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OVERVIEW

Nanomedicine and drug delivery systems in cancer and regenerative medicine

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Abstract

Nanomedicine and drug delivery technologies play a prominent role in modern medicine, facilitating better treatments than conventional drugs. Nanomedicine is being increasingly used to develop new methods of cancer diagnosis and treatment, since this technology can modulate the biodistribution and the target site accumulation of chemotherapeutic drugs, thereby reducing their toxicity. Regenerative medicine provides another area where innovative drug delivery technology is being studied for improved tissue regeneration. Drug delivery systems can protect therapeutic proteins and peptides against degradation in biological environments and deliver them in a controlled manner. Similarly, the combination of drug delivery systems and stem cells can improve their survival, differentiation, and engraftment. The present review summarizes the most important steps carried-out by the group of Prof Blanco-Prieto in nanomedicine and drug delivery technologies. Throughout her scientific career, she has contributed to the area of nanomedicine to improve anticancer therapy. In particular, nanoparticles loaded with edelfosine, doxorubicin, or methotrexate have demonstrated great anticancer activity in preclinical settings of lymphoma, glioma, and pediatric osteosarcoma. In regenerative medicine, a major focus has been the development of drug delivery systems for brain and cardiac repair. In this context, several microparticle-based technologies loaded with biologics have demonstrated efficacy in clinically relevant animal models such as monkeys and pigs. The latest research by this group has shown that drug delivery systems combined with cell therapy can achieve a more complete and potent regenerative response. Cutting-edge areas such as noninvasive intravenous delivery of cardioprotective nanomedicines or extracellular vesicle-based therapies are also being explored.

This article is categorized under:

Therapeutic Approaches and Drug Discovery > Emerging Technologies

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KEYWORDS

drug delivery systems, nanomedicine, nanotechnology, regenerative medicine

1 | INTRODUCTION

Drug delivery technologies nowadays play pivotal roles in medicine, facilitating better treatments than conventional drug products in cancer or tissue regeneration. In particular, nanotechnology may completely change the way we detect and treat cancer in the near future (Peer et al., 2007; van der Meel et al., 2019). A general overview of the current clinical landscape can be found at Anselmo and Mitragotri (2019). Nanoparticles (NPs), due to their nanometric size, are able to overcome biological barriers, accumulate preferentially in tumors, and specifically recognize single cancer cells for detection and treatment (Blanco, Shen, & Ferrari, 2015; Peer et al., 2007). In this regard, nanomedicines increase the therapeutic index of compounds by allowing target cancer therapy and/or by minimizing their accumulation in healthy tissues. Although the cancer nanomedicine field has grown exponentially in recent years and the vast majority of nanomedicines currently in preclinical and clinical use are for targeting malignant tumors, the application of nanomedicine to noncancer conditions such as cardiovascular diseases has also increased (Dormont, Varna, & Couvreur, 2018). Additionally, one of the most active research areas in the field of drug delivery is the application of innovative drug delivery technologies to regenerative medicine. Current tissue engineering strategies with clinical success have been recently summarized by Hoffman, Khademhosseini, and Langer (2019). Specifically, biomaterials can solve one of the greatest challenges in the formulation and delivery of protein and peptide biotherapeutics, which is their fast degradation (Mitragotri, Burke, & Langer, 2014). In this regard, drug delivery systems have been designed to protect biomolecules from degradation in biological environments and to improve their potency while reducing toxic side effects. Something similar is what happens through the combination of drug delivery systems and stem cells to improve their delivery, since a major focus in this field has long been to increase transplanted cell survival, differentiation, and engraftment (Madl, Heilshorn, & Blau, 2018). Latest advances in cell delivery using biomaterials showed promise in overcoming these difficulties. Nowadays biomaterials with tuneable properties can be tailored to influence the fate of transplanted cells and to manipulate the host environment. Moreover, as continuous progress is made, the use of more complex drug delivery technologies would allow the development of platforms for growing complex tissues for improved in vivo regeneration. Looking further forward, they will allow us to make fully implantable organs tailored to meet the patient's needs.

This review summarizes the most important steps carried out by Prof Blanco-Prieto's group in the area of drug delivery technologies for cancer and regenerative medicine (Figure 1). The overall goal is to bring together the most important contributions of her research trajectory and to discuss the most exciting scientific discoveries to facilitate the delivery of difficult-to-deliver molecules and to treat the deadliest diseases around the world, such as cardiovascular diseases or cancer using drug delivery systems. Her most recent advances in drug delivery, theragnosis, and biomedical engineering are also discussed. The group led by Prof Blanco-Prieto has actively contributed to the area of nanomedicine to improve anticancer therapy. In particular, both conventional lipid nanosystems such as solid–lipid nanoparticles (LNs) and more advanced lipid nanosystems based on squalenoyl technology loaded with edelfosine, doxorubicin, or methotrexate have demonstrated great anticancer activity in preclinical settings of lymphoma, glioma, and pediatric osteosarcoma. In the area of regenerative medicine, a major focus has been the development of drug delivery systems for brain and cardiac repair. In this area, different microparticle (MP)-based technologies loaded with therapeutic proteins have demonstrated efficacy in clinically relevant animal models such as monkeys and pigs that closely resemble human anatomy. The latest research by this group has demonstrated that the combination of drug delivery systems with cell therapy can achieve a more complete and potent regenerative response. Finally, cutting-edge areas such as noninvasive intravenous delivery of cardioprotective nanomedicines or extracellular vesicle (EV)-based therapies are also being explored.

2 | NANOMEDICINE AND CANCER

2.1 | Lipid-based nanosystems for lymphoma and glioma treatment

The aim of nanomedicine is to resolve problems associated to therapies or diseases, increasing their efficacy and diminishing the inherent toxicity of the employed treatments. The application of nanotechnology to the field of cancer is particularly intended to achieve important innovations in the monitoring, diagnosis, and treatment of cancer. For the last decade, nanomedicine has been extensively investigated in cancer therapy. Midst all types of nanoparticulated delivery systems, LNs have been contemplated as a new step: the drug is delivered in biocompatible and biodegradable colloidal carriers comprised

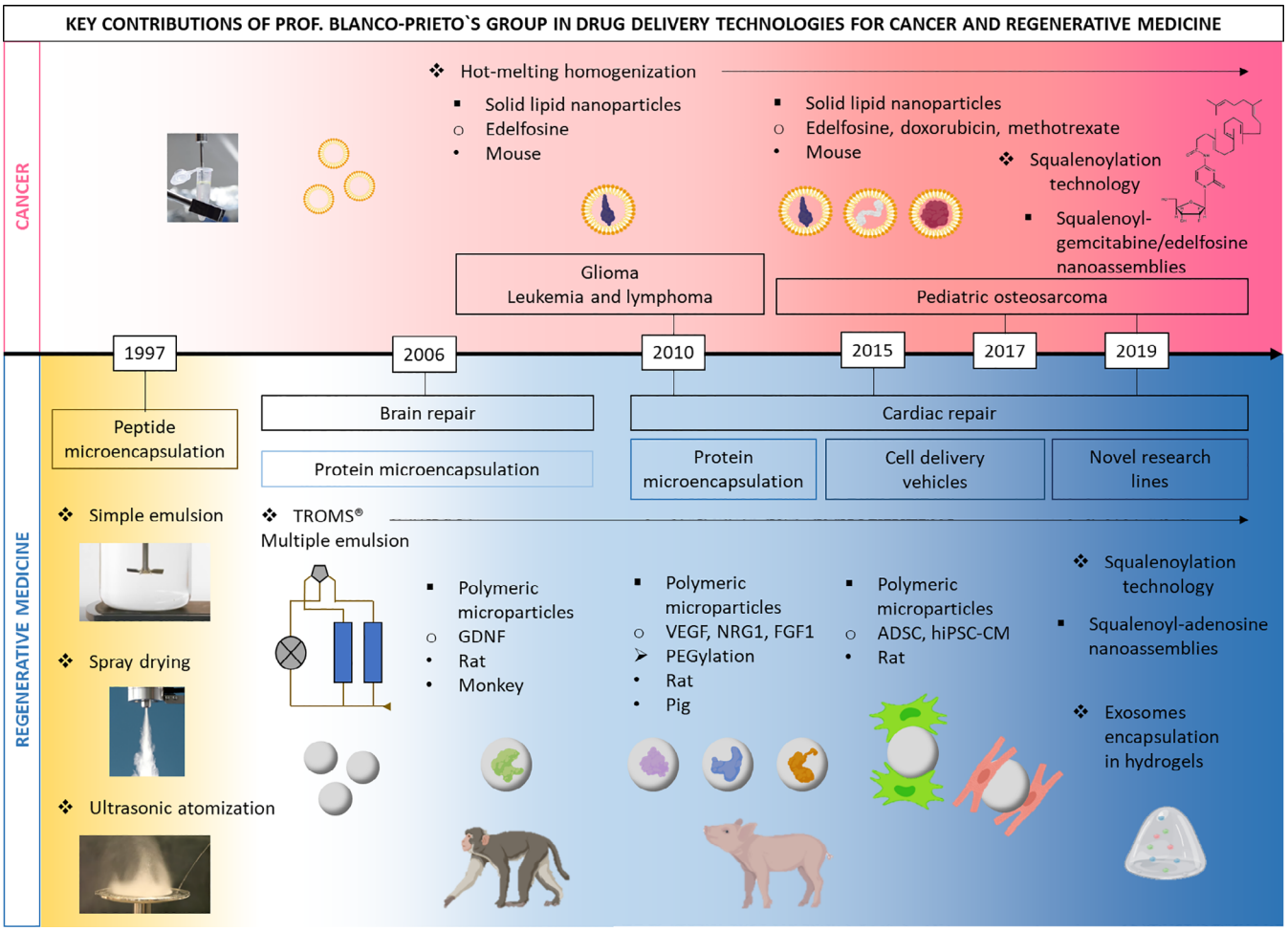


FIGURE 1 Timeline representing key contributions performed by the group of Prof Blanco-Prieto in the field of nanomedicine and drug delivery

of lipid matrices that stay solid at room temperature. These LNs combine advantages such as excellent tolerability, protection of incorporated labile drugs from degradation, physical stability, and controlled release while lessening possible drawbacks associated with other delivery systems like liposomes, such as drug leakage, limited physical stability of the liposomal dispersions, and nonspecific clearance by the mononuclear phagocytic system (Estella-Hermoso de Mendoza, Campanero, Mollinedo, & Blanco-Prieto, 2009). LNs present other different interesting features, like the ability to solubilize lipophilic drugs, as well as the absorption-enhancing effect they provide in the gastrointestinal tract owing to their nanometric size and their capability to penetrate between the villi of the intestinal mucosa and reach the bloodstream, as well as their lymphatic absorption (Bargoni et al., 1998; Estella-Hermoso de Mendoza et al., 2012). Another interesting advantage of the use of these nanocarriers for the delivery of anticancer drugs is the size-dependent specificity for the tumor site for NPs of sizes between 200 nm and 4 μm (Yuan et al., 1995), which is possible due to the enhanced permeability and retention effect and, hence, the reduced toxicity (Golombek et al., 2018). In this regard, extensive research of our group focuses on the use of LN for the delivery of anticancer drugs. In the last few years, the increase in cancer research has led to the discovery and further study of a new type of antineoplastic drugs. Edelfosine is the first-in-class of a new group of synthetic anticancer drugs called antitumor lipids, which may be administered orally in contrast to most antineoplastic drugs and presents an alkyl lysophospholipid structure that exerts its action at the level of the cell membrane, inducing selective apoptosis in malignant cells, but sparing normal ones (Mollinedo, Gajate, Martin-Santamaria, & Gago, 2012). However, the inherent properties of the drug made it difficult for its long-term oral or intravenous administration, due to its gastrointestinal and hemolytic side effects. Therefore, edelfosine vectorization in drug delivery systems was the path to follow. Due to the amphiphilic properties of edelfosine, the drug was encapsulated into LN by a warm microemulsion followed by high shear homogenization and ultrasonication method. Precirol® ATO 5 and Compritol® 888 ATO were the lipids used to encapsulate the drug (Estella-Hermoso de Mendoza, Rayo, Mollinedo, & Blanco-Prieto, 2008). Our initial experiments showed that this formulation

technique provided LN of size below 150 nm with an encapsulation efficiency of nearly 85% and significantly increased bioavailability after single oral administration (Estella-Hermoso de Mendoza et al., 2012). Interestingly, the Tween® 80 used in the composition of the formulation increased the brain delivery of these LNs and their presence in lymphatic nodes in a significant way after their oral administration (Estella-Hermoso de Mendoza et al., 2012; Estella-Hermoso de Mendoza, Préat, Mollinedo, & Blanco-Prieto, 2011). The efficacy of edelfosine-loaded LN was therefore next assessed in two different xenograft in vivo models of bearing mantle-cell lymphoma and glioma xenograft.

For the study of efficacy against lymphoma, an in vivo experiment was performed with mice bearing mantle-cell lymphoma. Free edelfosine solution was orally administered daily and LNs were administered orally every 4 days to mice. Three weeks after the oral administration of 30 mg/kg of the drug, either free or encapsulated into LN, the mice were sacrificed and tumors were collected for the determination of their weight and volume. Furthermore, along with the tumors, axillary, mesenteric, and inguinal lymph nodes were isolated and macroscopically analyzed for the study of efficacy against metastasis.

It is important to note that a daily administration of 30 mg/kg edelfosine was needed to reduce the tumor volume of mantle-cell lymphoma-bearing mice as much as Precirol and Compritol LN did after the treatment every 4 days. What is more, a daily oral administration of drug solution to mice was not successful enough to eradicate all the lymphatic metastases, and a certain degree of metastasis could still be observed. However, the administration of edelfosine-loaded LN every 4 days completely removed the metastasis process, with no sign of metastasis detected in any mice (Estella-Hermoso de Mendoza et al., 2012). This whole inhibition of the metastases was credited to the lymphatic absorption, and therefore accumulation of LN in the thoracic duct acting as a reservoir, which continuously releases them throughout the lymph nodes.

Another significant effect of LN along with the accumulation of edelfosine in lymph nodes due to the lymphatic absorption is the accumulation of edelfosine in brain tissue (Estella-Hermoso de Mendoza et al., 2011). As a result, the prospective efficacy of LN was next investigated in a xenograft C6 rat glioma-bearing mouse model, for which we first assessed the cytotoxicity of drug-loaded LN against the C6 rat glioma cell line that was going to be further tested in mice. The free drug showed a very high IC50 value (27.5 μg/mL) implying resistance of this cell line to edelfosine after 72 hr. However, when edelfosine was incorporated into either Precirol or Compritol LN, the IC50 value decreased noticeably to 7.5 and 5 μg/mL 72 hr after treatment, while edelfosine-free LN vehicles did not cause significant cytotoxicity against C6 cell line. This intense drop in the IC50 value can be ascribed to both the P-glycoprotein inhibiting properties of Tween 80 (Constantinides & Wasan, 2007; Estella-Hermoso de Mendoza et al., 2011) and the small size of the LN, which make intracellular uptake possible.

