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Evaluation of Alginate Microspheres for Mesenchymal Stem Cell Engraftment on Solid Organ

E. Trouche,* S. Girod Fullana,†‡ C. Mias,* C. Ceccaldi,†‡ F. Tortosa,*†
M. H. Seguelas,* D. Calise,* A. Parini,*† D. Cussac,*†[1] and B. Sallerin*†[1]

*INSERM U858, Toulouse, France
†Université Toulouse, UPS, Faculté des Sciences Pharmaceutiques, Toulouse, France
‡CIRIMAT, UPS-INPT-CNRS 5085, Toulouse, France

Mesenchymal stem cells (MSCs) may be used as a cell source for cell therapy of solid organs due to their differentiation potential and paracrine effect. Nevertheless, optimization of MSC-based therapy needs to develop alternative strategies to improve cell administration and efficiency. One option is the use of alginate microencapsulation, which presents an excellent biocompatibility and an in vivo stability. As MSCs are hypoimmunogenic, it was conceivable to produce microparticles with [alginate-poly-L-lysine-alginate (APA) microcapsules] or without (alginate microspheres) a surrounding protective membrane. Therefore, the aim of this study was to determine the most suitable microparticles to encapsulate MSCs for engraftment on solid organ. First, we compared the two types of microparticles with 4×10^6 MSCs/ml of alginate. Results showed that each microparticle has distinct morphology and mechanical resistance but both remained stable over time. However, as MSCs exhibited a better viability in microspheres than in microcapsules, the study was pursued with microspheres. We demonstrated that viable MSCs were still able to produce the paracrine factor bFGF and did not present any chondrogenic or osteogenic differentiation, processes sometimes reported with the use of polymers. We then proved that microspheres could be implanted under the renal capsule without degradation with time or inducing impairment of renal function. In conclusion, these microspheres behave as an implantable scaffold whose biological and functional properties could be adapted to fit with clinical applications.

Key words: Mesenchymal stem cells; Alginate; Microspheres

INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent stem cells derived from bone marrow stroma. They have the ability to form a variety of mesenchymal tissues including bone, fat, and cartilage, and non-mesenchymal tissues like neuron, kidney, and heart (13,15,27,36,55). Recent studies have shown that most of the therapeutic effects of MSCs are related to the secretion of paracrine factors (17,47). Systemic administration is easy but there is little quantity of cells reaching the organ. These findings supported the idea that direct intraparenchymal injection would allow to concentrate the paracrine factors produced by MSCs within the injured organ. However, the intraparenchymal administration is characterized by early death of grafted cells (23,29,38,44,48) and in the case of their survival they potentially differentiate in unsuitable phenotypes.

A way to protect MSCs and take advantage of their paracrine properties could be their inclusion in a biomatrix. This could permit to 1) prevent mechanical stress, 2) diminish the negative influence of the injured environment on grafted cells, 3) easily manipulate and deliver the cells near the organ, and 4) better follow the phenotype of the grafted cells. This goal may be achieved through the microencapsulation of MSCs in biomatrices and implantation of this scaffold. The design of the microparticles has to be optimized for permeability, stability, biocompatibility, and easiness of injection and integration. The advantage of natural polymers in comparison to synthetic polymers is that they induce less cytotoxicity or inflammatory reactions (21).

Alginate is the most commonly employed polymer for cell encapsulation because of its excellent biocompatibility and an in vivo stability (6,40,53). Alginate is a polysaccharide isolated from brown algae found in

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[1]These authors provided equal contribution to this work.
Address correspondence to Brigitte Sallerin, I2MR, INSERM U858, CHU Rangueil, BP 84225, 31432 Toulouse Cedex 4, France. Tel: 33-5-65-25-68-43; Fax: 33-5-65-25-98-16; E-mail: sallerin@cict.fr

coastal waters around the globe (19). It is a linear copolymer composed of β-D-mannuronic acid and α-L-guluronic acid that can be easily transformed into a gel by binding the guluronic acids with a divalent cation such as calcium (41). Classically, cells are encapsulated in alginate microcapsules with a semipermeable membrane, generally polylysine (PLL), which allows nonautologous cells to be implanted. This membrane protects the cells from immune mediators and allows the release of beneficial factors from the microcapsule.

Previous results from our laboratory have shown that adrenal medullary bovine chromaffin cells could be encapsulated in microcapsules of alginate-poly-L-lysine (APA) with a liquefied inner core (28). This procedure was associated to an increase in the viability of functional cells. However, in clinical applications of MSCs, a semipermeable membrane may not be necessary as these cells are poorly immunogenic (1,7,16,49). We hypothesized that alginate microspheres could also be suitable for MSC encapsulation. However, little is known concerning viability and functionality of MSCs in microspheres. In addition, the use of alginate microsphere graft for cell therapy of solids organs has not been extensively investigated. Indeed, microspheres present different permeability and mechanical behavior than microcapsules that could consequently impact the implantation procedure and in vivo scaffold integrity.

Thus, we designed a strategy based on the encapsulation of MSCs in alginate microspheres or microcapsules in order to determine which one was the most appropriated for cell therapy. We first evaluated the stability and mechanical parameters of microparticles. We then tested MSC viability and functionality in microparticles. We also determined the effect of alginate on differentiation of MSCs. Finally, we studied the feasibility of grafting alginate-encapsulated MSCs under the rat renal capsule.

