Knee Surg Sports Traumatol Arthrosc (2012) 20:1681–1688
DOI 10.1007/s00167-011-1722-7

EXPERIMENTAL STUDY

Host serum is not indispensable in collagen performance in viable meniscal transplantation at 4-week incubation

Pablo Eduardo Gelber · Raúl Torres · Natalia Garcia-Giralt · Juan Erquicia · Ferran Abat · Juan Carlos Monllau

Received: 27 June 2011 / Accepted: 11 October 2011 / Published online: 22 October 2011

Abstract
Purpose Viable meniscal transplantation has been criticized as an expensive and logistically demanding technique. The purpose was to compare the standard culture medium with another culture medium that is more widely available and easier to work with and to assess the collagen net ultrastructure architecture and the capacity of the preserved cells to produce proteins.
Methods Ten fresh lateral menisci were harvested. Each meniscus was divided into three parts; control group, fetal-bovinum-serum group and Insulin-Transferrin-Selenium group during 4 weeks. Cell metabolism was assessed with the gene expression of type I collagen, type II collagen and aggrecan. Collagen ultrastructure was assessed with transmission electron microscopy. The Collagen Meniscal Architecture scoring system was used to evaluate the degree of meniscal disarray.
Results Type I collagen was expressed more in the fetal-bovinum-serum group than in the ITS group ($P = 0.036$). No differences were found between cultured samples and control groups. Type II collagen showed decreased expression in both cultured groups compared with the control group. No differences were observed in the gene expression of aggrecan in either group. No differences were observed when the Collagen Meniscal Architecture scoring system was applied.
Conclusions Insulin-Transferrin-Selenium-supplemented medium is at least as effective as the fetal-bovinum-serum-supplemented medium to preserve the net architecture of the meniscal tissue. Gene expression of the studied proteins was similar in the Insulin-Transferrin-Selenium group to that observed in the control group at 4 weeks. Insulin-Transferrin-Selenium might be a better alternative and might be used instead of fetal-bovinum-serum or an autologous host serum in order to preserve meniscal tissue, which precludes the necessity of obtaining host serum previously. Thus, viable meniscal transplantation would logistically be less complicated to perform.

Keywords Meniscus · Insulin-Transferrin-Selenium · Allograft · Viable meniscal transplantation · Fetal bovine serum · Menisci preservation

P. E. Gelber (✉) · F. Abat · J. C. Monllau
Department of Orthopaedic Surgery, Hospital de Sant Pau, Universitat Autònoma de Barcelona, Sant Antoni María Claret, 167, 08025 Barcelona, Spain
e-mail: pablogelber@gmail.com

P. E. Gelber · J. Erquicia · J. C. Monllau
ICATME-Institut Universitari Dexeus, Universitat Autònoma de Barcelona, Sabino de Arana 5-19, 08017 Barcelona, Spain

R. Torres
Department of Orthopaedic Surgery, Parc de Salut Mar, Universitat Autònoma de Barcelona, Passeig Marítim 25-29, 08003 Barcelona, Spain

N. Garcia-Giralt
URFOA-IMIM. Universitat Autònoma de Barcelona, Doctor Aiguader 88, 08003 Barcelona, Spain

Introduction

Isolated allograft meniscus transplantation was first performed in 1984 by Milachowky et al. [16]. Since then, it has become an increasingly used option for the treatment of knee pain after total meniscectomy.

Controversies still exist relative to meniscal transplantation. Regarding meniscal preservation techniques,

different methods are currently used to keep menisci allografts in an acceptable condition until transplantation. Lyophilization is no longer used as it has been shown to alter the size and characteristics of the meniscal tissue leading to poor clinical results [24]. Freezing has been accepted as a simple and reliable way of preserving those tissues that only have to retain mechanical and some biochemical properties. It has been shown, in an animal model, to maintain the ultrastructure of the collagen net [8]. However, Gelber et al. [11] have more recently refuted this affirmation showing that, when ultrastructurally studied, the freezing process led to severe architectural disarray. Conversely, cryopreservation has been shown not to alter its biomechanical properties [12]. In addition, the main theoretical advantage of cryopreservation might be that it does not destroy cells. This was also recently questioned, as different ranges of cell survival have been observed with this technique [12, 23].

Regardless, fresh allografts may be advantageous as not only does it not destroy cells but it also keeps them viable by producing proteoglycans and collagen fiber structures. Thus, a normal or nearly normal cellular function can be expected from the moment of implantation [1, 4, 23]. Verdonk and Khon [23] showed that menisci can be safely kept at 4°C in sterile tissue culture medium solution for up to 15 days without a remarkable loss of cell viability.

