and cultured up to 28 days. At different time-points alcian blue staining was performed. Bullet-hBMP2 transduction in ATDC5 cells increased proteoglycan production up to 7.5-fold at day 10 as compared to Bullet-eGFP transduced control cells (Fig. 5A).

### 3.4. Effects of Bullet-hBMP2 on chondrogenesis in primary chondrocytes

Isolated articular chondrocytes as used in autologous chondrocyte transplantation (ACT) typically show a time-dependent dedifferentiation to a fibroblastic phenotype during the proliferation process. On the one hand this is essential to increase cell number for later transplantation, on the other hand cell proliferation rate has to decrease afterwards to allow

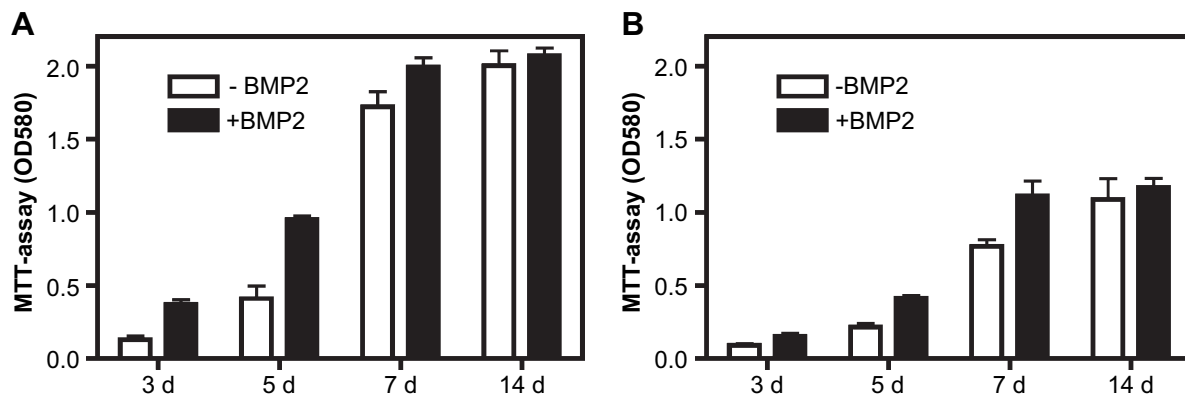


Fig. 4. Analyses of proliferation rates by MTT-assay in ATDC5 (A) and primary chondrocytes (B). After retroviral vector transduction, cells were incubated and MTT-assays were performed at indicated time-points (means  $\pm$  SD,  $n = 3$ ). +BMP2: BMP2 retrovirally transduced cells; -BMP2: eGFP retrovirally transduced cells.

