

## The Compromised Anti-microbial Function of Mesenchymal Stem Cells in Diabetic Patients

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**Category:** Basic Sciences/Biologics

**Keywords:** diabetes, mesenchymal stem cells, bacteria, infection

**Introduction/Purpose:** Diabetic foot infection (DFI), including skin infection and osteomyelitis, is a severe complication of late-stage diabetes. Mesenchymal stem cells (MSCs) facilitate bacterial clearance. In bacterial infection, MSCs, via paracrine mediators, regulate the host cell metabolism and inflammatory response. Particularly, MSCs augment the antibacterial function of neutrophils. It is generally believed that hyperglycemia in diabetes is toxic to MSCs/progenitors and detrimental to their regenerative function. It is unknown, however, whether the antibacterial function of MSCs is compromised in diabetes.

**Methods:** Bone marrow samples from 6 diabetic and 4 non-diabetic patients (approved by IRB) were used for MSC isolation.

1. MSCs from both diabetic and non-diabetic patients were treated with lipopolysaccharides (LPS), a bacterial wall component, for 6 hours. The tissue culture media were collected as conditioned medium. *E. coli* from a single colony were cultured with addition of the conditioned medium generated by either diabetic or non-diabetic MSCs and inoculated on LB-agar plates overnight. Bacterial colonies were counted.
2. Human macrophages were isolated from umbilical cord blood and co-cultured with either diabetic or non-diabetic MSCs, in a trans-well system, for 24 hours. The macrophages were then cultured with heat-inactivated *E. coli* for one hour. After extensive washing, macrophage and bacteria were stained with Pappenheim method. Bacterial phagocytosis of macrophages, after co-cultured with diabetic or non-diabetic MSCs, was assessed under a microscope.

**Results:** There was no statistical difference in the number of *E. coli* colonies when regular medium produced by diabetic and non-diabetic MSCs was added into the bacterial culture. When the diabetic and non-diabetic MSCs were treated with LPS and the conditioned medium was collected and added into bacterial cultures, *E. coli* colonies increased in the diabetic group, about 3 fold, as compared with the non-diabetic group ( $p < 0.05$ ). Macrophages were counted in defined areas of the Petri dishes and designated as infected or uninfected, according to the presentation of bacterial bodies or not. While the infection rate of macrophages co-cultured with non-diabetic MSCs was 85% ( $\pm 5.5\%$ ), it was 70% ( $\pm 6.6\%$ ) when macrophages were co-cultured with diabetic MSCs ( $p = 0.006$ ).

**Conclusion:** MSCs-produced paracrine factors suppressed the growth of *E. coli* but diabetic and non-diabetic MSCs had no difference in such a function. Activation with LPS did not augment the non-diabetic MSCs but weakened diabetic MSCs in suppression of bacterial growth. MSCs regulate macrophages in bacterial phagocytosis. Diabetic MSCs, however, had a limited role in regulation of macrophages. This study demonstrated that MSCs in diabetic patients are compromised in anti-bacterial infection. The results not only deepen the understanding of bacterial infection in diabetes but also open up new strategy to control bacterial infection in diabetic patients.