in muscle tissue. Therefore, the precise visibility of tendons and connective tissue bands is strongly reduced. Imaging advantages in characterizing the pulleys have been attributed to secondary and tertiary gaze positions. Hence, ocular movement amplitudes larger than those investigated in our current publication are necessary to provide better insight.

At present, spatial resolution of the published images generally is not high enough to separate horizontal extraocular muscles into functional subunits, such as the global and the orbital layers. Clear spaces between tissue borders may also be an expression of the water–fat shift of the MR signal.

The strong interest in the technique motivates us to further invest in it. Especially in the MR technique there is potential to improve the image quality and therefore even smaller changes may be visible in the future.

To summarize, motion-encoded MRI in its current technical state neither supports nor contradicts the active pulley hypothesis.

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Donor Cell Survival in Corneal Grafts

I read with great interest the two excellent articles from the Swedish research group headed by Lagali et al.1,2 about the survival of donor cells in human corneal transplants. They showed that there is great variability in the extent of donor cell replacement in corneal transplants, leading to a significant early loss of donor cells in some cases and significant long-term survival of donor cells in others. In addition, they demonstrated that successful, transparent grafts are compatible with both persistence of donor cells and complete replacement of donor cells by recipient cells. On the other hand, graft failure was observed despite donor cell survival in other cases. Apparently, the number of endothelial cells and not their cellular origin is important for a successful transplant.

However, I think that the authors placed too much emphasis on the possible survival of variable proportions of donor cells, confirming old dogma and prejudice regarding donor cell survival. Also, in my view the results of the two recent studies support the results of our similar studies conducted 10 years ago,3,4 but their studies provide a broader database and allow a more differential view.

From the beginning of keratoplasty, there has been a debate on the fate of the cells in the corneal transplant, and extensive efforts have been made to prevent graft rejection to ensure donor cell survival.5 The mainstream consensus, although never supported by facts, has been that the donor epithelium is replaced by the recipient cells, the donor keratocytes may be replaced, and the donor endothelium is never replaced.6 Ten years ago, using the same technique as was used in the recent Swedish studies, we showed that endothelial cell replacement is possible. At the time, this observation was a revolutionary finding, and it has been ignored by most experts to this day.5 The present Swedish studies have clearly confirmed that complete endothelial donor cell replacement can occur and is compatible with a clear graft (cases 15, 23, and 27 and basically also cases 25 and 48 in the first study1 and cases 2 and 11 in the second study2). This finding should no longer be ignored, and the dogma regarding endothelial cell survival in transparent grafts can no longer be upheld. It seems that the success of the transplant depends on the vitality of both the recipient cells and the donor cells, and the relative contribution of the two cell populations may differ depending on their vital function.

I would like to ask a few questions and add some comments:

1. It is unclear to me how Lagali et al.1,2 determined the donor’s sex, especially in cases with a long postoperative time interval of several decades. Did they have donor information files or did they deduce the donor’s sex from the sex-related signals that they found in the epithelium? If the latter is true, did they also check the epithelium in the second study,2 where they used only a monolayer of endothelial cells?

2. In both studies, it is stated that there was no correlation between donor cell survival and graft age and that the postoperative time interval did not have an influence on the occurrence of donor cell replacement.1,2 On the other hand, it is mentioned in the second paper, in which the filter membrane technique was used, that there was a significantly reduced proportion of donor endothelium in grafts older than 5 years relative to younger grafts.2

I think the absence of clear, statistically significant proof of donor cell replacement as a function of postoperative time or graft age in this series can be easily explained by the great variability between the individual cases, which would require a large number of samples to find statistically significant parameters. Obviously, postoperative time is one of the factors involved, because there was some reduction in the initial 100% presence of donor endothelial cells in all cases (except in case 7 after 3 months from the first series1) and a reduction by at least 50% in 21 of 35 in the first series1 and in 29 of 36 cases in the second series.2

Of course, time is only a formal descriptive parameter, not a cause. It is interesting that in autokeratoplasty, as well, a rapid postoperative decline in the endothelium has been reported—55.5% in the two first postoperative years.7 Donor cell attrition may be caused simply by the unnatural conditions in the graft which is surrounded by a circular scar, the neurovascular changes, the absence of stem cells, the cellular aging, the chronic inflammation, and the increase in intraocular pressure, among other possible causes. In some cases, chronic subclinical rejection may play a role. In our study, only two patients had an episode of graft rejection.8 Do Lagali et al. know which or how many cases in their series had previous episodes of graft rejection?

