Mesenchymal stem cells encapsulated in alginate: microspheres vs microcapsules

E. Trouche[1], F. Tortosa[1], C. Mias[1], MH. Seguelas[1], A. Parini[1], D. Cussac[1*] S. Girod[2] and B. Sallerin[1]
[1]INSERM I2MR, Toulouse, France (Daniel.Cussac@inserm.fr), [2]CIRIMAT, Toulouse, France

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, cartilage and non-mesenchymal tissues like neuron, kidney and heart (Herzog et al., 2003; Kale et al., 2003) that make them particularly promising in view of cell therapy (Humphreys et al., 2007; Minguell et al., 2006). Recent studies have suggested that a major part of the beneficial effects of MSCs administration are due to the production and secretion of paracrine factors that improve tissue regeneration and/or decreased endogenous cell death (Kinnaird et al., 2004; Mias et al., 2008; Togel et al., 2007). Despite strong evidences on the efficiency of the cell therapy, several points remained to be highlighted. One question concerns the administration of MSC into target tissue. Indeed, one of the major limits of the intraparenchymal route of administration is the extensive early death of grafted cells. Different studies performed in solid organs showed that more than 80-90 % of grafted cells die within 72 hours after injection and different mechanisms have been involved in this early death including oxidative stress, hypoxia and inflammation (Maurel et al., 2005; Suzuki et al., 2004). Based on these results, it becomes evident that approaches to allow an easy and safe deposition of the MSC near the injured area and the concentration of secreted cytokines would significantly improve the beneficial effects of cell therapy. So, we designed a strategy consisting in the incorporation of MSC in a biocompatible bio-matrix in order to 1/ manipulated and grafted them more easily 2/ concentrated them and 3/ protect them against deleterious effects of the target tissue. We hypothesized that alginate, a biocompatible polymer, could be a useful tool to encapsulate MSC because alginate beads could protect MSC from oxidative stress or inflammation process undergoing at the time of graft while permitting exchanges with the tissular environment and more particularly a secretion of the paracrine factors. Two types of microparticles: with or without external membrane have been produced in order to see which is the more appropriated for our cells.

Material and methods

MSC isolation and labeling. MSC where isolated from the bone marrow of Lewis rats using their adherent properties and where labeled with red fluorescent nanocristals (QD) (Mias et al., 2008).

MSCs-alginate constructs. Rat MSC, at passage 3, were lifted with trypsin, counted and centrifuged. Microspheres and microcapsules were produced by the method of Goosen et al., with modifications (Goosen et al., 1985), under sterile conditions. Cells were suspended in this sodium alginate solution at a density of 4 x 10^6 cells/ml alginate. The microspheres correspond to microparticles gelled in CaCl2 and the microcapsules correspond to microspheres coated by incubating in poly-l-lysine (PLL). In this case, an outer alginate layer was subsequently applied and microcapsules were treated with sodium citrate to liquefy the inner alginate core. Microspheres and microcapsules were incubated in culture medium at 37°C in 5% CO_2 and 95% humidity.

Particles morphology and stability over time. Microparticles size and morphology were routinely examined with a light microscope. The stability of empty particles 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. When their membrane becomes disrupted, their shape change: they are no longer spherical but flattened and wrinkled because their content is released in the outer medium.

Mechanical resistance. The mechanical resistance of the particles was evaluated on spheres or capsules obtained with a 0.8 mm needle according to the same protocol. At defined time intervals, the beads were submitted to a standardized compression test in a TA-XT2 texture analyzer (Stable Microsystems, UK). The integrity of the beads after the test was assessed by microscopic observation.

Cell viability assay. The viability of encapsulated cells was assessed using a LIVE/DEAD^R Viability/Cytotoxicity kit (FluoProbes, France) according to manufacturer's instructions. Briefly, after several washes with PBS/MEM, the microparticles were then incubated for 30 min with a solution containing 2 µM ethidium homodimer-1 and 1 µM calcein AM. Green fluorescence is emitted from intracellular esterase-converted calcein in live cells and red or yellow-orange fluorescence indicates dead cells or cells exhibited apoptotic transformation respectively. The cell viability was estimated by the ratio of green pixels to the total number of lightened pixels.

Animals, transplantation and histology. Lewis rats (Harlan, France) weighting 180-200g were used for allogenic recipients of MSC-alginate. For transplantation, the rats were anesthetized with isoflurane/oxygen inhalation (3/97). A total of 10 MSC-alginate were transplanted under the renal capsule. Sham-operated animals were subjected to the same surgical procedure without transplantation. Kidney sections were collected 1 month after MSC injection. Specimens were fixed in paraformaldehyde 4% and then they were dehydrated and subsequently embedded in paraffin. Sections (6µm) of kidneys were stained with hematoxylin/eosin using standard methods.

Results and discussion

Morphology and stability of the alginate microparticles. Two kinds of microparticles were tested: microspheres and microcapsules. Observed by optical microscopy, all cell-loaded microparticles had a uniform and spherical morphology as shown in Figure 1. In the case of microcapsules a smooth refringent ring, corresponding to the continuous transparent alginate-PLL membrane, could be observed.

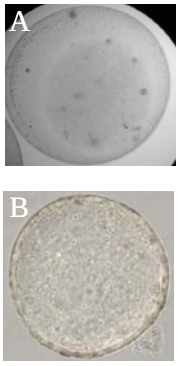

Fig 1: Encapsulated MSC morphology. Microspheres (A) and microcapsules (B) observed with optical microscope.

