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Response of Human Engineered Cartilage Based on Articular or Nasal Chondrocytes to Interleukin-1β and Low Oxygen

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Previous studies showed that human nasal chondrocytes (HNC) exhibit higher proliferation and chondrogenic capacity as compared to human articular chondrocytes (HAC). To consider HNC as a relevant alternative cell source for the repair of articular cartilage defects it is necessary to test how these cells react when exposed to environmental factors typical of an injured joint. We thus aimed this study at investigating the responses of HNC and HAC to exposure to interleukin (IL)-1β and low oxygen. For this purpose HAC and HNC harvested from the same donors ($N=5$) were expanded *in vitro* and then cultured in pellets or collagen-based scaffolds at standard (19%) or low oxygen (5%) conditions. Resulting tissues were analyzed after a short (3 days) exposure to IL-1β, mimicking the initially inflammatory implantation site, or following a recovery time (1 or 2 weeks for pellets and scaffolds, respectively). After IL-1β treatment, constructs generated by both HAC and HNC displayed a transient loss of GAG (up to 21.8% and 36.8%, respectively) and, consistently, an increased production of metalloproteases (MMP)-1 and -13. Collagen type II and the cryptic fragment of aggrecan (DIPEN), both evaluated immunohistochemically, displayed a trend consistent with GAG and MMPs production. HNC-based constructs exhibited a more efficient recovery upon IL-1β withdrawal, resulting in a higher accumulation of GAG (up to 2.6-fold) compared to the corresponding HAC-based tissues. On the other hand, HAC displayed a positive response to low oxygen culture, while HNC were only slightly affected by oxygen percentage. Collectively, under the conditions tested mimicking the postsurgery articular environment, HNC retained a tissue-forming capacity, similar or even better than HAC. These results represent a step forward in validating HNC as a cell source for cartilage tissue engineering strategies.

Introduction

In the past two decades, autologous chondrocytes implantation (ACI) techniques have been widely used to restore damaged articular surfaces.[1–4] In these cell-based techniques, autologous chondrocytes are enzymatically isolated from a small biopsy of healthy articular cartilage obtained in arthroscopic surgery from non-weight-bearing zones. However, the approach is characterized by relevant drawbacks possibly affecting the reproducibility of clinical outcome: (1) limited chondrocytes redifferentiation after *in vitro* expansion,[5,6] (2) reduced chondrogenic potential of chondrocytes from aged donors,[7] and (3) morbidity to a healthy area of the articular surface for the biopsy.[3,8–10]

As alternative cell source to overcome the previously cited drawbacks of ACI and potentially improve its results, we and others have advocated the use of human nasal septum chondrocytes (HNC). In particular, this strategy would make it possible (1) to obtain cartilage biopsy from the patient with a procedure that is less invasive than removing tissue from specific areas of the joint, (2) to reduce the donor site morbidity, and (3) to reduce complications associated with the harvesting of the biopsy. Moreover, several studies have indicated that as compared to human articular chondrocytes (HAC), HNC (1) proliferate faster and have a higher and more reproducible chondrogenic capacity, both *in vitro* and in an ectopic model *in vivo*,[11–13] and (2) can generate tissues in a quality that does not appear to be dependent on the age of the donor.[14]

Once implanted in the joint, HNC would be exposed to an environment that is biochemically and biomechanically markedly different from their native one. We have previously reported that HNC can respond *in vitro* to physical forces resembling joint loading similarly to HAC.[15] However, for HNC to be considered for the repair of articular cartilage defects, it is also crucial to explore their response to biochemical signals typical of the injured or postsurgery joint. In particular, cartilage covering the articular surfaces is

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exposed to a low oxygen tension, because of the lack of vascularity,[16] and, in case of intraarticular bleeding following injury or surgery, to a transient high level of proinflammatory chemokines such as interleukin (IL)-1β.[17]

Thus, the goal of this study was to compare the responses of HNC and HAC to IL-1β, a catabolic factor normally present in injured and postsurgical joints, at normoxic (i.e., 19%) or more physiologic, low oxygen (i.e., 5%) conditions. For this purpose, considering the typically large interindividual variability in chondrocyte function,[7] HAC and HNC were isolated from the same individuals. Cells were expanded in monolayers and transferred to different 3D culture systems that had previously been used as models to investigate chondrocyte redifferentiation and synthesis of cartilage-specific extracellular matrix proteins, namely micromass pellets and collagen-based 3D porous scaffolds.

Materials and Methods

Cartilage biopsies, chondrocyte isolation and expansion

Healthy articular and nasal cartilage tissues were harvested postmortem, respectively, from full-thickness biopsies of the femoral condyle and from the nasal septum of five individuals (mean age: 54 years, range 37–84 years, male:female = 2:3), in accordance with the Local Ethical Committee. HAC and HNC were isolated by 22 h of incubation at 37°C in 0.15% type II collagenase and resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 4.5 mg/mL D-glucose, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 mM HEPES buffer, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.29 mg/mL L-glutamate (complete medium, CM). HNC and HAC were plated in culture dishes at a density of 10^4 cells/cm^2 and expanded in CM supplemented with 1 ng/mL of transforming growth factor β1 and 5 ng/mL of fibroblast growth factor 2. When subconfluent, cells were detached by sequential treatment with 0.3% type II collagenase and 0.05% trypsin/0.53 mM EDTA, replated at 5×10^3 cells/cm^2, and cultured until again subconfluent, corresponding to a total of about 8 to 10 population doublings. Chondrocytes were subsequently cultivated in 3D scaffolds or in pellets as described below.

Culture on porous 3D scaffolds

This model was selected because it is close to a clinically relevant approach as per cell-scaffold combination. HNC and HAC from two donors were cultured into type I collagen meshes (6-mm-diameter, 2-mm-thick disks, Ultrafoam®, Davol) at a density of 70×10^6 cells/cm^3. Cell-scaffold constructs were cultured in CM supplemented with 0.1 mM ascorbic acid, 10 μg/mL insulin, and 10 ng/mL transforming growth factor-β3 (TGF-β3), with medium changes twice a week. After 2 or 4 weeks of culture, constructs (respectively defined as "*immature*" or "*mature*") were exposed to 50 pg/mL human recombinant interleukin-1 beta (IL-1β) (Sigma Chemical, St. Louis, MO) for 3 days, in a range previously demonstrated to mimic postsurgical joint environment,[18] and then assessed or cultured for additional 2 weeks in the absence of IL-1β (see Table 1 for a detailed description of the experimental groups). Resulting tissues were analyzed histologically, immunohistochemically, biochemically, and by quantitative real time RT-PCR as described below.

