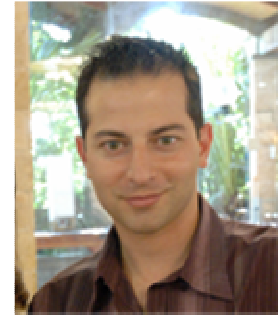
Polymeric system entrapping genetically engineered stem cells for cancer therapy



A. Goren[1] and M. Machluf[2]
[1] The Department of Biotechnology & Food Engineering. Technion – Israel Institute of Technology (gamit@tx.technion.ac.il) [2] Technion – Israel Institute of Technology, Haifa, Israel (machlufm@tx.technion.ac.il)

Introduction

Cell microencaspulation is one of the most important approaches for cell-based therapy and the continuous delivery of drugs and proteins. This approach is based on the entrapment of viable cells within the confines of a semi permeable membrane. The cells are genetically engineered to produce a therapeutic factor. The uniqueness of this system is that the membrane selectivity enables the entrance of nutrients into the cells and release of the therapeutic factor to the surroundings. On the other hand, it disables the entrance of larger molecules like antibodies and cells of the immune system (Uludag, 2000).
The cell encapsulation technique is known for years but still it is facing several drawbacks which prevent this application from clinical trails. One of the major problems with this system is the immunogenecity of the encapsulated cells (De Groot, 2004). By using adult stem cells our system offers a new type of cells which is naïve, and less immunogenic (Barry, 2005). Also these cells can be genetically modified for the use in cancer therapy. Therefore, our research goal is to develop a polymeric system which encapsulates genetically engineered mesenchymal stem cells (MSCs) for anti-angiogenic cancer therapy. The microcapsules are implanted in adjacent to the tumor site and the encapsulated stem cells secrete the anti-angiogenic factor which inhibits tumor development.

Material and Methods

Cell culture: human MSCs (hMSCs) were kindly given by Erella Livna from the Department of Anatomy & Cell biology at the Technion Medical School (under Helsinki approval). All experiments were conducted between passages 1-6.
Cell micro-encapsulation: Alginate Poly-L-Lysine (PLL) microcapsules were prepared using the extrusion droplet method with slight modifications.
Encapsulated cells viability and proliferation assays: Encapsulated hMSC viability and proliferation was tested using the AlamarBlue and radioactive [H^3] Thymidine assays respectively.
Surface markers analysis: The encapsulated hMSC cell markers were tested using different cell markers antibodies (CD29, CD44, CD105, CD90, CD34, CD133, CD31 and CD144) (Alhadlaq, 2004). The encapsulated cells were retrieved and analyzed using flow cytometry analysis.
Encapsulated cells differentiation assays: The encapsulated hMSCs were differentiated into the 3 mesoderm lineages: adipoblasts, chondroblasts and osteoblasts. The encapsulated hMSCs were cultured in the specific differentiation medium and after the incubation time the cells were retrieved and analyzed for differentiation using the specific staining techniques.
Encapsulated cells biocompatibility assay: For the biocompatibility assay *in vivo*, C57BL mice were injected with different capsule groups. The encapsulated hMSC immunogenicity was evaluated by RT-PCR using specific primers to IL-1β and TNF-α and also by histology analysis.
Genetically engineering of hMSC: hMSC were genetically modified to express the anti-angiogenic factor PEX, using the Virapower lentivirus plasmid kit (Invitrogen). The presence of PEX was analyzed using PCR and RT-PCR analysis. Also the bioactivity of PEX was evaluated using proliferation inhibition assay.