MATERIALS AND METHODS

Cell Culture

Marrow aspirate was obtained from femurs cavity of Lewis rats (Harlan, France) weighing 180–200 g. Bone marrow from the femur cavity was flushed with MEM medium (ABCYs, France) containing 10% FCS and 1% penicillin/streptomycin (Invitrogen, USA) and the cell suspension was centrifuged ($400 \times g$, 5 min). Then, cells were plated in culture flasks (200,000 cells/cm^2). Nonadherent cells were removed after 72 h and MSCs were recovered by their capacity to adhere highly to plastic culture dishes. MSCs were then routinely cultured and were used for the experiments. Most adherent cells expressed MSC markers CD90, CD29, and CD106 and were negative for CD34 (hematopoietic marker), CD45 (B cells marker), and CD31 (vascular cell marker). They were able to differentiate into cells from mesodermal lineage: osteoblasts, chondrocytes, and adipocytes (data not show).

MSC Encapsulation

Rat MSCs (passage 3) were lifted with trypsin, counted, and centrifuged. Microspheres and microcapsules were produced by the method of Goosen et al. with modifications (10) under sterile conditions. We used sterile ultrapur sodium alginate with 54% β-D-mannuronic acid (type M alginate) with an apparent viscosity of 141 mPas.s (Pronova, SLM 100, Novamatrix, Norway). Briefly, a 1.4% w/v sodium alginate solution was prepared by dispersing alginate in NaCl 150 mM, buffered to pH 7.4 with 12.5 mM HEPES. Cells were resuspended in this sodium alginate solution at a density of 2.5×10^6 or 4×10^6 cells/ml alginate. Homogeneous alginate microspheres were produced by extruding through an encapsulator (Inotech IE-50R, Switzerland), equipped with a 300-μm vibrating nozzle, the alginate–cell suspension into a solution of 1% $CaCl_2$, $2H_2O$, 0.4% NaCl, 12.5 mM HEPES, pH 7.4, which was continuously swirled. Microspheres were gelled for at least 20 min and then rinsed with HEPES buffer and either transferred into culture medium (case of alginate microspheres) or coated by incubating in 0.1% w/v PLL, 150 mM NaCl, 12.5 mM HEPES, pH 7.4, under gentle agitation. In the latter case, an outer alginate layer was subsequently applied by 10-min incubation in a dilute (0.1% w/v) alginate solution in saline buffer under gentle agitation (case of APA microcapsules). Finally, microcapsules were treated with 55 mM sodium citrate to liquefy the inner alginate core, and washed extensively with saline buffer. Both types of microparticles—microspheres and microcapsules—were studied.

Microspheres and microcapsules were incubated in culture medium at 37°C in 5% CO_2 and 95% humidity. At specified days postencapsulation, they were collected and further analyzed. In order to characterize their morphology and properties, control microparticles (without cells) were also prepared according to the same protocol.

Morphology and Stability of Particles Over Time

Size and morphology of microparticles were routinely examined with a light microscope. The stability of control microparticles was studied for 35 days at 37°C in saline buffer both visually and by light microscopic observation. In the case of microcapsules, their integrity was assessed.

Scanning Electron Microscopy (SEM)

SEM analysis of the surface and cross section of dried control microparticles was performed with a scanning electron microscope (Leo 435 VP). The microparti-

cle samples were mounted on an aluminum sample mount and sputter coated with silver. The specimens were observed at a 10 kV accelerating voltage.

Mechanical Resistance

The mechanical resistance of the microparticles was evaluated on microspheres or microcapsules obtained with a 0.8-mm needle according to the same protocol. At defined time intervals, the microparticles were submitted to a standardized compression test in a TA-XT2 texture analyzer (Stable Microsystems, UK). Briefly, the compression resistance of the microparticles was determined as the main force (g) required to generate a 30% compression of a sample of microparticles. The apparatus consisted of a mobile probe moving vertically, up and down at constant and predefined velocity (0.5 mm/s). The force exerted by the probe on the microparticles was recorded as a function of the displacement, leading to a force versus strain curve. The results are expressed as the average maximal mechanical force in grams from at least five independent observations. The integrity of the microparticles after the test was assessed by microscopic observation.

Cell Viability Assay

The viability of encapsulated cells was assessed using a LIVE/DEAD® Viability/Cytotoxicity kit (FluoProbes, France) for 35 days following microencapsulation. Briefly, microparticles were rinsed twice with phosphate-buffered saline (PBS) and MEM (v/v). Microparticles were then incubated for 30 min with a solution containing 2 μM ethidium homodimer-3 and 1 μM calcein AM. Microparticles were then rinsed with PBS and observed using a confocal microscope (Leica Microsystems, Germany). Cell viability was determined from confocal images as previously described (26). After staining, cells appear with red nuclei and lived cells with green cytoplasm. Some population of MSCs exhibited apoptotic transformation so their nuclei looked yellow-orange, revealing the colocalization of fluorescence in green and red spectral regions. The cell viability was estimated by the ratio of green pixels to the total number of lightened pixels.

