Al-Azhar Bull. Sci. Vol. 19, No. 1 (June.): pp. 91-100, 2008.

SYNTHESIS OF NEW MICROCAPSULES FROM AGAROSE GEL AND THEIR MEDICAL APPLICATIONS

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Abstract

Prevention of rejection is critical to achieve successful pancreatic islet transplantation protection of islet cells from rejection by isolating the islets in artificial membranes has been used instead of immunosuppression treatment. In these study we investigated the microencapsulation of microencapsulated hamster islets in hydrophilic microencapsules made of agarose. The microencapsulated hamster islets were placed interaperitoneally in mice in which diabetes was induced by a single dose (150 mg/kgof body weight) of streptozotocin. Five groups were studied. The first group (5 mice) received free hamster islets (1000 islets).The second group (5 mice) received 1000 empty agarose microcapsules and 1000 free hamster islets. The third group (10 mice) received hamster islets microencapsulated in agarose (500 microcapsules). The forth group (10 mice) received 1000islet microcapsules. The fifth group (10 mice) received 1000 islet microcapsules cultured in CMRL-1066 medium for 4 weeks at 37°C . Mice of group 1 and group 2 failed to achieve normoglycemia. Recepient mice received miccroencapsulated islets group (3,4,5) maintained normoglycemia for a mean of 45 ± 5 days range (30 – 65 days). These cured mice had normal glucose tolerance tests, which indicates that islets in the microcapsules were functioning as if they are in an intact pancreas. Microcapsules, retrieved up to 30 days after transplantation, showed no evidence of tissue reaction. Our study indicate that agarose microcapsules can protect islet xenografts from rejection. These microcapsules may be suitable for human clinical islet xenotransplantation.

Introduction:

Pancreatic islets transplantation for the treatment of human diabetes has been limited by the inability to prevent islets rejection. Various approaches for preventing islet graft rejection and thus maintaining long term islet cell function has been investigated **(Lacy, 1993).** One of these approaches is the protection of the transplanted islet from recipient immune system by enclosing them in membranes that prevent inward diffusion by immune mediators, but allow free exchange of glucose and insulin **(De-Vas *et al.,* 2002 and Kabayashi et al., 2003)**

Previous studies had demonstrated that islet can be entrapped in viable state in alginate capsules which are characterized by a shell of alginate -polyethyleneimine

(Lim and Sun, 1980 and Stieger et al.,2006). The drawback of this technique is the fragile nature of such preparations and the instability of these microcapsules **(Soon-Shiong *et al.,* 1993 and Hobbs *et al.,* 2001).** When these microcapsules were placed interperitoneally they produced only temporary remission of hyperglycemia **(Fan *et al.,* 1990).**Another study had shown that encapsulation of human islets in acrylic-copolymer fiber could prevent rejection of the grafts without immunosuppression for a period of 2 weeks after subcutaneous implantation **(Scharp *et al.,* 1984).** Also, **Iwata *et al.,(*1994)** and **(Foster et al.,2007)** reported that the advantage of agarose for microencapsulation of islet allograft and xenograft. In these studies only long-term allograft survival with maintenance of normoglycemia was achieved.

Our study conducted to examine whether hamster islets enclosed in agarose microcapsules can survive in the peritoneal cavity of mice as xenograft transplantation. Also to examine whether these microcapsules can maintain normoglycemia and provide normal glucose tolerance test response.

Materials and Methods:-

Animals:

Golden Syrian hamsters, weighing 100-120 g were used as islet donors. Swiss mice were the recipients. Non fasting plasma glucose levels of the recipient mice were determined before the induction of diabetes. Blood sugar level were monitored via orbital sinus blood samples with Aqua trend sensor. The mice were made diabetic by a single intraperitoneal injection of streptozotocin (150 mg/ Kg bodyweight) and only those mice with serum glucose levels more than 350 mg/ dl were used for transplantation.

