Cytotechnology (2018) 70:819–829
https://doi.org/10.1007/s10616-018-0191-y

ORIGINAL ARTICLE

Impact of dexamethasone concentration on cartilage tissue formation from human synovial derived stem cells in vitro

Ryota Chijimatsu · Masato Kobayashi · Kosuke Ebina · Toru Iwahashi · Yosuke Okuno · Makoto Hirao · Atsunori Fukuhara · Norimasa Nakamura · Hideki Yoshikawa

Received: 1 August 2017 / Accepted: 11 January 2018 / Published online: 19 January 2018

Abstract Human synovial mesenchymal stem cells (hSMSCs) are a promising cell source for cartilage regeneration because of their superior chondrogenic potential in vitro. This study aimed to further optimize the conditions for inducing chondrogenesis of hSMSCs, focusing on the dose of dexamethasone in combination with transforming growth factor-β3 (TGFβ3) and/or bone morphogenetic protein-2 (BMP2). When hSMSCs-derived aggregates were cultured with TGFβ3, dexamethasone up to 10 nM promoted chondrogenesis, but attenuated it with heterogeneous tissue formation when used at concentrations over than 100 nM. On the other hands, BMP2-induced chondrogenesis was remarkably disturbed in the presence of more than 10 nM dexamethasone along with unexpected adipogenic differentiation. In the presence of both TGFβ3 and BMP2, dexamethasone dose dependently promoted cartilaginous tissue formation as judged by tissue volume, proteoglycan content, and type 2 collagen expression, whereas few adipocytes were detected in the formed tissue when cultures were supplemented with over 100 nM dexamethasone. Even in chondrogenic conditions, dexamethasone thus affected hSMSCs differentiation not only toward chondrocytes, but also towards adipocytes dependent on the dose and combined growth factor. These findings have important implications regarding the use of glucocorticoids in in vitro tissue engineering for cartilage regeneration using hSMSCs.

Keywords Dexamethasone · Human synovial · Mesenchymal stem cells · Chondrogenesis · Adipogenesis · Cartilage regeneration

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10616-018-0191-y) contains supplementary material, which is available to authorized users.

R. Chijimatsu · M. Kobayashi · K. Ebina (✉) · T. Iwahashi · M. Hirao · N. Nakamura · H. Yoshikawa
Graduate School of Medicine, Orthopaedic Surgery, Osaka University, 2-2 Yamadaoka, Suita, Osaka, Japan
e-mail: k-ebina@umin.ac.jp

Y. Okuno · A. Fukuhara
Graduate School of Medicine, Metabolic Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka, Japan

N. Nakamura
Institute for Medical Science in Sports, Osaka Health Science University, 1-9-27 Kita-ku Tenma, Osaka, Osaka, Japan

N. Nakamura
Center for Advanced Medical Engineering and Informatics, Osaka University, 1-1 Yamadaoka, Suita, Osaka, Japan

Introduction

Chondral injury does not usually heal spontaneously. Thus, implantation of autologous cartilage or chondrocyte-derived tissue has been conducted to repair

cartilage in clinical setting. However, these methods present limitations such as donor site morbidity and limited availability of chondrocytes, which have not been resolved (Huey et al. 2012). Therefore, engineering cartilaginous implants from other cell sources is promising for the future of cartilage regeneration.

To this end, mesenchymal stem cells (MSCs) have been expected as a promising cell source because they can be harvested with minimally invasive procedures and present proliferative and chondrogenic capacities (Huey et al. 2012). Many studies investigated the optimum conditions for induction of chondrogenic differentiation of MSCs (Inui et al. 2012). In most studies, dexamethasone is traditionally used with growth factors to drive the differentiation of MSCs toward chondrogenesis (Fellows et al. 2016). Dexamethasone is a synthetic glucocorticoid which binds to homometric glucocorticoid receptor, thereby affecting cell viability and differentiation of various cell types in vitro. In general, dexamethasone is commonly used for the differentiation of stem cells into several lineages, not only chondrocytes but also osteoblasts, adipocytes, and others (Dimarino et al. 2013).

To drive the differentiation of MSCs toward chondrogenesis, growth factors such as transforming growth factor-βs (TGFβs) and bone morphogenetic proteins (BMPs) have been often used. The synergistic effect of dexamethasone and TGFβs is well known (Bilgen et al. 2007; Derfoul et al. 2006; Hara et al. 2015; Johnstone et al. 1998). However, results vary among reports; some studies showed a drastic effect (Bilgen et al. 2007; Johnstone et al. 1998), while others showed only a marginal effect (Derfoul et al. 2006; Hara et al. 2015). Furthermore, the effect of the combination of dexamethasone with BMPs is conflicting among reports (Diekman et al. 2010; Park et al. 2005; Shintani and Hunziker 2011). Of note, dexamethasone is used in re-differentiation of dedifferentiated chondrocytes and stimulates the synthesis of cartilaginous matrices (Ahmed et al. 2014), while in a pre-chondrogenic cell line, dexamethasone inhibits chondrogenesis (Fujita et al. 2004; Naito et al. 2015). Altogether, these data suggest that the effect of dexamethasone on chondrogenesis may differ depending on the cell type (Randau et al. 2013; Shintani and Hunziker 2011), combined growth factors (Shintani and Hunziker 2007, 2011), and culture systems (Florine et al. 2013; Medrado et al. 2006; Park et al. 2005). Taken together, cell specific experiments will be required to apply dexamethasone on the induction of chondrogenesis from MSCs.

Among MSCs, synovial derived MSCs (SMSCs) have been expected as an attractive cell source for the engineering of cartilage. Several reports demonstrated that SMSCs have the greatest chondrogenic potential in vitro compared with MSCs derived from other tissues (Koga et al. 2008; Yoshimura et al. 2007). We also previously reported the successful formation of a cartilaginous implant from human SMSCs (hSMSCs) (Koizumi et al. 2016; Yasui et al. 2016). In our previous studies, only BMP2 was applied to induce chondrogenesis, but another group reported that the combination of TGFβ3, BMP2, and dexamethasone was the best condition to induce chondrogenesis and form cartilaginous tissue from hSMSCs (Shirasawa et al. 2006). Thus, the combination and the concentration of growth factors have often been examined for chondrogenic induction of MSCs (Awad et al. 2003; Inui et al. 2012; Lee et al. 2009; Sekiya et al. 2001; Shintani and Hunziker 2011; Shirasawa et al. 2006), while dexamethasone is routinely used at the concentration of 100 nM in chondrogenic medium in most previous reports (Ahmed et al. 2014; Bilgen et al. 2007; Derfoul et al. 2006; Diekman et al. 2010; Florine et al. 2013; Hara et al. 2015; Johnstone et al. 1998; Koga et al. 2008; Park et al. 2005; Randau et al. 2013; Shintani and Hunziker 2007, 2011; Shirasawa et al. 2006; Yoshimura et al. 2007). However, it is possible that dexamethasone concentration sensitively affects the chondrogenic differentiation of hSMSCs as shown in other cell types (Awad et al. 2003; Fujita et al. 2004; Tangtrongsup and Kisiday 2016). To further optimize the efficient conditions for chondrogenic induction and cartilage tissue formation from hSMSCs, we here investigated the influence of the dose of dexamethasone on TGFβ3- and/or BMP2-induced chondrogenesis and cartilaginous tissue formation from hSMSCs for the first time.

