Stem-Cell “Hype” in Tracheal Transplantation?

Composite tissue allotransplantation (CTA) involves the transplantation of various tissues as opposed to a single separate organ in conventional transplantation; CTA is a new therapeutic modality to reconstruct major tissue defects of the face and upper extremities (1, 2). Restoration of the blood supply and the administration of immunosuppressive drugs to prevent rejection are essential in CTA.

The trachea poses a special challenge in CTA because of difficulties in restoring the blood supply; therefore, the trachea has been transplanted repeatedly as an avascular graft. Unfortunately, allotransplantation of an avascular trachea in an immunosuppressed recipient will inevitably lead to necrosis of the transplant. Orthotopic revascularization of the trachea, in which the trachea is wrapped with well-vascularized tissue of the recipient, is not possible because of the continuous movement of the transplant during breathing, swallowing, and coughing and the exposure of ischemic mucosal lining to the aggressive environment of the airway lumen (3).

Revascularization of the trachea can only take place in a heterotopic position, where an immobile trachea is surrounded by well-revascularized tissue of the immunosuppressed recipient (4, 5). As with all other CTAs, the trachea can be transplanted when the blood supply is restored in an immunosuppressed host (5). Vascularized tracheal allotransplantation was recently presented with long-term computed tomographic documentation of tracheal allograft viability (5).

An article published in *Lancet* in 2008, reporting on a tissue-engineered tracheal transplant, challenged our current knowledge of composite tissue transplantation (6). Recipient stem cells were used; therefore, immunosuppressive drugs could be avoided, and restoration of the blood supply was not necessary. The sequence of steps followed in creating and using the transplant is summarized in the Figure 1.

FIGURE 1. (A) The native tracheal blood vessels are too small to allow for revascularization by microvascular anastomosis between the donor and recipient blood vessels (1). Transplantation of an avascular allotransplant into an airway defect of an immunosuppressed host will lead to necrosis (2). Orthotopic revascularization attempts, in which the allotransplant is wrapped with vascularized recipient tissue, will fail (3). (B) Because of the various enzymatic treatments, the donor respiratory epithelial cells and chondrocytes are washed away (1). Repopulation of the cartilage rings occurs by bone marrow stem cells, and the lining is reestablished by cultured airway epithelial cells (2). The tissue-engineered construct is placed into an airway defect with a reportedly successful outcome (3).

An enzymatically decellularized donor trachea was suspended with recipient cells, and the recipient bone marrow stem cells reportedly colonized the cartilaginous rings. Cultured recipient airway epithelium was used to repopulate the epithelial tracheal lining. However, it is difficult to understand the cellular repopulation in which stem cells find their way through a cartilaginous matrix to the empty lacunae of the enzymatically removed chondrocytes, and epithelial cells adhere to an avascular, enzymatically treated base. It seemed that this tissue construct was fully repopulated by recipient cells, so that it could be transplanted without immunosuppressive medication. It is rather miraculous that this tissue-engineered

construct could be transplanted without restoration of the blood supply. This potential for regeneration in the absence of any blood supply is new and has never been observed in medicine before.

Unfortunately, clear evidence for the repopulation process and for the survival of the tissue-engineered trachea was lacking (7). To evaluate the degree of cellular repopulation, the authors would need to provide histologic evidence of the tracheal transplant after the enzymatic treatment and the repopulation process. Posttransplantation coronal and axial computed tomographic scans through the reconstructed airway could be helpful to follow-up the healing process of the tissue-engineered transplant.

Two years have elapsed since the initial publication, and no additional evidence has been provided so far. Recently, an article published in *BMJ* reported on a new case days after transplantation of a tissue-engineered transplant in a child with a tracheal defect (8). An editorial in *Transplantation* was published in March 2010 reporting on the same case initially published in the *Lancet* without new information and without new evidence of how the transplant survived within the main bronchial defect (9).

I would like to urge Macchiarini and coworkers to provide more evidence. The medical community needs to be informed objectively on this new development. If this construct works, it will revolutionize tissue engineering and CTA. The engineering of other hollow tissues and organs such as the esophagus, bowel, and larynx will quickly follow, as the authors suggested (10). However, based on our current knowledge of wound healing and CTA, there is also a possibility that the avascular tissue-engineered transplant underwent necrosis, and the bronchial defect healed by

secondary intention and wound contraction induced by the surrounding vascular bed. Data on wound healing of the tissue-engineered transplant are urgently warranted to distinguish between survival and necrosis of the tissue-engineered construct within the airway defect.

