Development of Advanced Cell-Based Therapy by Regulating Cell–Cell Interactions

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Cell-based therapy for disease treatment involves the transplantation of cells obtained either from self or others into relevant patients. While cells constituting the body tissues maintain homeostasis by performing remarkable functions through complicated cell–cell interactions, transplanted cells, which are generally cultured as a monolayer, are unable to recapitulate similar interactions in vivo. The regulation of cell–cell interactions can immensely increase the function and therapeutic effect of transplanted cells. This review aims to summarize the methods of regulating cell–cell interactions that could significantly increase the therapeutic effects of transplanted cells. The first method involves the generation of multicellular spheroids by three-dimensional cell culture. Spheroid formation greatly improved the survival and therapeutic effects of insulin-secreting cells in diabetic mice after transplantation. Moreover, mixed multicellular spheroids, composed of insulin-secreting cells and aorta endothelial cells or fibroblasts, were found to significantly improve insulin secretion. Secondly, adhesamine derivatives, which are low-molecular-weight compounds that accelerate cell adhesion and avoid anoikis and anchorage-dependent apoptosis, have been used to improve the survival of bone marrow-derived cells and significantly enhanced the therapeutic effects in a diabetic mouse model of delayed wound healing. Finally, the avidin-biotin complex method, a cell surface modification method, has been applied to endow tumor-homing mesenchymal stem cells with anti-tumor ability by modifying them with doxorubicin-encapsulated liposomes. The modified cells showed excellent effectiveness in cell-based cancer-targeting therapy. The discussed methods can be useful tools for advanced cell-based therapy, promising future clinical applications.

Key words  cell-based therapy; cell–cell interaction; multicellular spheroid; adhesamine; cell surface modification

1. INTRODUCTION

Cells, specifically eukaryotic cells with plasma membrane, cytoplasm, and nucleus, are the structural and functional units of living organisms. Various kinds of cells, with unique properties, are used for the formation of tissues, in which they are appropriately arranged and assembled in three dimensions. The human body is composed of approximately 37 trillion cells, and there are more than 200 cell types. Most types of cells resemble one another; they are 10–50 µm in diameter and have a lipid bilayer membrane with a nucleus. Despite these similarities, their functions differ in various aspects. For instance, fibroblasts in the skin repeatedly proliferate and turn over, whereas pancreatic β cells that release insulin to maintain blood glucose levels are hardly regenerated once they are lost. When non-regenerative cells are lost or fail to function due to genetic disorders or external factors, transplantation of healthy cells into the patients can alter the dysfunctional cells and repair the diseased condition; this process is referred to as cell-based therapy.

Cell-based therapy, including red blood cell transplantation (blood transfusion) and islet transplantation, for the treatment of type 1 diabetes, has been a consistent clinical process since long. With the recent development of cell culture technology, including the establishment of induced pluripotent stem (iPS) cells and embryonic stem (ES) cells, many protocols for preparing tissue-specific cells have been developed, and cell-based therapies are expected to become more popular in future. To date, many clinical trials have been conducted for disease treatment, using various types of cells, including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), lymphocytes, dendritic cells, hepatocytes, endothelial cells, fibroblasts, cardiomyocytes, epithelial cells, pancreatic islets, and chondrocytes. Of the clinical trials performed, HSCs and MSCs account for approximately 60%, followed by lymphocytes and dendritic cells. Although iPSC and ES cells are currently used only in a few clinical trials, they are expected to lead rapid development of cell-based therapy and regenerative medicine. Although cell-based therapies have shown excellent therapeutic effects not only in basic research but also in clinical trials, there remains a lack of information regarding survival duration, tissue distribution, and therapeutic mechanism of the transplanted cells, implying that cell-based therapies are not fully optimized for disease treatment.

Cell–cell interaction, including both direct and indirect interactions between homologous or heterologous cells, coordinates cellular functions, biological homeostasis, and organismal development through many molecules, such as various types of ions, metabolites, nucleic acids, and proteins occurring on the cell membrane or secreted from cells and extracellular vesicles, thereby allowing the cells to adapt to changes in their microenvironment. Cell–cell interac-
tion generally refers to the direct interaction between cells; stable interactions, including tight junction, gap junction, and ligand-receptor interaction, are required for cell adhesion that controls the shape of cells or tissues as well as proper cellular functions. Immune cells, including leukocytes or macrophages, defend against bacteria and other foreign substances and exclude them through transient interactions as an immediate immune response. In some diseases, including cancers, proper cell–cell interactions have been reported to be lost or disrupted; important relationship of cell–cell interactions with disease onset have been revealed at genetic levels. Therefore, cell–cell interaction of transplanted cells is an important concern in cell-based therapy.

