Chapter 8

Encapsulation of Transgenic Cells for Gene Therapy

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Additional information is available at the end of the chapter

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Abstract

A major challenge to emerging cell-based medicine including gene therapy is the host immune rejection of transplanted donor cells or engineered tissue. One way to address this problem is to use drugs to achieve immunosuppression. However, suppressing the patient's immune system may put the patient at risk for many other diseases. An alternative is to encapsulate living cells in macro/microcapsules to achieve immunoisolation of the cells, thereby increasing cell viability in the patient's body following transplantation. The capsule's membrane protects the encapsulated cells from being damaged by both the host's immune system and mechanical stress while allowing free diffusion of nutrients and metabolic waste for the cells to survive. Moreover, the membrane could be designed to achieve controlled and/or sustained release of therapeutic products produced by the encapsulated transgenic cells to treat a variety of diseases such as cardiovascular disorders, anemia, wounds, bone fractures, and cancer.

Keywords: Cell microencapsulation, Encapsulation, Microcapsules, Gene therapy, Cell-based medicine

1. Introduction

Cell encapsulation is the process of entrapping cells into a matrix. In general, the matrix is spherical in shape and in the form of a polymeric hydrogel. Cell encapsulation technology has shown great promise for immunoisolation and controlled release of therapeutic products towards gene therapy. Figure 1 demonstrates the mechanism of encapsulated transgenic cells for gene therapy.

1.1. Encapsulation materials

Both natural and synthetic polymers have been utilized for cell encapsulation. Natural polymers that have been used include alginate, agarose, collagen, and hyaluronic acid, while synthetic polymers, including poly(vinyl alcohol), poly(lactic-co-glycolic acid), polyacrylates, HEMA-MMA-MAA, polyphosphazines, and polyepoxides, have been studied.[1] Natural polymers are more commonly used because of their biocompatibility and are easily accepted by the public. However, their product quality and characteristics can vary greatly between companies and batches compared to synthetic polymers. Alginate, agarose, and polylactide-co-glycolide (PLGA), the most commonly used encapsulation materials, are introduced here.

1.1.1. Alginate

Alginates, polysaccharides, are linear block polymers consisting of α-l-guluronic acid (G) and β-d-manuronic acid (M) blocks (Figure 2). Divalent cations, such as Ca^{2+}, Ba^{2+}, and Sr^{2+}, can link alginate molecules together (i.e. through ionic cross-linking) forming alginate hydrogel capsules while encapsulating cells inside. The G and M contents of the alginate molecules can affect the gel properties including mechanical strength, biocompatibility, and permeability.[2–6] Recently, it has also been shown that oligochitosan could be used as a cross-linker for polysaccharide-based gel formations.[7]

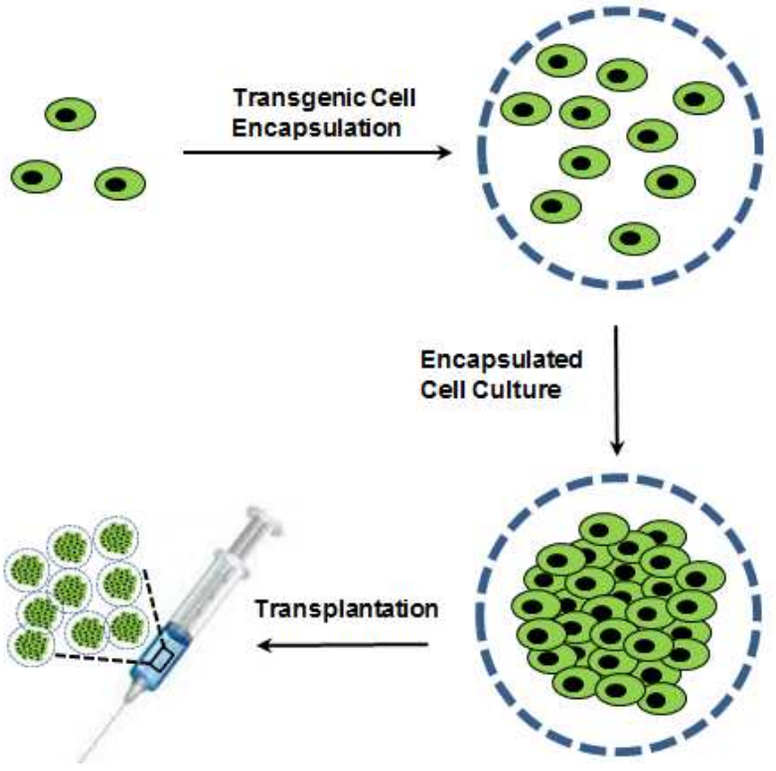

Figure 1. A conceptual schematic demonstrating cell encapsulation for gene therapy.