Materials and methods

Isolation and culture of human synovial MSCs (hSMSCs)

Adult human synovium (n = 3: donor 1; male, age 30, donor 2; male, age 18, donor 3; female, age 38) were obtained at the time of arthroscopic surgery of the

anterior crucial ligament reconstruction in accordance with a protocol approved by the Institutional Ethics Committee. For isolation of synovial MSCs, specimens were minced and digested with 0.25% trypsin/EDTA (Gibco, Carlsbad, CA, USA) for 30 min, and then floating adipose tissues were removed after centrifugation to obtain high chondrogenic potential cells (Katagiri et al. 2017; Mochizuki et al. 2006). Non-floating tissues were resuspended in DMEM (Gibco) containing 10%FBS (Sigma-Aldrich, St Louis, MO, USA) and supplemented with 400 unit/mL collagenase type A (Worthington, Lakewood, NJ, USA) and incubated at 37 °C. After 3 h, dissociated cells were collected, washed by centrifugation, and then cultured with high glucose-DMEM containing 10% FBS and 1% antibiotic-antimitotic (Sigma-Aldrich) (GM: growth medium) at 37 °C with humidified 5% CO_2. hSMSCs were expanded as previously described (Koizumi et al. 2016; Yasui et al. 2016) and used for experiments at passage 3 or 4.

The cells have been already characterized with FACS analysis and surface antigen profile was as follows: CD34 (−), CD45 (−), CD44 (+), CD73 (+), CD90 (+), CD105 (+). The results were consistent with a previous report (Jo et al. 2007) and there were no differences among donors. The details on FACS analysis were described in our previous report (Chijimatsu et al. 2017).

Chondrogenesis assays

To perform two-dimension micromass culture (Decker et al. 2014), hSMSCs harvested from passage 3 were suspended at a concentration of 1×10^7 cell/mL in GM; 10 µL drops (1×10^5 cells) were spotted onto the center of the well of 24 well tissue culture plates (Corning, Corning, NY, USA) and cultured for 3 h at 37 °C with humidified 5% CO_2. Then, 0.5 mL of chondrogenic basal medium (Shirasawa et al. 2006) (high glucose-DMEM (Gibco), 110 µg/mL sodium pyruvate (Gibco), 1% ITS + premix (Corning), 50 µg/mL ascorbic acid-2-phosphate (Sigma-Aldrich), 40 µg/mL L-proline (Wako, Osaka, Japan), 1% antibiotic-antimitotic) supplemented with or without 1 nM to 1 µM dexamethasone (Sigma-Aldrich, Cat# D4902), 50 ng/mL BMP2 (Medtronic, Dublin, Ireland), or 10 ng/mL TGFβ3 (Peprotech, Rocky Hill, NJ, USA) were added. Cells were maintained at 37 °C with humidified 5% CO_2. DMSO (1 µL/mL; Sigma-Aldrich) served as the vehicle control. Alcian Blue staining (pH 1.0) and Oil Red O staining were performed at day 7 (Enomoto et al. 2004).

For three-dimension pellet culture, 2×10^5 cells harvested from passage 4 were centrifuged in 96 deep well polypropylene plates (Evergreen Scientific, Vernon, CA, USA) and cultured in GM. The next day, the medium was changed to chondrogenic medium supplemented with the same factors used for the two-dimension micromass culture. The pellets were maintained with 0.5 mL medium at 37 °C with humidified 5% CO_2. The medium was replaced twice weekly.

The sulfated glycosaminoglycan (sGAG) in the pellets was measured by using Blyscan Glycosaminoglycan Assay Kit (Biocolor, Westbury, NY, USA) after lysis with papain. The cellularity was measured based on the double strand DNA (dsDNA) content using Qubit® 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit™ dsDNA HS Assay kit (Thermo Fisher Scientific).

Histology and immunohistochemistry

The samples were fixed in 4% paraformaldehyde, embedded in paraffin wax, and the 5 µm sections were used for Safranin-O/fast green staining (Safranin-O) or immunostaining. Stains were performed as previously described (Chijimatsu et al. 2017; Shirasawa et al. 2006). The following antibodies were used: anti-collagen type 2 (Kyowa Pharma Chemical, Toyama, Japan, Cat# F-57, 1:500 dilution) and anti-perilipin (CST, Danvers, MA, USA, Cat# 3470, 1:100 dilution). Type 2 collagen staining was automatically measured by Aperio Image Scope (Leica Biosystems, Wetzlar, Germany), and cells positive for perilipin were manually counted.

RNA and qRT-PCR

Total RNA was extracted with a Direct-Zol™ RNA kit (Zymo Research, Irvine, CA, USA) and reverse transcribed into complementary DNA using RevaTra Ace® qPCR RT Master Mix (TOYOBO, Osaka, Japan) according to the manufacturer's protocol. Quantitative RT-PCR was performed as previously described (Chijimatsu et al. 2017). Target gene transcriptional levels were normalized to the level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression. The expression levels of each

target gene was calculated by using the $2^{-\Delta Ct}$ method (Chijimatsu et al. 2017). The Taqman assays used are as follows: PPARγ, Hs00234592_m1; LPL, Hs00173425_m1; FABP4, Hs01086177_m1, Adiponectin, Hs00977214_m1; and GAPDH, Hs02758991_g1.

