Artificial Cell Microencapsulated Stem Cells in Regenerative Medicine, Tissue Engineering and Cell Therapy

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Abstract

Adult stem cells, especially isolated from bone marrow, have been extensively investigated in recent years. Studies focus on their multiple plasticity of transdifferentiating into various cell lineages and on their potential in cellular therapy in regenerative medicine. In many cases, there is the need for tissue engineering manipulation. Among the different approaches of stem cells tissue engineering, microencapsulation can immobilize stem cells to provide a favorable microenvironment for stem cells survival and functioning. Furthermore, microencapsulated stem cells are immunoisolated after transplantation. We show that one intraperitoneal injection of microencapsulated bone marrow stem cells can prolong the survival of liver failure rat models with 90% of the liver removed surgically. In addition to transdifferentiation, bone marrow stem cells can act as feeder cells. For example, when coencapsulated with hepatocytes, stem cells can increase the viability and function of the hepatocytes in vitro and in vivo.

Introduction

Stem cells from embryonic or adult sources have important roles in tissue and organ regeneration under physiological or pathophysiological states. Ethical controversy and availability of embryonic stem cells have directed attention toward the use of adult stem cells. Adult stem cells exist in various tissues including bone marrow, muscle, trabecular bone, dermis, adipose tissue, periosteum, pericyte, blood and synovial membrane.¹ Among these sources, the bone marrow, due to its easy accessibility and high yield, has been studied extensively in recent years. Adult stem cells are quiescent under normal physiological state, but when stimulated by specific factors, they can differentiate into the respective adult cell lineages, or can even transdifferentiate into other lineages. This plasticity of adult stem cells holds much promise for regenerative medicine, tissue engineering and cell therapy.

In this review, we briefly introduce use of artificial cell for cell encapsulation and also tissue engineering approaches using stem cells. We then illustrate this using the example of the encapsulation of bone marrow stem cells including in vitro and in vivo studies and therapeutic potentials in liver diseases.

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Cell Encapsulation

The principle, preparation and applications of artificial cells including applications for cell encapsulation are first reported in 1964.² One of the areas is its use for the encapsulation of living cells.²⁻⁴ Since then there has been much basic and clinical studies as well as industrial developments.⁵⁻⁷ Table 1 gives some examples of cells encapsulation and their applications.

Cell encapsulation provides a three dimensional environmental to enhance the cell-cell interactions, acts as niche-like microenvironment to maintain cells viability and function.^{3-5,7,29-31} Candidate cells for encapsulation includes a wide range of cell types, such as hepatocytes, islets and many others.⁵ Cells for encapsulation can be from autologous; allogeneic or xenogeneic source. Cells in microcapsules are immunoisolated from the host immune system, this renders the use of immunosuppressive unnecessary in allogeneic or xenogeneic transplantation. The original drop method for cell encapsulation²⁻⁴ has been extended to become the alginate poly-lysine alginate (APA) drop method⁶ that is now the most commonly used method for cell encapsulation.⁵

Adult Stem Cells and Their Plasticity

Adult stem cells are the immature tissue cells that are able to self-renew and differentiate into multiple cell lineages.³² These adult stem cells may not be as 'powerful' or diverse as embryonic stem cells, but obviously they have the advantages of ease of isolation and expansion, stable phenotype and compatibility with different delivery methods and formulations.³³ The conventional concept of adult stem cells being restricted to their own tissue has been challenged in the recent years, numerous reports indicated that adult stem cells can jump lineage barriers and differentiate into cells outside their own tissue, this process is called stem cell transdifferentiation.³⁴

Adult stem cells exist in various tissues, bone marrow is the most commonly used source of adult stem cells.³⁵ There are two main types of adult stem cells that reside in the bone marrow, the first category is hematopoietic stem cell (HSCs), which are the stem cells of each kind of blood cell, although some studies show the HSCs can transdifferentiate into other cell lineages, like hepatocytes, under either physiological or pathological conditions, this frequency is very low (10^{-4}), but they may provide cytokines and growth factors that promote other cells functions by paracrine mechanisms.³⁶ The second main category is mesenchymal stem cell (MSCs), which can differentiate into connective tissue cell types, such as adipocytes, chondrocytes, osteoblasts and hematopoiesis-supporting stroma cells. MSCs were first recognized by Friedenstein, who identified an adherent, fibroblast-like population that could regenerate rudiments of normal bone in vivo.³⁷⁻⁴⁰ Due to lack of definitive markers in vivo, MSCs are not clearly characterized in vitro by now.⁴¹

