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Application of cell encapsulation for controlled delivery of biological therapeutics ☆

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ABSTRACT

Cell microencapsulation technology is likely to have an increasingly important role in new approaches rather than the classical and pioneering organ replacement. Apart from becoming a tool for protein and morphogen release and long-term drug delivery, it is becoming a new three-dimensional platform for stem cell research. Recent progress in the field has resulted in biodegradable scaffolds that are able to retain and release the cell content in different anatomical locations. Additional advances include the use biomimetic scaffolds that provide greater control over material–cell interactions and the development of more precise encapsulated cell-tracking systems. This review summarises the state of the art of cell microencapsulation and discusses the main directions and challenges of this field towards the controlled delivery of biological therapeutics.

Contents

1. Introduction

For over four decades, different types of biomaterials extracted and fabricated from natural and synthetic polymers have been intensively used as drug delivery systems. Apart from designing new vehicles for drug, protein and growth factor delivery, a significant effort has been devoted to create artificial three-dimensional (3D) scaffolds that can hold, carry and protect cells from the external environment [1–5]. These scaffolds should provide an adequate microenvironment for the enclosed cells, promoting and controlling their viability, proliferation and release of therapeutic products. The latter has fueled the development of several cell-therapy strategies for drug and cell delivery, which are currently being used for organ replacement, tissue engineering and regenerative medicine purposes.

Cell microencapsulation represents one of these strategies that aims to overcome the present difficulties related to whole organ graft rejection including the scarcity of functional organs and the side-effects associated with the use of immunomodulatory protocols or immunosuppressive drugs [6–8]. In addition, this cell-based technology is gaining more attention from the scientific community due to its therapeutic potential in many other fields rather than organ replacement. Not surprisingly, this has renewed the excitement and hopes for this cell-based technology.

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

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For example, cell microencapsulation can be used to release proteins and morphogens for long-periods of time [9], becoming an interesting platform for medium and long-term drug delivery. In addition, the field has evolved towards the design and fabrication of active 3D scaffolds that can be used either to monitor enclosed cells and provide real-time protein delivery [10] or to develop biomimetic scaffolds by means of including peptides within the matrix that regulate the fate of the enclosed cells [11]. Interestingly, the field is also becoming a new tool for stem cell research. In fact, it is a cost-efficient way to study and promote stem cell growth and differentiation in a 3D environment [12] while, at the same time, it is an exciting platform for cell retention and delivery in different anatomical locations [13]. These new scientific avenues have demanded important biological, technological and pharmaceutical challenges including the development of both long-term durable but also fast-degradable 3D microcapsules, the fabrication of magnetocapsules for non-invasive cell monitoring, new genetic tools for improving the biosafety of the technology, reduced-sized particles for better access to the Central Nervous System (CNS) and the eye, and novel biomimetic particles for stem cell research.

Cell microencapsulation has encountered several hurdles in its quest towards application, but it is not an alien to the clinical experimental setting as different clinical trials have been performed or are currently ongoing. This has provided a range of promising therapeutic approaches for diabetes, anemia, cancer and CNS among others [14].

The rationale of this review is to describe and outline the current state of cell microencapsulation technology with respect to its potential

