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Surrogate testes: Allogeneic spermatogonial stem cell transplantation within an encapsulation device may restore male fertility

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A B S T R A C T

Toxic insult to the gonads by chemotherapy or radiotherapy can lead to permanent infertility. It's an important health concern because each year more than 4000 male patients are at risk of azoospermia in the United States due to gonadotoxicity of the regimens used. There are also several benign/genetic diseases whose natural course can result in infertility without gonadotoxic therapy. Considering the fact that most of these people are cured and survive with the advent of modern medicine, infertility is related to serious psychological and relationship implications and parenthood is a significant issue for those patients. Semen cryopreservation option is available for postpubertal adolescent and adult men, while children do not have this storing option since they do not have mature spermatozoa. However, their testes contain spermatogonial stem cells (SSCs), which are initiators of spermatogenesis. Promising findings in animal studies and human cell lines have encouraged scientists that SSCs may be hope for restoring fertility option of patients who cannot produce functional sperm and who have no other choice to preserve their future fertility. For this reason, several centers around the world already began to collect and cryopreserve testicular tissue or cells with anticipation that SSC-based therapies will be available in the near future; however, an optimal transplantation design in humans is yet to be developed. Here we propose an allogeneic testicular stem cell transplantation with an encapsulation device to restore fertility in patients with infertility. We endeavor to discuss the reliability of this method with the current literature and bring the evidence on its feasibility

Introduction

Currently, 7% of the male population worldwide is affected by infertility [1]. Genetic disorders, environmental factors and cancer treatments are the major contributors to the infertility. Sperm freezing is the standard practice for addressing this in adult males; however, there are no established fertility restoration options for those, such as pre-pubertal boys, who do not have mature spermatozoa [2]. There are a few experimental methods for restoring fertility in patients within this group, such as spermatogonial stem cell (SSC) transplantation and autologous testicular tissue engraftment.

More than 20 years ago, the first successful spermatogonial stem cell (SSC) transplantation in mice resulted in spermatogenesis and the production of offspring [3]. Several subsequent trials also demonstrated successful spermatogenesis and the production of offspring in other animal species; however, the only reported clinical study in humans had unsuccessful results. SSC transplantation in humans may be limited because of the insufficient co-transplanted niche for supporting SSCs and the unestablished scope of human SSC propagation in vitro.

Engraftment of testicular tissue may overcome these limitations. Recently, auto-transplantation of testicular tissue has demonstrated promising results in non-human primates [4]. However, the risk of re-introducing the malignant cells into the recipient is the major limitation of this procedure. Xeno-grafting and testicular tissue grafting under the back skin of immune-deficient mice may be effective in overcoming this issue and has resulted in success among mice, pigs, and non-human primates in obtaining offspring. However, it would be impossible to translate this technique into clinical situations due to the risk of infection by rodent retroviruses or undiscovered/unknown infections and possible immunoreactions [5–8].

Although, xeno-transplantation cannot be considered a viable method because of these risks, allogeneic-transplantation may be a useful tool for the growth of SSCs. Allogeneic transplantation has never been attempted in humans, although spermatogenesis in rhesus macaques was observed after autologous transplantation of SSCs [9].