Islet Isolation:

Hamster islet were isolated according to the method previously described **Getoh et al.,1985 and Avila et al.,2003** by intraduct injection of collagenase solution followed by digestion and extensive purification on Ficoll gradients. Hand-picked islets were cultured in CMRL-1066 medium containing 10% heat inactivated fetal calf serum (FCS) and antibiotic-antimycotic solution (1 ml/ 100 ml). The islets were then incubated at 37 °C for 3 days in a humidified atmosphere of 5% CO_2. Islets were stained for viability by diphenylthiocarbazone (DTZ).

Preparation of Agarose Microcapsules:

Pancreatic islet microcapsules were prepared according to the method previously described Jain et al.,1995 and Jain et al.,2007. Islets were washed by gravity sedimentation 3 times in CMRL-1066 medium containing only antibiotic-antimycotic solution. Agarose (Sigma Chemical Co., St. Louis, USA) in phosphate-buffered saline (PBS) without Ca and Mg salts at a concentration of 5% was autoclaved and stored at room temperature. Before use the gel was melted at 70 °C then cooled to 40 °C and mixed with islets suspended in an appropriate volume of growth medium. The mixture was poured into a 50 ml round-bottom glass centrifuge tube containing an equal volume of paraffin oil. The liquid was emulsified at room temperature with a vortex, to the desired bead size (~ 80-20 μm). the mixing vessel was then cooled in an ice bath for 5 minutes and 50 ml of cooled growth medium were added. The tube was centrifuged at 1200 g for 10 min. The oil phase was removed by suction and 50 ml of growth medium was added. After mixing, the suspension was recentrifuged and the remaining oil was removed. The microcapsules were transferred to cultivation Petri dish containing complete CMRL-1066 medium with 10% heat inactivated fetal calf serum and antibiotic-antimycotic solution (1 ml/ 100 ml). The microcapsules were then incubated overnight at 37 °C in a humidified atmosphere of air and 5% CO_2.

Transplantation of Microcapsules:

At transplantation, a midline incision (2 mm) was made in the abdominal cavity and islet microcapsules were injected through 1 ml syring without needle. A total of 40 mice were divided into 5 different treatment groups. Group 1 consisted of 5 mice grafted with 1000 free islets. Group 2 consisted of 5 mice grafted with 1000 free islets and 1000 empty agarose microcapsules. Group 3 consisted of 10 mice received 500 islet microcapsules. Group 4 consisted of 10 mice received 1000 islet microcapsules. Group 5 consisted of 10 mice received 1000 islet microcapsules that cultured in CMRL-1066 medium for 4 weeks at 37 °C.

Post-transplant Follow-up:

After transplantation, the mice were transferred to metabolic cages for daily examination. Non-fasting blood glucose levels of the recipients were monitored 3 times weekly for the first 3 weeks, then twice weekly thereafter. The mice were considered cured if exhibited the following criteria: a random plasma glucose less

than 200 mg/ dl, aglucosuria with steady weight gain. Rejection was considered when blood sugar concentration exceeded 200 mg /dl on two consecutive bleedings.

Glucose Tolerance Test:

Glucose tolerance test was carried out in transplanted mice that maintained a normoglycemia state for at least 30 days as previously described **Iwata et al.,1994**. For comparison the glucose tolerance test was also performed to 5 normal non-transplanted mice and 5 diabetic non-transplanted mice. Glucose solution 1 mg/ kg body weight was infused endogastrically through a polyethylene tube into mice that had been fasted overnight. Blood glucose levels were determined at 0, 30, 60, 90 and 120 min of glucose injection. The results were expressed as average blood glucose level ± SE. The degredation in glucose per minutes (k value) was calculated.

Stability of Microcapsules:

To assess the stability and islet preserving properties of microcapsules, groups of microcapsules (1000 microcapsules/ group) were cultured in CMRL-1066 medium and incubated for 4 weeks at 37 °C in a humidified atmosphere of 5% CO_2 (medium changed once per week). At the end of incubation period, the microcapsules were transplanted into diabetic mice according to **Hangmeichen et al.,2004.**

Results:

Immediately after separation, the viability of rat islets was investigated in DTZ dye. Direct-visualization of stained islets was achieved under inverted microscope. In DTZ dye **fig (1**). The viable islets were stained crimson red, while dead islet, acinar and ductal tissue failed to incorporate the stain. Approximately 90 % of islets were found viable. The same percentage of viable islets was found after one day of culture. Most cells were died during the subsequent 4 to 9 days of culture.