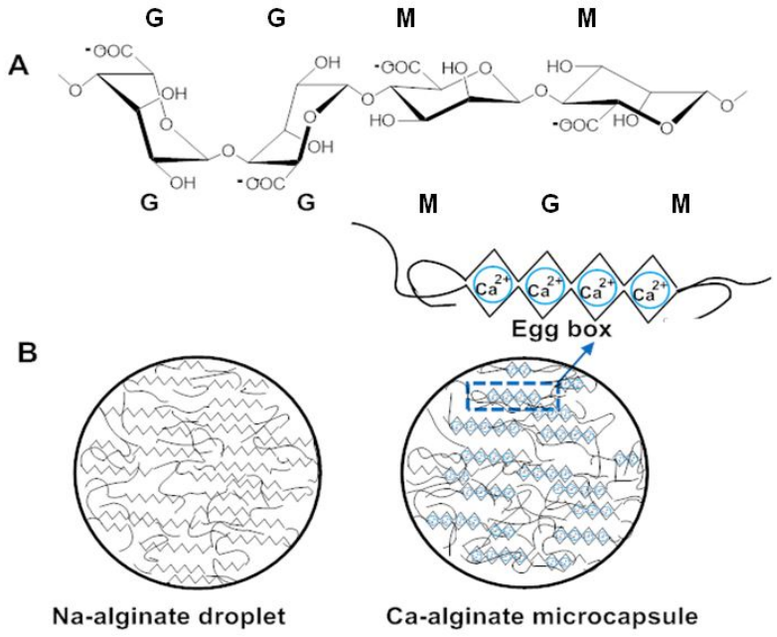

Figure 2. Chemical structure of alginate (A) and alginate-based hydrogel formation mechanism (B).

1.1.2. Agarose

Agarose, a thermal-responsive polymer, consists of β-d-galactopyranose and 3,6-anhydro-α-l-galactopyranose units which can undergo a sol–gel transition upon cooling (i.e. through thermal cross-linking) (Figure 3). Some agarose products have a transition temperature close to body temperature, making it a good candidate for cell encapsulation.[8]

1.1.3. Polylactide-co-Glycolide (PLGA)

PLGA polymers belong to aliphatic polyesters and are biodegradable (Figure 4). To prepare the capsules, PLGA is dissolved in methylene chloride, and then a second component is added to precipitate the polymer molecules (interfacial precipitation).[1,9]

1.2. Encapsulation technologies

Different technologies have been used for preparing macro/microcapsules, which include air-jet encapsulation, electrostatic spray, laminar jet breakup, and microfluidic channel/nozzle. Among them, electrostatic spray and microfluidic channel/nozzle are two of the most frequently used encapsulation approaches.[10]

1.2.1. Electrostatic spray method

The electrostatic spray method has a significant appeal due to its ease of operation, scale-up capabilities, negligible damage to cells, and allowance for sterile operation conditions.[10] The mechanism of cell encapsulation by using the electrostatic pray method is shown in Figure

