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# Donor and recipient endothelial cell population of the transplanted human cornea: a two-dimensional imaging study

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## ABSTRACT

**Purpose.** To elucidate the pattern of donor and recipient endothelial cell population in transplanted human corneas and determine the degree to which donor endothelial cells survive in the graft.

**Methods.** 36 corneal grafts were collected from recipients of opposite sex to the donor, at the time of re-transplantation for various indications. Cells from the endothelial side of the grafts were harvested, preserving their relative location on the endothelium. Fluorescent in situ hybridization of the sex chromosomes enabled each cell to be identified as donor or recipient-derived. Images of the graft endothelium were assembled to depict the pattern of cell population of the graft, and the proportion of donor cells present was estimated.

**Results.** Endothelial cells of donor origin were found in 26 of 36 grafts (72.2%), in one case up to 26 years after transplantation. The proportion of donor endothelium ranged from 2 – 99%, however, there was no significant correlation of this proportion with postoperative time ( $P = 0.19$ ). The mean annual rate of donor cell loss was negatively correlated to the time to graft failure by endothelial decompensation ( $P = 0.002$ ). Endothelial images indicated a highly variable pattern of recipient cell repopulation of the graft. A tendency towards donor cell retention in transparent, successful grafts was noted; however, this feature alone was not a reliable indicator of long-term graft transparency.

**Conclusions.** Two-dimensional imaging of the corneal graft endothelium revealed a variable pattern and extent of donor and recipient cell population, indicating the highly dynamic nature of the corneal endothelium after transplantation.

## **Introduction**

As the major refractive element in the eye, the cornea serves an essential function for vision. To provide this function, it is vital that the cornea remains transparent – a task for which the corneal endothelium plays a critical role. It is well established that the corneal endothelium functions to maintain corneal hydration, thickness, and transparency through a dual pump – barrier function.<sup>1</sup> In view of the limited ability of human corneal endothelial cells to divide mitotically,<sup>1</sup> endothelial trauma (surgical or otherwise) is accompanied by irreversible cell loss. Depending on the degree of endothelial cell loss, the pump – barrier function may be disrupted and present a risk for subsequent edema, clouding, and loss of vision. This risk is perhaps most apparent in the transplanted cornea, with a large proportion of grafts failing due to endothelial decompensation in the early or late postoperative phase.<sup>2,3</sup>

It is therefore of considerable interest to study the endothelial cell layer in the graft after transplantation, in an attempt to identify cellular activity that may contribute to the long-term transparency of the graft, or alternatively, to its decompensation and failure. To examine such questions the status of endothelial cells in the graft must first be assessed, accounting for the postoperative attrition of donor endothelial cells, cell migration, and replacement by recipient endothelium. Our understanding of these phenomena to date, however, is poor, and has been largely based on early animal studies using radioisotope labeling or sex chromatin as differential endothelial markers,<sup>4-9</sup> or has been gained by inference from specular microscopic observation of the human corneal endothelium.<sup>3,10-13</sup> As opposed to these earlier techniques, a more exact method for unequivocally identifying individual donor and recipient endothelial cells in the graft – namely fluorescent in situ hybridization (FISH) of the sex chromosomes in sex-mismatched corneal grafts – has only been proposed within the past decade,<sup>14</sup> and only two studies to date

have utilized this technique to examine human corneal endothelial cells.<sup>14,15</sup> In a recent study we observed for the first time that individual human donor endothelial cells survived in the graft for up to 32 years.<sup>15</sup> Both studies using the FISH technique, however, addressed the question of whether donor endothelial cells survive in the graft in the long term, and both used thin histologic corneal sections containing relatively few endothelial cells. To date, the pattern of donor and recipient endothelial cell population of the two-dimensional posterior graft surface at the level of the individual cells remains unknown.

Accordingly, as an initial step towards elucidating the dynamics of endothelial cell population of the graft, in this study we used the FISH technique in sex-mismatched human corneal grafts to observe the origin of cells (donor or recipient) and their respective location over the two-dimensional endothelial surface.

## **Materials and Methods**

### **Patients**

After obtaining approval from the Gothenburg University ethics committee and following the tenets of the Declaration of Helsinki, between September 2004 and December 2008 forty-nine corneal buttons were collected prospectively from patients with failed corneal transplants.

Buttons were retrieved in sex-mismatched cases (the patient and original donor were of opposite sex) at the time of re-operation by members of the Swedish Society of Corneal Surgeons.

Corneal button diameter varied from 7.25 to 8mm. Following further sample preparation, thirteen samples were deemed unsuitable for analysis leaving a final study sample of 36 corneal buttons.

The recipient population consisted of 23 female and 13 male patients, with a mean age of  $66 \pm 15$  y (mean  $\pm$  SD) at the time of reoperation. The age of the graft (time from initial penetrating keratoplasty to removal of the donor button at re-operation) ranged from 1 – 30 years. The most common indications for the primary transplant were keratoconus (12 cases) and bullous keratopathy/edema (10 cases). The main indication for re-operation was endothelial decompensation (27 cases), while nine grafts were removed for other reasons, 6 of which were substantially transparent at the time of removal. Details of recipient characteristics and the explanted buttons are given in Table 1.