Western Blot

For Western blot (WB), MSCs were extracted of microspheres through an incubation with citrate (50 μM) and centrifugation. Then MSC proteins were extracted from pelleted MSCs. WB analyses were performed with samples normalized for protein concentration. Membranes were probed with anti-bFGF (1:500; Santa Cruz Biotechnology, USA) or anti-ERK2 (1:1,000; Santa Cruz Biotechnology, USA) antibodies. Following several washes in Tris-buffered saline-Tween (0.2%), membranes were incubated to horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:10,000; Santa Cruz Biotechnology). Expression of bFGF is related to the expression of ERK2.

Chondrogenic Phenotype of Encapsulated MSCs

MSCs encapsulated in microspheres where cultured in high-glucose Dulbecco's modified Eagle's medium (ABCYs, France) with 1% fetal bovine serum (Invitrogen, USA), antibiotics, modified or not with ascorbate-2-phosphate (50 μg/ml, Sigma, France), proline (40 μg/ml, Sigma), pyruvate (3 mM, Sigma), ITS+ (1×, BD Biosciences, USA), dexamethasone (100 mM, Sigma), transforming growth factor-β3 (10 ng/ml, TGF-β3, R&D Systems, USA), and recombinant bone morphogenic protein-2 (200 ng/ml, R&D Systems). Medium was replaced twice weekly. On day 21, 31, 51, 57, and 61, MSCs were harvested from microspheres after incubation with citrate (50 μM). Cells were then centrifugated, resuspended with cultured medium, and then were seeded on slices to perform immunocytology.

Immunocytology

For in vitro differentiation, cells where stained with alizarin red or alcian blue coloration using standard methods. For chondrocyte detection, cells were incubated (1 h, RT) with an anti-PS100 rabbit antibody (1:200, ABCYs, France).

Animal Transplantation and Histology

Experimental animals were handled in accordance with the European animal care guidelines. Four Lewis rats (Harlan, France) weighing 180–200 g were used for allogenic recipients of MSC-alginate. For transplantation, rats were anesthetized with isoflurane/oxygen inhalation (3/97). A total of 18 microspheres containing 4×10^6 MSCs were transplanted under the renal capsule. Sham-operated animals were subjected to the same surgical procedure without transplantation. Kidney sections were collected 25 days after MSC injection.

After euthanasia, kidneys were collected, fixed in paraformaldehyde (4%), dehydrated, and then embedded in paraffin. Paraffin sections (10 μm) were stained with hematoxylin/eosin.

Statistical Analysis

Results are expressed as mean ± SEM. Statistical comparison of the data was performed using the *t*-test for comparison between two groups or one-way ANOVA and post hoc Tukey's test for comparison of more than two groups. A value of $p < 0.05$ was considered significant.

RESULTS

Morphology and Stability of the Alginate Microparticles

Morphology and stability of two types of microparticles, microspheres and microcapsules, were examined using optical and electron microscopy. Under optical microscopy, all cell-loaded microparticles had a uniform and spherical morphology (Fig. 1A, B). Concerning microcapsules (Fig. 1B), a smooth refringent ring, corresponding to the continuous transparent alginate-PLL membrane, could be observed. All of these microparticles present an average diameter between 500 and 710 μm with alginate microspheres slightly bigger than microcapsules. The examination showed that MSCs appeared evenly distributed throughout the microparticles, which remained stable in saline buffer and in culture media over the study.

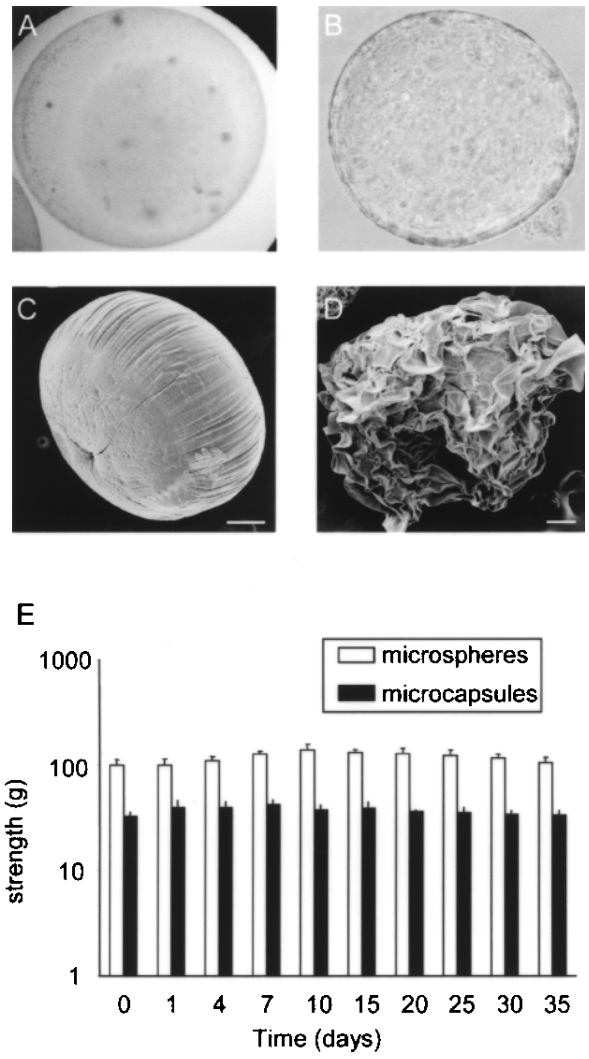


Figure 1. Characterization of alginate microparticles. (A, B) Microsphere (A) and microcapsule (B) size and morphology examined with a light microscope (objective 2× and 10×, respectively). (C, D) Scanning electron microscopy of microspheres (C) and microcapsules (D) Scale bars: 100 μm. (E) Measurement of the mechanical force needed to compress the beads of 30% of their height with time.