After assessing the efficacy of the edelfosine-loaded LN in vitro against glioma, our team aimed for the evaluation of their effectiveness in a xenograft C6 rat glioma-bearing mouse model. In order to do so, C6 glioma cell line-bearing mice were treated orally with 30 mg/kg of free edelfosine and edelfosine-loaded LN every 3 days. The oral administration of drug-loaded LN every 3 days for 14 days significantly reduced the tumor volume as compared to the constant increase in the tumor volume of the control group. After the third dose of LN, significant differences between the groups were observed (Figure 2). The oral administration of free edelfosine in solution every 3 days was not effective in tumor regression and these

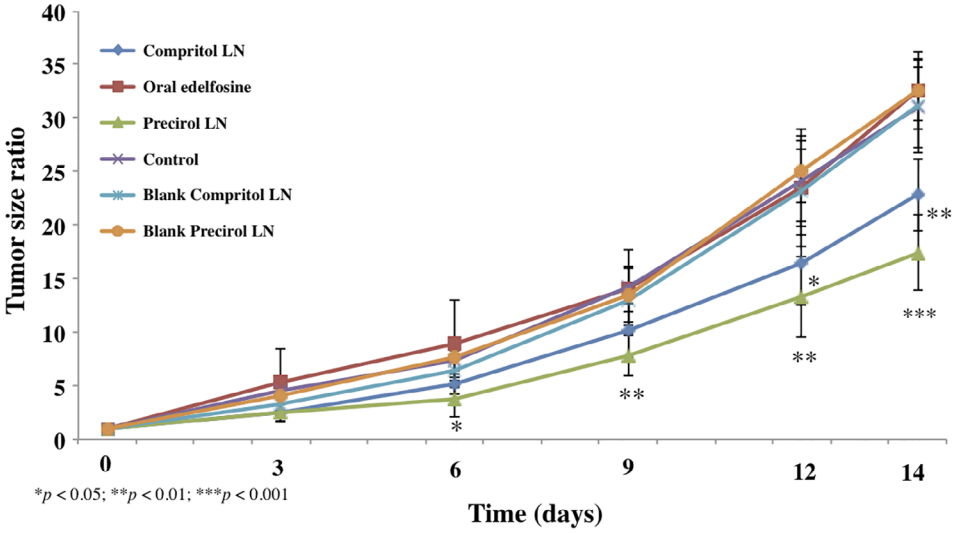


FIGURE 2 Tumor growth evolution in the C6 glioma model in mice, expressed as fold-increase ratio compared to tumor initial size, after treatment with edelfosine-loaded LN (30 mg/kg) and free edelfosine (30 mg/kg) every 3 days. *$p < .05$; **$p < .01$; ***$p < .001$ levels by Student's t test compared to the control. Reprinted with permission from Estella-Hermoso de Mendoza et al. (2011). Copyright 2011 Elsevier

tumors grew as fast as the controls. As a result, this in vivo antitumor efficacy of drug-loaded LN proved their effectiveness as appropriate delivery carriers against glioma (Estella-Hermoso de Mendoza et al., 2011).

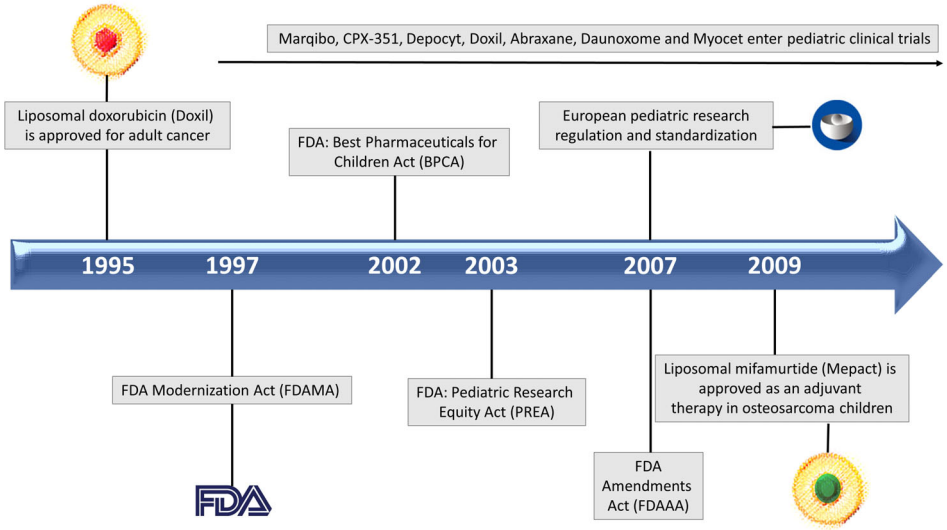
This LN loaded with edelfosine also proved to be an effective therapy against leukemia. In vitro results in leukemia cells showed similar or superior efficacy of the ET-LN over the free drug. Besides, even in the case of comparable efficacy, LNs offer benefits over the free drug in terms of protection against the drug's inherent toxicity. Indeed, toxicity studies have proven that LNs are a nontoxic vehicle for the oral administration of antitumor agents (Aznar, Lasa-Saracíbar, & Blanco-Prieto, 2014; Lasa-Saracíbar, Aznar, et al., 2014; Lasa-Saracíbar, Estella-Hermoso de Mendoza, Mollinedo, Odero, & Blanco-Príeto, 2013; Lasa-Saracíbar, Guada, Sebastián, & Blanco-Prieto, 2014).

2.2 | Lipid-based nanosystems for pediatric osteosarcoma therapy

More recently our group has focused on the investigation for novel strategies for the treatment of pediatric bone osteosarcomas (Box 1). Their aggressive behavior and propensity to spread to the lungs give this disease an unfavorable outlook, and it is one of the most lethal childhood cancer (Botter, Neri, & Fuchs, 2014; González-Fernández, Imbuluzqueta, Patiño-García, & Blanco-Prieto, 2015). To address these issues, lipid-based nanosystems were investigated with the aim of attaining tailored drug concentrations in the affected area, while reducing their exposure in healthy tissues.

BOX 1 NANOMEDICINES FOR PEDIATRIC CANCER

The most common cancer types in the pediatric population in terms of prevalence are hematological cancers, central nervous system tumors, non-central nervous system embryonal tumors, and musculoskeletal tumors. High-risk patients commonly receive high doses of chemotherapeutic drugs; however, these therapeutic cocktails are in many cases insufficient to effect cures and survivors can suffer long-term toxicities from their treatment (Gibson & Robison, 2015; Rodríguez-Nogales, Noguera, Couvreur, & Blanco-Prieto, 2019). In spite of the recent progress in the nanotechnology field for improving therapeutics in cancer management, such approaches are not applied in pediatric oncology. Thus, since Doxil® (liposomal doxorubicin) entered the market in 1995, some anticancer nanomedicines have been approved for adults but not for their use in pediatrics (Rodríguez-Nogales, González-Fernández, Aldaz, Couvreur, & Blanco-Prieto, 2018).



Historical timeline in the field of cancer nanomedicine and clinical research incentives in children. Reprinted with permission from Rodríguez-Nogales et al. (2018). Copyright 2018 American Chemical Society

2.2.1 | Solid–lipid nanoparticles

Distinct lipid-based nanocarriers were designed to encapsulate first and second-line treatments for osteosarcoma (i.e., methotrexate and doxorubicin) and also novel antitumor agents such as edelfosine. A modification of the above hot melting homogenization method was chosen to produce Precirol LNs around 100 nm, and with no need for using organic solvents in the formulation process. First, considering the high doses of methotrexate administered to these patients and the subsequent associated systemic toxicity, this antimetabolite was nanoencapsulated (González-Fernández, Zalacain, et al., 2015). Although LNs loaded with methotrexate were successfully designed and characterized, in vitro experiments revealed that the drug/matrix ratio required to exert an anticancer activity was not optimal. Of note, the encapsulation of the alkyl-lysophospholipid edelfosine in solid LN was able to ameliorate the efficacy of the drug against primary and metastatic osteosarcoma cells. This effect was thought to be caused by higher uptake of edelfosine when they were loaded into NPs.

The anticancer efficacy of doxorubicin-LN was afterwards investigated. This anthracycline is commonly given alone or in combination to osteosarcoma patients, but its clinical use is limited by the occurrence of multidrug resistances, as well as cardiovascular toxicities, among other factors. This time, doxorubicin was successfully encapsulated into LN and improved the efficacy of its nonloaded counterpart in vitro (González-Fernández et al., 2017). It was demonstrated that these NPs enhanced the cellular uptake of doxorubicin, bypassing an acquired resistance that is normally provoked by the overexpression of the P-glycoprotein 1 in resistant cells. On the other hand, free and encapsulated doxorubicin and edelfosine were shown to act synergistically. This suggested that a hypothetical combination of these antitumor agents may confer a more effective antitumor activity with reduced individual drug doses required.

The previous strategy was proposed as a reliable option to current therapies for refractory patients in successive works. Thus, the antitumor efficacy of orally administered edelfosine, edelfosine-LNs, and their combination with doxorubicin in two osteosarcoma orthotopic tumor models was investigated (González-Fernández, Brown, Patiño-García, Heymann, & Blanco-Prieto, 2018). In vivo, free doxorubicin and its combination with edelfosine-LN showed no significant antitumor effects than the nontreated group. Nevertheless, free and nanoparticulated edelfosine were found to detain the primary tumor growth. In that sense, after 27 days, the tumor volumes were significantly suppressed in comparison to the control group. Very importantly, free edelfosine mice group displayed an exponential tumor formation in lungs whereas LN loaded with edelfosine caused significant regression of lung metastases (Figure 3). It was shown that solid LNs were not only useful to diminish the inherent toxicity of edelfosine but also to prevent the metastatic spread from the primary tumor to the lungs.

2.2.2 | Squalenoyl nanoassemblies

A recent collaboration with the group of Patrick Couvreur in the University Paris-Sud resulted in the implementation of the "squalenization concept" to design multitherapy nanodevices in cancer therapy (i.e., pediatric osteosarcoma).

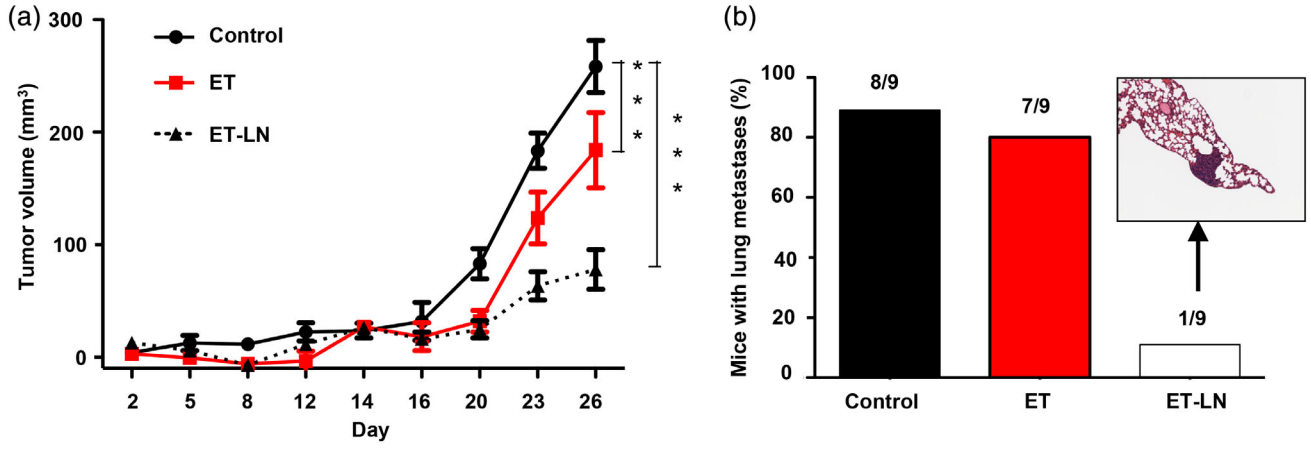


FIGURE 3 Edelfosine (ET) and edelfosine lipid nanoparticles (ET-LN) exhibited an antitumor effect in a 143 B-osteosarcoma induced model. 143 B-osteosarcoma cells were inoculated in intratibial site of nude mice. Six days after cell injection, mice were treated with ET (peroral, 30 mg/kg, three times/week), ET-LN (peroral, 30 mg/kg, three times/week), DOX (doxorubicin, intravenous, 2 mg/kg × 3 consecutive days every 21 days), their combination and PBS as control. (a) Effect of edelfosine (ET) and edelfosine-lipid nanoparticles (ET-LN) in primary OS tumor growth induced by 143 B cells. (b) After 26 days of treatment with ET and E-LN compared to the control group, macroscopic lung metastases were enumerated. ***$p \leq .001$ ($n = 9$, mean + SEM). Reprinted with permission from González-Fernández et al. (2018). Copyright 2018 Elsevier

This technology is based on the chemical linkage of drugs to squalenic acid, leading to lipid and biodegradable prodrugs able to form stable nanocomposites spontaneously (Couvreur et al., 2008). Squalenoyl nanoassemblies have attained promising results in a great variety of cancers. Among them, squalenoyl-gemcitabine nanoassemblies have been reported to ameliorate considerably the therapeutic index of the nucleoside analogue gemcitabine (Réjiba et al., 2011). We therefore chose it to design a new multidrug nanocomposite in combination with edelfosine (Rodríguez-Nogales, Sebastián, et al., 2019). Considering their amphiphilic nature, it was thought that combination antitumor agents may lead to the arrangement of a new anticancer nanomedicine.

The physical mixture of edelfosine and squalenoyl-gemcitabine at equimolecular concentrations led to the formation of homogenous nanoassemblies of 50 ± 4 nm without surfactants or polymers. In agreement with this, transmission electron microscopy images showed multilamellar and spherical nanoassemblies of 50 nm exhibiting a concentric disposition. This combination led to a diminished particle size and a different supramolecular organization (Figure 4), with better stability and more drug content in comparison with squalenoyl-gemcitabine nanoassemblies. It was thus likely that edelfosine acted as a complementary drug and as a particle stabilizer/surfactant. In vitro, squalenoyl-gemcitabine/edelfosine nanoassemblies avoided the hemolytic effect of edelfosine and displayed a better anticancer activity than squalenoyl-gemcitabine nanoassemblies in patient treated-derived metastatic osteosarcoma cells. The in-depth physicochemical characterization provided evidences into an easy formulation methodology of a novel multidrug nanomedicine with only drugs. In that sense, the main advantages of squalenoyl nanoassemblies over solid LNs mainly relies on a higher drug content, and the avoidance of using lipids, surfactants, or any other kind of excipient. Future experiments in vitro and in vivo will shed light on the clinic potential of this approach.