It has been demonstrated that cellular repopulation occurs in the meniscal allograft after transplantation even if there are no viable cells in the time of surgery [2, 17, 18, 25]. However, donor DNA in the viable human meniscal allograft has been detected for as long as sixty-four months after transplantation, indicating that donor cells survive for a long period of time [21]. Thus, the replacement process by host cells is probably slower in the human model, suggesting the superiority of a preserving system that is able to maintain the cell viability. In this controversial debate, other authors [3] have shown that sometime after transplantation the allograft meniscus nearly has only host DNA. Therefore, it suggests that cell viability is not as important as preservation of the collagen net architecture.

Fresh meniscus allograft has been shown to remain viable after 2 weeks in the Dulbecco modified Eagle's medium (DMEM; Gibco Invitrogen, Merelbeke, Belgium) supplemented with 20% of autologous serum [22, 23]. In in vitro studies, the autologous serum is replaced with fetal-bovinum-serum, which has shown its effectiveness in preserving the meniscal collagen net and cell viability for 2 weeks [20]. The exact composition of the fetal-bovinum-serum is not well known, and it has great variability depending on the source of the serum. Insulin-Transferrin-Selenium is a product that is commonly used to supplement chondrocyte culture mediums [7, 9, 26]. This is a controlled medium, available in any laboratory. Its composition is well known; it is a potential culture medium that can be used to maintain meniscal tissue for clinical use and avoids the need of host serum. On the other hand, Insulin-Transferrin-Selenium medium does not contain growth hormones, which in theory are of utmost importance to maintain a proper cell metabolism. However, specific growth hormones in a specific quantity can be easily added if needed. As far as we know, the use of the Insulin-Transferrin-Selenium solution to supplement mediums for meniscal tissue conservation has not been described. In addition, although the recommended 2-week window for safe transplantation after harvesting appears to work well according to previous studies [22, 23], it might not contribute to the widespread use of this technique for obvious time-relative limitations.

The aim of this study was to assess the meniscal tissue in terms of cell metabolism and collagen net ultrastructure architecture, comparing the standard laboratory culture medium supplemented with 20% of fetal-bovinum-serum with other medium supplemented with 1% of Insulin-Transferrin-Selenium after 4 weeks of the harvesting. Our first hypothesis was that an Insulin-Transferrin-Selenium-supplemented medium would maintain the collagen meniscal architecture and cell viability for up to 4 weeks after harvesting. The second hypothesis was that this ability of the Insulin-Transferrin-Selenium solution would be at least as good as that of a fetal-bovinum-serum-supplemented medium.

Materials and methods

Meniscal harvesting

Ten fresh human lateral menisci were harvested in sterile conditions during total knee replacement procedures (6 women, 4 men). The study group had a median age of 70 years (range 64–79). Informed consent was obtained from each donor following the guidelines laid down by our local Ethics Committee. Five of the menisci were obtained from the right side, and the other five from the left side. Radiographic evaluations as well as clinical intraoperative assessments were performed in order to ascertain the indemnity of the lateral femorotibial compartment. Cases with more than 50% of lateral joint space narrowing in the standing 45° posteroanterior radiograph position, macroscopic degeneration or even minimal calcification were all excluded from the study. Culture analysis was performed for each graft, and if positive, they were also excluded. Eight menisci did not meet these criteria, so they were excluded, and another eight were consecutively harvested. Collagen and cell distribution vary between the inner, the central and the outer section of the meniscus [6]. Thus,

only the central region of the body of each meniscus was used. The inner and the outer region of each corresponding body of the menisci were discarded. This central zone of each meniscus was divided into six sections in the surgical theatre in sterile conditions. One of these sections was then sectioned into 1 mm^3 slices and preserved in a 2.0% glutaraldehyde solution. At no more than 2 h of harvesting, a pathologist prepared the samples for analysis with transmission electron microscopy. This section was used as a control group. The other five pieces of the meniscus were placed in a sterile receptacle with DMEM medium, and they were sent to the laboratory.

Meniscus culture

One of the pieces of each meniscus was diced and incubated within an hour of harvesting with RNAlater (Qiagen) overnight at 4°C, and then, it was cryopreserved at –80°C for gene expression assessment. This sample (not cultured) was also used as a control group. The other four sections were placed into a 15-ml polystyrene centrifuge tube, and the culture medium was added up to the total volume required to cover the sample. The menisci were cultured with DMEM and supplemented either with 20% fetal-bovinum-serum or with 1% Insulin-Transferrin-Selenium and 50 µg/ml ascorbic acid at 37°C in a 5% CO_2 humidified atmosphere during 28 days. The medium solution was changed every 3–4 days.