#### Sample preparation

Immediately following surgery, an *en face*, two-dimensional sample of endothelial cells was obtained from the explanted button by blotting the endothelial side with a sterile filter membrane (Millicell 0.4  $\mu$ m, 10 mm dia. sterilized culture plate insert; Millipore Corp., Bedford, MA, USA). The membrane was gently pressed onto the button and after a few seconds was carefully lifted to harvest endothelial cells, which adhered to the membrane surface. The membrane with cells was allowed to dry in air for a few minutes and was then transported to the Department of Obstetrics and Gynaecology at Gothenburg University for further laboratory analysis. In the laboratory, endothelial cells adhering to the membrane surface were immediately fixed by immersing the entire membrane in 95% methanol for 5 minutes. Upon removal, the membrane was allowed to dry in air at room temperature. Samples were subsequently pre-processed for fluorescent in situ hybridization (FISH) analysis of the sex chromosomes of endothelial cells by re-fixing in a 3:1 Et-OH/acetic acid solution at room temperature for 15 min. The samples were allowed to dry in air, after which the entire filter membrane with adherent cells was mounted cell

side up on a glass microscope slide. FISH was then performed in an identical manner as has been described previously.<sup>15</sup>

#### Evaluation of FISH signals

FISH signals were observed using a Nikon fluorescent microscope equipped with a digital camera for image capture. An X-chromosome centromere exhibited an orange signal, a Y q-arm exhibited a green signal and the endothelial cell nucleus was counter stained with DAPI-blue. A triple bandpass DAPI/FITC/TRITC filter (360/490/570nm) was used to view all three fluorescent signals simultaneously. Samples were viewed using a Nikon Plan Fluor 10× objective lens (NA 0.30) to determine the relative location of cells on the filter membrane and a Nikon Plan Fluor 20× objective lens (NA 0.50) to determine the origin of the cells (donor or recipient). Endothelial cells were classified as being male- or female-derived based upon the presence of two orange signals (chromosomes X and X, female) or an orange and a green signal (chromosomes X and Y, male) within a single nucleus (blue). Only cells with two distinct signals in the nucleus and with distinct nuclear borders were classified.

Thousands of endothelial cells were typically lifted from each corneal button, and covered an area of the filter approximately 7mm in diameter. With a 20× objective lens (the lowest magnification required to identify individual FISH signals), only signals present in a  $360 \times 260\mu\text{m}$  (w × h) field of view could be observed simultaneously. The distribution of cells of donor and recipient origin was therefore determined by manually translating the sample stage in a raster fashion while recording the approximate location and origin of cells in the form of a hand-drawn image. Cells of female and male origin were recorded by means of red and green dots, respectively. Once each image was drawn, the proportion of male and female cells in the image was estimated. All hand-drawn images and estimates of cell proportions were made by a single



observer. Additionally, the final sample in the series (Patient 36) was selected for a more detailed microscopic analysis.

### Statistical Analysis

The proportion of donor endothelial cells present in each sample was recorded in a spreadsheet (Excel 2003, Microsoft Inc., Redmond, WA), by matching the gender of each recipient with the proportion of cells of opposite sex identified in the excised button. Linear relationships between age of the graft and proportion of donor endothelium were analyzed using the Pearson product moment correlation coefficient. Comparison of the proportion of donor endothelium present across different patient subpopulations was conducted using the nonparametric Mann-Whitney rank sum test. In all cases a P-value of  $< 0.05$  was considered significant. All statistics were performed using commercially-available statistical software (SigmaStat 3.5 for Windows, Systat Software Inc., Chicago, IL).

### Results

A large number of endothelial cells were lifted from the corneal button by the filter membrane. For a single case (Patient 36; Table1) a composite image of an entire filter membrane after FISH was assembled from 72 separate microscope image fields taken in fluorescence mode under low magnification (10 $\times$ ), indicating the distribution of endothelial cell nuclei on the membrane (Figure 1).

FISH signals from X and Y chromosomes, however, could only be visualized under higher magnification (20 $\times$ ; Figure 2). In the central cornea, the most densely populated image field at 20 $\times$  magnification contained 110 cell nuclei, corresponding to a maximum endothelial cell density of 1175 cells/mm<sup>2</sup>. Other image fields however, contained substantially fewer cells.

Although the small field of view at 20× magnification made imaging of the entire filter membrane difficult, a central vertical region from Figure 1 was imaged by assembling 20 separate microscope fields to illustrate the distribution of cells in this region (Figure 3). In this case of a female recipient, 509 female-derived cells and 645 male-derived donor cells were counted in this central strip, which represented approximately 10% of the cells present on the membrane. The remainder of the membrane was assessed manually at 20× magnification (without recording images), and it was determined that approximately 70% of cells in the sample were of female (recipient) origin.