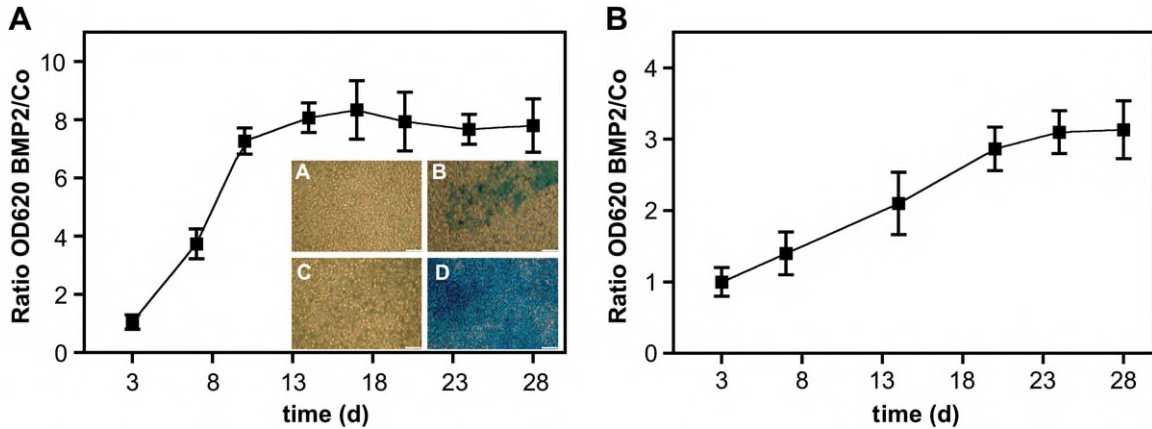


Fig. 5. Alcian blue staining in ATDC5 cells (A) and primary chondrocytes (B). After retroviral vector transduction (Bullet-*hBMP2*, Bullet-*eGFP*), *BMP2* expressing and control ATDC5 cells and articular chondrocytes were incubated, stained with alcian blue dye and quantified at the indicated times (means  $\pm$  SD,  $n = 3$ ). Values represent ratio of *BMP2* vs. *eGFP* transduced cells. For ATDC5 cells medium was changed every day, for articular chondrocytes every third day. Insert: Light microscopy showing alcian blue staining (cell culture 14 days), scale = 100  $\mu$ M. (A) control ATDC5 cells without retroviral vector transduction showing no staining, (B) ATDC5 cells treated with 10  $\mu$ g/ml insulin, 10  $\mu$ g/ml transferrin and  $3 \times 10^{-8}$  M sodium selenite with a low staining, (C) control ATDC5 cells transduced by Bullet-*eGFP* showing no staining and (D) ATDC5 cells transduced by pBullet-*hBMP2* with strong staining.

for redifferentiation of cells to chondrocytes. Therefore, the proliferation rate, the proteoglycan production and the morphology of Bullet-*hBMP2* transduced articular chondrocytes were compared to control cells (Bullet-*eGFP* transduced cells).

#### 3.4.1. MTT-assay

To analyse the potential influence of *hBMP2* expression in articular chondrocytes an MTT-assay was performed. This transduction increased the proliferation rate in articular chondrocytes in the first week. After two weeks the proliferation rate was similar to control cells (Bullet-*eGFP* transduced cells) (Fig. 4B).

#### 3.4.2. Proteoglycan production

To investigate if Bullet-*hBMP2* induces an increase in proteoglycan production Bullet-*hBMP2* transduced and control articular chondrocytes (Bullet-*eGFP* transduced cells) were seeded on 12-well plates and cultured for several days. At different time-points cells were fixed and stained by alcian

blue dye solution. Bullet-*hBMP2* transduction in articular chondrocytes increased proteoglycan production up to 3-fold in contrast to control cells (Fig. 5B).

#### 3.4.3. Morphology

During the first weeks after Bullet-*hBMP2* transduction morphology of articular chondrocytes was similar to control cells: they lost their chondrogenic round phenotype and showed a fibroblastic phenotype. However, at an average of four weeks of culture *BMP2* expressing cells lost again the developed fibroblastic phenotype and assumed a round shape in contrast to control cells (Fig. 6).

#### 3.5. Alkaline phosphatase production

Alkaline phosphatase (AP) is a widely recognized biochemical marker for osteoblast activity and hypertrophic chondrocytes. In addition, it is known that *BMP2* plays an important role in osteoblast function and bone remodelling [35]. Therefore, alkaline phosphatase (AP) activity in cell culture

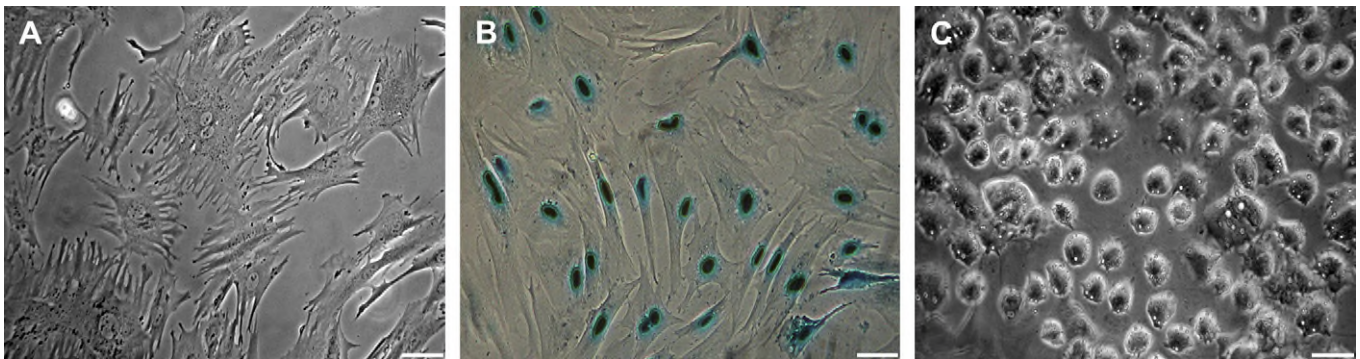


Fig. 6. Cell morphology of wild-type (A), Bullet-*nls lacZ* (B) and Bullet-*hBMP2* (C) transduced articular chondrocytes (light microscopy, scale = 50  $\mu$ M). Control and retroviral vector transduced articular chondrocytes were incubated for the indicated times and analysed in phase-contrast microscopy at different time-points. Photos show a typical morphology of the respective cells after culture for four weeks. Wild-type and Bullet-*lacZ* transduced articular chondrocytes show a typical fibroblastic shape, in contrast, Bullet-*hBMP2* transduced cells have no fibroblastic phenotype and are round.