The MSCs pharmacological and therapeutic importance is mainly related to these four aspects: they can secrete biologically important molecules and factors; they can express specific receptors; they can be genetically manipulated; and they are susceptible to molecules that modify their natural behavior.⁴² MSCs can differentiate in vitro and in vivo

into nonmesodermal cellular lineages such as hepatocytes, muscle cells, neurons and others. Table 2 shows the plasticity of the bone marrow MSCs.

Bone marrow MSCs are commonly isolated using adherent culture method. Although MSCs are rare in bone marrow, representing 1 to 5 in 10,000 nucleated cells, they can expand in vitro to hundreds of millions of cells from bone marrow aspirate^{63,67–70} as the following procedure. Firstly, whole bone marrow cells suspension is overlaid onto a percoll gradient (1,073 g/ml) and centrifuged. Mononuclear cells layer is collected and pelleted, then inoculated in defined expansion medium and cultured in 5% CO₂, 37°C incubator. The non adherent cells (non MSCs) will be removed when changing medium. As the adherent cells reach 80% confluence, they are passaged. Cells harvested after the third passage are usually used for further experiments. MSCs yield and phenotype after expansion vary with the age and condition of the donor and with the harvesting techniques.^{63,71–73}

Tissue Engineering of Bone Marrow Stem Cells

MSCs can be injected or delivered directly to the recipients, but in many circumstances, these free stem cells would not function properly after transplantation, so certain kinds of tissue engineering approaches should be used to optimize the application of stem cells.

Tissue engineering implies the use of organ specific cells for seeding a scaffold in vitro⁷⁴ and is a multidisciplinary field combining biology and engineering along with clinical application to design, manufacture, modify, grow and maintain living tissue.

Table 3 shows some approaches of stem cells tissue engineering in recent years.

Microencapsulation is one of the main approaches for stem cells tissue engineering, the main purpose of microencapsulation of MSCs is to maintain their viability and their phenotype, or their transdifferentiation capacity. Human bone marrow MSCs encapsulated in alginate beads, cultured in serum-free medium with the addition of transforming growth factor (TGF) beta1, dexamethasone and ascorbate 2-phosphate, after two weeks in culture, cells were characterized with substance positive in Safranin-O staining and immunohistochemistry for collagen type II at the periphery of cells. The reverse transcriptase-polymerase chain reaction (RT-PCR) revealed the expression of COL2A1 and COL10A1 which are the typical marker of chondrocytes and hypertrophy chondrocytes, respectively,⁷⁶ this indicates bone marrow MSCs can undergo endochondral ossification to begin mineralization in the alginate beads.^{76,90} In another study with human adult MSCs, using a defined mixture of collagen type I and agarose polymers, MSCs were encapsulated in a three dimensional microenvironments beads, the collagen Type I was incorporated in the matrix in order to promote cell spreading and osteogenesis. It was found there was clear effect of the collagen component of the bead matrix on the MSCs morphology. When collagen content increased, the MSCs increased spread morphologies over time in culture.⁷⁷ The cell shape can affect cell function⁹¹ and differentiation.⁹² Interestingly, when MSCs were allowed to spread on a 2D substrate, they underwent differentiation to osteogenesis, while they were kept rounded they became adipocytes. Using the 3D encapsulation microenvironment can control over cell shape and therefore regulate stem cell

differentiation.⁷⁷ The microencapsulation system can guide MSCs differentiation and be used as cell delivery vehicle in tissue repair applications. The bead microenvironment technology also has other potential applications, such as cell-based diagnostics and therapeutic protein production via biotechnology.⁷⁷

Coencapsulation of Bone Marrow Stem Cells with Hepatocytes to Enhance Hepatocytes Viability and Function