Pellet culture

This model was selected because it allows for multiple conditions testing. HNC and HAC from three donors were cultured in pellets using a defined serum-free medium as previously described.[7] Briefly, cells were suspended in DMEM supplemented with ITS + 1 (Sigma Chemical, St. Louis, MO), 0.1 mM ascorbic acid 2-phosphate, 1.25 mg/mL human serum albumin, 10^{-7} M dexamethasone, and 10 ng/mL TGF-β1. Aliquots of 5×10^5 cells/0.5 mL were centrifuged at 1000 rpm for 2 min in 1.5-mL polypropylene conical tubes (Sarstedt, Nümbrecht, Germany) to form spherical pellets, which were placed onto a 3D orbital shaker (Bioblock Scientific, Frenkendorf, Switzerland) at 30 rpm. Pellets were cultured for 7 or 14 days in a humidified incubator (37°C/5% CO_2) at either normoxic condition (19% O_2) or *low*, more physiological oxygen tension (5% O_2) as previously described.[19] Medium was equilibrated under 5% and 19% O_2 for

Table 1. Description of the Experimental Groups

	Groups	*Culture phase 1*[a]	*IL-1β exposure*[a]	*Culture phase 2*[a]
Scaffold culture				
Immature	ctr	2 weeks	No	No
	IL	2 weeks	3 days	No
	IL + recovery	2 weeks	3 days	2 weeks
Mature	ctr	4 weeks	No	No
	IL	4 weeks	3 days	No
	IL + recovery	4 weeks	3 days	2 weeks
Pellet culture				
Immature	ctr	1 week	No	No
	IL	1 week	3 days	No
	Recovery	1 week	3 days	1 week
Mature	ctr	2 weeks	No	No
	IL	2 weeks	3 days	No
	IL + recovery	2 weeks	3 days	1 week

[a]Scaffold cultures were performed at 19% oxygen and pellet cultures were performed either at 19% or 5% oxygen. ctr, control; IL, treatment with 50 pg/mL IL-1β; IL + recovery, samples treated with 50 pg/mL IL-1β and further cultured for the indicated time in the absence of IL-1β.

at least 6 h before each media change. After 1 (*immature*) or 2 (*mature*) weeks of culture, some of the pellets were exposed to 50 pg/mL IL-1β for 3 days and immediately assessed or cultured for one additional week at 19% or 5% oxygen in the absence of IL-1β (see Table 1 for a schematic description of the experimental groups). Resulting tissues were cut in halves and then analyzed histologically, immunohistochemically, and biochemically as described below.

Biochemical analyses

Constructs and pellets were digested for 15 h at 56°C with protease K (0.5 mL for pellets and 1 mL for scaffolds of 1 mg/mL protease K in 50 mM Tris with 1 mM EDTA, 1 mM iodoacetamide, and 10 μg/mL pepstatin-A, respectively). GAG amounts were measured spectrophotometrically after reaction with dimethylmethylene blue,[20] with chondroitin sulfate as a standard. DNA was measured spectrofluorometrically using the CyQuant cell proliferation assay Kit (Molecular Probes, Eugene, OR), with calf thymus DNA as a standard.[21] GAG contents were reported as % GAG/wet weight tissue (for constructs) or GAG/DNA (for pellets). GAG amounts were also quantified in media collected from cultured constructs.

Matrix metalloproteinases (MMPs) were quantified in media collected from cultured constructs and pellets by using the MultiAnalyte Profiling MMP base Kit (Fluorokine® MAP: LMP000) complemented with the specific MMPs (MMP-1: LMP901; MMP-13: LMP511, R&D Systems). The assays were performed on a Luminex 100™ analyzer following the manufacturer's instructions.

Real-time quantitative RT-PCR assays

RNA of pellets was extracted using Trizol (Life Technologies, Basel, Switzerland), according to the manufacturer's protocol. Pellets cultured in chondrogenic medium were first sonicated for 1 min while in Trizol. RNA was treated with DNase I using the DNA-free™ Kit (Ambion) and quantified spectrofluorimetrically. cDNA was generated from 3 μg of RNA by using 500 μg/mL random hexamers (Catalys AG, CH) and 1 μL of 50 U/mL Stratascript™ reverse transcriptase (Stratagene, NL), in the presence of dNTPs. PCR reactions were performed and monitored using the ABI Prism 7700 Sequence Detection System (Perkin-Elmer/Applied Biosystems, Rotkreuz, Switzerland). Cycle temperatures and times as well as primers and probes used for the reference gene (GAPDH) were as previously described.[7] Assays on-Demand (Applied Biosystem) were used to measure the expression of MMP-1 (Hs00233958_m1) and MMP-13 (Hs00233992_m1). For each cDNA sample, the threshold cycle (C_t) value of each target sequence was subtracted to the C_t value of GAPDH, to derive ΔC_t. The level of gene expression was calculated as $2^{\Delta Ct}$. Each sample was assessed at least in duplicate for each gene of interest.

Histological and immunohistochemical analyses

Generated pellets and constructs were rinsed with phosphate-buffered saline (PBS), fixed in 4% formalin, embedded in paraffin, and cross-sectioned (5 μm thick for pellets and 7 μm thick for constructs). Sections were stained with Safranin-O for sulfated glycosaminoglycans (GAG). Sections were processed for immunohistochemistry using an antibody against type II collagen (MPBiomedicals) or Aggrecan cryptical epitope-DIPEN (MD Biosciences) as previously described.[22]

Statistical analysis

For each experiment and donor, at least triplicate specimens were assessed and the values were presented as mean ± standard deviation of measurements. Statistical analyses were performed using the Sigma Stat software (SPSS Inc., Version 13). Differences between experimental groups were assessed by two-tailed Wilcoxon tests and considered statistically significant with $p < 0.05$.