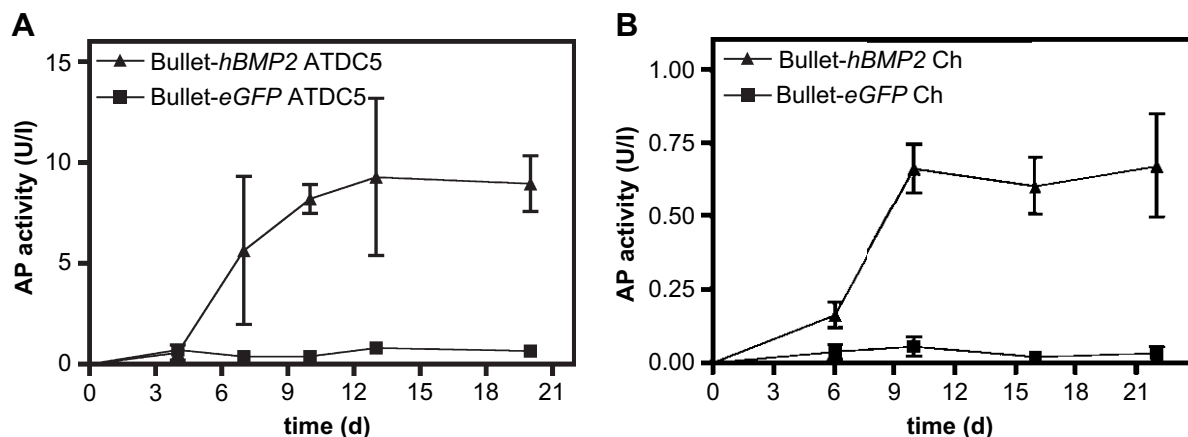


Fig. 7. Alkaline phosphatase detection in cell culture supernatants of ATDC5 cells (A) and articular chondrocytes (B). After retroviral vector transduction (Bullet-*hBMP2*, Bullet-*eGFP*), cells were incubated for the indicated times and alkaline phosphatase (AP) activity in supernatant was determined at different time-points (means  $\pm$  SD,  $n = 3$ ). For ATDC5 cells medium was changed every day, for primary chondrocytes every third day. AP activity detection was done directly before medium change.

supernatants of control (Bullet-*eGFP* transduced) and Bullet-*hBMP2* transduced ATDC5 cells and articular chondrocytes was detected to see if enzyme activity is affected. Bullet-*hBMP2* transduction induced in ATDC5 cell culture supernatants an increase of AP activity up to approximately 11-fold and in articular chondrocytes up to 20-fold (Fig. 7A, B).

#### 4. Discussion

The combination of gene therapy with tissue engineering methods has become a promising area of research [21,36]. The development of an efficient method for introducing a therapeutic gene into target cells is one key issue [24]. Non-viral vectors have some advantages over viral vectors, namely, lower toxicity, reduced immunogenicity and easier preparation. However, viral vectors are potentially more efficient in gene transfer and integrating vector types can lead to longer-lasting gene expression [3]. So far several investigators have demonstrated successful delivery of foreign genes to chondrocytes using non-viral [20], retroviral [4,14,29], adenoviral [25,26], lentiviral [28], and adeno-associated vectors [27]. Ikeda et al. described an adenoviral gene transfer of *lacZ* into cells with 82% positive cells after one week but only 55% positive cells after eight weeks, showing high efficiency of gene transfer, but no stable expression over time [26]. Carlberg et al. described a retroviral gene transfer into chondrocytes and mesenchymal stem cells in the field of cartilage repair with high initial transduction efficiencies, but long-term results of gene expression were not shown [14]. Furthermore, Hirschmann et al. showed a stable retroviral *eGFP* transduction for eight months in articular rabbit chondrocytes *in vitro* but initial transduction rates were low (18–43%) [29]. Therefore, an efficient and stable viral transduction system in the field of cartilage repair was not demonstrated until now.

