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# Design of a composite drug delivery system to prolong functionality of cell-based scaffolds

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ABSTRACT

Cell encapsulation technology raises hopes in medicine and biotechnology. However, despite important advances in the field in the past three decades, several challenges associated with the biocompatibility are still remaining. In the present study, the effect of a temporary release of an anti-inflammatory agent on co-administered encapsulated allogeneic cells was investigated. The aim was to determine the biocompatibility and efficacy of the approach to prevent the inflammatory response. A composite delivery system comprised of alginate-poly-L-lysine-alginate (APA)-microencapsulated Epo-secreting myoblasts and dexamethasone (DXM)-releasing poly(lactic-co-glycolic acid) (PLGA) microspheres was implanted in the subcutaneous space of Balb/c mice for 45 days.

The use of independently co-implanted DXM-loaded PLGA microspheres resulted in an improved functionality of the cell-based graft, evidenced by significantly higher hematocrit levels found in the cell-implanted groups by day 45, which was found to be more pronounced when higher cell-doses (100 μL) were employed. Moreover, no major host reaction was observed upon implantation of the systems, showing good biocompatibility and capability to partially avoid the inflammatory response, probably due to the immunosuppressive effects related to DXM. The findings of this study imply that DXM-loaded PLGA microspheres show promise as release systems to enhance biocompatibility and offer advantage in the development of long-lasting and effective implantable microencapsulated cells by generating a potential immunopriviledged local environment and an effective method to limit the structural ensheathing layer caused by inflammation.



## 1. Introduction

Microencapsulation of living cells is a promising approach for the continuous delivery of therapeutics. This technology is based on the immobilization of cells within a polymeric matrix surrounded by a semipermeable membrane. The inner cells release the therapeutic agent continuously, while the semipermeable membrane immunoprotects the cells from the host immune system allowing the exchange of nutrients, oxygen and waste products (Lee and Bae, 2000; Ricci et al., 2005).

Since the successful approach developed by Lim and Sun in 1980, using the APA system to entrap islets of Langerhans, extensive research has been carried out using microencapsulation technology as an alternative treatment for a wide range of disorders and significant achievements with claims of remarkable success including a few non-human primates and human pilot clinical trials have been obtained (Lim and Sun, 1980; Elliott et al., 2005; Dufrane et al., 2006; Calafiore et al., 2006; Hernández et al., 2010).

Nevertheless, if scalability of the technology into clinical practice is aimed, an optimal composite system must be designed. Failure to achieve optimal biocompatibility and immune acceptance has often been ascribed to the inflammatory response eventually evoked towards the transplanted microencapsulated cells leading to limited immunobarrier competence, hypoxia and finally encapsulated cell apoptosis due to the great distance between the encapsulated cells and the blood supply (De Groot et al., 2004; Orive et al., 2006; de Vos et al., 2009). As far as biocompatibility is concerned, implantable devices can elicit a foreign body reaction. An acute inflammatory response, characterized by neutrophils as the primary cellular infiltrate, is followed by a chronic inflammation characterized by monocytes and lymphocytes. Monocytes, differentiated into macrophages, lead into the

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granulation tissue development (Babensee et al., 1998; Anderson and Langone, 1999).

In spite of interesting and significant advances in the field already achieved, some challenges still remain unsolved. Optimal biocompatibility of the cell-based system upon *in vivo* implantation seems to be a pending issue, both in allogeneic and specially in xenogeneic approaches. Hence, the development of a temporally immunoprotected transplantation microenvironment might be a newsworthy approach. Considering the toxicity and side effects related to the implementation of general immunosuppression, the use of a temporal protocol locally administered in order to generate an immunopriviledged environment, represents an interesting alternative approach (Calafiore and Basta, 1999). Moreover, the labor-intensive constant administration of anti-inflammatory drugs needed to reduce host response against transplanted microcapsules could be avoided and hence a lower dose of drug permitted compared to its systemic administration, with favorable impact on typical treatment side effects due to chronic exposure (Safley et al., 2008).

Several different strategies have been considered to overcome the harmful effects related to systemic immunosuppression, including the use of high purity (pyrogen and endotoxin-free) polymers (already optimized by most researchers in the field) and combined cell microencapsulation systems where a secondary anti-inflammatory drug release system is incorporated along with the cells (Omer et al., 2003; Bunger et al., 2005; Baruch et al., 2009). However, considering the possible decrease in pH as a result of the biodegradation of PLGA, the incorporation of PLGA microspheres within the cell-loaded capsules was discarded (Baruch et al., 2009).

In the present study, an independent composite system was developed comprising APA microcapsules embedding Epo-secreting $C_2C_{12}$ myoblasts and PLGA microspheres loaded with DXM as a model anti-inflammatory drug, hence creating an immunopriviledged environment.

Over the past years, much interest has been focused on the development of PLA, PGA and PLGA copolymer microspheres as delivery carriers of interesting pharmacological agents (Benny et al., 2008; Bae et al., 2009; Anderson and Shive, 1997) due to their potential usefulness in increasing efficacy (Panyam and Labhasetwar, 2004), reducing enzymatic degradation (Rosler et al., 2001) and controlling release rates (Jain, 2000).

Dexamethasone is a clinically widely used glucocorticoid anti-inflammatory and immunosuppressive agent. It is considered a safe drug, being associated with a relatively low risk of adverse gastrointestinal effects and renal effects at anti-inflammatory doses (Brunton et al., 2006). Glucocorticoids are used to prevent or suppress the inflammatory response given by many irritating phenomena such as radiant, mechanical, chemical, infectious and immune stimulus.