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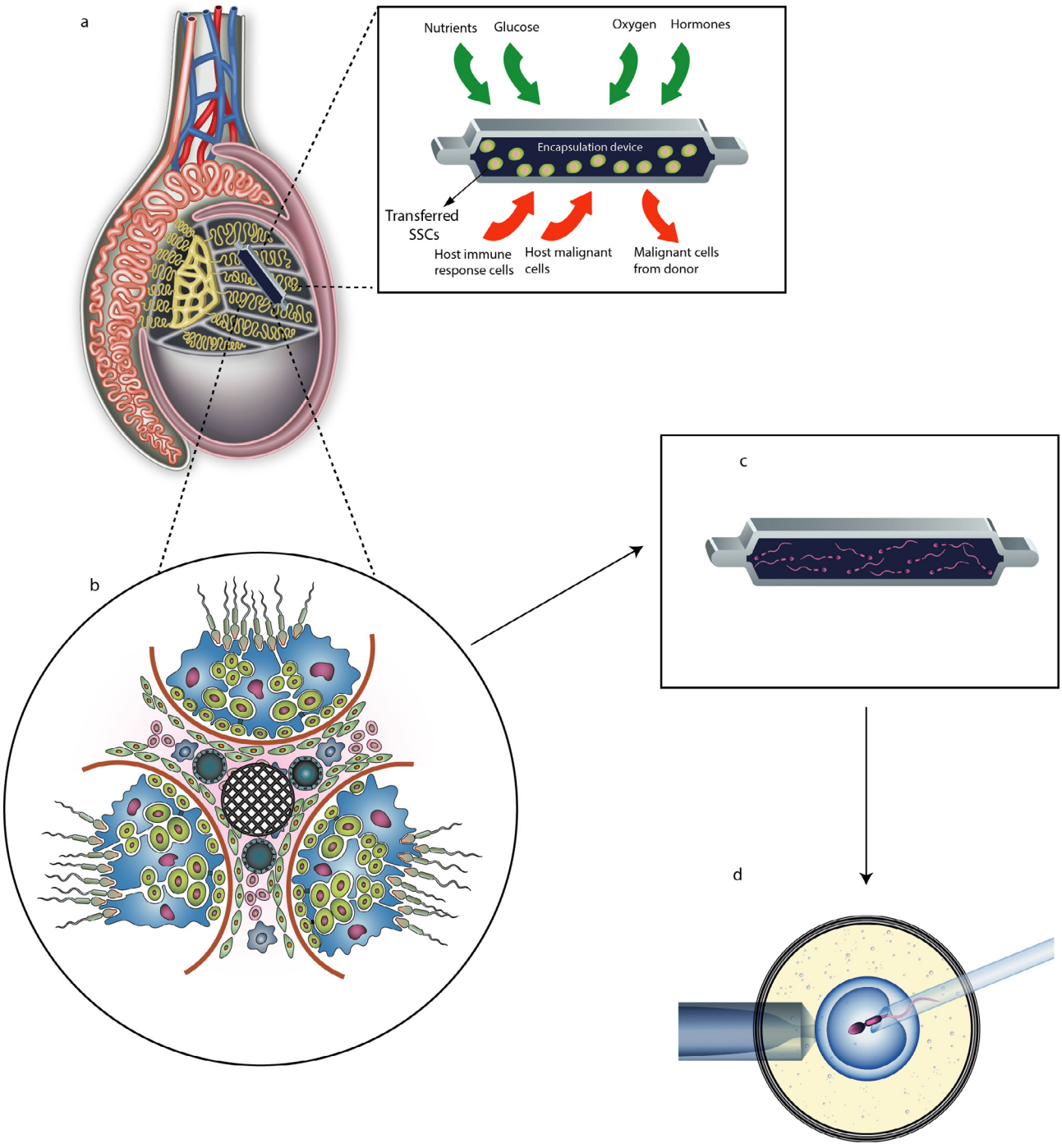

Fig. 1. Demonstrations of steps of allogeneic stem cell transplantation and *in vivo* spermatogenesis. a: Partial-sagittal cutaway of testis and characteristics of encapsulation device. b: Magnified area of testicular microenviroment and its relationship with encapsulation device. Grid area represents the contact area between interstitium and encapsulation device. c: In vivo spermatogenesis within the encapsulation device. d: Assisted reproductive techniques are used to generate embryo from the sperm obtained by encapsulation device.

Hypothesis

An alternative way of proliferating and differentiating SSCs *in vivo* would be allogeneic transplantation of SSCs/testicular grafts to the testes of healthy recipients, using an encapsulated device that provides effective immune protection and presents sufficient mass transfer between the outside environment and the encased SSCs/testicular grafts (Fig. 1).

Evaluation of the hypothesis

Allogeneic haematopoietic stem cell transplantation (AHSCT) is the most frequently used allogeneic stem cell transplantation in clinics and has been being successfully implemented for patients with several haematological cancers. Disease-directed or cytoreductive therapies, such as whole-body radiation or chemotherapy, are necessary before AHSCT to eliminate as many cancer cells as possible. In these cases, the aim is for the recipient to recover; however, for our hypothesis, the aim is for the patient to survive the engraftment first and then to generate

spermatogenesis in the host testis. The mature spermatozoa of the donor could subsequently be retrieved and then used to generate embryos with assistive reproductive techniques such as in vitro fertilization or intra cytoplasmic sperm injection.

