Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2021 Oct 16; 9(F):492-504.
https://doi.org/10.3889/oamjms.2021.5850
eISSN: 1857-9655
Category: *F - Review Articles*
Section: *Systematic Review Article*

Enhancement of Chondrogenesis in Hypoxic Precondition Culture: A Systematic Review

Sholahuddin Rhatomy[1,2,3]*, Riky Setyawan[3], Michael Aaron Romulo[4]

[1]Department of Orthopaedics and Traumatology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia; [2]Department of Orthopaedics and Traumatology, Sport and Adult Reconstructive Division, Dr. Soeradji Tirtonegoro General Hospital, Klaten, Indonesia; [3]Department of Orthopaedics and Traumatology, Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia; [4]Soeradji Tirtonegoro Sport Center and Research Unit, Dr. Soeradji Tirtonegoro General Hospital, Klaten, Indonesia

Edited by: Eli Djulejic
Citation: Rhatomy S, Setyawan R, Romulo MA. Enhancement of Chondrogenesis in Hypoxic Precondition Culture: A Systematic Review. Open Access Maced J Med Sci. 2021 Oct 16; 9(F):492-504. https://doi.org/10.3889/oamjms.2021.5850
Keywords: Stem cell; Cartilage; Hypoxic; Oxygen level
***Correspondence:** Sholahuddin Rhatomy, Department of Orthopaedics and Traumatology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. KRT. Dr. Soeradji Tirtonegoro Street Number 1, Tegalyoso, South Klaten, Klaten, Indonesia 57424. E-mail: sholahuddin.rhatomy@mail.ugm.ac.id
Received: 06-Feb-2021
Revised: 02-Oct-2021
Accepted: 06-Oct-2021

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Cartilage tear has begun to be treated with stem cells. However, stem cell oxygen level culture has not been evaluated for the best environment to enhance chondrogenesis.

AIM: The purpose of this review is to focus on the hypoxic oxygen level of stem cells culture as a treatment for cartilage tear.

METHODS: A literature search was systemically conducted on PubMed (MEDLINE), OVID, EMBASE, the Cochrane Library, Scopus, Web of Science, Science Direct, Wiley Online Library, Google Scholar, and bibliography of selected articles with the terms ("culture") AND ("stem cell" OR "mesenchymal stem cell" OR "MSC") AND ("hypoxic" OR "hypoxia") AND ("cartilage" OR "chondro*") as the main keywords. A total of 438 articles were reviewed. Thirty-six articles were considered relevant for this systematic review.

RESULTS: The result of this review supports stimulation effects of hypoxic oxygen level stem cell culture in chondrogenesis process. Most studies used 5% oxygen concentration for culture, both of *in vivo* and *in vitro* studies. Due to the heterogeneity nature of the included studies, meta-analysis was unable to be conducted.

CONCLUSION: Hypoxia state seems to play an important role in chondrocytes proliferation, differentiation, and matrix production.

Introduction

Cartilage has many important roles to support the normal joint function. It provides the gliding between two bone surfaces and as shock-absorber [1]. Cartilage is incapable to repair and regenerate once it is damaged [2]. Treatment of choice in cartilage damage varies from pharmacologic therapy to surgery. The latest surgical therapy methods are marrow stimulating technique, osteochondral transplantation, and autologous chondrocyte implantation [2], [3], [4]. Cell-based therapy is currently used to treat focal cartilage defects [5].

Stem-cell-based therapy has been used as an additional treatment because of its self-renewal properties, differentiation potentials, and immune-modulatory activities [6]. Stem cells can generate more fibrous cartilage to construct its biomechanical property and have higher durability [4]. After a 6-month follow-up, hyaline cartilage was regenerated after Mesenchymal Stem Cells (MSCs) were injected into the joint [7].

Stem cell culture is done to collect enough cells for transplant process. The previous researches did not consider the oxygen level condition in stem cell culture. Room air oxygen level (21%) is widely used to culture stem cells [8], [9]. Normally, cartilage is relatively avascular with a range of 1–7% of oxygen concentration [9]. Hypoxic state can induce sex-determining region Y-box9 (SOX9) expression that is an important transcription factor for chondrogenesis [9]. Stem cell proliferation and multipotency are maintained in hypoxic condition [5], [9], [10], [11]. Hypoxic state also increases extra-cellular matrix synthesis by chondrocytes [9].

However, there is still not enough data to confirm the level of hypoxic condition medium for stem cell culture. A systematic review from existing preclinical studies is needed to consider the safety and efficacy, and to guide future studies. The main purpose of this review is to summarize the *in vitro* studies regarding the hypoxic level of stem cell culture as a treatment for any cartilage damages.

Methods

Eligibility criteria

The inclusion criteria for this review consist of the following:

- Study design: Controlled laboratory study (*in vitro* study)
- Study group: Stem cell isolation from human or animals
- Interventions: Any application of hypoxic level condition to the study groups
- Outcomes: Main outcomes were any chondrogenic marker, cell size, and gene expression
- Language: English.

Non-English studies, duplicates, review articles, and irrelevant articles were excluded from the study.

Literature search and study selection

A comprehensive search was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [12]. The search was conducted from 9 sources including PubMed (MEDLINE), OVID, EMBASE, the Cochrane Library, Scopus, Web of Science, Science Direct, Wiley Online Library, Google Scholar, and bibliography of selected articles on July 1, 2020. The date range was restricted to all studies conducted through July 1, 2020. The term ("culture") AND ("stem cell" OR "mesenchymal stem cell" OR "MSC") AND ("hypoxic" OR "hypoxia") AND ("cartilage" OR "chondro*") was used as the search keyword.

Two authors (R.S. and S.R.) independently screened the title and abstracts for eligibility by reading the full texts, therefore using it to apply the inclusion and exclusion criteria. Additional searches were done to further include studies mentioned in the reference lists. Discussion was done to resolve any disagreements between the two authors.

Methodological quality assessment and risk of bias

ROBIS systematic review tool was used to assess the methodological quality of the included