Two of the sections of the menisci were used for RNA quantification. One sample was cultured in a medium supplemented with fetal-bovinum-serum, and the other sample was cultured with a medium supplemented with Insulin-Transferrin-Selenium. They were diced and incubated with RNAlater overnight at 4°C prior to storage at −80°C. The menisci used for transmission electron microscopy analysis were fixed with 2.0% glutaraldehyde solution. Figure 1 summarizes the transmission electron microscopy and gene expression protocol for each harvested meniscus.

Total RNA isolation and gene expression quantification of cartilage matrix proteins

The 2 sections of the menisci that were used for RNA quantification and cultured in a medium supplemented with fetal-bovinum-serum and in another supplemented with Insulin-Transferrin-Selenium were suspended in 1 ml of Tri Reagent (Molecular Research Center, inc.) and homogenized using a T8 Ultra-Turrax homogenizer (IKA), and finally, the RNA was extracted according to Tri Reagent manufacturer instructions. Seven ul of total RNA was used to synthesize the DNA complementary strain according to the protocol of TaqMan® Reverse Transcription Reagents (Applied Biosystems, Foster City, USA). TaqMan probes and primers for all

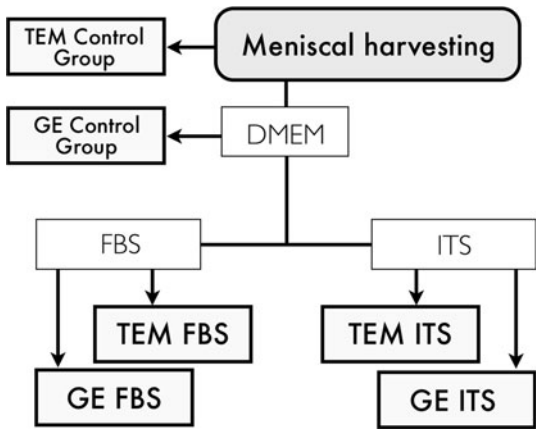

Fig. 1 Harvesting and processing protocol for each harvested meniscus. *DMEM* Dulbecco modified Eagle's medium, *TEM* sample sent to be studied with transmission electron microscopy, *GE* sample sent to be for gene expression evaluation of type I and II collagen and aggrecan, *FBS* sample to be cultured with 20% of fetal-bovinum-serum medium, *ITS* sample to be cultured with 1% of Insulin-Transferrin-Selenium medium

genes were obtained as inventoried assays from Applied Biosystems. The product was diluted by half with RNAse-free pure water, and 1 µl of the resultant solution was used to determine gene expression, aggrecan (AGC1), type I collagen (COL1A1) and type II collagen (COL2A1), using quantitative real-time PCR (all reagents were obtained from Applied Biosystems). Quantitative real-time PCR and its subsequent analysis were performed according to the manufacturer instructions. Real-time PCR was conducted in a volume of 20 µl containing gene-specific Assay on Demand primers, TaqMan-MGB probe and 10 µl TaqMan Universal PCR MasterMix 2X (Applied Biosystems) reacted in the following sequence: 2 min at 50°C, 10 min at 95°C followed by 40 cycles of 15 s at 95°C and 60 s at 60°C each cycle in 384-well plates with the ABI PRISM 7900 HT Detection System (Applied Biosystems). The results were analyzed using SDS TM Software 2.3 (Applied Biosystems), and the expression levels were calculated against 18S expression and then normalized to an internal sample (relative quantification) using arbitrary units. All real-time PCRs for each sample were performed in triplicate. Real-time PCR for 18S was carried out under the same conditions using an 18S endogenous control Assay on Demand (Applied Biosystems) for normalization purposes. Relative quantification of gene expression was performed using the comparative Ct method (ΔΔCt method) with one sample from the experiment as a reference sample (one sample cultured in Insulin-Transferrin-Selenium). The *Repeatability* (short-term precision or intra-assay variance) of the method has a Ct SD among replicates lower than 0.3. The *Reproducibility* (long-term precision or interassay variance) of the technique has a max SD in Ct interassay of 2.5 (Biosystems A. User Bulletin #2. ABI Prism 7700 Sequence Detection System. In; December 11, 1997; updated 10/2001).

Transmission electron microscopy procedure

The architectural state of the menisci was analyzed with transmission electron microscopy. The changes observed were quantified and qualified according to the previously described scoring system [7, 8].

Due to the fact that parties obtaining the initial menisci samples were also executing the final analysis of the histologic sections, a double-blinded study design was performed so as to minimize possible biases.

Forty sections of 1 mm^3 from each sample of the menisci were immediately fixed in a 2.0% glutaraldehyde cacodylate buffer solution. Before dehydration, post-fixation in osmium tetroxide was done in increasing concentrations of ethanol. Next, the menisci sections were treated with propylene oxide and placed in progressive concentrations of epon. The most representative zone was chosen from five different 1-μm-thick toludine blue-stained sections with the help of a light microscope (SMZ-10A, Nikon, Japan; 40× magnification). Ninety nanometer sections from the selected zone were finally stained with metal salts (uranyl acetate and lead citrate) and were analyzed with a transmission electron microscope (Philips, model #CM100, Holland). For each cross-section, four transmission electron microscopy photographs were randomly taken.