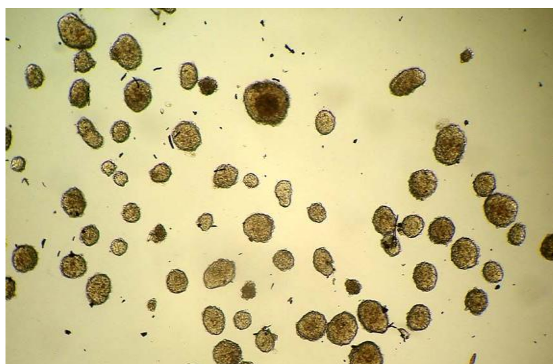


Fig (1) Light photograph showing hamster islets stained with DTZ. Islets appear healthy with viable α,β,δ cells

The islet microcapsules were purified by hand picking to be devoid of contamination at acinar cells, debris or empty microcapsules. Most microcapsules enclosed one islet and few enclosed two or more islets **fig.(2**). The result of islet microcapsule transplantation are summarized in table 1. Mice transplanted with agarose pancreatic microcapsules (group 3,4,5) became normoglycemic and remained so for 30 to 65 days with gain in body weight. To confirm that encapsulation of islets xenografts in microcapsules prevented islet graft rejection, free hamster islets were transplanted interaperitoneally in 5 mice (group 1). None of these mice became normoglycemic. Similarly, when the hamster free islets were transplanted together with empty agarose microcapsules 5 mice (group 2), none of these mice became normoglycemic. To determine the ideal recommended number of islets required to achieve normoglycemea 500 or 1000 microcapsules were tried (10 mice / group). Although euglycemia was achieved in both groups, blood glucose readings were higher with flactuated values when less than 1000 microcapsules were used **fig (3).**

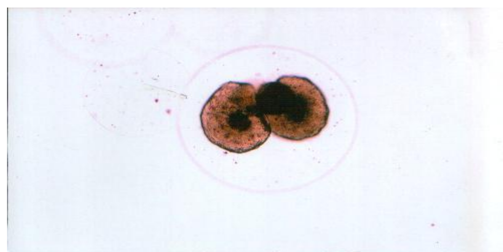
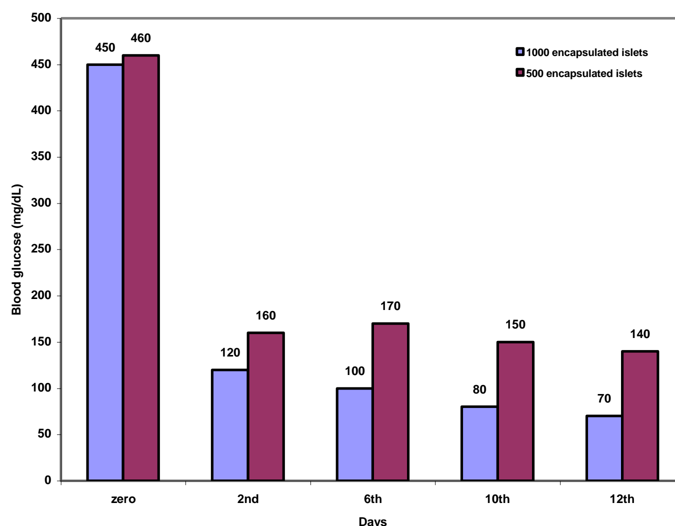


Fig (2) Light photograph showing hamster islets transplanted in agarose gel.