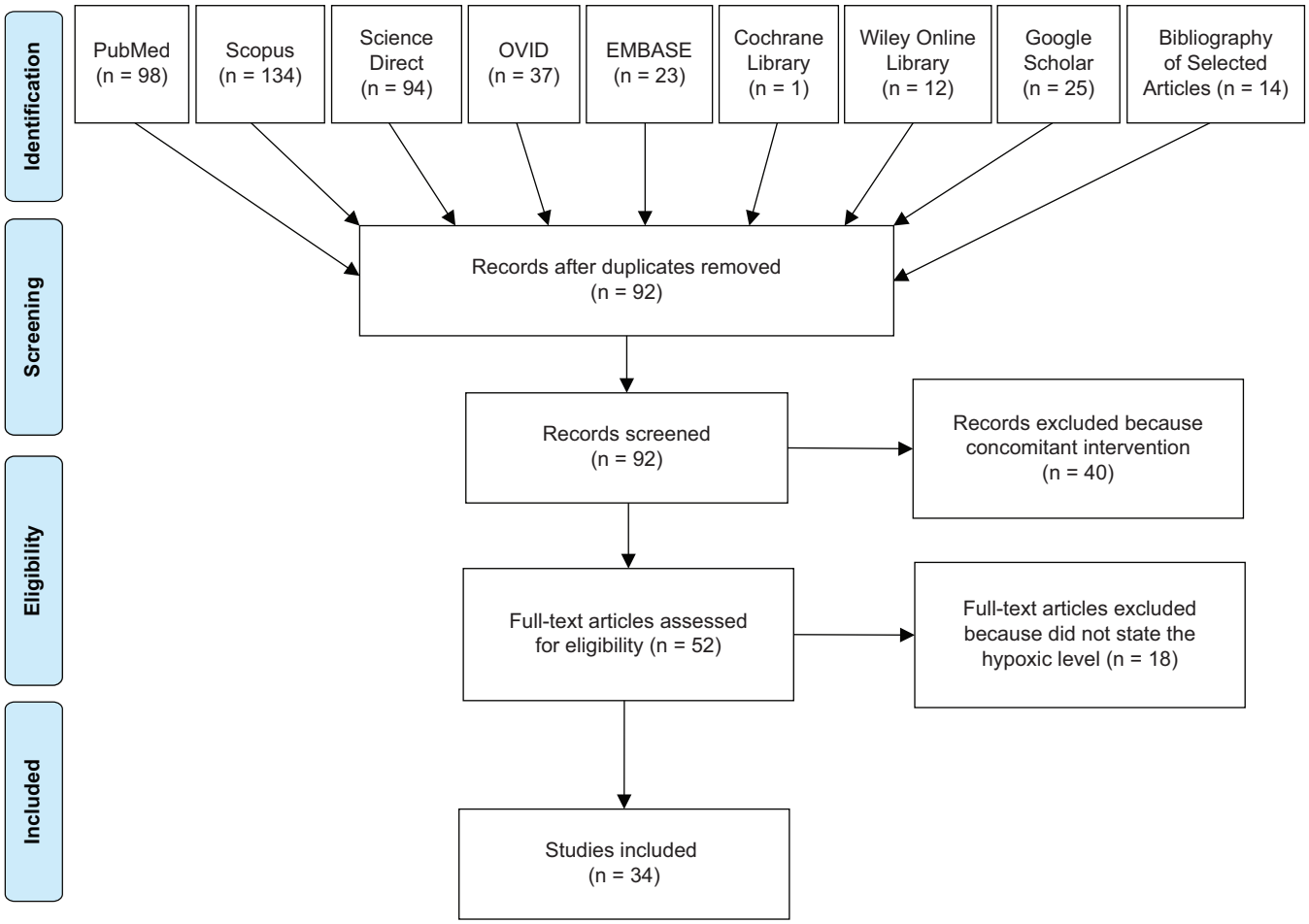

Figure 1: Flow chart of study process selection

Table 1: *In vitro* study result

Author	Type of MSC (Donor)	Donor characteristics	Cells preparation	Intervention to the main group	Control(s)	Duration	Scores/Results
Adesida *et al*. (2012)	BM-MSC (Human)	Iliac crest of 6 donors (1 female 34 y.o. and 5 male 43-62 y.o.)	α-MEM supplemented with 10% heat inactivated FBS, penicillin-streptomycin, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium pyruvate and 5 ng/ml bFGF or FGF2 at 37 °C with 5% CO_2	Hypoxia (oxygen tension 3%)	Normoxia (oxygen tension 21%)	14 days; 21 days	1. CFU: ig>cg 2. Cell surface markers (CD13, C29, CD44, CD73, CD90, CD 105, CD151) ig=cg 3. Extracellular matrix (GAG*): ig>cg 4. Chondrogenic gene and protein expression (ACAN*; Col2a1*; Sox9*; Col1a2): ig>cg (Col10a1*): ig<cg 5. Expression of TGFß receptor proteins (TGFß-RI; TGFß-RII*): ig>cg 6. mRNA expression (HIF-1α; HIF-2α*): ig>cg
Bae *et al*. (2018)	SDSC (Human)	Synovium tissue from 5 female osteoarthritis patients (66-72 y.o.) with 4thgradeKellgren Lawrence classification and undergone TKA	LG-DMEM with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/ amphotericin at 37°C with 5% CO_2	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	14 days and 21 days	1. CFU*: ig>cg 2. Expression of TGFß: ig>cg 3. Gene Expression (ACAN*; Sox9*; Col2a1*): ig>cg (Col10a1*): ig<cg 4. Extracellular matrix (GAG*): ig>cg
Bornes *et al*. (2015)	BM-MSC (Bovine)	Iliac crest from 6 skeletally mature, female Suffolk sheep with mean age 3.3±0.8 years	α-MEM supplemented with 10% heat inactivated FBS, penicillin-streptomycin, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium pyruvate and 5 ng/ml bFGF or FGF2 at 37 °C with 5% CO_2	Hypoxia (oxygen tension 3%)	Normoxia (oxygen tension 21%)	14 days	1. CFU*: ig<cg 2. Gene Expression (ACAN, Col1, Col2, Col10, COMP) ig=cg 3. Extracellular matrix (GAG*, DNA quantification): ig>cg 4. Cell scaffold construct size*: ig>cg
Cicione *et al*. (2013)	BM-MSC (Human)	Three patients who underwent total hip replacement with mean age 64 year	DMEM supplemented with 20% FBS and 1% penicillin-streptomycin with 5% CO_2	Severe Hypoxia (oxygen tension 1%)	Normoxia (oxygen tension 21%)	14 days	1. Gene Expression (ACAN, Col1): negative in cg (Sox9, Col2a1): ig<cg
Duval *et al*. (2012)	BM-MSC (Human)	Iliac crest of adult donors (ages 54-75 y.o. with median 68 y.o.)	α-MEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 1 ng/ml FGF-2, and antibiotics	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	7 days	1. Gene Expression (Col2*; ACAN*, Lsox5*; Sox6; Sox9*): ig>cg (Col10a1*; Cbfa1*): ig<cg 2. HIF-1α induced Sox9 promotor gene to produce Sox9 in hypoxia cultivation
Felka *et al*. (2009)	BM-MSC (Human)	Twenty-eight patient undergoing total hip replacement with age range from 45 to 83 y.o.	LG-DMEM supplemented with 5% human FFP, 10^5/ml platelets, 2 mM glutamine, 1000 IU heparin sodium, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37 °C with 5% CO_2	Hypoxia (oxygen tension 2%)	Normoxia (oxygen tension 21%)	28 days	1. IL-1ß expression: ig>cg 2. dimension size of cartilage: ig>cg 3. Gene expression (Col1a2*; Col2a1*; Col10*; ACAN*; CD-RAP*, Sox6*; Sox9*; BMP-2*): ig>cg
Gale *et al*. (2019)	SM-MSC and BM-MSC (Horse)	Synovium was harvested in standing horses or during arthroscopic procedure in 5 horses	N/A	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	28 days	1. Extracellular matrix BM-MSC (GAG, GAG/ DNA): ig>cg SM-MSC (GAG, DNA): ig<cg 2. Gene Expression BM-MSC (Sox9; ACAN; Col2b; Col10a1): ig>cg SM-MSC (Sox9; Col2b): ig> cg; (ACAN; Col10a1): ig<cg
Galeano-Garces *et al*. (2016)	AMSC and chondrocyte(Human)	Lipoaspiration was obtained from 3 consenting healthy donors Human primary chondrocytes were obtained from healthy donors undergoing amputation procedures for congenital limb deformity	Advanced MEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C with 5% CO_2	Hypoxia (oxygen tension 2%)	Normoxia (oxygen tension 21%)	14 days	1. Gene Expression in AMSC (ACAN*; Sox9*; HAPLN1*; Col1a1*; HIST2H4*; Col10a1*): ig>cg Chondrocyte (Sox9*; HIST2H4*; HIF-1α; Col2a1*; DCN; GLI1; IHH): ig>cg

(Contd...)

