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Topographic variation in redifferentiation capacity of chondrocytes in the adult human knee joint

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Summary

Objectives: The aim of this study was to investigate the topographic variation in matrix production and cell density in the adult human knee joint. Additionally, we have examined the redifferentiation potential of chondrocytes expanded *in vitro* from the different locations.

Method: Full thickness cartilage-bone biopsies were harvested from seven separate anatomical locations of healthy knee joints from deceased adult human donors. Chondrocytes were isolated, expanded *in vitro* and redifferentiated in a pellet mass culture. Biochemical analysis of total collagen, proteoglycans and cellular content as well as histology and immunohistochemistry were performed on biopsies and pellets.

Results: In the biochemical analysis of the biopsies, we found lower proteoglycan to collagen (GAG/HP) ratio in the non-weight bearing (NWB) areas compared to the weight bearing (WB) areas. The chondrocytes harvested from different locations in femur showed a significantly better attachment and proliferation ability as well as good post-expansion chondrogenic capacity in pellet mass culture compared with the cells harvested from tibia.

Conclusion: These results demonstrate that there are differences in extra cellular content within the adult human knee in respect to GAG/HP ratio. Additionally, the data show that clear differences between chondrocytes harvested from femur and tibia from healthy human knee joints exist and that the differences are not completely abolished during the process of de- and redifferentiation. These findings emphasize the importance of the understanding of topographic variation in articular cartilage biology when approaching new cartilage repair strategies.

Key words: Human, Knee, Articular cartilage, Topographic variation, Redifferentiation.

Introduction

Articular cartilage is a specialized tissue functioning as a load-bearing surface in the synovial joints and has unique biomechanical properties in order to resist stress in compression, tension and shear during normal locomotion. The ability of articular cartilage to perform its physiological functions arises from the specialized structure, composition and integrity of the extra cellular matrix (ECM) components. The ECM in articular cartilage is produced by the chondrocytes and primarily consists of water, proteoglycans, and collagen. The relationship between biomechanical characteristics of articular cartilage with the constitution of the tissue has been subjected to various studies[1–3]. Articular cartilage composition and structure are locally conditioned by different mechanical environment and show a topographical variation in the joint[2,4,5]. Areas in the joint that are subjected to a higher level of compression stress show higher content of proteoglycans, which makes these areas stiffer than areas subjected to low levels of stress[6–8]. Additionally, the elastic properties of articular cartilage show a site-related correlation with the proteoglycan to collagen (GAG/HP) ratio in the canine knee[2]. A topographic variation in biomechanical properties of articular cartilage has furthermore been demonstrated to significantly differ between sites of high and low incidence of lesions[9].

Due to the limited self-repair in cartilage, lesions to the articular surface represent a major challenge for orthopedic surgeons and the clinical need for cartilage reconstruction is paramount. Numerous strategies, such as cell based therapies and tissue engineering approaches, have therefore emerged for cartilage reconstruction. Most of these techniques require an initial phase of *in vitro* expansion of autologous chondrocytes, which is either followed by a direct injection of the cells at the site of lesion[10], or used to engineer implantable grafts[11–13]. During the *in vitro* expansion the chondrocytes dedifferentiate and lose their round shape; produce less cartilage specific matrix proteins, such as collagen type II; and become fibroblast like and produce increasing amounts of collagen type I[14,15]. To obtain

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adequate tissue once transplanted back into the defect, the *in vitro* expanded cells have to redifferentiate and regenerate a tissue with sufficient mechanical properties, i.e., cartilage with hyaline specific proteins.

However, there is a lack in knowledge of the pattern of the ECM at different anatomical positions in the human knee joint. Increased knowledge would provide valuable insight in normal cartilage physiology and help to guide new emerging cartilage repair approaches. A better understanding of site specific ECM composition would also be of importance when designing scaffolds for cartilage tissue engineering. Additionally, more site specific information of the post-expansion chondrogenic capacity used in cell based therapies and tissue engineering may help in the design of therapeutic strategies for the treatment of articular cartilage defects. Therefore, the specific objects of this study were to examine if cartilage, in respect to total collagen, proteoglycans and cellular content, has a topographic variation in the adult human knee joint and if chondrocytes isolated from these locations possess similar ability to redifferentiate after *in vitro* expansion.

Materials and methods

EXPERIMENTAL DESIGN

A flowchart illustrating the experimental set-up of analysis of the biopsies, the *in vitro* expansion and the analysis of the chondrogenic capacity, is shown in Fig. 1.

