Nanoparticle Improved Stem Cell Therapy for Erectile Dysfunction in a Rat Model of Cavernous Nerve Injury

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Abbreviations and Acronyms

ADSC = adipose-derived stem cell

BCNC = bilateral cavernous nerve crush

CC = corpus cavernosum

ED = erectile dysfunction

GAPDH = glyceraldehyde-3-phosphate dehydrogenase

ICI = intracavernous injection

ICP = intracavernous pressure

MAP = mean arterial pressure

MPG = major pelvic ganglion

Nano-ADSC = NanoShuttle magnetic nanoparticle ADSC

PBS = phosphate buffered saline

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Purpose: Recently intracavernous injection of stem cells has garnered great interest as a potential treatment of erectile dysfunction. However, most stem cells are washed out immediately after intracavernous injection. The goal of this study was to investigate using NanoShuttle™ magnetic nanoparticles to maintain stem cells in the corpus cavernosum after intracavernous injection, thereby improving stem cell therapy of erectile dysfunction in an animal model.

Materials and Methods: Adipose derived stem cells were magnetized with NanoShuttle magnetic nanoparticles to create Nano-adipose derived stem cells. A total of 24 rats underwent bilateral cavernous nerve crush and were randomly assigned to 3 groups, including adipose derived stem cells, Nano-adipose derived stem cells and Nano-adipose derived stem cells plus magnet. Cells were tracked at days 1, 3, 5 and 9 after intracavernous injection. Another 40 rats with bilateral cavernous nerve crush were randomly assigned to 4 groups, including bilateral cavernous nerve crush, bilateral cavernous nerve crush plus adipose derived stem cell intracavernous injection, bilateral cavernous nerve crush plus Nano-adipose derived stem cell intracavernous injection and bilateral cavernous nerve crush plus Nano-adipose derived stem cell intracavernous injection plus magnet. Functional testing and histological analysis were performed 4 weeks after intracavernous injection.

Results: In the *in vitro* study 1) NanoShuttle magnetic nanoparticles were successfully bound to adipose derived stem cells and 2) Nano-adipose derived stem cells migrated toward the magnet. In the *in vivo* study 1) cell tracking showed that Nano-adipose derived stem cells were successfully retained in the corpus cavernosum using the magnet for up to 3 days while most adipose derived stem cells were washed out in other groups by day 1 after intracavernous injection, and 2) intracavernous pressure/mean arterial pressure, and αSMA (α-smooth muscle actin) and PECAM-1 (platelet endothelial cell adhesion molecule 1) expression in the Nano-adipose derived stem cell group was significantly higher than in the other groups.

Conclusions: Magnetization of adipose derived stem cells with NanoShuttle magnetic nanoparticles kept adipose derived stem cells in the corpus cavernosum and improved adipose derived stem cell therapy of erectile dysfunction in an animal model.

Key Words: penis, erectile dysfunction, peripheral nerve injuries, magnetite nanoparticles, stem cells

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PROSTATECTOMY is the recommended procedure for patients with low and intermediate risk localized prostate cancer.[1] ED is the most common complication[2] with a prevalence of 20% to 90%.[3] For those patients with ED earlier penile rehabilitation has been widely practiced to protect erectile function before the cavernous nerves recover to baseline function, which can take 2 years or even longer.[4] Early intervention with phosphodiesterase type 5 inhibitors, vacuum erectile devices, or intracavernous or transurethral administration of vasodilators is recommended. However, the reported recovery rate varies and patient compliance is low due to cumbersome application and ineffectiveness.[5] Therefore, there is a great need to explore novel rehabilitation modalities.

