

REGENERATIVE MEDICINE FOR CONGENITAL MALFORMATION: NEW OPPORTUNITIES FOR THERAPY

De Coppi P

Reader and Consultant Paediatric Surgeon, Head of Surgery Unit at UCL Institute of Child Health and Great Ormond Street Hospital, London, UK

Corresponding Author: Paolo De Coppi, MD, Ph.D; e-mail: Paolo.DeCoppi@gosh.nhs.uk

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ABSTRACT

Congenital malformations are major causes of disease and death during the first years of life and most of the time functional replacement of the missing or damaged organ (or tissue) remains an unmet clinical need. It is estimated that congenital diseases are responsible for over a third of all pediatric hospital admissions, and for up to 50% of the total cost of pediatric hospital treatment. This is likely to have an influence into adult care as well since affected patients can now survive to adulthood requiring continued therapy. With advancement of prenatal diagnosis, pregnant women received details information regarding the condition affecting their fetus. However, options are usually termination of pregnancy, or delivery of an affected baby. Prenatal therapies adopting regenerative medicine options such as stem cell and gene therapy or tissue engineering could however change in a radical way the outcome of those fetuses. Tissue and organ regeneration will change the treatment of the congenital diseases, as we know it now. It will be particularly interesting to observe the evolution of surgical approaches to congenital malformation and the totally new possibility of correcting them using autologous tailored-made functional tissues.

INTRODUCTION

Regenerative medicine combines tissue engineering and cell transplantation for the replacement or repair of damaged tissues and organs¹. While most attention has focused on degenerative diseases such as cardiac failure, Parkinson's disease or Alzheimer's disease, very little has been achieved in the treatment

of congenital conditions². However, advancement in stem cell biology and material science have lead to new ways of repairing or replacing injured organs which ultimately could have a major impact in the treatment of congenital malformation³.

Congenital malformations are major causes of disease and death during the first years of life and most of the time functional replacement of the missing or damaged organ (or tissue) remains an unmet clinical need. Many congenital diseases have a relatively low prevalence, but collectively they represent a large burden of disease⁴. It is estimated that congenital diseases are responsible for over a third of all pediatric hospital admissions, and for up to 50% of the total cost of pediatric hospital treatment⁴. This is likely to have an influence into adult care as well since affected patients can now survive to adulthood requiring continued therapy. With advancement of prenatal diagnosis, pregnant women received details information regarding the condition affecting their fetus. However, options are usually termination of pregnancy, or delivery of an affected baby. Prenatal therapies adopting regenerative medicine options such as stem cell and gene therapy or tissue engineering could however change in a radical way the outcome of those fetuses.

There are indeed several reasons to support regenerative medicine in the early phases of development. *In utero* therapies could: 1) prevent disease damage before birth since some life-threatening congenital diseases have their onset during fetal life, so that irreversible organ damage has already occurred when the baby is born; 2) target more efficiently stem cells which are more numerous in the fetus, rapidly expand, and migrate in different niche during gestation; 3) be more efficient in lower number because of the small size of fetus and its immunological immaturity. Additionally, for structural congenital abnormalities, repair *in utero* may pro-

vide a more efficient surgical outcome because of more efficient wound healing and remodelling. In utero correction could also interfere with the pathological process as recently demonstrated for the *in utero* treatment of spina bifida⁵. Some of the benefits discussed above can also be taken in consideration for neonatal treatments. Most of congenital structural abnormalities do not necessarily need to be treated before birth but certainly right after birth. Cardiac circulation for example change completely at birth and therefore interventricular defects which are well tolerated by the fetus because of the absence of pulmonary circulation can dramatically affect the neonate and prosthesis are necessary to cover the defect and allow the separation of the pulmonary from the systemic circulation. A functional cardiac patch engineered during the gestation and transplanted soon after birth would be ideal for the treatment of some of these cardiac conditions. This would require the harvesting during the gestation of cell capable to efficiently differentiated towards specialized phenotype such as myogenic, endothe-

lial, neuronal. These cells could therefore be potentially stored and used later on or used when a malformation has been identified at the routine scans.

AMNIOTIC FLUID STEM CELLS

This is now a concrete option since it is now possible to derive multi and pluripotent stem cells from the amniotic fluid. Amniotic fluid stem cells (AFSC) can be derived from routine amniocentesis, amnioreduction or at birth during caesarean section⁶. Sampling of amniotic fluid (AF) is ideal for prenatal/neonatal applications with the advantages of being (i) relatively easy to perform; (ii) at low risk both for the mother and the foetus; (iii) widely accept method for prenatal diagnosis. Amniocentesis is a safe procedure with a miscarriage rate as low as 1 in 769, which usually takes place from 15 weeks of gestation⁷⁻⁸, while amnioreduction is usually performed later in gestation to reduce the amniotic fluid in case of polydramios, which can be associated to some congenital malformations such as oesophageal, duodenal or other intestinal atresias. AFS cells have intermediate characteristics between embryonic and adult stem cells⁹⁻¹¹. They