Thus, the objective of this study was to develop an independent composite drug delivery system secreting DXM to enhance and prolong the functionality of the cell-loaded graft. The composite system was evaluated for different microencapsulated Epo-secreting cell-doses with the aim of achieving more physiological hematocrit levels to test its therapeutic efficacy.

## 2. Materials and methods

### 2.1. Preparation of microspheres

Poly (DL-lactide-co-glycolide) (PLGA) (Resomer® RG 752H) with a copolymer ratio of 75:25 (lactic/glycolic (%)) was provided by Boehringer Ingelheim (Germany). Dexamethasone was purchased from Fagron Iberica (Barcelona, Spain). Poly (vinyl alcohol) (PVA; average MW = 30,000–70,000) was obtained from Sigma (St. Louis, USA).

Microspheres were prepared by modification of a previously described technique (Garcia et al., 2009). PLGA microspheres loaded with dexamethasone were prepared by oil-in water (O/W) emulsion/solvent evaporation technique. The organic phase consisted of 200 mg PLGA (75:25) and 40 mg dexamethasone dissolved in 1 mL of methylene chloride. This organic phase was sonicated for 1 min (Branson Ultrasonic Sonifier® 250, CT, USA). The resultant dispersion was added to 2.5 mL 1% PVA aqueous solution and homogenized at 8000 rpm for 2 min (Ultra Turrax T25, IKA-Labortechnik, Staufen, Germany). Then, 5 mL of 0.1% PVA aqueous solution was added to the obtained emulsion and sonicated again for 1 min. The final O/W emulsion was added to 50 mL of 0.1% PVA aqueous solution and stirred on a magnetic stir plate at room temperature for 3 h to complete evaporation of the solvent. The resulting microspheres were collected by centrifugation at 10,000 × *g* (Sigma 3-30K), washed three times with distilled water to remove any remaining solvent or PVA and finally, freeze-dried for 24 h (LyoBeta 15, Telstar, Tarrasa, Spain). Microspheres without dexamethasone, were prepared using the same method and parameters described above.

### 2.2. Characterization of microspheres: particle size analysis and morphological evaluation

The mean particle diameter and size distribution were determined by laser diffractometry with a Coulter Counter LS 130 (Amherst, MA, USA). Microsphere morphology and surface characteristics were examined by scanning electron microscopy (SEM; Jeol® JSM-7000F).

### 2.3. Dexamethasone loading efficiency

5 mg of microspheres were dissolved in 10 mL of acetonitrile. This solution was filtered and analyzed by high performance liquid chromatography (Alliance 2795 Waters) coupled to an UV detector. The analytical column was Nucleosil 120 C18 (15 cm × 4 mm, 5 μm, Technocroma) and mobile phase consisted of acetonitrile:water:phosphoric (30:70:0.5 (v/v/v)) at pH 6. The injection volume was 20 μL, the flow rate was 1 mL/min and UV/visible absorbance detector was set at 238 nm. The retention time of DXM was 7 min at room temperature (Zolnik and Burgess, 2008; Splanger and Mularz, 2001). The assay was linear over DXM concentrations ranging from 5 μg/mL to 60 μg/mL.

### 2.4. In vitro release studies

The release profile of DXM from PLGA microspheres was determined by incubating 5 mg of microspheres in a test tube containing 1 mL of PBS 20 mM (pH 7.4) and shaking with a rotator shaker at 25 rpm at 37 ± 0.5 °C. At defined time intervals, all release medium was removed by centrifugation and replaced with 1 mL of fresh medium. The amount of DXM released in the supernatant was determined by HPLC, using the same method described above. The release test was performed in triplicate and protected from direct light exposure. DXM release profiles were generated for this microsphere formulation in terms of cumulative DXM release *versus* time.

### 2.5. Cell culture

C3H skeletal muscle derived $C_2C_{12}$ myoblasts genetically modified to deliver murine Epo (mEpo) were kindly provided by the Institute des Neurosciences (Ecole Polytechnique Federale of

Lausanne, EPFL, Lausanne, Switzerland). Cells were grown in Dulbecco's modified Eagle medium supplemented with 10% foetal bovine serum, L-glutamine to a final concentration of 2 mM, 4.5 g/L glucose and 1% antibiotic/antimycotic solution. Cell cultures were plated in T-flasks, maintained at 37 °C in a humidified 5% $CO_2$/95% air atmosphere standard incubator and were passaged every 2–3 days. All reagents were purchased from Gibco BRL (Invitrogen S.A., Spain).

### 2.6. Cell encapsulation

$C_2C_{12}$ myoblasts genetically engineered to secrete murine Epo were entrapped into APA microcapsules using an electrostatic droplet generator (800 V) with brief modifications of the procedure designed by Lim and Sun (Lim and Sun, 1980). Low viscosity and high guluronic (LVG) alginate purchased from FMC Biopolymer (Norway) and poly-L-lysine (PLL; hydrobromide Mw: 15,000–30,000) obtained from Sigma (St. Louis, USA) were employed. Cells were suspended in 1.5% (w/v) LVG-alginate sterile solution (from now on alginate), obtaining a cell density of $5 \times 10^6$ cells/mL alginate. This suspension was extruded through a 0.35 mm needle using a 10 mL sterile syringe from a peristaltic pump (flow rate: 5.9 mL/h). The resulting alginate beads were maintained in agitation for 10 min in a $CaCl_2$ solution (55 mM) (Sigma, St. Louis, USA) for complete ionic gelation and were ionically crosslinked with 0.05% (w/v) PLL for 5 min, followed by a coating with 0.1% alginate for additional 5 min. Microcapsules were prepared at room temperature, under aseptic conditions and cultured in complete medium. The diameters and overall morphology were characterized using inverted optical microscopy (Nikon TSM).