Cells form tissues by closely interacting with neighboring cells and extracellular matrices, and perform excellent functions in the body, as described above. For example, pancreatic islets are three-dimensional tissues consisting of four types of cells, including α cells and β cells, extracellular matrices, and small arteries and veins. These cells are closely related to one another and adjust the blood glucose levels by releasing insulin and glucagon in response to changes in glucose levels. Importantly, when the three-dimensional structure of the pancreatic islet was disrupted and cells dispersed, the glucose-dependent insulin release decreased greatly. These experimental facts suggested that the surrounding environment of cells is quite important for cellular function. In general, single type of cells are prepared as a monolayer for cell-based therapy and transplanted into patients in the dispersed or suspended form. This conventional cell preparation method, however, hardly reflects the in-vivo cell–cell interactions, and the transplanted cells may not be able to exert their intrinsic functions. This could eventually result in insufficient therapeutic outcomes of cell-based therapy. Therefore, the regulation of cell–cell interaction during the process of cell transplantation is vital in order to achieve excellent cellular function and highly effective cell-based therapy. Till date, we have developed various regulatory methods for cell–cell interactions, including multicellular spheroid formation, the use of low molecular weight compounds, and cell surface modification, and demonstrated all of them to be useful for enhancing the therapeutic effects of cell-based therapy as well as for developing a new cell-based therapeutic modality.

2. MULTICELLULAR SPHEROIDS FOR CELL-BASED THERAPY

Multicellular spheroids are spherical three-dimensional cell aggregates formed by the adhesion of a large number of cells to one another. The cells forming a multicellular spheroid can perform in-vivo tissue-like functions better by closely interacting with one another, contrary to the monolayer cultured cells. This is because the three-dimensional structure of the spheroid mimics the in-vivo environment better. Therefore, multicellular spheroids can also be called artificial tissues. Multicellular spheroids of cancer cells or hepatocytes have been reported to show cellular characteristics closer to living organisms than monolayer culture cells, and are useful for the screening of anticancer drugs and evaluation of liver function. Since cellular function can be significantly enhanced by assembling cells into a spherical form or constructing a tissue-like structure, the use of multicellular spheroids is expected to be applicable in cell-based therapy. However, there are very few reports regarding the application of multicellular spheroids in disease treatment.

Multicellular spheroids may be prepared in various ways, including non-adhesive surface cell culture, hanging drop cell culture, rotary cell culture, and application of micromolding technology. However, most of these methods hardly satisfy the conditions required for their application in cell-based therapy. The micromolding technology is applicable in creating uniform microwell sheets by pouring liquid material into a pre-designed mold with multiple micro-sized protrusions. By culturing cells in the microwell sheet, the cells may be made to adhere to one another in the microwells, forming multicellular spheroids. Agarose, acrylamide, and polydimethylsiloxane (PDMS) are frequently used as the materials for microwell sheets, because they are molded easily and quickly. Microwell sheets, created by micromolding technology, usually have arrays of many uniformly sized microwells, and can produce uniform multicellular spheroids in large quantities. Microwell sheets using silicon-based polymers, namely PDMS, are widely applied because they can be used repeatedly; however, they cannot completely inhibit cell adhesion due to their hydrophobic surface. Therefore, we selected non-adhesive and temperature-responsive poly(N-isopropylacrylamide) (PNIPAAm) as a coating polymer. PNIPAAm coating on

