Viability of human arterial grafts monitored by comet assay.

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Short title: Arterial grafts viability

Summary

Introduction

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An analytical method for studying DNA degradation by electrophoresis after cell lysis and visualization of DNA fragments with fluorescent dye, comet assay, was used to evaluate the viability of the endothelial layer of human arterial grafts with the aim of identifying the procedure that will least damage the tissue before cryopreservation.

Materials and methods

Four groups of samples were studied: cryopreserved arterial grafts that were thawed in two different ways, slowly lasting 2 hours or rapidly for approx. 7 minutes. Arterial grafts that were collected as part of multiorgan procurement with minimal warm ischemia time. Cadaveric grafts were taken as part of the autopsy, so they have a more extended period of warm ischemia. The HeadDNA (%) parameter and others commonly used parameters like TailDNA(%). TailMoment, TailLength, OliveMoment, TailMoment to characterize the comet were used to assess viability in this study.

Results

The ratio of non-decayed to decayed nuclei was determined from the values found. This ratio for cadaveric grafts was 0.63, for slowly thawed cryopreserved grafts 2.9, for rapidly thawed cryopreserved grafts 1.9, and for multi-organ procurement grafts 0.68.

Conclusions

The results of the study confirmed the assumption that the allografts obtained from cadaveric donors are the least suitable. On the other hand, grafts obtained from multiorgan donors are better in terms of viability monitored by comet assay.

Introduction

Pathological changes in the arteries are among the most common causes of sudden death or a life-threatening condition, such as arterial obturation or rupture in the case of an aneurysm. Vascular surgery treats given conditions and often uses artificial vascular prostheses to replace blood vessels. However, e.g., in case of infection, the replacement must be explanted and replaced with another infection-resistant biological graft [1]. Such a solution uses a vascular allograft, i.e., allogeneic arterial transplantation. The demand for arterial replacements and the development of the program of cryopreserved grafts in the Czech Republic brings questions related to the preservation of the quality of grafts through the process of cryopreservation and subsequent thawing, as well as the search for qualitatively optimal sources of these grafts - i.e., the comparison of cadaveric donor grafts with grafts obtained as part of multiorgan procurement [2].

Different experiences with the postoperative course, especially after the implantation of cadaveric grafts, were the impetus for research aimed at comparing the properties of fresh grafts taken immediately during multiorgan sampling, cryopreserved in a tissue bank after several years of storage in liquid nitrogen vapor after thawing, and cadaveric arterial allografts in terms of viability [3], morphology, physical properties, and thawing protocols. This work follows up with previous experimental transplantations in animals, which pointed to the essential factor of the length of ischemia of the graft for its quality and related rejection after its transplantation [4, 5].

This study aimed at the use of the comet assay to evaluate the viability of the endothelium of human arterial grafts of the three groups mentioned. The Comet assay (also known as Single cell gel electrophoresis - SCGE) is an analytical method for studying DNA degradation by electrophoresis after cell lysis and visualization of DNA fragments with fluorescent dye [6-9]. Depending on various factors such as autolysis, toxic substances effect, tissue ischemia, and the time since thawing or collection, the nuclear DNA breaks down, and its fragments travel in the electric field, creating a comet image with a head and tail of various sizes. The assumption is that the degree of degradation of nuclear DNA indicates cell viability and, thus, the usability of the graft, i.e., preserving its properties to the maximum extent.

Materials and methods:

We included arterial grafts explanted from the donors for the analysis as follows:

• Cryopreserved arterial grafts: 20 samples were thawed in two different ways, slowly lasting two hours or rapidly for approximately seven minutes. These grafts were taken as part of multiorgan procurement and frozen as soon as possible at -190°C through cryopreservation.

• Arterial grafts that were collected as part of multiorgan procurement: 12 samples, i.e with minimal warm ischemia time.

• Cadaveric grafts: 15 samples. The grafts were taken as part of the autopsy, so they have a more extended period of warm ischemia.