Isolated primary hepatocytes can maintain viability and function in vitro for 1–2 weeks under conventional culture conditions.⁹³ Various approaches have been employed to increase the viability in vitro. One commonly used approach is to cocultivate hepatocytes with other types of cells, “feeder” cells. The most commonly used feeder cells are epithelial- and fibroblast like cells.^{94–96} In recent years, we have used bone marrow stem cells as feeder cells to enhance hepatocytes viability. The unfractionated rat bone marrow stem cells are coencapsulated with hepatocytes and cultured, this significantly enhances the hepatocytes viability than that of encapsulated hepatocytes alone.^{30,31} Figure 1 shows the protocol of using bone marrow stem cells as feeder cells in maintaining hepatocytes in coencapsulation.

In the in vivo studies, coencapsulated hepatocytes and bone marrow stem cells from wistar rats were transplanted into normal rats. The transplanted microcapsules were recovered at different time intervals and hepatocytes viability was determined. From week 7 after transplantation, the coencapsulated hepatocytes viability is significantly higher than that of hepatocytes encapsulation alone.³¹ The microcapsules could be recovered 4 months after transplantation, at this time the hepatocytes still maintained a high level of viability.³¹

We carried out further studies to investigate the maintenance of the hepatocytes specific liver function by coencapsulation with bone marrow stem cells. These coencapsulated cells were cultured in the medium supplemented with different concentration of ammonium chloride solution to study the conversion of ammonium into urea by the hepatocytes. During the culture period, the ammonium chloride concentration was much lower from week 2 in the coencapsulation group than in the hepatocytes encapsulation group.¹⁴ In the in vivo function test, coencapsulated hepatocytes and bone marrow stem cells were transplanted into Gunn rats. Gunn rats are allogeneic of wistar rats with hereditary deficiency in the enzyme, uridine diphosphate glucuronyltransferase (UDPGT). This enzyme is necessary for the conjugation of bilirubin to bilirubin diglucuronide, so Gunn rats have lifelong hyperbilirubinemia. After the coencapsulated hepatocytes and bone marrow cells transplantation, the Gunn rat blood bilirubin levels decreased significantly more than that in the single hepatocytes encapsulation transplantation. The control group of encapsulated bone marrow cells transplantation did not show any decrease in systemic blood bilirubin level.¹⁴

Hepatocyte transplantation are studied for the treatment of various liver diseases and thought to be an effective alternative to the orthotopic liver transplantation. The implantation of coencapsulated hepatocytes and bone marrow stem cells transplantation promises to be a new approach of promoting hepatocytes function in hepatocytes transplantation therapy.

The mechanism of longer term hepatocytes phenotype and specific function maintenance in the coencapsulation experiments in vitro and in vivo has not been elucidated yet. The possible mechanism may involve two aspects, firstly, the cell-cell direct interactions between hepatocytes and bone marrow stem cells, secondly, the growth factors or cytokines produced by bone marrow stem cells. Bone marrow stem cells have various types of cells including stromal cells and blood cells as well as their respective progenitor cells or stem cells. Whether the stromal cell types or the blood cell types or both play key roles needs to be further investigated.³⁰

Therapeutic Effect of Encapsulated Bone Marrow Stem Cells on the Liver Failure Model

In 1980, Makowka et al intraperitoneally implanted syngeneic bone marrow cells into rat liver failure models induced by D-galactosamine, they found that the liver failure models survival was prolonged.⁹⁷ This early study indicated a new therapeutic potential of bone marrow cells for the liver failure, but no further detailed studies at that time on therapeutic mechanism. Injection of bone marrow cells obtained from another human or from animal sources would require immunosuppression to prevent rejection. We have therefore studied the use of encapsulating adult stem cells for the therapy of liver diseases.^{14,15} Rat bone marrow stem cells were encapsulated and then transplanted intraperitoneally into 90% hepatectomy induced acute liver failure rat model.¹⁵ In the two weeks follow up, survival of the encapsulated bone marrow stem cells transplantation group was significantly prolonged comparable to the hepatocytes transplantation group (Fig. 2H). However, unlike Makowka's findings,⁹⁷ we found that free bone marrow cells transplantation did not prolonged survival in our study.¹⁵ We carried out histology and immunocytochemistry study of the recovered encapsulated bone marrow cells. Some cells showed evidence of glycogen synthesis as indicated by positive PAS staining (Fig. 2C). Some of the cells express albumin, cytokeratin 8, cytokeratin 18 and alpha-fetoprotein (Fig. 2D to G) that are typical markers of hepatocytes.¹⁵

