[Frontiers in Bioscience S5, 774-785, January 1, 2013]

Mesenchymal stem cell therapy for injured growth plate

Awang B Shukrimi[1], Mohd H Afizah[1], Jacqueline F Schmitt[1], James HP Hui[1]

[1]Department of Orthopaedic Surgery, National University Health System, National University of Singapore, 1E, Kent Ridge Road, Singapore 119288

TABLE OF CONTENTS

1. ABSTRACT

The growth plate has a limited self-healing capacity. Fractures sustained to the growth plate of young children could cause growth disturbances like angular deformity or growth arrest. Established therapies for injured physis only address related complications. Mesenchymal stem cells (MSCs) are multipotent cells which are capable of differentiating into various cells of the musculoskeletal system. Various MSC types have been tested for physeal regeneration, through *in vivo* lapine, porcine and ovine models, for the duration of 4-16 weeks. The created defect sizes ranged from 7-50% of the growth plate area, to simulate clinically-encountered cases. In vitro models have also been investigated, as a means to screen potential treatments. The effects of MSCs gathered from these models have revealed its function in the prevention of bone bridge formation, with the subsequent development of organized physeal repair tissue. Possible influential factors like the number of implanted MSCs, preconditioned state, growth factors, chondrocyte-MSC interaction and scaffolds are discussed. Possible further studies to optimize physeal repair based on MSC therapy in articular cartilage are also included.

2. INTRODUCTION

This article discusses the reported and possible uses of MSCs and stimulatory elements that would assist in physeal defect repair. This was conducted by performing a search through PubMed, of related English collections over the past 2 decades, using associated terms like zonal, chondrocytes, repair, MSCs and growth factors. Some of the cited researches are of treatments carried out on articular cartilage, for the purpose of extrapolating MSC behaviour.

3. PHYSEAL DEFECTS AND MSC AS A POTENTIAL REPAIR SOURCE

3.1. Physeal injuries and treatment

The physis, also named as growth plate, is a band of hyaline cartilage located at the end of long bones between the epiphysis and metaphysis, and is responsible for bone lengthening during growth. The physis is prone to injuries due to its fragile nature but has limited self-healing capacity due to its avascular state (1). An estimated 15% of all pediatric fractures reportedly involve the physis and

15% of these lead to growth disturbance which would advance to progressive angular deformity or growth arrest (2).

Established therapies for the injured physis currently only address related complications. Growth plate fractures impair the well-orchestrated growth process (3), leading to a trilogy of vascular invasion, matrix mineralization and bone bridge formation. If the bone bridge affects only a localized section of the physis, the remaining physeal cartilage would continue to produce bone, thus causing disruptions in junction function which would eventually lead to limb angular deformity. These patients will require complex limb lengthening surgeries which is associated with many complications. Realignment surgery is routinely done to treat angular deformity (4), while growth arrest had been remedied by surgical epiphysiodesis or lengthening of the involved or contralateral bone. Over the last two decades, progress made in the area of cartilage repair through chondrocyte or stem cell-based therapies have reignited hope in this field. Although the studies are limited to animal models, they offer promising therapies which have the potential to be applied clinically.

Chondrocytes have been investigated in the treatment of physeal repair (5, 6). In rabbit studies, physeal defects were created at the medial half of the proximal growth plate to generate a growth arrest model. Bone bridge excision was carried out, followed by the implantation of chondrocyte-embedded agarose. Through this therapy, growth arrest with angular deformation of the tibia was prevented (6). Since then, the use of cultured chondrocytes as interpositional material after physeal bridge resection had been actively researched on (7, 8). The transplantation of physeal chondrocytes isolated from fetal sheep into surgically created physeal defects in lambs aged 5 to 13 weeks resulted in repopulation of the physeal tissue and the prevention of bone bridge formation (5). However, the use of chondrocytes as a tissue source would involve donor site morbidity. Another limitation with its usage is the phenotype loss which chondrocytes typically undergo after multiple passages (9), which makes it difficult to obtain high cell numbers sufficient for therapy. A population of cells with stem-like properties had been discovered in physeal tissue. The resting zone regenerated excised proliferative and hypertrophic zones within one week in rabbits (10). Chondroprogenitor Cells (CPCs) located in the resting zone was postulated to be the cellular source for the observed tissue repair. Unfortunately, the percentage of stem-like cells that can be retrieved from growth plate cartilage would be a fraction of the sparse population of chondrocytes.

3.2. MSCs for physeal repair

MSCs are easily isolated from a number of tissues including bone marrow (11), adipose tissue (12), periosteum, (13) synovium (14, 15), and muscle (16), and can be readily expanded under standard culture conditions as plastic adherent populations. MSCs are determined as $CD105^+$, $CD73^+$ and $CD90^+$, and $CD45^-$, $CD34^-$, $CD14^-$, $CD11b^-$, $CD79\alpha^-$, $CD19^-$ and HLA-DR^- cells (17). They are multipotent cells which under the right stimuli, have the ability to differentiate into various cells of the musculoskeletal system including chondrocytes, osteoblasts, adipocytes, and myoblasts among others, and will maintain their stem cell qualities over numerous cell doublings (18).

All organs and tissues have a resident population of MSCs. Hence it is not surprising to see that various tissue types have been tested as possible cell sources for physeal regeneration. These include the periosteum (13, 19, 20), bone marrow (20-24), adipose tissue (20) and synovium (15). The treatment of physeal disorders requires an MSC tissue source which can be obtained with little or no detrimental effects at the donor site. Bone marrow can be easily aspirated from the iliac crest while adipose tissue can be harvested from subcutaneous abdominal tissue. The sites from which the MSCs are harvested have been shown to affect their suitability as a source of cells for physeal repair. Varying percentage of MSCs have been found in tissue types, with adipose tissue containing higher frequency of MSCs than bone marrow (25) and bone marrow containing significantly greater MSCs compared to umbilical cord (26). We have compared MSCs from bone marrow, periosteum and adipose tissue for their ability to correct defects using the rabbit physis defect model (20). MSCs from all three tissue sources were found to significantly correct varus angular defects as well as leg length discrepancies and prevented bone bridge formation in comparison to the control tibiae injected with fibrin glue alone. However, significantly enhanced defect correction was attained in tibiae injected with bone marrow- and periosteum-derived MSCs only, where the MSCs were found to have differentiated into physeal chondrocytes which integrated with the host tissue while adipose-derived MSCs developed into randomly-arranged chondrocytes. It is possible that the chondrogenesis of adipose-derived MSCs have not been fully optimized.

MSCs' low immunogenicity, most likely conferred by the absence of MHC class 2 surface markers (27, 28), makes them suitable for allogeneic implantation and it is especially beneficial since physeal injuries afflict young, skeletally immature patients who are unlikely to be amenable to repeated clinical procedures. MSCs possess immunosuppressive properties, observed from their inhibitory effect on T-cell proliferation (29). Hence MSCs could potentially be utilised immediately after the detection of physeal injury as inflammation is the first stage in the physeal repair process, succeeded by fibrogenic, osteogenic and maturation phases (30). This is unlike CPCs, where their chondrogenic potential was unfortunately inhibited under inflammatory conditions (31). In a recent study, Planka *et al* compared the effect of implanting autogeneic and allogeneic BMSCs on physeal repair and did not detect any significant difference in the femur length, valgus deformity or phenotype of the newly formed cartilaginous tissues in rabbits transplanted with either MSC sources (21).

4. PHYSEAL DEFECT MODELS

4.1. *In vivo* model

From 1990 till now, there had only been 8 *in vivo* physeal-defect repair studies done where MSCs-embedded

scaffolds were implanted, one each into ovine (23) and porcine models (24), while the rest were conducted in rabbit models (13, 15, 19-22). It must be noted that small animal models like rabbits have excellent regeneration abilities but are limited in their ability to simulate human joint conditions as physeal thickness and chondrocyte behavior differs between species. Larger animal models such as goat and pig can better approximate human joints biomechanically (6, 19).

