

Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocompos...



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Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study

Describe some important laboratory and clinical work that supported this proof-of-concept study and challenges faced.

Tumours which emerge in the trachea can be surgically resected however it has always been a challenge treating inoperable tumours found at the time of diagnosis. It is therefore, essential for new therapeutic approaches to be identified, especially for complex malignancies of the airway. For patients who present poor prognosis, the outcome of the treatment would be greatly improved if a substitute for the trachea was to be designed, consisting of similar biomechanical features and physiological properties to the patient's trachea. Jungebluth *et al.* (2011) reported the clinical transplantation of the tracheobronchial airway with a stem-cell-seeded bioartificial nanocomposite on a patient who presented primary recurrent cancer of the distal trachea and main bronchi. Following complete removal of the tumour, the airway was replaced with a tailored bioartificial nanocomposite which had been previously seeded *ex vivo* with autologous bone-marrow mononuclear cells.

As part of the pre-transplantation preparation, they firstly constructed an artificial trachea. Using a method called extrusion phase-inversion, fabricated by Ahmed *et al.* (2011) they produced a nanocomposite polymer (POSS-PCU), using pre-operative chest CT and three dimensional volume rendered images. The reason why their study was important for Jungebluth's work was because, they manufactured several and different vascular grafts using a method named as, automated extrusion phase-inversion, with the

aid of POSS-PCU polymer. They demonstrated versatility, simplicity and reproducibility of this production method, and revealed that if this method was carried out to produce tracheobronchial grafts, it would lead to major advantages in treatment strategies for patients presenting aggressive tumours of the trachea. It was also helpful as appropriate biocompatibility is essential for transplantation. Ahmed and group used controlled concentrations of NaHCO_3 in order to explore a highly defined, interconnected pore structure and to meet clinical standards and grafts were constructed with the combination of porogen leaching with a phase inversion coagulation technique. Their study revealed that porosities of the graft were dependent of the concentration of NaHCO_3 used. Subsequently, it became evident that, by using this method, grafts with a range of internal diameters, wall thicknesses and lengths was possible to be manufactured in a reproducible manner. Two important features which must be incorporated into grafts are, they must be non-thrombogenic in order to avoid poor patency of the graft and they must also be non-immunogenic. In order for artificial grafts to be function successfully, they should be able to enable an adequate healing response and retain the appropriate mechanical properties such as biological compliance and the tolerance to withstand long-term haemodynamic stress.

Furthermore, Jungebluth and group, used previously gathered knowledge, regarding the physical and mechanical properties of human trachea and the patient's preoperative CT scan and subsequently used this information to develop a POSS-PCU nano-composite polymeric airway of an appropriate size and morphology, reproducing the exact dimensions of the patient's

tracheobronchial structure. Baiguera *et al.* (2010), for the first time, completely described and characterised the obtainment of human tracheal bioactive supports. A suitable tracheal substitute for replacement of the airway not only must be structurally specific but must also portray morphological characteristics and retain some mechanical properties of the normal trachea. They demonstrated ischemia as a result of poor vascularisation is a primary risk factor of airway transplantation, a graft that encourages quick and efficient angiogenesis is critical in the restoration of the normal function of the trachea.

Prior to the above study in 2010, Meezan *et al.* (1975) and Yang *et al.* (2009) showed that the detergent-enzymatic method (DEM), appears to be a superior decellularisation approach than other methods, as it achieves a complete decellularisation while preserving tissue matrix integrity and biomechanical properties. Therefore, Baiguera *et al.* (2010) explored a range of different DEM cycles (starting from 17 cycles), before identifying the suitable number of cycles for obtaining a suitable human acellular tracheal scaffold. Upon completing the investigation, they demonstrated that 25 DEM cycles generated an almost complete decellularised human trachea matrix. The histo-architecture of the decellularised trachea was retained, demonstrating that, during the 25 cycles of the decellularisation process, DEM had protected and conserved the three-dimensional structure of the ECM. In agreement to these findings, Macchiarini *et al.* (2008) and Asnaghi *et al.* (2009) reported that human tracheal matrices, decellularised upon using the DEM approach, support mesenchymal stem-cell-derived chondrocytes and epithelial cell adhesion and proliferation, and represent an ideal

environment for cells. Therefore, from this study, it became evident that 25 DEM cycles are the optimum point to obtain suitable, non-immunogenic human tracheal substitute with specific mechanical properties, which was appropriately reflected in this work by Jungebluth and group.

