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EDITORIAL

Keeping cells in their place: the future of stem cell encapsulation

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1. Introduction

Ever since the early part of the 20th century, cell encapsulation has been explored as a technique to protect transplanted cells from the host immune system. Since then, the process of entombing cells within a number of natural and synthetic matrices has been exploited for a wide range of applications. Materials used for cell immobilization include the natural polymers alginate, chitosan, agarose, cellulose, collagen, and zanthan and the synthetic polymers poly(ethylene) glycol, polyvinyl alcohol, polyurethane, and polypropylene. These are selected, designed, or modified to perform as semi-permeable membranes that enable the inward diffusion of nutrients, exchange of therapeutic proteins, and elimination of waste products in order to maintain cell survival and function. The major applications of cell encapsulation include (i) cell transplantation and therapeutic delivery; (ii) *in vitro* three-dimensional (3D) culture and cell modeling; and (iii) biofabrication of tissues and organs. Despite its long history of use, cell encapsulation has presented a number of limitations to which much endeavor has been carried out to overcome. This is especially true with the clinical use of encapsulated cells where it is paramount to maintain effective mass transfer of nutrients and secretory components, whilst avoiding host tissue responses. Cells, cell fragments, and the encapsulation matrix itself can initiate these responses, making careful design vital for clinical success. Additionally, matrices must have predictable long-term stability and be retrievable when necessary. Fulfilling all of these requirements is not trivial. The encapsulation matrix that has had the longest and most intense research to meet these requirements is the natural hydrogel alginate. For this reason, alginate will be focused on throughout this editorial. It will briefly cover the fundamental applications mentioned and discuss the impact of how recent evidence describing the cytoprotective effects of encapsulation during storage could impact future delivery, flexibility, and accessibility of live purposeful cells.

2. Cell encapsulation for therapeutic delivery

Cell-based therapies encompass a number of approaches that aim to regenerate damaged tissues, accelerate wound healing, and/or modulate dysregulated cellular systems in the body. Associated with the therapeutic delivery of cells, whether autologous, allogeneic, or xenogeneic, are a number of challenges to which cell encapsulation has surfaced as a feasible approach to resolve. These include avoiding direct contact with the host immune system to prevent rejection, increasing the retention of cells at sites where they are needed, and maintaining the survival and functional capacity of transplanted cells. Rather than directly repopulating damaged cells and tissues, emerging cell therapies are based upon the capacity for cells to produce bioactive factors in an environmentally responsive manner. Whilst advances have been made in the development of materials and approaches for controlled drug delivery, it is difficult to recapitulate the sophistication of the cell to orchestrate complex multi-stage physiological processes. Therefore, using encapsulated cellular 'biofactories' could be considered the smartest medicine available to deliver appropriate factors at the appropriate time during regeneration.

Exemplifying the use of encapsulated cells for dysregulated physiological processes is the encapsulation and transplantation of pancreatic islet cells for the management of type I diabetes mellitus. In order to maintain glycemic homeostasis, rapid minute-to-minute regulation is necessary and the delivery of insulin-producing cells encapsulated in alginate has been explored for a number of decades. Clinical trials undertaken to date have used either allogeneic or xenogeneic-derived islets with relative success [1], epitomizing the immunoisolatory benefits of encapsulation. There have, however, been concerns over the alginate itself initiating host responses thus limiting efficacy of treatment. This has led to a growing understanding of how alginate material properties (i.e. mannuronic acid/guluronic acid composition, surface roughness, charge, implant size, and stability) influence host tissue responses and islet survival. Through modulating these properties, recent studies have demonstrated long-term glycemic control (almost 25 weeks) in immune-competent mice with encapsulated stem cell-derived islet beta cells implanted intraperitoneally [2] signifying considerable progress in alginate-encapsulation for therapeutic delivery. Indeed, what we now know about alginate has led it to be widely considered the principle encapsulation polymer for clinical applications [3], as

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well as it being the only encapsulation matrix approved for human use by the U.S. Food and Drug Administration.

Just as islet cells sense blood glucose levels and respond accordingly, this principal forms the basis of mesenchymal stem cell (MSC)-based cell therapies for wound healing and tissue regeneration. Following injury, endogenous MSCs are activated. These cells survey the wound area and follow the progression of wound healing, responding accordingly by producing factors that guide other cells involved in the regenerative process [4]. It is in this manner that these highly sophisticated cells are able to sense the environment and deliver an appropriate restorative dose at the right time. The amazing capabilities of MSCs as responsive 'drug factories' have led to the use of exogenous MSCs in a considerable number of clinical trials for the treatment of a plethora of conditions [5]. There are, however, issues with cell survival, retention, and maintenance of paracrine effects at sites of damaged tissue. This has led to a number of improved delivery methods being sought. Among these, cell encapsulation has proved to be a realistic option. Studies have demonstrated that alginate-encapsulation of human bone marrow-derived MSCs maintained their survival, retention, and pro-angiogenic activity in murine models of hind limb ischemia resulting in a dramatic increase in treatment efficacy [6]. Maintenance of cell survival and paracrine function could be, in part, associated with the immunoisolatory barrier offered by encapsulation. This has been shown to be important recently where MSCs encapsulated in alginate with a controlled pore size hindered the penetration of pro-inflammatory cells and cytokines, resulting in increased MSC viability and regenerative capacity [7]. As well as being a method for implantation, alginate has also been used for delivering umbilical cord-derived MSCs topically for the healing of full-thickness skin wounds in mice [8]. This accompanied by the use of alginate in wound dressings for decades represents an intriguing potential for exploiting both the advantageous properties of alginate in maintaining a favorable environment at the wound bed, whilst delivering paracrine factors from encapsulated MSCs capable of accelerating wound healing. Although there are a number of different materials and methodologies examined for encapsulated cell delivery, alginate represents a timeworn biopolymer with ongoing possibilities in cell-based therapies.