In order to demonstrate that the microcapsules had a liquefied core, intact microparticles were dried and then observed by electron microscopy (Fig. 1C, D). In these conditions, microcapsules appeared no longer spherical, as they would appear if they had a solid core, but flattened with a highly wrinkled surface (Fig. 1D). Because they became fragile upon drying, some of them were broken, clearly showing that initial capsules were hollow and only constituted of a membrane surrounding a liquefied core.

Mechanical Resistance

In order to complete the study of microparticle morphology and stability, we determined their mechanical resistance. The resistance and durability of the microparticles were studied by submitting them to a standardized mechanical stress (28). This resistance to compression protocol is derived from the method of Orive et al. (3,31). The authors demonstrated that this technique permits differentiation among solid particles in order to choose the most resistant internal configuration. Moreover, it can be applied to both microcapsules and microspheres.

Figure 1E shows the evolution of the maximal mechanical force (g) required to compress the beads of 30% of their height with time. In all cases, until day 35 postencapsulation, resistance to compression of microparticles remained stable. As expected, forces required to compress microspheres are higher than microcapsules, although the latter exhibited a more elastic behavior.

These results show that mechanical resistance of the two types of microparticles was unchanged with time (until day 35). In contrast, their mechanical behavior appeared quite different and could play a role in microparticle behavior postimplantation. Although they support small deformations, microcapsules remain prone to membrane disruption, while microspheres are mechanically stronger: their use would obviously increase the durability of the transplant and improve the feasibility of its retrieval.

In Vitro Viability of Encapsulated MSCs

In order to evaluate the effect of the different microparticles on the viability of MSCs, microspheres and microcapsules were followed for 35 days postencapsulation after loading with 4×10^6 MSCs/ml of alginate. Microparticles were stained with ethidium homodimer-

3 and calcein followed by 3D reconstitutions performed using confocal microscopy. The viability of MSCs was quantified by automatic cell counting. This quantification shows that viability of MSCs was poorly affected in microspheres (Fig. 2A, D, green cytoplasm) until day 35 (85.6 ± 4.8%) whereas it significantly decreased (64.6 ± 3.4%, 49.3 ± 4.9% on day 5 and 35, respectively) in microcapsules (Fig. 2B, D). These results led us to select microspheres rather than microcapsules for the next steps of our study.

In Vitro Functionality of Encapsulated MSCs in Microspheres

Several studies have shown that MSCs are able to secrete a number of cytokines, which may explain their beneficial effects. Among these factors, we and others have shown that bFGF mediates part of the paracrine effects of MSCs (25). We examined the production of bFGF in microencapsulated MSCs 7 days postencapsulation (Fig. 3). Results showed that bFGF remains detectable in microencapsulated MSCs and the amount of protein was not significantly different from that of nonencapsulated MSCs. These results indicated that, as showed for cultured MSCs, viable microencapsulated MSCs are able to produce bFGF.

In Vitro Differentiation of Microencapsulated MSCs

Some studies have investigated the possible differentiation of matrix encapsulated MSCs into mesodermal lineages and more particularly into the chondrogenic and osteogenic phenotypes. We compared the degree of chondrogenic differentiation of MSCs harvested from