The experimental design employed was highly similar between the *in vivo* studies. They involved the defect creation sufficient to induce bone bridge formation, the excision of bone bridge 3 weeks after, and the implantation of scaffold, cell-embedded scaffolds (13,19-24), or scaffold-free 3-dimensional (3D) cell construct (15). Recent *in vivo* research using cells in physeal repair could be categorized into three kinds of defect models; traumatic physis injury at one side, iatrogenic injury in the center of physis, such as the injury caused by ACL reconstruction in juvenile patient, and Salter Harris Type II fracture, to mimic the types of commonly-occurring physeal fractures. However, research on the use of MSCs for physeal repair has only addressed the first two fracture types. Chen *et al*'s model is representative of the first category (13). This model was made at the medial half of the proximal tibial physis in 6 weeks old New Zealand White (NZW) rabbits by using #15 scalpel blade to damage the physis and small curette to remove the debris. In a larger 8 week old ovine model, 1 cm^2 defect with 0.5 cm height was created with a dental burr (23). All of abovementioned defects were created at the medial part of the proximal tibial physis. Planka *et al* created rabbit and porcine model defects, which are representative of the second category. A canal defect was created in the central lateral part of the distal femoral physis through a 3.5mm or 6 mm drill bit (21, 24). The defects created in the animal models were reported to be 7-9% (21, 24), 50% (13, 19) or greater than 50% (20) of the growth plate area.

Limb length and angular deformity were used as the criteria to evaluate the animal models as they are the two critical clinical changes caused by physeal injuries. Follow-up studies in the rabbit model were conducted at 4, 6, 8 and 16 weeks. Differences in limb length and angular deformity between therapy and control groups became significant beginning from 4 weeks postoperatively. This was observed to last up till 16 weeks post-implantation, after which the proximal tibia physis would close (32).

4.2. *In vitro* model

The development of a suitable *in vitro* model, despite lacking the dynamic physiological environment present *in vivo*, would provide an invaluable, controlled, cost-effective way of screening potential physeal treatments.

In our laboratory, we have observed the formation of zonal physeal cartilage from the differentiation of human bone marrow MSCs (33).This was achieved through seeding Bone Marrow Stem Cells (BMSCs) in an agarose scaffold, with access to the culture medium limited only to the scaffold surface. The cells were differentiated through the sequential addition of 10 ng/ml Transforming Growth Factor beta (TGFβ)3 followed by 100 ng/ml Bone Morphogenetic Protein (BMP2). The *in vitro* tissues developed a heterogeneous population of proliferating and hypertrophic chondrocytes. Histological sections revealed zonally-aligned chondrocytes, with less mature chondrocytes located at the base of the construct while hypertrophic chondrocytes and matrix mineralisation were observed closer to the surface of the construct.

A separate *in vitro* experiment studied MSC chondrogenesis for 2 weeks followed by hypertrophy induction through TGFβ3 withdrawal and the introduction of 1 nM of hormone triiodothyronine. MSC-derived chondrocytes went through a series of differentiation stages similar to those observed in the growth plate, with the potential for terminal differentiation (34).

In vitro models can be used to gain more information on mechanistic functions. The implantation of MSCs into physeal defects would result in the close proximity of implanted MSCs with host chondrocytes of the surrounding cartilage tissue. Recent MSC and chondrocyte co-cultures *in vitro* have shown that improved chondrogenesis was obtained when MSCs and chondrocytes were grown alongside each other (35, 36) to mimic the *in vivo* environment. *In vitro* co-culture techniques had been carried out to study the trophic effect of MSCs, where the replacement of 75% chondrocytes by MSCs still induced a robust chondrogenesis (35). The co-culture technique was also employed to form an *in vitro* MSC-derived osteochondral interface with calcified cartilage interface separating a non-calcified cartilage layer and an underlying bone layer (37).

5. EFFECT OF MSCS

5.1. Prevention of bone bridge formation

Bone bridge resection coupled with the insertion of various implants, was a technique introduced in 1967, to alleviate partial closure of the growth plate (38). Since then, a variety of materials have been investigated for this purpose. Examples include silica gel (39), cartilage (40), free growth plate (41), and vascularized growth plate (42), bone wax (43), fat (44) and periosteum (40). However, these interpositions could not halt the formation of bone bridges at the injured growth plate.

Scaffolds provide a physical infrastructure for cells like chondrocytes and MSCs. Its use in physeal repair had evolved from being inert interpositional material to bioactive vehicles. From very soft fibrin glue (20) to Gelfoam (22, 23), scaffolds of various mechanical characteristics have been used in physeal studies. The scaffolds which have been investigated in *in vivo* physeal repair model include Gelfoam (22, 23), agarose (13), sodium hyaluronate and type I collagen (21), chitin (19), and type I collagen with chitosan micro and nano fibers (24). Although no significant differences were observed between these materials, the scaffold's physical state could

influence cell containment. Cell leakage was encountered when a liquid-state 5% gelatin scaffold was used (22).

McCarty *et al* reported that the implantation of MSC-seeded gelatin-based Gelfoam into ovine physeal defect prevented formation of a bone bridge (23). However, bone bridge formation was still detected with the usage of Gelfoam in the rabbit model (22). The favourable outcome in the ovine model could have been due to a higher ($4x10^6$) cell number used. The use of scaffolds alone has mostly been observed to be insufficient in preventing the formation of bone bridges (13, 19-21, 24).

The manipulation of MSCs into a 3D structure to avoid the usage of scaffolds could not prevent the formation of bone bridges (15). In the instances when MSCs were implanted with scaffolds into physeal defects in both small and large animal models, the formation of bone bridges was consistently inhibited (13, 19-24). We postulate that MSCs play a greater role in bone bridge inhibition, but that scaffolds have an additive effect on the inhibitory process. The varus angulation of the animal models was also significantly corrected (13, 19-21, 24) compared to control groups without MSC implantation, where severe angular angulations of 30°-50° were observed (13, 19, 20, 22). The absence of bone bridge formation in the MSC-treated physeal defects compared to control was observed starting from 6 weeks post-implantation (22), and till up to 8 weeks (13, 19, 20). It could be longer but at the longest time point of 16 weeks, the disappearance of the physeal line did not allow any further conclusions to be reached in the rabbit models (19, 20). For the bigger-sized ovine and porcine models, the absence of bone bridge formation lasted up till 5 weeks (23) and 4 months (24), respectively.

Various factors could have contributed to the tissue repair observed. The state, number of implanted MSCs, matrix synthesized and bioactive factors released could have played a part in repopulating the defect and the prevention of bone bridge formation.

5.1.1. Number of Implanted MSCs

The numbers of MSCs implanted into the animal models were usually 1.6-2 $x10^6$ cells (20-22, 24), except for the ovine model (23), where $4x10^6$ cells were used. Despite the creation of large physeal defects amounting to 50% of the proximal physis (20), the seeding range seems adequate to prevent bone bridge formation. While proliferation of MSCs *in vitro* is optimal at low seeding density of 10^3 cells/cm^2 (45), the reverse is, at most part, true for *in vivo* implantations. A high seeding density could provide a condensed environment favourable for cell-cell and cell-matrix interaction, but necessary for chondrogenesis. Mechanical compaction of chondrocytes was found to result in concentrated matrix content and improved compressive properties of cartilage constructs (46). A seeding density of $3x10^5$ cells per 226mm^3 was found to be optimal for umbilical cord stem cells compared to $5x10^4$ cells, which led to a reduced osteogenic differentiation potential (47). This highlights that to achieve the best repair possible, the seeding density of MSCs would have to be optimized, depending on the defect size, location and possibly scaffold material.