In addition, they also developed a bioreactor that could satisfy the maturation requirements of the Y-shaped synthetic construct for the transplantation process. This design was based on a sterilisable rotating-construct bioreactor, previously validated but with novel elements to drive a recirculating fluid flow within and around the developing graft, enabling consistent and uniform delivery of cells, nutrients, gases, and hydrodynamic shear forces within the bioreactor (Go *et al.* , 2010). In this study, two cell types, epithelial cells and mesenchymal stem cell derived cells, were seeded simultaneously on pre-engineered matrices by using a bioreactor with a dual-chamber. This meant that the device provided continuous rotation of the graft, which exposed the cells to a covering film of alternating gas and liquid phases. One of the significant reasons why this study supported Jungebluth's work very effectively was because, it confirmed the suitability of the decellularisation, cell preparation, and efficacy of the techniques used for re-seeding, as well as the simplicity of tracheal bioreactor mechanism. In agreement to these previously acknowledged concepts, Jungebluth's results showed a selective reduction of MSCs and haemopoietic stem cells (HSCs) after the reseeded process, with a particular decrease in CD90 high and CD59 dim cells, using flow cytometric phenotyping of the bioreactor medium. This suggested that they were efficiently attaching to the scaffold and engrafted to it successfully. Thus, it was evident that, the graft and the re-

seeded cells functioned together harmoniously, to establish a successful environment of artificial trachea ready for transplantation.

Autologous bone marrow derived mononuclear cells were used for seeding of the graft, which meant that they were harvested from the patient undergoing the transplant. This was done by a process called density gradient separation and also sterility was ensured. The reason why they decided to use autologous MNCs was because previously, Go *et al.* (2010), has shown that both epithelial cells and mesenchymal stem cell-derived chondrocytes contribute to the survival and persistence of tissue-engineered airway transplants in pigs. By showing that seeding with these cells is essential for optimal survival of the grafts, they established the clinical potential of autologous cells and tissue engineered tracheal grafts.

More importantly, their results also clearly presented that, animals did not develop any rejection responses, even without immunosuppression medication. Therefore a similar principle and approach was incorporated into Jungebluth's work. Bone marrow harvests from the patient, were obtained twice, initially 2 days before transplantation and secondly, just before the transplantation took place. The first harvest was used to seed the synthetic graft before incubation in the bioreactor. The second harvest was used to re-seed the airway construct before transplantation and stem cells from MNCs were induced by conditioning the construct with growth factors; recombinant human transforming growth factor- β 3, granulocyte colony stimulating factor filgrastim and epoetin beta. Upon completion of tumour resection, using standard surgical techniques, the airway was then reconstructed by

implanting the reseeded nanocomposite from end-to-end; first to the right, then the left main bronchi and finally to the proximal trachea.

Previous studies in this field have shown that one of the biggest limitations in this type of transplantation has been, the length of time consumed for the decellularisation process, which is almost 15-20 days. Other limitations include the need for different sizes, as this differs with various individuals and finally the compulsory requirement of an organ from a donor, which can take a long time. A study by Mel *et al.* (2009) aimed to firstly, biofunctionalise a nanocomposite biomaterial, Polyhedral Oligomeric silsesquioxane modified polycarbonate urea-urethane (POSS-PCU), based small diameter vascular graft and secondly, to induce endothelialisation with EPC containing monocytes, which were extracted from peripheral blood. SEM was used to confirm endothelialisation and endothelial cell markers, CD34, CD31 and eNOS were detected by immunostaining. They demonstrated that endothelialisation from cells extracted from the peripheral blood, is rapid with a biofunctionalised nanocomposite polymer-based small diameter bypass graft.

This study had a significant impact on Jungebluth's group's decision to use an artificial POSS-PCU based nanocomposite material because, the quick endothelialisation of cells from their study indicated that this material could be produced rapidly and in a clinically appropriate timeframe. Another reason why they chose to use this material was to ensure biocompatibility, non-toxicity, and more importantly, a material that is inert, which means that immunoreactivity is avoided or is present at a negligible level. Other reasons for their decision to use a bioartificial nanocomposite, was because it has in-
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vivo stability. Seifalian *et al.* (2003) very importantly showed that, no signs of gross infection and in particular inflammation of the grafts or surrounding areas were evident in their study using a poly urethane graft. No infection or inflammation of the grafts or surrounding tissues could be detected. Also, macroscopically there were no indications of material curvature, radial expansion or chemical breakdown. These evidences clearly authenticate the long lasting biostability that is achieved when using poly (carbonate-urea) urethane (PCU) grafts.

A few challenges were faced by Jungebluth and team whilst carrying out this study. Two days after transplantation, the patient was diagnosed with pneumonia in the right upper lobe. Secondly, biopsy samples from bronchoscopy taken 1 week after surgery showed, necrotic tissue which was accompanied by fungi contamination. When inflammation takes place, there is a release of trauma cytokines which subsequently upregulate epoetin receptors, but tissue protection is inhibited by reduced epoetin production and antiapoptotic downstream pathways which favour cell apoptosis (Jungebluth *et al.* 2011). Therefore to avoid early postoperative absence of local epoetin production, they administered regenerative (500 UI/Kg) epoetin doses for early local tissue protection and regeneration to be triggered. They were also faced with shortage of material which prevented them from analysing cell division, however some of the observed cells were found to be dividing and cells which were exposed to the bioreactor aggregated in dense clusters, which indicated that clonal expansion took place.

