Submitted: 01/13/2021

Research Article

[1]Department of Bioengineering, McGill University, Montreal, QC, Canada

Keywords

Mesenchymal stem cell; Liver disease; Cell delivery, Critical literature review

Email Correspondence

zhiyue.zhu@mail.mcgill.ca

Zhiyue Zhu[1]

Comparative analysis of delivery methods for stem cell therapy in liver diseases

Abstract

Background: Mesenchymal stem cell transplantation is an emerging therapy for treating acute and chronic liver diseases as an alternative for patients who are not transplant candidates. The potential of this treatment depends on its therapeutic efficiency and safety, which have been investigated and evaluated in both preclinical and clinical settings. However, there are still some risks associated with the delivery methods, such as low long-term retention rate and the possibility of arousing cancer or rejection.

Methods: During the review process, previous papers mentioning "stem cell treatment" and "liver diseases" were searched. We described different up-to-date approaches for stem cells from various origins to be delivered to the liver and compared respectively their pros and cons for clinical applications. We also proposed several potential techniques for future studies.

Summary: An efficient and safe stem cell delivery could be enabled via Alginate-Polylysine-Alginate (APA) microencapsulation, lipid-conjugated coating or the use of nanoparticles. Their efficacy will be improved through tissue engineering and microrobots as the delivery is sustained and targeted with fewer rejection responses.

Introduction

Acute and chronic liver injury, such as that arising from viral infection, alcohol abuse, metabolic disorders and toxins, can lead to liver cirrhosis or failure (1). Liver transplantation has been deemed the gold standard therapy for liver failure. However, the procedure still carries considerable risks and limitations. Donor organ supply shortage, graft failure, and severe transplant rejections make this an impractical option for many patients (2, 3).

Alternative approaches other than organ transplant have been pursued for decades before the advent of liver stem cells therapy. Regenerative therapy with granulocyte colony-stimulating factor (G-CSF) has become a lucrative option for improving transplant-free short-term survival by stimulating the proliferation and migration of bone marrow stem cells (4, 5). However, most clinical trials on G-CSF have been conducted without adequate knowledge on the pathway that promotes quantity and durable restoration of existing healthy hepatocytes or regeneration and formation of new liver cells (4). Thus, the potential risks regarding the utility of G-CSF in treating patients with liver diseases remain unclear. On the other hand, prosperous studies on transplantation of engineered mesenchymal stem cells have shown promising results in preclinical and clinical experiments, which could offer a potentially unlimited source of cells for hepatocytes (6). MSC therapy for liver has been shown to be effective via their immunomodulation, differentiation, and anti-fibrosis properties. Though, it is noticeable that stem cell therapy in liver failure is not yet standardized, as every laboratory has their own methods, in terms of the type of cells and delivery mechanisms.

In this review, we highlight the current findings from peer-reviewed journals on various stem cell delivery methods, which have ensured an efficient stem cell accumulation in liver for clinical treatments according to previous studies (1-3, 6). Some possible modifications could further increase the safety of liver pathology therapy by lowering immune rejection or inflammation.

Available Approaches

We summarized and examined currently available approaches for liver stem cell therapy published in precious academic journals.

Artificial Cell Microencapsulation

In 1957, Chang invented artificial cells which are ultra-thin polymer membrane microcapsules that can encapsulate various materials (7). The team showed that microencapsulation can immobilize stem cells to provide a favorable microenvironment for stem cells survival and functioning, as well as make the stem cells immuno-isolated after transplantation (8-10). Bone marrow stem cells (BMCs) were flushed out from the femurs of Wistar rats; hepatocytes were isolated and collected from the rat's liver (8-10). Alginate-Polylysine-Alginate (APA) encapsulation method was used to encapsulate the BMCs and hepatocytes (9, 10). This method led to cross-linking of anionic alginate with cationic poly-L-lysine (PLL) which allows a more controlled pore size of the microcapsules (11). This enables the controlled release of cell secretions from the microcapsule.

