Fibronectin-alginate microcapsules improve viability and protein secretion of encapsulated FIX-engineered human mesenchymal stem cells

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INTRODUCTION AND OBJECTIVES

Hemophilia B is an X-linked bleeding disorder caused by lacking of functional circulating FIX, affecting about 1 in 30,000 live male births. Being a monogenic disorder, hemophilia is an excellent candidate for gene therapy. Cell-therapy technologies offer a variety of safety advantages for hemophilia treatment. Engineered Mesenchymal Stem Cells (MSCs) are an attractive target cell population for cellular therapy because they are readily accessible, are suitable for *ex vivo* genetic modification, and more importantly, they display capabilities for modulating the host immune response. Current cell-therapy strategies for hemophilia treatment are limited due to the compromised viability of the implanted recombinant cells. The transplantation of FIX-engineered MSCs for the treatment of hemophilia B has been proposed (Coutu DL 2010) using local injection of MSCs in scaffolds; yet, an optimal delivery system that improves the viability and functionality of these cells remains to be identified. Encapsulation of recombinant cells within a semi-permeable membrane is a strategy for continuous delivery of FIX. This could reduce or even eliminate the chronic administration of immunosuppressants while allowing a sustained and controlled delivery of therapeutic products. Alginate is the most widely employed material for cell encapsulation, but success has been limited due largely to the low viability resulted from the lack of attachment site for anchorage-dependent MSCs in alginate microcapsules. Long-term survival of these cells and release of secreting protein are still major challenges in this field. It is well known that cell-adhesive molecules play central roles at the cell-surface interface through interacting with integrins for mediating survival signals.

Here, we explore the effect of fibronectin as a cell-adhesive molecule on viability and protein secretion of encapsulated MSCs within alginate-based microcapsules. The objectives of this study are three-fold: First, to assess if the incorporation of fibronectin within alginate matrix can manipulate the hydrogel microcapsules for enhanced viability and FIX secretion level of umbilical cord blood-derived (CB) MSC. Second, to investigate how the different concentrations of fibronectin can affect cell viability, proliferation and FIX secretion. Third, to induce differentiation of the encapsulated CB MSC in fibronectin-alginate microcapsules into osteogenic, chondrogenic and adipogenic lineages in order to examine the effect of differentiation status of the encapsulated cells on cell viability and FIX secretion.

MATERIALS AND METHODS

Tissue culture conditions:
Cord blood-derived human MSCs, engineered with lentivirus for FIX secretion, were grown in monolayer cultures in IMDM (10% FBS and 1% P/S).

Encapsulation technique:
MSCs were suspended in a 1.56% (w/v) alginate solution supplemented with 10, 100, or 500µg/ml human plasma fibronectin. Microencapsulation was performed with an encapsulator (Nisco Engineering Inc.). Alginate microcapsules were then coated with 0.05% (w/v) PLL and an outer layer of alginate 0.03% (w/v) and incubated *in vitro*. The same encapsulation procedure was performed on control experiments.

Viability and FIX secretion: Cell viability and prolifération was determined by LIVE/DEAD viability asay. FIX secretion was measured using a FIX ELISA kit.

Cell-matrix interactions:
Encapsulated cells were fixed, processed and stained for confocal microscopy and TEM analysis.

Statistical analysis: ANOVA was carried out. Student's t-test was conducted as a post hoc test. Data are expressed as means ± SD, $p<0.05$.

RESULTS AND DISCUSSION

The results of LIVE/DEAD viability assay confirm that incorporation of either 100 or 500µg/ml fibronectin into the alginate matrix significantly improves the viability and proliferation of the encapsulated MSCs. However incorporation of 10µg/ml fibronectin does not have a significant effect on viability and proliferation of the encapsulated cells (Figure 1, A,B).

As a cell adhesion molecule, fibronectin has the advantage of providing the cells with matrix cues for enhanced interaction and subsequent survival and proliferation. MSCs are able to attach to fibronectin through several integrins. Therefore, it is speculative, yet conceivable that the enhancement of MSC proliferation and viability observed in this study is modulated by MSCs interacting directly with fibronectin.

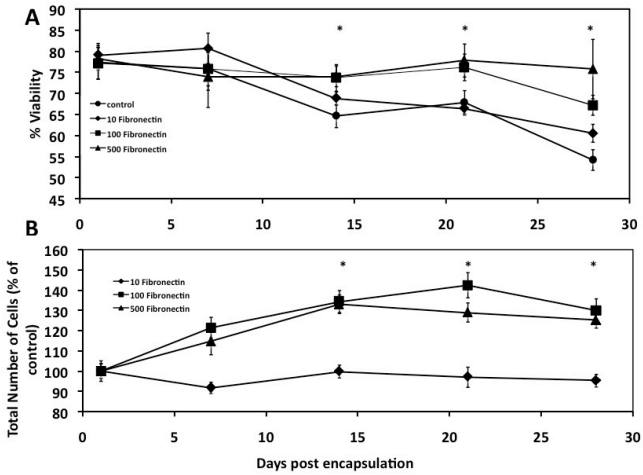

Figure 1. (A)%Viability and (B) proliferation of MSCs in microcapsules cultured *in vitro.* *Significant difference from control, P<0.05.