3. 3D cell culture and biofabrication

The use of biomaterials for 3D cell culture systems has ever-increasing interest for the study of relevant tissue physiologies, toxicity testing, and tissue engineering applications. Through manipulating physiochemical and biological cues within a spatial context, the native extracellular matrix can be recapitulated introducing physiologically relevant conditions in cell culture. This is especially important when studying mechanisms of disease in cell models, as well as pharmacological and toxicity testing where existing two-dimensional culture platforms influence drug susceptibility, cell survival, gene and protein expression, and differentiation [9]. Perhaps, the most exciting recent developments that take advantage of fabricating matrices for cell control are in 3D bioprinting. Using cell-laden hydrogels such as alginate as 'bioinks', this manufacturing technology promises the potential for personalized fabrication of tissues and organs to replace those that are lost or damaged. Currently, printing is being used as a strategy for the fabrication of complex 3D biological structures for maxillofacial applications, as well as in the generation of internal organs and vascular tissues [10]. Cell encapsulation has also been employed for a number of years as a method for scalable upstream bioprocessing of cells, including stem cells, for cell therapy applications [11]. Owing to the large hydrodynamic forces exerted during agitation in bioreactors, encapsulation offers a method by which cells can be shielded from these forces, be efficiently expanded, and be easily retrieved for downstream processing.

4. Encapsulation for cell preservation

A major challenge in the downstream bioprocessing of cells is how to store them. Whilst liquid (hypothermic) storage of cells is associated with a limited shelf life, cryopreservation often involves the use of potentially toxic cryoprotective agents and complex, non-flexible, expensive logistics for cell distribution. Introducing an exciting paradigm for the use of cell encapsulation is its ability to protect cells during hypothermic storage. Recent research undertaken in our laboratory demonstrated how alginate-encapsulation of adipose-derived stem cells, MSCs derived from fat, provided a method whereby cells could be stored at hypothermic temperatures for extended periods whilst preserving cell viability and functionality [12]. The ability to maintain cells in suspended animation opens up many avenues for the storage and application of cells in their liquid state. This has obvious implications in overcoming many of the logistical constraints associated with the cell therapy supply chain, but also any process that could benefit from holding cells outside the tissue culture incubator without the need for cryopreservation.

5. Expert opinion

Cell encapsulation represents a methodology with an abundance of applications: ameliorating therapeutic cell delivery; creating *in vitro* 3D culture systems for the physiologically relevant study of disease, pharmacological screening, and toxicity testing; 3D cell printing and tissue fabrication; and upstream bioprocessing of cells. It is only when you combine these applications with the ability to preserve (store) cells within the same encapsulation matrix that a really powerful tool is created.

Within this editorial, much has been said about the clinical delivery of encapsulated therapeutic cells, with alginate being highlighted as a medically viable matrix due to its history of use, understanding and development. Whilst great strides have been made toward the clinical application of alginate-encapsulated cells, the journey has been tortuous with a number of lessons being learnt. The biggest of these is probably the effect of alginate composition on initiating the host immune response, the exact determinants only recently being realized. It is now imperative to assess safety and efficacy in human trials, a number of which are either ongoing or completed. Examples for the potential of

cell encapsulation for therapeutic use have been drawn from two major examples: (i) islet cell delivery for diabetes and (ii) MSC-based therapies. Whilst human trials for encapsulated-islet delivery have been well documented, with a lot of activity currently underway, trials for encapsulated MSCs are still to be realized. This is surprising due to the high number of MSC-based trials that exploit the paracrine activity of this versatile cell type, and the recognized concerns about poor cell retention and survival. For the treatment of certain disorders however, where there is poor accessibility to the implant site, retrievability may be a limitation. There also remains limited preclinical studies exploring the effect of encapsulated MSCs on regeneration and wound healing, this should be of future focus for us to understand their true potential for a variety of disorders.

With growing interest in encapsulation for cell delivery, the capacity to store therapeutic cells within alginate at hypothermic temperatures could dramatically increase the clinical translation of cell-based therapies. Specifically, the same matrix that is used to immobilize cells for therapy would also preserve cell viability and function prior to treatment. This would not only aid in the distribution of cells from sites of manufacture to the clinic, but also increase time windows to create flexibility at their point of use. This increased flexibility could be paramount in limiting variability and wastage associated with cells stored in their liquid state due to transport delays or inadequate patient /clinician scheduling. Accessibility would also be heightened in countries where the infrastructure to produce or receive cell therapy products may not be available, or areas where there is a remote point of clinical need. Collectively, preserving cells in a form suitable for treatment offers exciting prospects for cell-based therapies.

The same principal can also be applied to some of the other applications mentioned. Whether this is for repeated toxicity or pharmacological testing in 3D cell culture models, or increasing hold-times in 3D biofabrication or post-upscale bioprocessing of cells, preservation within the encapsulation matrix enhances both portability and flexibility.

In order that emerging technologies can be truly translatable to the clinic, employing approaches that not only bear functional therapeutic benefits but also have the capacity for preserving cells during short-term storage overcomes many of the technical and logistical challenges associated with new cell-based technologies and cell therapy products. This opens up exciting possibilities for the future of cell encapsulation.

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Declaration of interest

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