Aorta or artery samples were stored in Custodiol® HTK Solution after collection or thawing until processing in the laboratory. The detailed characteristics of the examined grafts are in the tab. 1-3.

Vessels were first cut and thoroughly rinsed in HBSS Modified. The endothelium was obtained by peeling off an amount of endothelial layer of approximately 2×1 mm, which was gently cupped. Places without atherosclerotic changes were searched on vessels that were affected by atherosclerosis. A portion of the endothelial blank was incubated for 30 minutes in 3 ml of HBSS with Ca2+ and Mg2+ with the addition of collagenase I in a ratio of 1:2 at a temperature of 37 °C. After digestion, the visible remnants of the endothelial blank were removed, and the cell suspension was centrifuged for 15 minutes at 4000 rpm (Digital Minifuge Centrifuge). The incubation solution was aspirated, and the sedimented cells were rinsed twice with HBSS and centrifuged again for 15 and 5 minutes at 4000 rpm (Digital Minifuge Centrifuge).

After thoroughly aspirating the HBSS after the last centrifugation, 10 µl of PBS and 40 µl of low melting agarose were added to the cell pellet. The suspension was gently mixed, and 2 × 5 µl of this mixture was then dropped onto an NMA-coated microscope slide prewarmed to 37 °C. The endothelium suspension was processed by the alkaline comet test method. Briefly, we present the procedure: after dropping the cell suspension mixture in low-melting agarose onto microscope slides and covered with coverslips, the preparation was cooled on ice. When the LMA had solidified, the coverslips were torn off, and the preparation was placed in histological cuvettes with lysis solution for 1 hour at a temperature of 4 °C. The unfolding phase of DNA followed after lysis in the electrophoretic buffer for 20 minutes at laboratory temperature.

Electrophoresis was performed in a BIO-RAD Sub-Cell GT, PowerPac, at 25 volts, 300 mA for 20 minutes. Due to the heating of the electrolyte (avoidance of gradual heating of the low melting agarose), the electrophoresis was performed in the refrigerator at a temperature of 4 °C. After electrophoresis, and the gels were briefly rinsed in neutralization buffer and stained with 1-2 µl of SYBR Gold staining solution. Preparations were viewed in an Axiomat fluorescence microscope, Carl Zeiss, with a 450-490/520 nm excitation/emission filter at 200× magnification. The resulting comets were photographed with a Lucia QI825 camera and evaluated with the Lucia Comet Assay Analysis software (Laboratory Imaging s.r.o., Prague)

Statistical analysis

Descriptive statistics and ICC (intraclass correlation)estimates were conducted using STATA 16 [10] statistical package. Within-level and between-level covariance matrices were estimated using the lavaan library [11]in R. The results were considered significant if $p<0.05$.

Results

Present data were hierarchically structured. Nuclei at level-1 (within-level) were nested within samples at level-2 (between-level). The degree of dependency in data was measured by the intraclass correlation coefficient ICC. The values of ICC are between 0 and 1 and express the amount of

variability situated at level-2 compared to the total variability. The values of ICC close to 0 (0.05 or lower) indicate no variability between samples, and data can be disaggregated to the within-level and use a traditional single-level approach. Similarly, the values of ICC close to 1 (0.95 or higher) show no variability observed at the within-level, and data can be further aggregated to the between-level as means, medians, or percentages and use a common single-level approach at level-2.

A total of 7 non-redundant variables were observed at level-1: The values of these variables are associated with nuclei and vary within a given sample. We adopted terminology from the latent variable modeling methodology. According to this paradigm, viability is considered a latent variable (theoretical construct) reflected in mentioned seven manifest variables (empirical indicators). Thus, it is assumed that all seven variables were highly correlated. We followed the Muthén's approach [12] and decomposed the total covariance matrix to the within-level and between-level covariance matrices. Moreover, respective correlations were calculated and presented as joined covariance-correlation matrices.