All patient samples were assessed manually by the approximate method. A pictorial representation of the distribution of endothelial cells in the excised button in several cases from Table 1 is given in Figure 4. The distribution of donor and recipient cells varied considerably among samples and in most cases, no discernable pattern of recipient cells within the graft could be detected; however, in a few cases recipient cells were found principally in the graft periphery with only donor cells occupying the central graft. In total, in 25 of 36 cases (69.4%), at least some endothelial cells of recipient origin were found in the central graft, with graft age in these cases varying from 1 – 30 years and donor cell proportion ranging from 0 – 99%.

The proportion of donor endothelium in the excised buttons is given in Table 1, and is illustrated graphically in Figure 5. Overall, there was no significant correlation of the donor cell proportion at the time of graft removal with the age of the graft (Pearson coefficient -0.22,  $P = 0.19$ ).

Correlations between graft age and donor cell survival were further tested by sub-grouping samples based on graft transparency, recipient age (above or below the median age of 70y), recipient sex, and indication for primary transplantation (keratoconus or non-keratoconus). In all cases, no significant correlation of donor cell survival with graft age was found.

Donor cell survival varied widely across samples – this was particularly evident in grafts present within a recipient for less than 10 years. Complete replacement of donor endothelium by cells of recipient origin occurred in 10 of 36 grafts (27.8%). In one case, total replacement occurred as early as 1.5 years after transplantation. Of the six transparent grafts, two exhibited complete donor cell replacement while the remaining four (removed due to intractable astigmatism) had a substantial proportion of surviving donor endothelium, ranging from 30 – 99%. In one case, 50% of endothelial cells were of donor origin in a transparent graft removed 26 years after transplantation.

The proportion of donor endothelium present was compared across patients grouped by sex, keratoconic versus non-keratoconic eyes, and graft age (above or below the median age of 5 years). The only significant difference found was a reduced proportion of donor endothelium in grafts older than 5 years relative to younger grafts ( $P = 0.046$ , Mann-Whitney rank sum test).

The indication for initial transplantation was also considered. The proportion of donor endothelium surviving in conditions where peripheral recipient endothelial cell density would be expected to be high (keratoconus, lattice dystrophy, herpes keratitis, trauma, or chemical injury) was compared to conditions with a lower expected recipient endothelial cell density (endothelial dystrophy, bullous keratopathy and edema). No significant difference in the proportion of donor cells surviving in the graft could be found between the two groups ( $P = 0.94$ , Mann-Whitney), however, median graft survival time in the lower expected cell density group was significantly shorter than in recipients with a better-preserved endothelium ( $P = 0.002$ , Mann-Whitney). No significant correlation of graft age with donor cell survival (i.e., replacement of donor endothelium over time) could be found in either group of indications.

The rate of decline in the proportion of donor endothelial cells in the graft was additionally considered. Based on an assumption of a constant rate of donor cell loss after transplantation, the mean annual rate of decline in donor cell proportion was determined by dividing the proportion of donor cells lost at graft failure by the age of the graft in years (results in Table 1). In grafts which failed due to edema from endothelial decompensation, a significant correlation between the mean annual rate of proportional donor endothelial cell loss and the time to graft decompensation was found (Pearson coefficient: -0.56,  $P = 0.002$ ). In a likewise manner, we analyzed the results from our earlier study<sup>15</sup> and again found a significant correlation between the rate of donor endothelial cell loss and the time to graft decompensation (Pearson coefficient: -0.56,  $P < 0.001$ ).

## **Discussion**

Tracking the location and origin of endothelial cells across the two-dimensional posterior corneal graft surface by FISH analysis has revealed that a substantial proportion of donor-derived endothelial cells can survive in the graft for long periods, in some cases indefinitely.

Considerable variability exists, however, in the replacement of donor endothelium. Our findings support the results of our earlier study where relatively few endothelial cells were analyzed in thin cross-sections of the central graft.<sup>15</sup> Our results differ most notably from those of Wollensak and Green, who used FISH analysis of thin corneal sections to show complete endothelial replacement by recipient cells in all 14 grafts examined, with graft age ranging from 11 months to 30 years.<sup>14</sup> In that study, however, only failed grafts were examined, tissue samples were obtained retrospectively, and the analysis was limited by the small number of endothelial cell nuclei that could be examined in thin tissue sections.