BIOPSY HARVESTING

With approval from the Ethics Commission in Warsaw cartilage biopsies were harvested from three deceased donors, age 26–43 years (with a mean age of 37 years). Four cylindrical full thickness cartilage–bone biopsies were harvested from seven different anatomical positions in the human joint. Biopsies were harvested from the surface of the patellar groove of femur (FPG), from weight bearing (WB) and non-weight bearing (NWB) areas of the medial and lateral condyles of femur (FMW, FLW, FMNW, FLNW), and from WB area of the medial and lateral condyles of tibia (TMW, TLW) from each donor (see Fig. 2). The biopsies were harvested with a 5-mm diameter punch perpendicular to the surface of the articular cartilage of the left knee from human donors that had macroscopically intact cartilage and no known cartilage defect history. The International Cartilage Repair Society (ICRS) score was used to standardize the biopsy harvesting. The biopsies were put into physiological saline with antibiotics for transportation. Three of the biopsies from the same anatomical position were allocated for biochemical analysis and for cell culture and the fourth biopsy was fixated for histology/immunohistochemistry (see Fig. 1). The wet weight of the biopsy was measured and about 1/6th of the biopsy was taken for biochemical analysis and the rest to cell culture.

INCLUSION CRITERIA

The Mankin score was performed as described previously[16,17]. The average of the Mankin score was calculated out of all the seven different anatomical positions of the human knee joint and donors with an average of the Mankin score below 2[17], were included in the study.

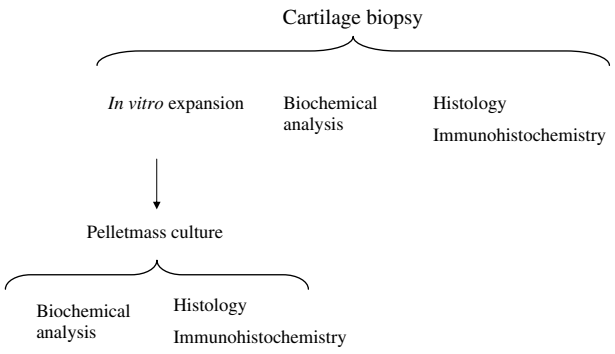

Fig. 1. Experimental set-up. For a detailed description, see Material and methods.

CELL CULTURE

Chondrocytes were isolated by mechanical mincing followed by over night collagenase digestion (type II clostridial collagenase, 0.8 mg/ml, Worthington Biochemicals, Lakewood, New Jersey) at 37°C and 7% CO_2/93% air[10]. Isolated chondrocytes were cultured in Dulbecco's modified eagle's medium (DMEM)/F12 (Invitrogen, Paisley, UK) supplemented with 0.025-mg/ml L-ascorbic acid (Apotekets produktionsenhet Umeå, Sweden), 1× Penicillin–Streptomycin (PAA Laboratories), 2-mM L-Glutamine (Gibco) and with 10% human serum. Generally, the first medium change was made at day 6, at day 7 a photo was taken and thereafter the media was changed twice a week. Subculture was made with trypsin-ethylene diamine tetraacetic acid (EDTA) solution [0.125% trypsin (Gibco) diluted in 0.1-M phosphate buffered saline with 0.2-g/l EDTA] when the cells had reached 80% confluence. At the subculture number of cells was counted and the time to reach 80% confluences noted.

PELLET CULTURE

The matrix production capacity of the expanded chondrocytes was analyzed in a 3D pellet culture system as described earlier[18,19]. Briefly, 2.0×10^5 cells from passage 1, were placed into polypropylene conical tubes with 0.5 ml of defined medium, consisting of DMEM-High glucose (PAA Laboratories, Linz, Austria) supplemented with insulin-transferrin-selenium (ITS+) (Life Technologies, Sweden), 5.0-μg/ml linoleic acid (Sigma-Aldrich, Stockholm, Sweden), 1.0-mg/ml human serum albumin [HSA (Equitech-Bio, TX, USA)], 10-ng/ml transforming growth factor beta 1 (TGF-β1) (R&D Systems, UK), 10^{-7}-M dexamethasone (Sigma), 14-μg/ml ascorbic acid (Sigma) and 1× Penicillin–Streptomycin (PAA Laboratories). The cells were centrifuged at 500 *g* for 5 min and maintained in 37°C in 7% CO_2/93% air. Six to eight pellets were cultured from each biopsy for 14 days and the medium was changed twice a week. Four to six pellets were further analyzed with biochemical methods and two pellets were fixed for supplementary histological and immunohistochemical analyses.