### 2.7. Metabolic cell activity

The cellular activity of the entrapped myoblasts was evaluated *in vitro* during 4 weeks post-encapsulation. The viable cell number *per* microcapsule was determined by the Cell Counting Kit-8 (CCK-8 assay) (Fluka, Buchs, Switzerland). CCK-8 allows convenient assays using WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon bioreduction in the presence of an electron carrier, 1-Methoxy PMS. WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells. Briefly, 10 μL of the CCK-8 solution was added to a known number of microcapsules (around 40) placed in a 96-well cell culture cluster and incubated at 37 °C for 4 h in humidified conditions. After 4 h, the resulting solution was read on a microplate reader (Multiskan EX, Labsystems) at 450 nm with 690 nm as the reference wavelength. Results are expressed as mean ± standard deviation.

### 2.8. Mechanical stability: osmotic resistance test

The swelling behavior of the microcapsules was determined after 1% citrate solution (w/v) treatment. In short, 100 μL of microcapsule suspension (50–100 microcapsules) was mixed with 900 μL of phosphate-buffered saline (PBS) and placed in a 24-well cell culture cluster. Each group was run in quadruplet. The cell cluster was placed in a shaker at 500 rpm and 37 °C for 1 h. Following this, supernatants were eliminated, and 800 μL of a sodium citrate solution was added. The cluster containing the microcapsules was maintained at static conditions at 37 °C for 24 h. On the following day, the diameters of 20 microcapsules of each group were measured. The washing and shaking step with PBS and the static condition were repeated during the following days until a one week period was completed.

### 2.9. Surgical procedure: subcutaneous implantation of APA microcapsules and PLGA microspheres

Adult female Balb/c mice (Harlan Interfauna, Spain) were used as allogeneic recipients. Animals were housed in specific pathogen free facilities under controlled temperature and humidity with a standardized 12 h light/dark cycle and had access to food and water *ad libitum* upon recovery. Recipients were anesthetized by isoflurane inhalation and a total volume of 100 μL or 50 μL of cell-loaded microcapsules ($5 \times 10^6$ cells/mL) suspended in Hank's Balanced Salt Solution (HBSS) (to a final volume of 500 μL) was implanted subcutaneously using an 18-gauge catheter (Nipro Europe N.V., Belgium). Treatment groups also received 6.75 mg of DXM-loaded PLGA microspheres (1 mg DXM/mouse), based on previous reports (Hickey et al., 2002a; Zolnik and Burgess, 2008), co-administered with microencapsulated cells, suspended in HBSS. Two control groups were assayed. One of them consisted of 100 μL of empty APA microcapsules and the other control group received empty APA microcapsules along with empty PLGA microspheres (microspheres without DXM), all suspended in HBSS, by the same route. Before implantation, microcapsules were washed several times in HBSS. All experimental procedures were performed in compliance with protocols approved by the institutional animal care and use committee.

### 2.10. Hematocrit measurement

Blood was collected during 45 days (weekly during the first month) from the submandibular vein using safety lancets and collection vials (Sarstedt, Spain). Hematocrits were determined after centrifugation at 3000 rpm for 15 min of whole blood using a standard microhematocrit method. Results are expressed as mean ± standard deviation.

### 2.11. Histological and macroscopical analysis: evaluation of the immune reaction

At day 60 after implantation, 3 animals from each group were sacrificed and capsules were explanted and fixed in 4% paraformaldehyde solution for histological analyses. The overall evaluation of the immune reaction towards transplanted microcapsules was performed blindly by a pathologist. H&E stained slides of each sacrification time point and from each treatment group were evaluated. Masson's trichrome and alcian blue staining were also performed for further evaluation. Photographic images were taken using a Nikon D-60.

### 2.12. Statistical analysis

Data are presented as mean ± standard deviation. Data between control and experimental groups were analyzed for statistical significance. The Student's *t*-test was used to detect significant differences when two groups were compared. One-way ANOVA and post-hoc test were used in multiple comparisons. The Bonferroni or Tamhane post-hoc test was applied according to the result of the Levene test of homogeneity of variances. All statistical computations were performed using SPSS 17.0 (SPSS, Inc., Chicago, IL).

## 3. Results

Fig. 1 shows an schematic illustration of the immunopriviledged microenvironment generated in the subcutaneous space of Balb/c