5.1.2. Predifferentiated MSCs

Undifferentiated BMSCs were found to be capable of forming cartilage tissue postoperatively (13, 19, 20), but predifferentiation was an additional step undertaken by some groups (21-24), possibly to commit MSCs further into the chondrogenic lineage. Mature chondrocyte-derived cartilaginous tissues were found to be more resilient to inflammatory IL-1B and could induce chemokines transforming growth factor (TGFβ)-1 and monocyte chemoattractant protein-1 which are necessary for tissue repair (48). The MSC treatment seemed to succeed in preventing bone bridge formation regardless of a prior conditioning step, except in Yoshida *et al*'s study (15). Nonetheless, MSCs predifferentiated in a chondrogenic cocktail containing 10ng/ml TGFβ3 for a period of 10 days led to a better repair outcome in rabbit model (22), as seen from the absence of any significant varus deformity, and the histological evidence that irregularly-arranged chondrocytes were present in columns. Preconditioning of MSCs in chondrogenic medium for 1 day, at a higher concentration of 100 ng/ml TGFβ1 resulted in reduced angular deformity, but did not affect the limb length in a rabbit model (21). Experimental data taken from a similar study done in a minipig model, which exposed allogeneic porcine MSCs to chondrogenesis-inducing medium, successfully prevented the occurrence of angular deformity and increased limb length (24), independent of scaffold type. The improved repair outcome achieved could have been due to an accelerated repair obtained in the MSC-treated samples. Predifferentiated MSCs were also implanted into an ovine model but did not form neo-cartilage-like tissue, possibly due to a very short postoperative time frame of 5 weeks (23). In the abovementioned studies, the postoperative time point for analysis ranged from 6 weeks (22) to 4 months (21). Another possible reason could be that the exposure of MSCs to chondrogenic medium was only prior to implantation, unlike the 1 day (21, 24) and 10 days (22) noted in other studies.

5.1.3. Growth factor TGFβ

Proliferation and differentiation in the physis are tightly coordinated by hormonal and paracrine factors which work in concert. Chondrocytes respond to various growth-regulating hormones, peptide-signaling proteins and immunoregulatory cytokines (1). *In vivo*, exogenous growth factors modulate the proliferation, migration and differentiation of cells from edges of treated defects (49). *In vitro*, growth factors have similarly been shown to drive proliferation and differentiation of MSCs (50, 51).

The choice of TGF as the growth factor for predifferentiation in all 8 of the mentioned *in vivo* studies could be due to its constitutive expression in the growth plate. TGFβ1 can induce MSC proliferation (52) and TGFβ1-containing scaffolds were observed to result in significant cellular proliferation (53). TGFβ is also essential for MSC chondrogenesis. Although recruitment of MSCs into chondrogenesis has been shown by the

simultaneous addition of TGFβ1 and other factors (51), when used singly, it can drive MSCs into chondrogenesis (54). MSCs transfected with either TGF isoforms β1 (55) or β3 (56) were reported to induce chondrogenesis. Under serum-free chondrogenic differentiation medium, MSCs treated with either TGFβ1 or TGFβ3 underwent hypertrophy (57), similar to chondrocyte behaviour (58). The ability of MSCs treated with TGFβ1 or TGFβ3 isoforms to undergo hypertrophy was inversely proportional to the length of prior chondrogenic differentiation (58). The subcutaneous implantation of constructs containing TGFβ3 microspheres into nude mice led to the formation of hypertrophy at 8 weeks post-implantation. The absence of any correlation between TGFβ pre-treated cells and physeal repair could indicate that the usage of TGFβ alone in the predifferentiation cocktail is insufficient for subsequent *in vivo* physeal repair.

5.1.4. Extracellular matrix (ECM)

One of the explanations for the physeal repair observed was postulated to be due to the ECM-like nature of the scaffolds used. Chitin contains polyheterosaccharide which structurally resembles glycosaminoglycan found in cartilage (19) while Gelfoam, a porcine-derived gelatinous material, has the ability to trap water, thus mimicking the function of ECM (23) which resists compressive forces.

MSCs can also synthesize cartilaginous ECM. The ECM is integral for cell-matrix communications, to compensate for the lack of cell-cell interaction due to the isolated nature of the chondrocytes. Integrins are heterodimeric transmembrane receptors that enable communication between MSCs and the ECM (59). Integrins specific for fibronectin, vitronectin, collagens and osteopontin were hypothesized to play important roles in the generation of ECM, which in turn influenced maintenance of chondrocytic phenotype during chondrogenic differentiation of BMSCs and cord blood stem cells (60). Integrin-ECM contacts change during hMSC differentiation and surfaces presenting defined peptide sequences could be used to target α5, αV, β1 and β3 integrins to indirectly influence hMSC differentiation (60, 61).

5.1.5. Trophic effect of MSCs

Tracing of the labelled MSCs in several of the reported *in vivo* physeal repair studies has revealed that MSCs remained *in situ* at the implanted defect site for up to 3 weeks (21, 22). The cellular source of repair was shown, through labelling with immunofluorescent DiI lipophilic tracer dye (22), CM-DiI and iron oxide nanoparticles Resovist (21), to have been from MSCs which underwent differentiation to form chondrocytes. However, the increased cartilage formation in MSC-chondrocyte cocultures was postulated to be due to MSCs' trophic effect, which stimulated chondrocytes to proliferate and deposit matrix, rather than differentiation into chondrocytes. This was based on the observation that human MSCs became undetectable in pellet cocultures of human MSCs and bovine primary chondrocytes (62, 63). In a separate MSC-chondrocyte coculture experiment, a mutually beneficial effect was seen, where MSCs were found to enhance the proliferation of chondrocytes while chondrocytes enhanced MSC chondrogenesis (64). Put together, data gathered from various research publications show that MSCs generate and could also influence the production of ECM which is conducive for the dynamic interaction between cells and its surrounding matrix.

5.2. Formation of organized physeal repair tissue from the use of MSCs

The prevention of bone bridge formation has to be accompanied with the formation of zonal physeal tissue, so as to allow a functional restoration. Proper physeal tissue regeneration could be measured from the degree of reconstruction achieved in the repair tissue. While the formation of the proliferative physeal zone was observed in a study by Ahn *et al* (22), histological analysis in four other rabbit studies (13, 15, 19, 20) also showed chondrocyte columnation strongly resembling the zonal physis tissue, despite the excision of more than 50% of the proximal physis (20). The reconstruction of columnar physis structure was obtained through the use of MSCs seeded into scaffold materials like agarose (13), chitin fiber mesh (19) and fibrin glue (20), which showed comparable function in supporting implanted cells. Gelfoam sponge, fabricated from gelatinous porcine skin also achieved physeal repair within 6 weeks in a rabbit model (22) although it must be noted that the cells at the repair site were not linearly arranged. Histological analysis of MSC-seeded Gelfoam scaffold implanted for 5 weeks in an ovine model showed repair tissue which was not zonally-arranged, devoid of chondrocytes but consisted of a predominantly dense fibrous tissue (23). A possible explanation is that the prevention of bone bridge formation seen at 5 weeks post-implantation occurs prior to tissue remodelling processes. Further studies could be carried out to investigate if physeal tissue regeneration could be achieved in the ovine model at later time points.