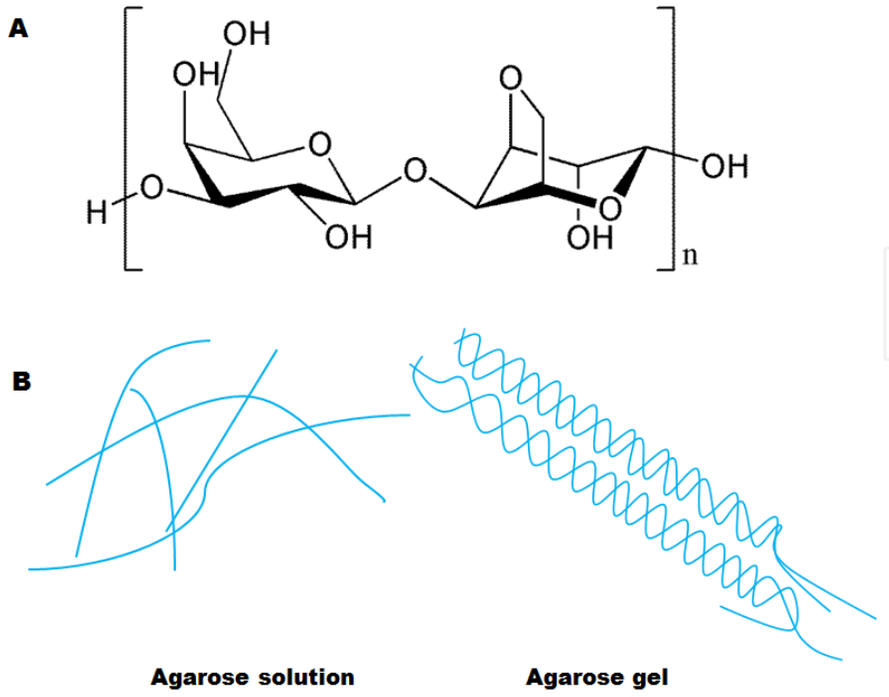

Figure 3. Chemical structure of agarose (A) and agarose-based hydrogel formation mechanism (B).

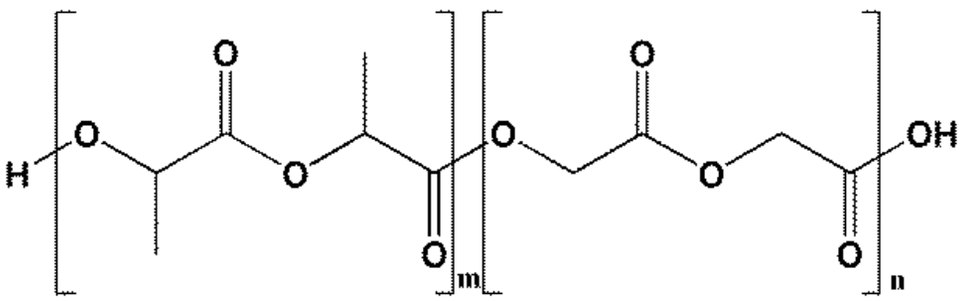

Figure 4. Chemical structure of PLGA.

5A. In general, a cell polymer mixture is extruded through a nozzle by using a pump or compressed air. The droplets are broken down into smaller ones under electrostatic force and/or other introduced forces (e.g. vibration). Once the droplets reach the gelling bath containing the cross-linkers, the cell-loaded hydrogel capsules form immediately through various forces, such as ionotropic reaction between divalent ions and alginate molecules. Moreover, the system could be modified to prepare the core-shell structure hydrogel capsules, as depicted in Figure 5B.[11]

1.2.2. Microfluidics channel/nozzle method

Microfluidics devices can be used to generate micrometer-scale droplets with a narrow size distribution and controlled morphology.[12–14] This method shows great promise for cell

encapsulation, especially for single cell encapsulation.[15] In general, capsules are formed by allowing a core fluid to be surrounded by a flowing sheath stream.[16] Recently, these devices have also been successfully applied for the generation of cell-loaded core-shell capsules (Figure 6).[14] Besides the relatively low encapsulation efficiency, a significant drawback of the current microfluidic technologies is that the oil used for shearing may leave a residual adhesive oil layer on the capsule which affects subsequent coating processes.[10,17]