Results

Effect of dexamethasone on two-dimension chondrogenic culture of hSMSCs

Initially, to clarify the effect of dexamethasone on chondrogenesis of hSMSCs, two-dimension micromass aggregates were cultured with various concentrations of dexamethasone (1 nM to 1 μM) in chondrogenic medium supplemented with or without BMP2 (50 ng/mL) and TGFβ3 (10 ng/mL) for 7 days. Unexpectedly, adipocyte-like oil droplets were recognized in microscopic observation when the cell aggregates were treated with 100 nM dexamethasone alone. Moreover, similar droplets emerged even at 10 nM dexamethasone in the presence of BMP2 (Fig. 1a). Based on this phenomenon, combined staining with Alcian Blue and Oil Red O was performed to assess chondrogenesis and adipogenesis (Enomoto et al. 2004). In the absence of growth factors, 10 nM dexamethasone induced nodule formation stained with Alcian blue which implied the origin of chondrogenesis (Oh and Chun 2003); however, Oil Red O positive droplets which implied adipogenesis, were also detected in these nodules. When dexamethasone was used at concentrations over 100 nM, nodule formation and Alcian blue staining faded, while an increase in oil droplets was detected (Fig. 1b). When combined with BMP2, nodule formation was induced at 10 nM dexamethasone, but a larger number of oil droplets was detected compared to that observed when dexamethasone was used alone. Notably, BMP2 sometimes induced adipogenesis without dexamethasone (Fig. 1c). On the other hands, TGFβ3 treatment thickened the micromass as a whole and strong Alcian blue staining was detected consist with previous report (De Bari et al. 2001; Park et al. 2005). However, combination with dexamethasone decreased this effect in a dose dependent manner, and few oil droplets were sometimes observed when dexamethasone concentration was beyond 100 nM (Fig. 1d). mRNA levels (Fig. 1e) of adipocyte and adipogenesis-associated genes (PPARγ, LPL, FABP4, and adiponectin) were significantly upregulated after dexamethasone treatment, and that was potentiated by BMP2. Consistent with the staining results, TGFβ3 suppressed the induction of adipocyte- and adipogenesis-associated genes by dexamethasone in hSMSCs.

The effect of dexamethasone on chondrogenesis of hSMSCs depends on the dose and combined factors in three-dimension chondrogenic culture

To assess the effect of dexamethasone on cartilaginous tissue formation from hSMSCs, three-dimension pellet culture was conducted with various concentrations of dexamethasone (1 nM to 100 nM) in the presence of TGFβ3 (10 ng/mL) or BMP2 (50 ng/mL). Without such chondrogenic factors, cartilaginous tissue formation failed as indicated by the lack of safranin-O staining (data not shown). When cultured with TGFβ3 for 4 weeks, less than 10 nM dexamethasone enhanced the synthesis of proteoglycan stained with safranin-O compared to in those slight staining of vehicle control (Figs. 2a and S1). Although, type 2 collagen was clearly detected in only one donor, it was increased by addition of dexamethasone (Figs. 2a and S1). However, 100 nM dexamethasone disturbed cartilaginous tissue formation, and remarkably, a cavity-like space was observed in the pellet center (Fig. 2a, black arrow). There were less matrices in the area, but cells existed with elongated shape, unlike chondrocytes.

When cultured with BMP2 for 4 weeks, proteoglycan and type 2 collagen expression with many chondrocyte-like lacunas were observed only when less than 1 nM dexamethasone was used (Figs. 2a–c and S1). However, such BMP2-induced cartilaginous tissue formation was drastically disturbed when more than 10 nM dexamethasone was used. Notably, many adipocyte-like cells were detected in these pellets (Figs. 2b, arrow heads and S1) consistent with the micromass culture results (Fig. 1c). Since BMP2 is often used at a high dose over 200 ng/mL for chondrogenic induction of MSCs (Koizumi et al. 2016; Shintani and Hunziker 2007, 2011; Shirasawa et al. 2006; Yasui et al. 2016), we additionally examined the combination of 250 ng/mL BMP2 and dexamethasone. Similarly, chondrogenesis was disturbed over 10 nM dexamethasone supplementation (data not shown).