**Biography**

Dr. Kosuke Kusamori was born in Hokkaido, Japan, in 1983. He earned his Ph.D. in Pharmacy from Kyoto University in 2013 under the supervision of Professor Yoshinobu Takakura. He worked as an Assistant Professor (or as a transient Postdoctoral fellow in 2016) in the Department of Biopharmaceutics, Kyoto Pharmaceutical University, Kyoto, Japan, under the supervision of Professor Akira Yamamoto from 2013 to 2017. He has been working at the Laboratory of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan, under the supervision of Professor Makiya Nishikawa since 2017 till present. During this time, he worked at the Division of Developmental Biology, Cincinnati Children’s Hospital Medical Center, OH, U.S.A., under the supervision of Professor James M. Wells in 2019. He received several awards for his research, including the Pharmaceutical Society of Japan Award for Young Scientists from the Pharmaceutical Society of Japan (2021). His research focus is on developing optimal drug therapeutic and drug delivery systems, including those involving low-molecular-weight compounds, peptides, proteins, nucleic acids, and cells. At present, he is developing a safe and effective cell-based therapeutic system.
microwell sheets have succeeded in producing multicellular spheroids of most types of cells examined, including mouse fibroblast NIH3T3 cells, human hepatoblastoma HepG2 cells, mouse aortic endothelial cells (MAEC), mouse colon adenocarcinoma colon26 cells, and mouse macrophage-like cell line RAW264.7 cells (Fig. 1). RAW264.7 spheroids could not be produced by conventional PDMS-based microwell sheets, owing to cell adhesion to the surface of microwell sheets. Since uniform multicellular spheroids need to be produced in large quantities in order to obtain highly reproducible therapeutic effects in cell-based therapy, the micromolding technology seems to be the most appropriate production method. Our PNIPAAm coating technique can further expand the range of application in the preparation of multicellular spheroids using micromolding technology. The micromolding technology can freely adjust the size of multicellular spheroids by designing the shape of the mold, thereby optimizing their size, viability, and cellular functions.

Previous studies have explored the use of multicellular spheroids in cell-based therapy. Bartosh et al. prepared MSC spheroids using a hanging-drop method. They demonstrated a high release of tumor necrosis factor α-stimulated gene/protein 6 (TSG-6), an anti-inflammatory protein, from MSC spheroids and an excellent therapeutic effect in a mouse model of peritonitis. However, the conventional methods, including the one in this report, have not succeeded in producing uniform multicellular spheroids in large quantities. To accurately evaluate the cellular function and obtain a reproducible therapeutic outcome, we prepared uniform multicellular spheroids using micromolding technology and used them in cell-based therapy. First, we selected mouse insulinoma NIT-1 cells as insulin-secreting cells. PDMS-based microwell sheets produced uniform-sized NIT-1 spheroids with a diameter of approximately 300 µm, which was almost the same as that of the microwell. PNIPAAm coating on the surface of the PDMS-based microwell sheets did not substantially affect the size, viability, and quantity of NIT-1 spheroids, but remarkably improved the collection procedure. Owing to the adhesion of multicellular spheroids to the surface of PDMS-based microwell sheets, their collection required excessive pipetting, which could reduce their yield and viability. Thus, the PNIPAAm coating is useful for the preparation and collection of multicellular spheroids. NIT-1 spheroids prepared by this method showed higher therapeutic effect than dispersed NIT-1 cells after transplantation into the renal capsule of streptozotocin-induced diabetic mice, although the insulin secretion from NIT-1 spheroids and monolayered NIT-1 cells was comparable. To understand the mechanism underlying the improved therapeutic effect of NIT-1 spheroids in diabetic mice, the cells remaining in the renal capsule after transplantation were detected by ex vivo imaging. The survival of NIT-1 spheroids was significantly higher than that of dispersed NIT-1 cells after transplantation. The spheroids achieved this increased survival possibly by avoiding anchorage-dependent apoptosis (anoikis) via adhesion of cells to one another. These results show that the prolonged survival of transplanted cells can contribute to the high therapeutic effect and that multicellular spheroids are a useful tool for cell-based therapy. However, it was unexpectedly found that the insulin secretion from NIT-1 cells was not improved by spheroid formation. We hypothesized that it was important to mimic the structural properties of islets to improve the insulin secretion from insulin-secreting cells. We then prepared mixed multicellular spheroids consisting of mouse pancreatic β cell line MIN6 cells, which possess high glucose-responsive insulin release ability, and NIH3T3 fibroblasts or MAEC endothelial cells using PNIPAAm-
coated PDMS-based microwell sheets. Uniform-sized MIN6, MIN6/NIH3T3, MIN6/MAEC spheroids with diameters ranging from about 280 to 330 μm were obtained, and MIN6/NIH3T3 and MIN6/MAEC spheroids showed higher insulin secretion than MIN6 spheroids in response to high glucose concentrations. Importantly, the monolayer cultures of the two different types of cells (MIN6/NIH3T3 or MIN6/MAEC) did not upregulate the insulin release, suggesting that different types of cells including insulin-secreting cells need to be included and cultured in three dimensions to achieve high cellular functions. These results show the usefulness of multicellular spheroids for cell-based therapy, and the multicellular spheroid preparation methods are expected to be used for the generation of more complex artificial organs in the future. Recently, Mizukami et al. evaluated the cell distribution of mixed multicellular spheroids consisting of various types of cells and demonstrated that the cell distribution in the mixed multicellular spheroid mainly depends on the characteristics of cells. This result suggests that the well-organized regulation of cell distribution in the mixed multicellular spheroids might optimize cellular function through improved cell–cell interactions.