HISTOLOGY OF BIOPSIES AND PELLETS

Biopsies and pellets were fixed in Histofix™ (Histolab products AB, Gothenburg, Sweden), dehydrated and embedded in paraffin. Five micrometer sections were cut and placed onto microscope slides (Superfrost Plus, Menzel-Gläser, Germany), deparaffinized and stained with Alcian blue/van Gieson, Safranin-O or immunoassayed with anti-type I collagen and anti-type II collagen as described below. The stained sections were analyzed by a Nikon Optiphot2-pol microscope (Nikon Instruments Inc, Melville, NY, USA) and digital pictures were taken with automatic camera tamer (ACT-1) software (Nikon, Japan).

The chondrogenic capacity of the pellets was further analyzed with the Bern Score system[20]. Briefly, the sections were graded according to intensity of the matrix staining, cell density/extent of matrix produced and cell morphology.

IMMUNOHISTOCHEMISTRY OF BIOPSIES AND PELLETS

Immunohistochemistry was performed, with a modified method, as described previously[19]. Briefly, sections from cartilage biopsies and pellets were deparaffinized, dehydrated, and digested with hyaluronidase, 8000 U/ml (Sigma-Aldrich) and further with chondroitinase AC, 0.05 U/ml (Sigma-Aldrich). Endogenous peroxidase activity was quenched with 3% H_2O_2 and the sections were blocked with 3% bovine serum albumin (BSA) (Sigma-Aldrich). Primary antibodies raised against Collagen types I and II (ICN Biomedicals, Aurora, OH, USA) were incubated 1 h at room temperature or over night at 4°C. A horse radish peroxidase (HRP) conjugated secondary antibody (Jackson Laboratory, Maine, ME, USA) was added to the sections for 1 h and visualized using the TSA-Direct Cy3 kit (Perkin Elmer, Boston, MA, USA) according to the manufacturer's instructions. Nuclei were stained with 4′, 6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich). The sections were analyzed with Nikon fluorescence microscope and digital pictures were taken with ACT-1 software.

BIOCHEMICAL ANALYSIS OF PELLETS AND BIOPSIES

The biochemical content was studied in respect to the seven distinct anatomical positions as well as the ratio between NWB to WB areas, femur to tibia, and medial to lateral side. Biochemical analysis of the biopsies and pellets was performed as described earlier[21]. Briefly, the biopsies and pellets were digested in papain (Sigma) solution [0.3-mg papain/ml sodium phosphate buffer (20 mM) with 1-mM EDTA and 2-mM dithiothreitol] over night

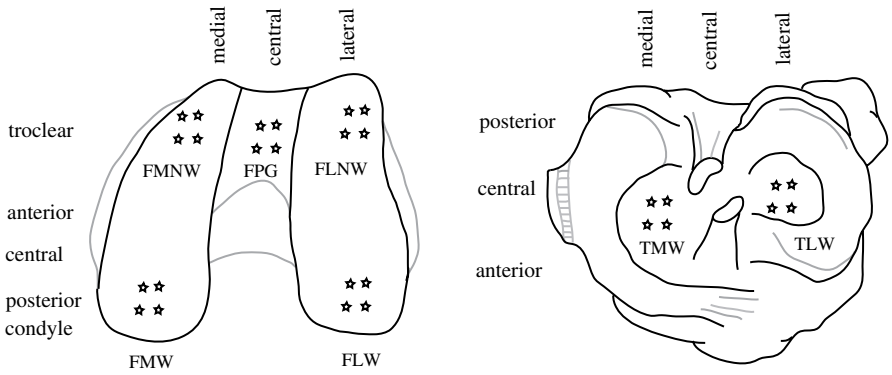

Fig. 2. Schematic picture of biopsy harvesting adapted from the ICRS score. To standardize the biopsy harvesting the ICRS score was used with kind permission from ICRS. ✛Indicating one biopsy. FMNW and FLNW: NWB areas of the medial and lateral condyles of femur, respectively; FMW and FLW: WB areas of the medial and lateral condyles of femur, respectively; FPG: femur patellar groove; TMW and TLW: WB areas of the medial and lateral condyles of tibia, respectively.

or for 90 min at 60°C, respectively. The digested samples were then further analyzed for DNA, proteoglycan (GAG) and hydroxyproline (HP) content. The amount of DNA was measured with Hoescht 33258 (Sigma), according to the manufacturer's instruction and the GAG and HP content as previously described[21]. All biochemical analyses were made in duplicates from duplicate or triplicate samples.