The microbeads of microscopic dimensions (less than 2 mm in diameter) can provide a high surface-to-volume ratio, which allows for good mass transfer of oxygen and nutrients as the theoretical diffusion across a membrane is proportional to the total surface area and inversely proportional to its membrane thickness (12). This ensures cell viability, while also acting to protect the cells from immune-rejection (7). In particular, hepatocytes have been shown to maintain 65% viability at Day 28 when co-cultured or co-encapsulated with bone marrow cells compared to 18% viability at Day 28 for hepatocyte in single encapsulation (8). Chang et al. and Zhang et al. proposed that the preservation of hepatocyte functions seemed to be dependent on cell-cell contact interactions. Transplanted stem cells will produce cytokine and secrete several potentially beneficial growth factors, such as the hepatocyte growth factor (HGF). HGF is an important factor in liver regeneration and stimulating the transdifferentation of BMCs into hepatocytes (13, 14). Hepatic stimulatory substance (HSS) released from free or microencapsulated hepatocytes can pass through the APA membrane to stimulate the remnant liver to regenerate (10, 15, 16). The most promising application of this technique is in acute liver failure such as hepatic coma, as it can provide immediate liver support (17). These bioencapulated BMCs could be retained in the peritoneal cavity such that secreted HGF is drained into the portal circulation to reach the liver, compared to free BMCs which will be rapidly removed from the peritoneal cavity; this may contribute to the higher efficiency of microencapsuled BMCs than the free ones (10).

The application of stem cell encapsulation is promising from these preliminary results. However, some challenges stop the technology to deliver its promise. The first contact of the capsule with the host could elicit

host immune response and cells in capsules might produce proteins that can provoke inflammatory responses in immune cells (7, 18). Research shows that this is because the thin and poorly formed membrane in the standard method allowed cells to be protruded and entrapped within the micro-capsular membrane matrix (19). These cells might activate rejection from body and degradation enzymes might be released from activated leukocytes of lysing cells, which can further accelerate the erosion of micro-capsular membrane (19, 20). These problems have now been solved by the use of a novel two step method of preparation resulting in membrane that does not allow the entrapment or protrusion of cells across the membrane (20).

Lipid-conjugated Heparin Coating

For acute liver failure (ALF) arising from acetaminophen (APAP) overdose, Hwang et al. investigated on the therapeutic effects of human adipose-derived stem cells (hADSCs), which could recover the metabolic rates of the liver cells to over 60% after one-day treatment (21). An enhanced delivery and longer retention of the cells was enabled by applying coating of lipid-conjugated heparin; the coating of lipid-conjugated heparin on hADSCs also enhanced the anti-inflammatory effects on the damaged liver to increase the recovery efficiency (21-23).

Many ALF patients with APAP overdose develop infectious complications with inflammatory response that can progress to multi-organ failure (24). hADSCs are a promising candidate to treat APAP liver injury as their secretomes have therapeutic effects by enhancing hepatocyte regeneration and inhibiting liver stress and inflammatory signaling (21-23). To increase the delivery and engraftment efficiency of the administered cells to the target tissue, cell surface engineering that decorates a target-oriented ligand on the cell surface, has been employed (25-27). It was reported by Tae et al. that the cell surface of hADSCs can be engineered with lipid-conjugated heparin (28). Heparin is an anionic glycosaminoglycan which has many distinct bioactivities including anticoagulant activity, immune suppression activity, and strong binding affinity hADSCs (29). Therefore, the interaction between heparin and cell membrane will contribute to the coating of it across the cell surface. However, free heparin itself did not induce stable coating on hADSCs (28). Thus, lipid-conjugation to heparin was necessary to induce the effective presence of heparin on the cell membrane. Sinusoid capillary structures within the liver are abundant with heparin receptors Stabilin on cell membrane, and this increases the amount of trapped heparin-coated ADSCs on the capillary wall, which might provide more chances for the cells to migrate into the parenchyma parts of liver from bloodstream (30-32).

For the synthesis of lipid-conjugated heparin, carboxyl-group activated heparin dissolved in deionized water (DIW) was mixed with the 1, 2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) lipid solution at 65 °C, and the mixture was then incubated for 20 h. Heparin was modified using its carboxylate group to ensure sufficient contact of the heparin (hydrophilic with a highly negative charge from sulfate groups) with the hydrophobic lipid (33). The obtained lipid-conjugated heparin was then added into human ADSCs (with 100% confluency) seeded one day prior to heparin coating at 250 μg/ml in 1% (v/v) fetal bovine serum (FBS). The harvested heparin-coated cells were intravenously injected to mice through the tail vein. Distinct changes in biodistribution by heparin coating could be seen with an increased accumulation of ADSCs in the liver (1.8 times more) and a reduced accumulation of the cells in the lung (59% less) compared to the control group with uncoated ADSCs (28). A 2.4-fold increase in heparin-coated ADSCs was also observed in the spleen of the mice. As spleen has been used as alternative cell transplantation site to treat acute and chronic liver diseases, this accumulation of ADSCs in spleen could also provide better therapeutic effects (28).

