Advanced Drug Delivery Reviews 67-68 (2014) 111–130

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Advances in biocompatibility and physico-chemical characterization of microspheres for cell encapsulation☆

Anne Mari A. Rokstad [a,e,1,*], Igor Lacík [b,1,**], Paul de Vos [c,1], Berit L. Strand [a,d,e,1]

[a] Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology (NTNU), Prinsesse Kristinasgt. 1, N-7491 Trondheim, Norway
[b] Department for Biomaterials Research, Polymer Institute of the Slovak Academy of Sciences, Dubravska cesta 9, 845 41 Bratislava, Slovakia
[c] Immunoendocrinology, Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, EA11, 9700 RB Groningen, The Netherlands
[d] Department of Biotechnology, NTNU, Sem Saelandsvei 6/8, N-7491 Trondheim, Norway
[e] The Central Norway Health Authority (RHA), Trondheim, Norway

ARTICLE INFO

Available online 20 July 2013

Keywords:
Biocompatibility
Biotolerability
Cell encapsulation
Cell therapy
Complement
Host response
Microbeads
Microcapsules
Microspheres
Physico-chemical characterization
Permeability

ABSTRACT

Cell encapsulation has already shown its high potential and holds the promise for future cell therapies to enter the clinics as a large scale treatment option for various types of diseases. The advancement in cell biology towards this goal has to be complemented with functional biomaterials suitable for cell encapsulation. This cannot be achieved without understanding the close correlation between cell performance and properties of microspheres. The ongoing challenges in the field of cell encapsulation require a critical view on techniques and approaches currently utilized to characterize microspheres. This review deals with both principal subjects of microspheres characterization in the cell encapsulation field: physico-chemical characterization and biocompatibility. The up-to-day knowledge is summarized and discussed with the focus to identify missing knowledge and uncertainties, and to propose the mandatory next steps in characterization of microspheres for cell encapsulation. The primary conclusion of this review is that further success in development of microspheres for cell therapies cannot be accomplished without careful selection of characterization techniques, which are employed in conjunction with biological tests.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Cell encapsulation and drug delivery".

* Correspondence to: A.M. Rokstad, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Prinsesse Kristinsgt. 1, N-7491 Trondheim, Norway. Tel.:+47 72825353, +47 93084332; fax: +47 72571463.
** Corresponding author. Tel.: +421 232294308; fax: +421 232294319.
E-mail addresses: anne.m.rokstad@ntnu.no (A.M.A. Rokstad), igor.lacik@savba.sk (I. Lacík), p.de.vos@umcg.nl (P. de Vos), berit.l.strand@ntnu.no (B.L. Strand).
[1] These authors contributed equally.

0169-409X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.addr.2013.07.010

1. Introduction

Cell microencapsulation is a multidisciplinary research field where polymers, biomaterials, surface chemistry and engineering meet biology, immunology, medicine and surgery. Most researchers entering the field are fascinated by the opportunities for application and, at glance, the simplicity of production of hydrogel microcapsules and microbeads in conjunction with cell encapsulation. However, as the complexity in transplantation of encapsulated cells is associated with a wide variety of factors involving (i) transplanted cells, (ii) biomaterials and (iii) heterogenic recipient properties, over the years it has become apparent that entering the clinics with therapy based on immunoprotected cells by encapsulation is a challenging task.

Research on microencapsulation of pancreatic islets dominates the studies on immune protection of transplanted cells. This is mainly driven by the high number of diabetic patients and benefits of intrahepatic islet transplantation in terms of freedom from endogenous insulin, controlling the blood glucose levels, reducing the diabetic complications and improving quality of life [1]. Immunoisolation of cells by encapsulation, *i.e.*, transplantation of cells without immunosuppression, would be a major breakthrough towards safer and widely applicable cell therapy in diabetes treatment. Recent reviews on islet encapsulation describe the current situation in transplantation of encapsulated islets and provide insight in factors determining successes and failures of transplanted immunoprotected islets [2–4]. Examples of other cell therapies, which would benefit from transplantation of encapsulated cells, include the treatment of neurological and sensory diseases [5], treatment of liver disorders by bioartificial liver devices [6] and cardiac repair [7]. Common for all these therapies is the requirement of safe (in terms of recipient) and functional (in terms of encapsulated cells) microspheres to provide long-term immunoprotection of cells capable of treating the specific disease.