Table 1: ***(Continued)***

Author	Type of MSC (Donor)	Donor characteristics	Cells preparation	Intervention to the main group	Control(s)	Duration	Scores/Results
Gomez-Leduc *et al.* (2017)	UCB-MSC (Human)	Samples collected at Obstetrics and Gynecology Unit from normal delivery cases with the informed consent from mothers	LG-DMEM supplemented with 20% fetal calf serum, 10^{7}M dexamethasone and incubated at 37^{0}C in a humidified 5% CO_2 atmosphere	Hypoxia (oxygen tension <5%)	Normoxia (oxygen tension 21%)	7, 14, 21 days	1. Chondrogenesis occurs in the presence of BMP-2 and TGF-ß1: ig=cg 2. Gene Expression (Col1a1; Col2a1; Col10a1, MMP-13): ig<cg
Gong *et al.* (2017)	MSC (Murine)	The murine mesenchymal cell line C3H10T1/2 was purchased from ATCC (Manassas, VA, USA)	DMEM supplemented with 10% FBS at 37°C in a humidified 5% CO_2 atmosphere	Hypoxia (oxygen tension 2%)	Normoxia (oxygen tension 21%)	14 days	1. Gene Expression (Sox9*; Col10a1*; Col2a1*, ACAN*): ig>cg 2. Reduction of miR-124*: ig<cg
Henrionnet *et al.* (2017)	BM-MSC (Human)	Six patients (3 men and 3 women) undergoing total hip arthroplasty with mean age 64.8±6.3 y.o.	LG-DMEM supplemented with 10% FBS, 1% glutamine, 1% penicillin-streptomycin and 1 ng/ml bFGF at 37°C in a humidified 5% CO_2 atmosphere	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	14, 28 days	1. Continous hypoxia (Col2a1*; ACAN*; Sox9*; COMP*): ig>cg (VCAN*; ALP* RUNX2*; BGLAP*): ig<cg
Huang *et al.* (2017)	Human articular chondrocyte; hMSC; co-culture hAC/ hMSC (Human)	Human primary chondrocytes were derived from healthy looking and full thickness cartilage, and dissected from knee biopsies of three patients (age: 60±3 y.o.) undergoing total knee replacement hMSCs were isolated from human bone marrow aspirates	hAC: DMEM supplemented with 10% FBS, 1×non-essential amino acids, ascorbic acid 2-phosphate (0.2 mM), proline (0.4 mM), penicillin (100 U/mL) and streptomycin (100 µg/mL) hMSC: a-MEM supplemented with 10% FBS, 1% L-glutamax, ascorbic acid (0.2 mM), penicillin (100 U/mL), streptomycin (100 µg/mL) and bFGF (1 ng/mL)	Hypoxia (oxygen tension 2.5%)	Normoxia (oxygen tension 21%)	35 days	1. Gene Expression (Col2a1*; ACAN*): ig>cg (RUNX2*; Col10a1*; ALPL*; Col1a1): ig<cg
Hung *et al.* (2011)	BM-MSC (Human)	Iliac crest of 15 healthy donors	IMDM and 10% FBS supplemented with 10 ng/ml FGF2, 100 U penicillin, 1000 U streptomycin, and 2mM L-glutamine	Hypoxia (oxygen tension 1%)	Normoxia (oxygen tension 21%)	28 days	1. Size of pallets: ig<cg 2. Mature chondrocyte morphology: ig<cg 3. Gene Expression (Col2a1*; COMP*; ACAN*): ig<cg
Kalpakci *et al.* (2014)	DIAS (Goat)	Full-thickness skins from the abdomens of seven adult goats were obtained from a local abattoir	DMEM and 10% FBS supplemented with 4.5 g/L glucose and L-glutamine, 1% penicillin/streptomycin/fungizone, and 1% non-essential amino acids	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 20%)	14 days	1. CFU*: ig<cg 2. Cell growth*: ig<cg 3. Total Collagen production*: ig>cg 4. Collagen type II: ig>cg 5. GAG content*: ig>cg 6. Histological evaluation (Collagen type 1 staining*): ig>cg
Kanichai *et al.* (2008)	BM-MSC (Rat)	Three-month old Wistar rats (250–350 g) with the femur and tibia were cut at both epiphyses and marrow was flushed into a 50 ml tube using 5 ml supplemented DMEM and a 25-gauge needle.	DMEM supplemented with 10% FBS; 100 U/ml penicillin/streptomycin; 2 mM Glutamax; 1 mM L-glutamine and 1% non-essential amino acids at 37°C in a humidified 5% CO_2 atmosphere	Hypoxia (oxygen tension 2%)	Normoxia (oxygen tension 20%)	21 days	1. Chondrogenic Growth Factors (Proteoglycan*; Col2a1*): ig>cg 2. Nuclear expression* and HIF-1α activity*: ig>cg 3. Phosphorylation of Akt*: ig>cg 4. HIF-1α siRNA inhibits proteoglycan deposition
Khan *et al.* (2007)	IPFP-SC (Human)	Three patients (aged 67, 69 and 72 y.o.) undergoing total knee replacement for osteoarthritis	DMEM supplemented with 20% (v/v) FCS, 100 U/ml penicillin and 100 µg/ml streptomycin, with 2mM l-glutamine at 37°C in a humidified 5% CO_2 atmosphere	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 20%)	14 days	1. Cell aggregates*; GAG content* and proteoglycan content*: ig>cg 2. Gene expression (Sox9*; Sox5*; Sox6*; HIF-2α*; ACAN*; VCAN*; Collagen type II*; Collagen type IX*; Collagen type X*; Collagen type XI*): ig>cg

(Contd...)

Table 1: ***(Continued)***

Author	Type of MSC (Donor)	Donor characteristics	Cells preparation	Intervention to the main group	Control(s)	Duration	Scores/Results
Khan *et al.* (2010)	BM-MSC (Human)	The bone marrow was extracted following fully informed consent of three 18–40 y.o. patients	MSC media supplemented with 5 ng/mL rh-FGF-2 at a density of 166,000 cells per cm in a T25 cell culture flask. Nonadherant cells were removed after 24 h by washing twice in Dulbecco's phosphate buffered saline (DPBS) and changing the medium	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 20%)	14 days	1. Cell aggregates*; GAG accumulation*; Proteoglycan content*: ig>cg 2. Gene Expression (Sox9*; Collagen type II*; Collagen type XI*; ACAN*): ig>cg
Kishimoto *et al.* (2009)	ATDC5 Cell/ Chondroproenitor Cells derived from murine embryonic carcinoma (Murine)	RIKEN cell Bank (Tsukuba, Japan)	1:1 mixture of DMEM and Ham's F-12 (Invitrogen) supplemented with 5% FBS and antibiotics (Invitrogen: penicillin 50 U/ml, streptomycin 50 mg/ml; Expansion medium) at 37°C in a humidified 5% CO_2 atmosphere	Hypoxia (oxygen tension 1%)	Normoxia (oxygen tension 20%)	7 days	1. Gene expression (sox9; HIF-1α): ig=cg 2. Cell expansion: ig=cg 3. Increment Chondrogenic matrix (BMP4; ITS+; ACAN; Col2a1): ig=cg
Koay *et al.* (2008)	ESC (Human)	The National Institutes of Health (NIH)-approved H9 line (WiCell, Madison, WI, USA) was cultured according to standard protocols using a defined medium (www.wicell.org) and a gamma-irradiated CF-1 (Charles River Laboratories, Wilmington, MA, USA) mouse embryonic fibroblast (MEF) feeder layer on T75 culture plates (Nunc, Rochester, NY, USA). Frozen ESCs at passage 33 (p33) were thawed according to standard protocol and sub-cultured.	HG-DMEM, 10^7-M dexamethasone, ITS+ Premix (6.25-ng/ml insulin, 6.25-mg transferrin, 6.25-ng/ml selenious acid, 1.25-mg/ml bovine serum albumin, and 5.35-mg/ml linoleic acid), 40-μg/ml L-proline, 50-mg/ml ascorbic acid, 100-mg/ml sodium pyruvate, and 1% FBS at 5% CO_2	Hypoxia (oxygen tension 2%)	Normoxia (oxygen tension 20%)	21, 49 days	1. Construct diameter*: ig<cg 2. Construct thickness*: ig<cg 3. Collagen 1* and Collagen 2*: ig>cg 4. Tensile strength; compressive property; and relaxed moduli*: ig>cg
Lee *et al.* (2013)	BM-MSC (Human)	Three male Asians with age ranged from the third to fifth decade, who received a spine surgery for spinal disorders	a-MEM supplemented with 16.6% FBS, 100 units/mL penicillin, 100 mg/mL streptomycin, and 2 mM L-glutamine at 37°C, 5% CO_2	Hypoxia (oxygen tension 1%)	Normoxia (oxygen tension 21%)	7, 14 days	1. Gene expression (Sox9*; Col2a1*; ACAN*): ig>cg (Col10a1*; RUNX2*): ig<cg 2. Hypoxic culture suppressed chondrogenesis-induced apoptosis 3. Hypoxic condition inhibited activation of caspase-8 and caspase-3 during chondrogenesis
Lee *et al.* (2015)	cAMSC (Dog)	4-month-old beagle dogs (n = 5)	DMEM supplemented with 1% penicillin streptomycin and 10% FBS and maintained in a humidified incubator at 5% CO_2 and 37°C	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	21 days	1. Survival Gene Expression (HIF-1α*; VEGFA*): ig>cg 2. Chondrogenic gene expression (Col2a1*; ACAN*): ig>cg
Mahyudin *et al.* (2018)	BM-MSC (Rabbit)	Bone marrow of healthy male New Zealand rabbit strain	α -MEM with 1-glutamine, without ribonucleside or deoxyribinucleside; FBS; 1 Glutamine, 200 mM (Initrogen); Penicillin G (10,000 units/mL) and streptomycin sulfate (10,000 μg/mL) in 0.85% NaCl solution; Ficol-Paque; Phospate buffered saline (PBS), without Ca or Mg^{2+}, pH 7.4; Tripsin (0.25%)-EDTA 4 NA (0.38 g/dL)	Hypoxia (oxygen tension 1%)	Normoxia (oxygen tension 21%)	35 days	1. Gene Expression (Collagen type II*; Sox9*): ig>cg (RUNX2*): ig<cg (RUNX2 with additional Chondrogenic medium*): ig>cg