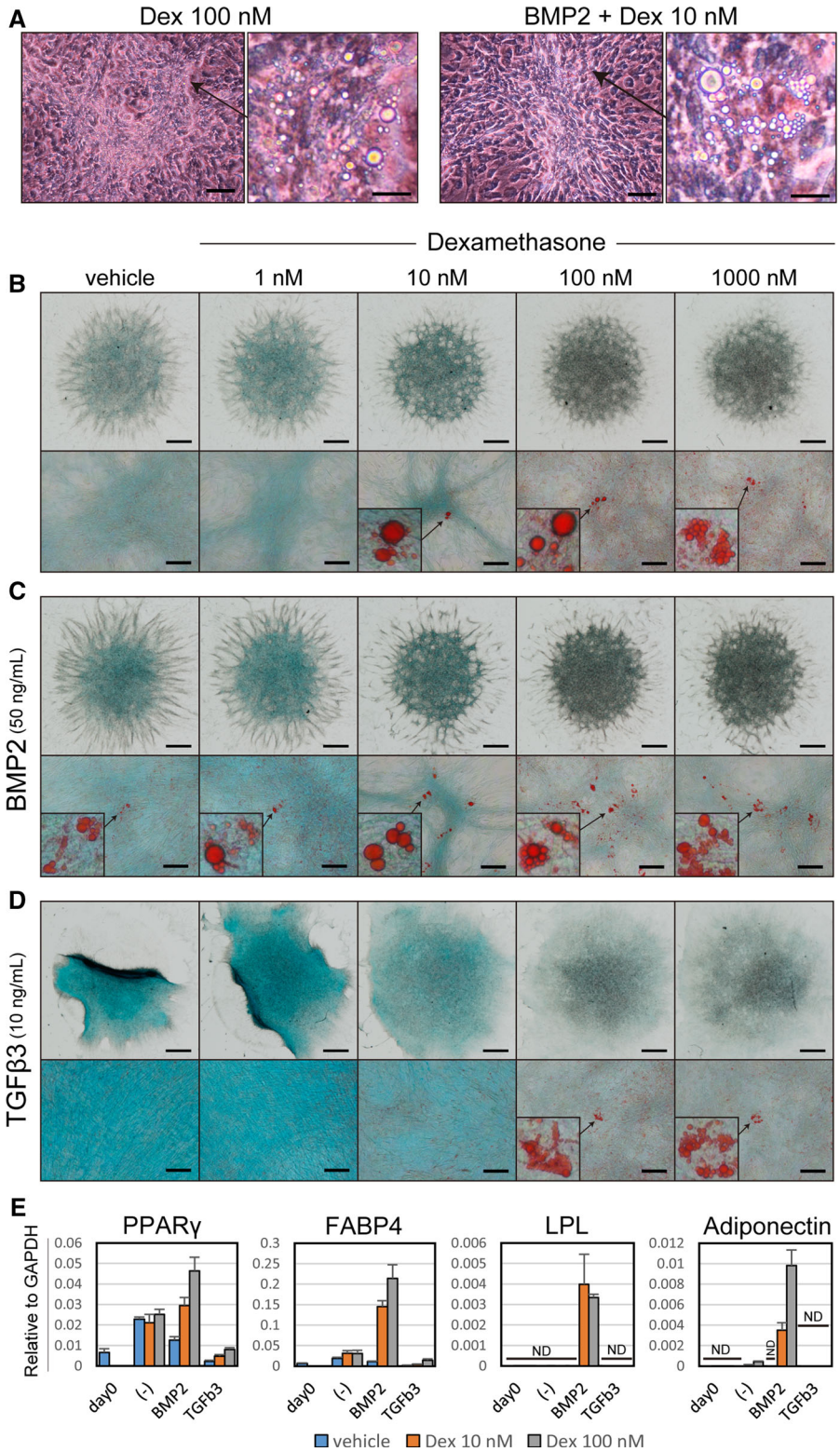

Fig. 1 Effect of dexamethasone on two-dimension micromass chondrogenic culture of hSMSCs. Representative data from three independent donors are shown. **a** The microscopic image of two-dimensional micromass culture in 100 nM dexamethasone or BMP2 + 10 nM dexamethasone on day 7. Arrows indicate the presence of oil droplets. Scale bars = 100 µm (left panels) and 20 µm (right panels). **b–d** Bright field images of Alcian blue and Oil Red O staining of micromass cultured with various concentrations of dexamethasone (1–1000 nM) with or without TGF#3 (10 ng/mL) and BMP2 (50 ng/mL) on day 7. Blue color indicates glycosaminoglycan deposition and red color indicates oil droplets of cytoplasm. Scale bars = 1 mm (upper panels) and 100 µm (lower panels). **e** Quantitative mRNA expression analysis of adipogenic genes (PPARγ, FABP4, LPL, and Adiponectin) in the micromass cultured with dexamethasone (0, 10, 100 nM) with or without TGFβ3 (10 ng/mL) and BMP2 (50 ng/mL) at day 7 (mean ± SD for three micromass). *ND* not detected. (Color figure online)

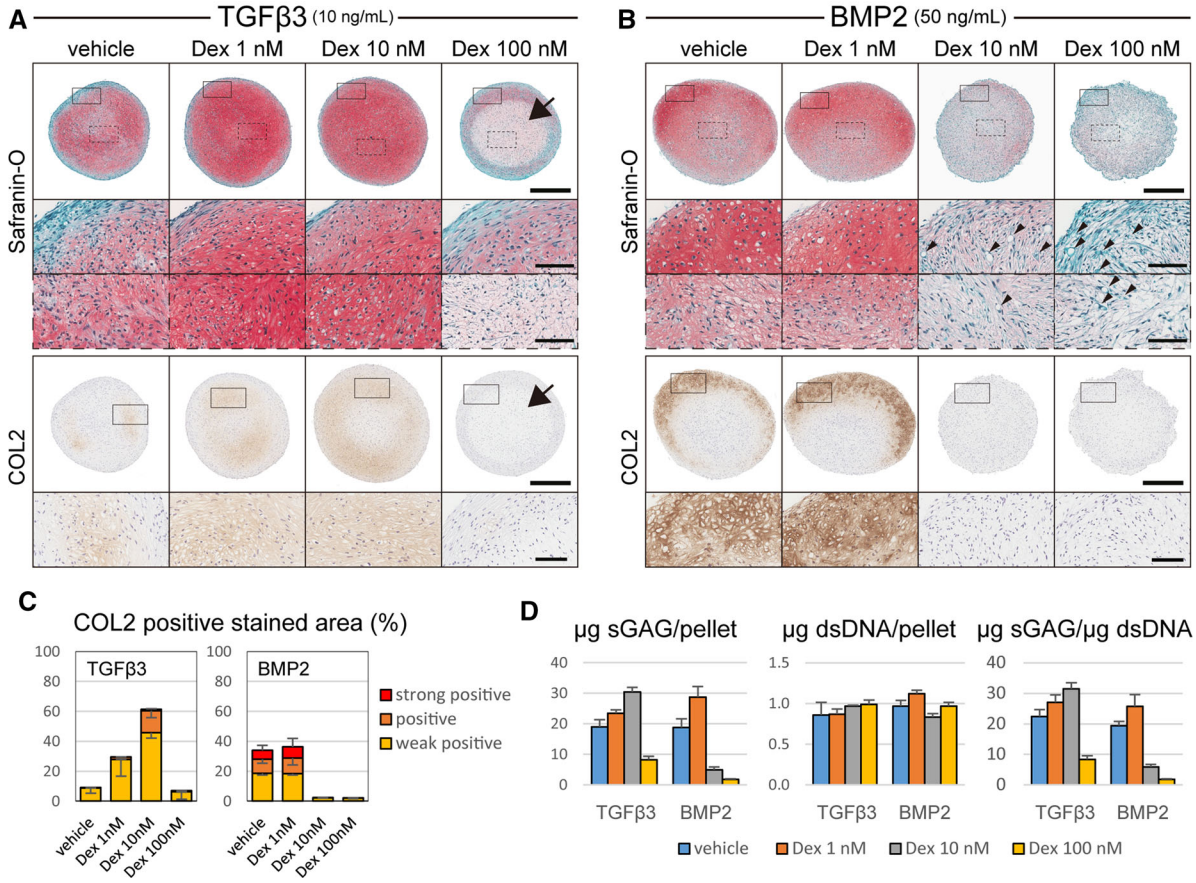

Fig. 2 Differential effect of dexamethasone on three-dimension chondrogenic culture of hSMSCs dependent on the dose and combined factors. The data of donor 2 from three independent donors are shown as representative results. The data obtained from the other two donors are shown in supplementary data. **a**, **b** Safranin-O staining and immunostaining for type 2 collagen of the pellets cultured with dexamethasone (0, 1, 10, 100 nM) and TGFβ3 (10 ng/mL) or BMP2 (50 ng/mL) for 28 days. The area of the periphery and center of Safranin-O staining and featured area of COL2 staining were magnified in the lower panels. Scale bars = 500 μm (whole pellet images) and 100 μm (magnified images). Arrows indicate the cavity-like area shown in only the condition of 100 nM dexamethasone and TGFβ3. Arrow heads indicate adipocyte-like oil droplets. **c** Quantitative area analysis of immunostaining for type 2 collagen (mean ± SD for three pellets). **d** The content of sulfated GAG and double-strand DNA of chondrogenic pellets on day 28 (mean ± SD for three pellets). (Color figure online)

Consistent with histological assessment, the sulfated GAG content of pellets decreased in the presence of high doses of dexamethasone, but the dsDNA content was not altered, suggesting that dexamethasone did not affect chondrogenesis by influencing cell viability (Fig. 2d).