Results

Anabolic responses of HAC- and HNC-based constructs to IL-1β

HNC and HAC isolated from one donor were cultured on Ultrafoam® meshes for 2 weeks (*immature*) or 4 weeks (*mature*) and the resulting tissues exposed to IL-1β. The following statistically significant trends were observed: (1) higher

Table 2. Biochemical Results of Constructs Generated by Human Nasal Chondrocytes and Human Articular Chondrocytes from Donor 1

	GAG/DNA (μg/μg)	*GAG/wet weight (μg/mg)*	*GAG loss (ctr vs. IL)*	*DNA/wet weight (μg/mg)*	*DNA loss (ctr vs. IL)*
I: HAC					
Immature, ctr	3.0 ± 0.2	1.93 ± 0.15		0.65 ± 0.08	
Immature, IL	2.5 ± 0.3*	1.51 ± 0.21*	21.8%	0.61 ± 0.01	6.1%
Mature, ctr	6.3 ± 0.4	3.80 ± 1.19		0.60 ± 0.05	
Mature, IL	6.1 ± 0.6	3.44 ± 0.48	9.5%	0.56 ± 0.04	6.7%
II: HNC					
Immature, ctr	4.2 ± 0.3#	2.96 ± 0.48#		0.71 ± 0.06	
Immature, IL	2.9 ± 0.1*,#	1.87 ± 0.11*,#	36.8%	0.65 ± 0.11	8.4%
Mature, ctr	8.9 ± 0.5#	5.62 ± 0.58#		0.63 ± 0.06	
Mature, IL	7.2 ± 0.5*,#	4.48 ± 0.48*,#	20.3%	0.62 ± 0.05	1.6%

*$p < 0.05$ from ctr (same cell source).
#$p < 0.05$ vs. articular chondrocytes (same maturation extent).
ctr, control; IL, treatment with 50 pg/mL IL-1β; HNC, human nasal chondrocytes; HAC, human articular chondrocytes.

accumulation of GAG in *mature* vs. *immature* tissues, more pronounced by HNC-based constructs, (2) higher GAG loss in response to IL-1β by HNC-based constructs vs. HAC-based constructs, and (3) superior GAG content after IL-1β exposure in HNC-based constructs compared to HAC-based constructs. Only minor differences in the amounts of DNA among all experimental groups were observed (Table 2).

Immature and *mature* cartilaginous tissues, generated as previously described with HAC and HNC from another donor, were cultured for an additional 2 weeks (recovery