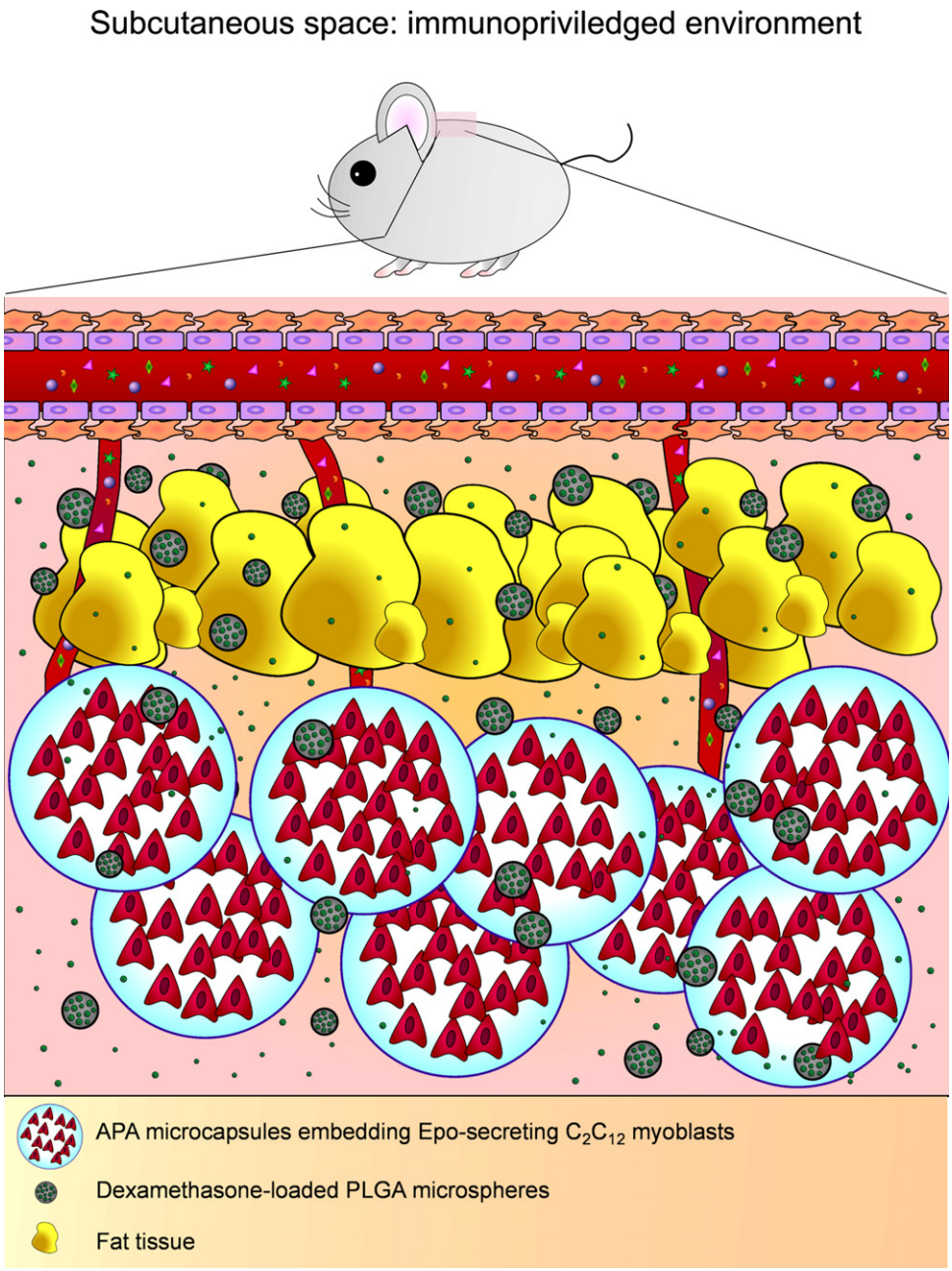


**Fig. 1.** Schematic illustration of the immunomodulatory environment created in the subcutaneous space of implanted mice.

mice after implantation of Epo-secreting encapsulated cells and dexamethasone-releasing microspheres.

### 3.1. APA microcapsule morphology evaluation

All cell-loaded microcapsules had a uniform and spherical morphology without irregularities on their surface and a narrow size distribution as shown in Fig. 2A. Previous studies have reported the relevance of the materials employed in the elaboration of microcapsules to obtain biocompatible microcapsules (Ríhová, 2000; Santos et al., 2010). However, not only the materials used but also the spherical and smooth shaped morphologies of the microcapsules have been observed to be of great importance to elude the host's immune response (Santos et al., 2010; Ponce et al., 2006), leading to the conclusion that in this study, enclosed cells were correctly adapted to the surrounding polymer scaffold.

### 3.2. Preparation and characterization of PLGA microspheres

The mean particle size for the obtained microspheres was $11 \pm 0.3\,\mu m$ for DXM loaded microspheres and $13 \pm 0.2\,\mu m$ for empty microspheres. When observed under scanning electron microscopy (SEM) the spheres appeared spherical with a smooth and uniform surface (Fig. 2B). Considering that the theoretical loading was 20%, the loading efficiency for the developed formulation was 74%. Total DXM loading was 15.6%.

### 3.3. Cell functionality and stability of microcapsules

The metabolic activity of the encapsulated cells was analyzed *in vitro* over the course of 30 days. CCK-8, being nonradioactive, allows a sensitive colorimetric assay for the determination of viable cells in cell proliferation. As seen in Fig. 3A, $C_2C_{12}$ myoblasts showed similar viabilities over the 30 day assay period, supporting the idea

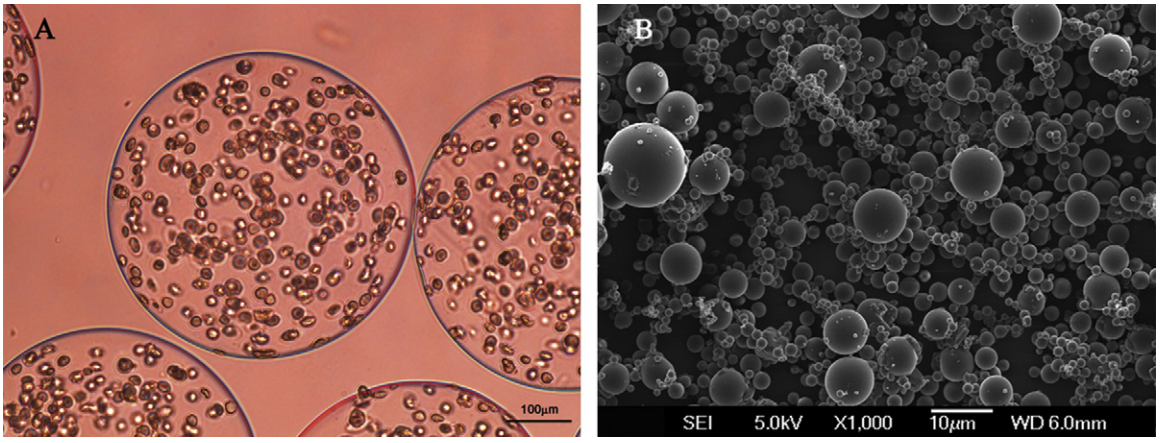


**Fig. 2.** Morphological evaluation of (A) alginate microcapsules and (B) PLGA microspheres.

that the diffusion of oxygen and nutrients was not influenced by an inappropriate membrane behavior. A slight increase in viability was observed after the third week, probably due to a slight proliferation of the enclosed myoblasts.