Results and Discussion

Development and characterization of the encapsulation system: hMSCs were encapsulated with Alginate Poly-L-Lysine (PLL) using the droplet extrusion technique. The polymeric system was optimized considering encapsulated cell viability, proliferation, density and also the microcapsule size and integrity. The encapsulated hMSCs viability and proliferation were measured and related to a known encapsulated cell line (NIH3T3). The encapsulated hMSCs were viable and proliferated for more than 70 days. Furthermore, the encapsulated hMSCs exhibited higher viability values than the NIH3T3 cells (Fig 1).
Characterization of the encapsulated hMSCs: One of the important aspects considering the entrapment of hMSCs is their ability to sustain their undifferentiated stage even inside the microcapsule. Therefore, the encapsulated hMSCs were characterized for their mesenchymal stem cell markers (CD105, CD90, CD44 and CD29) and for other non mesenchymal markers including, endothelial and hematopoietic markers (CD31, CD133, CD34 and CD144). Our results show that even after 2 months post-encapsulation the cells sustained their stem cell properties (Fig 2).

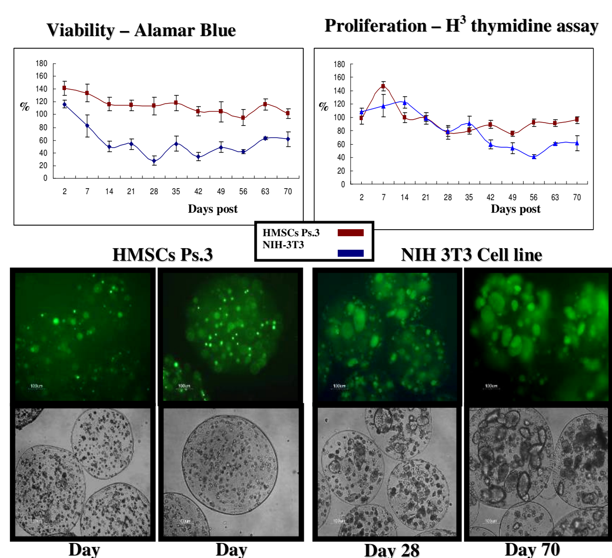


Figure 1: Encapsulated hMSCs characterization. The viability, proliferation and cell morphology of the encapsulated hMSCs was related with encapsulated NIH3T3 cell line. Pictures were taken by light and fluorescence microscopy. (n=4).

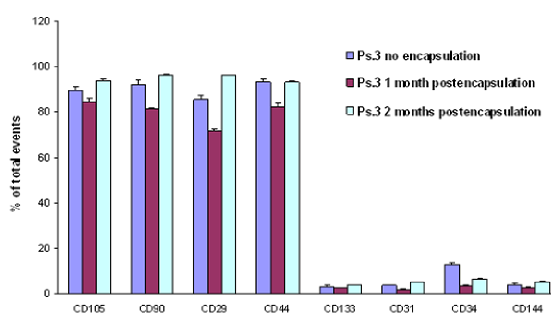


Figure 2: Encapsulated hMSCs sustained their undifferentiated stage even 2 months post encapsulation. The cells were retrieved 1 and 2 months post-encapsulation and immuno fluorescence was performed using Flow cytometry analysis. (n=4).

Differentiation of the encapsulated hMSCs: Another common method to prove the stemness of the encapsulated hMSCs is by differentiating them into the 3 mesoderm lineages: Osteoblasts, Chondroblasts and Adipoblasts. The encapsulated hMSCs were cultured with the appropriate differentiation medium. After the differentiation time the encapsulated hMSCs were either directly stained or first retrieved and than stained. The results show that the encapsulated hMSCs differentiated into the 3 lineages as expected (Fig 3).
The biocompatibility of the encapsulated hMSCs: The biocompatibility of the encapsulated hMSCs was evaluated *in vivo*. C57BL mice were injected with different capsule groups and followed for 8 weeks. After 1, 2, 4 and 8 weeks from injection the animals were scarified and lymph nodes were harvested. The immune reaction was measured by the levels of the inflammatory

cytokines TNF-α and IL-1β and also by histology. The RT-PCR results show that the MSCs groups were less immunogenic than the cell line group (Hek293 cell line). Also the histology reveals the same behavior (Fig. 4).