Recently ICI of stem cells has shown some potential to treat erectile dysfunction in animal models.[6–9] Many studies have shown functional and structural improvements with ICI of stem cells. However, groups have described difficulty in finding injected stem cells in the corpora cavernosa after ICI. Because most stem cells went to bone marrow and other places in the body, it was proposed that ICI of stem cells is systemic therapy in nature and paracrine factors may have an important role in stem cell therapy.[10–13] However, ICI is local therapy and systemic therapy is never the aim of ICI of stem cells. It is much more logical that retaining stem cells in the local target area would avoid systemic effects. Thus, there is a demand for methods to retain stem cells in the CC after ICI for ED therapy.

To address this need, we took a novel approach to keeping stem cells in the CC after ICI by magnetizing cells and using magnetic forces to retain them in the CC. Specifically we magnetized cells with NanoShuttle, a biocompatible magnetic nanoparticle assembly (approximately 50 nm) consisting of gold nanoparticles, iron oxide and poly-L-lysine. NanoShuttle magnetizes cells by electrostatically and nonspecifically attaching to cell membranes via poly-L-lysine (approximately 50 pg per cell)[14–18] with no effect on cell proliferation or viability. It does not interfere with fluorescence imaging.[19–21] After trypsinization and resuspension in medium these magnetized cells can be directed, aggregated and held using magnetic forces. In this specific case we sought to magnetize stem cells, inject them via ICI and use magnetic forces to retain them in the CC.

ADSCs have previously been used as experimental ED treatment.[13,22,23] ADSCs are an abundant stem cell source that can be isolated and autologously transplanted on the same day.[12,24] Therefore, ADSCs were chosen for our study.

We hypothesized that magnetization with NanoShuttle would keep ADSCs in the CC for a long time after ICI so that the ADSCs could exert a local therapeutic function and improve erectile function. This hypothesis was tested in the BCNC animal model, a widely used cavernous injury model that mimics ED after radical prostatectomy. The results of this study would greatly expand the current array of stem cell based therapies of ED.

MATERIALS AND METHODS

Animals

Eight-week-old male Sprague Dawley® rats were obtained from Harlan Laboratories, Dublin, Virginia. Care and treatment were approved by the institutional animal care and use committee at our institution. The rats were allowed time to become accustomed to the new environment as required by our institutional animal care and use committee before the study.

ADSC Isolation and Culture

ADSCs were isolated from the inguinal fat of rats. The rats were anesthetized by isoflurane inhalant and a midline abdominal incision was made. The inguinal fat was identified, excised and placed in PBS on ice. The harvested fat was minced into small pieces and incubated in digestion buffer consisting of PBS, bovine serum albumin and collagenase type I for 1 hour at 37C with shaking every 20 minutes. The digested solution was centrifuged at 1,000 $\times$ gravity for 10 minutes at room temperature and the supernatant was removed. The remaining cells were suspended in 10 ml Dulbecco's modified Eagle's medium/F-12 with 1% antibiotic-antimycotic solution and 10% fetal bovine serum (Life Technologies®). The cells were plated on cell culture plates and cultured at 37C in 5% CO_2 with 95% humidity with medium exchanged daily. ADSCs were used at the third passage for the following study.[13]

Nano-ADSC Preparation for ICI

ADSCs were cultured to 70% to 80% confluence and incubated with NanoShuttle (1 μl/10,000 cells)[25] overnight to allow for cell binding. The next day the cells were washed of excess NanoShuttle, trypsinized and resuspended in fresh medium. To track cells all cells to be used for transplantation were labeled for 30 minutes with CellTracker™ Green CMFDA before ICI. Approximately 1×10^6 labeled cells in 0.2 ml PBS were used for each ICI.

Intracavernous Injection

Seven days after BCNC the rats were anesthetized with isoflurane inhalant. ADSCs or Nano-ADSCs were injected in the CC. In the Nano-ADSC plus magnet group 2 N52 magnets (0.05-inch outer diameter $\times$ 0.25-inch high, 100 G) were placed outside the bilateral CC of the penis immediately after injection and removed 6 hours later.

Animal Model and Groups

A total of 24 Sprague Dawley rats underwent BCNC. In this procedure the rat was anesthetized and disinfected as

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