Fibril collagen measurements and histologic classification

Four hundred collagen fibrils were recorded and measured in longitudinal and transversal sections from every meniscus. Based on recent studies [6, 7], the analyzed photographs were set at 19,000× magnification. All measurements were determined by one independent observer with the help of an electronic digital caliper (ProMax, Fowler; USA Range 0–150 mm, resolution 0.02 mm) and then multiplied by 19,000 to get the corresponding measure in nanometers.

According to the published Collagen Meniscal Architecture scoring system [6], the collagen's periodicity and degree of disruption, loss of banding, degree of collagen packing, fibril size variability, and its intrafibrilar edema were evaluated. On this scale, the meniscus scored from 0 to 7. Following the established criteria, they were classified by two pre-instructed independent observers and then averaged in grades ranging from a normal state (grade I 0–2 points) to severe disarray (grade III 5–7 points). The interclass reliability for these two observers was finally calculated.

Statistical analysis

Cell viability variables are presented as mean ± SD, median, 25th and 75th percentiles, maximum and minimum and were compared using the nonparametric paired Wilcoxon signed rank test. The meniscus' ultrastructure data have been presented as mean ± SD for transversal and longitudinal variables. The one-way ANOVA with Tukey′s multiple comparison correction was used for comparisons. Interobserver agreement was analyzed using the interclass correlation coefficient (ICC). Statistical analysis was performed using SPSS 15 (SPSS Inc., Chicago, Illinois, USA). Statistical significance was set at 0.05.

Results

Gene expression measurements

Table 1 shows the descriptive and statistical data of each studied protein (type I collagen, type II collagen, and

Table 1 Cell viability assessed with genetic expression (gene expression unit) of three extracellular proteins of the meniscus

		Control group	FBS	ITS	*P* value[a] Cg versus FBS	*P* value[a] Cg versus ITS	*P* value[a] FBS versus ITS
AGG	Mean (SD)	1.4 (1.1)	1.3 (1.1)	1.3 (0.9)	n.s	n.s	n.s
	Med (P25–P75)	1.3 (0.4–1.9)	1.3 (0.2–2.4)	1.2 (0.4–2.0)			
	Min–Max	0.2–4.0	0.0–2.7	0.0–3.1			
Type I Col	Mean (SD)	3.3 (4.5)	4.8 (4.2)	1.2 (1.1)	n.s	n.s	0.036
	Med (P25–P75)	1.5 (0.6–5.6)	5.3 (0.4–8.2)	1.0 (0.7–1.3)			
	Min–Max	0.2–13.0	0.1–10.8	0.1–4.0			
Type II Col	Mean (SD)	2.1 (2.4)	0.7 (1.3)	0.2 (0.5)	n.s	0.012	n.s
	Med (P25–P75)	0.7 (0.2–3.8)	0.0 (0.0–0.9)	0.0 (0.0–0.2)			
	Min–Max	0.0–7.3	0.0–4.1	0.0–1.4			

AGG aggrecan, *type I Col* type I collagen, *type II Col* type II collagen in the three groups of the study; *FBS* meniscus cultured with 20% of fetal-bovinum-serum medium, *ITS* meniscus cultured with 1% of Insulin-Transferrin-Selenium medium

[a] Nonparametric Wilcoxon signed rank test

Table 2 Median diameter in nanometers of the collagen fibers in longitudinal and transverse section observed in the different culture mediums

	Transversal	Longitudinal
Control group	14.4 ± 4.1	12.3 ± 3.8
ITS	14.6 ± 3.3	13.1 ± 3.8
FBS	15.3 ± 4.4	12.6 ± 3.3
P value[a]		
Control group versus ITS	n.s	0.005
Control group versus FBS	0.002	n.s
FBS versus ITS	0.025	n.s

FBS menisci cultured with 20% of fetal-bovinum-serum medium, *ITS* menisci cultured with 1% of Insulin-Transferrin-Selenium medium

[a] *P* value from Tukey's multiple comparison correction

aggrecan) in the three groups: gene expressions in the control group, in the samples cultured with a supplement of fetal-bovinum-serum, and in the samples cultured with a supplement of Insulin-Transferrin-Selenium medium. As data obtained showed great variability with large standard deviations, comparisons were performed with medians and percentiles. No differences were observed in relation to the aggrecan gene expression comparing the three groups. Regarding the expression of type I collagen, a statistical difference ($P = 0.036$) was observed comparing Insulin-Transferrin-Selenium medium with fetal-bovinum-serum medium. No differences were detected when both mediums were compared with the control group. Regarding the type II collagen, a great decrease in the expression of this protein was observed in both mediums.

