CREATION OF BIOARTIFICIAL ORGANS

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Abstract:

Regenerative and reparatory medicine is one of the most attractive therapeutic options of the coming years, and the power to replace entire damaged organic structures or organs is, in our opinion, the most innovative possibility in this field.

In relation to the creation of bioartificial organs in particular, hearts, lungs, livers, kidneys, ovaries, intestines, pancreas and corneas have already been generated, all in the experimental field. However the most innovative development is, undoubtedly, that these experiments have already reached the clinical field, since in two patients with tracheal stenosis due to different causes, it has been possible to replace the affected trachea with a new one created bioartificially; it was shown that after transplant of the new organ, both patients recovered their respiratory capacity and were consequently able to resume their normal life.

Summary:

Regenerative and reparatory medicine is one of the most attractive therapeutic options of the coming years, and the power to replace damaged organ structures or entire organs is, in our opinion, the most innovative possibility in this field.

In relation to the creation of bioartificial organs in particular, hearts, lungs, liver, kidneys, ovaries, intestines, pancreas and corneas have already been generated, all in the experimental field. However the most novel development is undoubtedly that these experiments have already reached the clinical field, after two patients with tracheal stenosis due to different causes were able to have the affected trachea replaced with a

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new one created bioartificially; it was shown that after transplant of the new organ, both patients recovered their respiratory capacity and were consequently able to resume their normal life.

Key words: bioartificial organs, bioartificial organic structures, tissue engineering, regenerative and reparatory medicine.

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Introduction

Regenerative and reparatory medicine is one of the most attractive therapeutic possibilities of the coming years. In this respect, the United States Department of Health and Human Services has published the statement "2020: A New Vision- A Future for Regenerative Medicine", in which it highlights that regenerative medicine may be the vanguard of twenty-first century health care[1]. Moreover, the National Health Institutes and various National Academies in the United States recognise that regenerative medicine holds the major therapeutic promise of modern medicine[2,3], in which cell therapy, tissue engineering and the creation of bio-organs are the main instruments for its application.

Two strategies can be used within cell therapy: a) to potentiate the regenerative capacity of the tissues themselves, by stimulating this capacity with various growth factors or b) to use stem cells to replace the damaged organs. Embryonic stem cells[4], adult stem cells[5] or iPS cells[6,7] can be used for this purpose.

The use of embryonic stem cells for therapeutic purposes has been very limited to date[8], and in fact at present there is only one authorised Phase I clinical trial[9], the objective of which is to treat patients with a recent spinal cord injury. Although it is intended to

enrol 10 to 12 patients in the trial, only one has been included so far[10,11]. For the time being, the main aim of the trial is not to assess the possible beneficial effects, but to discard negative side effects.

With respect to adult stem cells, there are more than 3000 ongoing clinical trials at present[12]. These data suggest that the use of adult stem cells is currently the most widely used means for attempting to develop effective cell therapy. However, it should be noted that up to now, the beneficial effects obtained have been modest, with most ongoing trials in the area of autoimmune and vascular diseases, particularly cardiac conditions[13].

The achievement of cell reprogramming[6] and obtaining of autologous iPS cells from humans[14] has opened up an encouraging therapeutic avenue[15]. Indeed preclinical experiments have been conducted in some pathologies, such as sickle cell anaemia[16], Parkinson's disease[17], haemophilia A[18] and myocardial infarction[19] using these types of cells.

Creation of tissues and organ constructs

In 1987, the US National Science Foundation defined tissue engineering as the use of principles and methods of biological engineering aimed at understanding normal and pathological tissue structures and the creation of bioartificial organs to maintain or improve damaged organs[20].

The initial attempts to obtain tissues began in the early nineties, obtaining skin, cartilage and vascular grafts[21,22,23].

In order to create functioning bioartificial organs which could be used to repair damaged parts of different tissues or organs, the first stage was the creation of various organ scaffolds, developing functioning three-dimensional constructs which worked in

an artificial medium, designed in accordance with the shape and size of the organ which might require them.

In this respect, various organ constructs have been obtained. The following have been described, although the list does not claim to be exhaustive: obtaining of urinary bladder tissue using allogenic bladder submucosa, subsequently recovered with cells[24]; arteries, obtained by decellularising native arteries which were then re-seeded to obtain useful organ constructs for transplant[25]; skin, created to be grafted in damaged epidermal areas[26]; aortic valves, also obtained by decellularising porcine aortic valves which were then re-seeded[27]; ureters, using a decellularised matrix subsequently re-seeded with uroepithelial cells and mononuclear cells from bone marrow[28]; oesophagus, likewise by re-seeding a previously decellularised oesophageal matrix[29] and also by decellularising dog oesophagi to later re-seed them and be able to use them in the consolidation of oesophageal anastomosis[30]; cornea, using a pig cornea after being re-seeded with keratocytes from the patient itself[31].