5.2.1. Morphogen gradients

Chondrocytes in the physis are encased in extracellular milieu, where components vary according to the cartilage zone, with type II collagen and type X collagen constituting the predominant portion of the proliferating and hypertrophic zones respectively. The ECM has been discovered to contain positional signals, where morphogen gradients are established (65, 66) through proteoglycans (66). Proteoglycans are glycoproteins with an architecture consisting of glycosaminoglycan subunits linked to a protein core, and could influence morphogen receptor, binding affinity and cell response to secreted proteins. Gradients enable the diffusion of morphogens, which form polarized tissue and would develop into distinct cartilage zones observed in physis (67). The ability of MSCs to reproduce cartilaginous ECM is advantageous for physeal repair (13, 19-22, 24). MSCs were also found to synthesize chondrogenically superior ECM containing longer core protein and chondroitin sulphate (CS) chains than chondrocytes from age-matched or younger cartilage (68). The presence of two major cartilaginous ECM components, CS and hyaluronic acid (HA) in scaffolds accelerated the chondrogenic

process, which facilitated a significant up-regulation in expression of chondrogenic transcription factor SOX9 (69). ECM also optimized the effect of TGFβ1 in inducing chondrocyte differentiation (70).

5.2.2. Growth plate orientation factor (GPOF) and thyroid hormones

Growth plate orientation factor (GPOF) was mentioned by Abad *et al* in 2002 to describe the morphogen hypothesized to have the capacity in directing the alignment of proliferative chondrocytes into columns perpendicular to the growth plate within one week (10). Although the morphogen was not characterized, thyroxine, a thyroid hormone present in serum was recognized as a driving force behind columnar formation of cartilage tissue from chondrocytes (71). The addition of 50nM thyroxine to BMSCs induced the further differentiation of BMSCs into hypertrophic chondrocytes (72). Triiodotyronine, which contains one less iodine atom compared to thyroxine, was observed to similarly stimulate MSC hypertrophy (73), although the effect seemed to be dose-dependent, as 10 pM dose led to osteogenic differentiation of rat MSCs, but 1 pM had a reduced osteogenic effect and 10^5 pM dose reversed the effect (74). Although the zonal formation was not seen in MSCs treated with thyroid hormones, further research on its use with other factors could possibly reveal effects on columnar formation.

5.2.3. Scaffolds and pore sizes

The pore sizes in a scaffold could influence the outcome of MSC differentiation. Chitin (19) and chitosan (24) scaffolds with 100-300 μm-sized pores were used in physeal repair. Columnar-organised tissue was reported in the rabbit model (19). Although this was not seen in the pig model, the defect site was filled with hyaline cartilage (24). The construction of polymer scaffolds containing pore size gradients was found to influence the zonal organization within tissue-engineered cartilage constructs. *In vitro* cell seeding showed that pore-size gradients promoted anisotropic cell distribution highly resembling articular cartilage zones (75). Recently, microsized pores of 27-29μm were seen to be more beneficial for chondrogenesis compared to nanosized pores (76).

5.3. Further research: Possible further studies to optimize physeal repair, based on MSC therapy in articular cartilage repair

5.3.1. MSC seeding onto scaffold

Conventional diffusion-based *in vitro* culturing of MSCs has been found to result in uneven cellular distributions at the scaffold periphery. MSCs seeded onto prepared scaffolds have been observed to remain aggregated at the scaffold surface (22). This could be further optimized, as dynamic rotational culturing encouraged cellular migration, which penetrated throughout the whole scaffold (77). In another study, the usage of rotating bioreactors to provide a dynamic laminar flow was found to lead to a better overall tissue structure and composition for engineered cartilage, compared to static culture (78).

5.3.2. Fibroblast growth factor (FGF)

The usage of TGFβ alone has been shown to be inadequate to repair physeal defects. However, perhaps TGFβ's effects could be improved on, possibly through the inclusion of other complementary growth factors. An example is Fibroblast growth factor (FGF)-2 also known as basic FGF, which is secreted by growth plate chondrocytes (79, 80). Fibroblast growth factor (FGF) influences cell proliferation and differentiation of many cell types during development and tissue repair. The administration of FGF2 through an osmotic pump for one day was found to be successful in the recruitment of MSCs to induce articular cartilage repair (81). MSCs treated with 1ng/ml FGF2 during expansion showcased significantly improved cellular proliferation, and maintained chondrogenic ability up till 46 (82) and 50 (83) population doublings. At a higher dosage of 10ng/ml, a similar pattern was observed, although the extent was reduced to 30 population doublings (84). It was recently reported that 1ng/ml of FGF2 led to a better upregulation in Sox9 levels compared to 10ng/ml of FGF2, but both conditions eventually led to an earlier initiation of chondrogenesis in human bone marrow-derived MSCs (85). It was discovered that FGF2 selectively encourages, within the heterogeneous pool of MSCs, the survival of a subset of progenitor cells which possess longer telomere lengths. This characteristic had been linked to a superior chondrogenic capacity (82). FGF2 also primes MSCs for chondrogenesis by increasing Sox9 protein levels in both proliferating and non-proliferating hMSCs (86) and by preventing cellular senescence (87). The favourable effect of FGF on chondrogenesis in the abovementioned studies was observed after its usage during MSC expansion, and could be useful for physeal repair. It must be noted however, that FGF2 treatment during chondrogenic differentiation eliminated the chondrogenic effect brought about by BMP6 and TGFβ3 in MSCs derived from adipose tissue (88). FGF is also involved in the induction of chondrocyte hypertrophic differentiation, and is a BMP antagonist (89).

5.3.3. Dynamic compression

Growth plate chondrocytes are not spared from the compressive and tensile forces experienced in the knee. Mechanobiology conditioning like hydrostatic pressure, osmotic pressure, shear and ultrasound and compression affects the physeal tissue (90). Although the effect of dynamic compression on MSCs implanted into physeal defects is unknown, MSCs subjected to dynamic compression in articular cartilage defects responded by exhibiting temporal and spatial changes in the expression of cartilage marker genes (91-93). MSCs are sensitive to mechanical loading only after they have undergone chondrogenesis (94, 95). It was also noted that the core region of the constructs was more conducive for chondrogenesis than the annulus, evident from gene expression and ECM synthesis (96). These raise the possibility that predifferentiated MSCs further subjected to dynamic compression, could have a greater capacity to repair physeal defects, compared to the current practice of using predifferentiated cells only. Care must be taken in the designing of construct, to ensure even distribution of compressive forces.

5.3.4. Hypoxia

Chondrocytes found in the native physeal cartilage exist in a typical, low-oxygen environment. MSCs reside under hypoxic conditions *in vivo*, between 4% and 7% oxygen (97) and have shown excellent chondrogenic potential even under hypoxic conditions of 3% oxygen (98). When the hypoxia-expanded MSCs were embedded in *in situ* solidifying gelatin hydrogels, the chondrogenesis was further enhanced, suggesting that MSCs, cultured and differentiated in hypoxic conditions managed to undergo chondrogenesis (99). Hypoxia inducible factor (HIF) is reportedly necessary for chondrocytes to survive extremely low oxygen tension (100). HIF's involvement in MSC chondrogenesis was also proven when rat MSCs with siRNA knockdown of HIF-1alpha did not undergo chondrogenesis (101). A study demonstrated that continuous exposure to low oxygen tension is a more potent pro-chondrogenic stimulus than 1h/day of dynamic compression for porcine MSCs embedded in agarose hydrogels (102). The combination of hypoxia, with inflammatory IL-1beta also did not dampen chondrogenesis (103). The protocol for MSC culture could include hypoxic conditions to further enhance MSC chondrogenic ability, prior to implantation into physeal defects.