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Stem-Cell “Hype” in Tracheal Transplantation? A Response

We thank Delaere et al. (1) and congratulate them on their contribution to tracheal replacement. Delaere (2) pleads for more evidence and suggests that our grafts necrose and heal by secondary intent. We agree with the former and deny the latter. Although our clinical results to date show promise, a much “reverse translational” science is required to understand the mechanisms by which regenerative techniques result in “in situ” regeneration. We are working with scientists in appropriate fields, including scaffold, stem-cell biology, nanotechnology, and angiogenesis. Naturally, these studies are time consuming, and we cannot present results until the data are mature. However, relevant studies are reported in other tissues and species.

Essential point of Delaere is that successful tracheal replacement can only succeed if there is restoration of vascular supply. He is correct. However, he addresses this only at the macroscopic level: nowhere does he refer to angiogenesis or, more pertinently, arteriogenesis (3). He states that revascularization can only take place in a heterotopic position

because of movement of the graft if placed orthotopically and the “aggressive environment of the airway lumen,” but without explaining in what way the lumen might be “aggressive.” In our pig and human (one published and two undergoing follow-up) experience, revascularization occurred orthotopically within weeks with direct revascularization (4) or by local regeneration (5–8). The trachea is an excellent target for this approach as chondrocytes require low oxygen tensions (which may also direct stem-cell differentiation), while epithelial cells are in direct continuity with air (9). Thus, a wait of some days before the first microvessels arrive (as published in Refs. 5,9), is tolerable: it is not “miraculous,” although pleasing, that this occurs. It is surprising to suggest that neovascularization has never been observed in medicine before because it is the norm in healing areas of where appropriate signals exist (10). The question is rather how efficient such blood supply becomes (3) and how best we can optimize this for tissue engineered grafts (10).

Delaere pleads for long-term follow-up and computed tomographic studies of the reconstructed airways. We reported our first adult (5) and child (article in preparation) early at 6 months and 6 days, respectively, not by choice: press leaks forced early announcements. We agree that long-term studies will be the final arbiter of the value of our approach: acknowledged successful tissue engineered bladder replacements of Atala et al.’s (11) also used autologous cells on a simple scaffold, but results were not published correctly for a mean of 46 months. The absence of further reports on our own patients reflects our similar belief in the importance of long-term follow-up, and not, as Delaere seeks to imply, a technical failure; all patients are well. We thank the author for highlighting the importance of our complete avoidance of immunosuppression in graft recipients. We observed no sign of rejection serologically or clinically in any patient grafted to date. A comparison of allograft and regenerative techniques is presented in Table 1.

TABLE 1. Comparison of allograft and regenerative medicine approaches with tracheal replacement

	Tracheal allotransplantation (1)	Regenerative medicine approach to tracheal replacement (4)
Donor	Requires matching; a 9-mo wait in reported case.	Only size match necessary; immediate pool of donors at short notice.
Graft	First placed heterotopically. Half underwent necrosis.	First placed orthotopically. No evidence of necrosis.
No. recipient operations	Two major operations, plus eight admissions for exploration of heterotopically placed tissue.	One major operation.
Immunosuppression	Required (weaned after 8 mo).	Not required at any time.
Follow-up	At 1 yr, patient "satisfied," normal lung function at 1 year.	At 6 mo, patient work full time. Normal lung function at 2 yr.
Length of trachea replaced (cm)	3.5 ^a	7

^a Up to 4cm is resectable with conventional techniques.

Delaere finds it "difficult to understand cellular repopulation," which he describes in mechanical terms. This misses the point. The key factor here is not complete replication of native tissues by exogenous cells but rather the use of such cells, especially undifferentiated mesenchymal stem cells, to stimulate in situ host regeneration by the supply of appropriate signals and recruitment of mobilized circulating and local stem cells. Further signals support angiogenesis and arteriolar genesis. Furthermore, unpublished proteomic studies of our airway scaffolds demonstrate the retention of key angiogenic and other signaling molecules: they are not "completely decellularised," but rather processed to remove those cellular elements that would normally initiate a rejection response. This observation opens a new, exciting field of study in itself and may explain the readiness with which applied epithelial cells adhere and migrate (6) (another of Delaere's questions). At least nine different proteins were identified, which are known to act in this way.

We warmly welcome debate on this topic. We agree wholeheartedly with Delaere when he states that "data on wound healing of the tissue engineered transplant are urgently warranted." This is the subject of our present investiga-

tions, with reverse translation proceeding simultaneously with, and feeding off, our emergent regenerative medicine clinical protocols.