High dexamethasone dose causes adipogenesis dominant in chondrogenic culture of hSMSCs

To detect adipocytes in paraffin embedded samples, perilipin, which forms the membrane of oil droplets, was detected by immunostaining. As shown in Fig. 3a, perilipin positive adipocytes were detected in the whole pellets cultured with BMP2 and 10–100 nM dexamethasone, and increased dependent on the dose of dexamethasone. Of note, although 1 nM dexamethasone did not disturb BMP2-induced chondrogenesis, few adipocytes were detected in the formed cartilaginous tissues in all three donors (Fig. 3b). Furthermore, perilipin positive cells were detected when hSMSCs were cultured with TGFβ3 and 100 nM dexamethasone, and the location was biased in the cavity-like area, implying a relationship between adipogenesis and cavity-like tissue formation. In the condition of TGFβ3 and less than 10 nM

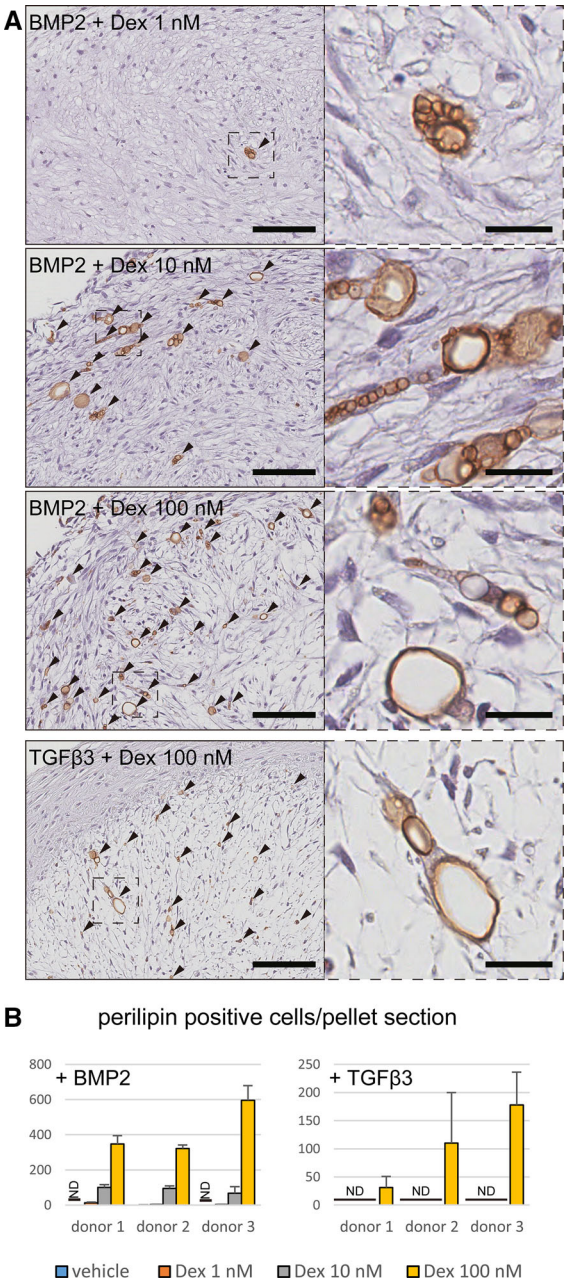

Fig. 3 Dexamethasone induces adipocyte differentiation in the chondrogenic pellets. **a** Immunostaining for perilipin of chondrogenic pellets magnified from Fig. 2a (TGFβ3 and 100 nM dexamethasone) and Fig. 2b (BMP2 and 1–100 nM dexamethasone). The featured area was further magnified in their right panels. Arrow heads indicate positive stained cells. Scale bar = 100 µm (left panels) and 20 µm (right panels). **b** Count for perilipin positive cells in histological section of each condition (dexamethasone and BMP2 or TGFβ3) as shown with arrow heads in Fig. 3a. The results from three independent donors are shown (mean ± SD for three pellets). *ND* not detected. (Color figure online)

dexamethasone, perilipin positive cells were not detected in all three donors (Fig. 3b).

Dexamethasone causes adipocyte contamination of tissue engineered cartilage

The combination of TGFβ3 and BMP2 is considered an optimal condition to induce chondrogenesis of hSMSCs (Shirasawa et al. 2006). Consistently, the combination of TGFβ3 and BMP2 effectively induced the formation of cartilaginous tissues, which were rich in proteoglycan and type 2 collagen compared with those cultured with only TGFβ3 or BMP2 (Figs. 4a and S2). Dexamethasone further promoted cartilaginous tissue formation as assessed by weight, sulfated GAG content, and homogenous organization of type 2 collagen (Fig. 4b–d). Although, 100 nM dexamethasone increased tissue volume without sensitively influencing the sulfated GAG content, formed tissues were often heterogeneous with a fibrotic center (donor 1: N = 3/3, donor 2: N = 2/3, donor 3: N = 0/3), similar to that observed in the combination of TGFβ3 and 100 nM dexamethasone. Furthermore, perilipin-positive cells were also detected in all tissues obtained from all donors only with 100 nM dexamethasone supplementation. They were mainly detected in the fibrotic area, but few adipocytes were observed in the area rich in proteoglycan and type 2 collagen (Figs. 4e, black arrow heads, f and S2).

Discussion

Dexamethasone is one of the major glucocorticoids used to induce chondrogenesis. In the present study, we investigated the optimal concentration of dexamethasone on chondrogenic induction and engineering of cartilaginous tissue from hSMSCs combined with TGFβ3 and/or BMP2. Although the supplementation of dexamethasone showed the best results in term of cartilaginous tissue generation, it also induced adipogenesis when used at 100 nM, the generally used dose, revealing issues in engineering cartilage using hSMSCs.

Previous reports demonstrated that dexamethasone affects human bone marrow and synovial derived stem cells via glucocorticoid receptor (GR) (Derfoul et al. 2006; Florine et al. 2013; Gossye et al. 2009), and activated GR signaling has been known to upregulate