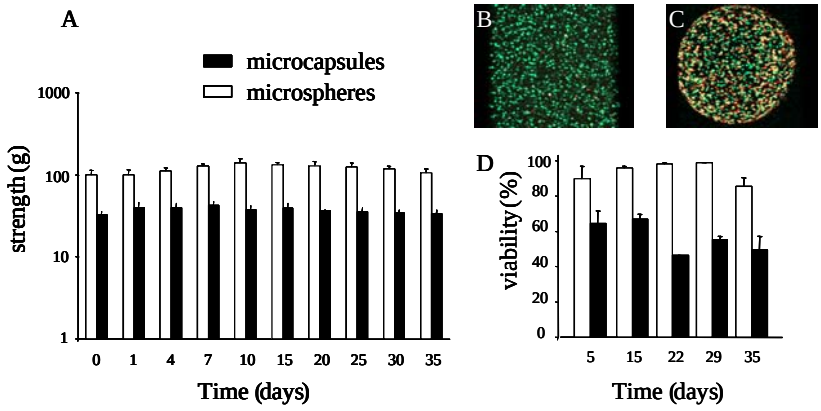

Fig 2: Mechanical resistance of microparticles and *in vitro* viability. Measurement of the mechanical force needed to compress the beads of 30% of their height with time (A). Resulting confocal image of encapsulated MSC in microspheres (B) and in microcapsules (C) after 2 µM ethidium homodimer-3 (red fluorescence) and 1 µM calcein (green fluorecence) loading. Quantitative analysis of confocal images (D).

They all present an average diameter between 500 and 710 µm, with alginate microspheres slightly bigger than microcapsules. Optical microscopic examination of the microparticles showed that MSC appeared evenly distributed throughout the beads and that they remained stable in saline buffer and in culture media over the study.

Mechanical resistance. The resistance and durability of the beads was studied by submitting them to a standardized mechanical stress. Figure 2A shows the evolution of the maximal mechanical force (in g) required to compress the beads of 30% of their height with time. This test of resistance to compression was chosen to evaluate the microparticles mechanical resistance, as it has been demonstrated to be more sensitive to small variations than a simple rupture test (Orive et al., 2003). In all cases, until day 35 post-encapsulation the microparticles resisted compression without rupture. As expected, forces exerted to compress microspheres are higher than microcapsules, but the latter exhibit more elastic behaviour.

***In vitro* viability of encapsulated MSC.** Microspheres and microcapsules have been stained and using confocal microscope 3D reconstitutions have been performed. The MSC viability has been quantified by automatic cell counting. This quantification shows that the majority of MSC in microspheres are alive (green cytoplasm) until day 35 (85,6%) whereas the viability of MSC is more reduced (64,6% on day 5 to 49,3% on day 35) in microcapsules (Fig 2B,C,D).

Implantation of encapsulated MSC. In order to evaluate the feasibility of implanting microparticles in a tissue, we have developed a model based on a deposition of the microparticles under the renal capsule (Fig 3). Twenty five days after the graft, microspheres and microcapsules are easily identified and have maintained their integrity. Prior to encapsulation, MSC were labeled with QD which allowed to track them and to reflect their viability. Furthermore, histological analysis shows that QD positives MSC are observed in microcapsules.

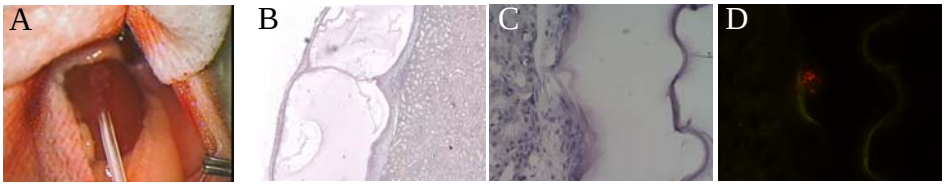

Fig 3: Implantation of microparticles.
Injection of microparticles under the renal capsule (A). Hematoxylin/eosine staining of renal histological sections showing microparticles 25 days after graft (B). Higher magnification of panel (B) showing grafted microparticles under optical (C) or fluorescent microscopy (D).

So, this result suggested that there still have living MSC in microcapsules 25 days after the graft. No evidences of malignant invasion or inflammation were found in any of the specimens but we observed an accumulation of cells around the microcapsules which seems to be a scar reaction. This phenomenon has been already demonstrated (Ma et al., 2005).

In this preliminary study, we have compared two kinds of microparticles: microspheres and microcapsules in order to define the most suitable to encapsulate MSC before grafting in a tissue. Our results show that microspheres were associated with a higher survival rate of MSC compared to microcapsules. This effect could be explained by the inability of some nutrients to be transferred across the membrane or by the spatial distribution (cell to cell interactions) of the cells inside the capsules. So, we will further modify the membrane permeability to determine its impact on cell viability. The *in vivo* study shows that it could be possible to graft microspheres and microcapsules and followed them during several weeks. Our results show that MSC are still alive in microspheres.

Further experiments will be needed to determine if alginate-encapsulated MSC always released paracrine factors and the effect of long-term culture on this release. We will perform an approach in a pathological model based on ischemia-reperfusion pattern. Finally, we will answer the question of the fate of MSC i.e. the differentiation of the cells in relation with the matrix/cell interaction.

Conclusion

With this study we are able to encapsulate viable MSC in alginate microspheres or microcapsules. The MSC have a better viability in the microspheres than in the microcapsules. Finally, these microparticles may be manipulated and grafted under the renal capsule as a model of solid organ cell therapy.

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