To learn more about biological effective concentrations and the time period in which certain growth factors are needed, it is indispensable to establish a highly efficient and stable vector system that allows investigation of the maximal effect of

a growth factor on cartilage healing. In our previous studies, non-viral gene transfer was not very efficient with approximately 10% cells expressing the marker gene *lacZ* [37]. The selection of transfected primary cells takes a long time, which is an obstacle when further *in vivo* application is intended. Therefore, we constructed and tested a VSV.G pseudotyped murine MoMLV retroviral vector as an alternative method for gene transfer into chondrocytes. Gene transfer by this VSV.G pseudotyped vector containing the *nslacZ* gene for the expression of *E. coli*  $\beta$ -galactosidase or the *eGFP* gene for the expression of green fluorescence protein (GFP) was very efficient, making a further selection step unnecessary. *In vitro* more than 92% of transduced rabbit articular chondrocytes expressed the marker gene *lacZ*, and more than 98% of transduced ATDC5 cells expressed *eGFP*. Isolated articular chondrocytes are primary cells and therefore not immortal. To study long-term expression in dividing cells, stable cell lines are needed. Because of their chondrogenic progenitor character after induction by insulin or growth factors, ATDC5 cells were used. With these cells it was shown that gene expression after VSV.G retroviral transduction is achieved in a very stable manner.

The first challenge of gene therapy in the field of cartilage tissue engineering is the induction of cell proliferation for a certain time period to get a critical amount of cells. BMP2 seems to be involved in the growth and differentiation of mesenchymal stem cells to chondroblasts and osteoblasts [14–16]. Proliferation and differentiation are opposed objects in cartilage repair. Therefore, it is necessary that proliferation is terminated after defect filling and differentiation processes can start. In addition, proliferation increase [38] and proliferation decrease [39] of cells after BMP2 treatment have been reported in literature. In our experiments, proliferation rate increased in Bullet-*hBMP2* transduced ATDC5 cells (until day five) and articular chondrocytes (until day seven), afterwards proliferation was comparable to controls, mimicking the intended missing *in vivo* proliferation effect of a growth factor after complete filling of a tissue defect. A parameter of cell

differentiation to articular chondrocytes is the proteoglycan production. Proteoglycans are typical compounds of the extracellular matrix in hyaline cartilage. The production of these macromolecules was strongly accelerated in Bullet-*hBMP2* transduced ATDC5 cells within two weeks and in articular chondrocytes up to three weeks in comparison to controls and outlasted proliferation increase in both cell types. Alkaline phosphatase (AP) is a widely recognized biochemical marker for osteoblast activity and hypertrophic chondrocytes. It is well known that BMP2 plays an important role in osteoblast function and bone remodelling [35] and that BMP2 increases alkaline phosphatase levels. Bullet-*hBMP2* transduction led to a strong increase in AP activity in cell culture supernatants of ATDC5 cells and articular chondrocytes. This shows in addition to the increase in proteoglycan production, that the expressed *BMP2* has a biological effect on ATDC5 cells and articular chondrocytes. However, alkaline phosphatase production is a marker of hypertrophic chondrocytes and therefore maybe counterproductive in cartilage repair. On the other hand hypertrophic chondrocytes may be useful for subchondral bone remodelling in osteochondral defects.

Admittedly due to their integrative character, Moloney derived retroviral vectors have been shown to exert severe side effects in three cases of clinical trials for SCID-X1 resulting in monoclonal lymphoproliferative disease [40]. For potential future application in humans safer vectors will be needed like self-inactivating (SIN) [41] retroviral vectors, e.g. SIN-lentiviral vectors to reduce risk of gene activation by insertional mutagenesis. Furthermore, vectors should allow for regulation of gene expression [37,42]. Temporarily, limited gene expression might be useful to prevent overproduction of growth factors potentially contributing to tumour formation.

## 5. Conclusion

The described vector system is useful for further *in vitro* experiments to identify promising factors in the field of cartilage repair/remodelling. In addition, it can be used for the stable expression of certain growth factors in cell lines like ATDC5. For example, it is possible after *BMP2* transduction to study desensitisation processes in signal transduction of serin/threonine kinases. The system was used in our previous study for the successful *in vivo* transduction of *nlslacZ* [4]. Taken together with the results of this study, the described retroviral vector seems to be an optimal system for *in vivo* experiments to test *BMP2* and other growth factors as proof of principle for cartilage remodelling.

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