Once the overall morphology of APA microcapsules was studied, the swelling behavior of the beads was evaluated and hence, their suitability and adaptability for the following *in vivo* assay was assessed. The swelling assay showed that microcapsules swelled and increased their size at approximately 10% after the first citrate treatment (Ponce et al., 2005). Nonetheless, after this initial accommodation of matrices, stability of their size was maintained during the one-week assay period (Fig. 3B). These results confirm the high chemical resistance of the developed microcapsules.

### 3.4. *In vitro dexamethasone release kinetic studies*

Fig. 4 shows the release profile of DXM from PLGA microspheres. The release profile was triphasic, with an initial burst of 40.1% of the total loaded protein. Between days 2 and 3, release was followed with a mean constant of 11.3 μg DXM/day/mg microspheres. From day 4 to the end of the release assay, a release rate of 1.54 μg DXM/day/mg of microspheres was observed.

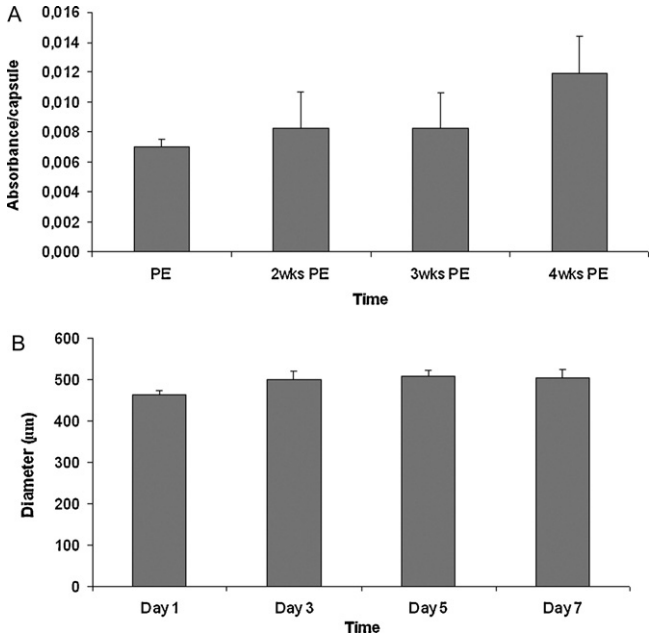


**Fig. 3.** (A) Viability evaluation of encapsulated cells (CCK-8). PE: post-encapsulation. (B) Osmotic pressure resistance of microcapsules after a one-week treatment with sodium citrate. The error bars on each point correspond to the standard deviation of the mean.

### 3.5. *Long-term functionality of subcutaneously implanted mEpo-secreting microencapsulated cells in Balb/c mice. Epo dosing evaluation and anti-inflammatory effect of dexamethasone on implants*

The anti-inflammatory effect of dexamethasone may ease to avoid the formation of pericapsular fibrosis, which is mainly responsible for the failure of the implanted devices. To address this issue, adult female Balb/c mice were used as recipients, and cell-loaded microcapsules were implanted in the subcutaneous space. As it is observed in Fig. 5, a significantly higher hematocrit level was observed in all the animals implanted with alginate microcapsules when compared with the control group ($P < 0.05$).

The use of an independent composite system resulted in improved functionality of the cell-based graft, which was found to be more pronounced in the 100 μL-dose group, from day 20 to the end of the study ($P < 0.05$).

### 3.6. *Microcapsule retrieval: cell functionality at explantation and histological analysis*

The implanted delivery systems of several mice from both control and treatment groups were removed at day 60 postimplantation. A macroscopic image of the implantation site can be observed in Fig. 6. Retrieval of microcapsules from the subcutaneous tissue revealed the formation of an irregular structure where capsules were mostly aggregated. Moreover, viability of the entrapped cells could be indirectly stated by the elevated hematocrit levels during the study period. The microcapsule network was easily harvested in one or two pieces after a small skin incision. Both the macroscopic (Fig. 6) and the histological analyses of the explanted microcapsules revealed some blood capillaries surrounding the microcapsule aggregates (Fig. 7, black arrow), mainly observed in the DXM-treated 100 μL-dose group. This might be due to the angiogenic effects reported for Epo (Murua et al., 2009b; Ribatti et al., 2007; Müller-Ehmsen et al., 2006; Benelli et al., 2006). Histological analyses revealed the formation of a mild fibrotic layer, specially in cell-implanted individuals not treated with dexamethasone. In order to assess the clinical-grade purity and transplantation suitability of the implanted devices alone, two control groups were included in the present study. As observed in Fig. 7, control groups showed no evidence of pericapsular overgrowth (they were practically free of inflammation), thus confirming the immuno