A further step in the production of organ constructs is the generation of biotubes or biovalves for transplant. In this respect, biotubes to replace parts of the damaged vascular tree[32, 33, 34, 35], as well as cardiac biovalves [36, 37, 38] have been developed. Sinus of Valsalva autologous valves have recently been created for the first time and then transplanted in dogs[39]. To obtain said objective, scientists developed a three-dimensional structure, using small silicon rods which mimic the architecture of the sinus of Valsalva. This artificial mould was then placed in the dorsal subcutaneous space of dogs and covered with autologous connective tissue, thus obtaining new biovalves which were then implanted in canine pulmonary arteries; the team were able to make the valves work properly, on observing that only minor regurgitation occurred.

Examination of the valves 84 days after the transplant showed that the surface was covered by endothelial cells and neointima.

The creation of cardiac tissue was also achieved, generated on extracellular matrices which were then covered with endothelial cells and activated with a growth factor. Once generated, they were transplanted to infarcted hearts, favouring the development of new vessels in the area adjacent to the infarct, which considerably improved the cardiac ischaemia area[40]. The experiments were conducted on 82 rats, in which an experimental infarction had been induced. The extracellular matrix used was a vitronectrin and collagen scaffold. After the vascular implant, the infarcted hearts of the rats showed neovasculogenesis, which helped to attenuate the negative organ consequences of the infarction and to improve ventricular function.

The creation of organ scaffolds has continued to progress relentlessly to the point where organ bioscaffolds of very different tissues have been obtained[41].

In relation to this, the same year PNAS dedicated an entire issue (volume 107) to the advances made in the field of tissue engineering. As Badylak and Neren[41] state, in the introduction to said issue, there has been a considerable advance in the production of organ tissues as diverse as skeletal muscle, bone, heart, blood vessels, cornea and nerves. In the aforementioned issue, five articles were published describing the use of stem cells in regenerative medicine, and six dedicated to the production of matrices upon which growth factors can be added to create, from them, functioning structures such as cartilage and spinal cord. However in our opinion, two articles related with the creation of bone tissue should be highlighted in the aforementioned PNAS issue.

In the first[42], a team from the Georgia Mechanical Engineering Institute in the United States managed to produce bone tissue using a polymer scaffold which was then re-seeded with stem cells. The bioartificial tissue created can be used to regenerate bone

mass which has been lost for any reason, and can replace or complement the use of bone grafts.

In preclinical studies, the bone structures created were used in bone fractures in rats which involved tissue loss, confirming their replacement. Adult mesenchymal stem cells from bone marrow and adult stem cells from foetal amniotic fluid were used as a source of stem cells.

In the second paper[43], the formation of new bone structures was induced in rats, *in vivo*, using microcapsules composed of poly-L-glutamic acid and poly-L-lysine, which transport specific growth factors that favour the regeneration of damaged bone.

Creation of bioartificial organs

Experimental studies

The transplant of organs (heart, lung, liver, kidney, etc.) continues to be the main therapeutic tool for treating serious degenerative diseases; however, the number of donated organs available is limited, so it appears necessary to continue developing other therapeutic options.

Of these, one of the most attractive is the creation of autologous bioartificial organs which can, partly or completely, replace the damaged organ.

In order to achieve this objective, the generic procedure used consists of decellularising donated organs and then recellularising or reseeding them, if possible with the patient's own stem cells.

Heart

The first attempt aimed at creating a bioartificial heart was made by Doris Taylor's group in 2008[44].

Prior to the experiments of Ott et al.[44], some attempts had already been made to construct a heart[20] artificially; however the objective was not fully achieved, since only contractile rings or cardiac tissue layers could be produced, which were subsequently transplanted in various types of animals[45-47]. In some cases, an improvement in ventricular function was obtained. However, as has been mentioned, it was Doris Taylor's group[44] who were first able to produce a fully functioning heart bioartificially, in this case a rat heart, and were then able to transplant it in another animal.

As the authors themselves state[44], in order to achieve their objective they had to solve three fundamental problems: to obtain a structure which could be used as a scaffold for the future heart, to reconstruct it cellularly and to make the new structure function as a cardiac pump.