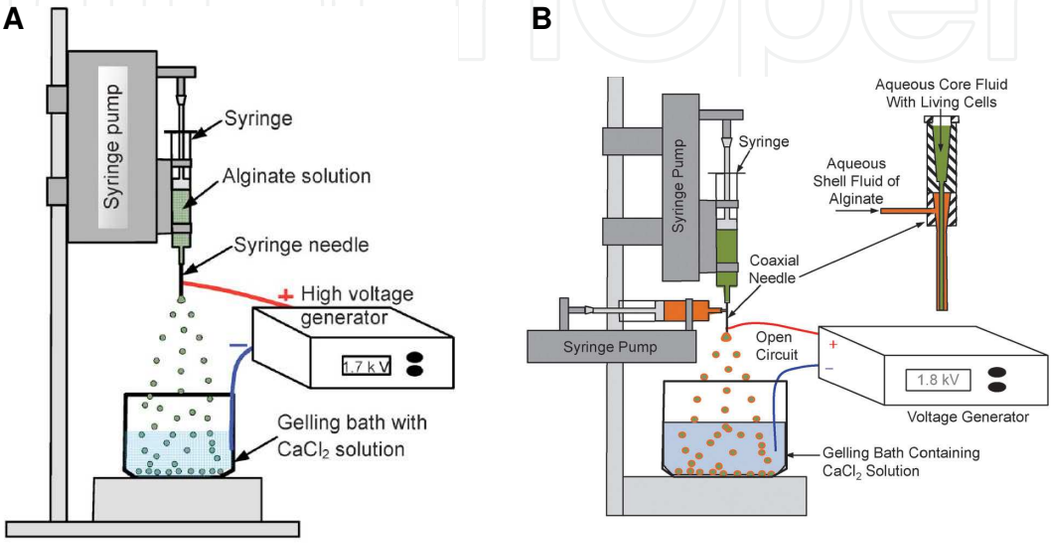

Figure 5. A sketch of the electrostatic spray device used for generating polymeric hydrogel capsules (A).[10] *Reproduced by permission of The American Society of Mechanical Engineering (ASME)*; A modified electrostatic spray setup for fabricating the core-shell structure hydrogel capsules (B).[11] *Reproduced by permission of The Royal Society of Chemistry.*

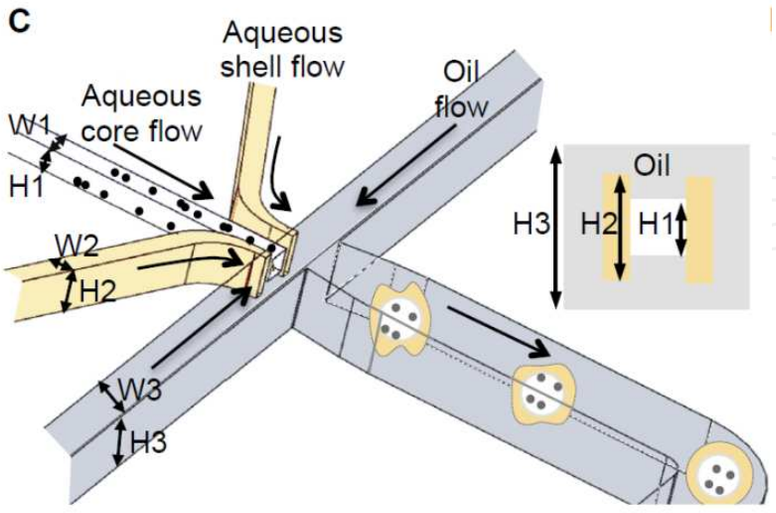

Figure 6. A sketch of the microfluidics device for generating core-shell hydrogel capsules. The core channel height (H1) is the lowest. H: height and W: width.[14] *Reproduced by permission of The Royal Society of Chemistry.*

2. Recent progress on transgenic cell encapsulation for gene therapy

Encapsulation of genetically modified cells has been conducted for the treatment of central nervous system diseases, cardiovascular disorders, mucopolysaccharidosis type VII (MPSVII) disease, wounds, bone fractures, and cancer.[18–30] Considering most genetically engineered cells are from allogeneic or xenogeneic sources, immunoisolation is a critical factor when using these cells.[5]

2.1. Bone-related diseases

Bone morphogenic protein-2 (BMP-2) is a member of the transforming growth factor-β (TGF-β) superfamily and has been widely reported to have osteoinductive activity. Ding *et al.* [31] studied the behaviour of BMP-2 gene-transfected bone marrow-derived mesenchymal stem cells in alginate-poly-l-lysine-alginate (APA) microcapsules. The results showed that encapsulated transfected cells could secrete BMP-2 proteins for at least 30 days and the APA microcapsules could be used for immunoisolation. Olabisi *et al.* [28] investigated microencapsulation of AdBMP-2-transduced MRC-5 cells (human diploid fetal lung fibroblasts) in poly(ethylene glycol) diacrylate (PEGDA) hydrogels. After injecting the encapsulated cells intramuscularly, the volume of the bone formed was about twice that of the control group (unencapsulated cells). Recently, rapid heterotrophic ossification by using cryopreserved PEGDA encapsulated BMP-2 expressing mesenchymal stem cells (MSCs) was also observed (as shown in Figure 7).[32] Additionally, human calcitonin delivered by microencapsulated recombinant myoblasts showed potential for allergenic gene therapy for postmenopausal osteoporosis. [33] Furthermore, transplantation of fibrin glue-compounding hepatocyte growth factor-transgenic MSCs is a promising novel method for avascular necrosis of the femoral head (ANFH) therapy.[34]