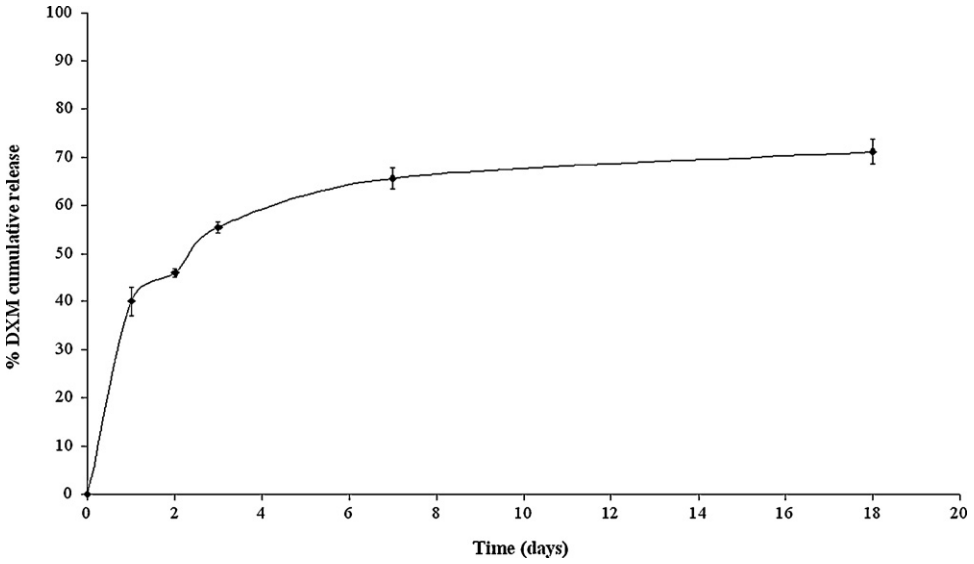


**Fig. 4.** *In vitro* dexamethasone release profile from PLGA microspheres at 37 °C in PBS buffer (pH 7.4). Values are represented as mean ± SD ($n = 3$).

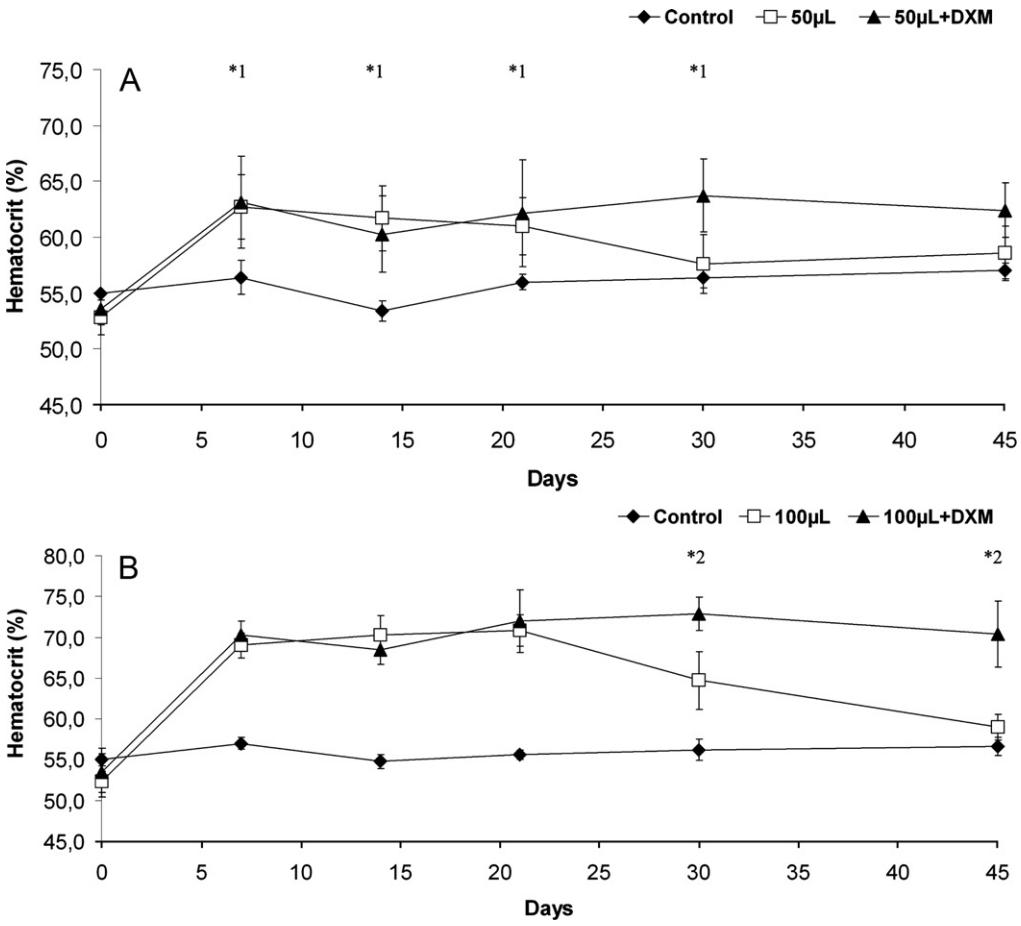


**Fig. 5.** Hematocrit levels of Balb/c mice over time (45 days). (A) 50 µL cell-microcapsule dose. *Control*: empty alginate microcapsules. (B) 100 µL cell-microcapsule dose. *Control*: empty alginate microcapsules + empty PLGA microspheres. Some groups received dexamethasone-loaded PLGA microspheres (+DXM) while others did not. Significance: $P < 0.05$; *1: control *vs.* cells. *2: No DXM *vs.* DXM group.

acceptance of the implanted drug delivery systems. Regarding the cell-implanted groups, even though no significant difference was observed (blindly analyzed by an independent pathologist) between the DXM-implanted and non-treated groups in terms of fibrotic reaction, there is a tendency towards a milder fibrotic overgrowth in DXM-treated groups as confirmed by the therapeutic outcome which resulted in enhanced functionality of the cell-based grafts.