(Contd...)

Table 1: ***(Continued)***

Author	Type of MSC (Donor)	Donor characteristics	Cells preparation	Intervention to the main group	Control(s)	Duration	Scores/Results
Markway *et al.* (2010)	BM-MSC (Human)	Ten ml bone marrow was taken from iliac crest of healthy donors	LG-DMEM supplemented with 10% FBS and 50 µg/ml gentamicin and placed in tissue culture flasks	Hypoxia (oxygen tension 2%)	Normoxia (oxygen tension 21%)	14 days	1. GAG: ig>cg 2. Detection of Collagen 1 and Collagen II in hypoxic cultivation 3. Gene expression (Sox9*; ACAN*; Collagen I*; Collagen II*; Collagen X*; RUNX2/CBFA1*): ig>cg
Merceron *et al.* (2010)	AMSC (Human)	Three different patients undergoing abdominal plastic surgery who had provided prior informed consent	DMEM containing 1% penicillin-streptomycin, 1% L-glutamine, and 10% FCS (control medium)	Hypoxia (oxygen tension <5%)	Normoxia (oxygen tension 20%)	28 days	1. GAG production occurred in D7, D14 and D28 2. Gene expression (ACAN*; Col2a1*): ig>cg
Meretoja *et al.* (2013)	Articular Chondrocyte and Bovine-MSC (Bovine)	Harvested from 7 to 10 day old calves and marrow isolated from tibiae and femoral bone of the bovine	DMEM, 10% FBS, 1% non-essential amino acids, 50 mg/mL ascorbic acid, 46 mg/mL L-proline, 20 mM HEPES, PSF	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 20%)	14 days	1. Gene expression (Collagen II*; CollagenII/I*; Collagen X): ig>cg (Collagen I*): ig<cg 2. DNA content: ig=cg 3. Hydroxyproline content: ig<cg 4. GAG synthesis: ig=cg 5. ALP: ig=cg
Munir *et al.* (2013)	Human AMSC (Human)	Four human donors adipose tissue was harvested using a tumescent technique with pump-assisted aspiration. Subcutaneous fat were collected from one female and three male donors, with fat from the abdomen and the hips as the primary source	α –MEM supplemented with 10% FCS in a standard humidified atmosphere containing 5% CO_2 at 37 °C	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	28 days	1. Matrix deposition and higher level of cellularity occurred peripherally in hypoxic cultivation 2. Gene expression (Sox9*; Collagen I*; Collagen II/I Ratio*; Collagen X*): ig>cg
Ohara *et al.* (2016)	SDSC (Human)	Human synovium was harvested during total knee arthroplasty from 33 donors diagnosed with knee osteoarthritis and some synovium were used for several experiments and the average age was 76±9 years old	10 mL a-MEM containing 10% FBS, 100 unit/mL penicillin and 100 mg/ml streptomycin	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	14 days	1. CFU: ig=cg 2. mitochondria number and morphology: ig=cg 3. GAG: ig=cg 4. attachment of SDSC: ig=cg
Portron *et al.* (2013)	Human AMSC (Human) Rabbit AMSC (Rabbit)	Human patients (hAMSC) undergoing liposuction and who had given written consent rAMSC harvested from the inguinal region of the Rabbit	Serum-free DMEM supplemented with 1% penicillin/streptomycin, 6.25 µg/mL insulin, 6.25 µg/mL transferrin, 6.25 ng/mL sodium selenite, 50 nM sodium L-ascorbate, 10^{8} M dexamethasone and 10 ng/mL TGF-ß1 with the cultivation in hypoxia 5% and normoxia 21%	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	21 days	rAMSC: (Col2a1*; ACAN*): ig>cg hAMSC: (Col2a1*; ACAN*): ig>cg
Ranera *et al.* (2013)	BM-MSC (Horse)	Bone marrow aspirates were obtained from a total of five castrated male horses	The cells were rinsed twice with PBS (Gibco), counted, and plated at 2 × 106 nucleated cells/cm^2 in 6-well plates (Becton Dickinson) in growth medium consisting of low glucose Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 10% Foetal Bovine Serum, 1% Glutamine (Sigma) and 1% Streptomycin/Penicillin.	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 20%)	21 days	1. sGAG content*: ig>cg 2. Gene expression (Col2a1*; ACAN*; LUM; COMP*; HIF-1α*): ig>cg

(Contd...)

Table 1: ***(Continued)***

Author	Type of MSC (Donor)	Donor characteristics	Cells preparation	Intervention to the main group	Control(s)	Duration	Scores/Results
Silva *et al*. (2020)	BM-MSC and SDSC (Human)	Bone marrow aspirates (healthy male 36 years) were obtained from Instituto Português de Oncologia Francisco Gentil, LisboaPotugal and an additional sample of fresh unprocessed bone marrow sample (Male 24 years) was purchased from Lonza (Basel, Switzerland) Synovium aspirates from donors undertaking routine arthroscopic surgery with no history of joint disease (healthy male 22 years and healthy male 28 years) were obtained from Centro Hospitalar de Lisboa Ocidental, E.P.E, Hospital São Francisco Xavier, Lisboa, Portugal	DMEM supplemented with 10% FBS and 1% antibiotics (penicillinstreptomycin, Pen-strep, Gibco) and cryopreserved in liquid nitrogen tanks until usage	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	21 days	1. Gene expression (Sox9*; ACAN*) BM-MSC: ig>cg SDSC: ig<cg
Tian *et al*. (2013)	BM-MSC (Human)	Iliac crests of 9 healthy volunteers (three males and five females)	LG-DMEM supplemented with 10% FBS, 1% penicillin Streptomycin and 2 mmol/L L-glutamine at 37 °C with 5% CO_2	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	21 days	1. Gene expression (ACAN*; Collagen type II*): ig>cg 2. Diameter*: ig>cg 3. GAG*: ig>cg
Wan Safwani *et al*. (2017)	AMSC (Human)	Adipose tissues were harvested from 6 healthy female donors aged 25e35 years who were undergoing Caesarean section with prior informed written consent	DMEM/F12 with 10% FBS, 200 μM indomethacin, 0.5 μM isobutyl-1-methyl xanthine, 1 μM dexamethasone and 10 μM insulin	Hypoxia (oxygen tension 2%)	Normoxia (oxygen tension 21%)	21 days	1. Cell number*: ig>cg 2. Gene expression (Col2*; Sox9*; ACAN*): ig>cg
Xu *et al*. (2007)	AMSC (Mice)	The inguinal fat pads from 3-week-old FVB mice were carefully dissected and washed sequentially in a Betadine and phosphate buffered saline (PBS) solution.	DMEM, 1% FBS, 1% penicillin/ streptomycin, 37.5 mg/mL ascorbate-2-phophate, ITS premix, and 10 ng/mL TGF-ß1	Hypoxia (oxygen tension 2%)	Normoxia (oxygen tension 21%)	11 days	1. Proliferation rate*: ig>cg 2. sGAG (proteoglycan accumulation)*: ig>cg 3. Gene expression (Collagen type II*): ig>cg (ACAN; Sox9): ig=cg

(Contd...)