STATISTICAL ANALYSIS

Statistical analysis was performed by the non-parametric Mann–Whitney *U* test. *P*-values less than 0.05 were considered significant.

Results

HISTOLOGY AND IMMUNOHISTOCHEMISTRY OF THE BIOPSIES

The inclusion criteria in this study were macroscopically intact cartilage, no known cartilage defect history, and an average Mankin score less than 2. The Mankin sore was performed as described previously[17]. The average Mankin score in the three patients was 1.6 ± 0.3. In general, the WB areas on the medial side of both femur and tibia had a slightly higher Mankin score than the corresponding lateral side.

The histological staining for proteoglycans, Alcian blue/van Gieson and Safranin-O, demonstrated high GAG content in all layers of the biopsies except in the surface zone (see Fig. 5). The biopsies harvested from tibia contained less amounts of proteoglycan than biopsies from femur as indicated by weaker Alcian blue/van Gieson and Safranin-O staining. The subtypes of collagen, types I and II, were qualitatively demonstrated by immunohistochemical staining with antibodies. In general, collagen type I was only detected in the surface of the biopsies whereas collagen type II was detected throughout all the layers. No topographic variation in the collagen type I or collagen type II expression was observed (see Fig. 5).

BIOCHEMICAL ANALYSIS OF THE BIOPSIES

Cellular content in the biopsies

To further characterize the biopsies, both DNA measurements and traditional cell counting with a hematocytometer were used to analyze the number of cells per mg wet weight biopsy. The two different methods showed good correlation (data not shown) and the average cell number per mg biopsy was 1483 ± 371 (see Fig. 3). The highest cellular content was detected in the condyles of femur. Moreover, the femur/tibia ratio showed a 55% higher cells/mg biopsy in femur than in tibia, i.e., $1643 \pm 257$and 1084 ± 143 cells/mg, respectively. A difference in cellular content was also observed between the NWB and WB areas, i.e., 12% higher cells/mg biopsy content in the NWB biopsies (Fig. 4).

ECM content in the biopsies

The proteoglycan to collagen (GAG/HP) ratio showed a site-dependent variation in all patients. In the NWB areas, the average of GAG/HP ratio was 38% lower than in the WB areas (see Table I). To examine whether the trend seen in the GAG/HP ratio was due to the proteoglycan or collagen content, the topographic variation of the matrix components was studied in respect to the wet weight of the biopsy. In NWB biopsies, the GAG content varied from 23 to 47 μg GAG/mg biopsy while in WB biopsies the corresponding values were 17–74 μg GAG/mg biopsy. The total collagen content, as seen by amount of HP, had a larger variance between the different locations of the knee joint in the three patients. The non-weight bearing areas ranged from 1.7 to 16.9 μg HP/mg biopsy and WB from 0.4 to 9.8. In average, the amount of GAG was 8% lower and the amount of HP was 78% higher in the NWB areas when compared to the WB areas.

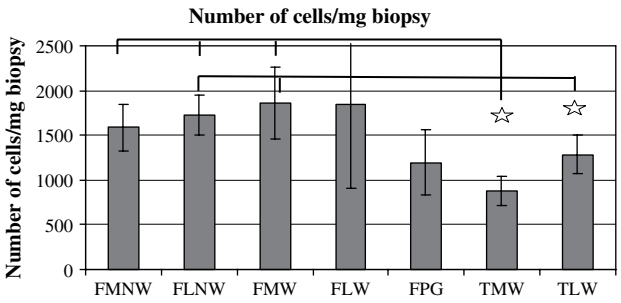

Fig. 3. A graph presenting number of cells per wet weight biopsy. The number of cells per wet weight biopsy in the different localizations at the time of isolation. *Indicates a significant difference with $P < 0.05$; $n = 3$.

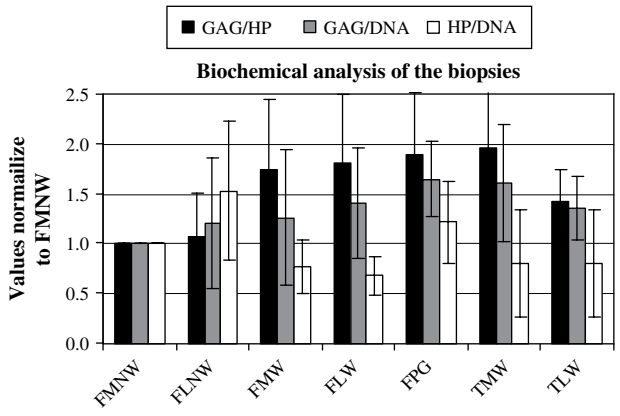

Fig. 4. Normalized levels of biochemical analyses of biopsies. Normalized biochemical analyses of the ECM and cellular content of biopsies harvested from different topographic locations in the human knee joint; GAGs-to-HP, GAG-to-DNA and HP-to-DNA ratio; $n = 3$.