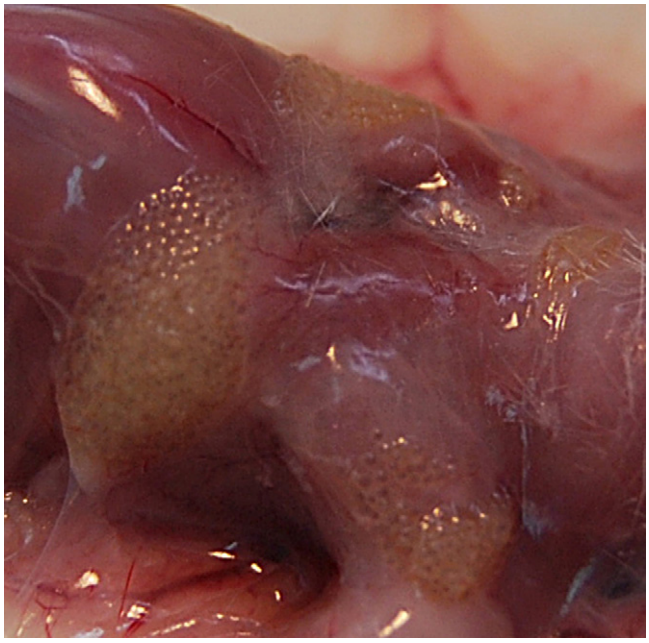

**Fig. 6.** Subcutaneous location of alginate microcapsules and PLGA-dexamethasone microspheres previous to their explantation on day 60.

## 4. Discussion

Extensive work has been carried out in recent years aiming at reducing or eliminating the immune reaction towards encapsulated cells implants. Many studies focused on improving the purity of the biomaterials employed in the elaboration of the microdevices and their adequate shape and morphology. In spite of the huge progress made in reducing the immune reaction, particularly in the case of xenotransplantation approaches, much work lies ahead. Short-term systemic immunosuppression has also been proposed as a possible alternative therapy towards eliminating the immune reaction from the host, by actively suppressing the inflammatory response generated against the transplanted encapsulated cells. However, the side effects derived from the systemic delivery of immunosuppressants cannot be avoided up to date so alternative locally secreted solutions need to be investigated (Weiss et al., 2006).

In the present study, we aimed to address this important issue by designing a composite drug delivery system, by the co-administration of PLGA-loaded dexamethasone microspheres. We hypothesized that the anti-inflammatory drug delivery system would provide a local and continuous release of the immunosuppresive agent to the transplantation site, thus decreasing the inflammatory reaction directed towards the microencapsulated cells and improve the system's long-term efficacy (Bhardwaj et al., 2007).

Dexamethasone was selected as a model anti-inflammatory drug due to its safety and wide clinical use (Ratner et al., 2004; Bunger et al., 2005). A temporal but continuous delivery was proposed to diminish the inflammatory response to subcutaneously implanted cell grafts. To suppress inflammation, glucocorticoids inhibit the production of different factors that are important to the emergence of the inflammatory response. DXM acts by decreasing the release of vasoactive and quimioatractive factors, the secretion of lipolytic and proteolytic enzymes, the extravasation of leukocytes into injury areas and finally fibrosis. It also decreases the expression of proinflammatory cytokines like COX-2 and NOS 2 (Ratner et al., 2004). Glucocorticoids are used in combination with other immunosuppressive drugs to treat transplant rejection. Overall, glucocorticoids, have anti-inflammatory effects in the cellular immune response. In addition, glucocorticoids, limit the allergic reactions that occur with other immunosuppressants.

To preserve scaffold functionality over weeks or months, it might result of paramount importance to minimize the immune response activity over the tissue environment surrounding the implanted device. The surgery, even if minor, the implantation of foreign materials (de Vos et al., 2002) and cytokine release from the encapsulated cells (Murua et al., 2009a) are involved in the immediate post-transplant inflammatory response and cannot always be avoided or controlled.

A major problem upon implantation of medical devices and other scaffolds is the tissue injury which triggers a cascade of inflammatory responses that may compromise their functionality in a short period of time (Hickey et al., 2002a).

The inflammation, wound healing, and foreign body reaction are generally considered as parts of the tissue or cellular host responses to injury. This immune response can be defined as the reaction of vascularized living tissue to local injuries that contain, dilute, neutralize, or wall off the injurious agent or process (Medzhitov, 2008; Barton, 2008; Hickey et al., 2002b; Dungel et al., 2008; Anderson, 2001; Jayant et al., 2009; Koschwanez et al., 2008).

Therefore, *in vivo* functionality of scaffolds can be significantly improved using immunosuppressive anti-inflammatory drugs, as dexamethasone (Patil et al., 2007). By using the local delivery of DXM, it is possible to avoid the peripheral side effects of chronic use (Kim and Martin, 2006).

In this work we investigated the potential of a composite drug delivery system to modulate the local microenvironment and provide an improved long-term response of a cell-loaded graft. The local release of DXM can prevent peripheral side effects that occur when immunosuppressive drugs are used by systemic administration. The efforts are targeted to achieve a local temporary release instead of a permanent release (Bunger et al., 2005; de Vos et al., 2002).

Interestingly, most of the DXM was released during the first day (~41%). Probably, the most important time frame for anti-inflammatory therapy may be day one after transplantation. Macrophages, the main cells involved in the early pericapsular overgrowth, are recruited during the first day and no changes occur until day 7, when a decrease is observed (de Vos et al., 2002).

Our research group has previously shown the efficacy of PLGA microspheres as drug release systems for the continuous delivery of therapeutics *in vitro* and *in vivo* (Gutierro et al., 2002; Mata et al., 2007). These synthetic polymers are the most extensively studied and used because of the number of advantages they provide. They have already been approved in medical implants and generated tremendous interest due to their excellent biocompatibility, biodegradability and their long term safety in humans (Ratner et al., 2004; Rajeev, 2000).