5.3.5. Scaffolds

Multiple properties of scaffolds, including biodegradability and stiffness, could be flexibly altered to suit the needs of different defects. One scaffold material which has not been tested in physeal repair but has shown potential in articular cartilage repair is Oligo (poly (ethylene glycol) fumarate) (OPF) hydrogel, a biodegradable scaffold with high water content (104). The implantation of biodegradable OPF hydrogel composites scaffolds alone resulted in zonally-organized hyaline-like tissue containing hypertrophic cartilage (105). The addition of cartilaginous proteins into scaffolds have shown encouraging articular cartilage repair. An enhancement in expression of transcription factor Sox9 mRNA and downregulation of type I collagen mRNA were detected upon addition of HA and CS to alginate scaffolds (66) and the crosslinkage of CS to type II collagen scaffold increased the gene expression of type II collagen and aggrecan (106). The combination of synthetic polymer polyethylene glycol (PEG) and natural biopolymers HA and CS have been reported to successfully create unique microenvironments which supported the differentiation of MSCs into zonal articular cartilage (107). In view of these findings, the usage of scaffolds incorporated with ECM components is another potential candidate that could be investigated in future physeal defect repair studies.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

Strides have been made in research on physeal cartilage repair. An early intervention to restore the biological function of damaged physis is imperative, to prevent the occurrence of any growth disturbance. The challenge is the total and precise removal of bone bridge (s) and to consistently achieve regeneration of a well-defined zonally organized cartilage. The highly complex tissue structure of physes would benefit from synergized collaborations across different disciplines, to tap on various expertises. Cell biologists are trained in biochemical cues, while mechanical engineers could design and build composite scaffolds with specific, controllable degradation rates and properties. Chemists and material engineers could provide insight into biochemical compatibility and clinicians are well-attuned to the disease pathology and pathway leading to clinical trial. Hopefully, this will pave the way to the development of long-awaited, clinically-tested and approved MSC treatments for physeal injuries.

7. ACKNOWLEDGEMENTS

The authors would like to thank Dr Ren Xiafei for his assistance in the writing of the initial draft.

8. REFERENCES

1. M. Ramachandran, D. L. Skaggs: Physeal injuries. In Green NE and Swiontknowski MF. Skeletal trauma in children, 4th ed. Elsevier, 19-39 (2009)

2. K. Ecklund, D. Jaramillo: Imaging of growth disturbance in children. *Radiol Clin North Am*, 39(4), 823-41 (2001)

3. E. Fischerauer, N. Heidari, B. Neumayer, A. Deutsch, A. M. Weinberg: The spatial and temporal expression of VEGF and its receptors 1 and 2 in post-traumatic bone bridge formation of the growth plate. *J Mol Histol*, 42(6), 513-22 (2011)

4. G. I. Pagenstert, B. Hintermann, A. Barg, A. Leumann, V. Valderrabano: Realignment surgery as alternative treatment of varus and valgus ankle osteoarthritis. *Clin Orthop Relat Res*, 462, 156-68 (2007)

5. B. K. Foster, A. L. Hansen, G. J. Gibson, J.J. Hopwood, G. F. Binns, O. W. Wiebkin: Reimplantation of growth plate chondrocytes into growth plate defects in sheep. *J Orthop Res*, 8(4), 555-64 (1990)

6. E. H. Lee, F. Chen, J. Chan, K. Bose: Treatment of growth arrest by transfer of cultured chondrocytes into physeal defects. *J Pediatr Orthop*, 18(2), 155-60 (1998)

7. X. B. Jin, Z. J. Luo, J. Wang: Treatment of rabbit growth plate injuries with an autologous tissue-engineered composite. An experimental study. *Cells Tissues Organs*, 183(2), 62-7 (2006)

8. S. K. Chow, K.M . Lee, L. Qin, K. S. Leung, W. H. Cheung: Restoration of longitudinal growth by bioengineered cartilage pellet in physeal injury is not affected by low intensity pulsed ultrasound. *J Biomed Mater Res B Appl Biomater*, 99(1), 36-44 (2011)

9. K. von der Mark, V. Gauss, H. von der Mark, P. Müller: Relationship between cell shape and type of collagen synthesised as chondrocytes lose their cartilage phenotype in culture. *Nature*, 267(5611), 531-2 (1977)

10. V. Abad, J. L. Meyers, M. Weise, R. I. Gafni, K. M. Barnes, O. Nilsson, J. D. Bacher, J. Baron: The role of the

resting zone in growth plate chondrogenesis: *Endocrinology*, 143(5), 1851-7 (2002)

11. H. Castro-Malaspina, R. E. Gay, G. Resnick, N. Kapoor, P. Meyers, D. Chiarieri, S. McKenzie, H. E. Broxmeyer & M. A. Moore: Characterization of human bone marrow fibroblast colony-forming cells (CFU-F) and their progeny. *Blood*, 56, 289−301 (1980)

12. P. A. Zuk, M. Zhu, H. Mizuno, J. Huang, J. W. Futrell, A. J. Katz, P. Benhaim, H. P. Lorenz & M. H. Hedrick: Multilineage cells from human adipose tissue: implications for cell-based therapies: *Tissue Eng*, 7(2), 211−228 (2001)

13. F. Chen, J. H. Hui, W. K. Chan & E. H. Lee: Cultured mesenchymal stem cell transfers in the treatment of partial growth arrest. *J Pediatr Orthop*, 23, 425-9 (2003)

14. C. De Bari, F. Dell'Accio, P. Tylzanowski & F. P. Luyten: Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum*, 44(8), 1928−1942 (2001)

15. K. Yoshida, C. Higuchi, A. Nakura, N. Nakamura, H. Yoshikawa: Treatment of partial growth arrest using an in vitro-generated scaffold-free tissue-engineered construct derived from rabbit synovial mesenchymal stem cells. *J Pediatr Orthop*, 32(3), 314-21 (2012)

16. P. Bosch, D. S. Musgrave, J. Y. Lee, J. Cummins, F. Shuler, S. C. Ghivizzani, C. Evans, P. D. Robbins & J. Huard: Osteoprogenitor cells within skeletal muscle. *J Orthop Res*, 18(6), 933−944 (2000)

17. M. Dominici, K. L. Blanc, I. Mueller, I. Slaper-Cortenbach, F. C. Marini, D. S. Krause, R. J. Deans, A. Keatings, D. J. Prockop, E. M. Horwitz: Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*, 8(4), 315-317 (2006)

18. S.P. Bruder, N. Jaiswal, S.E. Haynesworth: Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem*, 64(2), 278-94 (1997)

19. L. Li, J. H. Hui, J. C. Goh, F. Chen, E. H. Lee: Chitin as a scaffold for mesenchymal stem cells transfers in the treatment of partial growth arrest. *J Pediatr Orthop*, 4(2), 205-10 (2004)

20. J. H. Hui, L. Li, Y. H. Teo, H. W. Ouyang, E. H. Lee: Comparative study of the ability of mesenchymal stem cells derived from bone marrow, periosteum, and adipose tissue in treatment of partial growth arrest in rabbit. *Tissue Eng*, 11(5-6), 904-12 (2005)

21. L. Planka, P. Gal, H. Kecova, J. Klima, J. Hlucilova, E. Filova, E. Amler, P. Krupa, L. Kren, R. Srnec, L. Urbanova, J. Lorenzova, A. Necas: Allogeneic and autogenous transplantations of MSCs in treatment of the physeal bone bridge in rabbits. *BMC Biotechnol*, 12(8), 70 (2008)

22. J. I. Ahn, T. S Canale, S. D. Butler, K. A. Hasty: Stem cell repair of physeal cartilage. *J Orthop Res*, 22(6), 1215-21 (2004)

23. R. C. McCarty, C. J. Xian, S. Gronthos, A. C. Zannettino, B. K. Foster: Application of autologous bone marrow derived mesenchymal stem cells to an ovine model of growth plate cartilage injury. *Open Orthop J*, 23(4), 204-10 (2010)

24. L. Plánka, A. Necas, R. Srnec, P. Rauser, D. Starý, J. Jancár, E. Amler, E. Filová, J. Hlucilová, L. Kren, P. Gál: Use of allogenic stem cells for the prevention of bone bridge formation in miniature pigs. *Physiol Res*, 58(6), 885-93 (2009)

25. S. Kern, H. Eichler, J. Stoeve, H. Klüter, K. Bieback: Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells*, 24(5), 1294-301 (2006)