Table1. Results of nonencapsulated and encapsulated islet transplant

No	Transplanted group	Duration of normoglycemia (days)	Mean graft survival (days)
1	1000 free islets	**--------**	**---------**
2	1000 free islets + 1000 empty capsules	--------	---------
3	500 encapsulated islets	34,35,35,36,37,37,42,45,48,51	40 ± 3
4	1000 encapsulated islets	30,42,44,44,50,53,55,59,62,65	50 ± 3

5	1000 encapsulated and cultured islets	31,35,37,41,42,45,52,55,59,61	46 ± 3



Fig(3) The relation between blood glucose and days post transplantation

Glucose tolerance tests were carried out in transplantd mice that had maintained normoglycemic state for more than 30 days **fig.(4).** Five mice from each group were infused with 1 g /kg glucose after 30 days of transplantation. The mice were able to normalize their blood glucose. In normal mice and mice transplanted with islet microcapsules, Plasma glucose peaked at 30 minutes. And returned to base line levels by 120 minutes. Diabetic mice showed higher glucose levels after 120 minutes. Comparin the normal mice with transplanted mice, there was no significant difference in blood glucose levels at 0,30,60,90 and 120 minutes, fig (3). These mice normalize their blood glucose and exhibited euglycemia 120 minuts after glucose infusion. The rate of glucose degradation is measured by k value for the transplanted mice (2.5 ± 0.2)was higher than that of diabetic mice (0.85 ± 0.3). The k value for normal controles was (2.9 ± 0.2).

The stability and islets preserving properties of microcapsules were evaluated in vitro (group 5). Groups of 1000 microcapsules were incubated for 4 weeks at 37°C in CMRL-1066 medium. When these incubated microcapsules were transplanted

into diabetic mice, all mice became euglycemic and maintained normoglycemia for more than 30 days.

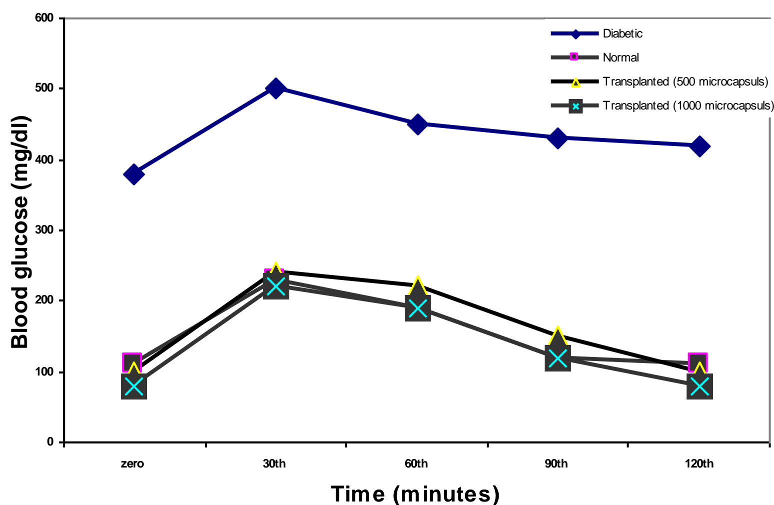


Fig (4) Blood glucose levels in response to infused glucose (1g/kg) at 30 days following transplantation

The microscopic examination of retained microcapsules 30 days after transplantation showed intact islet with neither tissue reaction nor adhesion **fig (5).** Moreover no evidence of inflammatory cells within or on the microcapsules were detected.

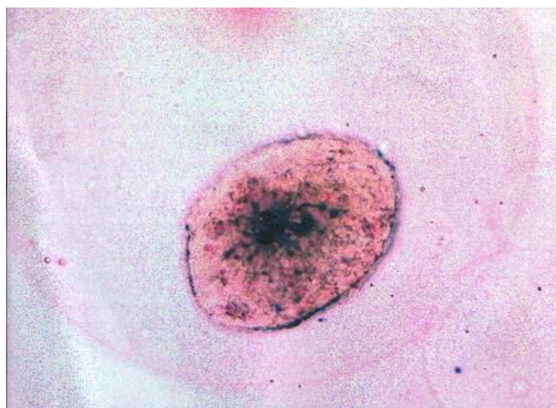


Fig (5) Light photograph showing microcapsule retained after 30 days of transplantation.There is no inflammatory cells within or on the microcapsules.