Two-dimensional images in this study have provided evidence of the dynamic nature of the endothelium after corneal transplantation. In some cases – as early as 18 months after transplantation – recipient endothelial cells completely replaced donor cells, after which graft failure due to endothelial decompensation invariably ensued. In these cases, the condition of the donor material, surgical trauma, or immunologic reaction may have facilitated the replacement.<sup>3,14,16,17</sup> In grafts where both donor and recipient cells co-existed, the pattern of recipient cell population of the graft was highly variable. In some cases, large regions of recipient endothelial cells appeared to invade the graft while in other cases isolated, single recipient cells appeared at disparate locations in the graft. In a few cases, a peripheral, circumferential repopulation of the graft by recipient endothelial cells occurred – as would be expected for a slow, orderly replacement of endothelium over time – however, these cases were exceptional. Our results notably contradict earlier findings of Ruusuvaara, who suggested very little migration of recipient endothelial cells into the graft.<sup>12</sup> Significant and rapid recipient endothelial cell migration into the graft can occur, apparently unimpeded by scar tissue at the recipient-to-graft interface. Endothelial cell division in human corneas is believed to be rare; instead, damaged endothelium is instead believed to heal by spreading and sliding of adjacent live cells.<sup>1,3</sup> Our observations of differing patterns of donor cell replacement and the presence of isolated recipient cells in the graft surrounded by donor cells is somewhat puzzling in this context. Recipient endothelial cells apparently do not always repopulate the graft *en masse*, and individual cells from the peripheral recipient endothelium appear to migrate far into the graft. While the possibility that individual, isolated recipient cells were of non-endothelial origin (eg., bone marrow-derived) cannot be excluded, the consistency of the nuclear size and morphology

of recipient cells with surrounding donor-derived endothelial nuclei and the distribution of the cells (see for example Figure 3) strongly suggests a corneal endothelial phenotype. In future studies, endothelial cell-specific markers could be used to confirm cell phenotype, or alternatively, specular microscope photographs of the central corneal endothelium (taken prior to explantation) could be used to examine cell density, morphology and phenotype. The presence, however, of ten grafts in this study and nine in our previous study<sup>15</sup>, all apparently exhibiting full replacement by recipient endothelium, suggests the ability of individual peripheral recipient endothelial cells to traverse the wound and migrate to the central cornea. Another possibility is that mitotic division, in addition to migration, contributed to the repopulation of graft endothelium. Although the mitotic potential of human corneal endothelial cells is believed to be limited<sup>1</sup>, evidence for mitosis in humans both in-vitro and in-vivo has been reported.<sup>18-20</sup> Moreover, studies of mitotic endothelial division in response to a corneal wound<sup>1</sup> may not adequately reflect in-vivo mitotic stimuli that may exist in the context of allotransplantation.<sup>20,21</sup> From our results, we speculate that a variable initial migration (and possibly division) of recipient and/or donor endothelium on the graft (dependent on factors such as donor tissue status and degree of surgical trauma), followed by endothelial cell attrition post-transplantation<sup>3</sup> (of both donor and recipient cells, presumably at various locations in the graft), may account for the varied patterns of endothelial replacement observed at the time of graft removal. The use of specific markers to determine the extent of endothelial cell migration or division, and the use of specular microscopy to record endothelial cell densities remain interesting possibilities for a more detailed future investigation of endothelial cell dynamics in explanted grafts.

Variable rates of donor endothelial cell replacement were found in grafts that failed due to decompensation. While a high rate of replacement (donor endothelial cell death) invariably resulted in early graft decompensation, some grafts with slow replacement of donor endothelium also decompensated early. This finding indicates that additional factors apart from donor cell survival alone, such as the absolute number of endothelial cells on the graft or the functional viability of the donor endothelium, may also impact the success of the graft.

In the four transparent, otherwise successful grafts removed in this study for astigmatic reasons, the rate of donor endothelial cell loss was low, indicating a tendency for donor endothelium to persist in transparent, successful grafts. A similar trend was also noted in our earlier study<sup>15</sup> and by others in animal studies.<sup>7,17,22,23</sup> In our two cases of transparent, but skewed grafts, no donor endothelium was present, however, these grafts were removed due to scar formation at the graft-to-recipient interface, which affected the visual axis due to the off-center location of the graft. Additionally, one of these grafts had a mild haze noted at the time of re-operation. In earlier studies in rabbits, Chi and colleagues noted that grafts with postoperative haziness followed by spontaneous clearing had very few donor endothelial cells present, and postulated that initially damaged donor endothelium was eventually replaced by recipient cells, which cleared the graft.<sup>22</sup> A similar effect was discussed in cases of late spontaneous clearing of human corneal grafts.<sup>24</sup> Although in this study we did not have information concerning the early postoperative transparency of grafts, it is apparent that in some cases, the retention of donor endothelial cells alone may not be a necessary condition for long-term graft transparency. This contention is supported by the observation in this study of a low proportion of donor endothelium in the three

cases of recurrent lattice dystrophy, where edema-free grafts were removed 13 to 30 years after transplantation.