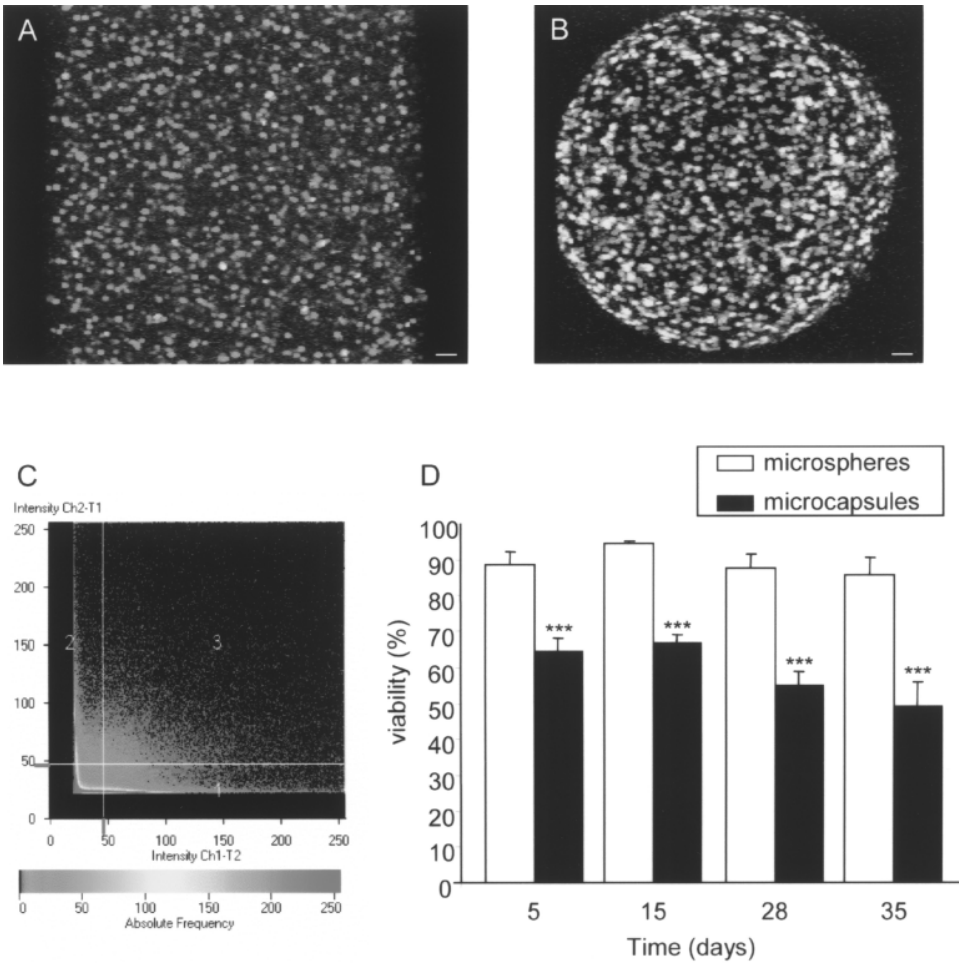


Figure 2. In vitro viability of MSCs in alginate microparticles. (A, B) Resulting confocal image of encapsulated MSCs in microspheres (A) and in microcapsules (B) after 2 μM ethidium homodimer-3 (red fluorescence) and 1 μM calcein (green fluorescence) labeling. Scale bars: 50 μm. (C) Representative correlation plot for images presented in (A) and (B). Pixels were counted to evaluate cell viability in gate 3. (D) Quantitative analysis of confocal images. ***$p < 0.001$ versus microspheres.

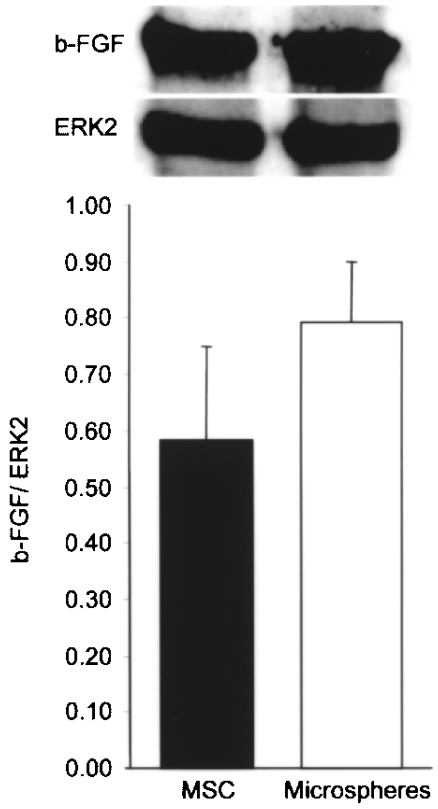


Figure 3. Production of cytokines by MSCs in alginate microspheres. In vitro expression of bFGF by the MSCs entrapped in microspheres. The same quantity of protein was loaded and the histogram shows the ratio of bFGF/ERK2.

microspheres (4×10^6 million MSCs/ml alginate) to MSC growth in chondroinductive medium. Results showed that in all conditions, MSCs extracted from microspheres at 21, 31, 51, 56, and 61 days after encapsulation and replaced in culture were negative for alcian blue staining (Fig. 4A) nor labeled for PS100 (Fig. 4B). Furthermore, 56 days after encapsulation and growth into standard medium, MSCs extracted from microspheres were also negative for alizarin red (Fig. 4C), a marker of osteogenic differentiation.

Impact of Cell Density on MSC Fate

Recent studies demonstrated that variations of cell density in various scaffold may influence chondrogenic differentiation of MSCs (14,42). According with this observation, we have verified whether cell density has an impact on MSC viability, functionality, and differentiation in alginate microspheres. In a first step, we produced alginate microspheres with 2.5×10^6 or 4×10^6 MSCs/ml of alginate (Fig. 5). Results showed no differences in viability and in differentiation between the two cell densities. Nevertheless, bFGF production was increased in higher concentrated microspheres. These results led us to choose higher concentrated microspheres for our study.

Implantation of Microencapsulated MSCs in Rats

As we have observed that microspheres are less elastic than microcapsules, we have evaluated the feasibility of manipulating and implanting microspheres at the surface of solid organ without degradation. Therefore, we have developed a model based on a deposition of microspheres under the renal capsule (Fig. 6A). Optical observations showed that microspheres are still intact and did not migrate out of the renal capsule 25 days after graft (Fig. 6B). Analysis of histological preparations stained with hematoxilin/eosin coloration showed detectable MSCs in microspheres 25 days after graft (Fig. 6C, dark arrow). No evidence of malignant invasion or inflammation was found in any of the specimens; however, long-term follow-up data are missing. We observed an accumulation of cells around microspheres with histological characteristics of a scar formation in the area under the renal capsule, but not in the cortical zone. This is in agreement with others results (22) and suggests that morphological characteristics of the renal parenchyma were not altered. Moreover, no fibrosis formation was observed around the microspheres (data not shown). In order to evaluate the effect of the graft on renal function, we compared plasma urea and creatinine of grafted and sham rats (Fig. 6D). Results indicated that no significant differences could be observed between the two groups until day 25 postgraft. Altogether, these results indicate that microspheres may be manipulated and grafted onto a solid organ, for a long period, without any significant degradation or impairment of the renal function.