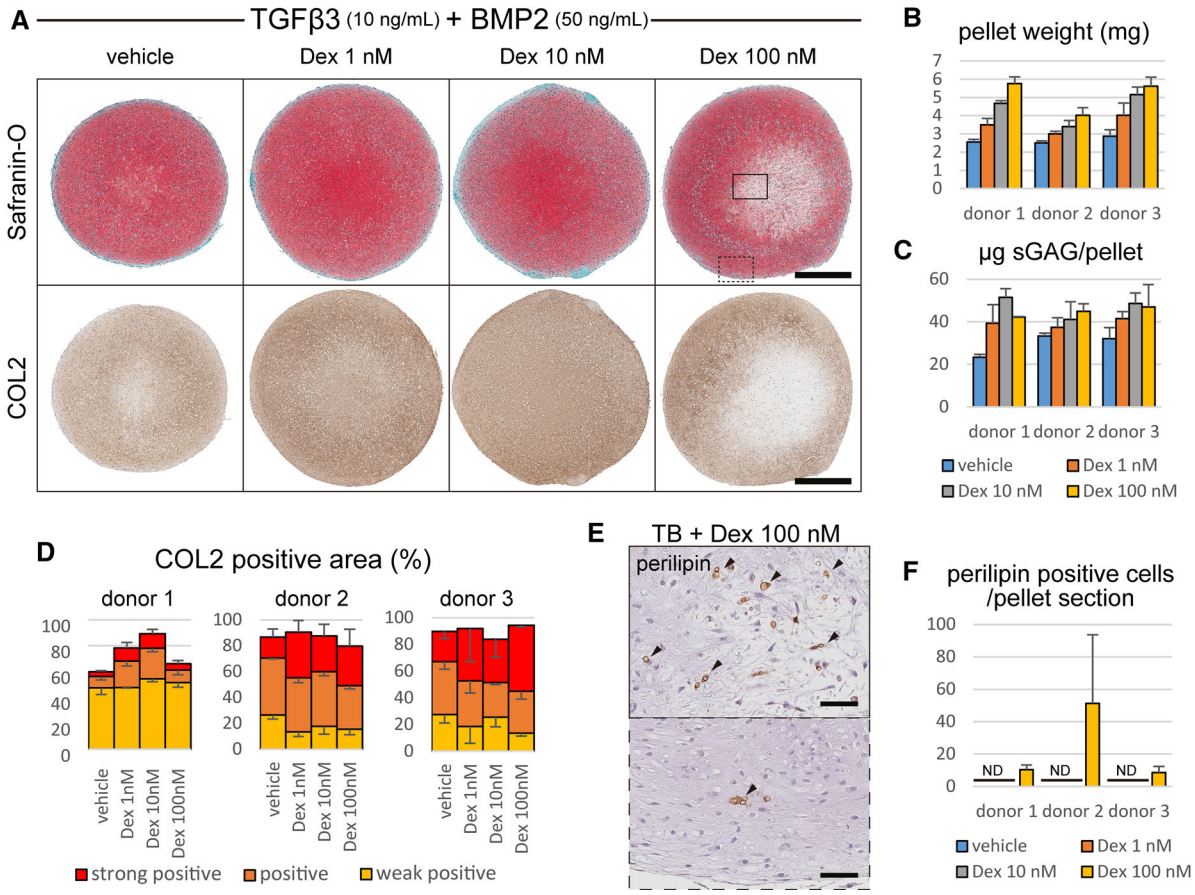

Fig. 4 Cartilaginous tissue formation induced by a combination of 10 ng/mL TGFβ3 and 50 ng/mL BMP2 (TB) is altered by supplementation with dexamethasone. The data of chondrogenic pellets on day 28 cultured with TB and/or dexamethasone are shown. **a** The images of safranin-O staining and immunostaining for type 2 collagen (donor 2). **b–d** Wet weight, sulfated GAG content, and quantitative analysis of immunostaining for COL2 (mean ± SD for three pellets). **e** Immunostaining for perilipin magnified from inset boxes in the image of safranin-O with the same frame. Scale bars = 50 μm. **f** Positive cell count for perilipin (mean ± SD for three pellets). *ND* not detected. Images related to (**b**) and (**e**) from the other two donors are shown in supplementary data. (Color figure online)

C/EBPs, leading to the expression of PPARγ, the master regulator of adipogenesis (Cristancho and Lazar 2011; Rosen and MacDougald 2006). Furthermore, BMP2 has also been known to potentiate adipogenesis of MSCs in adipogenic conditions when PPARγ is upregulated (Sottile and Seuwen 2000; Takada et al. 2012). As shown in Fig. S3, the combination of BMP2 with other glucocorticoids (prednisolone, betamethasone, and fluocinolone acetonide) also showed enhanced adipogenesis of hSMSCs. Collectively, glucocorticoids may trigger adipogenic differentiation of hSMSCs via GR signaling and may be strongly dominated by BMP2 stimulation, following interruption of chondrogenic differentiation.

Our data further demonstrated that the dose of dexamethasone sensitively affects the chondrogenesis in combination with not only BMP2, but also TGFβ3. It has been reported that dexamethasone promotes chondrogenesis of MSCs via enhancing TGFβs-induced phosphorylation of SMADs (Hara et al. 2015), and TGFβs inhibit adipogenesis of MSCs via blockade of C/EBPs expression (Cristancho and Lazar 2011; Rosen and MacDougald 2006). Consistently, TGFβ3 treatment suppressed dexamethasone-induced adipogenesis of hSMSCs and improved cartilaginous tissue formation when dexamethasone concentration

was no more than 10 nM. Although dexamethasone is typically used at a concentration of 100 nM for chondrogenic induction of many types of MSCs (Derfoul et al. 2006; Diekman et al. 2010; Fellows et al. 2016; Florine et al. 2013; Johnstone et al. 1998; Park et al. 2005; Randau et al. 2013), the effect was opposite in hSMSCs. Consistent with our results, heterogeneous tissue formation with cavity-like center was also observed in previous hSMSCs studies (Kim et al. 2011; Lee et al. 2009; Pei et al. 2008; Shirasawa et al. 2006), but the relationship with adipogenesis has not been mentioned. hSMSCs have high differentiation potential for adipogenesis as well as chondrogenesis (Mochizuki et al. 2006; Sakaguchi et al. 2005), and our results indicated that such two cell fates were competitive in the supplementation between dexamethasone and TGFβ3. Therefore, use of adipogenic inhibitors or increasing TGFβ3 dose may further improve chondrogenic differentiation and cartilaginous tissue formation.

Recently, fluocinolone acetonide, a glucocorticoid, was reported as a strong inducer of TGFβ3-associated chondrogenesis of bone marrow MSCs via effective enhancement of GR signaling when compared to dexamethasone (Hara et al. 2015). However, as shown in this study, GR signaling does not always act in favor of chondrogenesis, instead it needs to be adequately restricted for chondrogenic induction of hSMSCs. GR signaling is not only modulated by type and dose of glucocorticoids (Hara et al. 2015; Mushtaq et al. 2002), but also by intracellular environment. GR has two isoforms, and one of which is known to negatively regulate GR signaling (Chatzopoulou et al. 2015). Therefore, ratio of those expressions is closely involved in the sensitivity to glucocorticoids, that depends on type and state of cells (John et al. 2016). Thus, GR signaling should be carefully examined for specific cells when using a specific glucocorticoid as it may be important to regulate the differentiation of stem cells towards a specific lineage.

There are several limitations in this study. We examined hSMSCs from only three donors which may be insufficient to generalize the protocol for cartilaginous tissue formation. Moreover, intercellular mechanisms how GR signaling enhances or inhibits chondrogenesis on the interactions among PPARγ, SMADs, and other signalings was difficult to investigate in this study. Furthermore, we have to clarify the influence of such adipocytes contamination on clinical output of transplantation therapy.