Several methodological limitations in this study are worth noting. Although blotting the endothelial side of grafts using a filter membrane was an effective method of harvesting endothelial cells, in a few samples a substantial number of endothelial cells remained adherent to the graft after blotting. A second blot typically removed these remaining cells, and subsequent FISH analysis of second membranes indicated the same donor and recipient cell proportions (results not shown). Additionally, since the membranes used were flat, the concavity of the endothelial side of corneal buttons made sampling of cells at the outer edge of the graft difficult. In terms of imaging, our approximate method of drawing the pattern of donor and recipient cells in the graft could be improved by using an exact technique to provide data as illustrated in a partial region of a graft in Figure 3. The tedious nature of acquiring and assembling such large composite images could be aided by wide-field imaging techniques or an automated (motorized) sample scanning and image acquisition scheme. Finally, inclusion of a larger sample of transparent grafts would enable differences in the endothelium of successful and failed grafts to be meaningfully compared to isolate features significantly impacting transparency of the graft. In this study we presented the first picture of the two-dimensional pattern of donor and recipient endothelial cell population of the corneal graft. The speed and pattern of endothelial cell migration into the graft has not been directly observed in prior studies and our results suggest that the graft endothelium after keratoplasty is a much more dynamic environment than is currently realized. Significant endothelial cell migration across the graft – in some cases by single recipient cells – indicates a complexity not suitably explained by an orderly cell turnover or endothelial cell movement by expansion. Additionally, the variable response in donor cell



survival and cell migration patterns observed in this study indicates that factors beyond those considered – such as the degree of surgical trauma, postoperative complications, and the health and migratory (and possibly proliferative) potential of both donor and recipient endothelium – may be important determinants of the endothelial status of the corneal graft.

In summary, two-dimensional imaging of the posterior surface of corneal grafts has revealed a wide variation in the timing and pattern of replacement of donor endothelial cells by those from the recipient. There was no overall tendency towards replacement of donor cells in the long-term, indicating that in some cases donor endothelial cells may survive in the graft indefinitely. Grafts with a rapid rate of donor endothelial cell replacement by recipient cells, however, decompensated earlier than those in which donor cells were retained. Although a trend towards a higher retention of donor endothelium in transparent, successful grafts was noted, our observations in failed grafts indicate that retention of a high proportion of donor endothelial cells may not in itself be sufficient to prevent graft failure due to endothelial decompensation nor serve as a necessary condition for long-term graft transparency in all cases. Further studies investigating the interplay between recipient and donor cells on the endothelial surface after transplantation – specifically with a focus on elucidating the mechanisms of cell replacement – may help to illuminate the factors that determine the ultimate fate of the corneal graft.

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Table 1. Recipient details and results of endothelial cell analysis in removed corneal buttons.

Recipient	Sex	Age*	Indication for primary transplant	Compensated grafts†	Graft age‡ (y)	Donor cells (%)	Transparent grafts	Donor loss§ (%/y)
1	F	42	keratoconus		5	40		12
2	F		keratoconus	skewed graft	23	0	x	4
3	F	78	endothelial dystrophy		2.5	60		16
4	M	76	keratoconus	astigmatism	2.5	70	x	12
5	F	47	lattice dystrophy	recurrent lattice	13	5		7
6	F	80	bullous keratopathy		2	70		15
7	M	44	keratoconus		18	0		6
8	M	44	keratoconus	astigmatism	26	50	x	2
9	F	71	keratoconus		13	0		8
10	F	70	bullous keratopathy		1	5		95
11	F	43	scarring from trauma	skewed graft	2.5	0	x	40
12	F	70	lattice dystrophy	recurrent lattice	18	25		4
13	F		endothelial dystrophy		5	15		17
14	F	80	herpes keratitis		6	20		13
15	F	70	edema		2	10		45
16	M	74	bullous keratopathy		1	10		90
17	F	74	edema		1.5	0		67
18	F	72	endothelial dystrophy		4	5		24
19	M	58	keratoconus	astigmatism	3	99	x	0
20	M	57	trauma		5.5	80		4
21	F	69	Fuchs' dystrophy		6	0		17
22	F	88	bullous keratopathy		4	95		1
23	M	72	macula cornea		5.5	0		18
24	M	74	keratoconus		3	2		33
25	M		lattice dystrophy	recurrent lattice	30	0		3
26	M	60	edema		1.5	5		63
27	F	88	bullous keratopathy		5	2		20
28	M	36	keratoconus	astigmatism	12	30	x	6
29	M	55	keratoconus		2	55		23
30	F	63	keratoconus		5	45		11
31	F	85	endothelial dystrophy		9.5	15		9
32	F	84	bullous keratopathy		3.5	20		23
33	M	57	keratoconus		6	15		14
34	F		endothelial dystrophy		6.5	0		15
35	F	59	chemical injury		16	0		6
36	F	77	bullous keratopathy		2	30		35

\*age at time of re-operation. Age data was not available for four recipients.

†reason for explantation of well-compensated, non-edematous grafts. Astigmatism refers to intractable astigmatic refractive error. Skewed grafts caused defects in the visual axis due to their off-center corneal location.

