Encapsulated stem cells ameliorate depressive‑like behavior via growth factor secretion

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Abstract:
As prevalence of depression continues to rise around the world, there remains a stagnation of available treatments as the affected population grows. The subset of treatment-resistant depression also is on the rise highlighting the need for innovative treatments to address this issue. Mesenchymal stem cells (MSCs) have been reported to attenuate depression‑like behaviors, however, the effects of encapsulation of MSCs have yet to be investigated. Encapsulation of MSCs exhibited prolonged survival of exogenous cell injection accompanied with increased secretion of neurotrophic factors including vascular endothelial growth factor, ciliary neurotrophic factor, and others. The enhanced expression of these factors highlights the ability of encapsulated MSCs to upregulate the respective signaling pathways, which are associated with depression pathology and activation of neurogenesis. This treatment identifies a promising therapeutic option for depression, specifically treatment‑resistant depression. Further, evaluation of long-term effects of the treatment is warranted.

Keywords:
Animal model, depression, growth factors, mesenchymal, transplantation

Introduction
Depression is a disease that affects one in six individuals in America and may others worldwide.[1,2] Even with continuous progress in pharmacological treatments, only approximately one-half of the patients with clinical depression benefit from current therapies.[3] Thus, there is a pressing therapeutic need for research of treatments that target treatment-resistant depression.[4] Treatment-resistant depression consists of associated risk factors including congenital susceptibility to depression and resistance of common pharmacological therapies.[5] Several preclinical studies, Wistar Kyoto (WKY) rats, show elevated levels of congenital depression‑like behavior and exhibit resistance to common treatments, demonstrating their usefulness as a potential model for treatment-resistant depression.[4,6–8]

Mesenchymal stem cells (MSCs) exhibit potential as a therapy for neurodegenerative disease, including stroke, Parkinson’s disease, and multiple sclerosis.[9–11] Several transplantation methods have been investigated to evaluate its effect on depression. Intrahippocampal transplantation of MSCs enhanced neuroplasticity but failed to yield any antidepressant effects within a Lewis rat model.[12] Yet, when transplanted intraventricularly, MSCs produced...
antidepressant effects in Flinders sensitive line rats.\[^{13,14}\] However, the mechanism of action is currently unknown, specifically if the beneficial effects stem from contact between MSCs and host cells or MSC-secreted factors that alleviate depressive symptoms.

Several studies highlighted the encapsulation of a variety of cell lines, in which neurotrophic factors were suggested to be the therapeutic action in stem cells in numerous diseases.\[^{15-17}\] Secretion of MSC neurotrophic factors may provide the improved functional outcomes rather than direct survival and integration into the host by the MSC graft.\[^{18}\] The efficacy of encapsulated MSCs (eMSCs) as a treatment for depression has been relatively unknown up to this point. In this review, we will elucidate the possible mechanism of action and cognitive effects of eMSC in treatment-resistant depression.

**Cell Encapsulated Stem Cells and Depression**

Although pharmacological advances continue each day, only one-half of the patients who are clinically depressed benefit from current therapies. This highlights the necessity for further research and development of novel treatments, particularly targeting treatment-resistant depression. Despite the reported antidepressant properties of MSCs, the possible benefit of this treatment on treatment-resistant depression has yet to be studied. Cell encapsulation aims to increase survival of transplanted cells, yet the mechanisms and benefits of encapsulated MSCs are unknown. In this review, we discuss the enhanced therapeutic effects of encapsulating MSCs (eMSCs). The transplantation of eMSCs in the lateral ventricle amplified neurogenesis in the dentate gyrus (DG) and subventricular zone of the hippocampus as well as protecting against depressive-like behavior, compared to MSCs without encapsulation and when MSCs were transplanted into the striatum. eMSCs were able to release several neurotrophic factors over a prolonged period including brain-derived neurotrophic factor, ciliary neurotrophic factor (CNTF), fibroblast growth factor-2 and vascular endothelial growth factor (VEGF). Specifically, the transplantation of eMSCs into the lateral ventricle stimulated these pathways yielding the various neurotrophic factors. The use of eMSC transplantation as a therapy for depression, particularly treatment-resistant depression, is promising but further investigations should be considered to address the long-term effects associated with this treatment.

**Discussion**

This study showed that eMSCs had improved benefits over the transplantation of nonencapsulated MSCs for \emph{in vivo} treatment-resistant depression models.\[^{19}\] In prior study, MSCs transplanted in the lateral ventricle (MSC-LV) resulted in antidepressant effects and enhanced neurogenesis in animal models for depression.\[^{13}\] However, when administered in the present study, MSCs-LV failed to alter behavior or improve neurogenesis in the treatment-resistant depression animal model, although more cells were transplanted than the previously discussed study.\[^{13,19}\] In contrast, transplantation of eMSCs into the lateral ventricle (eMSC-LV), produced a behavioral as well as increased neurogenesis in rats with treatment-resistant depression.\[^{19}\] These results support that eMSCs may be an improved therapy when addressing this treatment-resistant depression modality, which is one of the strictest and likely represents a more advanced pathology then typically observed in a clinical setting. Yet, the eMSC-LV antidepressant profile is not common to conventional pharmacological treatments. Typical antidepressant drugs will enhance neurogenesis within the DG only, creating an antidepressant profile of enhanced Forced Swim Test without affecting open-field test, creating difficulty in behavioral assessments.\[^{20}\] The murky pathology of WKY rats accentuates the complexities of this problem. Future studies using other animal models for depression or battery of behavioral tests could provide further information.