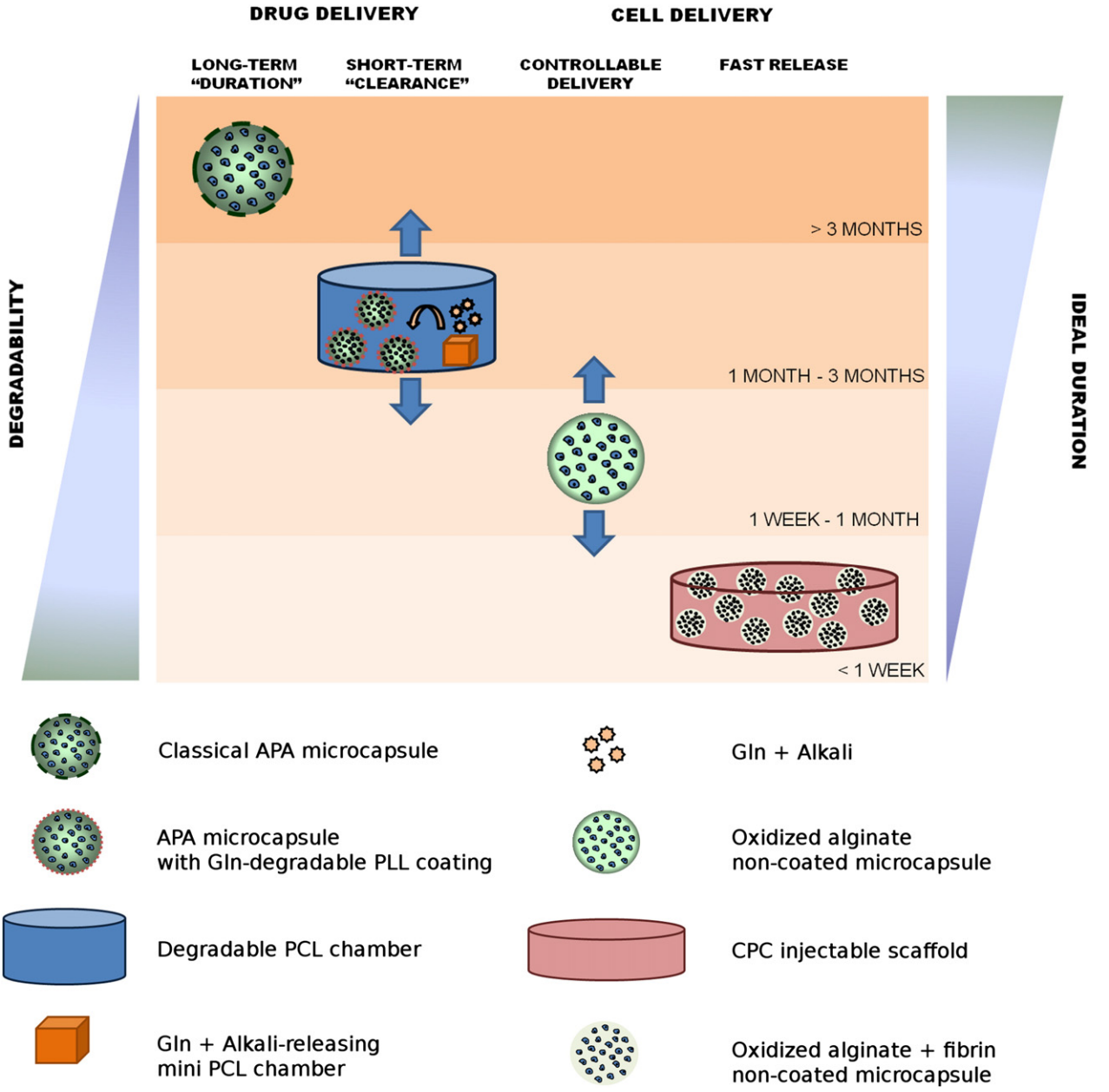

Fig. 1. Schematic illustration of the different degradation vs duration strategies according to the intended application. Drug delivery applications usually require microcapsules with higher durability. The classical APA design provides enough durability to sustain prolonged drug releases for several months, whereas the degradability of these biosystems is very low. For those drug delivery applications where degradability and final clearance of the scaffold is desired, it is proposed the use of newly formulated microcapsules coated with a glutamine (Gln)-degradable PLL which are introduced within a biodegradable poly(ε-caprolactone) (PCL) nanoporous chamber, together with glutamine releasing poy(lactic-co-glycolic acid) pellets. Cell delivery applications typically demand lower durability and higher degradation rates, so external coating is avoided in most cases. Alginate matrices are partially oxidated to create controllable numbers of functional groups susceptible to hydrolysis. Thus, the degradation rate may be adjusted by just varying the oxidation degree of the backbone. In case even higher degradability is required (i.e. when capsules are meant to release cells within injectable scaffolds such as calcium phosphate cements (CPC)), the use of oxidized alginate-fibrin microbeads is proposed, which are able to release the enclosed cells from the fourth day.

in the delivery of biological therapeutics including peptides, proteins, morphogens and cells. The therapeutic versatility of this strategy is mainly based on the duration/degradation rate of the particles, demanding more durable carriers in the case of drug delivery while needing more degradable scaffolds in the case of cell delivery and tissue engineering (Fig. 1). This therapeutic but also technological duality will be described and discussed in detail together with some of their most relevant applications. Finally, we will highlight the latest research, trends, opportunities and challenges to integrate biology, genetics, material science, medicine and pharmacy to develop cell-loaded microcapsules for controlled delivery of biological therapeutics.

2. Cell encapsulation as a platform for drug delivery

It is more than probable that the initial historical evidences of this technology correspond to the seminal reports from Bisceglie showing that cells transplanted in immunoprotective membranes could survive long enough to conclude that they were not destroyed [15]. Later on, Chang pioneered the term of "artificial cells" to describe how cell encapsulation could be used for the immunoprotection of transplanted cells [1]. The technology was successfully put in practice in the 1970s and 1980s to immobilize xenograft islet cells to aid in glucose control for diabetes in small animal models [16]. Since then, the technology has evolved not only from a technological point of view but also from a biological and pharmaceutical perspective.

Cell microencapsulation consists in the immobilisation of bioactive materials, mainly cells, within a microparticle, generally surrounded by a polymeric membrane. The latter permits the free passage of nutrients and oxygen and the egress of therapeutic protein products. The semipermeable membrane of the device would prevent high molecular weight molecules (>150 kDa), antibodies and other immunologic moieties from contacting the encapsulated cells and destroying them as foreign invaders [6,8]. Encapsulation allows the protection of the cell content from mechanical stress and in the case of allogeneic tissue also from host's immune response. In general, the size of the particles may range between 100 and 700 μm, although reduced-size particles are preferred due to their excellent surface-volume ratio and oxygen permeability properties. A suitable relation between the stability, biocompatibility, durability and diffusional properties of the immunoisolation device will guarantee the long-term functionality of the cells, thereby allowing long-term drug delivery and the treatment of chronic diseases [6–8].

Microcapsules are generally produced from hydrogels, which show a number of interesting properties including the high water content and the necessity of mild conditions for microcapsule preparation. The former is essential for the enclosed cells as it will provide the hydrated environment necessary to facilitate the biochemical, cellular, and physical stimuli that guide cellular processes such as differentiation, proliferation, and migration [17]. In addition, hydrogel-based particles can be easily prepared and scaled-up. The soft and pliable features of the hydrogels avoid, or at least reduce, the frictional or mechanical irritation to surrounding tissue, an outstanding issue when cell delivery or stem cell therapeutic approaches are needed. Other additional advantages of hydrogels include the biocompatibility and optimal permeability properties. In fact, hydrogels provide a high degree of permeability for low molecular weight nutrients and metabolites. Additionally, due to their hydrophilic properties, there is virtually no interfacial tension with surrounding tissues and fluids, minimizing cell adhesion and protein adsorption, and enhancing microcapsule biocompatibility [18]. Nonetheless, biocompatibility is still dependent on other factors such as the remaining impurities derived from the natural sources of these biomaterials.

One critical parameter when designing and fabricating cell-loaded microcapsules relies on their permeability properties. The permselective capsule environment has been shown to support cellular metabolism, proliferation, differentiation and cellular morphogenesis [18,19]. Many studies have evaluated the permeability properties of a wide range of cell-loaded microcapsules although no systematic approach has been taken to determine the permeability requirements of each cell type. In general, the molecular mass cut-off (MWCO; size of the largest molecule that is not substantially blocked by the semipermeable membrane) of the particle is determined to know the upper limit of capsule permeability. MWCO of the microcapsule should be tailored according to type of particle, material, cell and probably therapeutic application. For example, for most drug delivery applications, the preferred MWCO ranges from 70 to 150 kDa.

Immobilisation of therapeutically active cells offers important advantages when compared to the encapsulation of peptides, including the secretion of "de novo" produced therapeutic proteins and peptide delivery regulation as a function of physiological requirements. This enables not only enhanced chemical stability but also better efficiency. As a result, there is a wide range of experimental studies showing that after implantation, release of therapeutically active molecules from encapsulated cells can be prolonged and controlled from weeks to months, independently of the administration way or target tissue. In the following chapters, some of the most recent and interesting applications of cell microencapsulation as a tool for drug delivery in the treatment of different disorders ranging from CNS diseases to tissue repair and regeneration will be summarized.

3. Applications of drug delivery from microencapsulated cells

Many studies have demonstrated the pre-clinical feasibility of encapsulation as a means of delivering factors, proteins and morphogens during long-periods of time with the aim of treating or managing several disorders:

3.1. Treatment of chronic anemia

Anemia can appear due to many different factors including blood loss, kidney failure, nutritional factors, and inflammation. The management of anemia in chronic kidney disease patients has contributed to the understanding of treatment of anemia in many human disorders. The most relevant treatment consists in the administration of erythropoietin (Epo), a low-molecular (30.4 kDa) pleiotropic glycoprotein of 165 amino acids [20], which plays an hormonal role in the stimulation and maintenance of erythropoiesis (maturation of erythroid progenitor cells into mature red blood cells (RBC)) and erythrocyte differentiation [21,22]. Apart from this role, increasing evidences suggest a wider biological role of Epo/Epo-R. Many experimental and clinical studies have shown that low levels of Epo mRNA have also been detected in lungs, testes, enterocytes, breast gland and human milk, spleen, bone marrow macrophages, placenta, astrocytes, neurons and the mouse ischemic heart [23–31], suggesting that Epo is a multifunctional trophic factor, with many other physiological roles, a tissue-specific regulation and several mechanisms of action [32]. In addition, Epo and Epo-R have been found to be upregulated in the spinal cord and brain after injury and their protective role has been proven in ischemic animal models. Neuroprotective functions of Epo may be associated with antiapoptosis, antioxidation, neurotrophic action and angiogenesis, being therefore a promising candidate to be tested in the treatment of several neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, glaucoma and spinal cord injury [33].