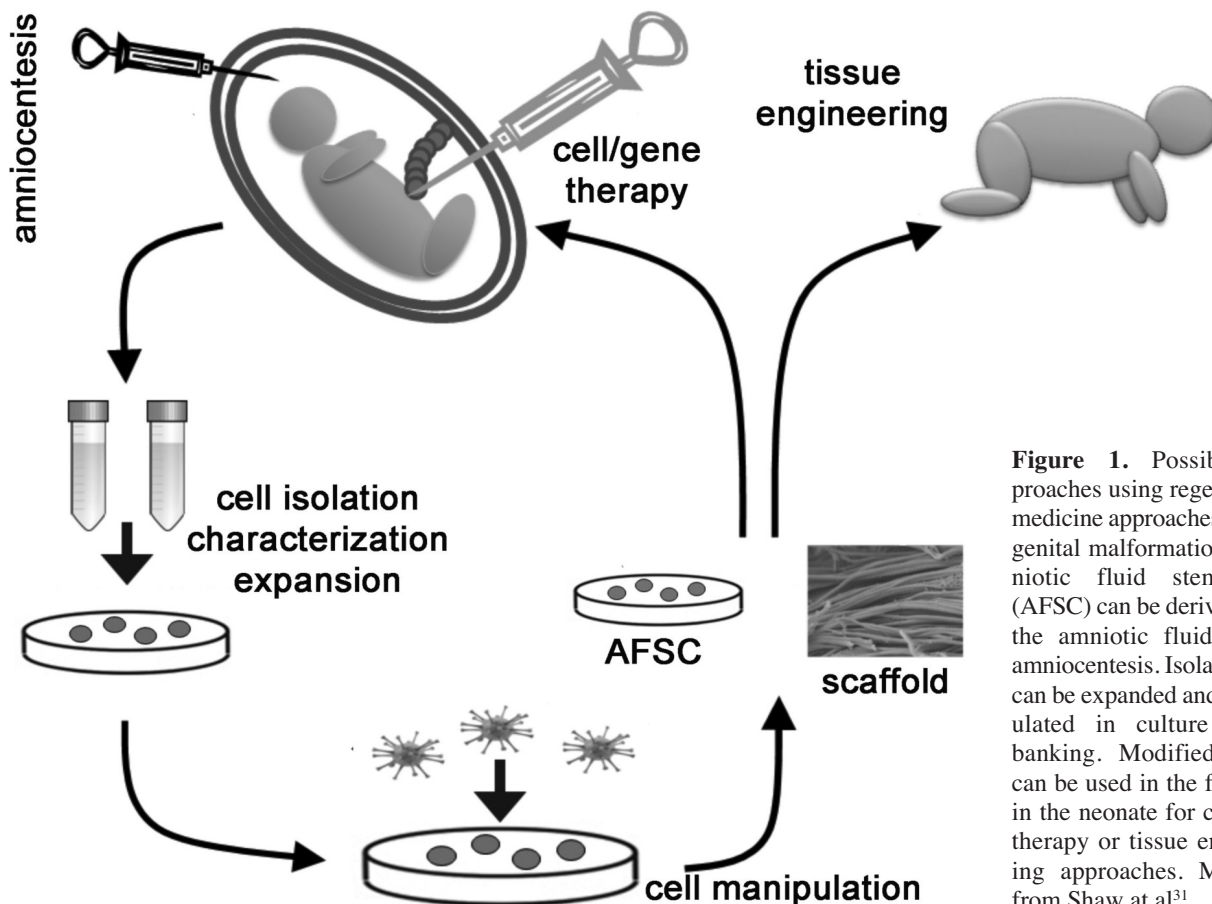


Figure 1. Possible approaches using regenerative medicine approaches to congenital malformations. Amniotic fluid stem cells (AFSC) can be derived from the amniotic fluid during amniocentesis. Isolated cells can be expanded and manipulated in culture before banking. Modified AFSC can be used in the foetus or in the neonate for cell/gene therapy or tissue engineering approaches. Modified from Shaw et al³¹.

displayed a multilineage hematopoietic potential¹² and they can be easily reprogrammed to a pluripotent status¹³. Human AFS cells have an estimated doubling time of 36 hours and maintain their potential up to 250 passages without telomerase length change when expanded in culture without feeder layers. However they did not show the teratoma formation, which remains the main concern and ethical issue in embryonic stem cells⁹. Moreover it has been shown that AFSC can be easily transduced without losing their potential in human, mice and sheep¹⁴⁻¹⁵. The surface antigen c-Kit (CD117) is known to be the receptor of stem cell factor and to play an essential role in gametogenesis, melanogenesis and hematopoiesis¹⁶. C-Kit positive cells are isolated by immunoselection using magnetic microspheres and then cultured⁹. Human AFS cells express surface markers of mesenchymal and/or neural stem cells origin, stage-specific embryonic antigen (SSEA)-4, a marker that is usually present in ES cells and Oct-4, a transcription factor⁹. Once cultured in adherence however, they do not however express markers of hemopoietic lineage such as CD45, CD34, and CD133 while they do express CD29, CD44, CD73, CD90 and CD105⁹. In addition AFSC, similarly to other fetal cells may represent the ideal source for therapy because, similarly to ES cells, they are still plastic and easy to expand, and, in common with the adult counterpart, they are less controversial, they are not tumorigenic and their use can be accomplished on an autologous setting². The latter is particularly important in neonatal surgery, in the context of congenital malformations. Surgical treatment of congenital anomalies is often complicated by insufficient available tissue at time of repair. Artificial materials are regularly the only option for reconstruction in those cases, with high morbidity rates².