Table 1: ***(Continued)***

Author	Type of MSC (Donor)	Donor characteristics	Cells preparation	Intervention to the main group	Control(s)	Duration	Scores/Results
Yodmuang *et al.* (2015)	ESC (Human)	ESC line H9 (Wicell Research Institute, Madison, WI) was expanded in 6-well plates on mouse embryonic fibroblast (MEF) feeders (Globalstem Rockville, MD)	DMEM-F12 supplemented with 10% Knockout™ serum replacement (KSR), 1 mM L-glutamine, 0.1 mM MEM amino acids, 0.1 mM 2-mercaptoethanol and 4 ng/ml bFGF	Hypoxia (oxygen tension 5%)	Normoxia (oxygen tension 21%)	7, 14, 21 days	1. Gene Expression (Sox9*): ig>cg (RUNX2): ig<cg 2. Transient hypoxia: (Col2a1*; DNA content*; GAG*): ig>cg (Col1a1*; Col10a1*): ig<cg

1. Regarding MSCs
   a. BM-MSC: Bone Marrow Mesenchymal Stem Cells
   b. SDSC: Synovium-derived Mesenchymal Stem Cells
   c. AMSC: Adipose Tissue Mesenchymal Stem Cells
   d. UCB-MSC: Umbilical Cord Blood-Mesenchymal Stem Cells
   e. hAC: Human Articular Chondrocyte
   f. hMSC: Human Mesenchymal Stem Cells
   g. SM-MSC: Synovial Membrane- Mesenchymal Stem Cells
   h. DIAS: Dermis Isolated Adult Stem Cells
   i. IPFP-SC: Infrapatellar Fat Pad-Stem Cells
   j. ESC: Embryonic Stem Cells
   k. cAMSC: Canine Adipose Derived-Mesenchymal Stem Cells
2. Charateristics
   a. y.o.: years old
   b. TKA: Total Knee Arthroplasty
   3. Related to preparation procedures:
   a. MEM: Minimum Essential Medium
   b. PBS: Phosphate Buffered Saline
   c. FBS: Fetal Bovine Serum
   d. DMEM: Dulbecco's modified eagle medium
   e. LG- DMEM: Low-Glucose Dulbecco's modified eagle medium
   f. HG-DMEM: High- Glucose Dulbecco's modified eagle medium
   g. IM-DM: Iscove's modified Dulbecco's medium
   h. FFP: Fresh Frozen Plasma
   i. FCS: Fetal Calf Serum
   j. HEPES: hydroxyethyl piperazineethanesulfonic acid
   k. PSF: Penicillin/streptomycin/amphotericin
   l. ITS: Internal Transcribed Space+
4. Score
   a. ig: intervention group
   b. cg: control group
   c. CFU: Colony-Forming Unit
   d. GAG: Glycosaminoglycan
   e. DNA: Deoxyribonucleic acid
   f. ACAN: Aggrecan
   g. Sox9: Sex-determining region Y-box 9
   h. Col1a1: Collagen 1 Alfa 1
   i. Col1a2: Collagen 1 Alfa 2
   j. Col2a1: Collagen 2 Alfa 1
   k. Col10a1: Collagen 10/ Collagen X Alfa 1
   l. COMP: Cartilage Oligomeric Matrix Protein
   m. HIF: Hypoxia Inducible Factor
   n. TGFß-RI: Transforming Growth Factor Receptor I
   o. TGFß-RII: Transforming Growth Factor Receptor I
   p. HAPLN1: Hyaluronan and proteoglycan link *protein* 1
   q. IHH: Indian Hedgehog Signaling Molecule
   r. GLI1: glioma-associated oncogene
   s. DCN: Decorin protein
   t. MMP-13: Matrix Metalloprotein-13
   u. VCAN: Versican
   v. ALP: Alkaline Posphatase
   w. RUNX2: Runt-related transcription factor 2
   x. BGLAP: Bone Gamma-Carboxyglutamate Protein
   y. ALPL: Alkaline Phosphatase, Biomineralization Associated
   z. Akt: Serine/Threonine Kinase
   aa. SiRNA: Small Interfering Ribonucleic Acid
   bb. BMP-2: Bone Morphogenetic Protein-2
   cc. BMP-4: Bone Morphogenetic Protein-4
   dd. ITS+: Internal Transcribed Space+
   ee. VEGFA: Vascular Endothelial Growth Factor A
   ff. CBFA1: Core-Binding Subunit Alpha-1
   gg. LUM: Lumican

studies [13]. Two authors (R.S. and S.R.) independently performed all the assessments. A thorough discussion was done to resolve discrepancies within authors.

Data extraction and synthesis

Two authors (R.S. and S.R.) recorded data from all included studies independently to extract the following data: Study design, type of cell donor, control and intervention given, duration of experiment, and result evaluation. Discussion was done to resolve any disagreements between the two authors.

Study outcomes are shown in Table 1. Meta-analysis could not be performed due to the heterogeneity of the data (i.e., source of MSCs, different hypoxic oxygen level, follow-up duration, and outcome measurement).

Table 2: *In vivo* study result

Author	Type of MSC (Donor)	Donor characteristics	Type of controlled laboratory experiments	Animals/cells preparation	Intervention to the main group	Control(s)	Duration	Scores/Results
Duval *et al.* (2012)	BM-MSC (Human)	Iliac crest of adult donors (ages 54-75 y.o. with median 68 y.o.)	*In vivo* (5-week-old fox n-1 nu/nu athymic mice)	Subcutaneous implantation in the dorsum of each mouse	Group 2 (received alginate beads with human chondrocytes cultured in hypoxia 5%)	Group 1 (received alginate beads containing stem cells cultured in normoxia 21%)	21 days	1. Macroscopic evaluation (hypoxia looked like hyaline cartilage): ig>cg 2. Histological examination (cartilage-like matrix): ig>cg 3. Immunohistological analysis (Col2): ig>cg 4. Cell expressing HIF-1α induced chondrocyte-like cell in normal expression: ig>cg
Portron *et al.* (2013)	Human ASC (Human) Rabbit ASC (Rabbit)	Adult female New Zealand White rabbits weighing 3 to 3.5 kg and 1-month-old female Swiss nude mice	*In vivo*	Rabbit: General anesthesia of rabbits was induced by intramuscular injection of ketamine (0.5 mL/kg) and xylazine (0.3 mL/kg) cocktail Nude mice: pre-medicated with morphine chlorhydrate (2 mg/kg) diluted into sterile saline solution and injected subcutaneously. General anesthesia was obtained in an induction chamber with isoflurane (2%) delivered in O_2 and prolonged through an individual mask	Group 1b: Implantation of rASC in rabbit articular defect Group 2b: Implantation of hASC in nude mice subcutis	Group 1a: Implantation of autologous rabbit nasal chondrocytes (RNCs) Group 2a: Implantation of horse nasal chondrocytes (HoNCs) in nude mice subcutis	48 days	rASC (rabbit): GAG and Col2a1 were detected in hypoxic precondition hASC (mice): formation of cell aggregates occurred and immune-reactive for type II collagen

5. Regarding MSCs
   a. BM-MSC: Bone Marrow Mesenchymal Stem Cells
   b. hASC: Human Adipose-derived Stem Cells
   c. rASC: Rabbit Adipose-derived Stem Cells
6. Charateristics
   a. y.o.: Years old
7. Score
   a. ig: Intervention group
   b. cg: Control group
   c. GAG: Glycosaminoglycan
   d. Col2a1: Collagen 2
   e. HIF: Hypoxia Inducible Factor

Results

Study selection

A PRISMA flow diagram (Figure 1) summarizes the study selection process. A total of 438 studies were identified. After screening of the titles and abstracts, 52 articles were considered eligible for further evaluation. After full-text assessment, 34 *in vitro* studies and two *in vivo* studies were included in this systematic review.