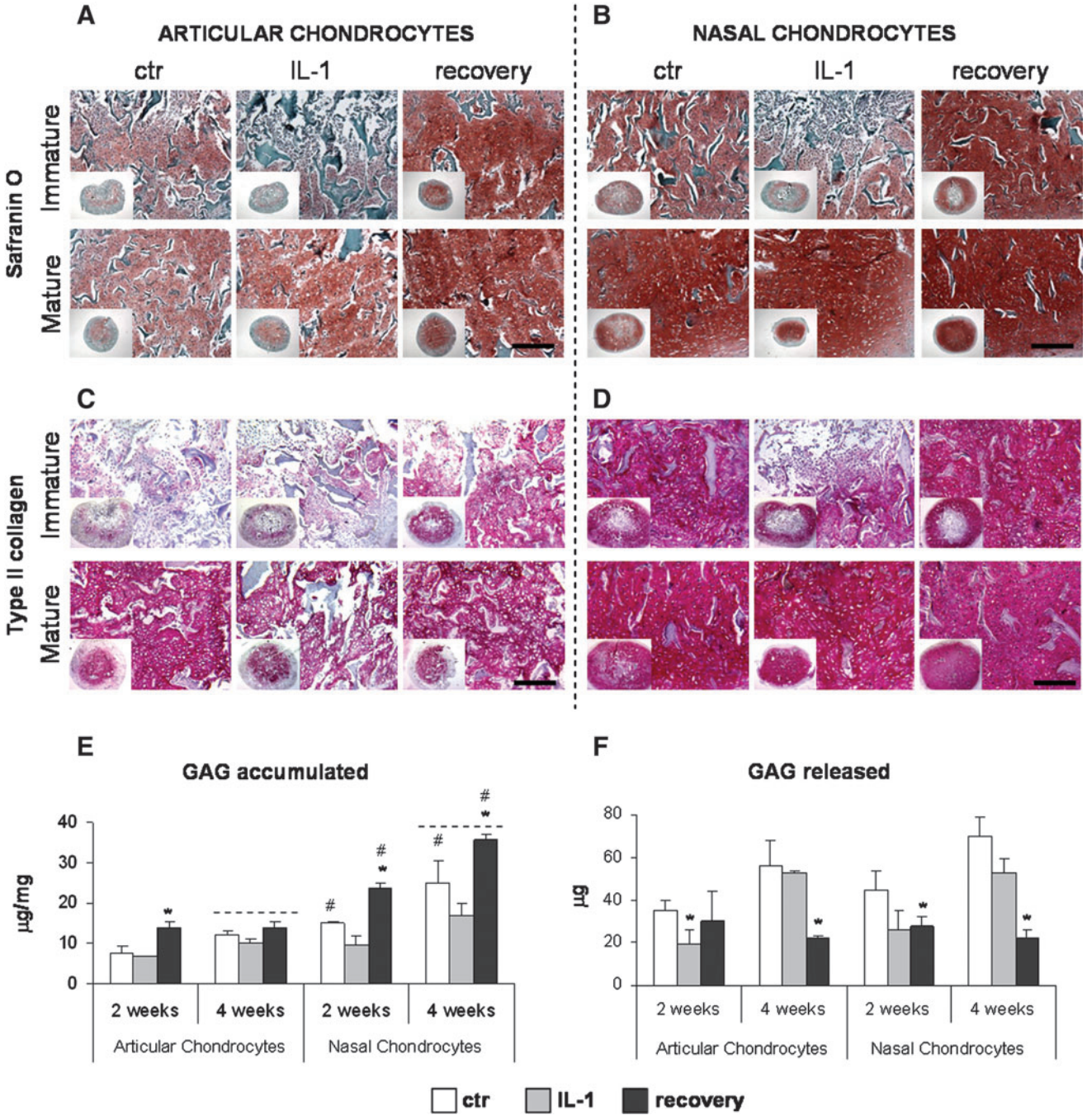

FIG. 1. Accumulation and release of cartilage matrix proteins by human articular chondrocytes (HAC)- and human nasal chondrocytes (HNC)-based scaffolds. **(A, B)** Safranin O and **(C, D)** type II collagen staining of representative constructs generated by HAC **(A, C)** or HNC **(B, D)** cultured in scaffold for a total of 2 weeks (*immature*) or 4 weeks (*mature*). Constructs were cultured for the last 3 days with (IL-1) or without (ctr) IL-1β and then maintained for an additional 2 weeks without IL-1β (recovery). Insets represent smaller magnifications of the constructs. Bars = 200 μm. **(E)** Sulfated glycosaminoglycan (GAG) content normalized to the construct wet weight. **(F)** GAG released in the culture medium. Values are mean ± SD of triplicate specimens obtained with cells isolated from one donor. The dashed line indicates the values displayed by samples cultured for a total of 6 weeks without IL-1β treatment. $^{*}p < 0.05$ from ctr (same cell source); $^{\#}p < 0.05$ vs. articular chondrocytes (same maturation extent). Color images available online at www.liebertonline.com/tea

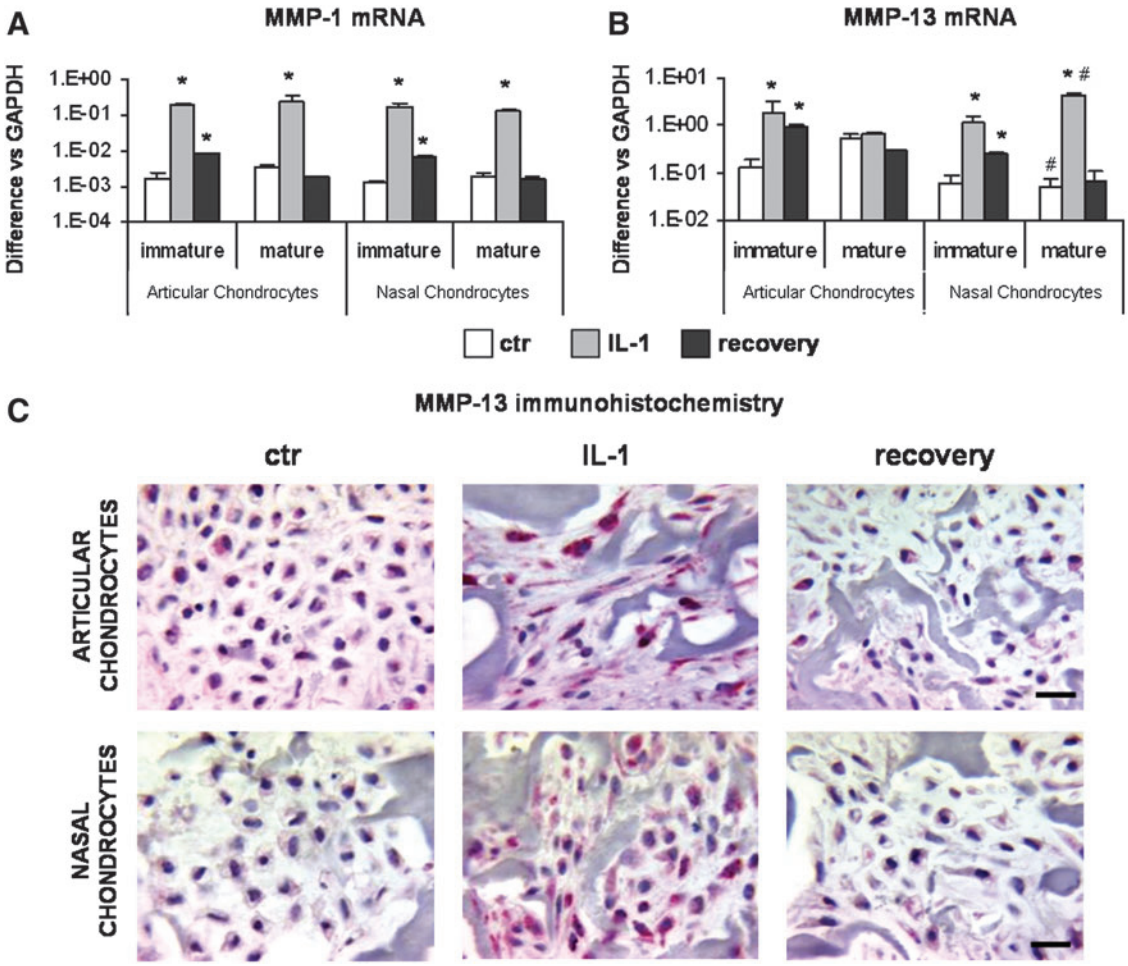

FIG. 2. Production of metalloproteases (MMP)-1 and MMP-13 by human articular chondrocytes (HAC)- and human nasal chondrocytes (HNC)-based scaffolds. **(A, B)** Real time RT-PCR analysis of the expression of type MMP-1 **(A)** and MMP-13 mRNA **(B)** by HAC and HNC cultured in collagen scaffolds for 2 weeks (*immature*) or 4 weeks (*mature*) (ctr), exposed to IL-1β (IL-1) for 3 days, and then maintained for an additional 2 weeks without IL-1β (recovery). Levels are expressed as fold of difference from glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Values are mean ± SD of measurements obtained from triplicate specimens obtained with cells isolated from one donor. **(C)** MMP-13 immunohistochemical staining of *immature* tissues. Bars = 400 μm. $^{*}p < 0.05$ from ctr (same cell source); $^{\#}p < 0.05$ from articular chondrocytes (same maturation extent). Color images available online at www.liebertonline.com/tea

time) after exposure to IL-1β. In agreement with the results of the former experiment, both HAC- and HNC-based constructs matured with culture time as observed by the stronger staining for GAG and type II collagen. Staining intensities for both macromolecules were, however, always higher in *immature* and *mature* HNC-based tissues compared to the corresponding HAC-based ones, and remained higher even following IL-1β exposure and the recovery time (Fig. 1A–D). The spatial distribution of GAG and type II collagen in both HAC- and HNC-based constructs was not modulated by the IL-1β exposure and differed between *immature* and *mature* tissues: both extracellular matrix molecules were mainly located in the outer region of *immature* specimens and more uniformly distributed through the tissue in *mature* ones (Fig. 1A–D).