In conclusion, the optimal conditions to efficiently induce chondrogenesis and cartilaginous tissue formation from hSMSCs may be combination of 10 nM dexamethasone with TGFβ3 and BMP2. Our study further suggests that optimizing GR signaling may be of importance for in vitro engineering of cartilage from MSCs.

Acknowledgements The authors thank Fumiko Hirayama and Yukiko Eguchi for their technical support.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest All authors have no conflict of interest to declare.

References

Ahmed N, Iu J, Brown CE, Taylor DW, Kandel RA (2014) Serum- and growth-factor-free three-dimensional culture system supports cartilage tissue formation by promoting collagen synthesis via Sox9–Col2a1 interaction. Tissue Eng Part A 20:2224–2233. https://doi.org/10.1089/ten.TEA.2013.0559

Awad HA, Halvorsen YD, Gimble JM, Guilak F (2003) Effects of transforming growth factor beta1 and dexamethasone on the growth and chondrogenic differentiation of adipose-derived stromal cells. Tissue Eng 9:1301–1312. https://doi.org/10.1089/10763270360728215

Bilgen B, Orsini E, Aaron RK, Ciombor DM (2007) FBS suppresses TGF-beta1-induced chondrogenesis in synoviocyte pellet cultures while dexamethasone and dynamic stimuli are beneficial. J Tissue Eng Regen Med 1:436–442. https://doi.org/10.1002/term.56

Chatzopoulou A, Roy U, Meijer AH, Alia A, Spaink HP, Schaaf MJ (2015) Transcriptional and metabolic effects of glucocorticoid receptor alpha and beta signaling in zebrafish. Endocrinology 156:1757–1769. https://doi.org/10.1210/en.2014-1941

Chijimatsu R, Ikeya M, Yasui Y, Ikeda Y, Ebina K, Moriguchi Y, Shimomura K, Hart DA, Hideki Y, Norimasa N (2017) Characterization of mesenchymal stem cell-like cells derived from human iPSCs via neural crest development and their application for osteochondral repair. Stem Cells Int 2017:1960965. https://doi.org/10.1155/2017/1960965

Cristancho AG, Lazar MA (2011) Forming functional fat: a growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol 12:722–734. https://doi.org/10.1038/nrm3198

De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP (2001) Multipotent mesenchymal stem cells from adult human

synovial membrane. Arthritis Rheum 44:1928–1942. https://doi.org/10.1002/1529-0131(200108)44:8%3C1928::AID-ART331%3E3.0.CO;2-P/abstract

Decker RS, Koyama E, Enomoto-Iwamoto M, Maye P, Rowe D, Zhu S, Schultz PG, Pacifici M (2014) Mouse limb skeletal growth and synovial joint development are coordinately enhanced by Kartogenin. Dev Biol 395:255–267. https://doi.org/10.1016/j.ydbio.2014.09.011

Derfoul A, Perkins GL, Hall DJ, Tuan RS (2006) Glucocorticoids promote chondrogenic differentiation of adult human mesenchymal stem cells by enhancing expression of cartilage extracellular matrix genes. Stem Cells 24:1487–1495. https://doi.org/10.1634/stemcells.2005-0415

Diekman BO, Estes BT, Guilak F (2010) The effects of BMP6 overexpression on adipose stem cell chondrogenesis: interactions with dexamethasone and exogenous growth factors. J Biomed Mater Res A 93:994–1003. https://doi.org/10.1002/jbm.a.32589

Dimarino AM, Caplan AI, Bonfield TL (2013) Mesenchymal stem cells in tissue repair. Front Immunol 4:201. https://doi.org/10.3389/fimmu.2013.00201

Enomoto H, Furuichi T, Zanma A, Yamana K, Yoshida C, Sumitani S, Yamamoto H, Enomoto-Iwamoto M, Iwamoto M, Komori T (2004) Runx2 deficiency in chondrocytes causes adipogenic changes in vitro. J Cell Sci 117:417–425. https://doi.org/10.1242/jcs.00866

Fellows CR, Matta C, Zakany R, Khan IM, Mobasheri A (2016) Adipose, bone marrow and synovial joint-derived mesenchymal stem cells for cartilage repair. Front Genet 7:213. https://doi.org/10.3389/fgene.2016.00213

Florine EM, Miller RE, Porter RM, Evans CH, Kurz B, Grodzinsky AJ (2013) Effects of dexamethasone on mesenchymal stromal cell chondrogenesis and aggrecanase activity: comparison of agarose and self-assembling peptide scaffolds. Cartilage 4:63–74. https://doi.org/10.1177/1947603512455196

Fujita T, Fukuyama R, Enomoto H, Komori T (2004) Dexamethasone inhibits insulin-induced chondrogenesis of ATDC5 cells by preventing PI3K-Akt signaling and DNA binding of Runx2. J Cell Biochem 93:374–383. https://doi.org/10.1002/jcb.20192

Gossye V, Elewaut D, Bougarne N, Bracke D, Van Calenbergh S, Haegeman G, De Bosscher K (2009) Differential mechanism of NF-kappaB inhibition by two glucocorticoid receptor modulators in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 60:3241–3250. https://doi.org/10.1002/art.24963

Hara ES, Ono M, Pham HT, Sonoyama W, Kubota S, Takigawa M, Matsumoto T, Young MF, Olsen BR, Kuboki T (2015) Fluocinolone acetonide is a potent synergistic factor of TGF-β3-associated chondrogenesis of bone marrow-derived mesenchymal stem cells for articular surface regeneration. J Bone Miner Res 30:1585–1596. https://doi.org/10.1002/jbmr.2502

Huey DJ, Hu JC, Athanasiou KA (2012) Unlike bone, cartilage regeneration remains elusive. Science 338:917–921. https://doi.org/10.1126/science.1222454

Inui A, Iwakura T, Reddi A (2012) Human stem cells and articular cartilage regeneration. Cells 1:994–1009. https://doi.org/10.3390/cells1040994

Jo CH, Ahn HJ, Kim HJ, Seong SC, Lee MC (2007) Surface characterization and chondrogenic differentiation of mesenchymal stromal cells derived from synovium. Cytotherapy 9:316–327. https://doi.org/10.1080/14653240701291620

John K, Marino JS, Sanchez ER, Hinds TD Jr (2016) The glucocorticoid receptor: cause of or cure for obesity? Am J Physiol Endocrinol Metab 310:E249–E257. https://doi.org/10.1152/ajpendo.00478.2015

Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU (1998) In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 238:265–272. https://doi.org/10.1006/excr.1997.3858