Fibril collagen size

Table 2 provides descriptive data regarding the size of the longitudinal and transversal sections of the collagen fibrils. Increases in the size of both sections in both culture mediums in comparison with the control group were observed. This increase has statistical significance in the Insulin-Transferrin-Selenium medium in the longitudinal sections ($P = 0.005$) and in the fetal-bovinum-serum medium in the transversal sections ($P = 0.002$), but in all cases, a tendency to an increase in the size in both sections was observed (Fig. 2).

Architectural degree and scoring

Following the Collagen Meniscal Architecture scoring system criteria, eight of ten samples were graded as I, the other two as II in the control group. In the fetal-bovinum-serum and Insulin-Transferrin-Selenium groups, seven of ten previously cultured menisci were classified as grade I and the remaining samples as II. Similarly, no differences were observed with respect to the scoring aspect of the scale. The control group averaged 1.5 points ±0.7, the fetal-bovinum-serum group 1.7 ± 0.9, and the Insulin-Transferrin-Selenium samples 1.9 ± 0.5 (ns). Interclass correlation between both observers was considered moderate with a single ICC 0.529 (95% IC 0.346–0.854).

Discussion

The main finding of this study was that the Insulin-Transferrin-Selenium-supplemented menisci showed a gene expression more similar to the control group than the fetal-bovinum-serum group and that the collagen meniscal net is not altered. This confirms our two hypotheses. However, type I collagen, the most important protein in the extracellular matrix [6], was significantly increased in the fetal-bovinum-serum group. It is not known whether this large amount of collagen production in the fetal-bovinum-serum group might lead to a fibrotic meniscus. Aggrecan is the most important proteoglycan in the extracellular matrix of the menisci. In the three studied groups, there were no differences in the aggrecan levels at 4 weeks. This is in contrast to previous studies performed with fetal serum [20, 23], in which the proteoglycan synthesis declined dramatically at 4 weeks, suggesting a recommendable window period from day 10 to day 14 for implantation of a viable meniscal allograft. In type II collagen, a protein found in low proportion in the extracellular matrix strongly decreased its expression in both the Insulin-Transferrin-Selenium and the fetal-bovinum-serum solutions. Type II collagen is mostly found in the inner part of the menisci [6]. However, the actual relevance of this protein for the extracellular matrix is not well known. The fetal-bovinum-serum-supplemented medium showed to have better induced type II collagen expression than the Insulin-Transferrin-Selenium-supplemented medium.

This is one of the first studies assessing the gene expression of the proteins after a period of culture. Verbruggen et al. [20] compared the cellular metabolism in human menisci cultured in a medium supplemented with 20% fetal calf serum and in a serum-free medium. They concluded that the menisci maintain their viable conditions up to 2 weeks with the fetal calf serum–supplemented medium. In longer periods of time, the levels of proteoglycan decrease considerably. To study the cell metabolism, they assessed the degree of sulphate that it is incorporated in the proteoglycan. Conversely, in the current study, fibroblast viability was assessed by evaluating the capacity to produce type I and II collagen and aggrecan. This method expresses the exact production of these proteins at a specific moment and therefore is a much more sensitive technique. This high sensitivity, the high cell and