Transplanted MSCs in neurogenerative disease rodent models often exhibit low graft survival and the present study was no different, as no MSCs were found in the lateral ventricle or adjacent brain tissue at 7 days post transplantation.\[^{21-23}\] Therefore, the overall engraftment rate of transplanted MSCs in the lateral ventricle is very mild in the best case. However, polymer encapsulation could shield the implanted cells from an endogenous immune system reaction.\[^{24}\] In the present study, eMSCs were demonstrated to survive until at least day 15 following injection, along with secretion of neurotrophic factors (brain-derived neurotrophic factor, CNTF, fibroblast growth factor 2 [FGF-2] and VEGF) from the transplanted eMSC.\[^{19}\] The encapsulation likely creates an indirect effect by increasing the survival of the MSCs graft, therefore mediating increased levels of neurotrophic factor secretion. As a result, encapsulation encouraged the improved antidepressant effects of transplanted MSCs and enhanced neurogenesis. However, due to the typical treatment length needed to address depression in the clinic the long-term effects of eMSCs are needed to elucidate the effects of long-term cell survival as well as multiple treatments.

The current study targets the lateral ventricle as the most effective site for transplantation over the striatum. As various secreted cytokines of MSCs are suggested...
to play a vital role in the beneficial effects of MSCs, the secretome of eMSCs-LV has a greater opportunity to reach distant sites than the secretions of eMSCs ingrafted in the striatum cannot.\textsuperscript{[18,25]} The many complexities of depression pathology remain unknown, and potential culprit lesions distant from the lateral ventricle can cause atypical pathology.\textsuperscript{[25]} The mechanism of delivery of the MSC secretome is an important aspect of the therapeutic potential stem cell transplantation in depression pathologies.

Targeting the efficiency of the secretome instead of MSCs graft survival could be the vital step to enhancing its antidepressant effects. When MSCs are transplanted into the lateral ventricle, they travel to the ipsilateral hippocampus, yet when the hippocampus is targeted for direct injection of MSCs, there are no significant antidepressant effects in rodent depression models.\textsuperscript{[12,13,26]} Evidence is shown that the administration of eMSCs-LV ameliorates depressive-like behavior, signifying that migration nor transplantation of MSCs into the hippocampus are necessary for the beneficial antidepressant effects to be present.

To further substantiate the roles of trophic factors in the eMSCs-mediated antidepressant effects, phosphorylation of FGRFR1, TrkB, and VEGFR2, which are receptors for FGF-2, BDNF, and VEGF, respectively were measured. eMSCs were responsible for secreting enhanced levels of BDNF, FGF-2, and VEGF and upregulating the phosphorylation related receptors for each factor.\textsuperscript{[19]} This suggests that eMSCs-LV stimulate the pathways of FGF-2, BDNF, and VEGF which notably are pathways related to pathological depression as well as regulating neurogenesis.\textsuperscript{[19,25,27-35]} The injection of any of the aforementioned factors into the lateral ventricle or the DG yield antidepressant effects.\textsuperscript{[33,36,37]}

Additional investigation focused on the relationship between depression and neurogenesis, utilizing CNTF, which is shown to affect depressive-like behavior, anxiety, and promote neurogenesis.\textsuperscript{[38-40]} To monitor the CNTF pathway, expression of STAT3 and phosphorylated STAT3 (pSTAT3) were monitored. STAT3 is a product of the CNTF pathway, and pSTAT3 is created by CNTF pathway activation.\textsuperscript{[41,42]} In the present study, eMSCs were found to release CNTF and upregulating pSTAT3 expression, indicating the CNTF pathway was stimulated by transplantation of eMSCs-LV. In a previous study, a capsule-releasing CNTF, injected into the lateral ventricle, rescued motor function in aged rats.\textsuperscript{[42]} The exhibited changes in behavioral deficits following eMSCs-LV treatment could involve the CNTF pathway and therefore implicate the Jak-STAT pathway as a positive autoregulatory loop.\textsuperscript{[33,43,44]} These pathways are likely only a portion of the many potential signaling pathways that regulated the neurogenic activity in depression pathology.

In lockstep with the examination of the function of neurogenesis in depression, eMSCs-LV were demonstrated to elevate the expression of CNTF, CNTFR-alpha (receptor of CNTF), VEGF, and VEGFR2.\textsuperscript{[19]} The enhanced expression of VEGF is similar to stroke animal models.\textsuperscript{[45,46]} When dental pulp cells, which have a similar phenotype as MSCs, are injected into the hippocampus they also increase the levels of VEGF and CNTF by the brain, suggesting the benefits of a conducive microenvironment within the brain to yield an antidepressant effect.\textsuperscript{[47]} The role of CNTF in an autocrine manner compared to VEGFR2, which is vital for neurogenesis, highlights the intimate relationship between neurogenesis and depressive-like behavior in creating an antidepressant effect.\textsuperscript{[38,39,48-50]}

Further studies need to address the inability to identify either neurogenesis, neurotrophic factors or potentially both as the chief component of the therapeutic effects of eMSCs-LV. Furthermore, evaluating the efficacy of the previously mentioned neurotrophic factors by directly injecting them into the lateral ventricle may elucidate their role and lead to a novel therapeutic modality for the depression model. In essence, eMSCs-LV fostered an antidepressant effect in the rodent treatment-resistant depression model. Encapsulation of MSCs prolongs the secretion of exogenous neurotrophic factors while eliciting the release of endogenous neurotrophic factors and promoting neurogenesis, altogether highlights the therapeutic potential of stem cell therapy as a treatment for depression.\textsuperscript{[51,52]}

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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