Katagiri K, Matsukura Y, Muneta T, Ozeki N, Mizuno M, Katano H, Sekiya I (2017) Fibrous synovium releases higher numbers of mesenchymal stem cells than adipose synovium in a suspended synovium culture model. Arthroscopy 33:800–810. https://doi.org/10.1016/j.arthro.2016.09.033

Kim JH, Lee MC, Seong SC, Park KH, Lee S (2011) Enhanced proliferation and chondrogenic differentiation of human synovium-derived stem cells expanded with basic fibroblast growth factor. Tissue Eng Part A 17:991–1002. https://doi.org/10.1089/ten.TEA.2010.0277

Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, Sekiya I (2008) Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: suitable conditions for cell therapy of cartilage defects in rabbit. Cell Tissue Res 333:207–215. https://doi.org/10.1007/s00441-008-0633-5

Koizumi K, Ebina K, Hart DA, Hirao M, Noguchi T, Sugita N, Yasui Y, Chijimatsu R, Yoshikawa H, Nakamura N (2016) Synovial mesenchymal stem cells from osteo- or rheumatoid arthritis joints exhibit good potential for cartilage repair using a scaffold-free tissue engineering approach. Osteoarthr Cartil 24:1413–1422. https://doi.org/10.1016/j.joca.2016.03.006

Lee S, Kim JH, Jo CH, Seong SC, Lee JC, Lee MC (2009) Effect of serum and growth factors on chondrogenic differentiation of synovium-derived stromal cells. Tissue Eng Part A 15:3401–3415. https://doi.org/10.1089/ten.TEA.2008.0466

Medrado GC, Machado CB, Valerio P, Sanches MD, Goes AM (2006) The effect of a chitosan-gelatin matrix and dexamethasone on the behavior of rabbit mesenchymal stem cells. Biomed Mater 1:155–161. https://doi.org/10.1088/1748-6041/1/3/010

Mochizuki T, Muneta T, Sakaguchi Y, Nimura A, Yokoyama A, Koga H, Sekiya I (2006) Higher chondrogenic potential of fibrous synovium- and adipose synovium-derived cells compared with subcutaneous fat-derived cells: distinguishing properties of mesenchymal stem cells in humans. Arthritis Rheum 54:843–853. https://doi.org/10.1002/art.21651

Mushtaq T, Farquharson C, Seawright E, Ahmed SF (2002) Glucocorticoid effects on chondrogenesis, differentiation and apoptosis in the murine ATDC5 chondrocyte cell line. J Endocrinol 175:705–713

Naito M, Ohashi A, Takahashi T (2015) Dexamethasone inhibits chondrocyte differentiation by suppression of Wnt/beta-catenin signaling in the chondrogenic cell line ATDC5.

Histochem Cell Biol 144:261–272. https://doi.org/10.1007/s00418-015-1334-2

Oh CD, Chun JS (2003) Signaling mechanisms leading to the regulation of differentiation and apoptosis of articular chondrocytes by insulin-like growth factor-1. J Biol Chem 278:36563–36571. https://doi.org/10.1074/jbc.M304857200

Park Y, Sugimoto M, Watrin A, Chiquet M, Hunziker EB (2005) BMP-2 induces the expression of chondrocyte-specific genes in bovine synovium-derived progenitor cells cultured in three-dimensional alginate hydrogel. Osteoarthr Cartil 13:527–536. https://doi.org/10.1016/j.joca.2005.02.006

Pei M, He F, Vunjak-Novakovic G (2008) Synovium-derived stem cell-based chondrogenesis. Differentiation 76:1044–1056. https://doi.org/10.1111/j.1432-0436.2008.00299.x

Randau TM, Schildberg FA, Alini M, Wimmer MD, el Haddouti M, Gravius S, Ito K, Stoddart MJ (2013) The effect of dexamethasone and triiodothyronine on terminal differentiation of primary bovine chondrocytes and chondrogenically differentiated mesenchymal stem cells. PLoS ONE 8:e72973. https://doi.org/10.1371/journal.pone.0072973

Rosen ED, MacDougald OA (2006) Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7:885–896. https://doi.org/10.1038/nrm2066

Sakaguchi Y, Sekiya I, Yagishita K, Muneta T (2005) Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum 52:2521–2529. https://doi.org/10.1002/art.21212

Sekiya I, Colter DC, Prockop DJ (2001) BMP-6 enhances chondrogenesis in a subpopulation of human marrow stromal cells. Biochem Biophys Res Commun 284:411–418. https://doi.org/10.1006/bbrc.2001.4898

Shintani N, Hunziker EB (2007) Chondrogenic differentiation of bovine synovium: bone morphogenetic proteins 2 and 7 and transforming growth factor beta1 induce the formation of different types of cartilaginous tissue. Arthritis Rheum 56:1869–1879. https://doi.org/10.1002/art.22701

Shintani N, Hunziker EB (2011) Differential effects of dexamethasone on the chondrogenesis of mesenchymal stromal cells: influence of microenvironment, tissue origin and growth factor. Eur Cell Mater 22:302–319

Shirasawa S, Sekiya I, Sakaguchi Y, Yagishita K, Ichinose S, Muneta T (2006) In vitro chondrogenesis of human synovium-derived mesenchymal stem cells: optimal condition and comparison with bone marrow-derived cells. J Cell Biochem 97:84–97. https://doi.org/10.1002/jcb.20546

Sottile V, Seuwen K (2000) Bone morphogenetic protein-2 stimulates adipogenic differentiation of mesenchymal precursor cells in synergy with BRL 49653 (rosiglitazone). FEBS Lett 475:201–204

Takada I, Yogiashi Y, Kato S (2012) Signaling crosstalk between PPARgamma and BMP2 in mesenchymal stem cells. PPAR Res 2012:607141. https://doi.org/10.1155/2012/607141

Tangtrongsup S, Kisiday JD (2016) Effects of dexamethasone concentration and timing of exposure on chondrogenesis of equine bone marrow-derived mesenchymal stem cells. Cartilage 7:92–103. https://doi.org/10.1177/1947603515595263

Yasui Y, Chijimatsu R, Hart DA, Koizumi K, Sugita N, Shimomura K, Myoui A, Yoshikawa H, Nakamura N (2016) Preparation of scaffold-free tissue-engineered constructs derived from human synovial mesenchymal stem cells under low oxygen tension enhances their chondrogenic differentiation capacity. Tissue Eng Part A 22:490–500. https://doi.org/10.1089/ten.tea.2015.0458

Yoshimura H, Muneta T, Nimura A, Yokoyama A, Koga H, Sekiya I (2007) Comparison of rat mesenchymal stem cells derived from bone marrow, synovium, periosteum, adipose tissue, and muscle. Cell Tissue Res 327:449–462. https://doi.org/10.1007/s00441-006-0308-z