26. S. A. Wexler, C. Donaldson, P. Denning-Kendall, C. Rice, B. Bradley, J. M. Hows: Adult bone marrow is a rich source of human mesenchymal 'stem' cells but umbilical cord and mobilized adult blood are not. *Br J Haematol*, 121(2), 368-74 (2003)

27. K. Le Blanc, O. Ringdén: Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*, 11(5), 321-34 (2005)

28. T. Deuse, M. Stubbendorff, K. Tang-Quan, N. Phillips, M. A. Kay, T. Eiermann, T. T. Phan, H. D. Volk, H. Reichenspurner, R. C. Robbins, S. Schrepfer: Immunogenicity and immunomodulatory properties of umbilical cord lining mesenchymal stem cells. *Cell Transplant*. 20(5), 655-67 (2011)

29. Y. Garfias, J. Nieves-Hernandez, M. Garcia-Mejia, C. Estrada-Reyes, M. C. Jimenez-Martinez: Stem cells isolated from the human stromal limbus possess immunosuppressant properties. *Mol Vis*. 18, 2087-95 (2012)

30. C. J. Xian, F. H. Zhou, R. C. McCarty, B. K. Foster: Intramembranous ossification mechanism for bone bridge formation at the growth plate cartilage injury site. *J Orthop Res*, 22, 417-426 (2004)

31. K. Pichler, B. Schmidt, E. E. Fischerauer, B. Rinner, G. Dohr, A. Leithner, A. M. Weinberg. Behaviour of human physeal chondro-progenitorcells in early growth plate injury response in vitro. *Int Orthop*, 36(9), 1961-6 (2012)

32. I. Masoud, F. Shapiro, R. Kent, A. Moses.: A longitudinal study of the growth of the New Zealand white rabbit: Cumulative and biweekly incremental growth rates for body length, body weight, femoral length, and tibial length. *J Orthop Res*, 4, 221–231 (1986)

33. J. F. Schmitt, S. K. Hua, Y. Zheng, J. H. Po, L. Hin: Sequential differentiation of mesenchymal stem cells in an agarose scaffold promotes a physis-like zonal alignment of chondrocytes. *J Orthop Res*, (2012)

34. M. B. Mueller, R. S. Tuan : Functional characterization of hypertrophy in chondrogenesis of human mesenchymal stem cells. *Arthritis Rheum*. 58(5), 1377-88 (2008)

35. V. Lettry, K. Hosoya, S. Takagi, M. Okumura: Coculture of equine mesenchymal stem cells and mature equine articular chondrocytes results in improved chondrogenic differentiation of the stem cells. *Jpn J Vet Res*, 58(1), 5-15 (2010)

36. V. V. Meretoja, R. L. Dahlin, F. K. Kasper, A. G. Mikos: Enhanced chondrogenesis in co-cultures with articular chondrocytes and mesenchymal stem cells. *Biomaterials*, 33(27), 6362-9 (2012)

37. H. W. Cheng, K. D. Luk, K. M. Cheung, B. P. Chan: In vitro generation of an osteochondral interface from mesenchymal stem cell-collagen microspheres. *Biomaterials*, 32(6), 1526-35 (2011)

38. G. Lonjon, P. Y. Barthel, B. Ilharreborde, P. Journeau, P. Lascombes, F. Fitoussi: Bone bridge resection for correction of distal radial deformities after partial growth plate arrest: two cases and surgical technique. *J Hand Surg Eur Vol,* 37(2), 170-5 (2012)

39. R. W. Bright: Operative correction of partial epiphyseal plate closure by osseous-bridge resection and silicone-rubber implant. An experimental study in dogs. *J Bone Joint Surg Am*, 56(4), 655-64 (1974)

40. T. Wirth, S. Byers, R. W. Byard, J. J. Hopwood, B. K. Foster: The implantation of cartilaginous and periosteal tissue into growth plate defects. *Int Orthop,* 18(4), 220-8 (1994)

41. A. Olin, C. Creasman, F. Shapiro: Free physeal transplantation in the rabbit. An experimental approach to focal lesions. *J Bone Joint Surg Am*, 66(1), 7-20 (1984)

42. C. V. Bowen, B. M. O'Brien, G. J. Gumley: Experimental microvascular growth plate transfers. Part 2--Investigation of feasibility. *J Bone Joint Surg Br,* 70(2), 311-4 (1988)

43. N. S. Broughton, D. R. Dickens, W. G. Cole, M. B. Menelaus: Epiphyseolysis for partial growth plate arrest. Results after four years or at maturity. *J Bone Joint Surg Br*, 71(1), 13-6 (1989)

44. B. K. Foster, B. John, C. Hasler: Free fat interpositional graft in acute physeal injuries: the anticipatory Langenskiöld procedure. *J Pediatr Orthop*, 20(3), 282-5 (2000)

45. T. Mochizuki, T. Muneta, Y. Sakaguchi, A. Nimura, A. Yokoyama, H. Koga, I. Sekiya: 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(3), 843-53 (2006)

46. E. Han, C. Ge, A. C. Chen, B. L. Schumacher, R. L. Sah: Compaction enhances extracellular matrix content and mechanical properties of tissue-engineered cartilaginous constructs. *Tissue Eng Part A*, 18(11-12), 1151-60 (2012)

47. H. Zhou, M. D. Weir, H. H. Xu: Effect of cell seeding density on proliferation and osteodifferentiation of umbilical cord stem cells on calcium phosphate cement-fiber scaffold. *Tissue Eng Part A*, 17(21-22), 2603-13 (2011)

48. S. Francioli, C. Cavallo, B. Grigolo, I. Martin, A. Barbero: Engineered cartilage maturation regulates cytokine production and interleukin-1β response. *Clin Orthop Relat Res,* 469(10), 2773-84 (2011)

49. J. F. Lafreniere, P. Mills, J. P. Tremblay, E. E. Fahime: Growth factors improve the in vivo migration of human skeletal myoblasts by modulating their endogenous proteolytic activity. *Transplantation*, 77(11), 1741-7 (2004)

50. R. Ramasamy, C. K. Tong, W. K. Yip, S. Vellasamy, B. C. Tan, H. F. Seow: Basic fibroblast growth factor modulates cell cycle of human umbilical cord-derived mesenchymal stem cells. *Cell Prolif*, 45(2), 132-9 (2012)

51. F. Ng, S. Boucher, S. Koh, K. S. Sastry, L. Chase, U. Lakshmipathy, C. Choong, Z. Yang, M. C. Vemuri, M. S. Rao, V. Tanavde: PDGF, TGF-beta, and FGF signaling is important for differentiation and growth of mesenchymal stem cells (MSCs): transcriptional profiling can identify markers and signaling pathways important in differentiation of MSCs into adipogenic, chondrogenic, and osteogenic lineages. *Blood*, 112(2), 295-307 (2008)

52. H. Jian, X. Shen, I. Liu, M. Semenov, X. He, X. F. Wang: Smad3-dependent nuclear translocation of beta-catenin is required for TGF-beta1-induced proliferation of bone marrow-derived adult human mesenchymal stem cells. *Genes Dev*, 20(6), 666-74 (2006)

53. H. Diao, J. Wang, C. Shen, S. Xia, T. Guo, L. Dong, C. Zhang, J. Chen, J. Zhao, J. Zhang: Improved cartilage regeneration utilizing mesenchymal stem cells in TGF-beta1 gene-activated scaffolds. *Tissue Eng Part A*, 15(9), 2687-9 (2009)

54. S. Weiss, T. Hennig, R. Bock, E. Steck, W. Richter: Impact of growth factors and PTHrP on early and late chondrogenic differentiation of human mesenchymal stem cells. *J Cell Physiol*, 223(1), 84-93 (2010)