Several covariate (independent) variables were considered at level-2: warm ischemia time, cold ischemia time, and dummy-coded groups. These variables didn't vary within samples and were joined to the between-level covariance matrix.

Descriptive statistics using the single-level approach are presented in table 4 (endothelial cells). Data are expressed as means and standard errors of the mean (SEM). The last columns of both tables are the ICC values. The results indicate that there is substantial variation located at both levels; thus, both levels must be considered here. Tables 3-6 represent covariances and respective correlations. ICCs can also be calculated from these matrices using relevant variance on the diagonals.

Another view on the results gives parameter HeadDNA (%). The interval of preservation of nuclear DNA in the interval of up to 90% [90,100], i.e., a decrease of no more than 10% of the total amount, was also chosen as a criterion. The ratio of non-decayed to decayed was established and it means the higher ratio, the better result.

Cryopreserved arterial grafts:

The preliminary single-level analysis results are presented first for better insight into the data. Seventeen cryopreserved arterial grafts were evaluated, of which 9/8 were performed using the slow/fast thawing method. In the group of rapidly thawed samples, 351 cores were evaluated, of which 66% were in the interval [90,100] and 34% in the interval [0,90), i.e., with the decay of more than 90%. The ratio of non-decayed to decayed was 1.9. In the slowly thawed group, 409 cores were evaluated, of which 74% were in the interval [90,100] and 26% in the interval [0,90) and the ratio of non-decayed to decayed was 2,9.

Multiorgan procurement arterial grafts.

A total of 10 samples were evaluated. Five hundred seventy-two nuclei were evaluated, of which 40% were in the interval [90,100] and 60% were in the interval [0,90). The ratio of non-decayed to decayed was 0,67.

Cadaveric arterial grafts

A total of 15 samples were evaluated. Three hundred eighty-seven nuclei were assessed, of which 39% were in the interval [90,100] and 61% were in the interval [0,90). The ratio of non-decayed to decayed nuclei was determined from the values found. This ratio for cadaveric samples was 0.63as far as for endothelial cells.

Tables 4-7

The degree of association between independent covariate variables and manifest variable at level-2 is presented in Table 10. Significant negative correlations between dummy-coded Fast thawed cryopreserved arterial grafts and slowly thawed cryopreserved arterial grafts groups and Head radius and Head area variables indicate significantly lower Head radius and Head area values within these groups. On the contrary, positive correlations between the multi-group and Head radius (0.60) and Head area (0.61) reflect significantly higher values of the Head radius within the multi-group compared to other groups. The significant differences in Head radius across groups were also confirmed using the nested ANOVA omnibus test with $p<0.05$ (results not shown).

fig

Discussion

SCGE is used as an analytical method in various scientific fields, e.g., pharmacology, to test the effectiveness and safety of drugs, genotoxicity, and ecotoxicology. It is also used to determine time since death [13], where DNA degradation was found to be dependent on time since death. The repair of cells after the harmful effect of various external agents, e.g. from donor exposure to irradiation, and application of drugs, is commonly studied using the comet test. DNA repair after exposure to these agents is reported to occur relatively rapidly.

The result of the study confirmed the assumption that the least suitable from the point of view of DNA degradation are arterial grafts obtained from the bodies of deceased persons. At the same time, the use of grafts from multiorgan procurement is slightly better. The best results can be obtained using cryopreserved, slowly thawed grafts, probably because of the shortest warm and cold graft ischemia times achieved. It is evident that if there is no suitable recipient of graft at the time of removal during the multiorgan collection of organs, the best is to perform cryopreservation instead of keeping it, e.g., in a cardioplegic solution for a longer time.

This study is part of large project where other methods are simultaneously used on the same samples (mechanical testing, Cell Viability Assessment Using Fluorescence Vital Dyes and Confocal Microscopy, thawing protocols testin in arterial samples etc.)