The above findings indicate the transdifferentiation of the bone marrow stem cells to the hepatocyte-like cells in the 90% liver failure model. Transdifferentiation of the bone marrow stem cells is important to maintain the liver function and the life of the hepatectomized rats. However, they have another mechanism that is responsible for preventing the death of these rats in the first week. For most rat model, death occurred within 3 days post surgery in the hepatectomy control group, but the transdifferentiation needs time and thus can not provide immediate liver support at the beginning. The possible mechanism might be that the growth factors or cytokines secreted from the bone marrow stem cells have effects on increasing the regeneration of remnant liver and also these growth factors and cytokines alleviate apoptosis of the remnant liver cells. Bone marrow MSCs can express HGF and HGF receptor/c-met.^{98–101} In the microencapsulated MSCs transplantation in the 90% hepatectomy rat models, the plasma HGF level is significantly increased than in the control groups in the first three days post transplantation.¹⁵ HGF is a potent mitogen for hepatocytes in normal liver. In vitro and in vivo studies show HGF promotes DNA and protein synthesis during liver regeneration after hepatectomy.¹⁰² In addition, extensive hepatectomy can cause increased

remnant liver cells apoptosis,^{103,104} this is the main cause of mortality in extensive hepatectomy,^{104–106} HGF can prevent hepatocytes apoptosis after liver injury, such as partial hepatectomy.¹⁰⁷ HGF have effects on the MSCs proliferation, migration and differentiation.^{15,108} HGF can induce differentiation of bone marrow cells into a hepatocyte lineage in vitro.¹⁰⁹ The later transdifferentiation into hepatocyte like cells is the second mechanism that is important in the recovery of the liver function after the acute stage. Figure 3 shows the mechanism schematic of therapeutic effect of microencapsulated bone marrow stem cells on 90% hepatectomized liver failure models.

Conclusions and Future Studies

Encapsulation of stem cells has four main implications, to provide a novel stem cells immobilization and delivery approach; to provide favorable microenvironment for stem cells survival and function; to be recovered easily for tracing and studying stem cells after transplantation; and to prevent stem cells from immunorejection after transplantation.

Cellular microencapsulation has been proved to be an effective tissue engineering approach in the past years. Adult stem cells have wide plasticity and are new source of cellular transplantation.

Our preliminary results show the promising application of stem cells encapsulation, however some questions should be answered to improve this approach before its full clinical application. These include questions both from encapsulation technique as well as the stem cells research. What is the optimal encapsulation procedure for the stem cells, what is the stem cells density in encapsulation and what is the optimal passages of stem cells being encapsulated. If using xenogeneic stem cells, there should be no pathological agents transmitted during transplantation. Another issue is to develop high biocompatible polymeric membrane, with sufficient durability and appropriate permeability. Purification of the alginate used in alginate-PLL-alginate(APA) capsular membrane not only reduces the total amount of impurities, but also avoids antibody response when microcapsules are transplanted. More studies should focus on the molecular and cellular mechanism of microencapsulated bone marrow stem cells therapy.

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Figure 1.

The schematic protocol of the use of bone marrow stem cells to enhance hepatocytes viability and specific function. The bone marrow stem cells and hepatocytes were coencapsulated, this enhanced the hepatocytes viability both in culture and in transplantation into rats. This coencapsulation also enhanced hepatocytes' specific function of ammonium conversion in culture and of lowering blood bilirubin after implantation into hyperbilirubinemia Gunn rats.

Figure 2.