DISCUSSION

Microencapsulation of cells could be a promising strategy to improve cell survival after graft on solid organ. Indeed, it allows avoiding intraparenchymal injection of cells, protecting them from mechanical stress, oxidative and inflammatory environment. Various polymers have been tested for microencapsulation such as alginate, agarose, fibrin, or collagen (12). Among all, alginate remains the most dominantly applied because of its well-documented biocompatibility, depending of its purity and composition (32). We have chosen ultrapure type M alginate as De Vos et al. observed that microcapsules prepared with purified M alginate remained free of any significant foreign body response for prolonged periods of time after implantation (6), while microcapsules prepared with high-G alginate were consistently associated with low recovery rates and extensive overgrowth of inflammatory cells.

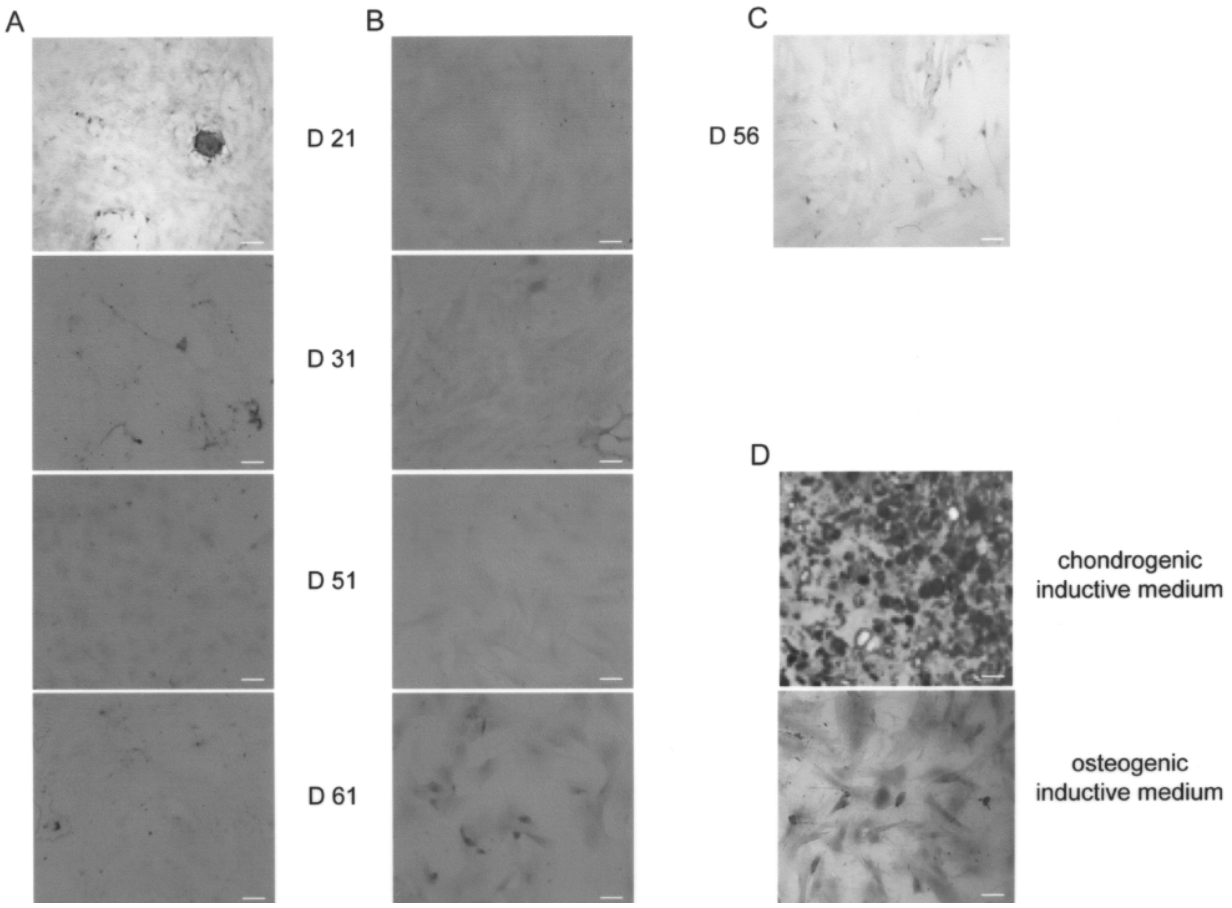


Figure 4. In vitro differentiation of MSCs in microspheres. Immnunocytochemistry on MSCs extracted from microspheres and replaced in culture. (A) Alcian blue staining at day 21, 31, 51, 61. (B) PS100 immunostaining at the same days. (C) Alizarin red staining at day 56 postencapsulation. (D) MSCs cultivated in a chondrogenic (upper panel) or osteogenic (lower panel) medium were used as a positive control for alcian blue and alizarin red staining. Scale bars: 50 μm.