Discussion:-

Islets of Langerhans have been considered extremely immunogenic sometimes surviving only for one or more days if transplanted across a strong histocompatibility barrier **Barker,et al., 1980.** A bioartificial pancreas has been proposed as a promising approach for treatment of insulin dependent diabetic patients **Prokop, et al., 2001.**The development of microencapsulation xenogeneic islet in a semipermiable membrane is particularly attractive, since it might permit the use of Xenografts to overcome the obstacle of scarcity of transplantable human pancreatic tissue without the need of any immunosuppression **Schneider, et al., 1961; Mullen, et al., 2000 and Sun, et al., 1989.**

The data of our study showed that islets transplanted alone or with empty agarose microcapsules failed to normalize the blood glucose of the recipient mice, on the other hand, mice transplanted with agarose transplanted islets enjoyed normoglycemia for maximum 65 days also when the microcapsules were cultured for 4 weeks before transplanted, all diabetic mice became normoglycemic. This indicates that these microcapsules continued to release insulin in the medium for 4 weeks and then continued to function in vivo. These data suggest that microencapsulation can be used as a method for the storage of islet prior to transplantation. Although successful encapsulated islet cells have been reported **Sun, et al., 1989** and **Takagi, et al., 1994.** there are still many reports of encapsulated islet graft failure due to pricapsular fibrosis **Wijsman, et al., 1992**. In our study examination of encapsulated islet by light microscopy showed intact islets with no

evidence of cellular infiltration around the microcapsule wall up to 4 weeks after transplantation.

Our experiments demonstrate that agarose pancreatic islets microcapsule fulfill the properties required of an immunoisolatory device for pancreatic islet cell transplantation. These microcapsules are biocompatible, maintain viability and normal function of islets, prevent rejection, achieve normoglycemia in the recipient. Besides they can easily and safety peritoneally implanted. It should also be emphasized that, despite the ability of the microcapsules to protect islets from immune destruction, the use of adequate number of highly purified viable islets is critical to achieving successful xenografting.

In summery the results of these study indicates that it is time to proceed with the development of microcapsules suitable for transplantation of islet xenografts in larger animals and ultimately in humans. In addition such microcapsules may be useful in treating conditions caused by an impaired functioning or the loss of other secretory cells. Such as disorders of a growth factor or any other hormone. Also, they may provide means for relatively long-term storage and preservation of islet prior to transplantation.

References:

1. Avilla,J.; Tsujumura,T.; Oberhalzer,J.; Churctell,T.; Salihi,P.; Janes,A.M.; Lakey,J.R.T. (2003): Improvement of pancreatic islet isolation outcomes using Glutamine perfusion during isolation procedures. Cell Transplant., 12: 1230.
2. Barker, C.,Naji, A. and ilivas, W. (1980): Immunoogic problems in islet transplantation. Diabetes, 29:86.
3. De-Vos, P.; Hamel, A. F. and Kiewice, K.T. (2002): Consideration for successful transplantation of encapsulated pancreatic islets. Diabetologia, 45 (2): 159.
4. Fan, M. Y.; Lum, Z. P.; Fu,X.; Evesque, L.; Tai, L. T. and Sun, A. (1990): Reversal of diabetes in BB rats by transplantation of encapsulated pancreatic islets. Diabetes, 39: 519.
5. Getoh, M.; Maki, T. and Kiyoiami, T. (1985): An improved method for isolation of pancreatic islets. Transplantation, 40 (4): 437.
6. Hangmeichen,W.; Jones,M.; Metz,T.; Haque,T,.; Martoni,C.; Prokash,S., (2004) :Artificial cell microcapsules for oral delivary of live bacterial cells for Therapy : desigen, preparation ,and in vitro characterization. J.Pharma Sci.,7(3):315.