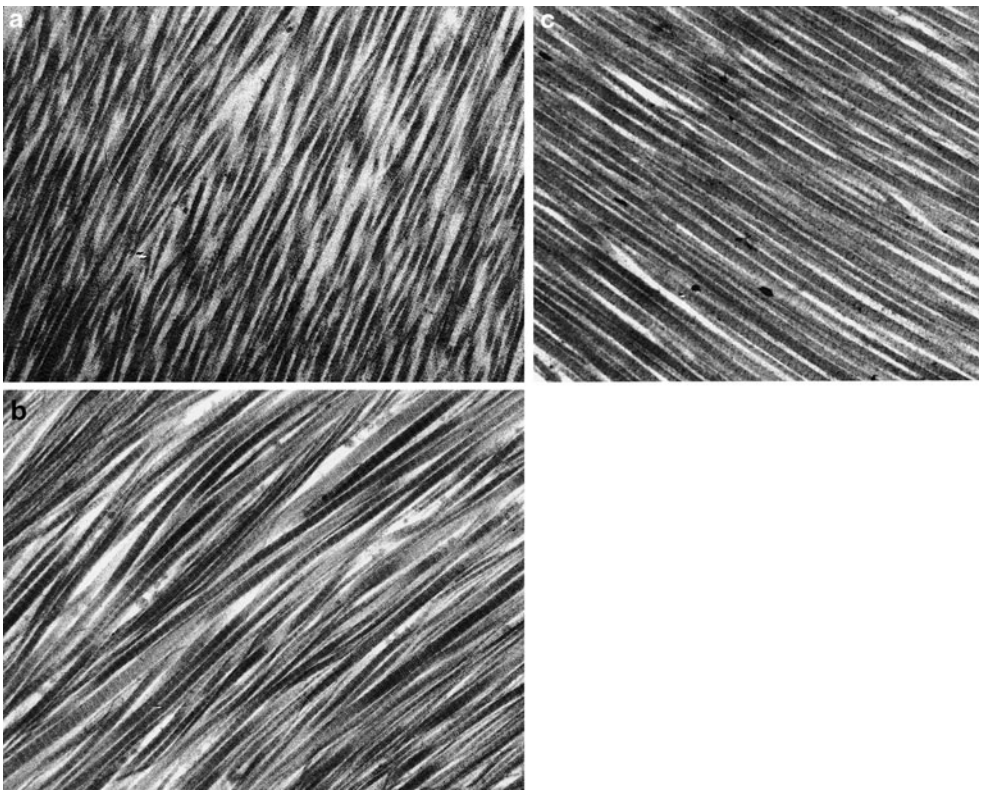

Fig. 2 Transmission electron microscopy photographs of a meniscus classified as grade I on the CMA scoring system. The cultured sections showed a slightly increase in the collagen fiber diameters. **a** Control section. **b** Section cultured with 20% of fetal-bovinum-serum medium. **c** Meniscus cultured with 1% of Insulin-Transferrin-Selenium medium

protein distribution's variability between menisci and even in different zones of each meniscus [6] and finally the fact that we did not assess the protein gene expression at 2 weeks as Verbruggen et al. did [20] limit precise comparisons between these investigations.

There are several studies describing the distribution of the different collagen types in the menisci [5, 6, 13, 15, 19]. Collagen proteins represent 70% of the dry weight of the meniscus. Type I collagen represents about 70% of the total collagen weight. Although there is no clear role for type II collagen, the specific location of this protein in the inner one-third of the meniscal tissue, possibly as a consequence of mechanical stimulation, might suggest a more important function in the net architecture than was initially thought. The low (almost absent) type II collagen gene expression at 4 weeks in both supplemented mediums in the current study might be interpreted as an important handicap regarding the ability to maintain a normal collagen net architecture.

The collagen net architecture was assessed both with the Collagen Meniscal Architecture score and by measuring the collagen fibers diameter in transversal and longitudinal sections of the menisci. Interobserver reliability calculated in this study was only moderate in contrast to a better coefficient calculated in a previous study [12]. There were no differences in the three studied groups regarding the scoring and the sizing in both sections, suggesting a good preservation of the collagen net in both mediums. However, a slight increase in the fibers sizes in the fetal-bovinum-serum- and Insulin-Transferrin-Selenium-supplemented mediums in both sections was observed. This might be a logical consequence of the collagen fibers being embedded in an aqueous medium for 28 days. Consistent with other studies [20, 22], it seems the net collagen architecture is not ultrastructurally altered at 4 weeks.

Cryopreservation and deep-frozen are two different techniques that are widely used to preserve meniscal tissue. It seems that cryopreservation keeps the net collagen architecture intact and produces a variable amount of cell survival [12]. With regard to the deep-frozen technique, there are conflicting studies [8, 11]. The key factor for the ultimate functional success of meniscal transplantation is the ability of the cells that repopulate a meniscal allograft to synthesize extracellular matrix [25]. Cells functioning adequately will remodel and maintain the extracellular matrix and thus maintain the biomechanical properties of the meniscus. There are contradictory studies relative to the time necessary to effectively repopulate the graft.

Arnozcky et al. [2] showed that this phenomenon is slow and might even be incomplete. They showed that the core of the meniscus remained acellular after 6 months from the transplantation of a deep-frozen meniscal autografts. In addition, Verdonk et al. [21] showed that donor DNA in the viable human meniscal allograft can be detected for as long as sixty-four months after transplantation. Although this donor DNA was found at the inner edge of the menisci, the region in where most biologic activity is retained, the aforementioned indicates that donor cells survive for a long period of time [21]. In contrast, Jackson et al. [14] suggested that host cells rapidly repopulate the transplanted meniscus and that there is no evidence that donor cells maintain the extracellular matrix of the meniscus on a long-term basis. Thus, no justification for using grafts with living cells was concluded. Long-term studies of meniscal transplantation survival using deep-frozen or cryopreserved menisci had not shown remarkable differences in comparison with viable transplanted menisci [9, 22–24]. In addition, when an allograft meniscus is being transplanted, the quality relative to cell and collagen components is unknown. However, many different variables inhomogeneously distributed in those studies make them non-comparable.