However, cell viability using SCGE is rarely tested, and it needs to be well-known to what extent the cell can regenerate degraded DNA. Collins [14] states that only cells should be used for experiments with comet assay cells where DNA degradation is zero or no more than 10% before the damage. Bankoglu states that, depending on the concentration of the agent inducing DNA degradation, the percentage of DNA in the tail of the comet increases, and non-vital cells increase, as well as the number of lost cells and apoptotic cells. Interestingly, however, after the DNA-degrading agent's removal, at least partial DNA repair occurred within a few hours. These and similar studies are

performed on cell cultures by adding hydrogen peroxide, etoposide, methyl methanesulfonate, or irradiation. All this experiments use standard and well defined experimental conditions. However, no analysis has been performed on human material under different tissue preservation conditions. According to our opinion degradation of nuclear DNA monitored by comet assay on fresh peripheral blood mononuclear cell is quite fast due to autolysis. The autolysis of samples from cadavers and probably of samples from multiorgan procurement even they stay in Custodiol is main problem. The autolysis is the process which hardly can be controlled or stopped. This represents limitation of this study. In our experiment, samples of grafts were used - cryopreserved arteries, collected during multiorgan procurement and dead bodies (cadaveric), from which endothelium was isolated [15]. Both non-decayed and decayed nuclear DNA in varying degrees of decay were detected in all samples. The presence of degraded endothelial nucleus can be caused by autolytic processes, especially in cadaveric and multiorgan allografts, due to the time between the removal of the vessel from the body to the beginning of laboratory processing (cold ischemia). It may simulate the time which elapses until the graft is implanted to the patient after its removal from the donor. The assumption is that samples where non-decayed nuclei predominate are more suitable for allograft implantation. It means the time from collection to processing of samples according the comet assay plays a valuable role.

Conclusion.

Based on the results of our study, it can be concluded that the most suitable grafts for clinical use are cryopreserved grafts that have been slowly thawed. In this subgroup we can see the shortest ischaemic time of the saples. The lowest degree of endothelial damage is also associated with a lower degree of rejection after vascular graft transplantation. There is no disruption of the basement membrane and no exposure of the tunica media antigenic layer, which are powerful triggers of rejection. In addition to the origin of the allogeneic grafts, the handling of the grafts in the period before the actual transplantation is also very important as we can see comparing groups of slowly and rapidly thawed samples.

Abbreviations.

DABCO: 1,4-Diazabicyclo[2.2.2]octane

DMSO: Dimethyl sulfoxide

HBSS: Hank's Balanced Solution with calcium, with magnesium without phenol red

LMA: low gelling agarose

NMA: normal melting agarose

PN: product number,

PBS: phosphate buffer solution

SDS: sodium dodecyl sulfate

Used chemicals.

Collagenase I: Sigma-Aldrich, PN SCR103)

DABCO: ROTH, PN 0718.1

DMSO: Sigma-Aldrich, D4540

EDTA disodium: Sigma Aldrich, PN E5513

Glycerine: Roth, PN 7533.1

HCL: LACH:NER, PN UN1789

KCL: Sigma-Aldrich, PN P5405

LMA: Sigma-Aldrich, PN A9414

NMA: Sigma-Aldrich, PN A9539

Na_2HPO_4 : Sigma-Aldrich, PN S5136

SDS: Amresco, PN 0227

NaOH: Penta, PN 15760-31000

SYBR™ Gold Nucleic Acid (10,000X Concentrate in DMSO, Thermo Fisher Scientific PN: S11494).