Biochemical analyses confirmed that despite a superior IL-1β-mediated GAG loss by HNC-based tissues (vs. HAC-based ones), as evidenced by a more pronounced reduction in the GAG contents (2.8- and 1.8-fold, respectively, for *immature* and *mature* tissues), GAG content remained at higher amounts in the tissues following IL-1β exposure (1.5- and 1.7-fold, respectively, for *immature* and *mature* tissues). In addition, HNC-based tissues better recovered following IL-1β withdrawal, so that the GAG contents after an additional 2 weeks of culture without IL-1β were 1.7- and 2.6-fold higher (respectively, for *immature* and *mature* tissues) compared to the corresponding HAC-based tissues (Fig. 1E). DNA contents of both HAC and HNC tissues did not substantially differ after IL-1β exposure or recovery time (data not shown). Analysis of culture supernatants indicated comparable trends between HAC- and HNC-based constructs in the release of GAG. In particular, GAG release decreased following IL-1β exposure of *immature* tissues (up to 1.8-fold) and after the recovery time of *mature* tissues (up to 2.4-fold) (Fig. 1F).

Overall, these results indicated that both HAC- and HNC-based tissues respond to IL-1β by reducing transiently the production (accumulation and release) of cartilage matrix

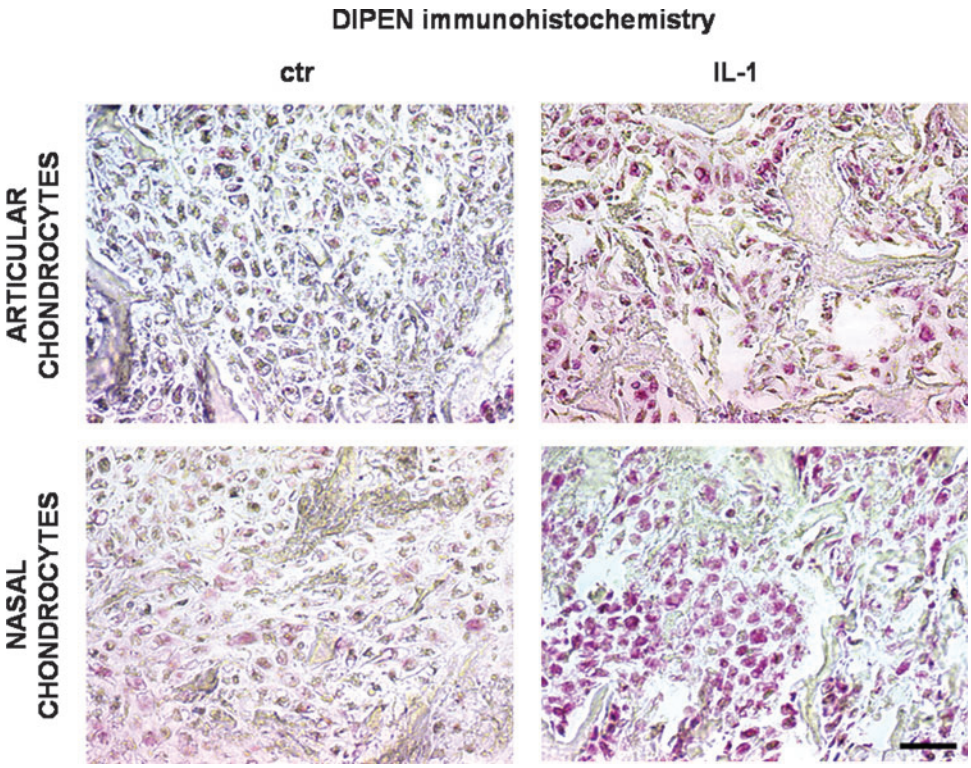

FIG. 3. Accumulation of Aggrecan cryptical epitope (DIPEN) by human articular chondrocytes (HAC)- and human nasal chondrocytes (HNC)-based scaffolds. Immunohistochemical detection of DIPEN by representative constructs cultured for 2 weeks (ctr) and exposed to IL-1β (IL-1) for 3 days. Bar = 200 μm. Color images available online at www.liebertonline.com/tea

proteins but promptly recover following IL-1β withdrawal. Both cartilage matrix loss and recovery were more pronounced with HNC-based tissues.

Catabolic responses of HAC- and HNC-based constructs to IL-1β

We first compared the expression of MMP-1 and -13 mRNA by HAC- and HNC-based tissues under different culture conditions. Both tissues expressed similar basal levels of MMP-1 and up-regulated this catabolic gene in response to IL-1β to a similar extent (up to 131- and 77.4-fold in *immature* and *mature* tissue, respectively). MMP-13 mRNA expression levels were generally lower in HNC- vs. HAC-based tissues under standard conditions (Fig. 2B). The expression of both MMPs strongly decreased after 2 weeks of recovery and reached levels measured before IL-1β exposure. Only *immature* HAC-based constructs after the recovery time expressed levels of MMP-13 higher than the basal ones (Fig. 2A and B). Immunohistochemical staining for MMP-13 overall confirmed the RT-PCR results (Fig. 2C). To evaluate the downstream effect of MMP action, we analyzed immunohistochemically the tissues for the cryptic cleaved fragment of aggrecan (DIPEN). HAC- and HNC-based samples displayed the following similar pattern of expression of this fragment: DIPEN expression (1) was at higher levels in *immature* vs. *mature* tissues (data not shown), (2) increased after exposure of *immature* tissues to IL-1β (Fig. 3) and decreased after the recovery culture (data not shown), and (3) remained at low levels in *mature* tissues after IL-1β exposure (data not shown).

These results indicate that HAC- and HNC-based tissues similarly respond to IL-1β by transiently activating the production of cartilage degradative enzymes.