Fetal-bovinum-serum is a medium supplement widely used to preserve meniscal tissue in laboratory studies [20, 22, 23]. Its composition is not well known because it is produced from bovine serum and depends on the animal killed. This medium cannot be used when the menisci have to be transplanted to a human knee. In these cases, we need to obtain host serum to supplement the medium of the menisci. It is difficult to set up fresh menisci banks because there is only a two-week window to transplant the meniscus. In addition, the host patient must be determined in advance in order to obtain his/her serum. Conversely, the Insulin-Transferrin-Selenium has a well-known composition, and it is widely used to preserve other human tissues [7, 10, 26]. With the data obtained in this study, it seems the Insulin-Transferrin-Selenium might be used to maintain meniscal tissue instead of the host donor or bovine serum at least at 4 weeks from harvesting.

The main limitation to the present study is the great variability observed in each meniscus. This might be due to the fact that meniscal tissue was harvested in aged patients. Although the menisci were obtained in good condition, menisci in 70-year-old patients had already initiated a degenerative process. Even in the clinical setting, a high variability in the collagen and cell components of the allografts is expected. However, the same specimens were used as experimental and control groups to thereby minimize any possible bias. Another possible explanation for the great variability observed might be that cell viability was assessed with gene expression instead of the total amount of protein synthesized during the 4 weeks of culture. Another limitation perceived was that although the fibril collagen diameters were measured with the help of a precise electronic caliper, this was done manually. It could have been solved with the help of image analysis software previously described [4].

The results of the study suggest that Insulin-Transferrin-Selenium might be used instead of fetal-bovinum-serum or autologous host serum in order to preserve meniscal tissue. It has been shown that collagen net architecture and protein metabolism of type I collagen and aggrecan are maintained at 4 weeks, regardless the practical absence of type II collagen. Hence, the Insulin-Transferrin-Selenium solution might be considered a good supplement to preserve meniscal tissue. Animal studies should be the next step in order to find out whether fresh menisci banks may be created using Insulin-Transferrin-Selenium as a medium supplement. This might extend the time between the harvesting of the meniscus and its placement in a host knee. Thus, viable meniscal transplantation would be logistically less complicated to be performed.

It is generally accepted that an intact collagen net architecture is a key factor for the graft to act as a proper scaffold. Currently, it is no possible to state that viable meniscal allografts have more clinical benefit than cryopreserved or deep-frozen allografts. However, a graft without cells maintaining the collagen ultrastructure might lead to temporary collagen net disarray due to the fact that repopulation occurs later on. Theoretically, this makes viable meniscal graft a step forward relative to other meniscal preservation techniques.

The clinical relevance of this study is that an Insulin-Transferrin-Selenium-supplemented medium might be used instead of fetal-bovinum-serum or an autologous host serum to preserve meniscal tissue, which precludes the necessity of obtaining host serum previously.

This might make viable meniscal transplantation less complicated logistically to perform.

Conclusion

The Insulin-Transferrin-Selenium-supplemented medium is at least as effective as the fetal-bovinum-serum-supplemented medium in preserving the net architecture of the meniscal tissue. The gene expression of the studied proteins was similar in the Insulin-Transferrin-Selenium group to that observed in the control group at 4 weeks. From a logical point of view, Insulin-Transferrin-Selenium might be a better alternative as a medium supplement for preserving meniscal tissue than fetal-bovinum-serum or host serum in vivo.

