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How can microsphere-mediated delivery of small molecules serve as a novel tool for engineering tissues from stem cells?

Stephanie Michelle Willerth*,¹

¹Department of Mechanical Engineering & Division of Medical Sciences, University of Victoria; School of Biomedical Engineering & International Collaboration on Repair Discoveries, University of British Columbia, Victoria, BC, V8W 2Y2 Canada; Vancouver, BC, V5Z 1M9, Canada

*Author for correspondence: Tel.: +1 250 721 7303; Fax: +1 250 721 6051; willerth@uvic.ca

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Stem cell-based tissue engineering

The fields of tissue engineering and regenerative medicine have made incredible advances due to the adoption of stem cells as a powerful tool for these applications [1]. Stem cells are defined by their ability to generate additional stem cells as well as to differentiate into multiple mature cell phenotypes [2]. One of the major challenges when engineering tissues from stem cells is how to control the process of differentiation to ensure that these cells become the desired mature phenotype with appropriate functionality for a particular application.

Common techniques for achieving the desired stem cell differentiation include utilizing growth factors as well as genetic manipulation. Both of these methods induce changes in the differentiation state of stem cells by altering the expression of different genes and, in turn, protein expression levels to yield the target population of cells based for the end application. Growth factors work by binding to receptors on the cell surface and activating intracellular cascades that alter the patterns of protein expression inside of the cell [3]. However, growth factors are expensive and must be handled carefully to ensure that they do not lose their activity when being encapsulated for controlled release applications [4].

Another commonly utilized tool for directing stem cell behavior is genetically modifying the cells, which alters their gene expression patterns by targeting the regulation of selected genes [5]. Genetic manipulation methods, including the use of gene editing tools, can be imprecise and lead to undesirable mutations, making these techniques difficult to translate for clinical applications. Thus, researchers have been examining more desirable methods for controlling stem cell differentiation that maintain efficacy while avoiding these aforementioned issues.

Small molecules as a tool for tissue engineering

One promising method utilizes small molecule morphogens as an alternative to alter stem cell behavior. The molecular weight cutoff for classifying a small molecule is usually considered to be under 900 Daltons as this size enables these molecules to be taken up readily through oral administration [6]. Similarly, the small size makes them likely to be endocytosed by cells, which are maintained in an *in vitro* culture system. Small molecule mediated control of cellular behavior has been gaining attention in recent years due to the increased interest in stem cell biology as well as the field of direct cellular reprogramming, in which one mature cellular phenotype is converted into the target phenotype [7,8,9]. These molecules offer several advantages when compared with growth factors

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or genetic manipulation as they are often cheaper to manufacture and easier to encapsulate into controlled drug delivery systems and usage does not have integration issues associated with genetic editing.

Microsphere-mediated delivery of small molecules

Our group had previously shown how multiple small molecules, including retinoic acid, purmorphamine and guggulsterone, could serve as powerful enhancers of mature neuronal differentiation when utilized to treat induced pluripotent stem cells (iPSCs), which are cells that can become any type of cell in the body [10,11]. Our studies then sought to develop novel biomaterial strategies to deliver these molecules in a controlled fashion to engineer neural tissue from pluripotent stem cells.

Our 2015 paper demonstrated that we could encapsulate retinoic acid into microspheres that were small enough to be incorporated into aggregates of human iPSCs [12]. Microspheres are tiny composites of a biodegradable polymer that can be formulated to encapsulate small molecules. Encapsulated microspheres can then slowly and continuously release these small molecules as they degrade, offering controlled delivery of the molecules homogeneously throughout the aggregate structure. Here, controlled delivery of retinoic acid promoted the formation of large neural aggregates, suggesting this strategy has a potential benefit for tissue engineering.

Our follow-up study demonstrated that, for the first time, we could successfully encapsulate and deliver the small molecule guggulsterone, which is normally utilized as an anticancer drug, in a controlled fashion over 44 days [13]. We then followed a similar process where these novel drug delivery tools were combined with human iPSC-derived neural aggregates, and we demonstrated that they promoted increased mature differentiation of stem cell-derived neural tissues, as indicated by the production of the neuronal marker Tuj1, which was higher than that of the control cultures, including aggregates treated with soluble guggulsterone.