Therefore, lipid-conjugated heparin coating on hADSCs might function to enhance the therapeutic effect of intravenously administered stem cells on liver diseases as cytokines or growth factors are secreted from the delivered hADSCs. This helps to recover the liver injury by improving liver tissue regeneration (21). The coating of lipid-conjugated heparin on hADSCs also enhanced the anti-inflammatory effects on the damaged liver (34). Despite the advantages of heparin-coated ADSCs to treat ALF, their benefits might not be applied successfully to other chronic liver diseases due to the relatively low retention rate (50% remaining after four days) of the stem cells (21). This might result from the lack of supporting matrix after hADSCs are trapped in liver (35). In addition, ALF might cause high oxidative stress (36), leading to an alteration or loss of stem cell functions (37). Unfortunately, heparin coating might not be efficient enough for antioxidant effects, which probably requires genetic modification of the stem cells such as overexpressing miR-210 (37, 38). From the work of Xu et al., MSCs transfected with agomiR-210 were exposed to H2O2 in vitro, which induces MSC apoptosis via oxidative stress, but miR-210 overexpression could attenuate the c-Met activity repression induced by H2O2, alleviate accumulation of ROS, as well as decrease the apoptosis rate of MSCs in H2O2 (41.52% less than non-modified MSCs (37). This indicated their antioxidant effects. However, in vivo research has not been continued, and thus the antioxidant effects of miR-210 mutations in liver disease environments may require further investigation.

Nanoparticles

Nanoparticles are nanosized structures in which at least one of its phases has a dimension ranging from 1 to 100 nm. Magnetic nanoparticles with paramagnetic characterization because of the unpaired electrons have been used in stem cell therapy for stem cell homing and tracking in the attempts of treating liver diseases (39).

MSCs homing is the delivery process of the cells to the site of injury, but its mechanism is not totally understood yet (40). There are different factors that are thought to affect this process, such as the expression of homing regulating molecules C-X-C chemokine receptor 4 (CXCR4) and Stromal cell-derived factor 1 (SDF1) (41). Yu Meng and his team showed that MSCs labelled with super-paramagnetic iron oxide nanoparticles (SPIONs) lead to efficient homing in vivo when exposed to an external magnetic field (EMF) generated by permanent magnets (42). In Yu Meng's experiment, Wharton's Jelly-derived MSCs (WJ-MSCs) were transfected using SPIONs with a final concentration of 25 μg/mL, and the labelled MSCs were hypodermically injected (hypodermis) in mice. By applying an external magnetic field (0.5 T, 1.5 cm away from the tissue, 6h/day), the direct movement of magnetized cells to the target tissues was accelerated with a significant reduction in displacement, area and signal-to-noise ratio (SNR) parameters by more than 50% (42). Yun et al. suggested that these SPIONs would not affect MSCs properties such as viability, proliferation and differentiation in vitro and in vivo in short-term experiments. Via the internalization of rhodamine B (IRBs) in SPIONs, it also showed an increase in CXCR4 as the nanoparticles stimulated the signaling receptor of CXCR4-SDF1 axis, which is essential in MSCs' homing (43).

In vivo stem cell tracking and visualization of the transplanted stem cell is an essential means for monitoring their tissue localization (39). MRI has the utility to track the homing and trajectory of SPION-labelled MSCs in the liver (44). This can help to predict the therapeutic potential of certain treatment since the success of cell therapy will depend on the local availability of stem cells for tissue. The combination of iron oxide nanoparticle along with MRI provides a means to deliver cells and immediately verify whether the cells have indeed grafted within the target organ (45).

Beyond the application of magnetic nanoparticles for MSC homing and tracking, there is also administration of other nanoparticles to increase the efficiency of stem cell delivery by lowering the possible inflammation. IL-1-induced intracellular signaling pathway could be blocked by IL-1Ra, and this will exhibit an anti-inflammatory effect. As shown previously, MSCs are promising in treating ALF by transdifferentiating into hepatocytes. Consequently, the co-administration of IL-1Ra with MSC transplantation has great potential of effectively treating ALF, as well as maintaining MSCs' functions against oxidative stress (36, 46). However, due to the high cost and short biological half-life of IL-1Ra, its clinical application is seriously restricted (47). Xiao et al. proposed the use of IL-1Ra loaded in lactosylated chitosan nanoparticles instead of using large dose of IL-1Ra directly. FITC-1L-1Ra lactosylated chitosan nanoparticles were prepared by dissolving lactose acyl chitosan in diluted acetic acid solution (0.3 mg FITC and a small amount of polyethylene oxide [PEO]) (46). Lactose acyl group on chitosan surface could specifically target the nanoparticles to hepato-