Consistent with improved viability and proliferation of cells encapsulated in 100 and 500µg/ml fibronectin-alginate and as measured by ELISA, FIX secretion from 100 500µg/ml fibronectin-alginate microcapsules is above 3000 ng/ml (capsules)/24 hr on day 14 and remains above 2000 ng/ml (capsules)/24 hr after 28 days of *in vitro* culture. On the other hand, FIX secretion from 10µg/ml fibronectin-alginate and control groups is approximately 2000 ng/ml (capsules)/24 hr on day 14 and decreases to around 1000 ng/ml (capsules)/24 hr on day 28 (Figure 2).

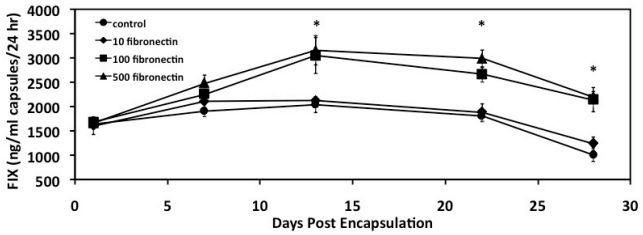

Figure 2. FIX Secretion from microcapsules. *Significant difference from control, P<0.05

For an in-depth look at the cell-matrix interactions, encapsulated MSCs were examined using TEM. In both 100 and 500µg/ml fibronectin-alginate, cells clearly demonstrated the presence of filopia-like membrane extensions into the surrounding matrix (Figure 3, E-H) which were lacking in control and 10µg/ml fibronectin-alginate groups (Figure 3, A-D).

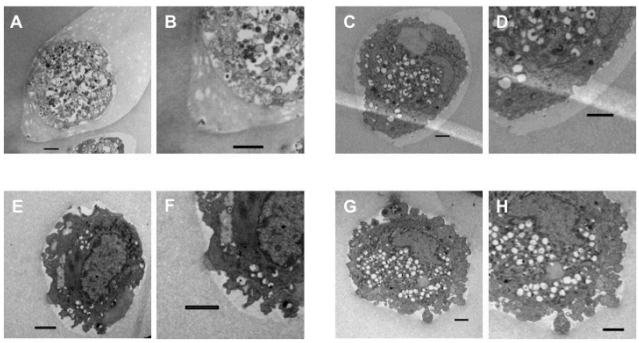

Figure 3. TEM images of CB MSCs encapsulated in control (A,B); 10 (C,D); 100 (E,F); 500 µg/ml-fibronectin-alg microcapsules (G,H).

The encapsulated MSCs were also evaluated for their ability to differentiate into the 3 mesoderm linages, osteoblasts, chondrocytes, and adipocytes in microcapsules. As shown in Figure 4, even with no stain, calcium deposit is evident in the microcapsules grown in osteogenic culture. Calcium deposits are further confirmed by Alizarin red, 3 weeks post encapsulation. However, the encapsulated cells grown in chondrogenic or adipogenic media were not successfully differentiated into chondorcytes or adipocytes.

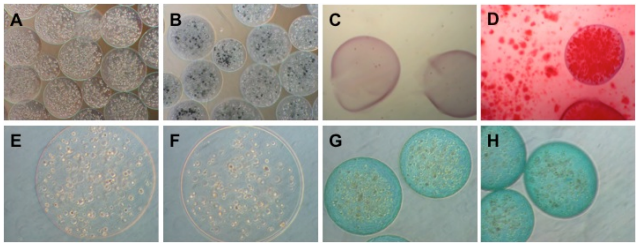

Figure4. Differentiation potential of encapsulated CB MSCs. Unstained in basal (A) and osteogenic (B) medium, stained with Alizarin Red in basal (C) and osteogenic medium (D), stained with Oil Red-O in basal (E) and adipogenic medium (F), stained with Alcian Blue in basal (G) and chondrogenic medium (H).

CONCLUSION

100 Fibronectin and 500 Fibronectin were shown to have a significant effect on enhancement of cell viability, proliferation and subsequent FIX secretion. 10 Fibronectin was comparable to non-supplemented microcapsules (control) and did not significantly affect cell viability or protein secretion. Differentiation studies confirm that while cultured in appropriate media, the novel 100 Fibronectin alginate microcapsules support osteogenic but not chondrogenic or adipogenic differentiation of the encapsulated MSCs grown in suspension culture.

REFERENCES

- Coutu DL et al. 2010 *Hierarchical scaffold design for mesenchymal stem cell-based gene therapy of hemophilia B.* Biomaterials. 2010;32(1):295-305.

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