Study characteristics

This review is presented in Tables 1 and 2 to specifically explain about the type of MSC used, cell preparation, control and intervention groups, duration of observation, and study results.

Most of the studies utilized stem cells from human (22 studies). Six studies used bovine, murine, and horse stem cells, with two studies for each stem cell type. Human and rabbit, rabbit, mice, rat, goat, and dog were used in the past six studies.

The most common MSC type was from bone marrow (14 studies), followed by AMSC (7 studies), ESC (two studies), and SDSC (two studies). With one study each, the others used BM-MSC and SDSC, UCB-MSC, SM-MSC and BM-MSC, murine MSC, bovine MSC, hAC/hMSC, DIAS, IPFP-SC, and Chondroprogenitor derived cells.

Hypoxia as intervention group varied from 1% to 5% oxygen level compared to normoxia with 21% oxygen level. Treatment duration varied from 7 to 49 days. Most of studies evaluated chondrogenic gene and protein expression including Col1a1, Col1a2, ACAN, Sox9, COMP, and RUNX2.

In vitro study outcomes

In vitro study outcomes are summarized in Table 1. According to most studies, there were higher chondrogenic gene and protein expression including ACAN, Col1a1, Col1a2, Col2a1, and Sox9 [5], [9], [10], [11], [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35], [36], [37], [38], [39], [40], and higher extracellular matrix including Glycosaminoglycan (GAG) [5], [9], [10], [18], [19], [24], [25], [29], [31], [33], [38], [41], [42], higher expression of HIF-1α and HIF-2α [5], [17], [18], [22], [29], [36], [39], GAG production [5], [9], [10], [18], [19], [24], [25], [29], [31], [33], [41] and presence of Transforming Growth Factor-ß (TGF-ß) [5], [9].

The presence of TGF-ß1 and Bone Morphogenetic Protein (BMP)-2 had no effect on chondrogenesis activity [40]. There was no difference between hypoxic and normoxic group in cell surface

markers including CD13, CD29, CD44, CD73, CD90, CD105, and CD151 [5]. IL-1ß was higher in hypoxic group [37]. Expression of miR-124 [14] and hydroxyproline content [26] was lower in hypoxic group than in normoxic group.

Evaluation of culture size diameter [10], [31], [37], cell number [32], proliferation rate [33], histological evaluation (collagen type 1 staining) [41], and phosphorylation of Akt [17] was higher in hypoxic group. However, study by Hung *et al*. stated that the size of pallets and mature chondrocyte morphology was lower in hypoxic group [16]. Kalpakci *et al*. also stated that cell growth was lower in hypoxic group than normoxic group [41]. Mitochondria number and morphology [43], cell expansion [20], and ALP [26] had no difference in between two groups.

Study by Koay *et al*. was evaluated for construct diameter and thickness of the cultured cell. The hypoxic group was more inferior to normoxic group. However, the tensile strength, compressive property, and relaxed moduli were found higher than normoxic group [34]. There was inconsistent result in CFU [5], [9], [10], [41], [43]. Adesida *et al*. and Bae *et al*. stated that CFU was higher in hypoxic group compared to normoxic group [5], [9]. Ohara *et al*. stated that there was no CFU difference between normoxic group and hypoxic group [43]. However, Bornes *et al*. and Kalpakci *et al*. concluded that there was lower CFU amount in normoxic group compared to hypoxic group [10], [41].

In vivo study outcomes

In vivo study outcomes are summarized in Table 2. There were only two studies that evaluate *in vivo* study. Study by Duval *et al*. revealed that there were superior results in macroscopic evaluation, histological examination, immunohistological analysis, and chondrocyte-like cell induction after HIF-1α expression in hypoxic group compared to normoxic group [36]. Study by Portron *et al*. concluded that there were GAG and Col2a1 detection in hypoxic precondition, and formation of cell aggregates for collagen type 2 [28].

Hypoxic condition (oxygen concentration)

Thirty-four *in vitro* studies used various oxygen level, 18 studies (52.9%) used 5% oxygen, eight studies (23.5%) used 2% oxygen, five studies (14.7%) used 1% oxygen, two studies (5.9%) used 3% oxygen, and one study (2.9%) used 2.5% oxygen (Table 1).

Two *in vivo* studies all used 5% oxygen concentration (Table 2).

Discussion

There were many studies that evaluated the chondrogenic genes and proteins. Collagen type II contributes more than 80% of normal articular cartilage extracellular matrix. It provides tensile strength and cartilaginous scaffold [40]. ACAN is a proteoglycan in articular cartilage that constitutes 80–90% of all articular cartilage proteoglycan [40]. It serves as fluid regulator in the cartilage matrix and has elastic and compressive strength in articular cartilage [5], [40]. Transcriptional factor Sox9 was found to be an essential factor for chondrogenesis gene including Col2a1 [5]. Collagen type 10 has more osteogenic than chondrogenic differentiation process and also has hypertrophic property marker for chondrocyte [5], [9], [15]. Some studies stated the downregulation and upregulation of this gene. RUNX2 is one of osteogenic mRNA [15]. Expression of Bone Morphogenetic Protein-2 (BMP-2) alone or BMP-2 with TGF-ß can increase chondrogenesis process [9], [40]. Adesida *et al*. wrote that there was improvement of TGF-ß Receptor II (TGF-ß RII) significantly and TGF-ß Receptor I in hypoxic culture condition [5].

Hypoxia state seems to play an important role in chondrocytes proliferation, differentiation, and matrix production [17], [18]. There was no specific consensus for the best hypoxic condition in chondrocyte scaffold culture. In this review, the hypoxic state varied from 1% to 5% oxygen level. Most of *in vitro* studies showed that hypoxic condition can induce production of ACAN, Collagen type II, and Sox9. There was still inconsistent result in improvement of BMP-2, TGF-ß, TGF-ß RI, TGF-ß RII, and reduction of Collagen type 10, RUNX2. Most studies that evaluated GAG extracellular matrix showed that there were more production of GAG in hypoxic state of stem cell culture compared to normoxic group [5], [9], [10], [18], [19], [24], [25], [29], [31], [33], [41].

Cell proliferation rate, cell number, cell diameter, and CFU were still unable to be determined due to inconsistent results. Some studies revealed superior result and the others showed inferior result compared to normoxic group. Hypoxic state can induce cartilage formation and chondrocyte proliferation. Normoxic oxygen level state may be stressful to MSC and may induce an oxidative stress response [33].