Several types of microspheres have recently been used in clinical trials for diabetes treatment [8–11] as well as for neural and sensory diseases [5]. However, a recipe for perfect and unquestionably clinically applicable microspheres is so far not available. The reason for this situation originates, at least partially, from the inability to specifically point out the factors contributing to success or failure of the transplanted device. This is at least partially due to the high numbers of variations in the way microspheres can be produced. However, in spite of this, the development of successful microspheres for cell therapies has been associated with a number of successful cases. But, up to now, the final set of recommendations with the general validity could not be provided because, similarly as in the case of failures, the reasons for the success could not be completely understood. A few reasons for uncertainties can be provided. Primarily, the polymer characterization, including composition, molecular weight and purity, is not always given for the encapsulation system, as well as the details in protocols for microsphere formation are missing as recently reviewed [12]. Although a terminology for microspheres is regularly given, as e.g. APA for alginate–poly-L-lysine (PLL)–alginate microspheres, the same type of microspheres can have complete different properties. This is caused by variation in alginate composition and molecular weight, gelling ions, non-gelling ions, solubilized or gelled core, PLL molecular weight, size of microspheres, exposure and washing times [13]. Hence, care should be taken in comparing studies and drawing conclusions from these comparisons as different microspheres might be applied. The properties of microspheres are usually determined before implantation and the correlation of performance of encapsulated cells with the properties of microspheres after exposure to the *in vivo* environment is missing. This is seen as an incorrect approach as the properties of microspheres may change after implantation [14]. In addition, the quality and viability of encapsulated cells are important for success. This is especially the case with pancreatic islets, where cell viability varies considerably due to donor variations and differences in efficacy of the enzyme-driven isolation process. Furthermore, the presence of encapsulated cells may influence microsphere properties such as the mechanical stability [15]. These are obviously only a few examples from the number of variables which have to be controlled and documented as a part of the encapsulation and transplantation protocols.

The study of the correlation between microsphere design, the protocol of encapsulation and the *in vitro* and *in vivo* performance requires a series of characterization methods. Many *in vitro* [16,17] and *in vivo* [14,18] approaches have been considered as appropriate to identify the factors determining the functional properties of microspheres. The methods regularly employed for characterization of microspheres are described in a recent monograph [19] and review paper [12]. The aim in applying various methods is to understand the mutual relationship between microsphere characteristics and performance in the presence and absence of cells, and to identify the important contributors to an "optimal window" [20] for the conditions providing reproducible graft function *in vivo*.

The characterization of microspheres with encapsulated cells is carried out on two mutually related platforms: (i) physico-chemical and (ii) biocompatibility. The complexity of parameters involved in microsphere characterization towards safe and functional performance is depicted in Fig. 1. The microsphere is manufactured from a biomaterial which requires characterization of the selected polymers. The mechanical stability, permeability and morphology characteristics of the microcapsules are important for the *in vivo* performance. The microsphere surface properties are highly important for the interaction with the host proteins and cells. The characterization methods are recommended for application in order to get a deeper understanding of the microsphere physico-chemical properties, since these properties are tightly linked to the biocompatibility and the functional performance of the microspheres. The biocompatibility assessments represent the second part of Fig. 1, and include aspects of immune compatibility, cell compatibility as well as characteristics connected to the transplantation site and the recipient.

Also the term "biocompatibility" needs to be considered. "Biocompatibility" has been used as a term within the last 40 years to describe the performance of a material after implantation [21]. The "biocompatibility" definitions are debated [21,22], and may hold different meanings depending on the type of applications and strategy [21,23]. In the field of microspheres, the "biocompatibility" term has been used to describe apparently contradictory outcomes, *i.e.*, microspheres both with no cellular overgrowth and those causing blood vessel formation can be considered as biocompatible. The strategies for functional grafting of