References

1. Arnoczky SP, Milachowski KA (1990) Meniscal allografts: where do we stand? In: Ewing JW (ed) Articular cartilage and knee joint function: basic science and arthroscopy. Raven, New York, pp 129–136
2. Arnoczky SP, Dicarlo EF, O'Brien SJ, Warren RF (1992) Cellular repopulation of deep-frozen meniscal autografts: an experimental study in the dog. Arthroscopy 8:428–436
3. DeBeer P, Decorte R, Delvaux S, Bellemans J (2000) DNA analysis of a transplanted cryopreserved meniscal allograft. Arthroscopy 16:71–75
4. Chen J, Iosifidis M, Zhu J, Tatarinsev I, Wang J (2006) Vanadate ingestion enhances the organization and collagen fibril diameters of rat healing medical collateral ligaments. Knee Surg Sports Traumatol Arthrosc 14:750–755
5. Chevrier A, Nelea M, Hurtig MB, Hoemann CD, Buschmann MD (2009) Meniscus structure in human, sheep, and rabbit for animal models of meniscus repair. J Orthop Res 27:1197–1203
6. Cheung HS (1987) Distribution of type I, II, III and V in the pepsin solubilized collagens in bovine menisci. Connect Tissue Res 16:343–356
7. Chua KH, Aminuddin BS, Fuzina NH, Ruszymah BHI (2005) Insulin- Transferrin- Selenium prevent human chondrocyte dedifferentiation and promote the formation of high quality tissue engineered human hyaline cartilage. Eur Cells Mater 9:58–67
8. Fabbriciani C, Lucania L, Milano G, Schiavone Panni A, Evangelisti M (1997) Meniscal allografts: cryopreservation versus deep-frozen technique. An experimental study in goats. Knee Surg Sports Traumatol Arthrosc 5:124–134
9. Flattar M, Dhollander A, Verdonk R, Almqvist FK, Verdonk P (2011) Twenty-six years of meniscal allograft transplantation: is it still experimental? A meta-analysis of 44 trials. Knee Surg Sports Traumatol Arthrosc 19:147–157
10. García-Giralt N, Izquierdo R, Nogués X, Pérez-Olmedilla M, Benito P, Gómez-Ribelles JL, Checa MA, Suay J, Caceres E, Monllau JC (2009) A porous PCL scaffold promotes the human chondrocytes redifferentiation and hyaline-specific extracellular matrix protein synthesis. J Biomed Mater Res A 85:1082–1089
11. Gelber PE, Gonzalez G, Lloreta JL, Reina F, Caceres E, Monllau JC (2008) Freezing causes changes in the meniscus collagen net: a new ultrastructural meniscus disarray scale. Knee Surg Sports Traumatol Arthrosc 16:353–359
12. Gelber PE, González G, Torres R, García Giralt N, Caceres E, Monllau JC (2009) Cryopreservation does not alter the ultrastructure of the meniscus. Knee Surg Sports Traumatol Arthrosc 17:639–644
13. Gruber HE, Mauerhan D, Chow Y, Ingram JA, Norton HJ, Hanley EN Jr, Sun Y (2008) Three-dimensional culture of human meniscal cells: extracellular matrix and proteoglycan production. BMC Biotechnol 26:8–54
14. Jackson DW, Whelan J, Simon TM (1993) Cell survival after transplantation of fresh meniscal allografts. DNA probe analysis in a goat model. Am J Sports Med 21:540–550
15. Melrose J, Smith S, Cake M, Read R, Whitelock J (2005) Comparative spatial and temporal localisation of perlecan, aggrecan and type I, II and IV collagen in the ovine meniscus; an ageing study. Histochem Cell Biol 124:225–235
16. Milachowski KA, Kohn D, Wirth CJ (1989) Homologous meniscus transplantation. Experimental and clinical results. Int Orthop 13:1–13
17. Shibuya S (1999) Meniscus transplantation using a cryopreserved allograft. Histological and ultrastructural study of the transplanted meniscus. J Orthop Sci 4:135–141
18. Raht E, Richmond JC, Yassir W, Albright JD, Gundogan F (2001) Meniscal allograft transplantation. Two-to eight-year results. Am J Sports Med 29:410–414
19. Upton ML, Chen J, Setton LA (2006) Region-specific constitutive gene expression in the adult porcine meniscus. J Orthop Res 24:1562–1570
20. Verbruggen G, Verdonk R, Veys EM, Van Daele P, De Smet P, Van den Abbeele K, Claus B, Baeten D (1996) Human meniscal proteoglycan metabolism in long-term tissue culture. Knee Surg Sports Traumatol Arthrosc 4:57–63
21. Verdonk P, Almqvist KF, Lootens T, Van Hoofstat D, Van Den Eeckhout E, Verbruggen G, Verdonk R (2002) DNA fingerprinting of fresh viable meniscal allografts transplantated in the human knee. Osteoarthr Cartil 10(Suppl A):S43–S44
22. Verdonk P, Demurie A, Almpvist KF, Veys EM, Verbruggen G, Verdonk R (2005) Transplantation of viable meniscal allograft. Survivorship analysis and clinical outcome of one hundred cases. J Bone Joint Surg Am 87:715–724
23. Verdonk R, Khon D (1999) Harvest and conservation of meniscal allografts. Scand J Med Sci Sports 9:158–159
24. Wirth CJ, Peters G, Milachowski KA, Weismeier KG, Kohn K (2002) Long-term result of meniscal allograft transplantation. Am J Sports Med 30:174–181
25. Yamasaki T, Deie M, Shinomiva R, Yasunaga Y, Yanada S, Ochi M (2008) Transplantation of meniscos regenerated by tissue engineering with a scaffold derived from a rat meniscos and mesenchymal stromal cells derived from rat bone marrow. Artif Organs 32:519–524
26. Zhang L, Song H, Zhao X (2009) Optimum combination of insulin-transferrin-selenium and fetal bovine serum for culture of rabbit articular chondrocytes in three-dimensional alginate scaffolds. Int J Cell Biol 2009:747016