‡ time interval between initial transplantation and removal at re-operation

§ mean annual rate of proportional loss in donor endothelial cells, using a simplified assumption of a constant rate of post-transplantation cell loss

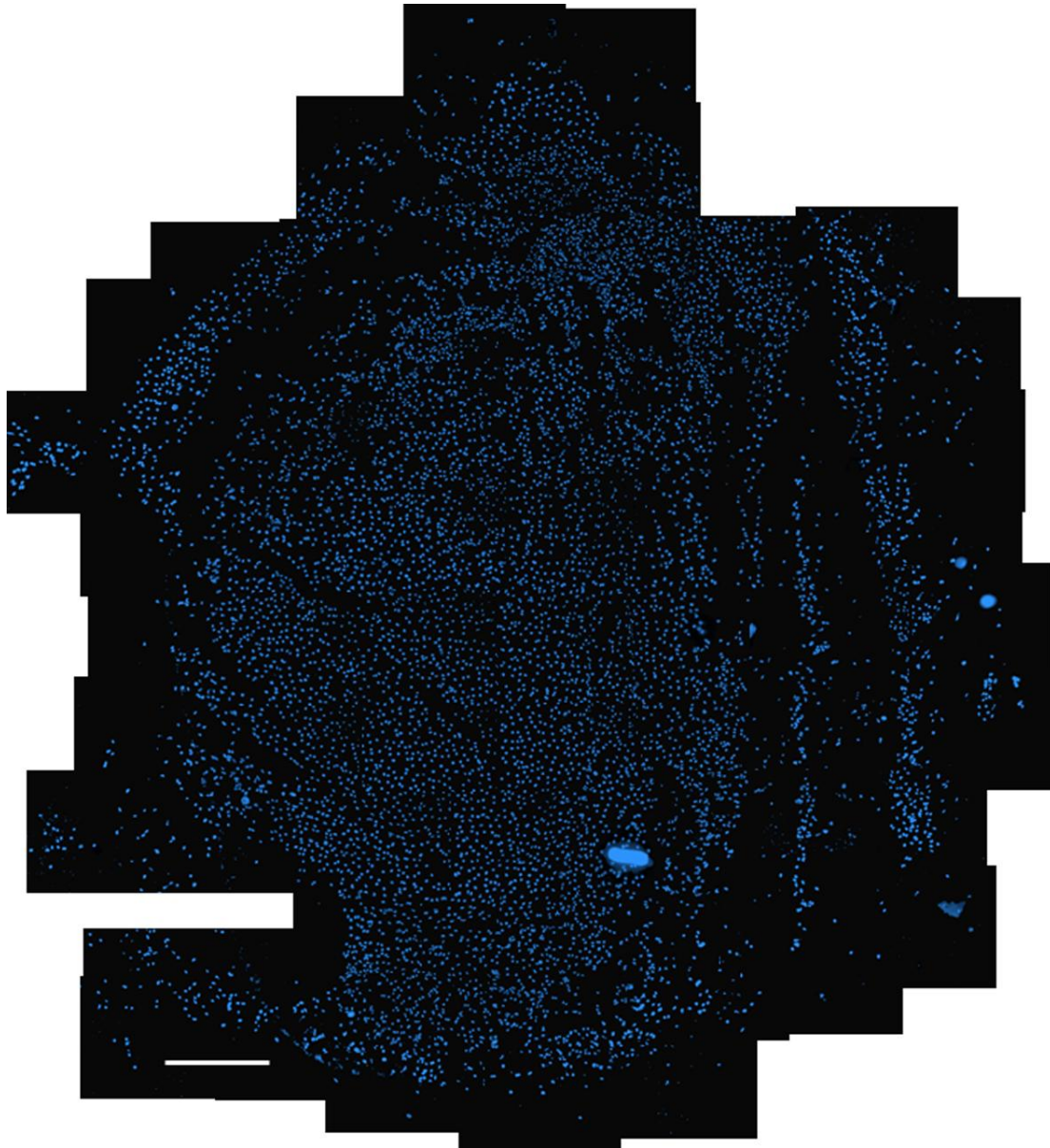


Figure 1. Composite image of endothelial cell distribution on a filter membrane after preparation for FISH analysis. Images were taken from an endothelial blot of a corneal button removed from a patient two years after initial transplantation. The image was constructed from 72 separate microscope image fields at 10× magnification in fluorescence mode, with endothelial cell nuclei stained blue (DAPI). Bar = 500 $\mu$ m.