TrisHCL: Amresco, PN 0234

Trisma base: Sigma-Aldrich 93362

Triton: ROTH, 3051.4

Software used:

Lucia Comet Assay Analysis software (Laboratory Imaging s.r.o., Prague, CZ)

Excel, Microsoft

STATA 16 SWInstruments:

Digital Minifuge Centrifuge GDC006, ES

Solution:

Hank's Balanced Solution Modified with calcium, with magnesium without phenol red (Sigma-Aldrich, PN 55037C)

PBS: 8,0 g NaCl+0,2 g KCl+0,25 KH2PO4+Na2HPO4 dissolve in dH2O, adjust on pH 7,4 and add to10000 ml.

NMA (0,75% solution): dissolve 188 mg NMA in 25 ml dH2O.

LMA (0,5% solution): dissolve 125 mg LGA in 25 ml PBS.

Lysing Solution- stock solution:

NaCl 146.1 g + EDTA 37.2 g + Trizma base 1.2 g dissolve in 700 mL dH2O and allow to dissolve while stirring, then add ~8 g NaOH; after dissolving, adjust the pH to 10 with either concentrated HCl or NaOH, then add to 890 ml of dH2O.

Lysing Solution- working solution:

Take 89 ml stock solution, add 1 ml Triton X-100 and 10 ml 10% sodium lauryl sarcosinate solution.

The solutions are kept in a refrigerator, and the working solution is always prepared fresh.

Developing Solution:

Electrophoretic buffer:

The stock solution I: dissolve 200 g NaOH in 500 ml dH2O.

Stock solution II: dissolve 14.89 g disodium EDTA in 200 ml dH2O and adjust NaOH to pH 10.

Electrophoretic buffer working solution: mix 30 ml Stock solution I and 5 ml Stock solution II, add to 1000 ml dH2O, and adjust the pH to 13.

Neutralization buffer:

0.4 M Tris - 48.5 g added to ~800 mL dH2O, adjust pH to 7.5 with concentrated HCl and fill in to 1000 mL with dH2O, store at room temperature.

Staining solution (Angelis K.J.: personal communication):

5 µl SYBR Gold was added into 20 µl of an antifade solution composed of 0,2 M Tris, 90 % glycerine and 2,33 % (w/v) DABCO.

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Table 1. Characteristics CRYOPRESERVED ARTERIAL GRAFTS n.1-20

GRAFT NO.	GENDER	AGE	GRAFT TYPE	STORAGE (MONTHS)	THAWING	WARM ISCHEMIA	COLD ISCHEMIA
1	F	56	thoracic aorta	81	slow	60 min	28 h 2 min
2	M	49	thoracic aorta	81	slow	60 min	28 h 40 min
3	M	27	thoracic aorta	82	fast	50 min	14 h 31 min
4	M	58	thoracic aorta	82	fast	60 min	21 h 50 min
5	F	56	thoracic aorta	81	slow	60 min	24 h 17 min
6	M	37	thoracic aorta	71	slow	1 h 35 min	24 h 40 min
7	M	38	thoracic aorta	72	fast	1 h 37 min	8 h 22 min
8	M	33	thoracic aorta	64	fast	60 min	12 h 5 min
9	M	27	abdominal aorta	81	slow	50 min	11 h 40 min
10	M	27	abdominal aorta	81	slow	50 min	11 h 50 min
11	F	36	a. femoralis	88	fast	1 h 47 min	9 h 3 min
12	M	61	a. femoralis	84	fast	60 min	17 h 18 min
13	M	65	thoracic aorta	72	slow	1 h 57 min	22 h 15 min
14	M	61	a. femoralis	84	slow	60 min	18 h 30 min
15	M	67	a. femoralis	84	slow	58 min	20 h 30 min
16	M	40	abdominal aorta	81	fast	1 h 40 min	15 h 48 min
17	M	40	abdominal aorta	81	fast	1 h 40 min	15 h 58 min
18	M	72	abdominal aorta	6	slow	60 min	19 h 47 min
19	F	73	abdominal aorta	2	fast	60 min	13 h 40 min
20	M	21	abdominal aorta	8	fast	38 min	6 h 57 min