Influence of low oxygen percentage on the anabolic and catabolic responses of HAC- and HNC-based pellets

HNC and HAC isolated from three donors were cultured in pellets for 1 week (*immature*) or 2 weeks (*mature*) and the resulting tissues exposed to IL-1β at different oxygen percentages. Safranin-O staining indicated that GAG accumulation (1) was generally higher in both *immature* and *mature* HNC-based pellets than in HAC ones, even after IL-1β exposure, (2) was strongly enhanced by exposure to 5% oxygen in HAC- but not in HNC-based pellets, and (3) recovered to a similar extent following IL-1β withdrawal (Fig. 4A and B). Biochemical analyses confirmed the histological results. At 19% oxygen, GAG amounts of both *immature* and *mature* HNC-based pellets were almost double those of the corresponding HAC-based pellets and remained higher even following IL-1β exposure. Low oxygen culture at 5% oxygen enhanced the accumulation of GAG mainly by HAC, so that no more difference in the content of this macromolecule was measured between the tissues generated by the two cell types. *Immature* tissues generally lost higher fractions of GAG as compared to *mature* counterparts in response to IL-1β exposure. Interestingly, the IL-1β-mediated loss of GAG was higher at 5% oxygen than 19% culture (34% vs. 20% and 42% vs. 25%, respectively, for HAC- and HNC-based pellets) (Fig. 4C and D). DNA amounts of pellets from both cell types were not significantly modulated by low oxygen culture or IL-1β exposure (data not shown).

Immunohistochemical analyses of *immature* pellets indicated that type II collagen was accumulated at higher amounts by HNC than HAC when cultured at 19% oxygen. After IL-1β exposure, type II collagen was almost absent in HAC-based pellets but still detectable in scattered areas of HNC-based

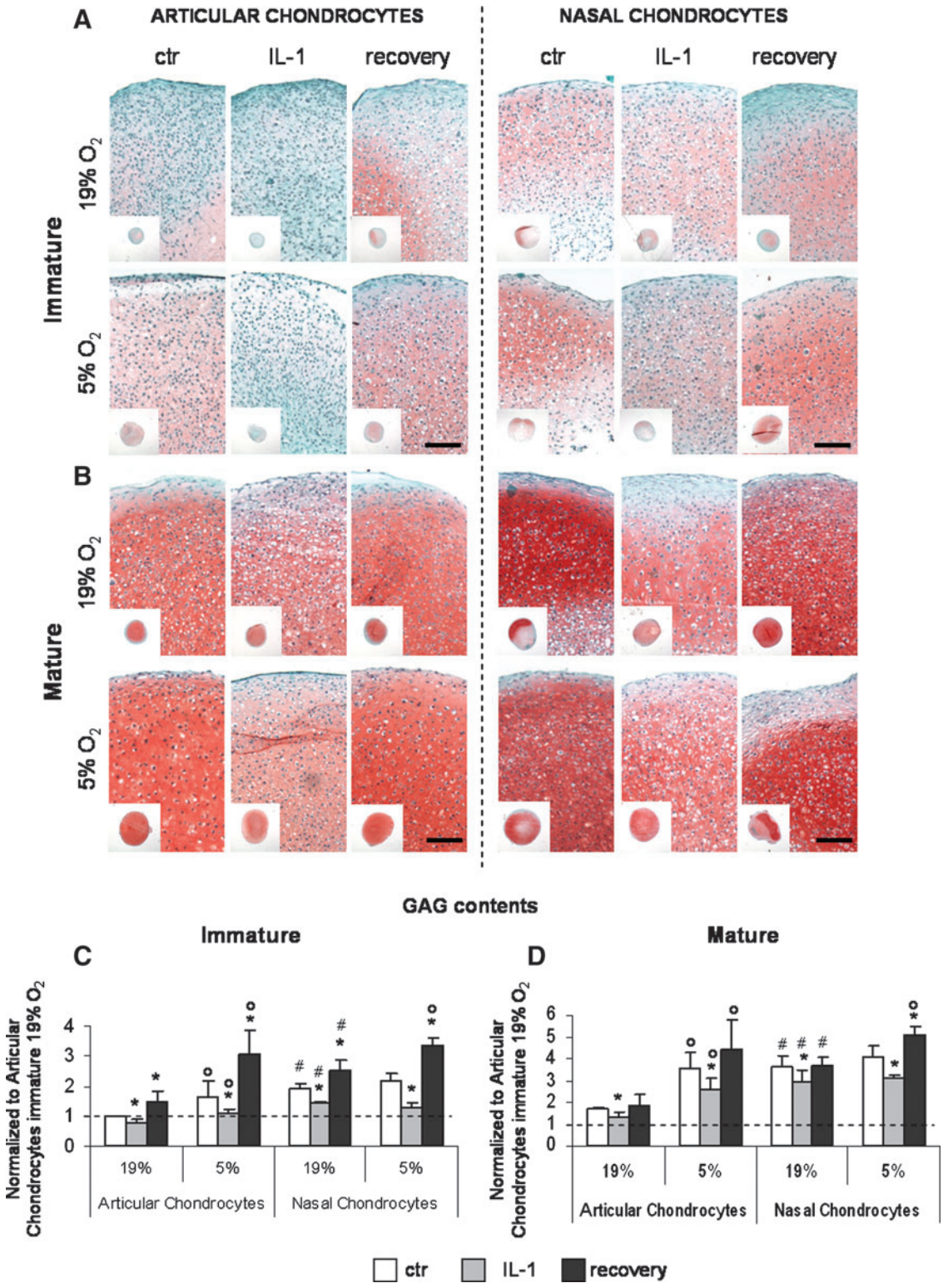

FIG. 4. Accumulation of glycosaminoglycans by human articular chondrocytes (HAC)- and human nasal chondrocytes (HNC)-based pellets. **(A, B)** Safranin O staining of representative pellets generated by HAC and HNC cultured at 19% or 5% oxygen percentage for 1 week (*immature*) (**A**) or 2 weeks (*mature*) **(B)**. Pellets were cultured for the last 3 days with (IL-1) or without (ctr) IL-1β and then maintained for an additional 2 weeks without IL-1β (recovery). Bars = 100 μm. **(C, D)** Sulfated glycosaminoglycan (GAG) content of *immature* **(C)** and *mature* **(D)** pellets. Levels are expressed as fold of difference from those measured in HAC-based *immature* pellets at 19% oxygen. Values are mean ± SD of three independent experiments (i.e., cells from three different donors). $^{*}p < 0.05$ from ctr (same oxygen percentage and cell source); $^{\circ}p < 0.05$ from 19% oxygen (same cell source and maturation extent); $^{\#}p < 0.05$ from articular chondrocytes (same oxygen % and maturation extent). Color images available online at www.liebertonline.com/tea