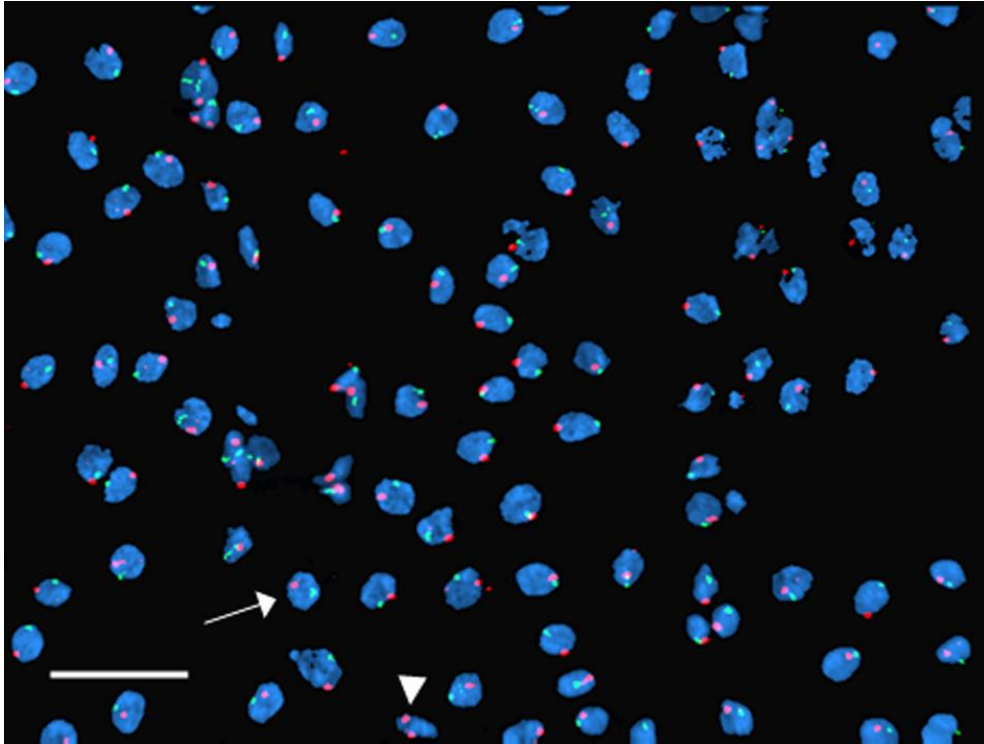


Figure 2. Higher magnification (20 $\times$ ) image taken from the central region of the filter membrane shown in Figure 1. FISH signals from X and Y-chromosomes are indicated by red and green dots, respectively. Cells of both donor (arrow) and recipient (arrowhead) origin were frequently observed adjacent to one another in the central cornea. Bar = 50 $\mu$ m.

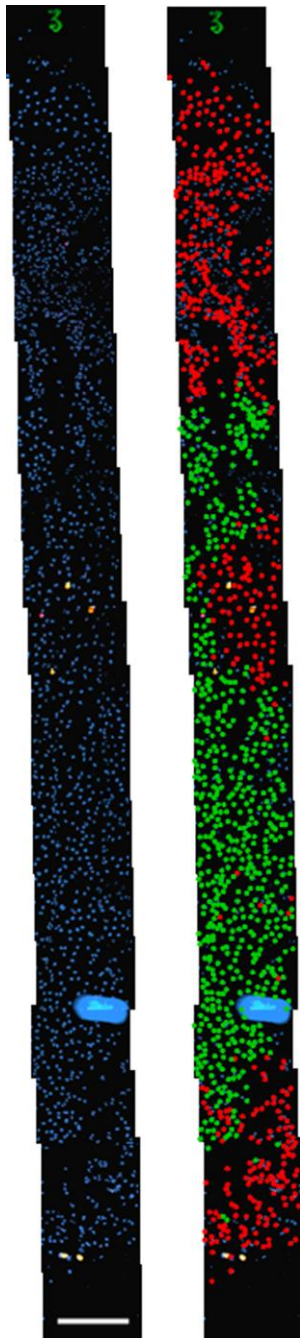


Figure 3. Composite images of endothelial cell distribution in a central vertical strip of the filter membrane shown in Figure 1. The image on the left was constructed from 20 separate image fields, taken at 20x magnification to enable the origin of individual endothelial cells to be determined. The image on the right represents the image on the left after placement of a red dot on each cell of female (recipient) origin and a green dot on each cell of male (donor) origin. Note the presence of recipient endothelial cells in the central cornea in this patient two years after transplantation. Bar = 250 $\mu$ m.