In these works, the improved safety and efficacy of LNs and squalenoyl nanoassemblies loaded with chemotherapeutic drugs were demonstrated in vitro and in vivo. Taking into consideration all these studies, edelfosine loaded LNs have been recognized as an attractive and promising therapeutic approach for the treatment of various neoplasms over the last years. We strongly believe that this successful nanoencapsulation is mainly due to its alkyl-lysophospholipid nature (both amphiphilic and lipid). Thus, our results in vitro and in vivo obliged us to be cautious about the feasibility of the encapsulation/co-encapsulation of other chemotherapeutic agents (e.g., doxorubicin and methotrexate) in solid LNs using the hot homogenization method.

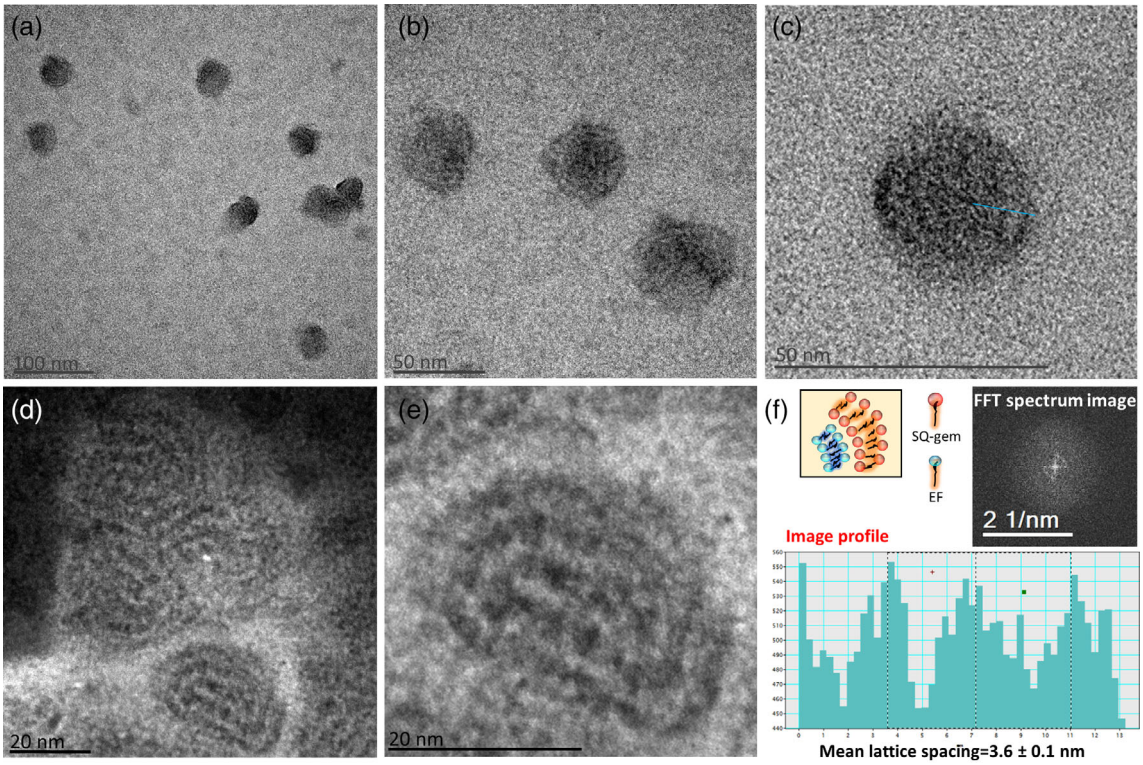


FIGURE 4 Transmission electron microscopy of SQ-gem/EF NAs (a–c). High angle annular dark field (HAADF) scanning transmission electron microscopy (STEM) of SQ-gem/EF NAs (d, e) (the signal corresponding to the organic material is inverted in comparison with TEM). Supramolecular organization diagram, Fourier transform image spectrum analysis and mean lattice spacing measurement of SQ-gem/EF NAs (f). Reprinted with permission from Rodríguez-Nogales, Sebastián, et al. (2019). Copyright 2019 Elsevier

2.3 | Ongoing investigations

Very recently, the group started a new line of research involving theranostics for glioma by co-encapsulating doxorubicin and magnetic NPs into poly(lactic-co-glycolic acid) (PLGA) nanosystems (Luque-Michel et al., 2016; Luque-Michel et al., 2017; Luque-Michel, Sebastian, Larrea, Marquina, & Blanco-Prieto, 2019). The research in theranostics is at a very early stage and there is a long way to go.

3 | DRUG DELIVERY SYSTEMS IN REGENERATIVE MEDICINE

3.1 | Peptide microencapsulation

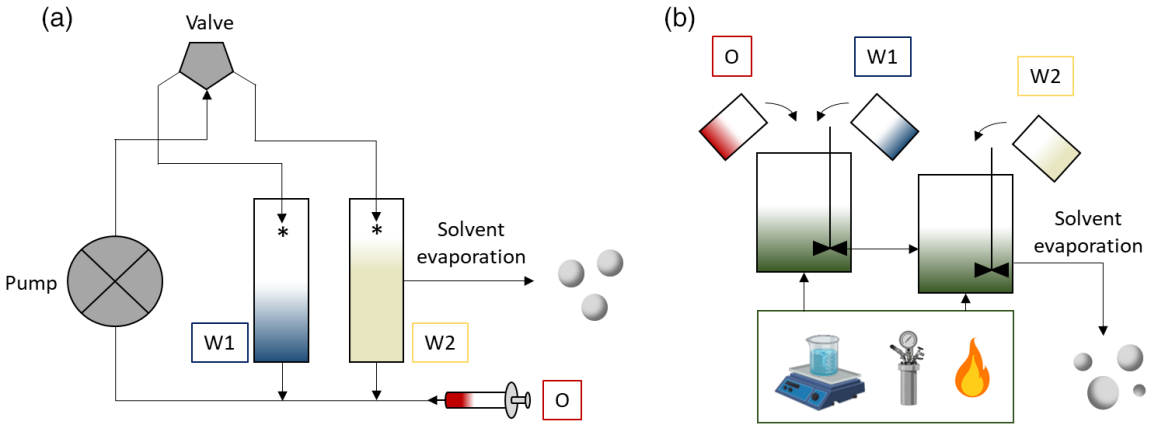
In the 1990s PLGA MPs were developed with the aim of entrapping small peptides (Couvreur, Blanco-Prieto, Puisieux, Roques, & Fattal, 1997). The first work concerned V3 BRU, a 33 amino acid peptide, which is a specific immunogenic fraction from the GP120 molecule of HIV. The aim was to administer the developed MPs as an oral adjuvant for HIV vaccination (Blanco Prieto et al., 1994). The MPs were prepared by the $W_1/O/W_2$ solvent evaporation method and different formulations were prepared and studied to obtain small PLGA microspheres with high encapsulation efficiencies. In addition, the peptide was not released in the gastric medium within 4 hr. Subsequently, the 7 amino acid peptide pBC 264, a highly potent agonist for CCK-B receptors with potential in the treatment of neurodegenerative diseases like Parkinson's disease (PD) was entrapped into the previously developed PLGA MPs. This peptide is rapidly eliminated from the brain and its entrapment into biodegradable MPs should increase its stability and allow us to study its long term biological and pharmacological effects. Our initial experiments encapsulating pBC 264 in the same conditions as V3 BRU led to a very low encapsulation efficiency (Blanco-Prieto et al., 1996, 1997) and the formulation had to be modified to achieve a high encapsulation efficiency and controlled release when the particles were administered into the brain (Blanco-Prieto et al., 2002). When MPs were locally implanted in the rat brain via stereotaxic surgery, in vivo release kinetics were almost identical to those observed in vitro, indicating that these MPs were useful for the slow delivery of neuropeptides in the brain. Finally, vapreotide, a somatostatin octapeptide analogue was encapsulated into PLGA MPs by spray-drying. Therapeutically, this peptide is of interest for the treatment of acromegaly and neuroendocrine tumors, among others. The overall aim of the study was the maintenance of sustained plasma levels over 2–4 weeks after a single administration in rats. With the best and optimized MP formulation, a twice-a-month injectable delivery system for vapreotide was developed (Blanco-Prieto et al., 1999, 2000; Blanco-Prieto, Campanero, Besseghir, Heimgatner, & Gander, 2004).

3.2 | Protein microencapsulation for brain repair

Following this initial research in peptide microencapsulation, this approach progressed in the following decade to protein microencapsulation for brain repair. Specifically, a lot of work was focused on the encapsulation of the most potent dopaminergic agent described so far, which is glial cell line-derived neurotrophic factor (GDNF) into polymeric MPs. GDNF is a 30 kDa protein considered promising therapeutic for PD treatment. PD is a complex neurodegenerative disorder characterized by the selective loss of nigral dopaminergic neurons and the presence of Lewy bodies and dystrophic neurites (Del Rey et al., 2018). Although several important contributions have transformed disease management, the development of novel disease-modifying therapies is one of the major unmet needs (Del Rey et al., 2018). GDNF has received great attention since this neurotrophic factor is able to enhance dopaminergic neuron survival (Kearns & Gash, 1995). However, GDNF displays a short biological half-life in circulation, degrades very easily, has difficulties crossing the blood–brain barrier and often presents serious side effects when administered systemically, making its brain delivery particularly challenging. Indeed, several clinical trials have failed to prove its efficacy in PD patients due to issues related to the delivery of this fragile molecule to the brain (Gill et al., 2003; Patel et al., 2005; Slevin et al., 2005). Hence, the need for novel therapeutic strategies able to enhance the sustained bioavailability of the protein to the brain directed our interest in its encapsulation into biodegradable MPs (Garbayo, Ansorena, Lanciego, Aymerich, & Blanco-Prieto, 2008). Various techniques have been reported for particle preparation, being the double emulsion solvent evaporation method the most common method used to encapsulate peptides and proteins as described before. Nevertheless, proteins may lose their bioactivity during the MP manufacturing process, which is one of the major concerns when working with these therapeutic molecules. To solve those issues, MPs were prepared by multiple emulsion solvent using the total recirculation one-machine system (TROMS). This technique avoids shear stress

being suitable for the encapsulation of therapeutic proteins, which are very fragile molecules (Garbayo et al., 2008) (Figure 5). Additionally, TROMS was developed to produce batches on a semi-industrial scale to facilitate the scale-up of the formulation. Regarding the material used to prepare the MPs, we thought of using PLGA since its biocompatibility with the brain has already been demonstrated. Moreover, this polymer has been often used in marketed protein-loaded drug delivery systems (Mitragotri et al., 2014; Park et al., 2019).

GDNF source is of critical importance when producing proteins for clinical use and therefore, the neurotrophic factor used in these studies was expressed and purified by our group in eukaryotic cells. This allows us to obtain protein with a mammalian glycosylation pattern similar to the endogenous one and to avoid some safety concerns observed in clinical trials when the unglycosylated GDNF was used as neutralizing antibody production (Ansorena et al., 2012; Ansorena, Garbayo, Lanciego, Aymerich, & Blanco-Prieto, 2010; Garbayo, Ansorena, Lanciego, Aymerich, & Blanco-Prieto, 2007). Initial studies by our group confirmed the behavioral recovery and the dopaminergic neuroprotection in parkinsonian rats (Garbayo et al., 2008, 2009; Garbayo, Ansorena, Lanciego, Blanco-Prieto, & Aymerich, 2011). Soon thereafter, pioneering work published in 2016 demonstrated the efficacy and safety of GDNF-MPs in parkinsonian monkeys given their similarity to humans (Garbayo, Ansorena, et al., 2016) (Figure 6a). Remarkably, this is one of the few studies carried out in more clinically relevant animal models like parkinsonian monkeys. In this study performed by our group, a single administration of GDNF-MPs within the brain induced motor function recovery (Figure 6b) and the restoration of the dopaminergic function (Figure 6b) without causing side effects. MPs were administered using brain stereotaxic surgery to locally deliver GDNF into the left *putamen* and to avoid side effects found when systemically administered. An increase in the number of striatal and nigral dopaminergic neurons was observed (Figure 6c,d). Notably, GDNF-MPs implantation into the left *putamen* resulted not only in the bilateral distribution of GDNF through the putamen but also in the retrograde transport of the neurotrophic factor to the *substantia nigra*. As discussed here,



	TROMS®	Conventional multiple emulsion and evaporation method
Shear stress	- Emulsion homogenization occurs due to the recirculation of the solution in the system	+ Emulsion homogenization requires external mechanical forces
Aggressive conditions	- Neither of both drug and particles are subjected to aggressive conditions during the entire process	+ High temperatures or long exposition of drugs to organic solvents is needed for emulsification
Homogeneous batches	+ Repeated recirculation through the system at the same flow and needles controls mean size and reproducibility	- Homogenization is difficult to control

FIGURE 5 Advantages of TROMS® versus conventional multiple emulsion-solvent evaporation method for the encapsulation of therapeutics. (a) Diagram representing TROMS technology. First a simple emulsion is formed by mixing W1 and O phases. Then a multiple emulsion is created by mixing the simple emulsion with W2. Neither shear stress nor aggressive conditions are need for drug encapsulation. * indicates where the needles used to mix the phases of the multiple emulsion are located. (b) Conventional multiple emulsion solvent-evaporation method. Simple emulsion is formed by mixing W1 and O phases. Then a multiple emulsion is created by mixing the simple emulsion with W2. Emulsification requires external mechanical forces such as agitators or pressure. Aggressive conditions are also normally needed for homogenization