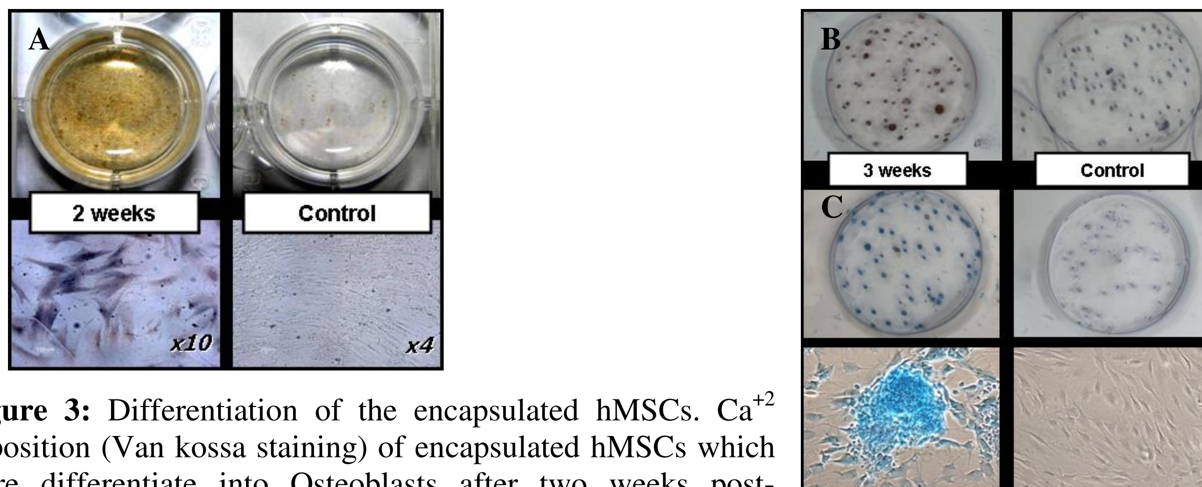


Figure 3: Differentiation of the encapsulated hMSCs. Ca^{+2} deposition (Van kossa staining) of encapsulated hMSCs which were differentiate into Osteoblasts after two weeks post-encapsulation compared to the control (A). Oil red O staining reveals the differentiation of the encapsulated hMSCs into Adipoblasts comparing to the control (B). Alcian Blue staining reveals the differentiation of the encapsulated hMSCs into chondroblasts comparing to the control (C). Pictures were taken by light microscopy.

Constructing genetically modified hMSCs for glioma cancer therapy: The next step was to use the encapsulated hMSCs for cell based therapy. To do so the hMSCs were genetically modified with M-cherry PEX plasmid using lentiviral transfection kit. M-cherry is a flour-chrome which is used to track the cells and PEX is a 29kD molecule, anti-angiogenic factor. Fig 5 shows the M-cherry positive hMSCs which express endogenous PEX as can be seen in the RT-PCR results. The bioactivity of the expressed PEX was evaluated by proliferation inhibition assay. The encapsulated PEX expressing hMSCs were able to inhibit the proliferation of U-87 glioma cells by 47%. We are currently evaluating the PEX anti angiogentic properties *in vivo*.

Conclusions

Our findings demonstrate for the first time the characterization of hMSCs encapsulated in Alginate PLL microcapsules. The encapsulated hMSCs were shown to proliferate well inside the microcapsules, exhibiting normal cell morphology. They exhibited positive mesenchymal markers and differentiated into the three mesoderm lineages. The biocompatibility *in vivo* assay showed that the encapsulated hMSCs were less immunogenic than the encapsulated 293 cell line.
By constructing the hMSCs to genetically express the anti-angiogenic factor PEX we were able to show that the encapsulated hMSCs inhibited the U87 proliferation by 47% *in vitro.*
To conclude, encapsulated hMSCs are excellent candidates for cell micro-encapsulation and can be used for the therapy of many illnesses including cancer malignancies by cell based therapy.