Our research group selected Epo, C_2C_{12} myoblasts and alginate microcapsules as proof-of-principle to analyse and optimize the technology of cell encapsulation more than 10 years ago (Fig. 2). To address this objective, we enclosed Epo-secreting C_2C_{12} myoblasts in alginate-poly-L-lysine-alginate (APA) microcapsules and implanted them in the peritoneum and subcutaneous tissue of syngeneic and allogeneic mice [9]. Data indicated that implantation of Epo-secreting cell-loaded microcapsules lead to high and constant hematocrit levels for more than 100 days in all implanted mice without implementing

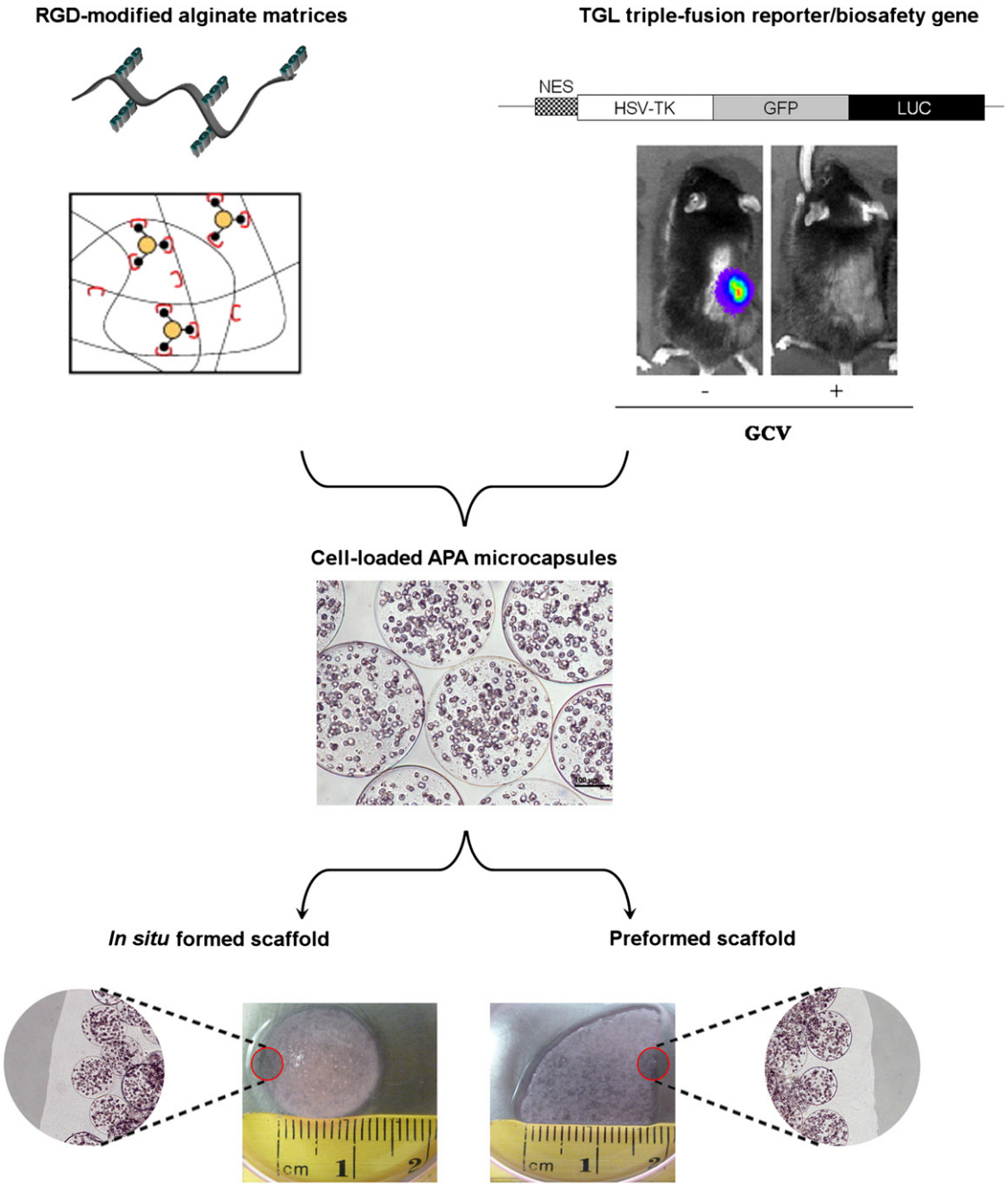

Fig. 2. Technological development of EPO-secreting advanced biosystems. The upper part of the figure represents the improvements regarding both biomaterial and biological aspects that have been implemented in cell microencapsulation (in the middle). Specifically, the upper left panel depicts the RGD-biofunctionalisation of the matrices in order to provide encapsulated cells with most suitable biomimetic microenvironment. Upper right panel shows the incorporation of TGL triple-fusion reporter/biosafety gene in the genome of enclosed cells with the aim of overcoming some of the existing biosafety concerns. Besides, the lower part of the figure illustrates different novel administration ways. In particular, APA microcapsules may be administered within either *in situ* formed (lower left panel) or preformed (lower right panel) hydrogel scaffolds in order to avoid the dissemination of the particles and make easier their retention and localisation for posterior extraction.

immunosuppressive protocols. Interestingly, it was observed that this molecule had additional biological effects for the graft. In fact, the angiogenic and immunomodulatory properties of Epo promoted the formation of a vascularised network surrounding the microcapsule graft and suitable biocompatibility, concluding that it may be an interesting approach for the long-term delivery of Epo. This proof of principle also helped to shed some light on several controversial topics. For example, the influence of the implantation site and the feasibility of using the same approach for allogeneic or xenogeneic transplantation. Furthermore, these studies served to emphasize the relevance of creating a vascularised milieu surrounding the immobilisation device in order to permit close contact between the encapsulated cells and the bloodstream and, thus, improve the long-term efficacy of the graft.