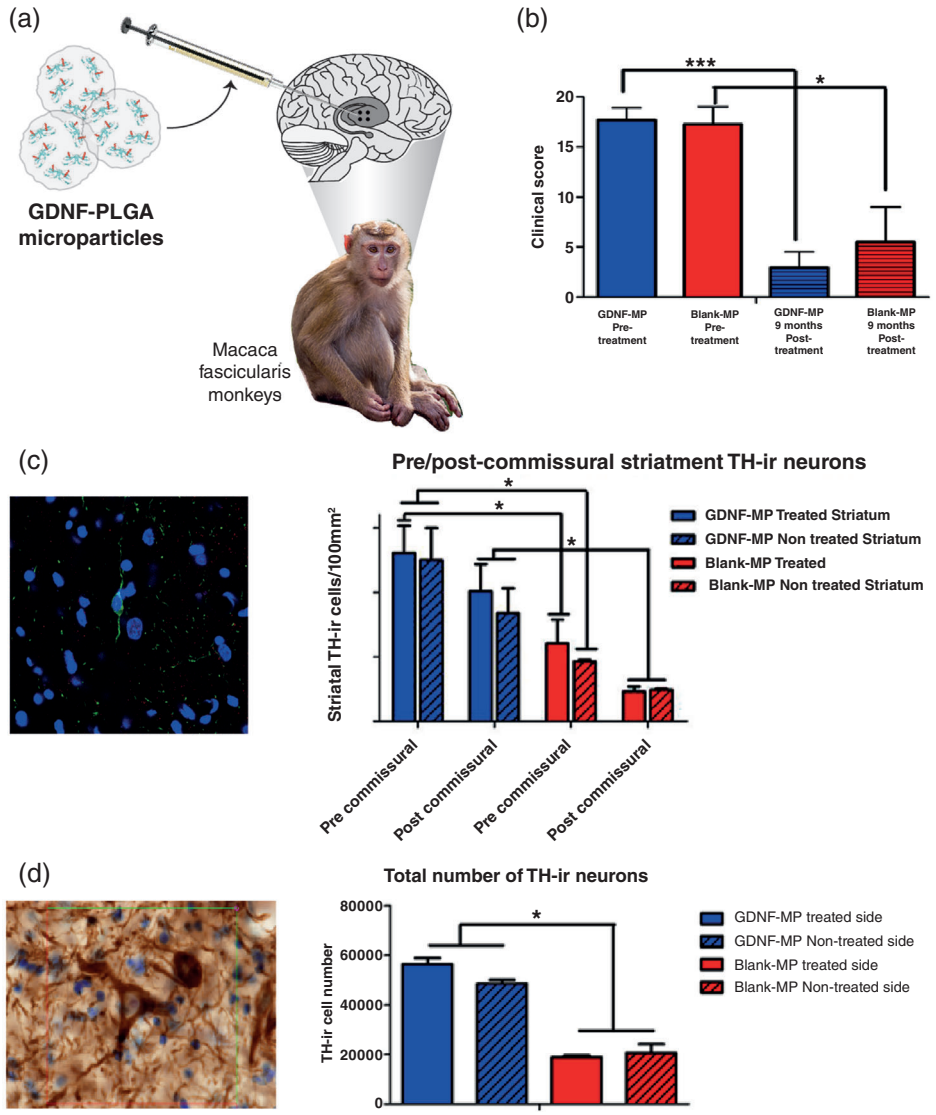


FIGURE 6 Efficacy of microencapsulated GDNF in parkinsonian monkeys. (a) GDNF-MPs were administered into the left *putamen* using stereotaxic surgery. (b) Motor function improvement was observed 9 months after treatment in GDNF-MP-treated monkeys (blue) compared to control (red) (***$p < .001$ vs. GDNF-MP pretreatment and *$p < .05$ vs. blank-MP pretreatment). (c) Therapeutic effect of GDNF-MPs in the *striatum*. Representative bipolar tyrosine hydroxylase-immunoreactive (TH-ir) neuron found in the *striatum* after treatment with GDNF-MPs. GDNF-MPs induced a significant increase in dopaminergic neuron density in the precommissural *striatum* 9 months after administration (*$p < .05$ vs. blank-MP treated and nontreated side). (d) Therapeutic efficacy of GDNF-MPs in the *substantia nigra*. Representative dopaminergic neuron found in the *substantia nigra* after treatment with GDNF-MPs. GDNF-MPs induced a significant bilateral increase in the total number of dopaminergic neurons in both hemispheres 9 months after treatment (*$p < .05$ vs. blank-MP treated and nontreated side). Reprinted with permission from Garbayo, Ansorena, et al. (2016). Copyright 2016 Elsevier; Torres-Ortega, Saludas, Hanafy, Garbayo, and Blanco-Prieto (2019). Copyright 2019 Elsevier

this pioneering example demonstrates that PLGA MPs are an efficient vehicle for GDNF brain delivery that leads to tremendous progress in the field of brain repair.

3.3 | Drug delivery systems for cardiac repair

3.3.1 | Protein microencapsulation for cardiac repair

Based on the promising results obtained in brain repair using microencapsulated peptides and proteins, we turned our attention to another disease area namely cardiac repair. Myocardial infarction (MI), also called heart attack, remains

the leading cause of morbidity and mortality worldwide (Benjamin et al., 2017). Moreover, the World Health Organization estimated that the number of deaths will increase within the next decades as a result of the rising prevalence of risk factors associated with this pathology (Benjamin et al., 2017). MI is caused by the blockage of a coronary artery, which restricts the blood flow to part of the heart muscle, leaving it without oxygen supply. This creates an infarcted area where all cardiac cells die, including cardiomyocytes—the heart cells responsible for heart contraction. Unfortunately, the heart muscle cannot recover from this injury and becomes weak, which can cause functional limitations, total heart failure and dead (Johansson, Rosengren, Young, & Jennings, 2017; Ruddox et al., 2017). Moreover, as in the case of PD, the damaged tissue area is very specific, being in this case only the part of the heart irrigated by the blocked artery. To tackle these issues, delivery systems constitute an optimal approach to locally deliver drugs and deal with the complications of injured cardiac tissue without triggering secondary and systemic effects (Pascual-Gil, Garbayo, Díaz-Herráez, Prosper, & Blanco-Prieto, 2015). In addition, current treatments are only palliative and cannot repair the already damaged cardiac tissue, being heart transplant the only real solution to recover heart functionality. However, the supply of donor hearts is extremely low compared with the number of MI patients, increasing the need to develop novel strategies for this disorder.

Since the death of cardiac cells occurs due to the lack of blood supply, promoting the formation of new vascular vessels in the infarcted heart tissue (angiogenesis) and the maturation of blood vessels (arteriogenesis) is an interesting approach for restoring normal cardiac environment (Formiga et al., 2012). Several growth factors have been used to this end so far, such as FGF1, NRG1, and VEGF (Formiga et al., 2012; Pascual-Gil, Garbayo, et al., 2015; Saludas, Pascual-Gil, Roli, Garbayo, & Blanco-Prieto, 2018). Among them, VEGF was one of the first characterized pro-angiogenic growth factors with clinical potential (Simón-Yarza et al., 2012). However, its use in different models of ischemia went hand in hand with limited clinical success as a result of the fast degradation and elimination of the injected cytokine (Formiga et al., 2012; Pascual-Gil, Garbayo, et al., 2015; Saludas et al., 2018). In the early 2000s, we contributed to the development and further optimization of TROMS® technology, proving that PLGA MPs with a diameter of around 5 μm (Figure 7a) were compatible with intramyocardial administration and remained in the cardiac tissue after 3 months postimplantation. Furthermore, no particle migration to other organs was reported (Formiga, Garbayo, et al., 2013). Successive studies demonstrated that encapsulated growth factors are protected against in vivo degradation, are released in a controlled manner over at least 3 months after MP injection, colocalized with MPs in the heart tissue and can activate biological receptors in cardiomyocytes (Pascual-Gil, Simón-Yarza, Garbayo, Prosper, & Blanco-Prieto, 2015) (Figure 7b,c). Taken together, these findings supported the use of PLGA MPs as vehicles for delivering growth factors for treating heart conditions such as MI, establishing a new research line in the group.

Remarkably, encapsulation of VEGF into PLGA MPs allowed a localized and sustained protein release over time in the heart tissue, as demonstrated in a rat model of MI (Formiga et al., 2010). This was correlated with an increase in angiogenesis in the heart, as well as with a greater ventricle wall thickness, indicating a stronger heart pumping capacity. Further studies confirmed these results and added evidence showing that VEGF PLGA MPs improve heart function 3 months after treatment (Simón-Yarza, Tamayo, et al., 2013). Although our group has not performed a direct comparison of PLGA MPs versus other drug delivery systems such as hydrogels, other groups have explored the possibility of using hydrogels to deliver VEGF to the myocardium showing an angiogenic effect after sustained cytokine treatment (Matsusaki, Sakaguchi, Serizawa, & Akashi, 2007). However, the control of the release rate of growth factors from hydrogels is not easy. A strong initial burst release is generally found, which has been associated with severe side effects such as hypotension (Matsusaki et al., 2007).

Although VEGF is a major contributor to angiogenesis, it primarily drives the formation of new capillaries, and arteriogenesis remains an issue (Stegmann, 1998). Combination of VEGF with other growth factors is therefore recommended to achieve proper revascularization. Synergistics effects and combination potential among different molecules must be addressed in these cases, since synergism may not always occur. For instance, when VEGF was coencapsulated with coenzyme Q10—an antioxidant agent with beneficial roles in cardiac function and clinical course of heart failure (Jankowski, Korzeniowska, Cieślewicz, & Jabłecka, 2016)—the combination therapy failed to offer any extra therapeutic outcomes (Simón-Yarza, Tamayo, et al., 2013).

Progress in protein therapeutics and a better understanding of mechanistic pathways allowed the identification of other growth factors with a more important role in cardiac repair, such as FGF1 and NRG1. Thus, FGF growth factors induce a more mature vascularization than VEGF (Slavin, 1995; Wallner et al., 2015), and NRG1 helps not only angiogenesis but is also essential for proper heart development, maintenance, and repair (Liu et al., 2006; Rupert & Coulombe, 2015; Yutzey, 2015). Based on this evidence, the efficacy of FGF1 or NRG1 loaded PLGA MPs was tested in a rat model of MI, reporting that the delivery of both growth factors resulted in a significant enhancement of cardiac

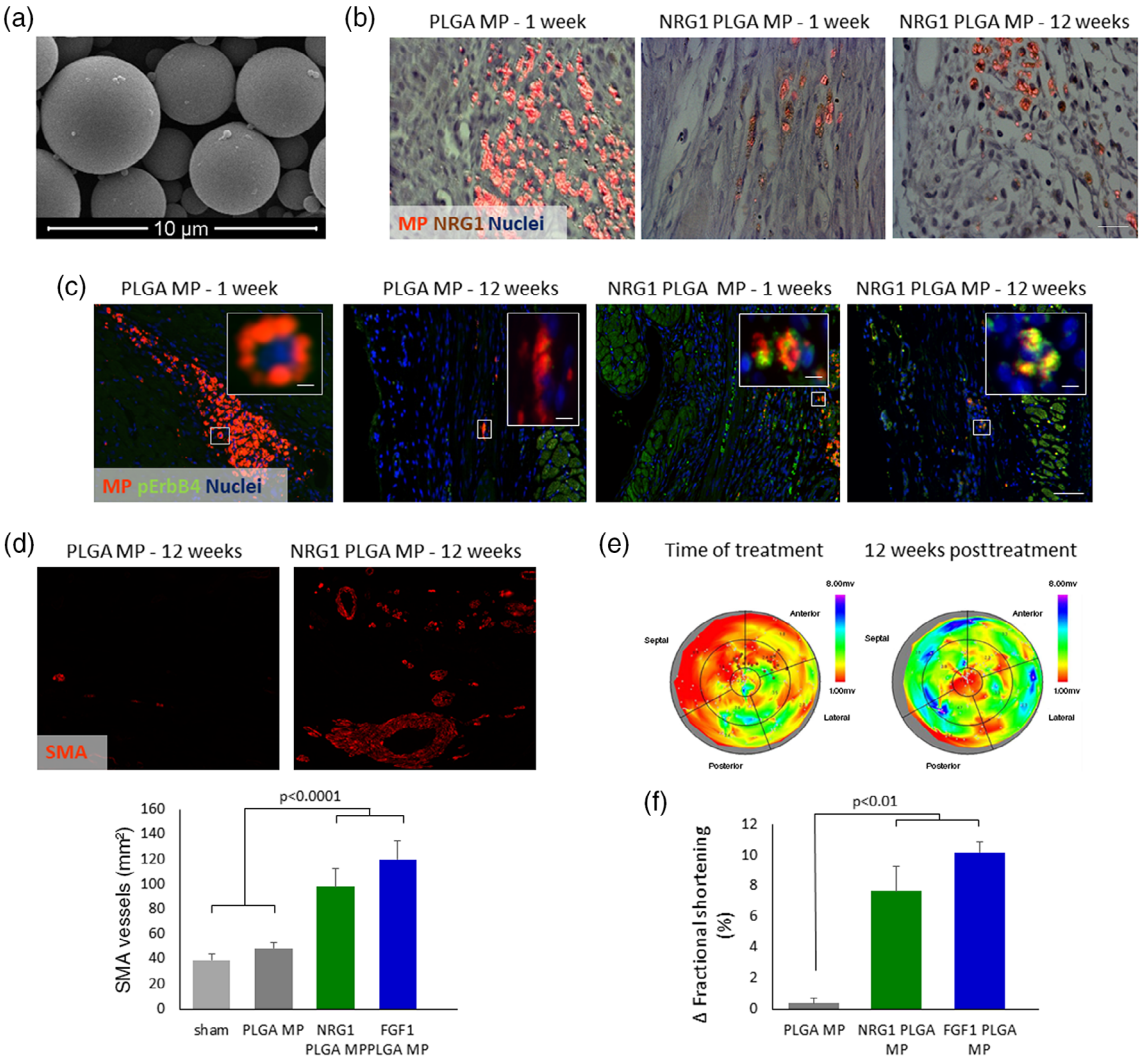


FIGURE 7 PLGA MPs as vehicles for the delivery of therapeutics to the cardiac tissue. (a) Scanning electron microscope imaging of PLGA MPs. (b) Representative images showing the in vivo release of NRG1 from PLGA MPs. NRG1 (brown precipitate) can be observed for up to 12 weeks after MP administration. Scale bar 50 μm. (c) Activated biological receptor for NRG1, called ErbB4, could be detected in its phosphorylated from during at least 3 months after NRG1 PLGA MPs administration. The activated receptor was not detected when non loaded MPs were administered. Scale bar 50 μm. Scale bar of magnification inserts 8 μm. (d) NRG1 PLGA MPs significantly increase blood vessel number in the cardiac tissue compared to non-loaded MPs. (e) Representative bipolar area NOGA maps taken at time of injection and 3 months after treatment administration in pigs. (f) Cardiac function measured by fraction shortening showing that both NRG1 and FGF1 PLGA MPs improve heart function 3 months after treatment administration in pigs. Reprinted with permission from Garbayo, Gavira, et al. (2016). Copyright 2016 Springer Nature; Pascual-Gil, Simón-Yarza, et al. (2015). Copyright 2015 Elsevier

function, smaller infarct size, lower fibrosis, and induction of angiogenesis and arteriogenesis (Figure 7d) 3 months after MP administration (Formiga, Pelacho, et al., 2013; Pascual-Gil, Simón-Yarza, et al., 2015). Moreover, cardiomyocyte proliferation and recruitment of stem cell were also reported in treated animals, encouraging studies with larger and more clinically relevant animal models of MI approximating human anatomy such as the pig. No relevant issues were found during the scale up of the microsphere protein delivery system. This is probably due to the use of TROMS to prepare the MPs since this machine allows us to produce batches on a semi-industrial scale facilitating the scale-up of the formulations. The cardiac administration of FGF1 or NRG1 PLGA MP in a model of ischemia–reperfusion in pigs using a minimally invasive percutaneous delivery approach proved to enhance cardiac function and to decrease infarct progression compared to the administration of nonloaded MPs (Garbayo, Gavira, et al., 2016) (Figure 7e,f). The improvement of cardiac function was associated with an increase of the heart vascularization and positive muscle remodeling, strongly supporting the use of MPs as a cardiac delivery system for growth factor

administration in cardiac regenerative strategies. It is important to highlight here that the realization of such a translational study required the expertise of a multidisciplinary team ranging from pharmaceutical, material, polymer, and medical sciences and bioengineering, an approach that holds the key to progress in drug delivery research.