Based on *in vivo* studies, there was supportive result in two studies. Portron *et al*. stated that there was an increment in production of GAG and collagen type II [28]. Study conducted by Duval *et al*. concluded that there were increments in macroscopic evaluation, histological examination, immunological analysis, and HIF-1α-induced chondrocyte like cell [36]. Low oxygen tension culture before implantation can enhance chondrogenesis in stem cells. These cells produce chondrocyte markers (type II collagen, ACAN) and

chondrogenesis marker (Sox9) [36]. Hypoxic oxygen level *in vitro* can influence the regenerative potential of cartilage after *in vivo* implantation [28]. Chondrogenic stimuli affect stem cells chondrogenesis and cartilage maturation tissue [11], [44].

There were several limitations to this study. The included studies were mostly done with *in vitro* study with only two studies that reviewed *in vivo* study. The included studies have several hypoxic oxygen level state, difference in stem cells used, different duration of study evaluation, and different end-point evaluation. Therefore, it was hard to perform a quantitative analysis. Further research is needed to evaluate the exact hypoxic oxygen level that produces the best chondrogenic properties in stem cell culture. More *in vivo* study is required to achieve better result from further studies.

Conclusion

Application of hypoxic oxygen level in stem cell culture is a promising method to trigger the chondrogenic lineage differentiation and proliferation. However, more pre-clinical studies are needed to further evaluate the exact hypoxic oxygen level to produce the most supportive environment for stem cell culture.

References

1. Carballo CB, Nakagawa Y, Sekiya I, Rodeo SA. Basic science of articular cartilage. Clin Sports Med. 2017;36(3):413-25. https://doi.org/10.1016/j.csm.2017.02.001
   PMid:28577703
2. Pruksakorn D, Pothachareoun P, Klunklin K, Nimkingratana P, Rohanastein S, Padongkiert S, *et al*. Articular cartilage injury treatment: History and basic science review. Orthop Muscul Syst. 2012;1(4):1-7. https://doi.org/10.4172/2161-0533.1000114
3. Rodriguez-Merchan EC. Articular Cartilage Defects of the Knee. Berlin, Germany: Springer; 2012. p. 1-113.
4. Wang M, Yuan Z, Ma N, Hao C, Guo W, Zou G, *et al*. Advances and prospects in stem cells for cartilage regeneration. Stem Cells Int. 2017;2017:4130607. https://doi.org/10.1155/2017/4130607
   PMid:28246531
5. Adesida AB, Mulet-Sierra A, Jomha NM. Hypoxia mediated isolation and expansion enhances the chondrogenic capacity of bone marrow mesenchymal stromal cells. Stem Cell Res Ther. 2012;3(2):9. https://doi.org/10.1186/scrt100
   PMid:22385573
6. Phinney DG, Prockop DJ. Concise review: Mesenchymal stem/multipotent stromal cells: The state of transdifferentiation and modes of tissue repair-current views. Stem Cells. 2007;25(11):2896-902. https://doi.org/10.1634/stemcells.2007-0637
   PMid:17901396
7. Nam Y, Rim YA, Lee J, Ju JH. Current therapeutic strategies for stem cell-based cartilage regeneration. Stem Cells Int. 2018;2018:8490489. https://doi.org/10.1155/2018/8490489
   PMid:29765426
8. Mas-Bargues C, Sanz-Ros J, Román-Domínguez A, Inglés M, Gimeno-Mallench L, El Alami M, *et al*. Relevance of oxygen concentration in stem cell culture for regenerative medicine. Int J Mol Sci. 2019;20(5):1195. https://doi.org/10.3390/ijms20051195
   PMid:30857245
9. Bae HC, Park HJ, Wang SY, Yang HR, Lee MC, Han HS. Hypoxic condition enhances chondrogenesis in synovium-derived mesenchymal stem cells. Biomater Res. 2018;22(1):28. https://doi.org/10.1186/s40824-018-0134-x
   PMid:30275971
10. Bornes TD, Jomha NM, Mulet-Sierra A, Adesida AB. Hypoxic culture of bone marrow-derived mesenchymal stromal stem cells differentially enhances *in vitro* chondrogenesis within cell-seeded collagen and hyaluronic acid porous scaffolds. Stem Cell Res Ther. 2015;6(1):84. https://doi.org/10.1186/s13287-015-0075-4
    PMid:25900045
11. Huang X, Hou Y, Zhong L, Huang D, Qian H, Karperien M, *et al*. Promoted chondrogenesis of co-cultured chondrocytes and mesenchymal stem cells under hypoxia using *in situ* forming degradable hydrogel scaffolds. Biomacromolecules. 2017;19(1):94-102. https://doi.org/10.1021/acs.biomac.7b01271
    PMid:29211452
12. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, *et al*. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. PLoS Med. 2009;6(7):e1000100. https://doi.org/10.1371/journal.pmed.1000100
    PMid:19621070
13. Whiting P, Savovic J, Higgins JP, Caldwell DM, Reeves BC, Shea B, *et al*. ROBIS: A new tool to assess risk of bias in systematic reviews was developed. J Clin Epidemiol. 2016;69:225-34. https://doi.org/10.1016/j.jclinepi.2015.06.005
    PMid:26092286
14. Gong M, Liang T, Jin S, Dai X, Zhou Z, Gao M, *et al*. Methylation-mediated silencing of miR-124 facilitates chondrogenesis by targeting NFATc1 under hypoxic conditions. Am J Transl Res. 2017;9(9):4111-24.
    PMid:28979686
15. Henrionnet C, Liang G, Roeder E, Dossot M, Wang H, Magdalou J, *et al*. Hypoxia for mesenchymal stem cell expansion and differentiation: The best way for enhancing TGFß-induced chondrogenesis and preventing calcifications in alginate beads. Tissue Eng Part A. 2017;23(17-18):913-22. https://doi.org/10.1089/ten.tea.2016.0426
    PMid:28385113
16. Hung SP, Ho JH, Shih YR, Lo T, Lee OK. Hypoxia promotes proliferation and osteogenic differentiation potentials of human mesenchymal stem cells. J Orthop Res. 2012;30(2):260-6. https://doi.org/10.1002/jor.21517
    PMid:21809383
17. Kanichai M, Ferguson D, Prendergast PJ, Campbell VA. Hypoxia promotes chondrogenesis in rat mesenchymal stem cells: A role for AKT and hypoxia-inducible factor (HIF)-1α. J Cell Physiol. 2008;216(3):708-15. https://doi.org/10.1002/jcp.21446
    PMid:18366089
18. Khan WS, Adesida AB, Hardingham TE. Hypoxic conditions increase hypoxia-inducible transcription factor 2α and enhance chondrogenesis in stem cells from the infrapatellar fat pad of osteoarthritis patients. Arthritis Res Ther. 2007;9(3):R55. https://

doi.org/10.1186/ar2211

PMid:17537234

19. Khan WS, Adesida AB, Tew SR, Lowe ET, Hardingham TE. Bone marrow-derived mesenchymal stem cells express the pericyte marker 3G5 in culture and show enhanced chondrogenesis in hypoxic conditions. J Orthop Res. 2010;28(6):834-40. https://doi.org/10.1002/jor.21043

PMid:20058274

20. Kishimoto KN, Oxford CL, Reddi AH. Stimulation of the side population fraction of ATDC5 chondroprogenitors by hypoxia. Cell Biol Int. 2009;33(12):1222-9. https://doi.org/10.1016/j.cellbi.2009.06.009

PMid:19524690

21. Lee HH, Chang CC, Shieh MJ, Wang JP, Chen YT, Young TH, *et al*. Hypoxia enhances chondrogenesis and prevents terminal differentiation through PI3K/Akt/FoxO dependent anti-apoptotic effect. Sci Rep. 2013;3:2683. https://doi.org/10.1038/srep02683

PMid:24042188

22. Lee J, Byeon JS, Lee KS, Gu NY, Lee GB, Kim HR, *et al*. Chondrogenic potential and anti-senescence effect of hypoxia on canine adipose mesenchymal stem cells. Vet Res Commun. 2016;40(1):1-10. https://doi.org/10.1007/s11259-015-9647-0