The histological analysis of the biopsies indicated a difference in the matrix composition between tibia and femur. This observation was confirmed with the biochemical analysis and in average all patients had about 44% higher μg GAG/mg biopsy and 85% μg HP/mg biopsy in femur compared to tibia. The GAG content in femur varied from 18 to 73 μg GAG/mg biopsy and in tibia from 16 to 42. The corresponding values for the HP content were 0.7–16.9 μg HP/mg biopsy for femur and 0.4–7.4 μg HP/mg biopsy for tibia. The large variance in ECM content was seen when comparing all three patients. Within each patient the variance was smaller, as seen in Table II.

IN VITRO EXPANSION

A clear difference in the expansion behavior of chondrocytes harvested from femur and tibia was observed. The cells isolated from femur showed good attachment and proliferation rate while most cells isolated from tibia biopsies showed low attachment and proliferation. In average, the cell doublings per day were 0.24 ± 0.02 for chondrocytes harvested from femur, whereas cells isolated from tibia had a cell doubling per day at 0.09 ± 0.14. A slight non-significant difference was noted between the NWB and corresponding WB position in femur, i.e., 0.26 ± 0.03 and 0.22 ± 0.00, respectively.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY OF THE PELLETS

To study the redifferentiation capacity of the *in vitro* expanded chondrocytes, the cells were placed into pellet mass culture in passage 1, i.e., after 3.8 ± 1 population-doublings. The cells were cultured as 3D-pellets in redifferentiation medium for 2 weeks and then harvested and analyzed. In general the pellets originating from biopsies harvested in femur were significantly larger and contained more matrix proteins than those from tibia. All pellets from femur had high proteoglycan and collagen type II expression and a modest collagen type I expression were detected in a few pellets. In contrast, the pellets produced with chondrocytes expanded from tibia contained low amounts of proteoglycans indicated by weak Alcian blue/van Gieson and Safranin-O staining. The tibia pellets contained collagen type I and modest amounts of collagen type II (see Fig. 7). The Bern score[20] of the pellets was significantly higher in the pellets originating from femur compared to the ones from tibia (7.1 ± 0.60 and 3.5 ± 0.04, respectively). No difference in the Bern score was detected between the different anatomical positions in femur.

BIOCHEMICAL ANALYSIS OF THE PELLETS

The proteoglycan content of the pellets was considerably higher in the pellets originating from chondrocytes harvested in femur compared to tibia, i.e., 4.9 ± 3 times higher (see Fig. 6). The GAG content in the pellets originating from femur varied from 16 to 76 μg GAG/pellet and in tibia from