2.2. Cancer

Both mouse myoblasts (C2C12 cells) and human embryonic kidney 293 (HEK293) cells were engineered to continuously secrete angiostatin, and were encapsulated into alginate-based microcapsules for cancer treatment. The *in vivo* experimental results demonstrated the potential for angiostatin-mediated cancer therapy by using an encapsulated transgenic cell-based approach.[35,36] Considering immunotherapies have been proven to be alternative strategies for malignancy treatment[37], combined immunotherapy (an interleukin 2 fusion protein, sFvIL-2) and antiangiogenic therapy (angiostatin) were tested. It was shown that transplantation of angiostatin expression and sFvIL-2-expressing C2C12 cells encapsulated in APA microcapsules improved the survival rate of experimental animals.[38] Recently, microencapsulation of therapeutic antibodies producing cells in APA microcapsules was tested for cancer treatment. [39] Additionally, with the advancement of stem cell research, there is an increased potential for cancer therapy by using encapsulated stem cells.[40]

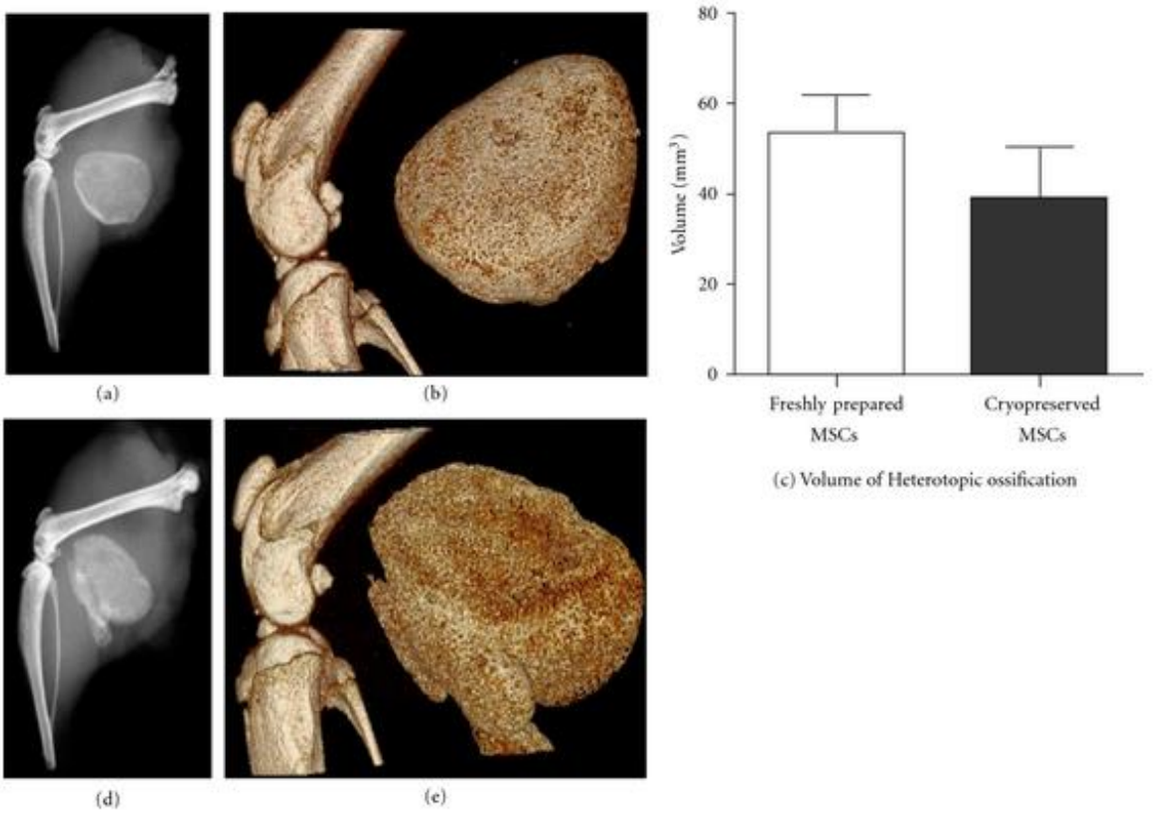

Figure 7. Microencapsulated BMP2-transduced MSCs in a mouse model for heterotopic ossification. X-ray and MicroCT images of the resulting heterotopic ossification for freshly prepared BMP2 microencapsulated MSCs (a and b) and for cryopreserved BMP2 microencapsulated MSCs (d and e).[32]