Beyond assessing the efficacy of different microencapsulated growth factors, MP optimization has also been a regular topic of discussion in our group. For instance, of particular interest are recent studies where poly (ethylene glycol) (PEG) coating of MPs was used to enhance their bioavailability. PEG coating (or PEGylation) has been broadly used to shield the particles surface from opsonization and phagocytosis, increasing their blood circulation half-time (Owens & Peppas, 2006; Suk, Xu, Kim, Hanes, & Ensign, 2016). Initial in vitro assays showed that PEGylation resulted in a significant decreased uptake of particles by macrophages (J774 and Raw 264.7 lines) (Simón-Yarza, Formiga, et al., 2013). In the heart, long-term in vivo studies in rats confirmed this trend and collectively demonstrated that PEGylation reduced MP phagocytosis in the cardiac tissue 3 months after their injection (Pascual-Gil, Simón-Yarza, et al., 2015). However, a deeper analysis of the phagocytic process revealed that both PLGA and PEGylated PLGA MPs were equally phagocyted in the heart tissue in the short term (1 week after administration) (Pascual-Gil, Simón-Yarza, et al., 2015) (Figure 8a). Consequently, the amount of therapeutic proteins carried and delivered in the cardiac tissue by PLGA and PEGylated PLGA MPs was the same in the short term, which corresponds to the therapeutic time window in which growth factors can arrest infarction progression. According to this, both types of MPs had similar efficacy in promoting cardiac function (Figure 8b) as well as in inducing angiogenesis and arteriogenesis (Figure 8c,d). Equal phagocytosis rates also suggested for the first time that particle clearance in solid tissues may not be dependent on opsonins. Importantly, the heart inflammatory response after MI has been recognized as a key regulatory step that dictates the fate of future reparative and healing processes (Epelman, Liu, & Mann, 2015; Lavine et al., 2014). In the heart there are two main types of macrophages: (a) inflammatory macrophages responsible for chronic inflammation and infarct progression, and (b) reparative macrophages associated with tissue homeostasis and reparative processes (Dick et al., 2019; Hulsmans et al., 2017; Lavine et al., 2014). Promoting the reparative phenotype indeed constitutes a novel and promising strategy for developing new therapies for treating MI (Bajpai et al., 2018). In a preliminary study, NRG1 PLGA MPs proved to induce polarization of macrophages (J774 line) toward the reparative phenotype in a similar way than IL-4 does (Pascual-Gil et al., 2019), which is a well-established reparative state inducer (Martinez & Gordon, 2014). In vivo assessment of the polarization potential of NRG1 PLGA MPs was done in a rat MI model, reporting a significantly higher presence of reparative macrophages in the vicinity of NRG1 PLGA MPs compared to more distant heart areas (Pascual-Gil et al., 2019) (Figure 8e). Although we have made significant progress in the development of this system, how to modulate macrophage polarization still represents a challenging field on which future projects should focus to develop more effective therapies for cardiac repair.

3.3.2 | MPs as delivery platforms for cells

Regenerating the heart after a MI is a complex multifactorial process in which numerous cell-signaling pathways play a key role. Reparative mechanisms such as protection of damaged cardiomyocytes from necrosis, stimulation of their proliferation, formation of new blood vessels, or matrix deposition and remodeling require the precise and timely activation of several interconnected intracellular cascades (Broughton et al., 2018; Uygur & Lee, 2016). Furthermore, the activation of cellular pathways should lead to a joint tissue response in all the affected myocardium where the regenerative stimuli needs to be properly synchronized and orchestrated to overcome consequences from the ischemic insult. As described in the above section, our group has a long and proven experience in the development and preclinical evaluation of therapies for heart repair focused on the encapsulation of therapeutic proteins (Formiga, Pelacho, et al., 2013; Garbayo, Gavira, et al., 2016). However, the group's latest research in this field hypothesized that a more complete and potent cardiac regenerative response could be achieved by the combination of the previously developed MP-based formulations with cell therapy (Figure 9). So far, most protein delivery systems have failed to deliver a cocktail of growth factors, each one with a specific dose, in a precise time and with distinct kinetics. By contrast, the administration of stem cells offers a solution to this drawback. Cells can interact and respond to signals of the host microenvironment and locally release a wide range of growth factors and cytokines (Baraniak & McDevitt, 2010; Hodgkinson, Bareja, Gomez, & Dzau, 2016). Stem cells have been traditionally delivered using hydrogels. However, we postulated that MPs could represent excellent delivery vehicles for cells as they provide three-dimensional support that favors cell retention and they could be easily injected using catheter systems (Saludas et al., 2018) (Figure 9).

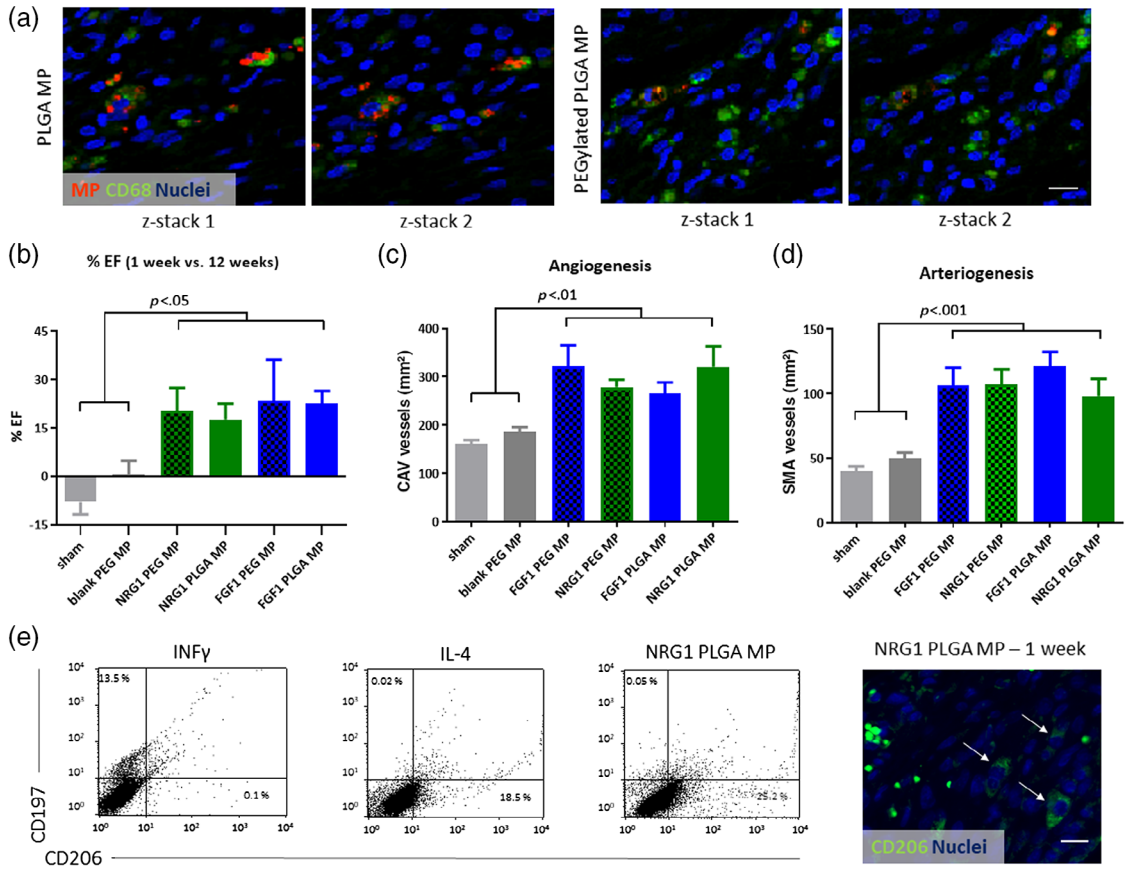


FIGURE 8 Functional comparison between PLGA and PEGylated PLGA MPs in the cardiac tissue. (a) Confocal fluorescent images representing the phagocytosis of PLGA and PEGylated PLGA MPs in the heart. Both MPs (in red) can be observed inside macrophages (in green) since red signal and green signal colocalize in consecutive z-stacks (5 μm length between consecutive stacks). (b) Cardiac function measured by ejection fraction showing that NRG1 or FGF1 loaded PLGA or PEGylated PLGA MPs improve heart function in rats 3 months after treatment administration. (c) NRG1 or FGF1 loaded PLGA or PEGylated PLGA MPs significantly increase the number of small caliber vessels in the cardiac tissue compared to controls 3 months after treatment. (d) NRG1 or FGF1 loaded PLGA or PEGylated PLGA MPs significantly induce the maturation of blood vessels (big caliber vessels) in the cardiac tissue compared to controls 3 months after treatment. (e) NRG1 PLGA MPs promote macrophage polarization toward a reparative phenotype in vitro and in vivo. Their effect on polarization is similar to that of IL-4. White arrows indicate $CD206^{+}$ macrophages in the cardiac tissue located close to the injection track. Scale bar 30 μm. Reprinted with permission from Pascual-Gil et al. (2019). Copyright 2019 Taylor Francis; Pascual-Gil, Simón-Yarza, Garbayo, Prósper, and Blanco-Prieto (2017). Copyright 2017 Elsevier

In the first study performed by our group, adipose-derived stem cells (ADSCs) were adhered to the previously developed biodegradable PLGA MPs releasing NRG (Díaz-Herráez et al., 2013) (Figure 10a). ADSCs are responsible for the secretion of a wide variety of molecules (e.g., VEGF, FGF, hepatocyte growth factor, etc.) but the secretion of NRG by these cells has never been described (Hong, Traktuev, & March, 2010), indicating that the combination of both strategies could entail complementary effects on cardiac healing. PLGA MPs were prepared using TROMS and functionalized with a biomimetic layer made of collagen and poly-D-lysine, which facilitates the interaction with cell membrane proteins. After the adhesion of cells, the administration of the complexes in a rat MI model showed good biocompatibility as they did not trigger an inflammatory reaction and they were well tolerated by the cardiac tissue. Furthermore, complexes could be detected in the injection site after 2 weeks, still supporting the retention and viability of transplanted cells (Díaz-Herráez et al., 2013) (Figure 10b). In a later efficacy study, it was successfully confirmed that MPs enhanced cell survival and engraftment in the ischemic tissue (Díaz-Herráez et al., 2017). In this sense, whereas cells injected alone could no longer be found in the tissue after 1 week, this was the first study describing the presence of ADSCs in the heart 3 months after their administration due to their adhesion to MPs (Figure 10b). As a result, infarct size and left ventricular wall thickness were significantly improved in the long term (Figure 10c). Importantly, the formation of blood vessels was stimulated, which is of key importance for alleviating the oxygen deficit in the tissue (Figure 10c).

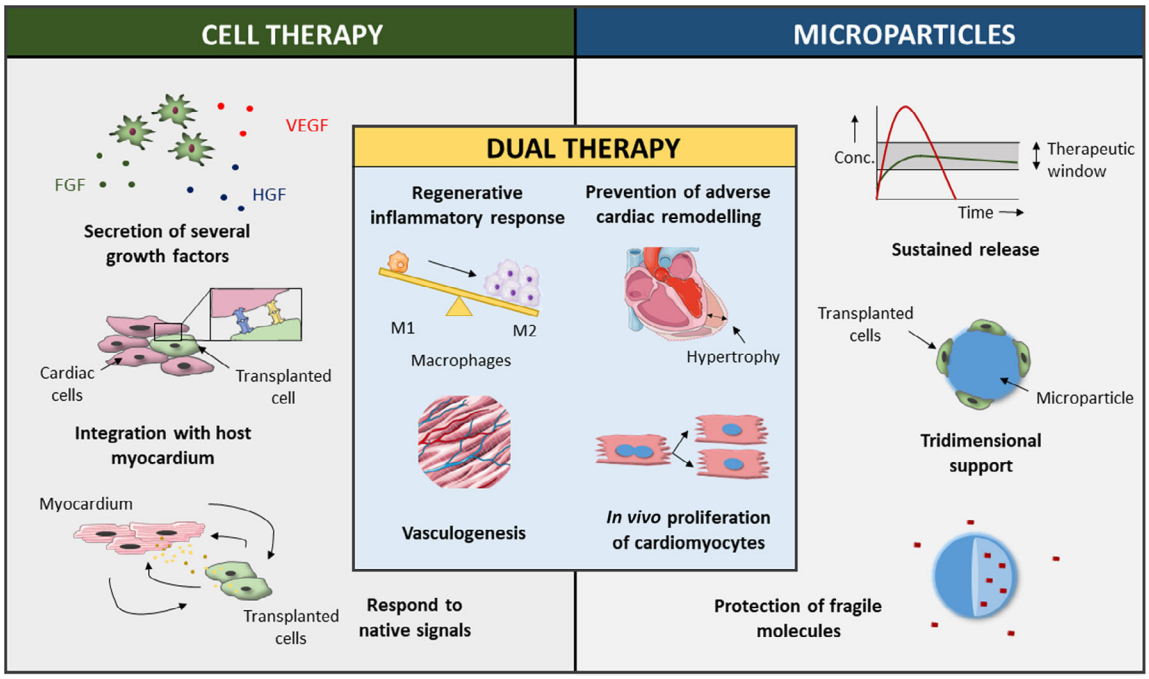


FIGURE 9 Schematic illustration of benefits and advantages of cell therapy and microparticle-based protein delivery as well as therapeutic outcomes obtained when both are combined in a single strategy for cardiac repair

When the mechanisms behind this beneficial effect were investigated, it was found that NRG stimulated the proliferation of cardiomyocytes (Figure 10d) while ADSCs stimulated a shift in macrophage polarization toward a regenerative phenotype (Díaz-Herráez et al., 2017). However, one obstacle found in the study was the lack of ADSCs differentiation to cardiomyocytes, an issue that remained controversial in several previous studies (Mazo et al., 2008). Therefore, after this successful proof-of-concept study, the efficacy of using these MP-based platforms to deliver cardiomyocytes obtained from human induced pluripotent stem cells (hiPSC-CM) to the infarcted heart was investigated (Saludas et al., 2019). The idea of administrating cardiac muscle cells directly emerged from the huge possibilities of these cells compared with nondifferentiated stem cells, as they could remuscularize the ischemic area, they integrate into the native myocardium and they are responsible for a potent paracrine secretion that mimics tissue requirements (Tachibana et al., 2017). As previously described, a combination of hiPSC-CM with MPs induced a two-fold increase in in vitro cell survival while cells were found in the mouse heart for up to 2 months. When the fate of transplanted cells was analyzed in the long term, these cells preserved their cardiac phenotype and they were interconnected with native tissue through gap junctions, which could be meaningful to avoid the appearance of arrhythmias (Figure 10e). This study was also of key importance to confirm that therapeutic effects resulted in cardiac repair since the functional performance of the heart was significantly improved (Figure 10e) (Saludas et al., 2019).