3. It is not correct to say that no compensated grafts were included in our series. Three compensated grafts without corneal edema or endothelial decompensation were included (cases 9, 10, and 11, with 9, 11 and 12 years’ postoperative time, respectively).3 These grafts were removed for recurrence of lattice dystrophy.

4. Long-term donor cell survival is possible to some extent, as was shown by Lagali et al. and by us. However, it seems that such survival is not often the case, because recurrences of corneal dystrophies demonstrating recipient cell repopulation are usually found only within the first 2 to 15 postoperative years, rarely later,9 and graft rejections demonstrating the persistence of donor cells also mostly occur within the first 5 postoperative years—seldom later.10 So, overall, long-term survival of significant amounts of donor cells seems to be possible but rather infrequent.

5. Lagali et al. have shown and confirmed that a clear successful graft is compatible both with and without signifi-
cant donor cell survival (3–5 of 10 in the first series¹ and 2 of 6 compensated grafts in the second series²). Often, a mosaic pattern is also observed. A gradual replacement of the donor cells does not lead to graft decompensation. Apparently, only the number of endothelial cells determines their functionality, not their cellular origin. The extent and the speed of the donor cell replacement seems to depend on factors affecting the vitality of the transplanted cells, such as cellular aging, the circular scar, the absence of stem cells, chronic inflammation, increased intraocular pressure, and so forth, and on the quality of the recipient cells at the corneoscleral margin.

6. Mathematically, a complete donor cell replacement in the graft by the corneoscleral recipient cells is possible even without cell proliferation, leading to a reduction of the cell density by approximately 50%.

If one assumes a corneal transplant radius of 4 mm (diameter: 8 mm) and a total corneal radius of 6 mm (diameter: 12 mm), one can calculate the area of the outer corneal ring that remains intact in corneal transplantation to be $\pi \times 20 \, \text{mm}^2$ by subtracting the area of the transplant ($\pi \times 16 \, \text{mm}^2$) from the area of the total cornea ($\pi \times 36 \, \text{mm}^2$). From this calculation, it follows that the ratio of the area of the outer corneal ring to the area of the total cornea is approximately 0.6, so that the recipient endothelial cells of the outer ring would have to spread on an area double the size and that the endothelial cell density would be reduced only by half, which is often observed in corneal transplants.⁵

In addition, however, endothelial cell proliferation seems possible. Endothelial mitotic activity can be observed in organ² and cell culture, and endothelial stem cells have been described recently.¹²,¹³

7. The two main reasons for assuming endothelial donor cell survival are the terrible experience of graft rejection and the alleged impossibility of endothelial cell proliferation. It is correct that a sudden and massive graft rejection leads to primary graft failure. However, gradual cell replacement within the corneal graft over years is probably unrelated to graft rejection and is compatible with endothelial cell function and a clear graft, as long as a minimum cell density⁶ (higher than 500 endothelial cells/mm²) is preserved.

As for the endothelium, cell mitosis is unusual but has been observed in organ¹¹ and cell culture, and endothelial stem cells have been described recently.¹²,¹³

8. I wonder whether there are any practical implications of the findings about the dynamics of the cellular changes within the transplant.¹⁻³ From large results of statistical studies, it is well known that the long-term prognosis for grafts is significantly better in keratoconus than in bullous keratopathy,⁵ as was confirmed by a recent Swedish study.¹⁴ Maybe this difference can be explained in part by the lower vitality of the recipient endothelium in Fuchs' dystrophy. Perhaps the use of a central limbal keratoplasty with an eccentric trephination of the donor cornea would provide endothelial-like stem cells similar as for the epithelium,¹⁵ making this graft type more like a bone marrow transplant in contrast to a blood transfusion. In cases with good recipient endothelium, as in keratoconus, dystrophies, or trauma, deep anterior lamellar keratoplasty (DALK)¹⁶ or small transplants may be advantageous over transplants with a large diameter, in that more recipient endothelium would be preserved.

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References


Author Response: Donor Cell Survival in Corneal Grafts

We welcome the comments regarding our two recent articles¹,² detailing our observations of donor and recipient endothelial cell populations in the human corneal graft and are pleased to have the opportunity to further discuss and clarify our reported findings.

In both studies, we used fluorescent in situ hybridization (FISH) of the sex chromosomes in sex-mismatched corneal grafts, a mode of investigation pioneered by Wollensak et al.³,⁴ In the first such study published by that group in 1999, FISH analysis of paraffin-embedded sections from 14 corneal buttons revealed the persistence of a proportion of donor keratocytes (15%–26%) for at least 4.5 years, whereas no donor endothelial cells were detected in any sample.⁵ The authors acknowledged an individual variabili-