cytes, and this could be observed as concentration of IL-1Ra in liver tissue (147.15 pg/ml) was significantly higher than that in serum (25.65 ± 9.59 pg/ml), kidney (42.65 ± 9.79 pg/ml), and heart (46.58 ± 10.18 pg/ml). IL-1Ra will be released from biocompatible and biodegradable chitosan through self-diffusion, therefore, the time of drug release and effect will be significantly extended (48). IL-1Ra chitosan nanoparticles thus can not only lower the cost using IL-1Ra via controlled drug release but also show significant liver targeting ability via employing lactose acyl on chitosan surface. Combined therapy with IL-1Ra chitosan nanoparticles and MCS transplantation exhibited great synergistic effects for ALF treatment through suppression of inflammation for a period of time with 200% extension, and this can significantly improve the survival rate of transplanted cells. In addition, this therapeutic strategy can promote hepatocyte proliferation (46).

The implementation of nanoparticles in stem cell therapy has a great effect in enhancing the efficiency of stem cell-based liver diseases treatment, however, the relatively short duration of investigation might not be sufficient to conclude the long-term effects of magnetic field and nanoparticles on the viability and differentiation potential of the labelled MSCs (42). Nevertheless, experimental settings as the concentration of nanoparticles and the strength of the applied EMF are not standardized, which requires further research to allow the optical therapy effects.

Comparison and Summary

The basic principle, method, advantages and disadvantages of each aforementioned approach is compared and summarized in **Table 1**.

efficiency and safety, as well as solve some risks associated with using stem cell treatments. For example, tissue engineering and microrobots allowed a more sustained and targeted cell delivery to liver.

Tissue Engineering

Tissue engineering has been a promising technique to promote stem cell homing by providing a bioactive material scaffold and include some necessary biochemical signals within the structure. Hydrogels could serve the purpose of extracellular matrices (ECMs) which assist in cell adhesion, proliferation, and differentiation.

Das et al. developed a 3D bioprinting tissue analog using silk fibroin-gelatin (SF-G) bioink encapsulating tissue-derived mesenchymal progenitor cells (50). The hydrophobic interaction of silk fibroin macromolecules could be altered by tyrosinase and sonication, which changes the crosslinking of the hydrogel. The effect of optimized rheology and other printing parameters were assessed to achieve maximum cell viability and multilineage differentiation of the encapsulated hMSCs. Lee et al. and his team showed that by including hepatocytes within the bioink, the survivability and functionality of HCs could also help with the transdifferentiation of stem cells (51).

Xu et al. transplanted mouse ADSC-seeded and BMSC-seeded regenerated silk firoin (RSF) electrospun matrix on the injured liver surface of a mice (35). RSF scaffolds were excellent in biological effect and biocompatibility; the degradation products of RSF are amino acids that could be conducive to cell growth, which make RSF an effective biomaterial for liver regenera-

Table 1 Liver diseases that can be treated with stem cell therapy and the applied delivery technique

Method	Role	Disease	Advantages	Disadvantages	Author
APA membrane microencapsulation	APA encapsulation for BMCs and hepatocytes to maintain a longer viability of hepatocytes. BMCs secrete HGF for liver regeneration and transdifferentiate into hepatocytes within the capsule	Acute liver failure Ex: Hepatic coma	a. Provide a favorable environment for stem cells survival and function b. Recovered easily c. Prevent immuno-rejection	Applicable for liver regeneration especially for short-term uses	Chang et al. (7-10, 12, 15-17, 19, 20, 49)
Lipid-conjugated heparin coating	Lipid-conjugated heparin can interact with hACSCs' cell membrane and be recognized by liver capillary receptor to ensure efficient stem cell delivery	APAP-induced acute liver failure	a. Could be recognized by Stabilin receptors on the liver capillary to ensure a higher distribution of stem cells in liver b. Heparin also has anti-inflammatory effects, which enhance the liver disease treatment	a. Low retention rate for long-term treatment due to lack of support matrix b. Cannot withstand oxidative stress	Hwang et al. (21, 33) Kim et al. (28, 29)
Nanoparticles	Magnetic SPIONs used for stem cell homing by applying external magnetic field and tracking by using MRI	Diverse liver diseases Ex: liver fibrosis, liver cirrhosis and liver cancer	a. SPIONs with EMF can enhance homing b. MRI and nanoparticle can monitor stem cell trajectory to target	a. Unknown long-term effects of magnetic fields and SPIONs on MSCs b. Design setting not optimized	Meng et al. (42) Bos et al. (45)
	IL-1Ra chitosan nanoparticles used to exhibit extra anti-inflammatory effects and protect MSCs from oxidative stress	Acute liver failure	a. Cost efficient for controlled release of IL-1Ra, which also helps with ALF treatment b. Lactosylated chitosan could specifically target the NPs to target	Unsure long-term effects of IL-1Ra and chitosan on MSCs' viability and functions	Xiao et al. (46)