To obtain the heart scaffold, they decellularised a rat heart using a detergent solution, thereby obtaining the desired heart scaffold, in which they preserved some of the cardiac structures such as blood vessels, pericardium and valves. Cellular reconstruction of the cardiac scaffold obtained was by intramural injection of cardiac cells, and by perfusion of endothelial cells into the residual blood vessels; they observed that after four days the new heart began to contract and after eight, following electrical stimulation, it worked as a cardiac pump, with an activity approximately equivalent to 2% of that of an adult heart and 25% of that of a 16-week rat foetal heart.

They also applied this technique to pig hearts, and were able to decellularise them, which demonstrated that the method could be used in hearts of a similar size and complexity to human hearts. They also managed to decellularise other organs, such as lungs, liver, kidneys and muscles. The first step towards obtaining bioartificial organs had been taken.

In a further step along the pathway of the clinical application of biohearts, the first laboratory in the world dedicated to the creation of these types of bioorgans was founded in Hospital Gregorio Marañón, Madrid. This project is being developed in association with Dr. Doris Taylor of the University of Minnesota and the Spanish National Transplant Organisation. The aim is to have heart matrices available which can be used for future transplants. The hearts will be obtained from discarded hypertrophic hearts from transplants. To date, it appears that nine decellularised human heart scaffolds have already been generated[48].

Lungs

Lung diseases are the cause of around 400,000 deaths per year in the United States. Furthermore, lung tissue is difficult to regenerate, so the only means of replacing damaged lung tissue is a lung transplant; however, the number of lungs available for this purpose is limited. Indeed, in 2005, only 1 in every 4 patients who were waiting for a lung in the United States got one[49]. Moreover, even if the transplant is consolidated, long-term success is not always achieved, especially due to rejection complications or to the adverse effects of immunosuppressant therapy[50], which means that only 10 to 20% of transplant patients survive more than 10 years[51]. For these reasons, the creation of autologous bioartificial lungs may be a promising solution for treating lung disease patients who require a transplant.

In this respect, a research team from Yale University[52] has taken the first step towards obtaining functioning lung tissue, having produced lung tissue which is capable of exchanging gases and consequently functioning like normal lungs, although previous rather unsuccessful attempts had been made[53].

The technique used by the Yale researchers to decellularise the rat heart was similar to the one used by Ott et al[44], but in this case using lungs from adult rats which were

decellularised until a matrix was obtained which conserved the alveolar microarchitecture and the pulmonary vascular system. The organ scaffold obtained was reconstituted with a mixture of epithelial and endothelial cells from neonatal lungs, observing that the lung tissue generated could exchange oxygen and carbon dioxide, as well as being able to oxygenate blood haemoglobin.

This could be the first step in the regeneration of fully functioning lungs in larger animals and even in man, although there is still some way to go to transfer these experiments to human medicine[54].

The study by Petersen et al[52] was published in July 2010. Then, as early as August of the same year, another team led by JP Vacanti published another article[49] in which, using the same technique that had already been used by Taylor's group[44], they decellularised a rat lung to obtain a lung scaffold which retained a perfusible vascular system and a proper alveolar structure. To prove that this technology could be applied in the future to human lungs, they also used it in larger animals such as pigs, calves and primates.

Pancreas

In September of the same year, another organ, the pancreas, joined the family of bioartificially created organs. However, unlike the heart and lungs, which are produced *in vitro*, in the case of the pancreas, this involves *in vivo* bioproduction. Indeed, a team composed of several Japanese universities and a London university has developed an ingenious technology to create a murine pancreas[55]. For its production, they used reprogrammed mouse pluripotent cells, which they injected into mutated mouse blastocysts, carriers of a genetic mutation which prevented them from developing a functioning pancreas. The hybrid obtained developed a normal pancreas. The

aforementioned experiment opens the door to the possibility of creating organs *in vivo* using pluripotent cells from a particular donor, which in the more or less near future could be used to generate whole organs using pluripotent cells from the patient himself.

Liver

The definitive treatment for liver failure is liver transplant, but the number of organs available, as happens with other organs, cannot cover the clinical requirements in most countries. In the United States alone, the deficit is around 4000 livers per year[56]. In order to resolve this problem, the creation of bioartificial livers has been proposed. To this end, Uygen et al[57], using Ott's technique[44], were able to develop a bioartificial liver by decellularising a donated organ, to obtain a liver matrix which preserves the microvascular network. The liver scaffold generated was then recellularised with hepatocytes. The new livers generated can be transplanted into rats.

Technically, preserving the liver scaffold is fundamental for the consolidated implantation of the hepatocytes and for the long-term function and survival of the new organ, a function which includes albumin secretion, urea synthesis and expression of cytochrome P450, to reach levels which are comparable to those found in normal liver *in vitro*.

Furthermore, the conserved vascular network enables the circulation of the new liver to be restored, facilitating the oxygenation and supply of nutrients post-transplant.