The size of multicellular spheroids is an important consideration for their application. Previous studies have reported increased apoptosis in larger multicellular spheroids (diameter >400 μm) because of the lack of oxygen and nutrients, whereas smaller spheroids may not be suitable for obtaining highly functional cells because of limited cell–cell interactions. However, the effect of spheroid size on their functions has not been evaluated in detail. We evaluated the albumin release and gene expression in HepG2 spheroids with various diameters. Uniform-sized HepG2 spheroids with various diameters (about 195, 320, 490, and 550 μm) and high viability were prepared using PNIPAAm-coating PDMS-based microwell sheets with different well diameters. Interestingly, the cell viability of larger spheroids (diameter >400 μm) was maintained at approximately 90% by frequent medium changes, although the cells within the spheroids were under hypoxic conditions. Albumin release and the gene expression of CYP1A1, a member of the CYP superfamily, of HepG2 spheroids varied depending on the size of the spheroids. Therefore, further studies are needed to determine the size of HepG2 spheroids that are highly homologous to the liver in the body. Separately, we evaluated the efficiency of tumorigenesis in the lung after intravenous administration of colon26 spheroids. Although the expression of cell adhesion molecules, including integrin β1, CD44, and fibronectin tended to increase with increasing size of colon26 spheroids, the highest efficiency of tumorigenesis in the lung was observed in small colon26 spheroids with a diameter of about 170 μm compared with that of dispersed colon26 cells and medium-sized colon26 spheroids with a diameter of about 240 μm. These results show that the size of multicellular spheroids is strongly correlated with cellular function and affects the tissue distribution and survival duration of multicellular spheroids after transplantation. Based on these findings, we are currently attempting to prepare MSC spheroids and apply them to lung disease treatment.

We also prepared the multicellular spheroids of macrophages, which do not generally form aggregates, using PNIPAAm-coating PDMS-based microwell sheets. Cells of the mouse macrophage-like cell line J774.1 were forcibly polarized into inflammatory macrophages, and J774.1 spheroids greatly suppressed the proliferation of co-cultured cancer cells. These results show the potential of multicellular spheroid-based cancer treatments. Overall, the regulation of cell–cell interactions through constructing tissue-like threedimensional structures improved cellular functions and therapeutic effects. Cell-based therapy using multicellular spheroids can achieve higher therapeutic effects than those of conventional dispersed cell transplantation.

3. LOW-MOLECULAR-WEIGHT COMPOUNDS FOR REGULATING CELL–CELL INTERACTION OR MODIFYING THE CELL SURFACE CHARACTERISTICS

The low and short survival of transplanted cells have been serious concerns in cell-based therapy because the prolonged survival of transplanted cells is closely related to the effectiveness of cell-based therapy. There are several reasons for the low and short survival of transplanted cells: environmental changes from the in vitro condition to the in vivo condition, attack by immune cells, insufficient nutrients and oxygen due to shortage of angiogenesis, physical stress, and anoikis. To improve cell survival and enhance the therapeutic effect of cell-based therapy, many researchers have developed the methods to prolong the cell survival. A representative example is cell encapsulation using biocompatible polymers such as sodium alginate which can suppress the immune attack and physical stress. Alginate capsule has been applied to cell-based therapy for a long time, and pancreatic islets encapsulated alginate capsules demonstrated prolonged cell survival after transplantation and long-term therapeutic effects in diabetic patients. In recent years, a new type of alginites including alginate analogues which avoids the recognition by macrophages has been developed, and there are high expectations for their application to cell-based therapy. By contrast, cell encapsulation generally needs complicated operations and tends to increase particle size (0.5–2 mm), making it difficult to administer by injection. Separately, apoptosis inhibitors have been shown to improve the survival of transplanted cells, but the effect was temporary. Accordingly, easy-to-use and versatile methods to improve the survival of transplanted cells are highly required for cell-based therapy.