PMid:26661466

23. Mahyudin F, Utomo DN, Martanto TW, Hidayat AR, Putri LM. Effect of decellularized cartilage bovine scaffold and hypoxic condition on stem cell differentiation to chondrocyte: An *in vitro* study. J Biomim Biomater Biomed Eng. 2018;35:67-76. https://doi.org/10.4028/www.scientific.net/jbbbe.35.67

24. Markway BD, Tan GK, Brooke G, Hudson JE, Cooper-White JJ, Doran MR. Enhanced chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells in low oxygen environment micropellet cultures. Cell Transplant. 2010;19(1):29-42. https://doi.org/10.3727/096368909x478560

PMid:19878627

25. Merceron C, Vinatier C, Portron S, Masson M, Amiaud J, Guigand L, *et al*. Differential effects of hypoxia on osteochondrogenic potential of human adipose-derived stem cells. Am J Physiol Cell Physiol. 2010;298(2):355-64. https://doi.org/10.1152/ajpcell.00398.2009

PMid:19940068

26. Meretoja VV, Dahlin RL, Wright S, Kasper FK, Mikos AG. The effect of hypoxia on the chondrogenic differentiation of co-cultured articular chondrocytes and mesenchymal stem cells in scaffolds. Biomaterials. 2013;34(17):4266-73. https://doi.org/10.1016/j.biomaterials.2013.02.064

PMid:23489925

27. Munir S, Foldager CB, Lind M, Zachar V, Søballe K, Koch TG. Hypoxia enhances chondrogenic differentiation of human adipose tissue-derived stromal cells in scaffold-free and scaffold systems. Cell Tissue Res. 2014;355(1):89-102. https://doi.org/10.1007/s00441-013-1732-5

PMid:24178804

28. Portron S, Merceron C, Gauthier O, Lesoeur J, Sourice S, Masson M, *et al*. Effects of in vitro low oxygen tension preconditioning of adipose stromal cells on their *in vivo* chondrogenic potential: Application in cartilage tissue repair. PLoS One. 2013;8(4):e62368. https://doi.org/10.1371/journal.pone.0062368

PMid:23638053

29. Ranera B, Remacha AR, Álvarez-Arguedas S, Castiella T, Vázquez FJ, Romero A, *et al*. Expansion under hypoxic conditions enhances the chondrogenic potential of equine bone marrow-derived mesenchymal stem cells. Vet J. 2013;195(2):248-51. https://doi.org/10.1016/j.tvjl.2012.06.008

PMid:22771146

30. Silva JC, Han X, Silva TP, Xia K, Mikael PE, Cabral JMS, *et al*. Glycosaminoglycan remodeling during chondrogenic differentiation of human bone marrow-/synovial-derived mesenchymal stem/stromal cells under normoxia and hypoxia. Glycoconj J. 2020;37(3):345-60. https://doi.org/10.1007/s10719-020-09911-5

PMid:32086666

31. Tian HT, Zhang B, Tian Q, Liu Y, Yang SH, Shao ZW. Construction of self-assembled cartilage tissue from bone marrow mesenchymal stem cells induced by hypoxia combined with GDF-5. J Huazhong Univ Sci Technolog Med Sci. 2013;33(5):700-6. https://doi.org/10.1007/s11596-013-1183-y

PMid:24142723

32. Wan Safwani WK, Choi JR, Yong KW, Ting I, Mat Adenan NA, Pingguan-Murphy B. Hypoxia enhances the viability, growth and chondrogenic potential of cryopreserved human adipose-derived stem cells. Cryobiology. 2017;75:91-9. https://doi.org/10.1016/j.cryobiol.2017.01.006

PMid:28108309

33. Xu Y, Malladi P, Chiou M, Bekerman E, Giaccia AJ, Longaker MT. *In vitro* expansion of adipose-derived adult stromal cells in hypoxia enhances early chondrogenesis. Tissue Eng. 2007;13(12):2981-93. https://doi.org/10.1089/ten.2007.0050

PMid:17916040

34. Koay EJ, Athanasiou KA. Hypoxic chondrogenic differentiation of human embryonic stem cells enhances cartilage protein synthesis and biomechanical functionality. Osteoarthritis Cartilage. 2008;16(12):1450-6. https://doi.org/10.1016/j.joca.2008.04.007

PMid:18541445

35. Cicione C, Muiños-López E, Hermida-Gómez T, Fuentes-Boquete I, Díaz-Prado S, Blanco FJ. Effects of severe hypoxia on bone marrow mesenchymal stem cells differentiation potential. Stem Cells Int. 2013;2013:232896. https://doi.org/10.1155/2013/232896

PMid:24082888

36. Duval E, Baugé C, Andriamanalijaona R, Bénateau H, Leclercq S, Dutoit S, *et al*. Molecular mechanism of hypoxia-induced chondrogenesis and its application in *in vivo* cartilage tissue engineering. Biomaterials. 2012;33(26):6042-51. https://doi.org/10.1016/j.biomaterials.2012.04.061

PMid:22677190

37. Felka T, Schäfer R, Schewe B, Benz K, Aicher WK. Hypoxia reduces the inhibitory effect of IL-1beta on chondrogenic differentiation of FCS-free expanded MSC. Osteoarthritis Cartilage. 2009;17(10):1368-76. https://doi.org/10.1016/j.joca.2009.04.023

PMid:19463979

38. Gale AL, Mammone RM, Dodson ME, Linardi RL, Ortved KF. The effect of hypoxia on chondrogenesis of equine synovial membrane-derived and bone marrow-derived mesenchymal stem cells. BMC Vet Res. 2019;15(1):201. https://doi.org/10.1186/s12917-019-1954-1

PMid:31200719

39. Galeano-Garces C, Camilleri ET, Riester SM, Dudakovic A, Larson DR, Qu W, *et al*. Molecular validation of chondrogenic differentiation and hypoxia responsiveness of platelet-lysate expanded adipose tissue–derived human mesenchymal stromal cells. Cartilage. 2016;8(3):283-99. https://doi.org/10.1177/1947603516659344

PMid:28618870

40. Gómez-Leduc T, Desancé M, Hervieu M, Legendre F, Ollitrault D, de Vienne C, *et al*. Hypoxia is a critical parameter for chondrogenic differentiation of human umbilical cord blood mesenchymal stem cells in Type I/III collagen sponges. Int J Mol Sci. 2017;18(9):1933. https://doi.org/10.3390/ijms18091933

PMid:28885597

41. Kalpakci KN, Brown WE, Hu JC, Athanasiou KA. Cartilage tissue engineering using dermis isolated adult stem cells: The use of hypoxia during expansion versus chondrogenic differentiation. PLoS One. 2014;9(5):e98570. https://doi.org/10.1371/journal.pone.0098570

PMid:24867063

42. Yodmuang S, Marolt D, Marcos-Campos I, Gadjanski I, Vunjak-Novakovic G. Synergistic effects of hypoxia and morphogenetic factors on early chondrogenic commitment of human embryonic stem cells in embryoid body culture. Stem Cell Rev Rep. 2015;11(2):228-41. https://doi.org/10.1007/s12015-015-9584-x

PMid:25618295

43. Ohara T, Muneta T, Nakagawa Y, Matsukura Y, Ichinose S, Koga H, *et al*. Hypoxia enhances proliferation through increase of colony formation rate with chondrogenic potential in primary synovial mesenchymal stem cells. J Med Dent Sci. 2016;63(4):61-70.

PMid:28049938

44. Angele P, Yoo JU, Smith C, Mansour J, Jepsen KJ, Nerlich M, *et al*. Cyclic hydrostatic pressure enhances the chondrogenic phenotype of human mesenchymal progenitor cells differentiated *in vitro*. J Orthop Res. 2003;21(3):451-7. https://doi.org/10.1016/s0736-0266(02)00230-9

PMid:12706017