Perspectives

In this section, we propose some intriguing techniques that have the potential to be incorporated with stem cell therapy to improve the delivery

tion. MSCs also exhibited good interaction with RSF and differentiated to hepatocyte-like cells in vitro. The transplanted stem cells could remain in tissue for up to 30 days and have been proved to lead to an improved recovery of the liver (35). This technique could enhance the retention rate of stem cells at the target for long-term treatments, but the requirement of an

open surgery renders it less attractive than minimally invasive treatment.

Microrobots

Magnetically actuated microrobots could facilitate cell/drug delivery in fluidic environments; due to their small size and wireless control, microrobots undergo littler distortion or attenuation in the body. Thus, they have several advantages as medical treatments, such as reduced pain, risk of infection, and trauma (52). If stem cells are transported via microrobots, cell function could be preserved by the devices; these cell-loaded microrobots can be guided toward some hard-to-reach tissue or human cavities, making various medical treatments possible (53). Therefore, fewer cells are needed, which lower the possible host immune response due to cytokine production.

Jeon et al. developed a biocompatible porous 3D microrobots and assessed their feasibility for delivery of stem cells in 3D culture using magnetic locomotion (52). With different shape designs, the microrobots exhibited different motions upon application of a rotating magnetic field: for spherical microrobot, it would be a rolling motion; for a helical microrobot, it would be a corkscrew motion. These designs showed higher propulsion efficiencies for a precise targeting of the transplanted stem cells. The porosity of the 3D scaffold could be customized by laser lithography; pore size of 15 μm was chosen to fit the size of stem cell, and nickel and titanium layers were deposited to increase the model's biocompatibility. Human stem cells could then be cultured within the microrobots. By applying an external magnetic field (EMF), the devices have shown precise movements along the direction of the applied magnetic field gradient. Real-time visualization and localization are required for a more accurate in vivo manipulation of the microrobots. In addition, the recycle of microrobots is another problem as this might arouse rejection response from body.

To reduce the potential immune activation and thrombi formation, degradable materials PEG diacrylate (PEGDA) were used to fabricate the microrobots (54). The degradation and swelling of the microrobot will help the cells to passively detach from the device surface. PA imaging was used to visualize the microrobots with a penetration depth reaching 2 cm to achieve a precise delivery of therapeutic cells. The penetration depth may not be optimal for stem cell transplantation in deep tissue; therefore, alternative tracking systems should be investigated. Similar to the use of magnetic nanoparticles, the effects of EMF on the functions of these stem cells need further research.

Comparison and Summary

The basic principle, advantages and disadvantages of each promising technique is compared and summarized in **Table 2**.

Conclusion

Many studies have been conducted to ensure an efficient and safe delivery of stem cells, including microencapsulation with APA membrane, lipid-conjugated heparin coating and administration of nanoparticles. They have shown promising results to treat both acute and chronic liver diseases. However, several concerns remain, regarding the poor long-term cell retention rate, and the risk of host rejection. We proposed several techniques to enhance the delivery efficacy. By including a 3D matrix, we could enhance cell homing and support long-term treatment. Tracking and targeted delivery could be achieved with degradable magnetic microrobots when applying external magnetic fields and MRI.

MSCs delivery should be easy to perform, less invasive, with limited side effects and high stem cell survival. It should also be capable of having a live tracking of the delivery process. Among the various potential modalities for stem cell delivery in liver disease as discussed in this review, each approach has its own peculiarities, so the choice of the modality should be based on the clinical settings. When the corresponding issues for those methods have been addressed, an optimal approach integrating stem cell biology, gene therapy, tissue engineering, and transplant medical devices can be pursued. This will lead to better treatment for liver diseases like ACLF, HBV reactivation with immunosuppression and alcoholic hepatitis, especially for patients who are in liver failure but are not transplant candidates.

References

1. Sharma A, Nagalli S. Chronic Liver Disease. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2020, StatPearls Publishing LLC.; 2020.