Current investigations regarding this technology are going a step further with the present experiments focused on elucidating the influence of the particle coating made of collagen and poly-D-lysine on cardiac stem cell paracrine secretion as well as the role of EVs secreted by these cells on the reparative response observed. These studies would shed light on cell therapy mechanisms. Furthermore, EV analysis could help to develop safer and improved treatments. In fact, EVs have been found to be more effective than their parent cells, to avoid the formation of teratomas, and to be less immunogenic (Adamiak et al., 2018; Ong & Wu, 2015).

3.3.3 | Ongoing investigations

Hydrogels as drug delivery systems for next generation of cell therapies

Recent work by M.J. Blanco-Prieto's team aligns with current research trends focused on cell-free therapies as the next generation of cell-based strategies. The demonstration that cell beneficial effects are a consequence of their paracrine secretion rather than cell differentiation is attracting the interest of many scientists in different fields (Mol et al., 2019).

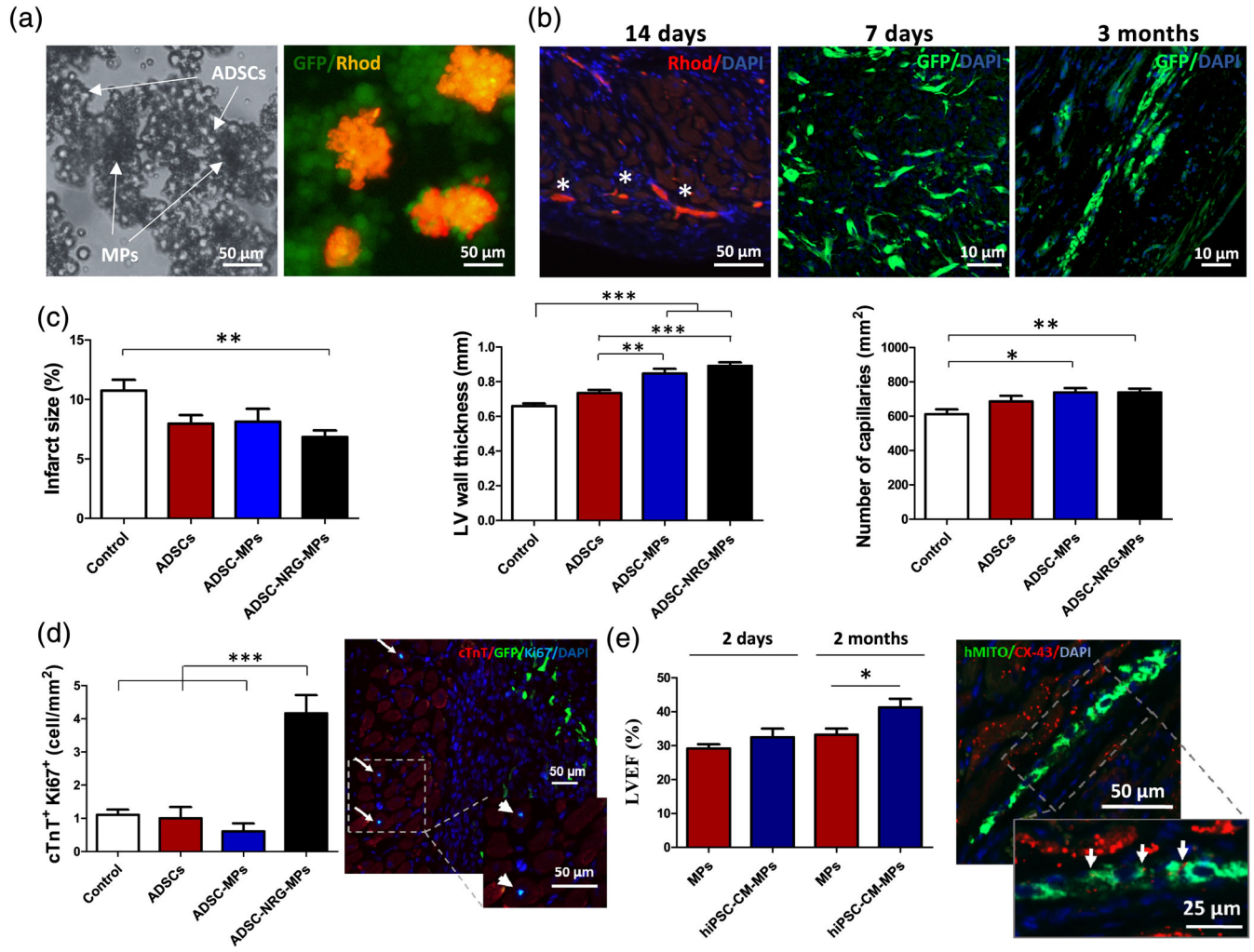


FIGURE 10 (a) Bright field and fluorescence images of GFP-ADSCs in combination with rhodamine-labeled NRG-MPs. (b) Retention of rhodamine-MPs in the ischemic myocardium after 14 days (asterisks) and GFP-ADSCs survival at 7 days and 3 months after their administration adhered to NRG-MPs in the heart (green). (c) Quantification of infarct size, left ventricular (LV) wall thickness and number of capillaries at 3 months showing that ADSC-NRG-MPs prevented adverse cardiac remodeling and induced angiogenesis. (d) Quantification of proliferative cardiomyocytes ($cTnT^+$ $ki67^+$) and representative fluorescence image reflecting the enhanced proliferation of cardiac muscle cells (arrows) after treatment with ADSC-NRG-MPs. (e) Cardiac function improvement at 2 months after treatment with CMs adhered to MPs (hiPSC-CM-MPs) and integration of transplanted cells (h-MITO, green) with other native/transplanted cardiomyocytes (arrows) reflected in the expression of connexin-43 (red). Data are mean ± SEM. *$p < .05$, **$p < .01$ and ***$p < .001$. Reprinted with permission from Díaz-Herráez et al. (2013). Copyright 2013 Elsevier; Díaz-Herráez et al. (2017). Copyright 2017 Elsevier; Saludas et al. (2019). Copyright 2019 Aspet

In one of its latest projects, the group is working on the therapeutic potential of EVs isolated from cardiac stem cells for cardiac repair. Furthermore, aiming to extend and boost the effect of EVs, a novel hydrogel is being developed for the sustained delivery of therapeutics as these drug delivery systems represent promising tools for cardiac delivery (Saludas, Pascual-Gil, Prósper, Garbayo, & Blanco-Prieto, 2017).

NPs for cardioprotection

In the context of the European project "Nanoheart," the group is also presently focused on the evaluation of the cardioprotective efficacy of squalene-adenosine NPs in a rat ischemia–reperfusion MI model. Although traditionally focused on the regenerative perspective, this initiative means a shift of paradigm toward a merely cardioprotective strategy. It is based on the squalenoylation technology, developed by Patrick Couvreur (Couvreur et al., 2006) and previously described for the administration of anticancer drugs (Rodríguez-Nogales, Sebastián, et al., 2019). Here, adenosine, a pharmacological compound with proven cardioprotective effects, is covalently linked to squalene, forming a prodrug that assembles into NPs in aqueous solution. The chemical encapsulation proposed in this study is advantageous as it

protects adenosine from degradation, avoids the burst release observed with physical encapsulation and guarantees a high drug loading. Current studies are evaluating the efficacy of this technology for cardiac application for the first time.

4 | CONCLUSION

Nanomedicine and drug delivery technologies are becoming a realistic prospect in modern medicine. Notably, a large part of the work carried out by the group of Prof Blanco-Prieto has been centered on these challenging areas, where a multidisciplinary translational research has been performed.

Some of the main achievements include significant progress in the development and preclinical evaluation of new anticancer engineered nanomaterials for lymphoma, glioma, and pediatric osteosarcoma treatment. In these multidisciplinary projects it has been shown that LN loaded with antitumor agents not only decreased the primary tumor but also were able to inhibit the metastases, thus paving the way for better anticancer treatments. In the field of regenerative medicine, a large number of projects were initiated in collaboration with clinicians, pharmaceutical scientists, material engineers, and veterinarians leading to several successful delivery systems for PD and MI with demonstrated efficacy in clinically relevant animal models (monkeys and pigs). Taken together, the advances achieved in nanomedicine and drug delivery technologies offer the potential for novel therapeutic approaches for unmet medical needs (e.g., cancer, cardiovascular, and neurodegenerative diseases) and the impact might be extremely important.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Maria J Blanco-prieto: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization; writing-original draft; writing-review and editing. **Elisa Garbayo:** Conceptualization; supervision; visualization; writing-original draft; writing-review and editing. **Simon Pascual Gil:** Conceptualization; validation; visualization; writing-original draft; writing-review and editing. **Carlos Rodriguez Nogales:** Conceptualization; validation; visualization; writing-original draft; writing-review and editing. **Laura Saludas:** Conceptualization; validation; visualization; writing-original draft; writing-review and editing. **Ander Estella Hermoso de Mendoza:** Conceptualization; validation; visualization; writing-original draft; writing-review and editing.

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REFERENCES

Adamiak, M., Cheng, G., Bobis-Wozowicz, S., Zhao, L., Kedracka-Krok, S., Samanta, A., ... Zuba-Surma, E. K. (2018). Induced pluripotent stem cell (iPSC)-derived extracellular vesicles are safer and more effective for cardiac repair than iPSCs. *Circulation Research*, *122*(2), 296–309. https://doi.org/10.1161/CIRCRESAHA.117.311769

Anselmo, A. C., & Mitragotri, S. (2019). Nanoparticles in the clinic: An update. *Bioengineering & Translational Medicine*, *4*(3), e10143. https://doi.org/10.1002/btm2.10143

Ansorena, E., Casales, E., Aranda, A., Tamayo, E., Garbayo, E., Smerdou, C., ... Aymerich, M. S. (2012). A simple and efficient method for the production of human glycosylated glial cell line-derived neurotrophic factor using a Semliki Forest virus expression system. *International Journal of Pharmaceutics*, *440*(1), 19–26. https://doi.org/10.1016/j.ijpharm.2012.04.071

Ansorena, E., Garbayo, E., Lanciego, J. L., Aymerich, M. S., & Blanco-Prieto, M. J. (2010). Production of highly pure human glycosylated GDNF in a mammalian cell line. *International Journal of Pharmaceutics*, *385*(1–2), 6–11. https://doi.org/10.1016/j.ijpharm.2009.10.015

Aznar, M. Á., Lasa-Saracíbar, B., & Blanco-Prieto, M. J. (2014). Edelfosine lipid nanoparticles overcome multidrug resistance in K-562 leukemia cells by a caspase-independent mechanism. *Molecular Pharmaceutics*, *11*(8), 2650–2658. https://doi.org/10.1021/mp5000696

Bajpai, G., Schneider, C., Wong, N., Bredemeyer, A., Hulsmans, M., Nahrendorf, M., ... Lavine, K. J. (2018). The human heart contains distinct macrophage subsets with divergent origins and functions. *Nature Medicine*, *24*(8), 1234–1245. https://doi.org/10.1038/s41591-018-0059-x

Baraniak, P. R., & McDevitt, T. C. (2010). Stem cell paracrine actions and tissue regeneration. *Regenerative Medicine*, *5*(1), 121–143. https://doi.org/10.2217/rme.09.74

Bargoni, A., Cavalli, R., Caputo, O., Fundarò, A., Gasco, M. R., & Zara, G. P. (1998). Solid lipid nanoparticles in lymph and plasma after duodenal administration to rats. *Pharmaceutical Research*, *15*(5), 745–750. https://doi.org/10.1023/A:1011975120776

Benjamin, E. J., Blaha, M. J., Chiuve, S. E., Cushman, M., Das, S. R., Deo, R., ... Muntner, P. (2017). Heart disease and stroke statistics—2017 update: A report from the American Heart Association. *Circulation*, *135*(10), e146–e603. https://doi.org/10.1161/CIR.0000000000000485

Blanco, E., Shen, H., & Ferrari, M. (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nature Biotechnology*, *33*(9), 941–951. https://doi.org/10.1038/nbt.3330

Blanco Prieto, M. J., Delie, F., Fattal, E., Tartar, A., Puisieux, F., Gulik, A., & Couvreur, P. (1994). Characterization of V3 BRU peptide-loaded small PLGA microspheres prepared by a (w1/o)w2 emulsion solvent evaporation method. *International Journal of Pharmaceutics*, *111*(2), 137–145. https://doi.org/10.1016/0378-5173(94)00104-9

Blanco-Prieto, M. J., Besseghir, K., Orsolini, P., Heimgartner, F., Deuschel, C., Merkle, H. P., ... Gander, B. (1999). Importance of the test medium for the release kinetics of a somatostatin analogue from poly(D,L-lactide-co-glycolide) microspheres. *International Journal of Pharmaceutics*, *184*(2), 243–250. https://doi.org/10.1016/s0378-5173(99)00118-0

Blanco-Prieto, M. J., Besseghir, K., Zerbe, O., Andris, D., Orsolini, P., Heimgartner, F., ... Gander, B. (2000). In vitro and in vivo evaluation of a somatostatin analogue released from PLGA microspheres. *Journal of Controlled Release*, *67*(1), 19–28. https://doi.org/10.1016/s0168-3659(99)00289-8

Blanco-Prieto, M. J., Campanero, M. A., Besseghir, K., Heimgatner, F., & Gander, B. (2004). Importance of single or blended polymer types for controlled in vitro release and plasma levels of a somatostatin analogue entrapped in PLA/PLGA microspheres. *Journal of Controlled Release*, *96*(3), 437–448. https://doi.org/10.1016/j.jconrel.2004.02.015