Once the allogeneic model approach based on subcutaneous implantation of microencapsulated Epo-secreting cells was suitably characterized and biomedical grade biomaterials selected as the most biocompatible polymers [34,35], a complete morphological and mechanical evaluation of microcapsules containing Epo-secreting C_2C_{12} myoblasts was carried out. This was followed by a successful xenogeneic approach where Epo-secreting murine C_2C_{12} myoblasts were subcutaneously implanted for 14 weeks in Fischer rats, using transient Tacrolimus (FK-506) immunosuppression [36]. In a

posterior study, we evaluated whether creating 3D alginate scaffolds tailored with biological cues could improve the viability and long-term functionality of the graft as well as the mechanical properties of the encapsulation devices [11]. The search of an ideal extracellular-like environment (trying to mimick the extracellular matrix of tissues) has fueled the development of matrices and biomimetic scaffolds that incorporate integrin-mediated cell adhesion sequences including IKLLI, IKVAV, LRE, PDSGR, YIGSR and the most widely employed arginine–glycine–aspartic acid (RGD), which derives from fibronectin, a natural protein presented in ECM. These moieties trigger a cascade of intracellular signalling events through the focal contacts providing tight control over cell–matrix interactions. In a collaborative project with the research group of David Mooney, we fabricated RGD-enriched alginate capsules to immobilize Epo-releasing cells [11]. Cell integrin-mediated bindings to the RGD moieties acted as additional crosslinking molecules within the alginate matrix, augmenting the mechanical properties and resistance of the capsule against swelling, as well as the value of rupture force. In the same way, the addition of such oligopeptides resulted in a more natural environment for the enclosed cells that, as a consequence, improve their viability and long-term functionality in vivo.

The possibility of tracking cell-containing microcapsules, monitoring cell viability, and controlling the therapeutic activity (external control of the expression of the therapeutic product) represent important challenges for the field of cell encapsulation in its way to the clinical setting. We recently developed myoblasts-containing alginate microcapsules in which cells were transfected with the SFGNESTGL triple-fusion reporter retroviral vector. The latter contained green fluorescence protein (GFP), firefly luciferase and herpes simplex virus type 1 thymidine-kinase (HSV1-TK) [37]. Apart from facilitating capsule monitoring after implantation in animals, treatment of mice with the thymidine-kinase substrate ganciclovir caused death of microencapsulated myoblasts and inactivation of the therapeutic effects [37,38]. This tool may help to control cell location and viability in a non-invasive way. Cell death can be induced by administration of a drug, in case therapy needs to be interrupted.

Other interesting challenges to overcome include the improvement of the retention for posterior retrieval of the microcapsules in the tissue where they are implanted, while reducing post-transplant inflammation. Including the cell-loaded microcapsules within a hydrogel-based scaffold could result of help in these aspects. These hydrogel-based scaffolds could be used as implantable forms (preformed scaffolds) or injectable forms (in situ formed scaffolds), being the main differences between these two types of hydrogels the elaboration protocol and the administration way [39]. The histological analysis of the explanted microcapsules performed 2 months after administration showed that pericapsular overgrowth was reduced when cell-loaded microcapsules were enclosed in the hydrogels scaffolds, whereas hematocrit levels were maintained up to 80% for at least those 2 months [39].

3.2. Myocardial regeneration

Cellular therapy for cardiac repair, or the use of cells to improve cardiac function, has emerged as an alternative approach [40]. Indeed, promising results have been obtained delivering cells of various origins including skeletal myoblast [41], neonatal cardiomyocytes [42], and stem cells [43,44] into de damaged myocardium. However, the low rate of engraftment ("wash out") and survival of these transplanted cells following implantation remains a major challenge in such cases. To overcome massive losses one interesting strategy relies on enclosing cells within microcapsules and increasing the size of the cell-dose. In this way, the contractive forces of the hearth are unable to wash out the capsules into the bloodstream [45]. In a recent paper, Al Kindi et al. confirmed that in cellular cardiomyoplasty, microencapsulation may provide the tool to increase the initial retention of the injected cells. In fact, 200 μm and 400 μm APA microcapsules showed a fourfold increase in retention rate compared with 10 μm microspheres [46].

Biomimetic scaffolds could play a key role to replace the damaged cardiac ECM [47], as a matter of fact, RGD alginate microbeads have been used as a scaffold for hMSCs administration into an injured rat myocardium. Additionally, Yu et al. [48] reported the effectiveness of RGD modified alginate microcapsules for the delivery of stem cells to the myocardium. Results showed that the graft provided structural support to maintain left ventricular (LV) shape and prevented the negative remodeling of the LV following a myocardial infarction.

Recently, our research group has developed a cell encapsulation system based on porcine adipose tissue-derived stem cells (ASCs) encapsulated in APA microcapsules labeled with SPIO (superparamagnetic iron oxide nanoparticles, Endorem®) nanoparticles [49]. We investigated whether these capsules could act as a carrier for the cells, increase their retention and survival in the tissue, and facilitate the localisation and monitoring of the graft by MRI. To address this, cell-loaded particles were implanted in a myocardial infarct porcine model. Cells survived within the three-dimensional (3D) magnetocapsules and were easily localized *in vivo* during the study period (30 days).

3.3. Bone and cartilage defects

Significant progress has been achieved in bone tissue engineering through the combination of biomaterial scaffolds, cells, and biologically active molecules [50]. Although many different biomaterials have been used in cell microencapsulation for bone regeneration purposes, alginate is by far the most frequently employed hydrogel. This is in part due to its high water content, biocompatibility, biodegradability and potential.

Assuming the role that angiogenesis plays in the growth and repair of bone [51], Grellier et al. proposed a new design for bone repair combining human osteoprogenitors and endothelial cells within RGD-alginate microcapsules [52]. Immobilized cells promoted mineralisation after implantation in a long bone defect but results were significantly enhanced when osteoprogenitors were co-immobilized with endothelial cells (Fig. 3). In a similar approach a synergist effect was achieved by combining platelet-rich plasma (a plasma that has been enriched in platelets) and ADSCs microencapsulated in alginate [53]. The addition of platelet rich plasma (PRP) to this scaffold design will, ultimately, provide high quantities of autologous growth factors and cytokines promoting both angiogenesis and bone regeneration [54].

Cartilage tissue has minimal reparative capabilities owing to the decrease in chondrocyte metabolism as a result of disease, injury, and aging [55]. Current cell-based treatments use autologous chondrocytes, which present limitations such as donor site morbidity, inadequate cell numbers, cellular dedifferentiation when cultured in vitro, and the formation of fibrous repair tissue [56]. Mesenchymal stem cells (MSCs) are a potential alternative to autologous chondrocytes. Recently, Dashdart et al. [57] created bilateral full thickness cartilage defects in rabbits studied the cartilage repair potential of both chondrogenic pre-differentiated mesenchymal stem cells (CMSCs) and undifferentiated MSCs encapsulated in alginate particles. Results demonstrated that the use of either MSC or CMSCs provided superior repair potential when compared to cartilage defects that were untreated, and besides, both cells produced comparable treatment outcomes.