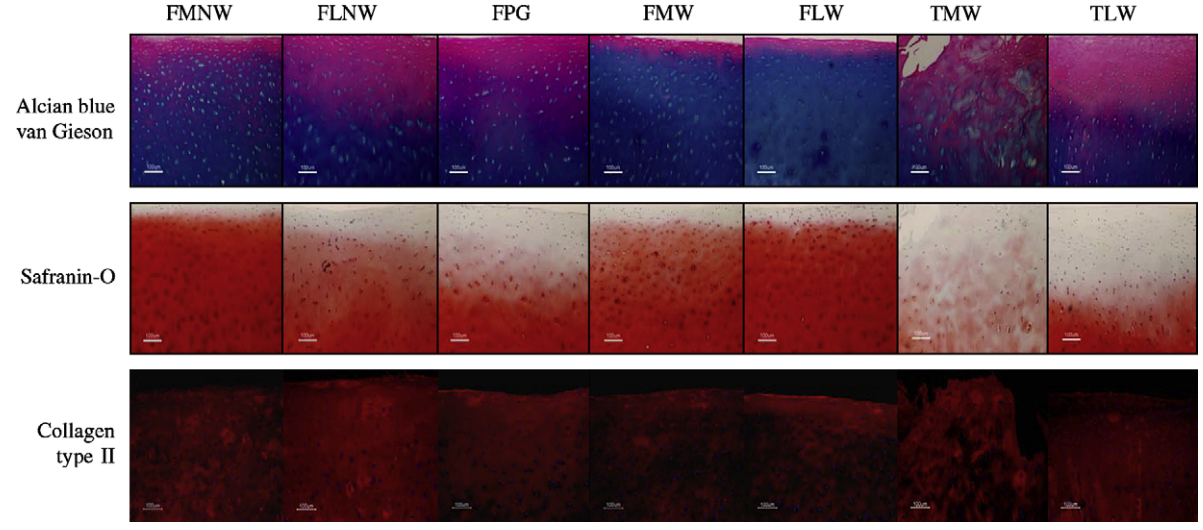

Fig. 5. Histology and immunohistochemistry of biopsies. Representative cartilage biopsies harvested from different topographic locations in the human knee joint stained with Alcian Blue/van Gieson (nuclei stained black, sulfated proteoglycans stained blue), Saffranin-O (nuclei stained black, sulfated proteoglycans stained red), and antibody raised against collagen type II (nuclei stained blue using DAPI and collagen matrix stained red).

Table I
Comparison of the ECM content in the joint. The ratio of femur vs tibia and non-weight bearing areas (NWB) vs weight bearing areas (WB) in respect to the ECM and cellular content in the adult human knee joint n = *3*

	GAG		GAG		HP	
	HP	std	DNA	std	DNA	std
Femur/tibia	1.00	±0.09	0.87	±0.16	0.80	±0.19
NWB/WB	0.62	±0.04	0.80	±0.04	1.56	±0.71

3 to 17 μg GAG/pellet. To further study the importance of the choice of sampling site, the redifferentiation capacity of chondrocytes harvested from different localizations in femur was investigated. The production of proteoglycans per cell was higher in the pellets originating from the NWB areas when compared to the corresponding WB area of femur. The GAG production per cell varied from 91 to 115 μg GAG/μg DNA in the NWB area and 26–94 μg GAG/μg DNA in corresponding WB site. The NWB areas had further a higher μg GAG/μHP, i.e., 19%, when compared with the corresponding WB site. Noteworthy, the values of the ECM components in respect to DNA were about 10 times lower in the pellets than in the cartilage biopsies and that the cells harvested from tibia of one of the donors did not proliferate at all.

Discussion

Articular cartilage from distinct anatomical locations in the joint of young and middle-age humans (26–43 years of age) were subjected to a thorough analysis of the ECM and cellular content. Additionally, the redifferentiation capacity of *in vitro* expanded chondrocytes harvested from the different localizations was examined. In this study we demonstrate that there are differences in ECM content within the adult human knee in respect to GAG/HP ratio. Additionally, the data show that clear differences between chondrocytes harvested from femur and tibia from a healthy human knee joint exists and that the differences are not completely abolished during the process of de- and redifferentiation.

Data regarding the biochemical composition of articular cartilage is useful for the understanding of tissue function in health and disease. The biochemical properties of articular cartilage vary considerably throughout the knee joint. The topographic variations are related to local differences in the type and degree of mechanical loading the area is subjected to[2,5]. Furthermore it has been reported that the equilibrium response of articular cartilage is governed by

Table II
*ECM and cellular content in distinct localization of the human knee. Summary of results of ECM in human articular cartilage from seven different locations in the adult human knee joint from a representative patient. *indicates* p < *0.05 in respect to *** n = *3*

	μg GAG		μg GAG		μg HP	
	μg HP	std	mg Biopsy	std	mg Biopsy	std
FMNW	4.6	±0.9**	27.0	±8.0*	6.2	±2.8
FLNW	4.4	±0.2*	40.7	±21.0*	10.8	±4.9
FMW	8.8	±2.2	73.5	±9.9*	7.4	±1.4
FLW	8.9	±1.1*	47.3	±9.2**	5.4	±1.5
FPG	11.6	±4.5*	47.4	±10.9	4.4	±1.5
TMW	9.1	±3.7*	42.3	±12.1*	4.9	±1.5
TLW	6.3	±1.5	26.2	±1.7*	5.0	±1.2

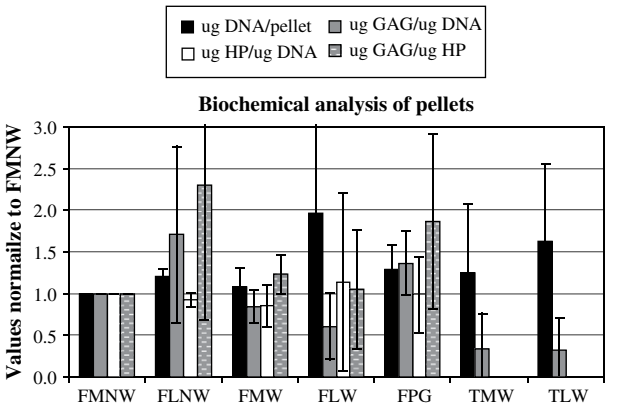

Fig. 6. Normalized levels of biochemical analyses of pellets. Normalized biochemical analyses of the ECM and cellular content of the pellets originating from different topographic locations in the human knee joint.