Histology and survival study of microencapsulated bone marrow stem cells transplantation into 90% hepatectomized rat model. A) microcapsules with bone marrow cells before transplantation. Original magnification $\times 40$. B) HE staining of microcapsules recovered 2 weeks post transplantation. Original magnification $\times 400$. C) PAS cytochemical glycogen stain for the recovered microencapsulated bone marrow stem cells. Positive cells showed pink staining in cytoplasm. Original magnification $\times 1000$. D to G) Immunocytochemistry staining of the recovered microencapsulated bone marrow 2 week post transplantation, for albumin D), cytokeratin 8 E), cytokeratin 18 F), alpha-fetoprotein G). Dark brown cells indicate the positive cells. Original magnification $\times 1000$. H) Kaplan-meier survival curve, at week 2 post transplantation, rats transplanted with microencapsulated bone marrow stem cells (encap BM) survived longer than the partial hepatectomy (PH) group, empty microcapsules (emp cap) group and the free bone marrow cells (free BM) group, comparable to the sham operation (sham OP) group, $n = 6$ in each group. (Reprinted with permission from John Wiley and Sons, Inc. Liu ZC, Chang TM. Transdifferentiation of bioencapsulated bone marrow cells into hepatocyte-like cells in the 90% hepatectomized rat model. *Liver Transpl* 2006; 12:566–572.)

Figure 3.

Mechanism of therapeutic effect of encapsulated bone marrow stem cells transplanted into 90% hepatectomized rats. The encapsulated bone marrow cells are immunoisolated from immunorejection by antibodies and inflammatory cells, whereas the nutrients and oxygen can pass freely through the capsular membrane, growth factors and cytokines secreted by the stem cells can diffuse out the microcapsules into peritoneal cavity, then were drained into portal venous system into the liver to enhance the liver regeneration.

Table 1

Some examples of cell encapsulation and their applications. The microcapsules can contain various cells for different therapeutic purposes

Liver failure, congenital liver diseases ^{3,4,8-15}
Diabetes ^{3-6,16-19}
Renal failure ²⁰
Secrete mGH for Dwarfism ²¹
Factor IX for haemophilia B ²²
Hybridomas antibody production ⁷
Secrete erythropoietin for anaemia ²³
β -endorphi for pain killing ²⁴
Tumor suppression ²⁵
Cholesterol removing ²⁶
Huntington's disease ²⁷
Secrete hNGF for parkinsonism ²⁸

Table 2

Plasticity of bone marrow MSCs

Plasticity	References
Liver	43–45
Neuron	46–49
Heart	50–53
Renal	54
Adipocyte	55,56
Astrocyte	49
Chondrocyte	55,57–59
Mesangial cell	60
Muscle	61,62
Osteoblast	55,63–65
synovial joint	33,66

Table 3**Bone marrow stem cells tissue engineering approaches**

Approaches	Applications	Study	Source of Stem Cells
Microencapsulation ^{14,15,75-77}	Liver diseases, Induction of chondrogenesis,	In vivo, in vitro	Rat, human
Embedded in atelocollagen ⁷⁸	Degenerative disc disease	In vivo	Rabbit
Hydroxyapatite (HA) ceramics ⁷⁹	Bone tumor therapy	In vivo	Human
Three-dimensional poly-lactic-glycolic acid (PLGA) scaffolds ⁸⁰	Induction of chondrogenesis, Engineered cartilage	In vitro	Rabbit
Porous silk scaffold ⁸¹	Cartilage tissue engineering	In vitro	Human
Alginate and collagen hydrogels ⁸²	Tendon repair	In vivo	Rabbit
Silk with covalently bound RGD sequences ⁸³	Engineering bone-like tissue	In vitro	Human
Collagenous matrix ⁸⁴	Cartilage defect repair	In vitro	Human
Polyglycolic acid (PGA) nonwoven mesh ⁸⁵	Chondrogenic differentiation	In vivo	Rabbit
Ascorbic acid-functionalized poly(methyl methacrylate) ⁸⁶	Osteogenic differentiation	In vitro	Human
Micropatterned polydimethylsiloxane (PDMS) substrates ⁸⁷	Connective tissue repair	In vitro	Human
3D nanofibrous scaffold ^{88,89}	Cartilage tissue engineering	In vitro	Human