Another parameter that could be modulated is the type of microparticle used. Depending on encapsulation process, various mechanical and mass transport properties of alginate microparticles should be obtained, both influencing scaffold integrity upon implantation and cells viability and functionality. APA microcapsules with a liquefied inner core, pioneered by Lim and Sun (20), have been the most frequently employed devices to transplant biomaterials to the host in the absence of immunosuppression. However the long-term in vivo success of APA microcapsules has been limited, principally due to the mechanical fragility of the membrane complex and to the difficult handling. Alginate microspheres are expected to be stronger, obviously increasing the durability of the transplant and improving the feasibility of its retrieval. But there is still an open question related to the optimal conditions during the formation of mechanically stable particles with defined porosity that can be applied to cell immobilization. The challenge is therefore not to increase stability but to determine what mechanical resistance is required for a specific application without changing other relevant particle characteristics (5,46). According to microparticle permeability, the relation between tissular environment and encapsulated cells could be modified (50). However, APA microcapsule cut-off is generally reported to be between 60 and 90 kDa (4), while the absence of membrane in microspheres enables an open macroporosity, permitting higher molecular weight molecules to diffuse (45).

In this study, we investigated both microcapsules and microspheres in order to define the most suitable for encapsulation of MSCs before grafting. Our results showed that they presented differences in their morphologies and their mechanical resistance but both remained stable over time. Moreover, our results indicated that micro-

spheres were associated with a higher survival rate of MSCs when compared to microcapsules. The higher survival of MSCs in microspheres could be explained by their need of adherence, as they are immobilized in the alginate matrix while they are in suspension in microcapsules. However, a deleterious influence of capsule membrane cut-off cannot be excluded. Furthermore, Tam et al. have showed that the extent of immunoglobulin adsorption on alginate microcapsules was highly dependent on the presence of the polylysine membrane, indicating that positive charges of the polycation are mainly responsible for the binding of immunoglobulin (35,43). Because IgG, IgM, and IgA are known to be opsonizing proteins that lead to complement activation upon their adsorption to foreign surfaces, we decided to proceed with alginate microspheres.

Altogether these results present an interest for future clinical approaches as microspheres are easier to obtain than microcapsules and would consequently facilitate a patient's graft. In order to evaluate functionality of encapsulated MSCs we measured MSC viability and the expression of bFGF, which is one of most abundant cytokines secreted by MCSs (25). Indeed, bFGF is an angiogenic and mitogenic factor that has been previously involved in renal protection and repair, mainly by stimulating angiogenesis and regeneration of renal cells (9,47). The fact that encapsulated MSCs are still functional in these microspheres is important because beneficial effects of MSCs are mainly due to their paracrine activity (54).

The last unknown data remained their ability to be grafted on solid organs. In a recent study, RGD-alginate

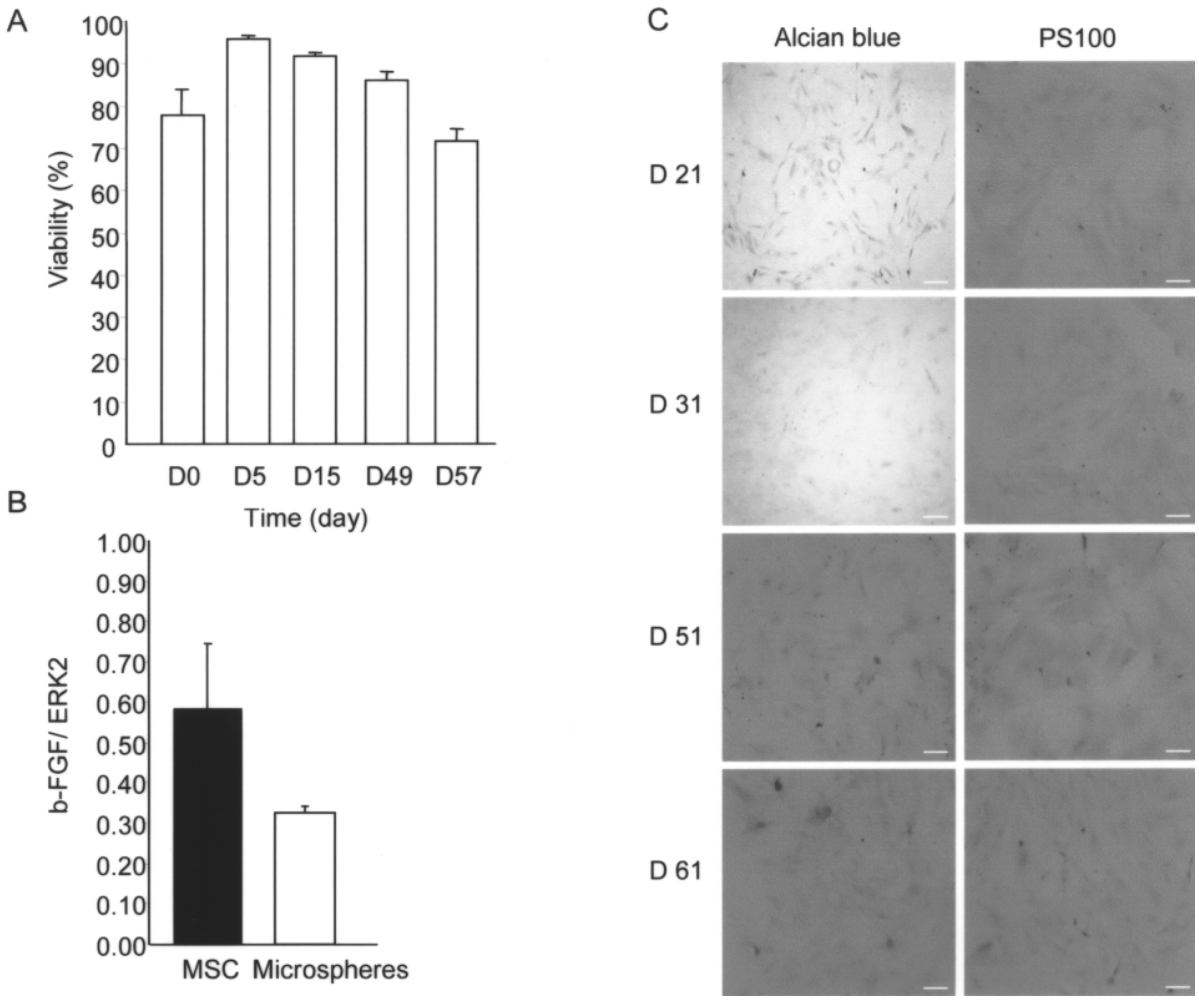
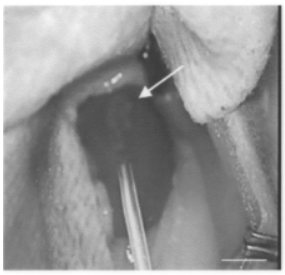