the elastic properties of the tissue. Jurvelin *et al.* have demonstrated that the Poisson's ratio, i.e., elastic property, of articular cartilage has a negative correlation to the collagen to proteoglycan content in the canine knee joint. Moreover this correlation showed a site-related variation[2]. This is in agreement with our data demonstrating that the GAG/HP ratio is lower in the NWB areas than in the WB areas of the human knee joint. However, in the WB areas our data did not correlate with the GAG/HP ratio found at the same anatomical locations in the canine joint[2]. This could be due to species variation and that the loading pattern differs between the species. Nevertheless, various studies have demonstrated that areas subjected to high levels of compression stress show a higher content of proteoglycans, which are in accordance to our data[2,3,6,8]. Furthermore our results demonstrate that the collagen content is higher in the NWB areas. These data are similar with previous published data showing a higher collagen content in areas subjected to intermittent peak loads compared to areas with constantly loading in standing and moving[6]. Our results show that there is no significant difference in GAG or HP content between the seven distinct anatomical positions, when comparing all patients, which confirms Garcia-Seco *et al.*'s results[22]. The values for total collagen and proteoglycan content varied greatly for a given measurement site. The large variability observed in the biochemical analysis of the different individuals could be due to factors of a variety of nature, such as lifestyle, previous drug treatment and sport activities. The large variation in the biochemical content has previously been observed in human articular cartilage[16].

In this study the cell yield from a cartilage biopsy harvested from femur was larger than in biopsies from tibia in the same patient. This suggests that the cell reduction process in articular cartilage may be faster and more dominant in tibia than femur. Additionally, our results show that the number of cells isolated from a cartilage biopsy harvested from the medial side of tibia is smaller than from the lateral side. It has previously been demonstrated that the medial compartment has a higher prevalence of osteoarthritis (OA) than the lateral compartment[23]. Taken together this indicates that an increase of acellularity of human articular cartilage might relate to its diminishing capacity for self-repair, since fewer cells become responsible for increasingly large areas of matrix.

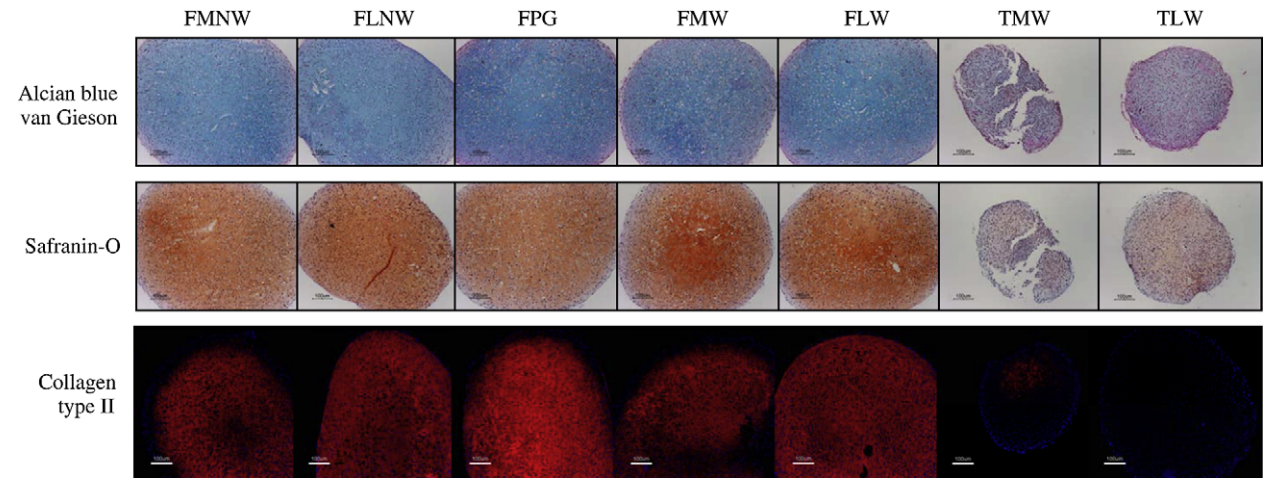

Fig. 7. Histology and immunohistochemistry of pellets. Representative pellets originating from the seven distinct localizations in the human knee joint stained with Alcian Blue/van Gieson (nuclei stained black, sulfated proteoglycans stained blue), Saffranin-O (nuclei stained black, sulfated proteoglycans stained red) and antibody raised against collagen type II (nuclei stained blue using DAPI and collagen matrix stained red).