2. Burki TK. Liver disease in Canada: the scale of the problem. The Lancet Gastroenterology & Hepatology. 2020;5(2):114-5.

3. Liu C-L, Soong R-S, Lee W-C, Chen D-H, Hsu S-H. A predictive model for acute allograft rejection of liver transplantation. Expert Systems with Applications. 2018;94:228-36.

4. Philips CA, Augustine P, Ahamed R, Rajesh S, George T, Valiathan GC, et al. Role of Granulocyte Colony-stimulating Factor Therapy in Cirrhosis, 'Inside Any Deep Asking Is the Answering'. J Clin Transl Hepatol. 2019;7(4):371-83.

5. Meng F, Francis H, Glaser S, Han Y, DeMorrow S, Stokes A, et al. Role of stem cell factor and granulocyte colony-stimulating factor in remodeling during liver regeneration. Hepatology. 2012;55(1):209-21.

6. Dahlke MH, Popp FC, Larsen S, Schlitt HJ, Rasko JE. Stem cell therapy of the liver--fusion or fiction? Liver Transpl. 2004;10(4):471-9.

Table 2 Promising techniques to enhance stem cells delivery efficiency and safety

Method	Role	Advantages	Disadvantages	Author
Tissue engineering	Promote stem cell homing by providing a scaffold and include some biochemical signals Generate a matrix to be transplanted in tissue where stems cells are seeded	a. Increase differentiation efficiency with the mechanical support from the biomaterials and inclusion of certain growth factors b. Increase stem cell retention rate for long-term treatment	May require an open surgery to transplant the scaffold or tissue	Das et al. (50) Xu et al. (35)
Microrobots	Magnetic SPIONs used for stem cell homing by applying external magnetic field and tracking by using MRI	a. Ensure a target transport of stem cells with externally applied magnetic fields b. Could monitor the cell movements by MRI and other visualization techniques c. Lower the number of stem cells required to reach therapeutic effects, and this decreases the potential of host rejection response	The effects of the microrobots materials and applied external fields on stem cell viability are unknown	Jeon et al. (52) Wei et al. (54)

7. Chang TM. Therapeutic applications of polymeric artificial cells. Nat Rev Drug Discov. 2005;4(3):221-35.

8. Liu Z, Chang TM. Effects of bone marrow cells on hepatocytes: when co-cultured or co-encapsulated together. Artif Cells Blood Substit Immobil Biotechnol. 2000;28(4):365-74.

9. Wong H, Chang TM. Bioartificial liver: implanted artificial cells microencapsulated living hepatocytes increases survival of liver failure rats. Int J Artif Organs. 1986;9(5):335-6.

10. Liu ZC, Chang TMS. Transdifferentiation of bioencapsulated bone marrow cells into hepatocyte-like cells in the 90% hepatectomized rat model. Liver Transplantation. 2006;12(4):566-72.

11. Orive G, Tam SK, Pedraz JL, Hallé JP. Biocompatibility of alginate-poly-L-lysine microcapsules for cell therapy. Biomaterials. 2006;27(20):3691-700.

12. Chang TMS. ARTIFICIAL CELL evolves into nanomedicine, biotherapeutics, blood substitutes, drug delivery, enzyme/gene therapy, cancer therapy, cell/stem cell therapy, nanoparticles, liposomes, bioencapsulation, replicating synthetic cells, cell encapsulation/scaffold, biosorbent/immunosorbent haemoperfusion/plasmapheresis, regenerative medicine, encapsulated microbe, nanobiotechnology, nanotechnology. Artificial Cells, Nanomedicine, and Biotechnology. 2019;47(1):997-1013.

13. Zhang Y, Chen X-M, Sun D-L. Effects of coencapsulation of hepatocytes with adipose-derived stem cells in the treatment of rats with acute-on-chronic liver failure. The International journal of artificial organs. 2014;37(2):133-41.

14. Banas A, Teratani T, Yamamoto Y, Tokuhara M, Takeshita F, Osaki M, et al. IFATS collection: in vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. Stem Cells. 2008;26(10):2705-12.

15. Kashani SA, Chang TMS. Release of Hepatic Stimulatory Substance from Cultures of Free and Microencapsulated Hepatocytes: Preliminary Report. Biomaterials, Artificial Cells and Artificial Organs. 1988; 16(4):741-6.

16. Kashani SA, Chang TMS. Effects of Hepatic Stimulatory Factor Released from Free or Microencapsulated Hepatocytes on Galactosamine Induced Fulminant Hepatic Failure Animal Model. Biomaterials, Artificial Cells and Immobilization Biotechnology. 1991;19(3):565-77.