Table 2. Characteristics of FRESHARTERIAL MULTIORGAN DONORŚ GRAFTS n.21-32

GRAFT NO.	GENDER	AGE	GRAFT TYPE	WARM ISCHEMIA	COLD ISCHEMIA
21	M	45	thoracic aorta	60 min	12 h 18 min
22	M	67	thoracic aorta	60 min	20 h 45 min
23	M	52	thoracic aorta	60 min	39 h 38 min
24	M	23	thoracic aorta	90 min	2 h 15 min
25	M	23	a. iliaca	90 min	2 h 20 min
26	M	23	a. iliaca	90 min	2 h 25 min
27	F	44	thoracic aorta	60 min	21 h 03 min
28	F	44	a. femoralis	60 min	21 h 08 min
29	F	44	a. femoralis	60 min	21 h 13 min
30	M	76	thoracic aorta	50 min	41 h 1 min
31	M	76	a. femoralis	50 min	41 h 11 min
32	M	76	a. femoralis	50 min	41 h 21 min

Table 3. Characteristics of CADAVERIC ARTERIAL GRAFTS n.33-47

GRAFT NO.	GENDER	AGE	GRAFT TYPE	WARM ISCHEMIA	COLD ISCHEMIA
33	M	46	a. iliaca	38 h 3 min	23 h 35 min
34	M	46	a. iliaca	38 h 3 min	23 h 40 min
35	M	46	abdominal aorta	38 h 3 min	23 h 45 min
36	M	76	thoracic aorta	63 h 45 min	23 h
37	M	70	thoracic aorta	36 h 23 min	22 h 50 min
38	M	70	abdominal aorta	36 h 28 min	22 h 50 min
39	M	76	a. iliaca	77 h 15 min	22 h 45 min
40	M	76	thoracic aorta	77 h 15 min	22 h 50 min
41	M	79	thoracic aorta	75 h 30 min	23 h 10 min
42	M	79	a. carotis	75 h 30 min	23 h 20 min
43	M	70	abdominal aorta	57 h 45 min	24 h 50 min
44	M	70	abdominal aorta	57 h 55 min	24 h 45 min
45	M	70	thoracic aorta	58 h 5 min	24 h 40 min
46	M	79	a. carotis	32 h 40 min	45 min
47	M	79	a. carotis	32 h 55 min	35 min

Table 4. Descriptive statistics, single-level, endothelial cells (35 clusters)

Variable	Fast (N=351)	Slow (N=409)	Multi (N=572)	Cadaver (N=387)	Total sample (N=1719)	ICC
Tail DNA (%)	29.8 (2.22)	21.9 (1.83)	35.0 (1.53)	31.9 (1.75)	30.1 (0.91)	0.436
Sum_intensities	381.0 (40.07)	333.0 (46.25)	425.9 (14.63)	571.3 (30.60)	427.4 (16.21)	0.361
Head radius	14.2 (0.90)	12.4 (0.66)	19.0 (0.28)	18.7 (0.42)	16.4 (0.28)	0.277
Tail lenght	39.5 (3.70)	28.8 (2.95)	49.0 (1.84)	39.2 (1.93)	40.1 (1.29)	0.581
Olive_moment	23.1 (2.28)	16.5 (1.80)	19.1 (1.08)	14.6 (1.02)	18.3 (0.76)	0.552
Head area	1135.8 (194.52)	772.0 (142.63)	1217.8 (38.61)	1233.7 (66.51)	1098.6 (55.96)	0.158
Tail area	3488.6 (404.80)	2236.6 (302.86)	2326.6 (105.47)	2164.1 (128.38)	2505.9 (119.20)	0.519
Time of warm ischemia	1.2 (0.02)	1.1 (0.02)	1.0 (0.01)	50.5 (0.96)	12.2 (0.54)	NA
Time of cold ischemia	14.9 (0.22)	20.5 (0.30)	25.2 (0.59)	15.8 (0.54)	19.9 (0.27)	NA