In 2009, Uesugi’s group discovered adhesamine, a low-molecular-weight compound that promotes cell adhesion, from a chemical library and reported that adhesamine promoted cell adhesion and proliferation by binding to heparan sulfate, a type of proteoglycan on the cell surface. Since adhesamine was found to promote the adhesion of suspended human T lymphocyte cell line Jurkat cells to culture plates, the acceleration of cell adhesion might suppress the death of adherent cells by anoikis after transplantation. In addition, adhesamine has superior characteristics; its mass production involves low cost, it exhibits low immunogenicity, and it is easy to use as a tool for cell-based therapy. First, we investigated the effect of adhesamine on the viability of firefly luciferase-expressing mouse melanoma B16BL6/luc cells and firefly luciferase-expressing NIH3T3/luc cells after transplantation into mice. Adhesamine significantly increased the viability of B16BL6/luc cells in the lung after intravenous injection and prolonged the survival of NIH3T3/luc cells in mice after
subcutaneous injection. These results suggest that adhesamine enhances the effect of cell-based therapy by improving the survival of transplanted cells. Based on these findings, we synthesized a water-soluble derivative of adhesamine with the RGDS peptide, a peptide with an integrin-binding motif. Interestingly, adhesamine with the RGDS peptide strongly inhibited anoikis of suspended NIH3T3 cells in vitro, whereas adhesamine or RGDS hardly inhibited anoikis. Next, we applied adhesamine with the RGDS peptide to bone marrow-derived cells (BMDCs) for the acceleration of wound healing. Adhesamine with the RGDS peptide significantly improved and prolonged the survival of green fluorescent protein (GFP)-expressing BMDCs after subcutaneous injection (Fig. 2) and significantly accelerated wound healing in diabetic mice. Therefore, these low-molecular-weight compounds promoting cell adhesion and inhibiting anoikis can serve as useful, safe, and easy-to-use tools for cell-based therapy. Recently, we also reported the functionalization of cells using adhesamine with the SFFK peptide, which exhibits a property of clustering with syndecan-4 on the surface of cells. This adhesamine derivative possesses the alkylchloride substituent at the edge of the compound, which can engage in strong binding with the Halo-tag. We demonstrated that subcutaneously injected NIH3T3 cells, which generally remained in the subcutaneous tissues, invaded the adjacent muscle tissue when the cell surface was modified with matrix metalloproteinase-2 (MMP2) using the adhesamine with the SFFK peptide and MMP2-Halo fusion protein. This finding suggests the possibility of a novel cell delivery method using functional low-molecular-weight compounds.

4. REGULATION OF CELL SURFACE CHARACTERISTICS BY CELL SURFACE MODIFICATION

Cell surface modification methods involve functionalizing cells by attaching functional compounds to the cell surface. These methods are broadly classified into the following categories based on the binding modes: covalent interactions, electrostatic or hydrophobic interactions. While the advantages of cell surface modification methods are that they can endow cells with new functions via easy and rapid manipulation, the conventional cell surface modification methods generally pose concerns in terms of cytotoxicity and short-term modifications (of less than a few days). The short modification period and temporal effectiveness of the modified compounds on cells limit their wide application in cell-based therapy. In addition, stable modification such as covalent binding requires a relatively long incubation time, which could cause cytotoxicity. Therefore, there is a need for the development of a more stable and rapid method for functionalized cell-based therapy via cell surface modification.