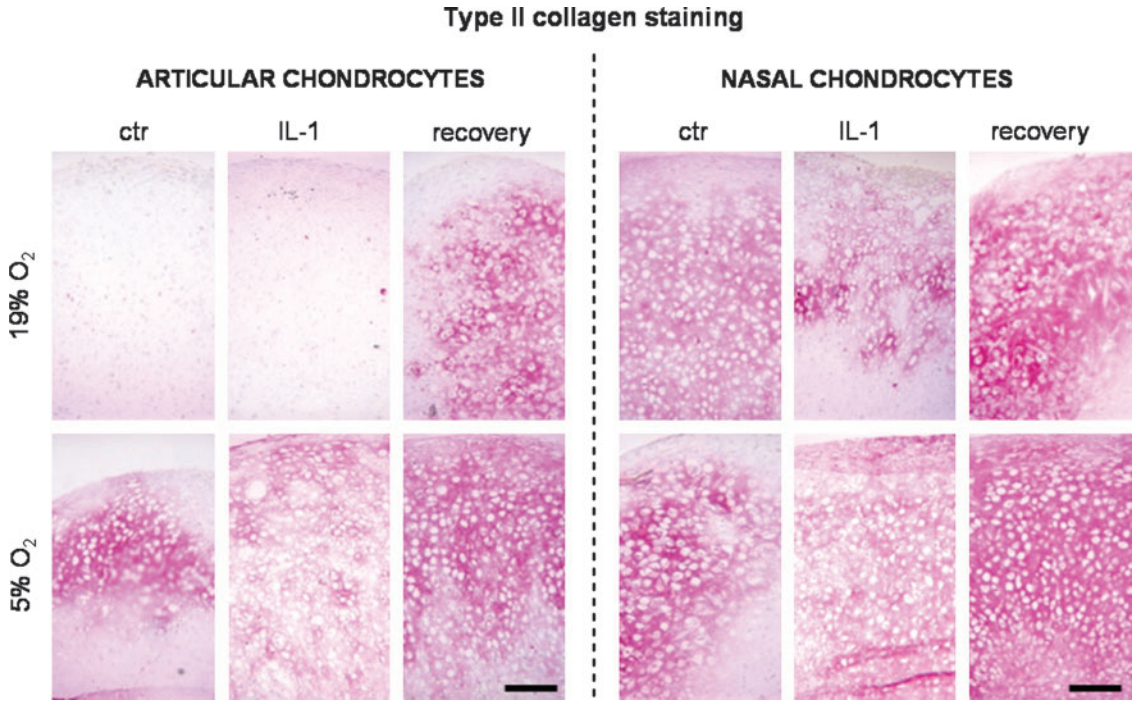

FIG. 5. Accumulation of type II collagen by human articular chondrocytes (HAC)- and human nasal chondrocytes (HNC)-based pellets. Type II collagen staining of representative pellets generated by HAC and HNC cultured at 19% or 5% oxygen percentage for 1 week (ctr), exposed to IL-1β (IL-1) for 3 days, and then cultured for an additional 2 weeks without IL-1β (recovery). Bars = 100 μm. Color images available online at www.liebertonline.com/tea

pellets, and remained stronger in the latter tissues after the recovery culture. At 5% oxygen, both cell types displayed an increase in type II collagen accumulation, with minimal differences among them (Fig. 5). *Mature* pellets were more intensely stained for type II collagen, with minimal differences between cell sources and culture conditions (data not shown).

The quantification of MMP-1 and MMP-13 in the supernatants of pellets revealed a similar pattern of MMPs release by HAC and HNC. Interestingly, the basal release of both catabolic enzymes for HAC was very low and close to the limit of detection but relatively higher for HNC. The released amounts of both MMPs increased following IL-1β exposure, were higher for HNC than for HAC (up to 2.9- and 10.4-fold, respectively, for MMP-1 and MMP-13), and after the recovery time decreased to levels comparable to those measured before IL-1β exposure. Almost identical trends in the MMPs release were observed at 5% and 19% oxygen (Fig. 6).

Discussion

In this study we compared the anabolic and catabolic responses of expanded human articular and nasal chondrocytes (HAC and HNC) to IL-1β and low oxygen percentage. Both cell types responded to IL-1β by a transient reduction of GAG and type II collagen accumulation and increased production of MMP-1 and -13. The extent of IL-1β-mediated cartilage matrix loss and recovery (following IL-1β withdrawal) was more pronounced by HNC and enhanced in HAC by a lower oxygen percentage (5% vs. 19%).

The response of articular chondrocytes to different interleukin isoforms has been previously investigated by various groups. It was demonstrated that bovine engineered cartilage does not grow when exposed to IL-1β after 14 days of culture, even after a recovery period or after physiological loading.[23,24] Similar results were also reported using porcine chondrocyte-based engineered cartilage exposed to whole blood, in order to duplicate the postsurgical intraarticular bleeding, which is likely to be the major source of IL-1β after implantation.[25,26] Although interesting, those results cannot be directly compared to those of the current study because of the use of tissues harvested from very young animals, as opposed to human chondrocytes from adult individuals.

The superior loss of GAG and type II collagen in response to IL-1β and the greater expression of MMP-1 and -13 by HNC- vs. HAC-based tissues suggested that nasal chondrocytes are more susceptible to IL-1β-induced damage. These data are in agreement with those of Kozaci *et al.*[27] indicating that the larger loss of cartilage matrix by bovine nasal vs. articular cartilage explants in response to IL-1α was accompanied by an enhanced MMP activity. This higher susceptibility to matrix degradation by nasal chondrocytes could be due to the production/activation of different degradative enzymes. Nevertheless, even if a larger amount of extracellular matrix proteins was lost by HNC in response to IL-1β, tissues generated by HNC contained more GAG and type II collagen after IL-1β exposure and better restored the matrix components following a recovery time.