Note. Data are expressed as mean (SEM). ICC = Intraclass Correlation Coefficient

Table 5. Within-level covariance-correlation matrix, endothelial cells

	Tail DNA	Sum int	Head radius	Tail lenght	Olive_moment	Head area	Tail area
Tail DNA (%)	**780,0**	*0,29*	*-0,30*	*0,79*	*0,81*	*-0,29*	*0,60*
Sum intensities	4424,7	**288482,1**	*0,27*	*0,52*	*0,45*	*0,19*	*0,70*
Head radius	-83,9	1468,0	**101,0**	*-0,26*	*-0,33*	*0,92*	*-0,21*
Tail lenghth	753,7	9614,7	-88,3	**1168,2**	*0,94*	*-0,32*	*0,88*
Olive_moment	474,9	5130,7	-69,1	678,3	**441,3**	*-0,36*	*0,86*
Head area	-17008,9	217792,1	19698,5	-23081,0	-16107,1	**4539461,2**	*-0,30*
Tail area	57441,8	1288205,0	-7136,9	102035,2	61358,7	-2157342,0	**11580227,0**

Note. Bold entries on the diagonal are within-level variances, covariances are in the lower-left triangle and respective correlations (italic) are in upper-right triangle. All correlations are significant at p<0.05 level

Table 6. Between-level covariance-correlation matrix, endothelial cells

	Tail DNA	Sum int	Head radius	Tail lenght	Olive_moment	Head area	Tail area
Tail DNA (%)	**603,7**	*0,68*	*0,60*	*0,91*	*0,88*	*0,56*	*0,75*
Sum intensities	6765,8	**163114,8**	*0,87*	*0,80*	*0,78*	*0,94*	*0,88*
Head radius	92,2	2186,7	**38,7**	*0,68*	*0,58*	*0,92*	*0,69*
Tail lenghth	896,3	13019,5	170,5	**1619,7**	*0,96*	*0,72*	*0,91*
Olive_moment	504,6	7315,0	83,6	903,4	**543,7**	*0,68*	*0,94*
Head area	12619,5	348738,1	5292,5	26635,1	14677,6	**851770,9**	*0,85*
Tail area	65276,0	1254985,0	15202,8	129701,2	77362,0	2784172,0	**12501433,0**