3.4. Neurological diseases

Encapsulated cell-based therapies are also under study for their attractiveness as potential treatments for a variety of neurological diseases. In many of the current neurodegenerative disorders, it is extremely necessary the local delivery of different therapeutic agents including neurothrophic factors, angiogenic proteins or combination of both. In addition, most of these molecules are characterized by their short lives and by eliciting significant side effects when delivered systemically. To overcome these drawbacks, neurothrophic factors can be delivered directly to the brain by implantation of encapsulated cells

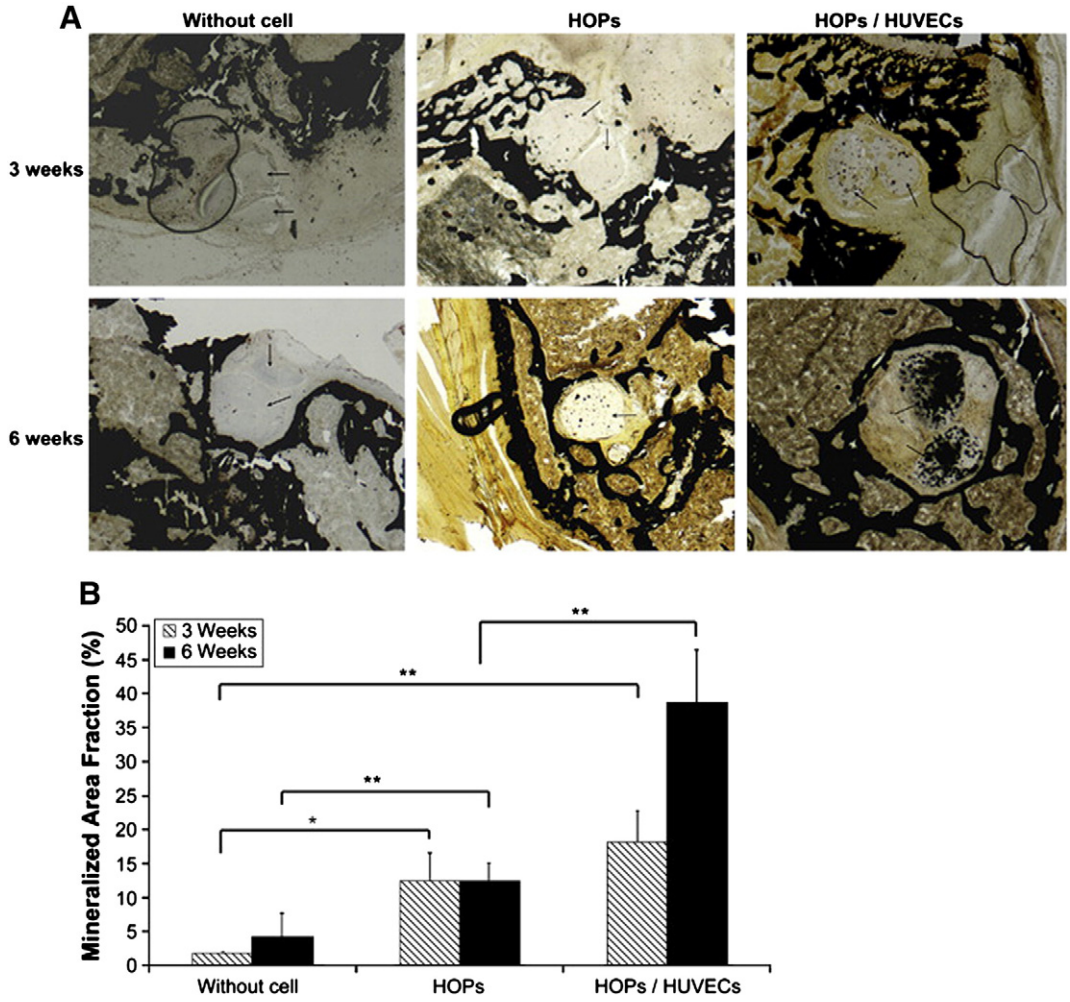

Fig. 3. von Kossa staining of regenerated bone defects and mineralisation quantification. Bone defects were performed in the femoral metaphysis of *nude* mice and RGD-alginate microspheres containing no cells, only human osteoprogenitors (HOPs) or HOPs co-immobilized with human umbilical vein endothelial cells (HUVECs) were implanted. Mineralisation was followed by von Kossa staining after 3 and 6 weeks. (A): Arrows show alginate beads. No mineralisation was observed when alginate microspheres containing no cells were implanted in the bone defects. When only immobilized HOPs were implanted, a few mineralisation nodules were generated 3 and 6 weeks post-implantation. However, co-immobilisation of HOPs and HUVECs led to mineralisation inside the microspheres at 3 weeks post-implantation and mineralisation deposition was clearly observed at 6 weeks post-implantation. Original magnification: ×4. (B): Quantification of mineralisation revealed by von Kossa staining. The presence of cells significantly increased mineralisation of bone defects after 3 and 6 weeks, compared to alginate microspheres implanted with no cells. After 6 weeks implantation of co-immobilized HOPs and HUVECs mineralisation was significantly higher than with HOPs immobilized alone. Significance was calculated by Mann–Whitney's *U* test ($^{*}p \leq 0.05$; $^{**}p \leq 0.01$). Reprinted from [52], © 2009, with permission from Elsevier.

engineered to produce them [58–60]. For example, MSCs releasing glucagon-like peptide 1 (GLP-1), an interesting candidate for the treatment of neurodegenerative disorders as it exhibits neuroprotective, neurotrophic activity [61], and anti-apoptotic effects on neurons, were immobilized in alginate capsules and implanted into the right lateral ventricle in an experimental traumatic brain injury model [62]. Hippocampal neuronal cell loss and neuronal and glial skeletal abnormalities were evaluated reaching to the conclusion that encapsulated cells expressing GLP-1 reduced damage in both cell populations. Based on these successful results, in 2011, a Phase I/II clinical trial in patients with intra-cerebral hemorrhage (ICH) was promoted [63]. In this study, alginate microcapsules containing allogeneic GLP-1-MSCs were implanted into the brain tissue cavity after neurosurgical evacuation of the hematoma. Capsules were removed by second surgery after 14 days of treatment. Although the trial is still ongoing, preliminary evaluation of the first 11 patients revealed no side effects from the surgical interventions and no implant-related side effects. In addition, a total of 30% of the retrieved cells survived the 2-week implantation period and they release GLP-1 after explantation [64].

Similar approaches based on intracerebroventricular injection of encapsulated GLP-1 producing MSCs have been or are under study for the treatment of different disorders including Alzheimer's disease [65] (AD) and amyotrophic lateral sclerosis ALS [66]. According to all these experimental findings, hMSCs enclosed in alginate capsules have demonstrated anti-inflammatory and neuroprotective properties, which seem to be improved using cells delivering GLP-1 peptide. Therefore, although additional studies evaluating the biosafety of the approach are necessary, GLP-1-secreting hMSCs capsules may result a promising alternative in several acute and chronic neurological diseases.

Vascular endothelial growth factor (VEGF) is under investigation as a therapeutic agent for the treatment of neurodegenerative disorders [67,68]. AD is a neurodegenerative disorder characterized by elevated levels of amyloid β-peptide (Aβ) in brain. Based on the hypothesis that the initial vascular damage plays a critical role in neuronal damage [69] and Aβ accumulates in brain as a result of such vascular injury [70], our research group implanted microencapsulated fibroblasts releasing VEGF in transgenic APP/Ps1 mice, a cross of the Tg2576 (over-expressing human AβPP695) and mutant PS1

(M146L) mice. The hypothesis was that VEGF-releasing cells would increase angiogenesis in the damaged brain and enhance the removal of Aß plaques. Results showed an improvement in Aβ clearance, and less apoptosis and cognitive deficits in treated mice [71] (Fig. 4). Additionally, this cell-based approach increased cellular proliferation in the hippocampal dentate gyrus [72], thus suggesting a novel alternative strategy for the treatment and prevention of brain amyloidosis.