Blanco-Prieto, M. J., Durieux, C., Daugé, V., Fattal, E., Couvreur, P., & Roques, B. P. (2002). Slow delivery of the selective cholecystokinin agonist pBC 264 into the rat nucleus accumbens using microspheres. *Journal of Neurochemistry*, *67*(6), 2417–2424. https://doi.org/10.1046/j.1471-4159.1996.67062417.x

Blanco-Prieto, M. J., Fattal, E., Gulik, A., Dedieu, J. C., Roques, B. P., & Couvreur, P. (1997). Characterization and morphological analysis of a cholecystokinin derivative peptide-loaded poly(lactide-co-glycolide) microspheres prepared by a water-in-oil-in-water emulsion solvent evaporation method. *Journal of Controlled Release*, *43*(1), 81–87. https://doi.org/10.1016/S0168-3659(96)01474-5

Blanco-Prieto, M. J., Leo, E., Delie, F., Gulik, A., Couvreur, P., & Fattal, E. (1996). Study of the influence of several stabilizing agents on the entrapment and in vitro release of pBC 264 from poly(lactide-co-glycolide) microspheres prepared by a W/O/W solvent evaporation method. *Pharmaceutical Research*, *13*(7), 1127–1129. https://doi.org/10.1023/A:1016087530812

Botter, S. M., Neri, D., & Fuchs, B. (2014). Recent advances in osteosarcoma. *Current Opinion in Pharmacology*, *16*, 15–23. https://doi.org/10.1016/j.coph.2014.02.002

Broughton, K. M., Wang, B. J., Firouzi, F., Khalafalla, F., Dimmeler, S., Fernandez-Aviles, F., & Sussman, M. A. (2018). Mechanisms of cardiac repair and regeneration. *Circulation Research*, *122*(8), 1151–1163. https://doi.org/10.1161/CIRCRESAHA.117.312586

Constantinides, P. P., & Wasan, K. M. (2007). Lipid formulation strategies for enhancing intestinal transport and absorption of P-glycoprotein (P-gp) substrate drugs: In vitro/in vivo case studies. *Journal of Pharmaceutical Sciences*, *96*(2), 235–248. https://doi.org/10.1002/jps.20780

Couvreur, P., Blanco-Prieto, M. J., Puisieux, F., Roques, B., & Fattal, E. (1997). Multiple emulsion technology for the design of microspheres containing peptides and oligopeptides. *Advanced Drug Delivery Reviews*, *28*(1), 85–96. https://doi.org/10.1016/S0169-409X(97)00052-5

Couvreur, P., Reddy, L. H., Mangenot, S., Poupaert, J. H., Desmaële, D., Lepêtre-Mouelhi, S., ... Ollivon, M. (2008). Discovery of new hexagonal supramolecular nanostructures formed by squalenoylation of an anticancer nucleoside analogue. *Small*, *4*(2), 247–253. https://doi.org/10.1002/smll.200700731

Couvreur, P., Stella, B., Harivardhan Reddy, L., Hillaireau, H., Dubernet, C., Desmaële, D., ... Cattel, L. (2006). Squalenoyl nanomedicines as potential therapeutics. *Nano Letters*, *6*(11), 2544–2548. https://doi.org/10.1021/nl061942q

Del Rey, N. L.-G., Quiroga-Varela, A., Garbayo, E., Carballo-Carbajal, I., Fernández-Santiago, R., Monje, M. H. G., ... Blesa, J. (2018). Advances in Parkinson's disease: 200 years later. *Frontiers in Neuroanatomy*, *12*, 113. https://doi.org/10.3389/fnana.2018.00113

Díaz-Herráez, P., Garbayo, E., Simón-Yarza, T., Formiga, F. R., Prosper, F., & Blanco-Prieto, M. J. (2013). Adipose-derived stem cells combined with Neuregulin-1 delivery systems for heart tissue engineering. *European Journal of Pharmaceutics and Biopharmaceutics*, *85*(1), 143–150. https://doi.org/10.1016/j.ejpb.2013.03.022

Díaz-Herráez, P., Saludas, L., Pascual-Gil, S., Simón-Yarza, T., Abizanda, G., Prósper, F., ... Blanco-Prieto, M. J. (2017). Transplantation of adipose-derived stem cells combined with neuregulin-microparticles promotes efficient cardiac repair in a rat myocardial infarction model. *Journal of Controlled Release*, *249*, 23–31. https://doi.org/10.1016/j.jconrel.2017.01.026

Dick, S. A., Macklin, J. A., Nejat, S., Momen, A., Clemente-Casares, X., Althagafi, M. G., ... Epelman, S. (2019). Self-renewing resident cardiac macrophages limit adverse remodeling following myocardial infarction. *Nature Immunology*, *20*(1), 29–39. https://doi.org/10.1038/s41590-018-0272-2

Dormont, F., Varna, M., & Couvreur, P. (2018). Nanoplumbers: Biomaterials to fight cardiovascular diseases. *Materials Today*, *21*(2), 122–143. https://doi.org/10.1016/j.mattod.2017.07.008

Epelman, S., Liu, P. P., & Mann, D. L. (2015). Role of innate and adaptive immune mechanisms in cardiac injury and repair. *Nature Reviews Immunology*, *15*(2), 117–129. https://doi.org/10.1038/nri3800

Estella-Hermoso de Mendoza, A., Campanero, M. A., Lana, H., Villa-Pulgarin, J. A., De La Iglesia-Vicente, J., Mollinedo, F., & Blanco-Prieto, M. J. (2012). Complete inhibition of extranodal dissemination of lymphoma by edelfosine-loaded lipid nanoparticles. *Nanomedicine*, *7*(5), 679–690. https://doi.org/10.2217/nnm.11.134

Estella-Hermoso de Mendoza, A., Campanero, M. A., Mollinedo, F., & Blanco-Prieto, M. J. (2009). Lipid nanomedicines for anticancer drug therapy. *Journal of Biomedical Nanotechnology*, *5*(4), 323–343. https://doi.org/10.1166/jbn.2009.1042

Estella-Hermoso de Mendoza, A., Préat, V., Mollinedo, F., & Blanco-Prieto, M. J. (2011). In vitro and in vivo efficacy of edelfosine-loaded lipid nanoparticles against glioma. *Journal of Controlled Release*, *156*(3), 421–426. https://doi.org/10.1016/j.jconrel.2011.07.030

Estella-Hermoso de Mendoza, A., Rayo, M., Mollinedo, F., & Blanco-Prieto, M. J. (2008). Lipid nanoparticles for alkyl lysophospholipid edelfosine encapsulation: Development and in vitro characterization. *European Journal of Pharmaceutics and Biopharmaceutics*, *68*(2), 207–213. https://doi.org/10.1016/j.ejpb.2007.06.015

Formiga, F. R., Garbayo, E., Díaz-Herráez, P., Abizanda, G., Simón-Yarza, T., Tamayo, E., … Blanco-Prieto, M. J. (2013). Biodegradation and heart retention of polymeric microparticles in a rat model of myocardial ischemia. *European Journal of Pharmaceutics and Biopharmaceutics*, *85*(3 Pt A), 665–672. https://doi.org/10.1016/j.ejpb.2013.02.017

Formiga, F. R., Pelacho, B., Garbayo, E., Abizanda, G., Gavira, J. J., Simon-Yarza, T., … Blanco-Prieto, M. J. (2010). Sustained release of VEGF through PLGA microparticles improves vasculogenesis and tissue remodeling in an acute myocardial ischemia-reperfusion model. *Journal of Controlled Release*, *147*(1), 30–37. https://doi.org/10.1016/j.jconrel.2010.07.097

Formiga, F. R., Pelacho, B., Garbayo, E., Imbuluzqueta, I., Díaz-Herráez, P., Abizanda, G., … Blanco-Prieto, M. J. (2013). Controlled delivery of fibroblast growth factor-1 and neuregulin-1 from biodegradable microparticles promotes cardiac repair in a rat myocardial infarction model through activation of endogenous regeneration. *Journal of Controlled Release*, *173*, 132–139. https://doi.org/10.1016/j.jconrel.2013.10.034

Formiga, F. R., Tamayo, E., Simón-Yarza, T., Pelacho, B., Prósper, F., & Blanco-Prieto, M. J. (2012). Angiogenic therapy for cardiac repair based on protein delivery systems. *Heart Failure Reviews*, *17*(3), 449–473. https://doi.org/10.1007/s10741-011-9285-8

Garbayo, E., Ansorena, E., Lana, H., Carmona-Abellan, M. D. M., Marcilla, I., Lanciego, J. L., … Blanco-Prieto, M. J. (2016). Brain delivery of microencapsulated GDNF induces functional and structural recovery in parkinsonian monkeys. *Biomaterials*, *110*, 11–23. https://doi.org/10.1016/j.biomaterials.2016.09.015

Garbayo, E., Ansorena, E., Lanciego, J. L., Aymerich, M. S., & Blanco-Prieto, M. J. (2007). Purification of bioactive glycosylated recombinant glial cell line-derived neurotrophic factor. *International Journal of Pharmaceutics*, *344*(1–2), 9–15. https://doi.org/10.1016/j.ijpharm.2007.04.003

Garbayo, E., Ansorena, E., Lanciego, J. L., Aymerich, M. S., & Blanco-Prieto, M. J. (2008). Sustained release of bioactive glycosylated glial cell-line derived neurotrophic factor from biodegradable polymeric microspheres. *European Journal of Pharmaceutics and Biopharmaceutics*, *69*(3), 844–851. https://doi.org/10.1016/j.ejpb.2008.02.015

Garbayo, E., Ansorena, E., Lanciego, J. L., Blanco-Prieto, M. J., & Aymerich, M. S. (2011). Long-term neuroprotection and neurorestoration by glial cell-derived neurotrophic factor microspheres for the treatment of Parkinson's disease. *Movement Disorders*, *26*(10), 1943–1947. https://doi.org/10.1002/mds.23793

Garbayo, E., Gavira, J. J., de Yebenes, M. G., Pelacho, B., Abizanda, G., Lana, H., … Prosper, F. (2016). Catheter-based Intramyocardial injection of FGF1 or NRG1-loaded MPs improves cardiac function in a preclinical model of ischemia–reperfusion. *Scientific Reports*, *6*, 25932. https://doi.org/10.1038/srep25932

Garbayo, E., Montero-Menei, C. N., Ansorena, E., Lanciego, J. L., Aymerich, M. S., & Blanco-Prieto, M. J. (2009). Effective GDNF brain delivery using microspheres – A promising strategy for Parkinson's disease. *Journal of Controlled Release*, *135*(2), 119–126. https://doi.org/10.1016/j.jconrel.2008.12.010

Gibson, T. M., & Robison, L. L. (2015). Impact of cancer therapy-related exposures on late mortality in childhood cancer survivors. *Chemical Research in Toxicology*, *28*(1), 31–37. https://doi.org/10.1021/tx500374k

Gill, S. S., Patel, N. K., Hotton, G. R., O'Sullivan, K., McCarter, R., Bunnage, M., … Heywood, P. (2003). Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nature Medicine*, *9*(5), 589–595. https://doi.org/10.1038/nm850

Golombek, S. K., May, J.-N., Theek, B., Appold, L., Drude, N., Kiessling, F., & Lammers, T. (2018). Tumor targeting via EPR: Strategies to enhance patient responses. *Advanced Drug Delivery Reviews*, *130*, 17–38. https://doi.org/10.1016/j.addr.2018.07.007

González-Fernández, Y., Brown, H. K., Patiño-García, A., Heymann, D., & Blanco-Prieto, M. J. (2018). Oral administration of edelfosine encapsulated lipid nanoparticles causes regression of lung metastases in pre-clinical models of osteosarcoma. *Cancer Letters*, *430*, 193–200. https://doi.org/10.1016/j.canlet.2018.05.030

González-Fernández, Y., Imbuluzqueta, E., Patiño-García, A., & Blanco-Prieto, M. J. (2015). Antitumoral-lipid-based nanoparticles: A platform for future application in osteosarcoma therapy. *Current Pharmaceutical Design*, *21*(42), 6104–6124. https://doi.org/10.2174/1381612821666151027152534

González-Fernández, Y., Imbuluzqueta, E., Zalacain, M., Mollinedo, F., Patiño-García, A., & Blanco-Prieto, M. J. (2017). Doxorubicin and edelfosine lipid nanoparticles are effective acting synergistically against drug-resistant osteosarcoma cancer cells. *Cancer Letters*, *388*, 262–268. https://doi.org/10.1016/j.canlet.2016.12.012

González-Fernández, Y., Zalacain, M., Imbuluzqueta, E., Sierrasesumaga, L., Patiño-García, A., & Blanco-Prieto, M. J. (2015). Lipid nanoparticles enhance the efficacy of chemotherapy in primary and metastatic human osteosarcoma cells. *Journal of Drug Delivery Science and Technology*, *30*, 435–442. https://doi.org/10.1016/j.jddst.2015.08.004

Hodgkinson, C. P., Bareja, A., Gomez, J. A., & Dzau, V. J. (2016). Emerging concepts in paracrine mechanisms in regenerative cardiovascular medicine and biology. *Circulation Research*, *118*(1), 95–107. https://doi.org/10.1161/CIRCRESAHA.115.305373

Hoffman, T., Khademhosseini, A., & Langer, R. (2019). Chasing the paradigm: Clinical translation of 25 years of tissue engineering. *Tissue Engineering – Part A*, *25*(9–10), 679–687. https://doi.org/10.1089/ten.tea.2019.0032

Hong, S. J., Traktuev, D. O., & March, K. L. (2010). Therapeutic potential of adipose-derived stem cells in vascular growth and tissue repair. *Current Opinion in Organ Transplantation*, *15*(1), 86–91. https://doi.org/10.1097/MOT.0b013e328334f074

Hulsmans, M., Clauss, S., Xiao, L., Aguirre, A. D., King, K. R., Hanley, A., … Nahrendorf, M. (2017). Macrophages facilitate electrical conduction in the heart. *Cell*, *169*(3), 510–522.e20. https://doi.org/10.1016/j.cell.2017.03.050

Jankowski, J., Korzeniowska, K., Cieślewicz, A., & Jabłecka, A. (2016). Coenzyme Q10 – A new player in the treatment of heart failure? *Pharmacological Reports*, *68*(5), 1015–1019. https://doi.org/10.1016/j.pharep.2016.05.012

Johansson, S., Rosengren, A., Young, K., & Jennings, E. (2017). Mortality and morbidity trends after the first year in survivors of acute myocardial infarction: A systematic review. *BMC Cardiovascular Disorders*, *17*(1), 53. https://doi.org/10.1186/s12872-017-0482-9

Kearns, C. M., & Gash, D. M. (1995). GDNF protects nigral dopamine neurons against 6-hydroxydopamine in vivo. *Brain Research*, *672*(1–2), 104–111. https://doi.org/10.1016/0006-8993(94)01366-p