7. Hobbs, H. A.; Kindall, W. F.; Darrable, M. and Opera, E. C. (2001): Prevention of morphological changes in alginate microcapsules for islet xenotransplantation. J. Investing. med., 49 (6): 572.
8. Iwata, H.; Kobayashi, T.; Takagi, T.; Oka, T.; Yong, H.; Ammiya, H.; Tsuji, T. and Ito, F. (1994): Feasibility of agarose microbeads with xenogeneic islet as a bioartifecial pancreas. J. Biomed Mater. Res.,28(9):1003.
9. Iwata, H.; Takagi, T. and Amemiya, H. (1992): Agarose for bioartifecial pancreas. J. Biomed. Materials Res., 26: 967.
10. Jain, K.; Yang, H.; Caj, B.; Haque, B.; Harvitz, A. Diehi, G.; Miyata, T.; Smith, B. H.; Stenzel, K.; Suthanthiran, M. and Rabin, A. L. (1995): Retrievable replaceable macrocapsulated pancreatic islet xenografts. Transplantation, 59 (3): 319.
11. Jayne,L.; Williams,G.; Lindy,J.; Bimaral,E. (2007): Differentiation of transplanted microcapsulated fetal pancreatic cells. Transplantation, 15 (11):1440.
12. Kanti,J.; Albert,R.; Barry,S. (2007): Preparation of agarose Coatd, solid agarose collagen beard containing Secretary cells. United state patent RE 39452
13. Kobayashi,T,; Amastsu,Y.; Iwata,H.; Kin,T.; Kanehiro,H.; Saihoka,H.; Nagao,M.; Nakaajima,Y.;(2003): Indifinit Islet protection from autoimmune destruction in non obese diabetic mice by agarose microcapsules without immunosuppression. Transplantation,15,78(5)
14. Lacy, P. E. (1993): Status of islet cell transplantation. Diabetes Rev., 1 (1):76.
15. Lim, F. and Sun, A. (1980): Microcapsulated islet as bioartifecial endocrine pancreas. Science, 210-908.
16. Multen, Y., Maruyama, M.and Smith, C. (2000): Current progress and perspectives in immunoisolated islet transplantation. J. hepatobilary Pancreat.Surg.; 7 (4); 347.
17. Prokop, A. 2001: Bioartrficial pancreas; Materials, devices, function and limitation, Diab.Technol..Ther., 3 (3);431.
18. Scharp, D.; Mason, N. S. and Sparks, R. E. (1984): Islet immunoisolation, the use of hybrid artifecial organs to prevent islet tissue rejection. World J. Surg., 8: 221.
19. Schmeider, S., Feilen, PSlotry.,V., Kampfner, D., Prenss, S., Breger, S., Beyer, J. and Pommersheim, R. (1961): Multilayer capsules: a promising microcapsulation system for transplantation of pancreatic islets. Biomaterials 001;22(14)
20. Soon Shoing, P.; Feldman, E. and Nelson R. (1993): Long-term reversal of diabetes by the injection of immunoprotected islets. Proc. Nat. Acad. Sci. USA., 90: 5843.
21. Stieger.P.; Stadlbane, ; Schaffeliner,S.; Halwachs,G.; Lackner,C.; Havser.O.; Lbever,F.; Tscheliessnigg,K.; (2006): Cryopreservation of insuline producing microcapsulated in sodium sulphate.Transplant.proc., 38(9):

22. Sun, A., Fan, M., Lum, Z., Fu, X., Noon, J., (1989): Bioartificial pancreas: reversal of diabetes in BB rats by transplantation of encapsulated pancreatic islets . Artif Organs; 13: 567.

23. Takagi, T., Iwata, H., Kabayashi, K., Oka, T., Tsuji, T., and Ito, F.(1994): development of a microcapsule applicable to islet xenotransplantation. Transplant. Proc., 26(2): 801

24. Wijsman, j., Atkison, P., Mazaheri, R.,Garcia, B., Paul, T., Vose, J., Oshea, G., and shiller, C. (1992): Histological and immunopathological analysis of recovered encapsulated allogenic islets from diabetic BB/W rats. Transplantation.