2.3. Neural diseases

Parkinson's disease (PD) belongs to a group of conditions called motor system disorders, resulting from the loss of dopamine-producing brain cells.[41] This disease could be amenable to gene product replacement strategies including implantation of encapsulated transgenic cells.[42] There are several publications regarding encapsulated cell biodelivery of glial cell line-derived neurotrophic factor (GDNF) for PD treatment; GDNF has been proven to have neuroprotective and neurotrophic properties on dopaminergic neurons.[26,43,44] Furthermore, encapsulated transgenic cells could be utilized in brain tumour treatment.[45,46]

Small capsules (<200 μm) have been developed for the delivery of gene products, secreted by encapsulated transgenic cells, to the brain, bypassing the blood–brain barrier (BBB). To date, several alginate-based microcapsule systems, Ca-alginate, APA, and alginate-chitosan-alginate (ACA), have been reported.[10,47,48] Encapsulation of transgenic cells has also been used for other disease treatments, such as mucopolysaccharidosis VII and myocardial infarction. Table 1 summarizes the recent gene therapy studies based on encapsulated transgenic cells, with the exception of bone-related and neural diseases and cancer treatment.

Disease	Therapeutic Product(s)	Cell Type	Encapsulation System	Ref.
Fabry disease	α-Galactosidase A	Chinese hamster ovary cells	Semipermeable Polymer Fiber	[49]
Mucopolysaccharidosis VII	β-Glucuronidase	Mouse 2A-50 fibroblasts	Alginate-poly-l-lysine	[50]
		Human amniotic epithelial cells	Polymer (polysulfon) Hollow fibers	[23]
Myocardial infarction and wound	Glucagon-like peptide-1	Human mesenchymal stem cells	CellBeads™	[51]
	Vascular endothelial growth factor	Chinese hamster ovary cells	Alginate-Poly-l-Lysine-Alginate Microcapsules	[27]
		Adipose stem cells	AP-PLL-brPEG microcapsules	[52]
		NIH3T3 cells	Alginate-barium microcapsules	[21]
		Human umbilical cord mesenchymal stromal cells	Alginate-barium microcapsules	[53]
		Human umbilical cord mesenchymal stem cells	Alginate-barium microcapsules	[54]
Polycythemic diseases	Erythropoietin	Mouse C2C12 myoblasts	Semipermeable polyethersulf hollow fibers	[55]
Hypertension and/or congestive heart failure	Atrial natriuretic peptide	Chinese hamster ovary cells	Polycaprolactone tubes	[56]
Acute skin flap ischemia	Basic fibroblast growth factor (FGF-2)	Mouse C2C12 myoblasts	Microporous polyethersulfone hollow fibers	[57]
Hemophilia B	Factor IX	Mouse C2C12 myoblasts mouse C2C12 myoblasts	Alginate-poly-l-lysine-alginate microcapsules	[58]
			Alginate-poly-l-lysine-alginate and alginate-poly-l-arginine-alginate microcapsules	[59]
Laron syndrome	Recombinant human IGF-1	Pig Sertoli cells	Alginate microcapsules	[60]

Table 1. Recent gene therapy studies by using encapsulated transgenic cells

3. Challenges and future direction

Recent clinical trials regarding gene therapy by using encapsulated transgenic cells are summarized in Table 2. For eventual clinical applications of encapsulated transgenic cells for gene therapy, however, there are still some issues that need to be addressed.[62,63]

1. Protrusion of encapsulated cells

Cell growth leads to protrusion of cells over time, which may cause the failure of immunoisolation following *in vivo* transplantation. Bhujbal *et al.* reported a novel multilayer immunoisolating encapsulation system aiming to prevent cell protrusion without compromising cell survival (Figure 8).[64]

2. Scaling-up cell microencapsulation

Cell encapsulation processes are usually performed at the lab scale. For successful clinical applications, massive production of encapsulated cells following good manufacturing practices (GMP) standardized procedures [65] for transplantation is critical. Different designs have been reported for scaling-up cell encapsulation. One design based on a 3D microfluidic approach, which contains a 3D air supply and multinozzle outlet, has been reported recently.[17]