17. Chang TM. Haemoperfusions over microencapsulated adsorbent in a patient with hepatic coma. Lancet. 1972;2(7791):1371-2.

18. Paredes Juárez GA, Spasojevic M, Faas MM, de Vos P. Immunological and Technical Considerations in Application of Alginate-Based Microencapsulation Systems. Frontiers in Bioengineering and Biotechnology. 2014;2(26).

19. Wong H, Chang T. The Microencapsulation of Cells Within Alginate Poly-L-Lysine Microcapsules Prepared with the Standard Single Step Drop Technique: Histologically Identified Membrane Imperfections and the Associated Graft Rejection. Biomaterials, Artificial Cells and Immobilization Biotechnology. 1991;19(4):675-86.

20. Wong H, Chang T. A Novel two Step Procedure for Immobilizing Living Cells in Microcapsules for Improving Xenograft Survival. Biomaterials, Artificial Cells and Immobilization Biotechnology. 1991;19(4):687-97.

21. Hwang Y, Kim JC, Tae G. Significantly enhanced recovery of acute liver failure by liver targeted delivery of stem cells via heparin functionalization. Biomaterials. 2019;209:67-78.

22. Kapur SK, Katz AJ. Review of the adipose derived stem cell secretome. Biochimie. 2013;95(12):2222-8.

23. Salomone F, Barbagallo I, Puzzo L, Piazza C, Li Volti G. Efficacy of adipos tissue-mesenchymal stem cell transplantation in rats with acetaminophen liver injury. Stem Cell Res. 2013;11(3):1037-44.

24. Fontana RJ. Acute liver failure including acetaminophen overdose. Med Clin North Am. 2008;92(4):761-viii.

25. Stephan MT, Irvine DJ. Enhancing Cell therapies from the Outside In: Cell Surface Engineering Using Synthetic Nanomaterials. Nano Today. 2011;6(3):309-25.

26. Abbina S, Mohtaram NK, Kizhakkedathu JN. Cell Surface Engineering. In: Jafar Mazumder MA, Sheardown H, Al-Ahmed A, editors. Functional Biopolymers. Cham: Springer International Publishing; 2018. p. 1-42.

27. Lee JK, Choi IS, Oh TI, Lee E. Cell-Surface Engineering for Advanced Cell Therapy. Chemistry – A European Journal. 2018;24(59):15725-43.

28. Kim JC, Tae G. The modulation of biodistribution of stem cells by anchoring lipid-conjugated heparin on the cell surface. Journal of Controlled Release. 2015;217:128-37.

29. Kim M, Kim YH, Tae G. Human mesenchymal stem cell culture on heparin-based hydrogels and the modulation of interactions by gel elasticity and heparin amount. Acta Biomaterialia. 2013;9(8):7833-44.

30. Harris EN, Baggenstoss BA, Weigel PH. Rat and human HARE/stabilin-2 are clearance receptors for high- and low-molecular-weight heparins. Am J Physiol Gastrointest Liver Physiol. 2009;296(6):G1191-9.

31. Mishima Y, Lotz M. Chemotaxis of human articular chondrocytes and mesenchymal stem cells. J Orthop Res. 2008;26(10):1407-12.

32. Ross MH, Pawlina W. Histology : a text and atlas : with correlated cell and molecular biology. 6th ed. ed: Philadelphia : Wolters Kluwer/Lippincott Williams & Wilkins Health; 2011.

33. Hwang SR, Seo D-H, Al-Hilal TA, Jeon O-C, Kang JH, Kim S-H, et al. Orally active desulfated low molecular weight heparin and deoxycholic acid conjugate, 6ODS-LHbD, suppresses neovascularization and bone destruction in arthritis. Journal of Controlled Release. 2012;163(3):374-84.

34. Mousavi S, Moradi M, Khorshidahmad T, Motamedi M. Anti-Inflammatory Effects of Heparin and Its Derivatives: A Systematic Review. Adv Pharmacol Sci. 2015;2015:507151-.

35. Xu L, Wang S, Sui X, Wang Y, Su Y, Huang L, et al. Mesenchymal Stem Cell-Seeded Regenerated Silk Fibroin Complex Matrices for Liver Regeneration in an Animal Model of Acute Liver Failure. ACS Applied Materials & Interfaces. 2017;9(17):14716-23.

36. McGill MR, Jaeschke H. Oxidative Stress in Acute Liver Failure. In: Albano E, Parola M, editors. Studies on Hepatic Disorders. Cham: Springer International Publishing; 2015. p. 199-214.