In the authors' opinion, the results of their study show that it is possible to generate liver tissue grafts which could have great therapeutic potential in various conditions which require transplant of this organ, although the generation of whole livers will require extensive further work, since the development of a whole liver requires the perfusion of cells other than hepatocytes, such as endothelial cells, hepatic sinusoidal cells, biliary epithelial cells and Kupffer cells.

Kidney

Decellularisation of rat kidneys has also been achieved[58] using Ott's technique[44], obtaining decellularised kidney scaffolds which preserve the glomerular, tubular and vascular architecture of the kidney. The aforementioned scaffolds were then re-seeded with murine embryonic stem cells which were injected either through the renal artery, or retrograde through the ureter. The injected cells proliferated within the glomerular, vascular and tubular structures which had been preserved in the decellularised kidney matrix. The renal tissue recreated expressed immunohistochemical markers typical of real tissue.

Ovary

Another possibility is the creation of bioartificial organs, not to be transplanted in a patient, but to carry out their function outside them. This could be the case of the development of an artificial ovary, capable of creating mature oocytes which can then be fertilised and the embryos transferred to an animal uterus.

The study, conducted by a team from Brown University and Rhode Island Women and Children's hospital[59], described how they seeded immature oocytes, granulosa cells and theca cells onto an agarose mould. Seventy-two hours after the construction of the artificial ovary, the theca cells completely covered the oocyte spheres, which were in turn covered by the granulosa cells. From this artificial ovary, they were able to extract mature oocytes which could then be fertilised.

However, one fact to take into consideration to properly assess this achievement is that a potentially important experiment was published in a low impact factor journal, which does not usually happen with outstanding scientific achievements.

Clinical applications

It would appear that taking the step from the experimental to the clinical in the field of bioorgans will take years. However, there have already been two cases in which bioartificial organs have been used in clinical medicine.

The first of these[60] was a few months after the publication of Doris Taylor's study[44]. It describes the transplant of a bioartificially-produced trachea in a patient with a serious respiratory disease. The patient was a 30-year old woman who in 2004 had suffered a tuberculous infection in the cervical part of the trachea which had residually caused stenosis of the trachea, preventing normal respiration. In March 2008, the patient was admitted with severe dysphonia which led to her being unable to carry out the most basic tasks, so transplant of a bioartificially-produced airway was considered. To that end, a 7 ms segment of trachea was obtained from a 51-year old donor who had died due to a brain haemorrhage. The entire tracheal connective tissue was removed and the trachea was placed in a detergent solution until a decellularised tracheal matrix was obtained. The matrix was recellularised with epithelial cells and mesenchymal stem cells obtained from the patient herself, and resulted in a functional airway. After the implant, the woman was able to normalise her respiratory function and improve her quality of live, maintaining this improvement for four months, a normality which she maintains to date[61]. These results show that respiratory behaviour can be produced by bioengineering, with mechanical properties which mimic normal behaviour and which do not have a risk of being rejected as the recellularisation has been carried out with autologous material.

It was two years later before a similar experiment was performed. In it, as described in The Independent[61], and in the British Medical Journal[63], the trachea of a 10-year old British boy with severe tracheal stenosis was replaced. The patient had been born with the condition, and had a one-millimetre lumen, which prevented him from breathing

without mechanical aid. To try to resolve the problem, he had undergone several unsuccessful surgical procedures, so in the opinion of his doctors, the only useful option was a trachea transplant, which they decided would be a biotrachea.

To this end, a trachea was obtained from a deceased child. The donated trachea was subjected to enzyme treatment until a fibrous collagen scaffold was obtained. This scaffold was then injected with bone marrow stem cells obtained from the child's rib, and the process was subsequently activated with various growth factors. The main difference between this and the previously described case is that in the former, the neotrachea produced was activated in the laboratory; however in this case, after being generated, the trachea was transplanted in the sick child, with his own body acting as a bioreactor. He is the first child in the world to which this revolutionary methodology has been applied. At present, the child is breathing well and leads a normal life.

As Dr. Martin Birchall, one of the members of the surgical team who operated on the sick child says, in the United Kingdom there are several hundred children who could benefit from the same technique, and likewise, it could be used for other types of patients, which would significantly widen its possibilities for use[62].

Conclusion

Thus far, we have reviewed the current situation in bioorgan production.

There is no doubt that it is still in a pre-experimental stage, and that transferring these experiments to the clinical field will undeniably take several years. However, the fact that bioorgans have already been used in two patients in a field as specific as that of tracheal stenosis, opens an encouraging door to the clinical use of this innovative medical technology.

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