3. Monitor and control the encapsulated transgenic cells

Once the therapy has reached its goal or when undesirable deleterious effects occur, noninvasive monitoring and deactivation/elimination of the encapsulated cells are critical for clinical practice.[63] Recently, Shen *et al.* [66] reported the encapsulation of recombinant cells by using a magnetized ferrofluid alginate for *in vivo* monitoring by magnetic resonance imaging (MRI). Moreover, magnetic field-controlled gene expression in encapsulated cells, coencapsulated with magnetic nanoparticles, has been reported. The cells were modified to produce therapeutic products under the control of a heat-inducible promoter. Heat induction could be achieved by elevating the temperatures of the capsules through coencapsulated magnetic nanoparticles subjected to a magnetic field (Figure 9).[67] Catena *et al.* reported an interesting and smart system which shows potential for monitoring encapsulated cells and selectively eliminating them at a specific moment by using the $SFG_{NES}TGL$ triple reporter system.[68]

Project	Therapeutic Product(s)	Target Disease(s)	Phase	Status
A study of encapsulated cell technology (ECT) implant for patients with late stage retinitis pigmentosa	Ciliary neurotrophic factor (CNTF)	Late-stage retinitis pigmentosa	II and III	Completed
A study of encapsulated cell technology (ECT) implant for	Ciliary neurotrophic factor (CNTF)	Early stage retinitis pigmentosa	II and III	Completed

Project	Therapeutic Product(s)	Target Disease(s)	Phase	Status
participants with early stage retinitis pigmentosa				
A Study of an Encapsulated Cell Technology (ECT) Implant for Patients With Atrophic Macular Degeneration	Ciliary neurotrophic factor (CNTF)	Macular degeneration	II	Completed
Pilot immunotherapy trial for recurrent malignant gliomas	Insulin-like growth factor receptor-1	Malignant glioma of brain	I	Completed
GLP-1 CellBeads® for the treatment of stroke patients with space-occupying intracerebral hemorrhage	Glucagon-like peptide-1	Intracerebral hemorrhage (ICH)	I and II	Terminated
CNTF implants for CNGB3 achromatopsia	Ciliary neurotrophic factor (CNTF)	Eye disease achromatopsia	I and II	Active
Retinal imaging of subjects implanted with ciliary neurotrophic factor (CNTF)-releasing encapsulated cell implant for early-stage retinitis pigmentosa	Ciliary neurotrophic factor (CNTF)	Early stage retinitis pigmentosa or Usher syndrome (type 2 or 3)	II	Recruiting
A phase 2 multicenter randomized clinical trial of CNTF FOR MacTel	Recombinant human ciliary neurotrophic factor	Macular telangiectasia type 2	II	Recruiting
MVX-ONCO-1 in patients with solid tumours	Irradiated autologous tumour cells	Solid tumour cancer	I	Recruiting
Study of the intravitreal implantation of NT-503-3 encapsulated cell technology (ECT) for the treatment of recurrent choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD)	Anti-VEGF therapy	Macular degeneration	I and II	Not yet recruiting
Encapsulated cell biodelivery of nerve growth factor to Alzheimer´s disease patients	Nerve growth factor (NGF)	Alzheimer's disease	I	Unknown

Table 2. Clinical trials of gene therapy involving encapsulated transgenic cells [61]