A 2018 study led by Laura de la Vega characterized the ability of poly caprolactone microspheres to deliver purmorphamine, a small molecule Sonic Hedgehog agonist, in a controlled fashion over 46 days [14]. Her work incorporated a mixture of retinoic acid and purmorphamine releasing microspheres into human iPSC-derived aggregates to determine their effect on differentiation. These microspheres were able to promote the differentiation of these neural aggregates into mature neural tissues that expressed the motor neuron marker HB9 on day 35 and the mature motor neuron marker, choline acetyltransferase.

Our three studies indicate how particle-mediated delivery of small molecules can promote mature differentiation of human iPSCs in comparison with traditional methods of application in the cell culture media. These strategies can also be directly translated for preclinical studies as these microspheres are directly incorporated into the tissue. This method may prove beneficial when transplanting these tissues into harsh micro-environments, such as after spinal cord injury, as the controlled small molecule release may be able to overcome the effects of an inhibitory micro-environment.

Additionally, studies have developed strategies to harness the power of small molecules for differentiating stem cells into mature, functional tissues for other applications. For instance, the controlled release of many different small molecules, including purmorphamine, hydroxycholesterol, dexamethasone, mevinolin, simvastatin, resveratrol, genistein, icariin, melatonin, metformin, alendronate and zoledronic acid, have been characterized for osteogenic differentiation of stem cells by activating various intracellular signaling pathways, such as those associated with bone morphogenic protein and MAP kinase [15].

Recently, the small molecule, tacrolimus, has been identified as a potential morphogen for engineering bone tissues from stem cells [16]. Thus, bone tissue engineering can benefit from utilizing particle-mediated controlled release of these aforementioned small molecules in a similar fashion. Additionally, an interesting recent study demonstrated a novel liposome-based particle formulation termed sterosome for delivering multiple hydrophobic molecules with silencing RNA [17]. These drug-delivering sterosomes promoted osteogenic differentiation of mesenchymal stem cells in 2D and 3D settings *in vitro* as well as in a mouse bone defect model, suggesting its potential for translational studies.

Two other recent studies have developed novel biomaterial strategies for delivering small molecules to promote the maturation of stem cells into two different types of target tissues: cardiac and cartilage. The first study encapsulated two different small molecules, a GSK3 inhibitor CHIR99021 and the Wnt inhibitor IWP2, into silica particles with the goal of utilizing controlled release of these molecules to promote cardiac differentiation of human iPSCs [18]. The controlled release of the molecules was able to promote cardiac differentiation as indicated by gene expression and the presence of calcium gradients. The authors suggest that utilizing these small molecule-releasing particles promotes consistent differentiation compared with adding these small molecules to the media directly.

Another study developed multifunctional nanoparticles, similar to microspheres but on the nanoscale, which can be utilized as a tool for tracking mesenchymal stem cells and delivering small molecules to promote controlled differentiation of stem cells [19]. The functionalities included having a Cys-Arg-Gly-Asp sequence to enable the particles to bind to the stem cells and a photocaged linker to enable the delivery of the small molecule kartogenin upon stimulation with infrared light while releasing an ultraviolet signal indicating the location of the cell. These nanoparticles promoted differentiation of mesenchymal stem cells into chondrocytes upon light stimulation while enabling the cells to be tracked *in vivo*, in which the cells promoted cartilage formation. Overall, the multifunctionality of the particles demonstrates how controlled small molecule release can be combined with technologies that are necessary when performing *in vivo* stem cell transplantation.

Conclusion

This editorial highlights recent studies that illustrate the wide potential of using particle mediated delivery of small molecules for engineering tissues from many different types of stem cells. While these methods demonstrate tremendous potential, several issues need to be addressed before these methods can be translated for clinical applications. While the mechanism of action is known for some small molecules in relation to what pathways they influence, this information needs to be characterized for other pathways. Another potential consideration is if these small molecules could have off-target effects if delivered to healthy tissues. A corresponding concern would be how to translate the dosages of small molecules needed for controlled release strategies from preclinical testing for clinical applications. Similar considerations would also be needed when translating these approaches for cellular reprogramming when performing *in vivo* tissue engineering. However, these studies only represent the first efforts of combining particle-mediated controlled delivery of small molecules with stem cells for tissue engineering. Work remains to be completed to exploit the full potential of therapeutic delivery of small molecules using particles for stem cell tissue engineering. Demonstrated in the study using multifunctional nanoparticles, different chemistries can be utilized to generate novel methods of release and these strategies can be utilized for engineering a wide variety of tissues from stem cells and to translate these engineered tissues for *in vivo* applications.

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