Trends in Biotechnology

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Review

Cell-laden Polymeric Microspheres for Biomedical Applications

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Microsphere technology serves as an efficient and effective platform for cell applications (*in vitro* cell culture and *in vivo* cell delivery) due to its mimicry of the 3D native environment, high surface area:volume ratio, and ability to isolate the entrapped cells from the environment. Properties of cell-laden microspheres are determined by the type of application and the cell. While high cell densities are preferable for large-scale therapeutic biomolecule production *in vitro*, an immunoprotective barrier is most important for allogeneic pancreatic islet transplantation into patients. Furthermore, the biological cells require a suitable microenvironment in terms of its physical and biochemical properties. Here, we discuss applications of cell-laden microspheres and their corresponding design parameters.

Trends

Polymers for cell-laden microspheres are discussed.

Fabrication techniques and key parameters regarding cell-laden microspheres for specific applications are explored.

Several uses of cell-laden microspheres (*in vitro* production of cells and biomolecules, as well as *in vivo* delivery of cells for tissue regeneration or therapeutic biomolecule secretion on-site) are discussed and summarized.

Using Microspheres Embedded with Live Cells

Microsphere technology has been exploited in many emerging biomedical applications, including cell, drug, biomolecule, and gene delivery. Notably, cell-laden microspheres have been developed for two main classes of application: (i) *in vitro* cell culture for cell expansion and biomolecule manufacturing and (ii) *in vivo* cell delivery for cell replacement or therapy. Microspheres are relatively easy to fabricate and handle, and provide a large surface area:volume ratio for cell culture and *in vitro* applications. For *in vivo* applications, microspheres can provide minimally invasive, localized delivery and protection from the immune systems of patients. Cells delivered by microspheres may secrete (either naturally or through genetic modification) therapeutic factors in a sustained manner, circumventing the need for multiple administrations of drugs.

The paradigm of cell culture has switched from a conventional monolayer culture to utilizing biomimetic 3D platforms. It is well recognized that prolonged monolayer culture results in **dedifferentiation** (see Glossary) [1,2]. However, macro-sized 3D platforms are not feasible for cell culture due to an oxygen diffusion constraint of a maximum 200 μm [3]. While some research groups focus on microvascularizing such macro-sized constructs, another scientific community aims to bypass the constraint via a bottom-up approach: creating micro-sized cellular constructs that may then be used as is, or put together to form macro-sized constructs. This has led to an increase in commercially available microspheres and microsphere generators, both of which are increasingly utilized by researchers.

Microspheres designed for cell adhesion or encapsulation, categorized as **microcarriers** or **microcapsules**, are generally spherical polymerized networks with a diameter of 100–400 μm to maintain cells within the oxygen diffusion limits. Microcarriers are usually fabricated to have cell-adhesive moieties, which cells are then seeded on. By contrast, microcapsules are typically

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fabricated by crosslinking a polymer–cell suspension so as to entrap cells within the core of the microsphere. However, the seemingly simple product requires much thought in its design and optimization to provide the appropriate microenvironment in which cells can survive, reside, and maintain their desired function. Microsphere design also needs to take into consideration the cell type and polymer porosity, mechanical strength, cytotoxicity, **immunogenicity**, degradation products, and rates of their formation. Given the intricate interplay of design parameters required to successfully encapsulate cells within microspheres and the quick pace of research advancement, here we this discuss the use of cell-laden microspheres in two main branches of therapeutic applications (*in vitro* culture and cell delivery) as well as important application-specific considerations to maximize efficacy and efficiency.

Designing Cell-laden Microspheres

Microspheres are designed with the end function in mind: their application and cell type. Porosity, cell microenvironment, and degradability are some primary considerations. Customizability to fit applications is not based so much on the choice of fabrication technique but on the polymers, crosslinking parameters (e.g., polymer concentration, temperature, crosslinker type, and duration of crosslinking), and postfabrication modifications. The fabrication technique is generally chosen based on the desired simplicity, scalability, size, and size distribution (see Figure S1 and Table S1 in the supplementary information online). For example, if uniformly sized microspheres are required for ease of monitoring *in vitro*, microfluidics and extrusion techniques are preferred to emulsions, because the microspheres thus produced have a maximum 5% variation in diameter [4].