In spite of recent progress associated with the use of cell encapsulation technology in the field of CNS disorders, one of the main challenges that remains to be solved is the excessive diameter of the microcapsules. Air coaxial flow [73] and flow focusing technologies [74], represent an appealing alternative to fabricate small-sized particles in the similar mild conditions that offer the traditional droplet generators. These reduced size microcapsules may be more appropriate to reach difficult targets such as those related with the CNS where injection of microcapsules into the skull cavity excludes large transplant volumes. Furthermore, it may open the door to new medical fields for cell microencapsulation such as ophthalmology [74].

4. Cell encapsulation as a platform for cell delivery

As stem cell-based cell therapy reaches new horizons for its applicability, the demand for more and more sophisticated scaffolds that enable a controllable delivery of these cells increases continuously [13,75,76]. As for this goal, capsules are not committed to play the typical immunobarrier role—and therefore there is no need for external coatings—but they should be designed to act more as a support scaffold that may facilitate the correct grafting of the implanted cells. Although the most typical scaffolds used for this purpose are based on in situ gelling injectable hydrogels, microcapsules offer additional advantages due to their large surface area, the capacity to entrap higher number

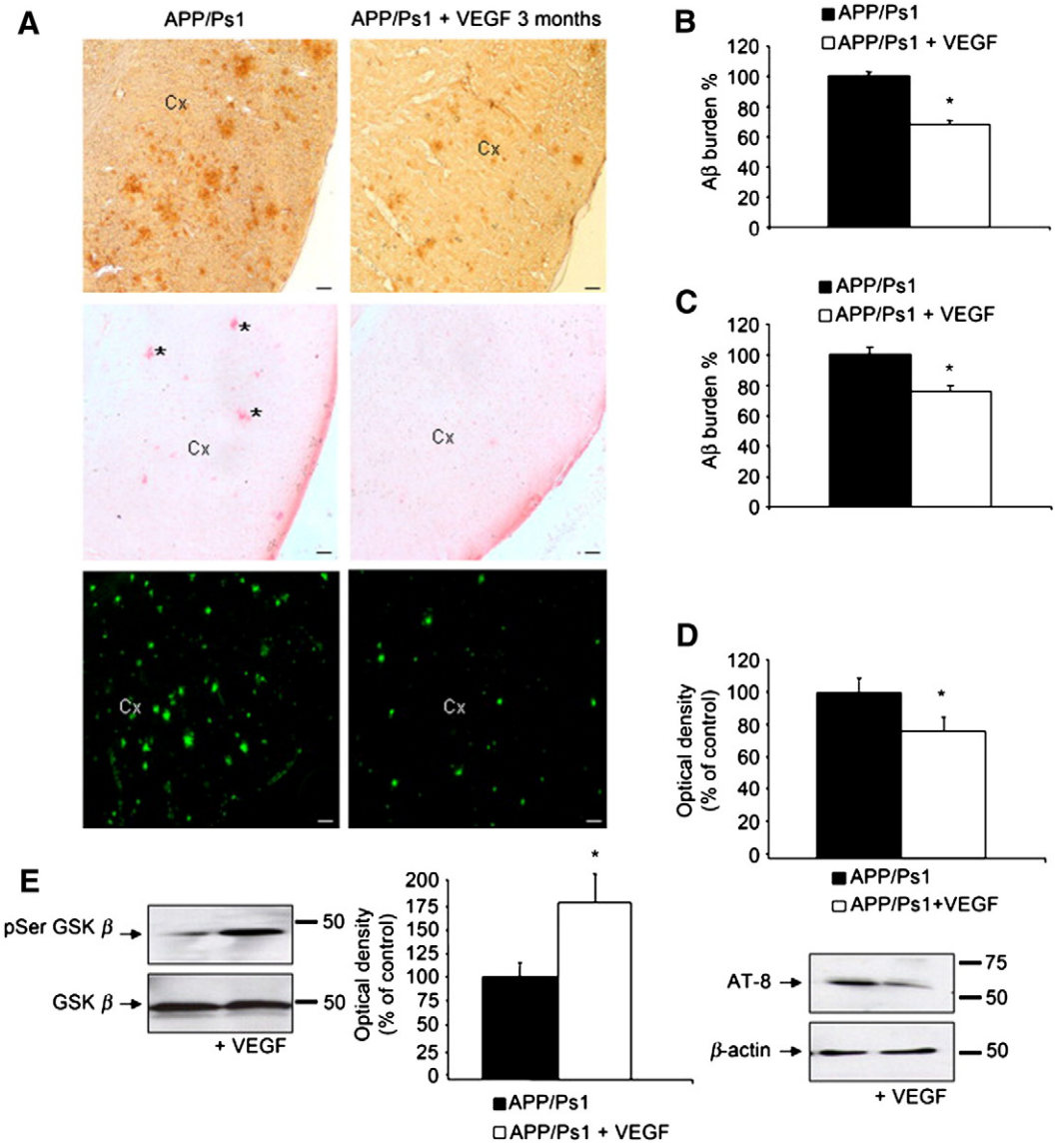

Fig. 4. Brain Aβ burden in APP/Ps1 mice after implantation of VEGF microcapsules. (A) Amyloid deposits in the cerebral cortex (Cx) of APP/Ps1 mice are reduced by VEGF microcapsules treatment. Murine and human Aβ were detected with an antibody recognizing both Aβ forms (top panels). Amyloid deposits (black asterisk) were also detected with Congo Red staining (middle panels). Thioflavin-S staining to detect amyloid deposits and thioflavin-positive aggregates are reduced after VEGF microcapsule treatment (botton panels). (Scale bar = 20 μm). (B) Cerebral cortex and (C) hippocampus Aβ burden is decreased in VEGF microcapsules-treated APP/Ps1 mice. Brain Aβ burden represents the percentage area covered by Aβ immunoreactivity. (D) Brain Hpf-tau levels significantly decreased after VEGF microcapsules implants in the cerebral cortex of APP/Ps1 mice. (E) Levels of pSer9GSK-3β are increased in the cerebral cortex of VEGF microcapsule-treated APP/Ps1 mice. Representative western blots and densitometry histograms are shown. (Data are expressed as mean ± SEM, $^{*}p < 0.05$ versus control APP/Ps1 mice, $n = 8$ for APP/Ps1 control group, $n = 7$ for VEGF microcapsule-treated APP/Ps1 mice). Reprinted from [71], © 2010, with permission from Elsevier.

of cells per volume unit [77,78] and the possibility of integration as part of more complex systems such as composed cell delivery systems and organ printing [77,79].