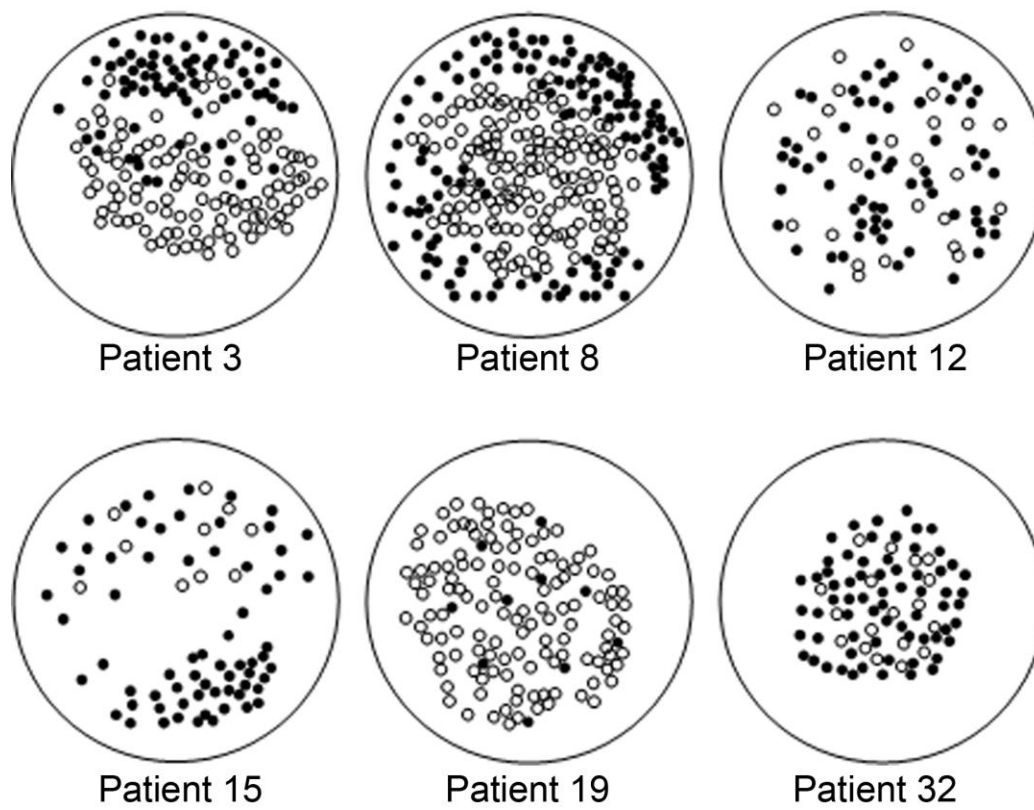


Figure 4. Computerized representation of hand-drawn images of the endothelium from six corneal buttons, indicating the relative population and location of donor and recipient endothelial cells after FISH analysis. Donor cells are indicated by open circles and recipient cells are indicated by filled circles. Note the variation in the relative proportion of donor cells and in the pattern of recipient endothelial cell re-population of the graft. Details of the clinical characteristics of the patients and grafts are given in Table 1. Note that the grafts removed from Patients 8 and 19 were fully transparent (removed due to astigmatism).



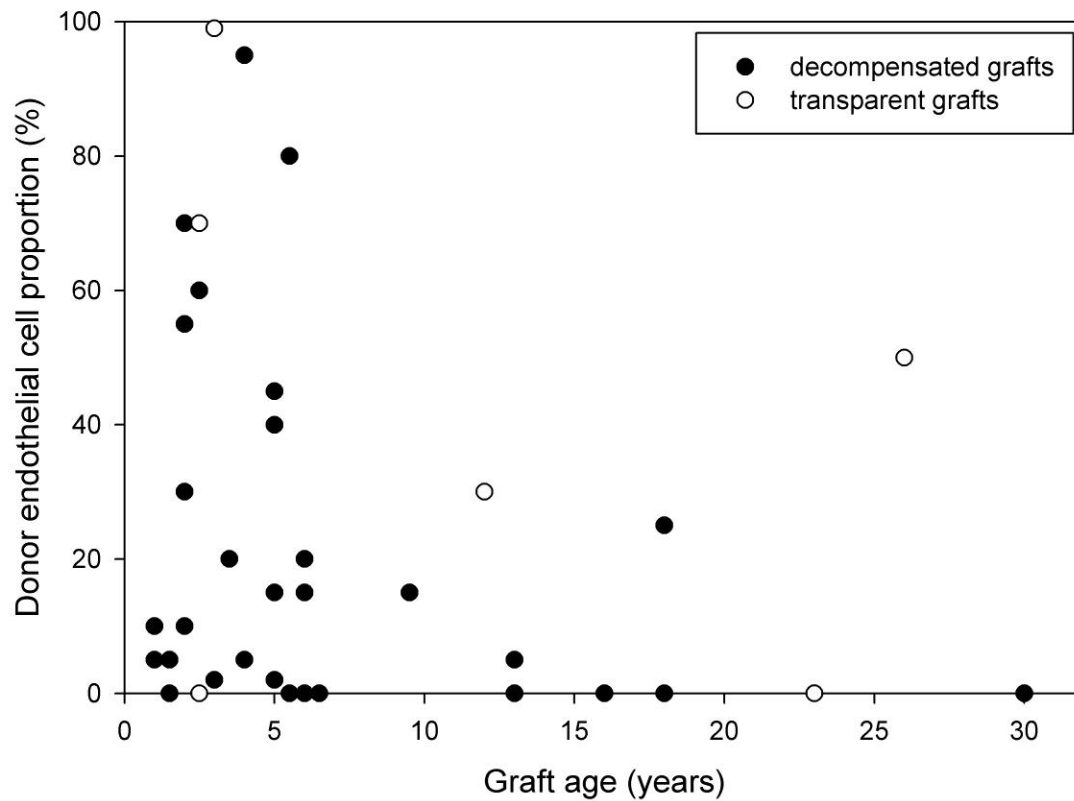


Figure 5. Donor endothelial cells as a proportion of total endothelial cells observed in each of 36 corneal grafts, plotted against graft age. A subset of six transparent grafts (removed for reasons other than endothelial decompensation) is indicated by open circles. No donor endothelial cells were found in 10 grafts. The proportion of surviving donor endothelial cells did not correlate with graft age.