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Short-Term Follow-Up of Radioembolization With Yttrium-90 Microspheres Before Liver Transplantation: New Perspectives in Advanced Hepatocellular Carcinoma

Liver transplantation is a well-established procedure in patients affected by hepatocellular carcinoma (HCC) within the Milan criteria (1) or the extended Milan criteria (2). However, orthotopic liver transplantation (OLT) is not considered for patients who exceed the extended Milan or Milan criteria (i.e., size of nodule >7 cm or vascular invasion) because of the high risk of recurrence. In these patients, OLT can only be planned after downstaging of the disease to within acceptable limits. Patients with HCC who are transplanted after downstaging have similar outcomes in terms of survival and recurrence-free survival as those who meet the accepted criteria at initial presentation (3). We report a case of advanced HCC with neoplastic portal vein thrombosis, which was downstaged after radioembolization with yttrium-90 (^{90}Y) microspheres and led to a successful liver transplantation.

In February 2007, a 62-year-old man with hepatitis C virus-related cirrhosis with a model for end-stage liver disease score of 10 was diagnosed with an unresectable advanced HCC in the right lobe (7.5-cm diameter) and an increased α -fetoprotein (AFP) level more

than 70,000 ng/mL. Macrovascular invasion of the right branch of the portal vein was confirmed by spiral computed tomography (CT) (Fig. 1) imaging. The clinical case was discussed by a multidisciplinary panel, and it was concluded that the patient was unsuitable for OLT because of the size of the tumor and vascular invasion.

In May 2007, the patient received whole-liver treatment with an intraarterial infusion of ^{90}Y resin microspheres (SIR-Spheres; Sirtex Medical Ltd., Sydney, Australia). The radioembolization activity administered was 1702 MBq. The treatment was well tolerated. Follow-up 1 month later revealed a positive tumor response. AFP level at 2 months after treatment was 15 ng/mL. CT imaging at 3 months postradioembolisation documented a complete radiologic response with total regression of the primary lesion and portal thrombus.

The patient was then regularly seen in the outpatient clinic for 22 months after treatment, during which time, no other lesions developed in the liver. In February 2009, the patient was reassessed and considered to be a suitable candidate for OLT. Transplant assessment performed by positron emis-

sion tomography, CT, and bone scans did not reveal new HCC lesions within and outside the liver. In March 2009, the patient was placed on the transplant waiting list. While waiting for transplantation, a CT scan showed a suspected hypervascularized lesion at the site of the previously treated lesion, measuring less than 1 cm. In May 2009, a second segmental-liver treatment with ^{90}Y resin microspheres was performed (1665 MBq). The patient developed liver dysfunction (ascites and cholestasis with bilirubin that peaked at 7 mg/dL), which resolved after 2 months of conservative treatment. In November 2009, the patient was transplanted successfully by the piggy-back technique without veno-venous bypass. Transplant surgery was unexpectedly more difficult than normal because of the presence of fibrous tissue between the inferior vena cava and the caudate lobe where the HCC was located probably due to the radioembolization and resulting edema. The postoperative course was uneventful, and the patient was discharged on day 14 on tacrolimus (Prograf; Astellas Pharma, Europe, Staines, UK) and prednisone. Pathologic examination of the explanted liver showed two 5-mm HCC nodules surrounded by the fibrous tissue. The lymph nodes were negative, and no portal vein thrombus was found. Eight months after OLT, the patient has no signs of HCC recurrence as showed by a CT scan, and the last AFP level was 10 ng/mL.

In the past 10 years, different techniques for downstaging HCC have been evaluated with encouraging results. However, radioembolization using intraarterial ^{90}Y -labeled microspheres has proved to be one of the most effective techniques. Approved in Europe for the treatment of unresectable liver tumors, radioembolization seems to be a safe and possibly more effective technique than transarterial chemoembolization for the downstaging of HCC (4, 5). The ^{90}Y -bound microspheres remain confined to the vascular rim of the tumor, where they deliver a high (>120 Gy) but localized

FIGURE 1. The computed tomography scan shows an hypervascular area corresponding to hepatocellular carcinoma with neoplastic right portal vein thrombosis before yttrium-90 microspheres treatment.

dose of β -radiation to the tumor compartment (6). The limited tissue penetration of β -radiation (mean 2.5 mm) (6) means that most of the normal liver tissue is spared (7, 8). Radioembolization can be used in the liver transplant setting (a) as a bridge to transplant (controlling tumor progression for those on the waiting list) or (b) for the downstaging of tumors and resolution of vascular invasion, thereby enabling access to the organ waiting list for those outside the conventional criteria for organ transplant.

In conclusion, radioembolization is an effective treatment of patients with unresectable HCC, allowing downstaging to OLT in selected cases. Although the post-OLT follow-up period is still too short to draw any definitive conclusions, we would encourage liver and transplant surgeons to share our experience and consider radioembolization as an alternative treatment of unresectable advanced and intermediate stage HCC.

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