55. X. Cao, W. Deng, Y. Wei, Y. Yang, W. Su, Y. Wei, X. Xu, J. Yu: Incorporating pTGF-β1/calcium phosphate

nanoparticles with fibronectin into 3-dimensional collagen/chitosan scaffolds: efficient, sustained gene delivery to stem cells for chondrogenic differentiation. *Eur Cell Mater*, 23, 81-93 (2012)

56. C. H. Lu, K. J. Lin, H. Y. Chiu, C. Y. Chen, T. C. Yen, S. M. Hwang, Y. H. Chang, Y. C. Hu: Improved Chondrogenesis and Engineered Cartilage Formation from TGF-β3-Expressing Adipose-Derived Stem Cells Cultured in the Rotating-Shaft Bioreactor. *Tissue Eng Part A*, (2012), [Epub ahead of print]

57. M. B. Mueller, M. Fischer, J. Zellner, A. Berner, T. Dienstknecht, L. Prantl, R. Kujat, M. Nerlich, R. S. Tuan, P. Angele: Hypertrophy in mesenchymal stem cell chondrogenesis: effect of TGF-beta isoforms and chondrogenic conditioning. *Cells Tissues Organs*, 192(3), 158-66 (2010)

58. R. Narcisi, R. Quarto, V. Ulivi, A. Muraglia, L. Molfetta, P. Giannoni: TGF β-1 administration during ex vivo expansion of human articular chondrocytes in a serum-free medium redirects the cell phenotype toward hypertrophy. *J Cell Physiol*, 227(9), 3282-90 (2012)

59. U. R. Goessler, P. Bugert, K. Bieback, J. Stern-Straeter, G. Bran, H. Sadick, K. Hörmann, F. Riedel: In vitro analysis of integrin expression in stem cells from bone marrow and cord blood during chondrogenic differentiation. *J Cell Mol Med*, 13(6), 1175-84 (2009) Retraction in: *J Cell Mol Med*. 13(9B), 4085 (2009)

60. J. E. Frith, R. J. Mills, J. E. Hudson, J. J. Cooper-White: Tailored Integrin-Extracellular Matrix Interactions to Direct Human Mesenchymal Stem Cell Differentiation. *Stem Cells Dev*, 21(13), 2442-56 (2012)

61. J. E Frith, R. J. Mills, J. J. Cooper-White: Lateral spacing of adhesion peptides influences human mesenchymal stem cell behaviour. *J Cell Sci*, 125(2), 317-27 (2012)

62. L. Wu, J. C. Leijten, N. Georgi, J. N. Post, C. A. van Blitterswijk, M. Karperien: Trophic effects of mesenchymal stem cells increase chondrocyte proliferation and matrix formation. *Tissue Eng Part A*, 17(9-10), 1425-36 (2011)

63. L. Wu, H. J. Prins, M. N. Helder, C. A. van Blitterswijk, M. Karperien: Trophic Effects of Mesenchymal Stem Cells in Chondrocyte Co-Cultures are Independent of Culture Conditions and Cell Sources. *Tissue Eng Part A*, 18(15-16), 1542-51 (2012)

64. C. Acharya, A. Adesida, P. Zajac, M. Mumme, J. Riesle, I. Martin, A.Barbero: Enhanced chondrocyte proliferation and mesenchymal stromal cells chondrogenesis in coculture pellets mediate improved cartilage formation. *J Cell Physiol*, 227(1), 88-97 (2012)

65. P. C. Salinas: Signaling at the vertebrate synapse: new roles for embryonic morphogens? *J Neurobiol. Review*, 64(4), 435-45 (2005)

66. E. E. Coates, C. N. Riggin, J. P. Fisher: Matrix molecule influence on chondrocyte phenotype and proteoglycan 4 expression by alginate-embedded zonal chondrocytes and mesenchymal stem cells. *J Orthop Re,* (2012)

67. C. E. de Andrea, P. C. Hogendoorn: Epiphyseal growth plate and secondary peripheral chondrosarcoma: the neighbours matter. Review. *J Pathol*, 226(2), 219-28 (2012)

68. P. W. Kopesky, H. Y. Lee, E. J. Vanderploeg, J. D. Kisiday, D. D. Frisbie, A. H. Plaas, C. Ortiz, A. J. Grodzinsky: Adult equine bone marrow stromal cells produce a cartilage-like ECM mechanically superior to animal-matched adult chondrocytes. *Matrix Biol*, 29(5), 427-38 (2010)

69. C. M. Murphy, A. Matsiko, M. G. Haugh, J. P. Gleeson, F. J. O'Brien: Mesenchymal stem cell fate is regulated by the composition and mechanical properties of collagen-glycosaminoglycan scaffolds. *J Mech Behav Biomed Mater*, 11, 53-62 (2012)

70. J. L. Allen, M. E. Cooke, T. Alliston: ECM Stiffness Primes the TGFβ Pathway to Promote Chondrocyte Differentiation. *Mol Biol Cell,* 23(18), 3731-3742 (2012)

71. R. T. Ballock, A. H. Reddi: Thyroxine is the serum factor that regulates morphogenesis of columnar cartilage from isolated chondrocytes in chemically defined medium. *J Cell Biol*, 126(5), 1311-8 (1994)

72. A. M Mackay, S. C. Beck, J. M. Murphy, F. P. Barry, C. O. Chichester, M. F. Pittenger: Chondrogenic differentiation of cultured human mesenchymal stem cells from marrow. *Tissue Eng. Winter,* 4(4), 415-28 (1998)

73. M. A. Mello, R. S. Tuan: Effects of TGF-beta1 and triiodothyronine on cartilage maturation: in vitro analysis using long-term high-density micromass cultures of chick embryonic limb mesenchymal cells. *J Orthop Res,* 24(11), 2095-105 (2006)

74. J. N. Boeloni, N. M. Ocarino, A. B. Melo, J. F. Silva, P. Castanheira, A. M. Goes, R. Serakides: Dose-dependent effects of triiodothyronine on the osteogenic differentiation of rat bone marrow mesenchymal stem cells. *Horm Res*, 72(2), 88-97 (2009)

75. T. B. Woodfield, C. A. Van Blitterswijk, J. De Wijn, T. J. Sims, A. P. Hollander, J. Riesle: Polymer scaffolds fabricated with pore-size gradients as a model for studying the zonal organization within tissue-engineered cartilage constructs. *Tissue Eng,* 11(9-10), 1297-311 (2005)

76. S. Shanmugasundaram, H. Chaudhry, T. L. Arinzeh: Microscale versus nanoscale scaffold architecture for mesenchymal stem cell chondrogenesis. *Tissue Eng Part A*, 17(5-6), 831-40 (2011)

77. C. T. Buckley, K. U. O'Kelly: Maintaining cell depth viability: on the efficacy of a trimodal scaffold pore architecture and dynamic rotational culturing. *J Mater Sci Mater Med*, 21(5), 1731-8 (2010)

78. B. Obradovic, I. Martin, L. E. Freed, G. Vunjak-Novakovic: Bioreactor studies of natural and tissue engineered cartilage. *Ortop Traumatol Rehabil*, 3(2), 181-9 (2001)

79. D. M. Ornitz: FGF signaling in the developing endochondral skeleton. FGF signaling in the developing endochondral skeleton. *Cytokine Growth Factor Rev,* 16(2), 205-13 (2005)

80. R. Sullivan, M. Klagsbrun: Purification of cartilage-derived growth factor by heparin affinity chromatography. *J Biol Chem,* 260(4), 2399-403 (1985)

81. H. Chuma, H. Mizuta, S. Kudo, K. Takagi, Y. Hiraki: One day exposure to FGF-2 was sufficient for the regenerative repair of full-thickness defects of articular cartilage in rabbits. *Osteoarthritis Cartilage*, 12(10), 834-42 (2004)

82. S. Yanada, M. Ochi, K. Kojima, P. Sharman, Y. Yasunaga, E. Hiyama: Possibility of selection of chondrogenic progenitor cells by telomere length in FGF-2-expanded mesenchymal stromal cells: *Cell Prolif*, 39(6), 575-84 (2006)