SCAFFOLDS FOR TISSUE ENGINEERING

While the cellular role is pivotal, in order to give rise to a new functional organ-like structure, it is crucial the provision of a three-dimensional growth structure termed "scaffold"³. Scaffolds are usually made by natural materials, which are essentially bioactive but lack mechanical strength, or synthetic materials, which lack inherent bioactivity but are mechanically strong and can be engineered with the desirable macro- and microstructure, and might possess desired bioactive properties to make possible cellular growth and organogenesis¹⁷. Although scaffolds could ultimately represent the exclusive tool for tissue engineering and several attempts to generate

whole organs, such as liver, have been done by developing structures with vascular channels to ensure an adequate network of vascular supply², major developments in the clinical scenario have been achieved in the last few years using relative simple scaffolds. In 2006 it was reported a series of 7 patients with myelomeningocele, aged 4-19 years, which successfully received an engineered bladder tissues¹⁸. After undergoing a bladder biopsy, urothelial and muscle cells were grown separately and subsequently seeded on a biodegradable bladder-shaped scaffold made of a composite of collagen and polyglycolic acid. The autologous engineered bladder were transplanted about 7 weeks after explant and wrapped in omentum¹⁸. In 2011 the same group described also a similar experience with 5 boys who, following trauma, underwent reconstruction using engineered urethras seeded with autologous cells which remain functional in a clinical setting for up to 6 years¹⁹. In parallel, following the successful decellularization of trachea in pigs, the first patient received in 2008 an engineered bronchus prepared using a cadaveric decellularised trachea seeded with the patient own bone marrow and epithelial stem cells. The engineered airway was successful transplanted in a 30-year old woman to substitute the narrow main left bronchus²⁰. Using a similar approach, we have recently reported the successful 2 years follow up of the first child who received a stem-cell-based trachea²¹. Intriguing, the trachea engrafted perfectly in the child maintaining its lumen even if it was transplanted right after seeding without bioreactor conditioning. Both systemic granulocyte colony stimulating factor and human recombinant erythropoietin, associated with topical human recombinant erythropoietin and transforming growth factor β were used to stimulate cellular mobilization, engraftment, growth and differentiation respectively²¹. More recently, a 10 year old girl with extra hepatic portal vein obstruction was transplanted with a decellularised donor iliac vein seeded with endothelial and smooth muscle cells differentiated from bone marrow²². The graft was initially successful but it was substituted after 1 year by a second tissue-engineered construct, which restored portal circulation. Decellularised tissue may in the future be utilized to engineer also for more complex organs, but this will require more time before being applied for therapy. Perfusion decellularization techniques for the liver and kidney generated scaffolds which maintained many characteristics of the original organ²³⁻²⁴. Rat decellularised livers have also been recellularized using

rat primary hepatocytes, showing promising hepatic function and the ability to heterotopically transplant these bioengineered livers into animals for up to 8 h²⁵. The decellularization approach was pioneered in the heart by Ott et al. which showed that was possible not only to generate a whole organ scaffolds using a perfusion decellularization system, but also that neonatal rat cardiomyocytes (delivered through transmural injection) and endothelial cells (injected through the aorta) could generate a contractile construct²⁶. Using a similar approach lungs have also been regenerated through the seeding of pulmonary epithelium and vascular endothelium on rat lung ECM²⁷. Among the unmet clinical needs, intestine engineered is particularly relevant in children with short bowel, which have a particularly poor outcome of intestinal transplantation. So far however ideal scaffolds for the intestine have not been available and only recently successful decellularization of the intestine was obtained²⁸. It is possible that in the future, intestinal stem cells, which reside in the base of Lieberkuhn crypts and express *Lgr5* could be used to engineered new intestinal units²⁹⁻³⁰.

CONCLUSIONS

We are living a very exciting time for the treatment of congenital malformation using tools offered by regenerative medicine. Tissue and organ regeneration will change the treatment of the congenital diseases, as we know it now. It will be particularly interesting to observe the evolution of surgical approaches to congenital malformation and the totally new possibility of correcting them using autologous tailored-made functional tissue. This will be translated in the near future to the patients, possibly using stem cells derived from the fetus and decellularised tissues which can mimic the extracellular matrix of the original organ².

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