Stroke is the only major disease without an effective treatment and is a major cause of adult disability in industrialized nations. We believe that effective recruitment of neural progenitor cells (NPCs) into the sites of brain injury is an essential component for the promotion of neurogenesis and mechanisms to mediate recovery and regeneration post-stroke; however, it has been demonstrated that the ability of basal neurogenesis post-stroke, while existent, is attenuated in aged rodent models[1], similar to the expected phenotype in aged stroke patients. The Segura group has successfully engineered an injectable formulation, composed of enzymatically-responsive nanocapsules loaded into an injectable hyaluronic acid (HA) hydrogel, which promoted angiogenesis in the avascular stroke cavity[2]. However, NPC migration was not achieved when solely promoting angiogenesis. Following injury, activated endothelial cells within cerebral vessels begin to secrete stromal cell-derived factor-1 (SDF-1)[3], which functions as a recruitment signal by recruiting progenitor/stem cells to sites of injury and has been implicated in NPC recruitment and neurogenesis[4]. We propose that through engineering the delivery of SDF-1 *in vitro* with enzymatically responsive nanocapsules, we can arrive at hydrogel formulations that can recruit NPCs to the stroke cavity. **We hypothesize that by using microfluidic generated gradients, we will be able to optimize SDF-1 gradient parameters to promote NPC migration and that this process can be modeled in a predictive kinetic model capable of guiding hydrogel design for future *in vivo* applications.** This hypothesis is based on the following observations:

First, biomolecular gradients are essential in a wide range of biological processes. At the tissue level, biomolecular gradients direct organizational patterning through dictating cellular differentiation and migration. This guiding system holds true with cellular responses within injury and disease states. Since biomaterials have been engineered to contain bioactive signals to direct native cellular mechanisms and facilitate tissue regeneration, we hypothesize that optimizing chemotactic gradients generated in therapeutic biomaterials will induce desirable cellular recruitment for enhanced healing responses at wound sites. Second, by utilizing an *in vitro* microfluidic system to generate predictable, reproducible, and easily quantifiable chemical gradients, we can streamline the

identification of favorable chemotactic gradients by eliminating the traditional method of step-wise checking of specific discrete concentrations. Third, gradient profile steepness has been demonstrated to differentially promote cell migration rate and distance in a variety of chemotactic responses[5]. Due to the micro-scale device architecture, discrete control of biomolecular presentation can produce well-defined concentration gradients[6], so as to inform us on NPC-specific responses to differential SDF-1 gradient steepness. After identifying the optimal SDF-1 gradient for NPC migration into our biomaterial, we will engineer new nanocapsules, in order to release SDF-1, and form optimized NPC-recruiting SDF-1 gradients within an injectable hydrogel. The research effort to obtain **optimal SDF-1 gradient parameters to promote NPC migration while guiding HA hydrogel design for future *in vivo* applications** will be focused on three aims:

Aim 1 will test our hypothesis that the concentration of SDF-1 affects NPC migration within a three-dimensional HA hydrogel, and that our microfluidic gradient platform can be used to identify SDF-1 concentrations which promote enhanced NPC migration through HA hydrogels. This will be accomplished by the design, development, and production of a microfluidic gradient generator to assess a continuous linear gradient across HA hydrogels and am working towards determining NPC migration as a function of SDF-1 concentration.

Aim 2 will test our hypothesis that SDF-1 gradients with differential slopes result in differing NPC migration. Gradient profile steepness can modify cell migration rate and total migration distance in a variety of chemotactic responses, we will build on results from **Aim 1** to determine a range of SDF-1 concentrations to assess in **Aim 2** across linear and nonlinear gradients across hydrogels. To accomplish this, I will generate a second type of microfluidic devices to assess SDF-1 gradient profiles role on increasing NPC migration, and elucidate NPCs response to differential SDF-1 profiles.

Aim 3 will test our hypothesis that enzymatically degradable nanocapsules can produce an optimized SDF-1 gradient to promote NPC migration. In this aim I will focus on generating computational models to determine release kinetics for SDF-1 gradient generation. These models will allow us to design SDF-1-releasing

nanocapsules and load the appropriate formulations of these nanocapsules within HA hydrogels. The Segura lab has produced nanocapsules which respond to proteolytic enzymes, resulting in the delivery of proteins with spatiotemporal control[2]. This technology is based on mild encapsulation of the proteins in nanocapsules using different protease-specific cleavable peptides, controlling the proteolytic kinetics. Tunable SDF-1 nanocapsules will be developed and incorporated within HA hydrogels. We will use these nanocapsules to recapitulate similar SDF-1 concentrations and gradients to those identified in **Aim 1 & 2** for improved NPC migration. We plan that each aim can be accomplished in approximately eight months, with focus solely on this project which the Ford Fellowship would afford me.

We believe our proposed work to investigate SDF-1 gradients on NPCs has applications beyond the scope of this project by assigning specific SDF-1 release profiles to be assessed in therapeutic trials of our HA hydrogels. In addition, this work will develop a platform and technique to investigate other chemotactic signals and the recruitment of various cell populations; improving early-stage development of clinically-relevant biomaterials.

I have chosen to complete the proposed work under the guidance of Professor Tatiana Segura at UCLA's Chemical Engineering Department. Professor Segura's lab has generated a variety of hydrogels for in situ instruction and manipulation of cells to promote healing responses and tissue regeneration[7–10]. In addition, the lab's development of nanocapsule systems[2,10] would allow me to generate hydrogels loaded with SDF-1 nanocapsules critical for my proposed work. Marrying my microfluidic experience with Professor Segura's expertise in hydrogel scaffolds will broaden my scientific training significantly. Additionally, this project's stroke/neural component will provide me the experience to pursue my career as a faculty member specializing in 3D microscale disease models with a focus on neurological disorders. I plan to accomplish this by using organ on-a-chip technologies to produce microengineered disease/disorder surrogate models to elucidate mechanisms resulting in or accelerating these conditions, and ideally help improve the development of novel therapeutics to target these conditions.