We were inspired to establish our hypothesis by two pre-clinical allogeneic transplantation studies. It has been over seven years since allogeneic SSC transplantation first led to successful production of offspring in non-human primates [9]. For this purpose, rhesus macaques were exposed to busulfan treatment for germ cell depletion prior to transplantation. This essential step allows donor cells to migrate to the basement membrane of the seminiferous tubules of the recipients and then settle down. The rhesus macaques were then treated with immunesuppressants to protect the donor cells from immune rejection. In the second study, allogeneic transplantation of the testicular cells of prepubertal monkeys into the testes of the irradiated post-pubertal monkey resulted in the development of de novo tubules in which the elements of complete spermatogenesis were shown [10]. The results of these two studies are inspiring, since they both demonstrated that if the transplanted testicular cells contact the microenvironments of the testes under convenient circumstances, they are capable of producing mature spermatids. In clinics, however, immune suppression to prevent the rejection of transplanted stem cells may involve the recipient patient encountering infection and malignancies. Obviously, then, these designs cannot be translated into human clinical trials. Nevertheless, an encapsulation device that can protect the cells/testicular grafts from immunoreactions while at the same time allowing them to grow or proliferate would be a viable option for allogeneic transplantation of SSCs/testicular grafts.

The use of encapsulation devices is not new. Although a wide range of study areas has been explored, such as the parathyroid, ovary and pancreas [11–13], beta cell replacement has attracted a growing interest as it may provide metabolic control without the need for exogenous insulin with the aid of an encapsulation device [14]. For the same reason, encapsulation and immune modulation strategies have been used for decades to achieve beta cell survival and protection from immune systems to cure diabetes mellitus. Although attempts demonstrated success with pancreatic grafts in animal models [15–18], the optimal encapsulation device has not yet been developed for humans.

Encapsulation technologies offer two types of encapsulation strategies: microencapsulation and macroencapsulation. Although microencapsulation can provide maximum surface area-to-volume ratios and improved nutrient exchanges [19], every cell needs to be encapsulated individually, which makes tracking those cells a challenge. Also, these devices have less control over membrane parameters, such as pore size and porosity. In addition, testicular tissue engraftment cannot be used with this approach due to its microscale. For these reasons, microencapsulation devices do not seem to be a reliable option for SSC/testicular grafts. Conversely, macroencapsulation devices offer more control over membrane parameters as well as additional space for more SSCs or tissue grafts to transplant. First attempts with macroencapsulation devices resulted in failure, mainly because of fibroblastic overgrowth of the graft, indicating the significance of the biocompatibility of the material used in encapsulation devices [20]. The material used to build a chamber is also very important in achieving immune-isolation and communication with micro environments. In the search for optimal material for macroencapsulation, a wide range of materials has been tested, from polymers, such as polycaprolactone and expanded polytetrafluoroethylene (ePTFE), to inorganic materials, including titania and silicon [21–23]. Although tight pore size distribution and more controllable membrane configuration are among the major advantages of inorganic materials, their rigid characteristics may lead to more fibrotic responses [24]. Conversely, good vascularization with limited fibrotic response has been shown with polymeric materials [22,23]. More recently, an encapsulation device was engineered by polycaprolactone using a non-templating technique resulted in rapid neovascularization adjacent to encapsulation device without foreign body response [22]. The combination design with nano and micro channels were also introduced to improve immunoprotection and survival of the transplanted cells [25]. With the advent of Hi-tech micro and nano-manufacturing systems, engineering the membrane with precise characteristics will become possible.

Lessons learned from these experiences encourage us to propose an optimal testicular encapsulation device with following features; (i) protection the host from the risk of stem cell-derived oncogenic transformation, (ii) having sufficiently permeable to the nutrients, glucose, oxygen and hormones to sustain transplanted SSCs/tissue graft viability and differentiation, (iii) protection the transplanted SSCs/tissue graft from host immune response, (iv) being biocompatible to cause almost non-fibrotic response (Fig. 1). The central role of a testicular encapsulation device should be facilitating the communication between grafted cells/tissue with the microenvironment of host tissue, while sustaining graft viability.

One of the limitations of many encapsulation strategies is the lack of sufficient oxygen to maintain cell function. To overcome this problem another port connected to encapsulation device may be left out of the scrotum (oxygen port) to fill the encapsulation device with oxygen intermittently. Secondly, foreign body response may lead to fibrotic encapsulation which may hamper the transplantation procedure; however, inflammatory response caused by biocompatible materials is possible to resolve without fibrosis which would allow nutrient and hormone exchange via vascular growth adjacent to the transplanted capsule.

Conclusion

Extensive efforts have been attempted to find optimal encapsulation design for cell-based microencapsulation and macroencapsulation technologies. We believe that current challenges would be overcome by multi-disciplinary approaches ranging from engineering to immunology which would revolutionize new encapsulation technologies and allow translation of encapsulated cell therapy from laboratory to the clinic in a very near future.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.109634.

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