PLGA microspheres have multiple benefits as local controlled drug delivery systems. A continuous and controlled drug concentration may be achieved in addition to reducing frequency of administration, dose dumping possibility and systemic effects (Hickey et al., 2002a).

Therefore, we decided to combine DXM-loaded PLGA microspheres as a sustained delivery system with APA microcapsules entrapping cells.

The use of an independent composite system resulted in an improved functionality of the cell-based graft, which was found to be more pronounced when higher cell-doses were implanted. On the basis of previously reported studies (Murua et al., 2007, 2009a; Orive et al., 2005), we estimated that 100 μL of cell-loaded microcapsules ($5 \times 10^6$ cells/mL alginate) might result in a therapeutic dose to provide significant increase in mice hematocrit levels over time. However, given the angiogenic and immunomod-

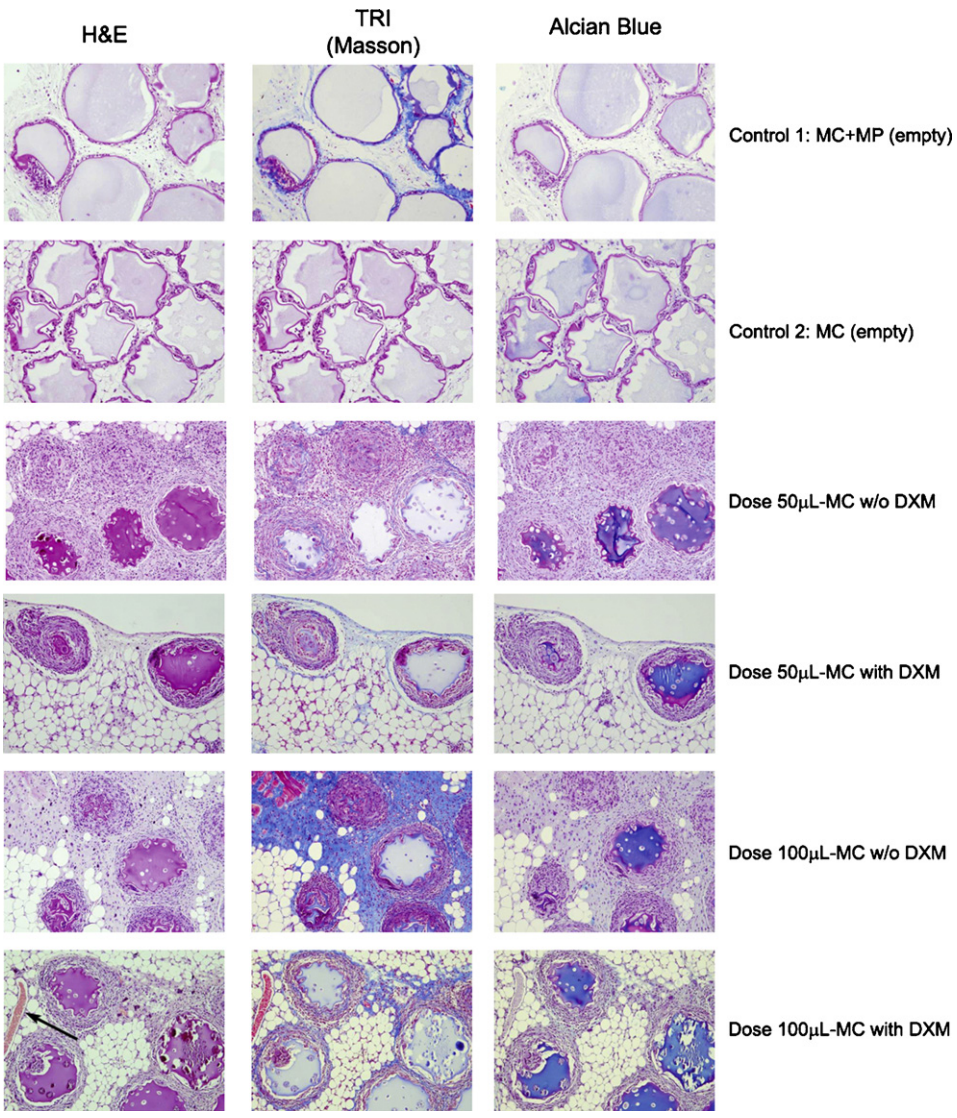


**Fig. 7.** Histological evaluation of subcutaneously implanted alginate microcapsules (MC) embedding Epo-secreting $C_2C_{12}$ myoblasts (with or without dexamethasone-loaded PLGA microspheres [MP]). Black arrow: blood capillary.

ulatory effects related to Epo, a tendency was also observed in a lower cell-dose (50 μL). Additionally, the systems showed good biocompatibility and capability to partially avoid the inflammatory response and the pericapsular cell overgrowth, probably due to the immunosuppressive effects related to DXM (Sorianello et al., 2002). This system may open doors to future new alternative composite systems.

## 5. Conclusions

Taking the aforementioned results altogether, it may be concluded that the co-administration of dexamethasone-loaded PLGA microspheres along with the encapsulation of Epo-secreting myoblasts may enhance performance of the encapsulation system and may hence be considered very promising and interesting to prevent inflammation leading to pericapsular fibrosis, which may reduce the probability of a successful graft. Further improvement of the composite system is required in order to provide a long-term efficacy of the system, with a suitable therapeutic effect employing lower cell-dose grafts. The release of dexamethasone from PLGA microspheres might provide a useful pharmacological way to prevent the acute inflammatory response due to both biomaterials and surgical manoeuvres employed during the implantation procedure. In a very fast-track developing area, such as bioartificial devices, this preliminary study might give venue to properly address strategies for cell-based therapies and tissue engineering.

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