HAC displayed a notable increase in synthetic activity under low oxygen tension, as already reported in the literature,[19,28–30] whereas HNC were less affected by low oxygen conditions. This finding is not in line with previously published work reporting a positive effect of low oxygen tension

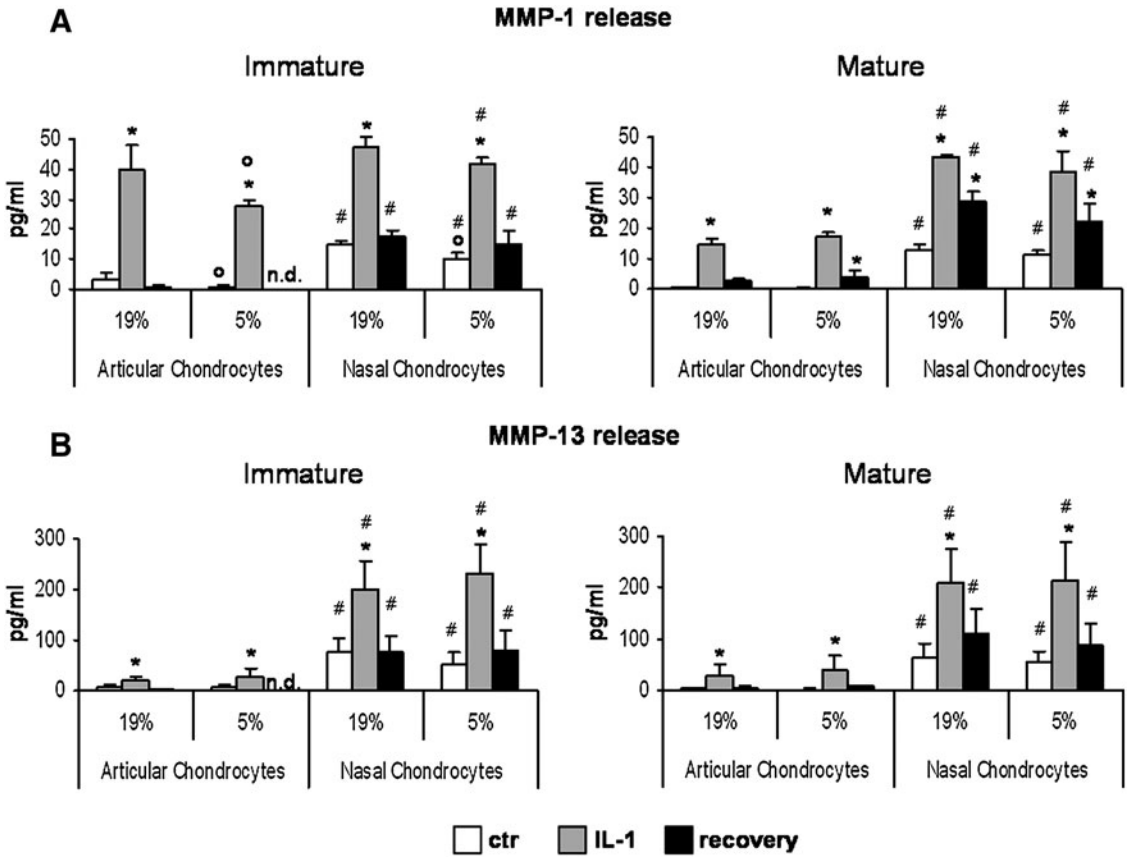

FIG. 6. Release of metalloproteases (MMP)-1 and MMP-13 by human articular chondrocytes (HAC) and human nasal chondrocytes (HNC) cultured in pellets. Amounts of MMP-1 **(A)** or MMP-13 **(B)** released by HAC- and HNC-based pellets cultured at 19% or 5% oxygen percentage for 1 weeks (*immature*) or 2 weeks (*mature*) (ctr), exposed to IL-1β (IL-1) for 3 days, and then cultured for an additional 2 weeks without IL-1β (recovery). Values are mean ± SD of three independent experiments (i.e., cells from three different donors). n.d., not detected. $^{*}p < 0.05$ from ctr (same oxygen percentage and cell source); $^{\circ}p < 0.05$ from 19% oxygen (same cell source and maturation extent); $^{\#}p < 0.05$ from articular chondrocytes (same oxygen % and maturation extent).

to HNC, with respect to GAG accumulation.[31] The difference could be related to the different culture conditions and experimental set-up, including the static environment as opposed to the bioreactor-induced hydrodynamic shear. However, in accordance with previously published work, our study highlights the potential importance of hypoxic preculture of the HAC-based engineered cartilage tissues prior to their implantation in order to optimize cell phenotype and cartilaginous tissue features.[30] The preconditioning could be relevant in order to mimic the anaerobic metabolism of chondrocytes in articular cartilage, especially within the deep layers.[16] It is also important to consider that under the culture conditions used in this study (i.e., static culture of the constructs in an incubator at 5% oxygen) the oxygen tension was likely not homogeneous throughout the tissue. Indeed, cells in the core are expected to be exposed to lower oxygen tensions than those in the outer regions. This could determine heterogeneity in cell metabolism and, therefore, in tissue organization. The culture of the constructs within perfusion-based bioreactor culture systems allowing for a more homogeneous mass transfer could represent a solution to overcome this limitation. [32]

In conclusion, as compared to HAC, HNC generated cartilaginous tissues of either similar (micromass culture at 5% oxygen) or superior (micromass culture at 20% oxygen or scaffold culture at both oxygen percentages) quality. Moreover, the stability of the tissues generated by HNC was higher upon exposure to environmental factors typical of an injured joint (i.e., IL-1β and low oxygen). Therefore, from a clinical standpoint, HNC-based engineered cartilage could have more favorable chances to successfully engraft into the joint and regenerate the articular surface. The relevance of these results is further strengthened by the fact that all HAC/HNC comparisons were performed between cells harvested from the same donor. Our findings represent an important step forward in validating HNC as a cell source for cartilage tissue engineering and prompt for further preclinical studies in large animal models.

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Disclosure Statement

No competing financial interests exist.

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