Recruitment of repair cells for integration and remodelling of the newly

transplanted material, is an important aspect of synthetic transplantation
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setting. Cells contributing to this process can be employed either the local tissue or from circulating progenitors. Jungebluth *et al.* (2011) observed HSC mobilisation together with increased amounts of circulating MSCs. However, these were contrasting with previous findings who have shown no mobilisation of MSCs, when G-CSF was used alone as a mobilising agent. However, it was believed that, the reason for the observed MSC mobilisation in their patient may have been due to inflammation from surgery and chemokine and anaphylatoxin release at the site of implantation. Nevertheless, this aspect can be explored further in future transplantations.

Their involvement in developing this procedure has been significant in transplantation approaches for successful tracheobronchial replacement. Previous attempts to replace the airways of patients with tracheal cancer with synthetic materials have been unsuccessful because of poor seeding of the graft, necrosis, infection and ultimately death of the patients. It has been deduced, that these drawbacks are clearly related to the fact that the trachea is not located in a mesenchymal environment and direct exposure to air that is inhaled increases the chances of contamination and infection. Thus, in this study they have shown that these factors can be avoided by using a bioreactor environment to reseed the bioartificial scaffold with autologous mononuclear cells. Therefore, results from this study provide clear evidence that a successful organ regeneration strategy has been achieved and through this approach they have shown, scaffolds constructed in this manner, can be used to replace airways that has been affected with complex defects.

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References

- Ahmed, M., Ghanbari, H., Cousins, B. G., Hamilton, G. and Seifalian, A. M. (2011) ' Small calibre polyhedral oligomeric silsesquioxane nanocomposite cardiovascular grafts: influence of porosity on the structure, haemocompatibility and mechanical properties', *Acta Biomater* , 7(11), pp. 3857-67.
- Asnaghi, M. A., Jungebluth, P., Raimondi, M. T., Dickinson, S. C., Rees, L. E., Go, T., Cogan, T. A., Dodson, A., Parnigotto, P. P., Hollander, A. P., Birchall, M. A., Conconi, M. T., Macchiarini, P. and Mantero, S. (2009) ' A double-chamber rotating bioreactor for the development of tissue-engineered hollow organs: from concept to clinical trial', *Biomaterials* , 30(29), pp. 5260-9.
- Baiguera, S., Jungebluth, P., Burns, A., Mavilia, C., Haag, J., De Coppi, P. and Macchiarini, P. (2010) ' Tissue engineered human tracheas for in vivo implantation', *Biomaterials* , 31(34), pp. 8931-8.
- de Mel, A., Punshon, G., Ramesh, B., Sarkar, S., Darbyshire, A., Hamilton, G. and Seifalian, A. M. (2009) ' In situ endothelialization potential of a biofunctionalised nanocomposite biomaterial-based small diameter bypass graft', *Biomed Mater Eng* , 19(4-5), pp. 317-31.
- Go, T., Jungebluth, P., Baiguero, S., Asnaghi, A., Martorell, J., Ostertag, H., Mantero, S., Birchall, M., Bader, A. and Macchiarini, P. (2010) ' Both epithelial cells and mesenchymal stem cell-derived chondrocytes contribute to the survival of tissue-engineered airway transplants in pigs', *J Thorac Cardiovasc Surg* , 139(2), pp. 437-43.
- Jungebluth, P., Alici, E., Baiguera, S., Le Blanc, K., Blomberg, P.,

Bozoky, B., Crowley, C., Einarsson, O., Grinnemo, K. H., Gudbjartsson, <https://assignbuster.com/tracheobronchial-transplantation-with-a-stem-cell-seeded-bioartificial-nanocomposite/>

T., Le Guyader, S., Henriksson, G., Hermanson, O., Juto, J. E., Leidner, B., Lilja, T., Liska, J., Luedde, T., Lundin, V., Moll, G., Nilsson, B., Roderburg, C., Stromblad, S., Sutlu, T., Teixeira, A. I., Watz, E., Seifalian, A. and Macchiarini, P. (2011) ' Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study', *Lancet* , 378(9808), pp. 1997-2004.

- Macchiarini, P., Jungebluth, P., Go, T., Asnaghi, M. A., Rees, L. E., Cogan, T. A., Dodson, A., Martorell, J., Bellini, S., Parnigotto, P. P., Dickinson, S. C., Hollander, A. P., Mantero, S., Conconi, M. T. and Birchall, M. A. (2008) ' Clinical transplantation of a tissue-engineered airway', *Lancet* , 372(9655), pp. 2023-30.
- Meezan, E., Hjelle, J. T., Brendel, K. and Carlson, E. C. (1975) ' A simple, versatile, nondisruptive method for the isolation of morphologically and chemically pure basement membranes from several tissues', *Life Sci* , 17(11), pp. 1721-32.
- Seifalian, A. M., Salacinski, H. J., Tiwari, A., Edwards, A., Bowald, S. and Hamilton, G. (2003) ' In vivo biostability of a poly(carbonate-urea)urethane graft', *Biomaterials* , 24(14), pp. 2549-57.
- Yang, M., Chen, C. Z., Wang, X. N., Zhu, Y. B. and Gu, Y. J. (2009) ' Favorable effects of the detergent and enzyme extraction method for preparing decellularized bovine pericardium scaffold for tissue engineered heart valves', *J Biomed Mater Res B Appl Biomater* , 91(1), pp. 354-61.