Lasa-Saracíbar, B., Aznar, M. Á., Lana, H., Aizpún, I., Gil, A. G., & Blanco-Prieto, M. J. (2014). Lipid nanoparticles protect from edelfosine toxicity in vivo. *International Journal of Pharmaceutics*, *474*(1–2), 1–5. https://doi.org/10.1016/j.ijpharm.2014.07.053

Lasa-Saracíbar, B., Estella-Hermoso de Mendoza, A., Mollinedo, F., Odero, M. D., & Blanco-Príeto, M. J. (2013). Edelfosine lipid nanosystems overcome drug resistance in leukemic cell lines. *Cancer Letters*, *334*(2), 302–310. https://doi.org/10.1016/j.canlet.2013.01.018

Lasa-Saracíbar, B., Guada, M., Sebastián, V., & Blanco-Prieto, M. J. (2014). In vitro intestinal co-culture cell model to evaluate intestinal absorption of edelfosine lipid nanoparticles. *Current Topics in Medicinal Chemistry*, *14*(9), 1124–1132. https://doi.org/10.2174/1568026614666140329225340

Lavine, K. J., Epelman, S., Uchida, K., Weber, K. J., Nichols, C. G., Schilling, J. D., … Mann, D. L. (2014). Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(45), 16029–16034. https://doi.org/10.1073/pnas.1406508111

Liu, X., Gu, X., Li, Z., Li, X., Li, H., Chang, J., … Zhou, M. (2006). Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy. *Journal of the American College of Cardiology*, *48*(7), 1438–1447. https://doi.org/10.1016/j.jacc.2006.05.057

Luque-Michel, E., Larrea, A., Lahuerta, C., Sebastian, V., Imbuluzqueta, E., Arruebo, M., … Santamaría, J. (2016). A simple approach to obtain hybrid Au-loaded polymeric nanoparticles with a tunable metal load. *Nanoscale*, *8*(12), 6495–6506. https://doi.org/10.1039/c5nr06850a

Luque-Michel, E., Sebastian, V., Larrea, A., Marquina, C., & Blanco-Prieto, M. J. (2019). Co-encapsulation of superparamagnetic nanoparticles and doxorubicin in PLGA nanocarriers: Development, characterization and in vitro antitumor efficacy in glioma cells. *European Journal of Pharmaceutics and Biopharmaceutics*, *145*, 65–75. https://doi.org/10.1016/j.ejpb.2019.10.004

Luque-Michel, E., Sebastian, V., Szczupak, B., Imbuluzqueta, E., Llop, J., & Blanco Prieto, M. J. (2017). Visualization of hybrid gold-loaded polymeric nanoparticles in cells using scanning electron microscopy. *Journal of Drug Delivery Science and Technology*, *42*, 315–320. https://doi.org/10.1016/j.jddst.2017.04.008

Madl, C. M., Heilshorn, S. C., & Blau, H. M. (2018). Bioengineering strategies to accelerate stem cell therapeutics. *Nature*, *557*(7705), 335–342. https://doi.org/10.1038/s41586-018-0089-z

Martinez, F. O., & Gordon, S. (2014). The M1 and M2 paradigm of macrophage activation: Time for reassessment. *F1000Prime Reports*, *6*. https://doi.org/10.12703/P6-13

Matsusaki, M., Sakaguchi, H., Serizawa, T., & Akashi, M. (2007). Controlled release of vascular endothelial growth factor from alginate hydrogels nano-coated with polyelectrolyte multilayer films. *Journal of Biomaterials Science, Polymer Edition*, *18*(6), 775–783. https://doi.org/10.1163/156856207781034160

Mazo, M., Planat-Bénard, V., Abizanda, G., Pelacho, B., Léobon, B., Gavira, J. J., … Prósper, F. (2008). Transplantation of adipose derived stromal cells is associated with functional improvement in a rat model of chronic myocardial infarction. *European Journal of Heart Failure*, *10*(5), 454–462. https://doi.org/10.1016/j.ejheart.2008.03.017

Mitragotri, S., Burke, P. A., & Langer, R. (2014). Overcoming the challenges in administering biopharmaceuticals: Formulation and delivery strategies. *Nature Reviews Drug Discovery*, *13*(9), 655–672. https://doi.org/10.1038/nrd4363

Mol, E. A., Lei, Z., Roefs, M. T., Bakker, M. H., Goumans, M., Doevendans, P. A., … Sluijter, J. P. G. (2019). Injectable supramolecular ureidopyrimidinone hydrogels provide sustained release of extracellular vesicle therapeutics. *Advanced Healthcare Materials*, *8*(20), 1900847. https://doi.org/10.1002/adhm.201900847

Mollinedo, F., Gajate, C., Martin-Santamaria, S., & Gago, F. (2012). ET-18-OCH3 (edelfosine): A selective antitumour lipid targeting apoptosis through intracellular activation of Fas/CD95 death receptor. *Current Medicinal Chemistry*, *11*(24), 3163–3184. https://doi.org/10.2174/0929867043363703

Ong, S.-G., & Wu, J. C. (2015). Exosomes as potential alternatives to stem cell therapy in mediating cardiac regeneration. *Circulation Research*, *117*(1), 7–9. https://doi.org/10.1161/CIRCRESAHA.115.306593

Owens, D. E., & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics*, *307*(1), 93–102. https://doi.org/10.1016/j.ijpharm.2005.10.010

Park, K., Skidmore, S., Hadar, J., Garner, J., Park, H., Otte, A., … Wang, Y. (2019). Injectable, long-acting PLGA formulations: Analyzing PLGA and understanding microparticle formation. *Journal of Controlled Release*, *304*, 125–134. https://doi.org/10.1016/j.jconrel.2019.05.003

Pascual-Gil, S., Abizanda, G., Iglesias, E., Garbayo, E., Prósper, F., & Blanco-Prieto, M. J. (2019). NRG1 PLGA MP locally induce macrophage polarisation toward a regenerative phenotype in the heart after acute myocardial infarction. *Journal of Drug Targeting*, *27*(5–6), 573–581. https://doi.org/10.1080/1061186X.2018.1531417

Pascual-Gil, S., Garbayo, E., Díaz-Herráez, P., Prosper, F., & Blanco-Prieto, M. J. (2015). Heart regeneration after myocardial infarction using synthetic biomaterials. *Journal of Controlled Release*, *203*, 23–38. https://doi.org/10.1016/j.jconrel.2015.02.009

Pascual-Gil, S., Simón-Yarza, T., Garbayo, E., Prosper, F., & Blanco-Prieto, M. J. (2015). Tracking the in vivo release of bioactive NRG from PLGA and PEG-PLGA microparticles in infarcted hearts. *Journal of Controlled Release*, *220*(Pt A), 388–396. https://doi.org/10.1016/j.jconrel.2015.10.058

Pascual-Gil, S., Simón-Yarza, T., Garbayo, E., Prósper, F., & Blanco-Prieto, M. J. (2017). Cytokine-loaded PLGA and PEG-PLGA microparticles showed similar heart regeneration in a rat myocardial infarction model. *International Journal of Pharmaceutics*, *523*(2), 531–533. https://doi.org/10.1016/j.ijpharm.2016.11.022

Patel, N. K., Bunnage, M., Plaha, P., Svendsen, C. N., Heywood, P., & Gill, S. S. (2005). Intraputamenal infusion of glial cell line-derived neurotrophic factor in PD: A two-year outcome study. *Annals of Neurology*, *57*(2), 298–302. https://doi.org/10.1002/ana.20374

Peer, D., Karp, J. M., Hong, S., Farokhzad, O. C., Margalit, R., & Langer, R. (2007). Nanocarriers as an emerging platform for cancer therapy. *Nature Nanotechnology*, *2*(12), 751–760. https://doi.org/10.1038/nnano.2007.387

Réjiba, S., Reddy, L. H., Bigand, C., Parmentier, C., Couvreur, P., & Hajri, A. (2011). Squalenoyl gemcitabine nanomedicine overcomes the low efficacy of gemcitabine therapy in pancreatic cancer. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *7*(6), 841–849. https://doi.org/10.1016/j.nano.2011.02.012

Rodríguez-Nogales, C., González-Fernández, Y., Aldaz, A., Couvreur, P., & Blanco-Prieto, M. J. (2018). Nanomedicines for pediatric cancers. *ACS Nano*, *12*(8), 7482–7496. https://doi.org/10.1021/acsnano.8b03684

Rodríguez-Nogales, C., Noguera, R., Couvreur, P., & Blanco-Prieto, M. J. (2019). Therapeutic opportunities in neuroblastoma using nanotechnology. *Journal of Pharmacology and Experimental Therapeutics*, *370*(3), 625–635. https://doi.org/10.1124/jpet.118.255067

Rodríguez-Nogales, C., Sebastián, V., Irusta, S., Desmaële, D., Couvreur, P., & Blanco-Prieto, M. J. (2019). A unique multidrug nanomedicine made of squalenoyl-gemcitabine and alkyl-lysophospholipid edelfosine. *European Journal of Pharmaceutics and Biopharmaceutics*, *144*, 165–173. https://doi.org/10.1016/j.ejpb.2019.09.017

Ruddox, V., Sandven, I., Munkhaugen, J., Skattebu, J., Edvardsen, T., & Otterstad, J. E. (2017). Atrial fibrillation and the risk for myocardial infarction, all-cause mortality and heart failure: A systematic review and meta-analysis. *European Journal of Preventive Cardiology*, *24*(14), 1555–1566. https://doi.org/10.1177/2047487317715769

Rupert, C. E., & Coulombe, K. L. (2015). The roles of neuregulin-1 in cardiac development, homeostasis, and disease. *Biomarker Insights*, *10*(Suppl 1), 1–9. https://doi.org/10.4137/BMI.S20061

Saludas, L., Garbayo, E., Mazo, M., Pelacho, B., Abizanda, G., Iglesias Garcia, O., … Blanco-prieto, M. J. (2019). Long-term engraftment of human cardiomyocytes combined with biodegradable microparticles induces heart repair. *Journal of Pharmacology and Experimental Therapeutics*, *370*(3), 761–771. https://doi.org/10.1124/jpet.118.256065

Saludas, L., Pascual-Gil, S., Prósper, F., Garbayo, E., & Blanco-Prieto, M. (2017). Hydrogel based approaches for cardiac tissue engineering. *International Journal of Pharmaceutics*, *523*(2), 454–475. https://doi.org/10.1016/j.ijpharm.2016.10.061

Saludas, L., Pascual-Gil, S., Roli, F., Garbayo, E., & Blanco-Prieto, M. J. (2018). Heart tissue repair and cardioprotection using drug delivery systems. *Maturitas*, *110*, 1–9. https://doi.org/10.1016/j.maturitas.2018.01.011

Simón-Yarza, T., Formiga, F. R., Tamayo, E., Pelacho, B., Prosper, F., & Blanco-Prieto, M. J. (2012). Vascular endothelial growth factor-delivery systems for cardiac repair: An overview. *Theranostics*, *2*(6), 541–552. https://doi.org/10.7150/thno.3682

Simón-Yarza, T., Formiga, F. R., Tamayo, E., Pelacho, B., Prosper, F., & Blanco-Prieto, M. J. (2013). PEGylated-PLGA microparticles containing VEGF for long term drug delivery. *International Journal of Pharmaceutics*, *440*(1), 13–18. https://doi.org/10.1016/j.ijpharm.2012.07.006

Simón-Yarza, T., Tamayo, E., Benavides, C., Lana, H., Formiga, F. R., Grama, C. N., … Blanco-Prieto, M. J. (2013). Functional benefits of PLGA particulates carrying VEGF and CoQ10 in an animal of myocardial ischemia. *International Journal of Pharmaceutics*, *454*(2), 784–790. https://doi.org/10.1016/j.ijpharm.2013.04.015

Slavin, J. (1995). Fibroblast growth factors: At the heart of angiogenesis. *Cell Biology International*, *19*(5), 431–444. https://doi.org/10.1006/cbir.1995.1087

Slevin, J. T., Gerhardt, G. A., Smith, C. D., Gash, D. M., Kryscio, R., & Young, B. (2005). Improvement of bilateral motor functions in patients with Parkinson disease through the unilateral intraputaminal infusion of glial cell line-derived neurotrophic factor. *Journal of Neurosurgery*, *102*(2), 216–222. https://doi.org/10.3171/jns.2005.102.2.0216

Stegmann, T. J. (1998). FGF-1: A human growth factor in the induction of neoangiogenesis. *Expert Opinion on Investigational Drugs*, *7*(12), 2011–2015. https://doi.org/10.1517/13543784.7.12.2011

Suk, J. S., Xu, Q., Kim, N., Hanes, J., & Ensign, L. M. (2016). PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced Drug Delivery Reviews*, *99*(Pt A), 28–51. https://doi.org/10.1016/j.addr.2015.09.012

Tachibana, A., Santoso, M. R., Mahmoudi, M., Shukla, P., Wang, L., Bennett, M., … Yang, P. C. (2017). Paracrine effects of the pluripotent stem cell-derived cardiac myocytes salvage the injured myocardium. *Circulation Research*, *121*(6), e22–e36. https://doi.org/10.1161/CIRCRESAHA.117.310803

Torres-Ortega, P. V., Saludas, L., Hanafy, A. S., Garbayo, E., & Blanco-Prieto, M. J. (2019). Micro- and nanotechnology approaches to improve Parkinson's disease therapy. *Journal of Controlled Release*, *295*, 201–213. https://doi.org/10.1016/j.jconrel.2018.12.036

Uygur, A., & Lee, R. T. (2016). Mechanisms of cardiac regeneration. *Developmental Cell*, *36*(4), 362–374. https://doi.org/10.1016/j.devcel.2016.01.018

van der Meel, R., Sulheim, E., Shi, Y., Kiessling, F., Mulder, W. J. M., & Lammers, T. (2019). Smart cancer nanomedicine. *Nature Nanotechnology*, *14*(11), 1007–1017. https://doi.org/10.1038/s41565-019-0567-y

Wallner, C., Schira, J., Wagner, J. M., Schulte, M., Fischer, S., Hirsch, T., ... Behr, B. (2015). Application of VEGFA and FGF-9 enhances angiogenesis, osteogenesis and bone remodeling in type 2 diabetic long bone regeneration. *PLoS One*, *10*(3), e0118823. https://doi.org/10.1371/journal.pone.0118823

Yuan, F., Dellian, M., Fukumura, D., Leunig, M., Berk, D. A., Jain, R. K., & Torchilin, V. P. (1995). Vascular permeability in a human tumor xenograft: Molecular size dependence and cutoff size. *Cancer Research*, *55*(17), 3752–3756.

Yutzey, K. E. (2015). Regenerative biology: Neuregulin 1 makes heart muscle. *Nature*, *520*(7548), 445–446. https://doi.org/10.1038/520445a

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