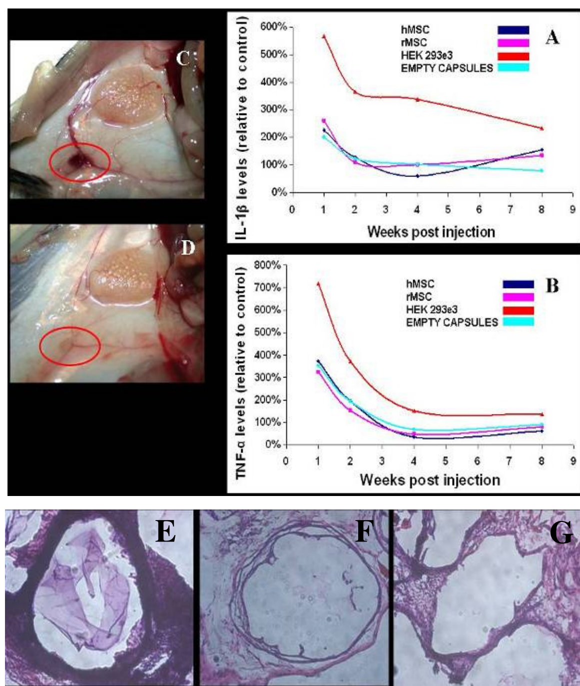


Figure 4: Encapsulated MSCs reduce immunogenecity significantly in comparison with a known cell line (hek293). Microcapsules entrapping MSCs, hek293 or no cells were injected into C57BL mice.1,2,4 and 8 weeks post injection the lymph nodes were harvested and RT PCR analysis was performed for IL-1β (A) and TNF-α (B). (n=6). Visual pictures of the lymph nodes (red circled) of the HEK-293 group (C) and the hMSC group (D). H & E histology staining of the microcapsule graft. The microcapsules were retrieved on week 4 post injection and paraffin molds were prepared. hek293 group (E), Empty capsules (F), hMSCs capsules (G).

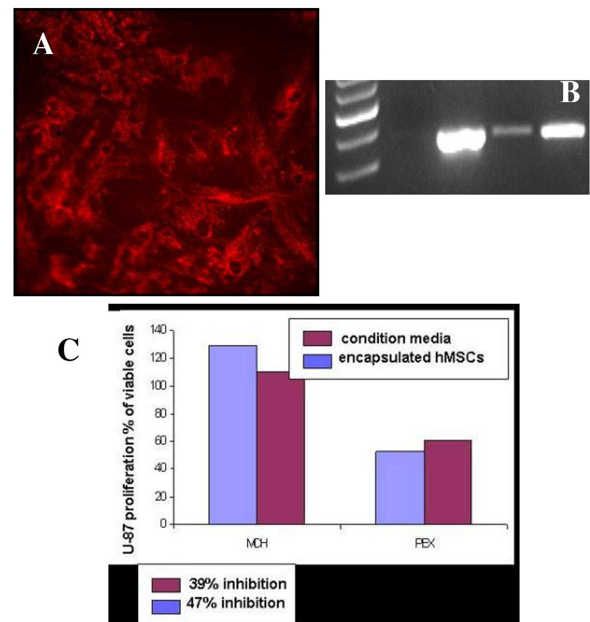


Figure 5: Genetically engineered hMSCs expressing PEX. hMSCs were transduced using lentiviral vector. The trasduced cells expressed M-cherry (A) and exogenous PEX lane 1: size markers, lane 2: control, lane 3: PCR analysis, lanes 4+5: RT-PCR analysis (B). Proliferation inhibition assay performed on U87 glioma cells reveled 47% inhibition in comparison with control.

References

H. Uludag et al. (2000) *Technology of mammalian cell encapsulation*. Adv Drug Deliv Rev 42, 29-64

M. De Groot et al. (2004) *Causes of limited survival of microencapsulated pancreatic islet grafts*. J Surg Res 121, 141-150

F.P. Barry et al. (2005) *Immunogenicity of adult mesenchymal stem cells: lessons from the fetal allograft*. Stem Cells Dev 14, 252-265

A. Alhadlaq et al. (2004) *Mesenchymal stem cells: isolation and therapeutics*. Stem Cell Dev 13, 436-448