37. Xu J, Huang Z, Lin L, Fu M, Gao Y, Shen Y, et al. miR-210 over-expression enhances mesenchymal stem cell survival in an oxidative stress environment through antioxidation and c-Met pathway activation. Science China Life Sciences. 2014;57(10):989-97.

38. Lapenna D, Mezzetti A, de Gioia S, Ciofani G, Marzio L, Di Ilio C, et al. Heparin: does it act as an antioxidant in vivo? Biochem Pharmacol. 1992;44(1):188-91.

39. Fahmy HM, Abd El-Daim TM, Mohamed H, Mahmoud E, Abdallah EAS, Mahmoud Hassan FE, et al. Multifunctional nanoparticles in stem cell therapy for cellular treating of kidney and liver diseases. Tissue Cell. 2020;65:101371.

40. Sohni A, Verfaillie CM. Mesenchymal Stem Cells Migration Homing and Tracking. Stem Cells International. 2013;2013:130763.

41. Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith

JE, et al. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. Blood. 2004;104(9):2643-5.
42. Meng Y, Shi C, Hu B, Gong J, Zhong X, Lin X, et al. External magnetic field promotes homing of magnetized stem cells following subcutaneous injection. BMC Cell Biol. 2017;18(1):24.

43. Yun WS, Choi JS, Ju HM, Kim MH, Choi SJ, Oh ES, et al. Enhanced Homing Technique of Mesenchymal Stem Cells Using Iron Oxide Nanoparticles by Magnetic Attraction in Olfactory-Injured Mouse Models. International Journal of Molecular Sciences. 2018;19(5):1376.

44. Faidah M, Noorwali A, atta H, Ahmed N, Habib H, Damiati L, et al. Mesenchymal stem cell therapy of hepatocellular carcinoma in rats: Detection of cell homing and tumor mass by magnetic resonance imaging using iron oxide nanoparticles. Advances in Clinical and Experimental Medicine. 2017;26:1171-8.

45. Bos C, Delmas Y, Desmoulière A, Solanilla A, Hauger O, Grosset C, et al. In vivo MR imaging of intravascularly injected magnetically labeled mesenchymal stem cells in rat kidney and liver. Radiology. 2004;233(3):781-9.

46. Xiao J-q, Shi X-l, Ma H-c, Tan J-j, Lin z, Xu Q, et al. Administration of IL-1Ra Chitosan Nanoparticles Enhances the Therapeutic Efficacy of Mesenchymal Stem Cell Transplantation in Acute Liver Failure. Archives of Medical Research. 2013;44(5):370-9.

47. Granowitz EV, Porat R, Mier JW, Pribble JP, Stiles DM, Bloedow DC, et al. Pharmacokinetics, safety and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healthy humans. Cytokine. 1992;4(5):353-60.

48. Nishikawa M, Miyazaki C, Yamashita F, Takakura Y, Hashida M. Galactosylated proteins are recognized by the liver according to the surface density of galactose moieties. Am J Physiol. 1995;268(5 Pt 1):G849-56.
49. Liu ZC, Chang TMS. Artificial cell microencapsulated stem cells in regenerative medicine, tissue engineering and cell therapy. Adv Exp Med Biol. 2010;670:68-79.

50. Das S, Pati F, Choi Y-J, Rijal G, Shim J-H, Kim SW, et al. Bioprintable, cell-laden silk fibroin–gelatin hydrogel supporting multilineage differentiation of stem cells for fabrication of three-dimensional tissue constructs. Acta Biomaterialia. 2015;11:233-46.

51. Lee JW, Choi YJ, Yong WJ, Pati F, Shim JH, Kang KS, et al. Development of a 3D cell printed construct considering angiogenesis for liver tissue engineering. Biofabrication. 2016;8(1):015007.

52. Jeon S, Kim S, Ha S, Lee S, Kim E, Kim SY, et al. Magnetically actuated microrobots as a platform for stem cell transplantation. Science Robotics. 2019;4(30):eaav4317.

53. Li J, Esteban-Fernández de Ávila B, Gao W, Zhang L, Wang J. Micro/nanorobots for biomedicine: Delivery, surgery, sensing, and detoxification. Science Robotics. 2017;2(4):eaam6431.

54. Wei T, Liu J, Li D, Chen S, Zhang Y, Li J, et al. Development of Magnet-Driven and Image-Guided Degradable Microrobots for the Precise Delivery of Engineered Stem Cells for Cancer Therapy. Small. 2020;16(41):e1906908.