Biocompatibility, specific porosity, cell-adhesive properties, or controlled degradation are conferred via choice of polymer (see also Table S2 in the supplementary information online) and their modifications, such as coatings and crosslinker choice (Figure 1). Both naturally derived and synthetic polymers have been used as cell-compatible materials, and each has unique advantages and disadvantages. Low-cost and biocompatible naturally derived polymers, such as alginate, suffer batch-to-batch variation and immunogenicity, whereas expensive and controllable synthetic polymers, such as poly(lactic-co-glycolic) acid (PLGA), have biocompatibility issues, especially with their degradation products. As such, naturally derived polymers require stringent and certified purification protocols to qualify as clinical-grade materials.

Porosity determines the diffusion and size of particles entering the microsphere. For immune isolation, the surface porosity of the microcapsule must prevent immunoglobulin, antibody, and immune cell intrusion without compromising the exchange of metabolites. Polycation poly (L-lysine) coating reduces the surface porosity, and is often masked by another alginate coating, because it can promote **inflammation**. Microcapsules of this kind are commonly known as alginate-poly-L-Lysine (PLL)-alginate (APA) microcapsules (Figure 1A) [5–7]. To date, there is no standardized porosity or molecular weight cut off (MWCO) for microcapsules, but scientists estimate a safe limit at approximately 150 kDa (corresponding to the MW of immunoglobulins). More in-depth studies must be performed to ascertain a safe MWCO for future clinically useable microcapsules. By contrast, for other applications that require infiltration of cells into microspheres, these may be fabricated with large pores either via double emulsion or stacked microfluidic devices.

The need for cell-adhesive moieties on microspheres depends on what cell type is to be encapsulated. Cells are mainly divided into **anchorage-** or **nonanchorage-dependent cells** (ADCs, nonADCs), the former requiring extensive adhesion to a substrate and displaying a spreading morphology, otherwise they undergo **anoikis**. Several naturally derived and most synthetic polymers lack cell-adhesive properties required for the survival of ADCs. This limitation can be overcome by blending, coating, or conjugating cell-adhesive moieties, which can be

Glossary

Allogeneic: transplantable product that is sourced from a different individual, but of the same species.
Anoikis: a homeostatic process in ADCs to undergo programmed cell death (apoptosis) when there is insufficient, inappropriate, or no adhesion to a substrate. This mechanism is the innate switch of a cell to undergo apoptosis upon detachment so as to avert dysplastic proliferation, a key step in tumor formation and metastasis.
Anchorage-dependent cells (ADCs): cells that require extensive adhesion to a substrate and exhibit a spreading morphology; includes most cell types, including endothelial, neuronal, and muscle cells.
Dedifferentiation: the process of cells losing their typical morphology and their cell-specific functions when cultured in a monolayer long-term.
Differentiation: the process of stem cells changing to a downstream, specialized cell type.
Foreign body response (FBR): end-stage inflammatory response characterized by fusion of macrophages on a biomaterial surface and fibrous capsule formation as part of wound healing.
Immunogenicity: the ability of a compound to induce a humoral (antibody-mediated) or cell-mediated immune response
Inflammatory response/ inflammation: a host response towards the implanted material that leads to immune cell activation and production of cytokines.
Lower critical solution temperature (LCST): the temperature below which the polymer changes from a gel into a solution.
Macroporous: a microcarrier that contains large pores, allowing cells to penetrate and settle inside it.
Microcapsule: a microsphere that has a protective capsule over its encapsulated biological cells or drugs.
Microcarrier: a microsphere that presents cells or drugs on its surface. The microsphere is usually fabricated before seeding of cells.
Nonanchorage-dependent cells (nonADCs): cells that do not require (extensive) adhesion to survive and typically have a rounded morphology. They may be colony forming in soft substrates (e.g., chondrocytes and pluripotent stem cells) or in