Figure 5. Effects of cell density on MSCs behavior in microspheres. Microspheres were produced with a density of 2.5×10^6 MSCs/ml. (A) Evaluation of MSC viability in microspheres. (B) In vitro expression of bFGF by the MSCs entrapped in microspheres. (C) Alcian blue staining and PS100 immunostaining at day 21, 31, 51, 61 postencapsulation. Scale bars: 50 μm.

A



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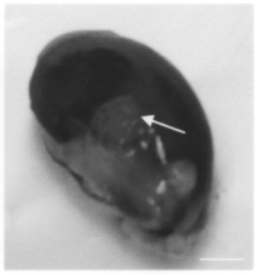

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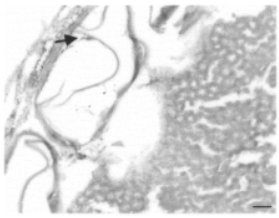

D

	Plasmatic urea (nmol/L)		Plasmatic creatinin (µmol/L)	
	Sham	Microspheres	Sham	Microspheres
D2	10.9 ± 1.36	9.5 ± 1.23	66.7 ± 5,6	51.6 ± 7.1
D17	6.9 ± 2.93	5.4 ± 0.47	38.4 ± 9.6	33.2 ± 2.0
D25	5.6 ± 0.15	5.0 ± 0.29	39.7 ± 1.5	31.8 ± 2.8

Figure 6. In vivo implantation of microspheres. (A) Injection of MSC microspheres (white arrow) under the renal capsule. (B) Localization of MSC microspheres (white arrow) under the renal capsules 25 days after graft. Scale bars: 5 mm. (C) Hematoxylin/eosin staining of renal histological sections showing microsphere (MSCs, arrow) 25 days after graft. Scale bars: 100 µm. (D) Plasmatic urea and creatinine concentrations in rats grafted with microparticles.

microspheres were grafted in bone (11) but nothing is known about their graft at the surface of solid organs like the heart or kidney. Another study has shown that intramyocardial injection of microcapsules could be done (52,56), but we have preferred avoiding intraparenchymal graft and the consecutive mechanical stress imposed to cells. So, we developed a protocol based on the graft of microspheres under the renal capsule (37). This site has been chosen as we have previously showed that MSC administration could have beneficial effects on kidney recovery, in a model of renal ischemia-reperfusion, through a paracrine activity (25). Furthermore, this graft site has already been validated for the transplantation of encapsulated pig islets with a better biocompatibility than for the intraperitoneal route (8).

Our in vivo approach shows that it is possible to graft microspheres and to follow them during several weeks without any microsphere degradation and impact on renal function. The small and uniform microspheres tested offer many advantages when compared to intraparenchymal injection or bigger scaffolds: a higher degree of biocompatibility, a reduced total implant volume, a better diffusion of cellular paracrine secretion, an improved cell oxygenation, a potential access to different implantation site (32,33), and a concentration of the grafted cells in a dedicated zone. Moreover, it would be possible to retrieve microspheres after graft or to play with alginate molecular weight and consecutive biodegradability to obtain cells release in the purpose of tissue engineering.

Encapsulating MSCs in alginate microspheres would be a promising approach to improve cell survival after their graft in a solid organ. However, concerning the heart, it could be difficult to implant microspheres. Recent studies have proposed to use patches to repair infarcted cardiac tissue (2,18,30,34,39,51). In our laboratory, we have already showed that intramyocardial graft of pharmacologically modified MSCs improves cardiac function (24). To further enhance MSC viability after graft and consequently MSC efficiency it could be interesting to combine the easiness of engineering highly biocompatible alginate scaffolds with beneficial effects of MSCs.

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