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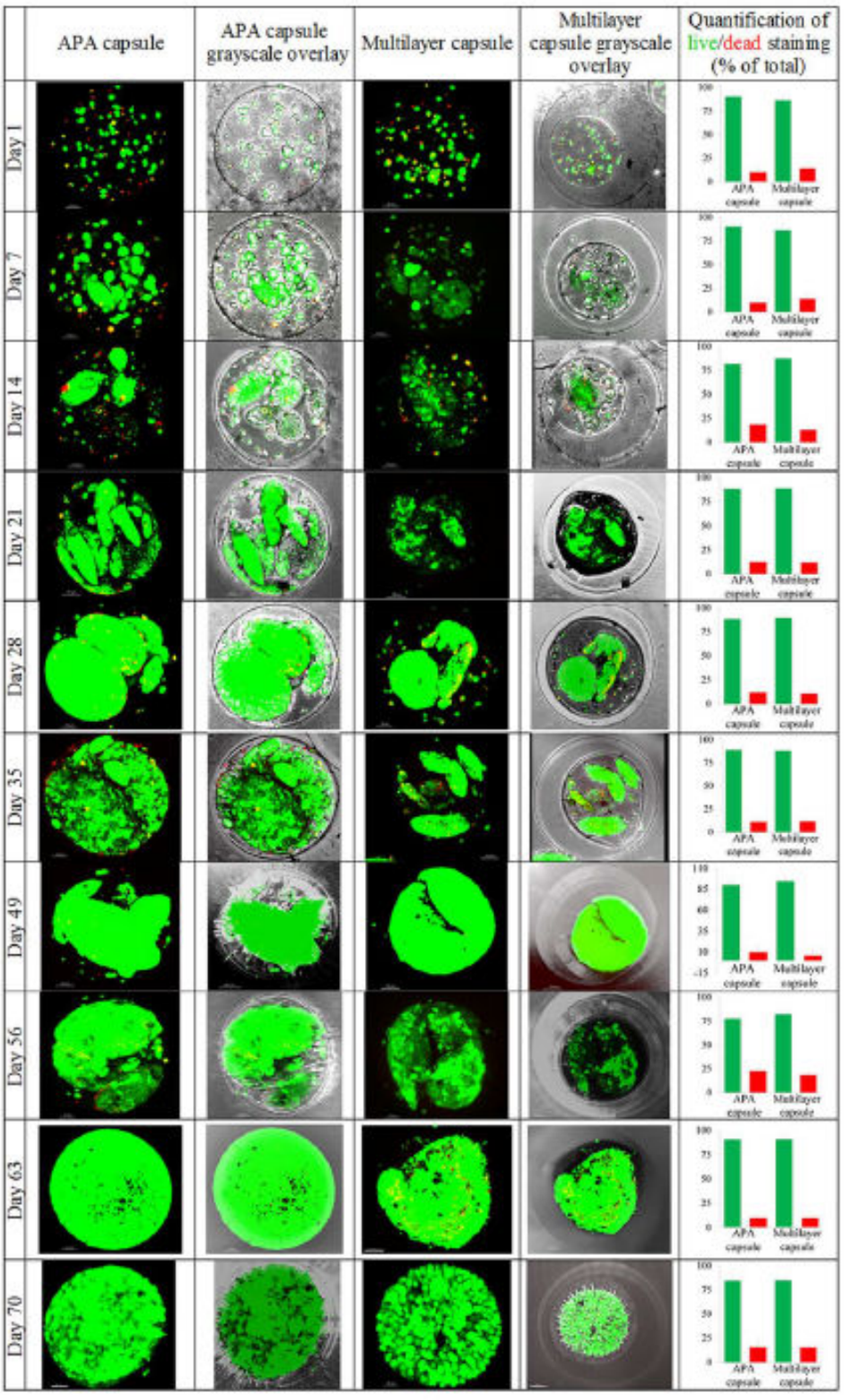

Figure 8. Cell growth within common APA capsules and multilayer capsules. Live cells were stained green while dead cells were stained red.[51]

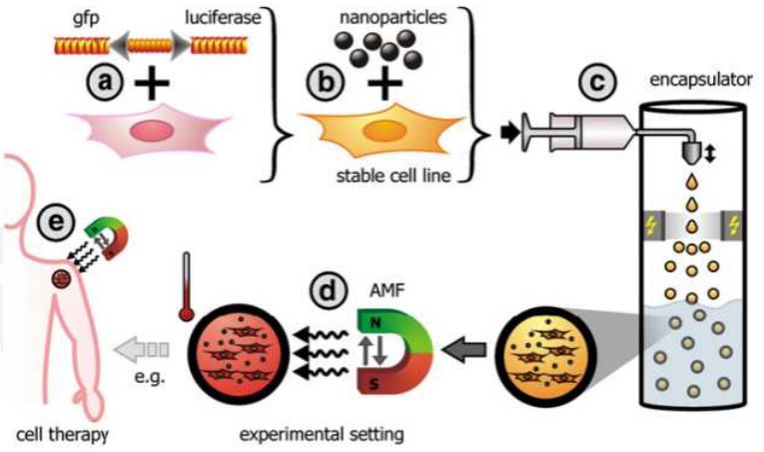

Figure 9. Schematic representation of the magnetic field-controlled gene expression in encapsulated cells.[67]

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