We applied the avidin-biotin complex (ABC) method to achieve stable and rapid cell surface modification. Avidin, a protein found in egg white, possesses four biotin-binding sites per molecule. Avidin and biotin form a strong non-covalent bond (dissociation constant of $10^{-15}$ M) in a short reaction time ($<10$ min). Therefore, the ABC method could overcome the shortcomings of conventional cell surface modification methods. Regarding the application of the ABC method to cell surface modification, little is known about the influence of the modification on cellular characteristics and the stability of the modification. We demonstrated that the ABC method...
method can modify the avidinated surface of mouse MSC line C3H10T1/2 with biotinylated GFP within 10 min with high efficiency. In addition, biotinylated NanoLuc luciferase (Nluc) remained bound to C3H10T1/2 cells for more than 14 d in vitro, without any significant changes in cellular characteristics, such as viability, migration, and differentiation. In vivo, Nluc modification to C3H10T1/2 cells using the ABC method was stable. In vivo imaging revealed that the luminescence of Nluc bound to C3H10T1/2 cells was detected for more than 7 d after intraperitoneal injection. Accordingly, the ABC method is a stable and rapid cell surface modification method and can serve as a useful tool for cell-based therapy. Based on this finding, we applied the ABC method to an MSC-based drug delivery system. MSCs are non-neoplastic pluripotent stem cells that can be isolated from the bone marrow, adipose tissue, and umbilical cord and have been approved for application in regenerative medicine owing to their excellent immunomodulatory and tissue-repairing effects. In addition, MSCs possess a unique tumor-homing property, and MSCs actively accumulate in tumor tissues by sensing tumor cells in the body. Thus, it is expected that this property can be used for the development of MSC-based cancer-targeting therapy. For MSC-based cancer-targeting therapy, MSCs need to be functionalized because of their low antitumor effect. Although there are several reports on the functionalization of MSCs, most of them are based on the chemical modification of the cell surface or gene transfer. We selected the ABC method to endow the MSCs with anti-tumor properties because the ABC method can rapidly modify the surface of MSCs with antitumor drugs and avoid cytotoxicity caused by antitumor drugs internalized by the cells during the modification process. Antitumor drug doxorubicin (DOX)-encapsulated liposomes (Lip) were biotinylated and added to avidinated C3H10T1/2 cells. The fluorescence of DOX-encapsulated liposomes (Lip) was observed on the surface of C3H10T1/2 cells and no significant changes in cellular characteristics, including viability and tumor-homing ability, were observed. Furthermore, DL-modified C3H10T1/2 cells significantly suppressed the survival of firefly luciferase-expressing mouse colon adenocarcinoma colon26/luc cells in both direct co-culture and indirect co-culture using the Transwell® system. Interestingly, the suppressive effect of DL-modified C3H10T1/2 cells on the survival of colon26/luc cells was higher in direct co-culture than in indirect co-culture. To explore the high antitumor effect in the direct co-culture, we observed the behavior of DL under the direct co-culture conditions using confocal laser microscopy and found that DL migrated directly from the surface of C3H10T1/2 cells to the interior of adjacent colon26/GFP cells. This result indicates that drugs modified on the surface of MSCs via the ABC method were actively and effectively delivered to adjacent cells. In addition, intravenous injection of DL-modified C3H10T1/2 cells almost completely suppressed tumor growth in a mouse model of lung cancer metastasis, which was prepared by the intravenous injection of colon26/luc cells. From these results, we suggest that antitumor drug modification on MSCs via the ABC method can be useful for MSC-based cancer-targeting therapy through direct intercellular drug delivery. Recently, we are currently developing an advanced MSC-based drug delivery system acting through multiple intercellular drug delivery pathways, and this intercellular drug delivery system may be valuable in cell-based drug delivery in the future. Polymer modification of the cell surface can regulate tissue distribution of intravenously injected cells, because most types of cells interact with the pulmonary capillary endothelial cells and form an embolus with blood cells after intravenous injection. We are currently trying to regulate the tissue distribution of MSCs after intravenous injection by polymer modification of the cell surface using the ABC method to enable better MSC-based disease treatment. Previous research shows that polymer modification of islets resulted in higher survival after transplantation, as these islets could avoid immune cells. Cell surface modification can result in novel cell functions and regulate cell–cell interaction between transplanted cells. This strategy can be developed into a useful tool for the advanced cell-based therapy.

5. CONCLUSION

Regulating cell–cell interaction can not only mimic tissue environments and realize intrinsic cellular function, but also endow a completely new function to cells. Cell-based therapy still has a lot of room for improvement in terms of therapeutic efficacy and new therapeutic applications. Most cell-based therapeutics is currently transplanted into patients without considering cell–cell interactions. On the other hand, drug delivery systems have better therapeutic outcomes, with few side effects, as the function and pharmacokinetics of drugs can be controlled. Likewise, controlling the functions and tissue distribution of transplanted cells would lead to optimized cell-based disease treatments. Regulation of cell–cell interactions will be one of the key strategies for optimized cell transplantation, and has the potential to advance cell-based therapies.

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