83. G. Bianchi, A. Banfi, M. Mastrogiacomo, R. Notaro, L. Luzzatto, R. Cancedda, R. Quarto: Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2. *Exp Cell Res*, 287(1), 98-105 (2003)

84. L. A. Solchaga, K. Penick, V. M. Goldberg, A. I. Caplan, J. F. Welter: Fibroblast growth factor-2 enhances proliferation and delays loss of chondrogenic potential in human adult bone-marrow-derived mesenchymal stem cells: *Tissue Eng Part A*, 16(3), 1009-19 (2010)

85. T. Cheng, C. Yang, N. Weber, H. T. Kim, A. C. Kuo: Fibroblast growth factor 2 enhances the kinetics of mesenchymal stem cell chondrogenesis. *Biochem Biophys Res Commun.* (2012)

86. A. M. Handorf, W. J. Li: Fibroblast growth factor-2 primes human mesenchymal stem cells for enhanced chondrogenesis. *PLoS One*, 6(7), e22887 (2011)

87. D. L. Coutu, M. François, J. Galipeau: Inhibition of cellular senescence by developmentally regulated FGF receptors in mesenchymal stem cells. *Blood*, 117(25), 6801-12 (2011)

88. F. Hildner, A. Peterbauer, S. Wolbank, S. Nürnberger, S. Marlovits, H. Redl, M. van Griensven, C. Gabriel: FGF-2 abolishes the chondrogenic effect of combined BMP-6 and TGF-beta in human adipose derived stem cells. *J Biomed Mater Res A*, 94(3), 978-87 (2010)

89. E. Minina, C. Kreschel, M. C. Naski, D. M. Ornitz, A. Vortkamp: Interaction of FGF, Ihh/Pthlh, and BMP signaling integrates chondrocyte proliferation and hypertrophic differentiation. *Dev Cell,* 3(3), 439-49 (2002)

90. D. Schumann, R. Kujat, M. Nerlich, P. Angele: Mechanobiological conditioning of stem cells for cartilage tissue engineering. *Biomed Mater Eng. Review*, 16(4) Suppl, S37-52 (2006)

91. M. G. Haugh, E. G. Meyer, S. D. Thorpe, T. Vinardell, G. P. Duffy, D. J. Kelly: Temporal and spatial changes in cartilage-matrix-specific gene expression in mesenchymal stem cells in response to dynamic compression. *Tissue Eng Part A*, 17(23-24), 3085-93 (2011)

92. R. L Mauck, B. A. Byers, X. Yuan, R. S. Tuan. Regulation of cartilaginous ECM gene transcription by chondrocytes and MSCs in 3D culture in response to dynamic loading. *Biomech Model Mechanobiol*, 6(1-2), 113-25 (2007)

93. P. Angele, D. Schumann, M. Angele, B. Kinner, C. Englert, R. Hente, B. Füchtmeier, M. Nerlich, C. Neumann, R. Kujat: Cyclic, mechanical compression enhances chondrogenesis of mesenchymal progenitor cells in tissue engineering scaffolds. *Biorheology*, 41(3-4), 335-46 (2004)

94. A. H. Huang, M. J. Farrell, M. Kim, R. L. Mauck: Long-term dynamic loading improves the mechanical properties of chondrogenic mesenchymal stem cell-laden hydrogel. *Eur Cell Mater*, 26(19), 72-85 (2010)

95. S. D. Thorpe, C. T. Buckley, T. Vinardell, F. J. O'Brien, V. A. Campbell, D. J. Kelly: The response of bone marrow-derived mesenchymal stem cells to dynamic compression following TGF-beta3 induced chondrogenic differentiation. *Ann Biomed Eng*, 38(9), 2896-909 (2010)

96. D. Pelaez, N. Arita, H. S. Cheung: Extracellular signal-regulated kinase (ERK) dictates osteogenic and/or chondrogenic lineage commitment of mesenchymal stem cells under dynamic compression. *Biochem Biophys Res Commun,* 417(4), 1286-91 (2012)

97. G. Pattappa, H. K. Heywood, J. D. de Bruijn, D. A Lee: The metabolism of human mesenchymal stem cells during proliferation and differentiation. *J Cell Physiol*, 226(10), 2562-70 (2011)

98. A. B. Adesida, A. Mulet-Sierra, N. M. Jomha: Hypoxia mediated isolation and expansion enhances the chondrogenic capacity of bone marrow mesenchymal stromal cells. *Stem Cell Res Ther*, 3(2), 9 (2012)

99. J. Müller, K. Benz, M. Ahlers, C. Gaissmaier, J. Mollenhauer: Hypoxic conditions during expansion culture prime human mesenchymal stromal precursor cells for chondrogenic differentiation in three-dimensional cultures. *Cell Transplant* (2011)

100. D. Pfander, T. Cramer, E. Schipani, R. S. Johnson: HIF-1alpha controls extracellular matrix synthesis by epiphyseal chondrocytes. *J Cell Sci*, 116(9), 1819-26 (2003)

101. M. Kanichai, D. Ferguson, P. J. Prendergast, V. A. Campbell: Hypoxia promotes chondrogenesis in rat mesenchymal stem cells: a role for AKT and hypoxia-inducible factor (HIF)-1alpha. *J Cell Physiol*, 216(3), 708-15 (2008)

102. E. G. Meyer, C. T. Buckley, S. D. Thorpe, D. J. Kelly: Low oxygen tension is a more potent promoter of chondrogenic differentiation than dynamic compression. *J Biomech,* 43(13), 2516-23 (2010)

103. T. Felka, R. Schäfer, B. Schewe, K. Benz, W. K. Aicher: Hypoxia reduces the inhibitory effect of IL-1beta on chondrogenic differentiation of FCS-free expanded MSC. *Osteoarthritis Cartilage*, 17(10), 1368-76 (2009)

104. T. A. Holland, E. W. Bodde, V. M. Cuijpers, L. S. Baggett, Y. Tabata, A. G. Mikos, J. A. Jansen: Degradable hydrogel scaffolds for in vivo delivery of single and dual growth factors in cartilage repair. *Osteoarthritis Cartilage*, 15(2), 187-97 (2007)

105. X. Guo, H. Park, S. Young, J. D. Kretlow, J. J. van den Beucken, L. S. Baggett, Y. Tabata, F. K. Kasper, A. G. Mikos, J. A. Jansen: Repair of osteochondral defects with biodegradable hydrogel composites encapsulating marrow mesenchymal stem cells in a rabbit model. *Acta Biomater*, 6(1), 39-47 (2010)

106. W. C. Chen, Y. H. Wei, I. M. Chu, C. L. Yao: Effect of chondroitin sulphate C on the in vitro and in vivo chondrogenesis of mesenchymal stem cells in crosslinked type II collagen scaffolds. *J Tissue Eng Regen Med* (2012).

107. L. H. Nguyen, A. K. Kudva, N. L. Guckert, K. D. Linse, K. Roy: Unique biomaterial compositions direct bone marrow stem cells into specific chondrocytic phenotypes corresponding to the various zones of articular cartilage. *Biomaterials*, 32(5), 1327-38 (2011)

Key Words: Mesenchymal stem cells, Growth Plate, Cartilage, Chondrogenesis, Bone Bridge, Zonal Arrangement, Growth Factors, Articular Cartilage, Chondrocytes, Cartilage Repair, Review

Send correspondence to: James Hui, Cartilage Repair Program, Therapeutic Tissue Engineering Laboratory, Department of Orthopaedic Surgery, National University Health System, National University of Singapore, 1E, Kent Ridge Road, Singapore 119288. Tel: 65-6772-4321, Fax: 65-6778-0720, E-mail: james_hui@nuhs.edu.sg