During the *in vitro* expansion of the chondrocytes, differences in the ability to attach and proliferate were seen between femur and tibia. The culture method used in this study is optimized for chondrocytes used in autologous chondrocyte transplantation (ACT) in which cells are harvested from a NWB area in femur[10]. To obtain a better attachment and proliferation of the chondrocytes harvested from tibia the culture conditions could be optimize for these cells by for example the addition of growth factors in the culture media or by coating of culture flasks. However, by having different culture methods during the expansion of the chondrocytes harvested from different locations in the human knee the post-expansion chondrogenicity could be due to either the diverse culture conditions used during the expansion or by the fact that the cells originate from different localizations in the joint. The ability of the chondrocytes to attach and proliferate could also be due to the age of the patients. It has been demonstrated that there is an age-related reduction of the proliferation rate of articular chondrocytes, which corresponds with our results[24,25]. Our results demonstrate that the chondrocytes harvested from tibia from the youngest patient (26 years) had a better ability to attach and proliferate than the other patients (40 and 43 years of age).

The redifferentiation capacity of the *in vitro* expanded chondrocytes did not correlate with the age as previously described by Barbero *et al.*[25]. A significant difference was seen in the post-expansion chondrogenic capacity between cells originating from femur and tibia. Even when the attachment and proliferation rate were similar, the post-expansion chondrogenic capacity between chondrocytes harvested from femur was significantly higher compared to cells from tibia. The pellets originating from NWB areas in femur had more ECM production per cell than corresponding WB site. Taken together, our results indicate that the dedifferentiation and redifferentiation did not completely abolish the differences of the chondrocytes originating from either femur or tibia. Cell based methods of surgical repair therapies of articular cartilage injuries, such as ACT, have made it possible for successful generation of a hyaline-like repair tissue with correct biomechanical properties of articular cartilage[10]. The findings of this study verify that harvesting a biopsy from a NWB area of the femur condyle will give the best opportunity for cartilage regeneration using *in vitro* expanded chondrocytes when performing an ACT. Moreover, an inverse correlation between the biopsies and pellets in femur with respect to GAG/HP and GAG/DNA was observed in this study. The amount of proteoglycan or collagen per cell was, however, about 10 times lower in pellets than in the biopsies they originated from. The difference of the chondrocytes ability of redifferentiate after *in vitro* expansion observed could possibly originate from their *in vivo* situation. It is well documented that articlular cartilage is an inhomogeneous tissue where the organization of collagen and proteoglycan concentration regulates the biomechanical properties of the tissue[4]. Chondrocyte phenotypic expression and biosynthetic activities may be altered by loading of the cartilage ECM[26]. The chondrocytes in the WB areas of femur produces more proteoglycans per cell indicating that these cells in average are more differentiated than cells found in corresponding NWB area. It has furthermore been suggested that articular chondrocytes need to dedifferentiate to a certain extent to achieve good redifferentiation in the pellet mass system[27]. During the *in vitro* expansion the cells from different localizations did approximately the same number of population-doublings. The different ability of the post-expansion chondrogenic capacity observed in this study might be due to what state of dedifferentiation the *in vitro* expanded chondrocytes had when put into pellet mass culture. Taken together these findings might designate that there is a distinction in the differentiation grade of the chondrocytes from diverse anatomical positions in the human joint. Moreover, our results indicate that these differences are not completely eliminated during the process of de- and redifferentiation.

Even though the number of knees and cartilage biopsies studied were small, our results demonstrated that there are differences in ECM content between the WB and NWB localizations within the adult human joint in respect to GAG/HP ratio. Knowledge of the biochemical properties of human articular cartilage is a useful basis for further investigation into the effects of different treatments of articular cartilage defects as well as understanding the progression of OA. Further, these findings highlight that the choice of sampling sites

may influence the outcome of cartilage repair and biochemical studies of articular cartilage and therefore must be interpreted with care. Additionally, we demonstrate that a clear difference in post-expansion chondrogenic capacity between chondrocytes harvested from femur and tibia from a healthy human joint exists and that these differences are not completely abolished during the process of de- and redifferentiation. These findings emphasize the importance of understanding the topographic variation of the articular cartilage biology when approaching new articular cartilage cell based and tissue engineering repair strategies.

Conflict of interest

The authors declare that they have no competing interests.

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