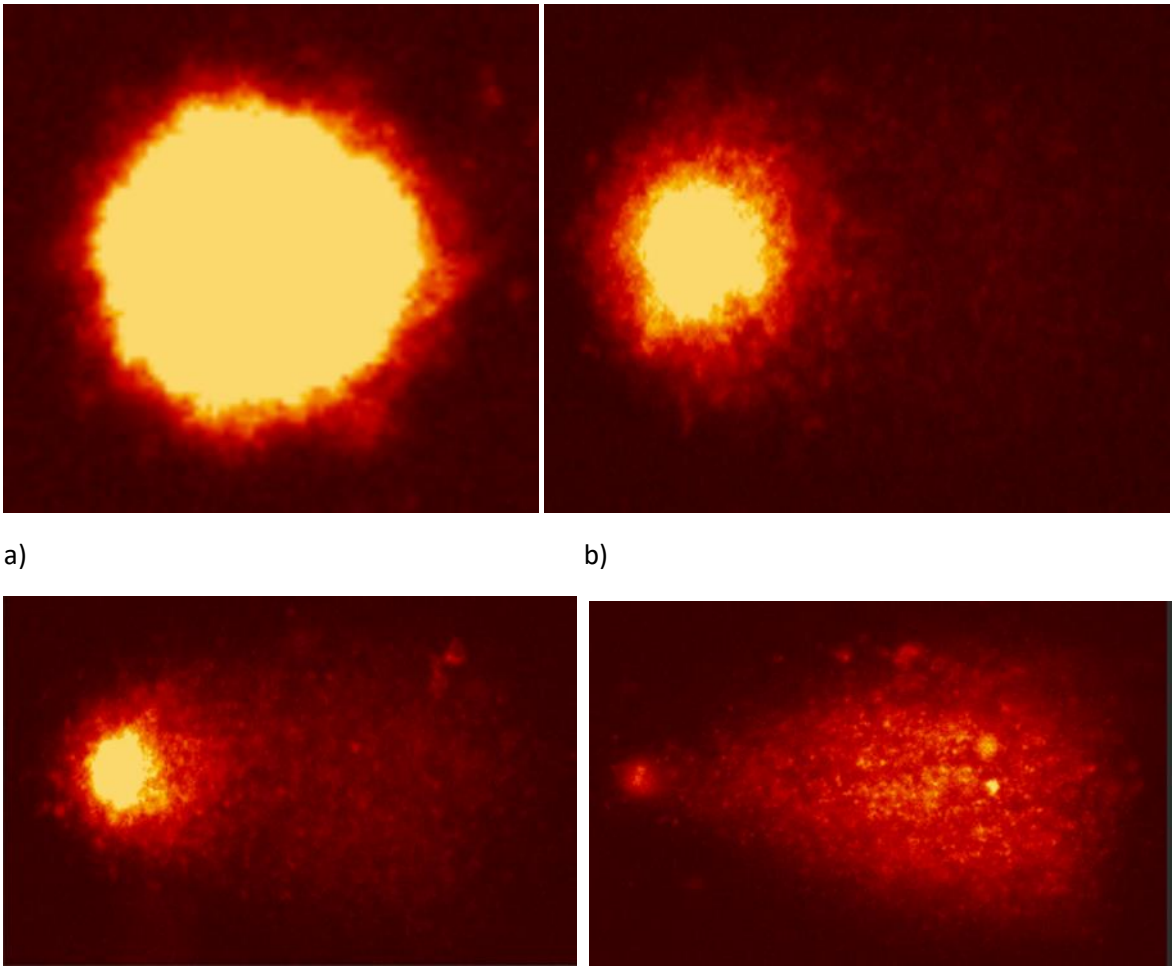
Note. Bold entries on the diagonal are within-level variances, covariances are in the lower-left triangle and respective correlations (italic) are in upper-right triangle. All correlations are significant at p<0.05 level

Table 7. Correlations at level-2 between independent variables and dependent variables

endothelial cells	Fast thawed	Slow thawed	Multiorgan donors	Cadaver donors	Warm ischaemia	Cold ischaemia
Tail DNA (%)	-0,07	-0,12	0,19	-0,01	-0,05	0,06
Sum intensities	-0,13	-0,08	0,04	0,16	0,11	0,10
Head radius	-0,25	-0,27	0,33	0,16	0,12	0,14
Tail lenghth	-0,07	-0,10	0,19	-0,03	-0,03	0,15
Olive_moment	0,05	-0,01	0,06	-0,10	-0,11	0,13
Head area	-0,05	-0,13	0,13	0,04	0,01	0,08
Tail area	0,08	0,00	0,00	-0,07	-0,07	0,13

Note. Red values are significant at p<0.05

Figure 1.



a) b)

c) d)

Fig. 1: typical shape of non-decayed nuclei (a) a decayed nuclei in different stage of degradation (b: mild, c: moderate, d: advanced).

	HeadDNA (%)	TailDNA (%)	Suma intenzit	Head radius	Tail lenghth	Tail moment	Olive moment	Head area	Tail area
a	98.97	1.03	302.25	18.11	7.12	0.07	0.19	1043.14	128..37
b	84.72	15.28	388.73	19.1	31.41	4.8	4.27	1232.45	1281.01
c	60.1	39.6	561	20.52	56.03	22.39	14.95	1390.29	2713.67
d	2.94	97.06	1245.9	11.74	97.8	94.93	57.09	412.91	6525.75