In cell delivery applications degradation rate serves a critical function and should be adjusted to the time required by grafted and host cells to replace the scaffold. During degradation, mentioned cells remodel their microenvironment as they deposit their own extracellular matrix (ECM). In addition, degradation is absolutely necessary for cell migration and may regulate the release of matrix-tethered biomolecules, which can promote different cellular functions [80,81]. Indeed, scaffold degradability may offer numerous advantages when it comes to gaining control over cell behaviour within the capsule [82,83]. It is fundamental to take into account that products from the degradation process must not be deleterious either for enclosed cells or for adjacent host tissues [84,85]. Likewise, in the search of the most proper degradation rate for a particular application, it is also important to keep in mind that degradation profiles obtained *in vitro* should not be assumed as valid for *in vivo* situations, as the hydrolysis is given in a different way depending on the water content of the implantation site [84].

Alginate, which is by far the most often employed polymer in the field of cell microencapsulation, may be rendered degradable by means of different protocols [86–88]. Among them, the best-known procedure is based on the creation of controllable numbers of functional groups, namely open chain adducts, susceptible to hydrolysis by partial oxidation of the polymer chains [88]. Thus, by just varying the oxidation degree of the alginates, it is possible to tune the degradation properties of the particles to release cells in a predictable way [83,84]. For example, fast cell deliveries (started from the fourth day) have been achieved by combining oxidized alginate and fibrin [89,90]. An alternative emerging strategy consists in allowing cellularly-driven matrix degradation by modifying the alginate backbone with specific sequences that are recognized and cleaved by metalloproteases (MMPs) secreted by cells [91,92]. This enables the entrapped cells to interact with the hydrogel and the neighboring cells in a dynamic form, resembling more realistically the physiological process given during wound healing or regeneration [93,94].

Similarly, biofunctionalisation of the matrices with adhesion moieties also provides necessary elements for cell delivery. It has been demonstrated that cell-adhesion not only avoids anoikis in adhesion-dependent cells, but it has also notorious effect on enhancing cell function and the migration capacity of encapsulated cells, being decisive in the final success of their release, proliferation and spreading [77,95,96]. For certain applications where microcapsules are administered within injectable scaffolds, this biofunctionalisation has been proved to be more effective when accomplished in the scaffold itself than in the particles, as cells tend to migrate towards RGD-containing areas [95].

Mesenchymal stem cells (MSCs), which can be derived from multiple sources, are probably the most interesting option as cellular model to build cell encapsulation platforms for therapeutic cell delivery aims in humans [64]. MSCs are multipotent progenitor cells with capability to self-renew and differentiate into specialized cells of the mesodermal germ layer [97]. These cells can be expanded *ex vivo* easily while maintaining their undifferentiated state, although they can be cultured only for a limited number of passages. In addition, and despite the controversy of genetically modified cells, MSCs can be engineered to express various genes and/or even to be immortalized [64,98–100]. In fact, the continuous progress in the technology of gene therapy encourages a promising future for the use of engineered cells in clinical applications. However, what makes MSCs especially attractive is their cell homing capacity and their hypoimmunogenic and immunomodulatory properties [101,102]. It is equally worthy to note that MSCs retain their stem-cell virtues when encapsulated, which means that due to their naive cell condition, they shed a very low amount of antigens [98,103].

5. Applications of cell delivery

Cell delivery has a great potential in the field of tissue engineering and regenerative medicine. Given that the process of tumour formation and wound healing share some similarities such as the release of signalling cues that prompt chemotaxis and proliferation of MSCs [104,105], one of the main applications lays on harnessing the cell homing capacity of MSCs. This strategy has been successfully carried out by implanting encapsulated and therapeutically engineered stem cells in the tumour resection cavity of rats to target tumour-selective migration and release of S-TRAIL (secretable tumour necrosis factor apoptosis inducing ligand) [13] (Fig. 5). Although the latter approach was carried out by using hydrogels, such strategy might be readily tackled by means of cell microencapsulation technology.

Besides, perhaps the most obvious applications of cell delivery are focused on tissue regeneration purposes. Enclosed cells may be released to damaged areas where they can act either by direct engraftment or by orchestrating the secretion of multiple cytokines and growth factors that guide the remodelling of the affected tissue [106,107]. Here, bone regeneration covers, undoubtedly, the main part of the so far performed approaches. For example, it has recently been reported that MSCs released from alginate-fibronectin microcapsules implanted in a calvarial bone defect were able to accelerate bone regeneration process [77]. Many of these studies with the same aim employ injectable scaffolds such as calcium phosphate cements (CPC) which are useful to fill the treated area of interest, avoid dissemination of the particles and make easier their retention and localisation [95,96,108]. In addition, microbeads act as porogens, leaving macropores in the CPC as they degrade. The same design may be also valid to be aimed towards different applications such as muscle tissue engineering [90].

MCSs have been also shown that may play a pivotal role in the angiogenesis and arteriogenesis processes [101,109]. The capacity to induce vascularisation is of a great importance, as this not only increases the regenerative properties of the therapy itself, but also results crucial for the adaptation of the implant the subsequent days after implantation, where the hypoxia suffered by the encapsulated cells the following days after implantation represents one of the main hurdles for graft survival [110].

6. Looking to the future, addressing current challenges

In the following years, cell microencapsulation is expected to achieve new important milestones. This biotechnology was firstly regarded as a platform to supply the lack of functional organs or target the sustained delivery of therapeutic factors by providing non-autologous cells with a vehicle serving as an immunobarrier for their transplantation. However, now, with the boom in tissue engineering and regenerative medicine, the therapeutic potential of cell microencapsulation has increased and its applicability broadened.

Definitely, alginate seems to be considered as the biomaterial of choice, although additional efforts are needed to overcome somehow the poor mechanical properties associated with its hydrogels. These limitations are leading to an increasing interest in covalently cross linked alginate matrices such as those based on photoinitiated polymerisations [82] or those based on developing alginate–tyramine conjugates to obtain both ionically and covalently crosslinkable capsules [111]. For example, by blending ionically crosslinked alginate and covalently crosslinked \polyacrylamide it may be also possible to introduce in the field of cell microencapsulation the recently proposed energy-dissipating mechanism for hydrogels [112]. More recent investigations are also bringing in new approaches to replace current coating polymers [113–115]. Among them, genipin—a naturally derived metabolite employed as a cytocompatible alternative to chemical crosslinkers such as glutaraldehyde—is increasingly being used to strengthen the mechanical properties of the external coatings [116–119].

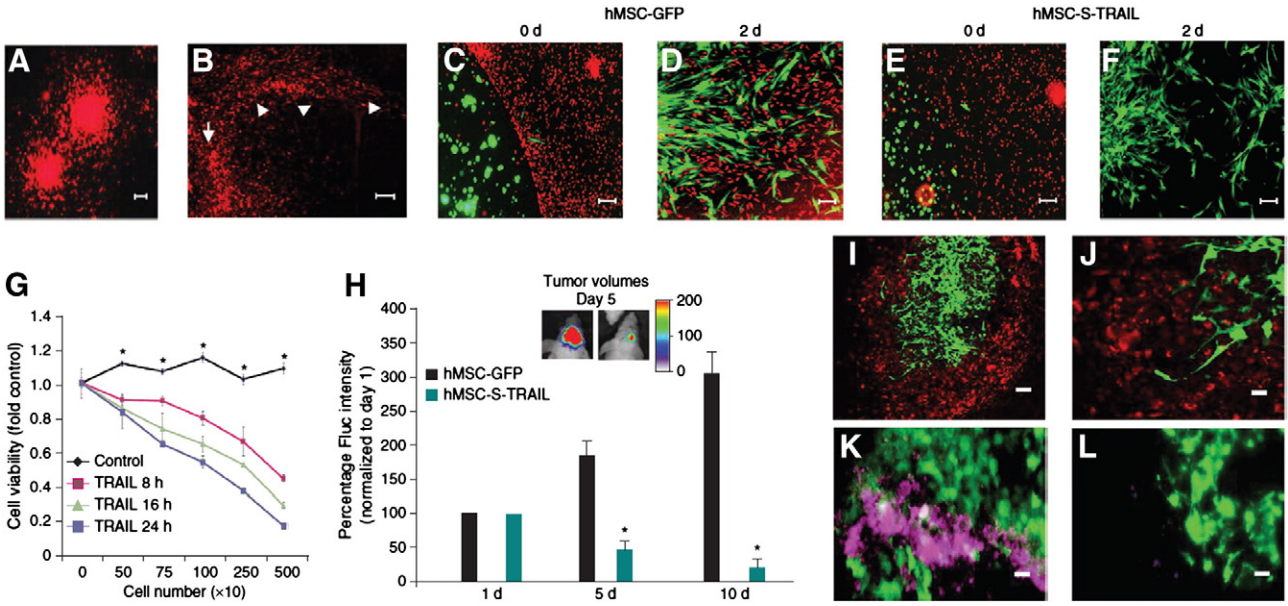

Fig. 5. sECM-encapsulated therapeutic human MSCs have anti-tumor effects on primary invasive human GBMs *in vitro* and *in vivo*. (A,B) Primary invasive GBM8-mCherry-Fluc cells grown as neurospheres in a collagen matrix (A) and brain section of mice bearing GBM8-mCherry-Fluc tumors, showing the highly invasive nature of GBM8 (B). Arrow, site of implantation; arrowheads, path of invasion. (C–G) hMSCs expressing GFP or S-TRAIL were encapsulated in sECM and placed in a culture dish containing human GBM8-Fluc-mCherry cells. hMSCs (green) were followed for migration out of sECM, and GBM8 cells (red) were followed for their response to S-TRAIL secreted by hMSCs. Photomicrographs show sECM-encapsulated hMSCs on the day of plating (C,E) and 48 h after plating (D,F). (G) GBM8 cell viability at different time points after culturing with varying numbers of either sECM-encapsulated hMSC-GFP (control) or hMSC-S-TRAIL (TRAIL) cells. *$P < 0.05$ versus TRAIL at 8 h, 16 h and 24 h. (H–J) Encapsulated hMSC-S-TRAIL or hMSC-GFP cells in sECM were implanted intracranially in the tumor resection cavity of mice bearing GBM8-mCherry-Fluc cells and mice were followed for changes in tumor volume by serial Fluc bioluminescence imaging and correlative immunohistochemistry. Plot and representative images show the relative mean Fluc signal intensity from mice bearing sECM-encapsulated hMSC-GFP or hMSC-S-TRAIL cells. *$P < 0.05$ versus control (H). (I,J) Low-magnification (I) and high-magnification (J) photomicrographs of serial brain sections of mice showing hMSCs (green) on day 5 after hMSC implantation in the GBM8 (red) resection cavity. (K,L) Representative images showing cleaved caspase-3 staining (purple) on brain sections from mice implanted with hMSC-S-TRAIL cells (green, K) and control cells (green, L) 5 days after treatment. Scale bars: 100 μm (A,C–F,I), 200 μm (B) and 50 μm (J–L). Data are mean ± s.e.m. Reprinted by permission from Macmillan Publishers Ltd: Nature, [13], © 2011.

An important challenge lays on the development of adequate tools to follow up and understand the behavior of encapsulated cells. This would become decisive to gain insight into the parameters affecting cell function and, thereby, design the particle that takes the most of the used cell type for the intended therapeutic goal. In this context, the way in which the biofunctionalisation is carried out is currently being debated, given that it is not clear whether short synthetic peptides like RGD or full ECM proteins like fibronectin or collagen are the most preferable options for this purpose [120]. Furthermore, even the sweeping statement that RGD offers therapeutic benefits still remains unclear, as unsuitable or uncontrolled cell-ECM interaction may produce undesired effects [120].

The biosafety concerns impeding the clinical translation of cell microencapsulation may be overcome by different ways. In drug delivery applications, the development of advanced biosystems capable of responding to external stimuli may be advantageous to exert dynamical control over the release of active compounds. This would allow, for example, switching between sustained/pulsatile releases as well as secreting multiple drugs sequentially [121]. Inducible tet-on/off systems [122] or physiologically inducible promoters may bring the tools to realize such ideas. On the other hand, cell delivery could also be controlled, for example, by means of active porous scaffolds, which deliver cells under applied magnetic fields [123]. Finally, the inclusion of suicide and reporter genes in the genome of encapsulated cells to inactivate the implant when necessary may be of a great interest for both drug delivery and cell delivery applications [37,38].

Administration and extraction procedures also need to be refined to be as minimally invasive as possible. The incorporation of the microcapsules within injectable scaffolds such as CPC [95] or hydrogel-based scaffolds [39] is providing important improvements in this field, as it avoids the dissemination of the particles out of the implanted area and makes easier their full recovery once the therapy reaches its final goal. The use of degradable microcapsules—even for drug delivery applications—may also be desirable to allow final clearance of the biosystems without the need of invasive extraction protocols, which becomes specially attractive in difficult-to-access areas such as the eye or the central nervous system [124]. Working in this direction, He et al. have designed novel microcapsules with a glutamine (Gln)-degradable PLL coating, which are administered within a biodegradable poly(ε-caprolactone) (PCL) nanoporous chamber, together with an additional mini PCL chamber containing Gln and alkali-releasing poy(lactic-co-glycolic acid) pellets [125]. Thus, the release of Gln and alkali leads to microcapsule degradation and cell death after a pre-defined time period. Final clearance of the whole system is carried out by the immune-system of the host.

Final considerations regarding the scale-up and clinical translation of this biotechnology should comprise the optimisation of the cost-effectiveness, together with establishing and satisfying a series of strict regulatory aspects. Indeed, likely one of the major challenges consists in moving from laboratory-based techniques to clinically acceptable large scale practices operating under reproducibility, safety and high-output requirements.

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