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Research paper

# Efficacy of intervertebral disc regeneration with stem cells — A systematic review and meta-analysis of animal controlled trials

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ABSTRACT

Management of intervertebral disc (IVD) degenerative disease is challenging, as it is accompanied by irreversible loss of IVD cells. Stem cell transplantation to the disc has shown promise in decelerating or arresting the degenerative process. Multiple pre-clinical animal trials have been conducted, but with conflicting outcomes. To assess the effect of stem cell transplantation, a systematic review and meta-analysis was performed. A comprehensive literature search was conducted through Week 3, 2015. Inclusion criteria consisted of controlled animal trials. Two reviewers screened abstracts and full texts. Disagreements were resolved by a third reviewer. Random effects models were constructed to pool standardized mean difference (SMD). Twenty two studies were included; nine of which were randomized. Statistically significant differences were found with the stem cell group exhibiting increased disc height index (SMD = 3.64, 95% confidence interval (CI): 2.49, 4.78; $p < 0.001$), increased MRI T2 signal intensity (SMD = 2.28, 95% CI: 1.48, 3.08; $p < 0.001$), increased Type II collagen mRNA expression (SMD = 3.68, 95% CI: 1.66, 5.70; $p < 0.001$), and decreased histologic disc degeneration grade (SMD = −2.97, 95% CI: −3.97, −1.97; $p < 0.001$). There was statistical heterogeneity between studies that could not be explained with pre-planned subgroup analyses based on animal species, study designs, and transplanted cell types. Stem cells transplanted to the IVD in quadruped animals decelerate or arrest the IVD degenerative process. Further studies in human clinical trials will be needed to understand if such benefit can be translated to bipedal humans.



## 1. Introduction

Low back and neck pain have a point prevalence of 19% of the world's population, a three-month prevalence of 31% in the United States (Strine and Hootman, 2007; Hoy et al., 2012, 2014). They are ranked the first and fourth most common causes for disability in the United States (Murray et al., 2013), and are associated with a tremendous expenditure of hundreds of billions ($80.1 billion to $91.8 billion) of US dollars annually (Martin et al., 2008). Intervertebral disc (IVD) degeneration is ubiquitous and increases with age, with a prevalence of over 70% in the age group of 50 years or younger, and over 90% in those older than 50 (Teraguchi et al., 2014). As an imaging finding, it is simply age-related change, and is frequently asymptomatic (Bogduk, 2012). There is a population, however, in which the disc becomes painful, often termed discogenic pain. Discogenic pain accounts for 25% to 80% of all low back and neck pain (Rogers, 2003; Manchikanti et al., 2009; Gilbert et al., 2013;

*Abbreviations:* IVD, intervertebral disc; SMD, standardized mean difference; 95% CI, 95% confidence interval; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT, randomized controlled trial; N-RCT, non-randomized controlled trial; CAMARADES, Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies; BMSC, bone marrow stromal cell; ADMSC, adipose-derived mesenchymal stem cell; ECM, extracellular matrix.

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Peterson et al., 2013). As a cause of back pain, discogenic pain is more common in relatively young patients (DePalma, 2011).

The degenerative intervertebral disc disease is characterized by cell death and degeneration of extracellular matrix (Urban and Roberts, 2003). Cell death occurs through an apoptosis process associated with aging, genetic propensity, and spinal loading (Lotz and Chin, 2000; Hunter et al., 2003; Livshits et al., 2011; Hirata et al., 2014; Yurube et al., 2014). The decrease of extracellular matrix synthesis and increase of extracellular matrix degeneration are associated with loss of cells and phenotype changes in the surviving cells secondary to local inflammatory responses (Trout et al., 1982; Urban and Roberts, 2003). As a result, the condition presents with dehydration of the nucleus pulposus, fissures of the annulus fibrosus, extrusion of the NP, and a cascade of inflammatory responses that perpetuate the cycle of loss of appropriate matrix (Urban and Roberts, 2003; Gilbert et al., 2013). Micro-environmental changes including neovascularization and nerve growth lead to clinical presentations of pain and altered biomechanical function (Adams et al., 1996; Freemont et al., 1997; Johnson et al., 2002; Chan et al., 2008; Purmessur et al., 2008; Hughes et al., 2012; Richardson et al., 2012).

The treatment of discogenic pain has been particularly challenging due to the irreversible loss of intervertebral disc cells. Current treatment modalities include pain medication (Koes et al., 1997; Van Tulder et al., 2000; Roelofs et al., 2008), therapies (Van Middelkoop et al., 2011; Jang and Lee, 2012), injections (Staal et al., 2009; Lu et al., 2014), nucleoplasty (Welch and Gerszten, 2002; Mirzai et al., 2007; Adam et al., 2013) and surgical discectomy (McCulloch, 1996; Soliman et al., 2014). None addresses the IVD degeneration. Because the extracellular matrix is synthesized and modulated by IVD cells, there has been significant interest in researching cell therapy utilizing stem cells and mesenchymal stem cells for the regeneration of the IVD (Trout et al., 1982; Kalson et al., 2008; Richardson et al., 2008; Henriksson et al., 2009; Benneker et al., 2014).

Multiple pre-clinical randomized controlled animal trials have been performed, but often suffered with small sample size, heterogeneous designs, and conflicting outcomes. Since a consensus on the effect of stem cell transplantation in animals is needed to justify human clinical trials, we conducted a systematic review and a meta-analysis. Specifically, the objective of this study was to evaluate intervertebral disc regeneration due to stem cell transplantation in controlled animal trials. Objective outcomes of disc regeneration included: changes in disc height, nucleus pulposus rehydration on T2 weighted MRI images, histologic disc degeneration grade, and expression of type II collagen regeneration.

## 2. Methods

The study protocol was finalized in advance of any data collection, which defined objectives, search strategy, inclusion/exclusion criteria, data extraction, outcomes of interest, and analytical approaches. The reporting of this systematic review complies with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2009).

### 2.1. Search strategy

We conducted a comprehensive search of seven databases, including Ovid Medline In-Process & Other Non-Indexed Citations, Ovid MEDLINE, Ovid EMBASE, Ovid PsycINFO, Ovid Cochrane Central Register of Controlled Trials, Ovid Cochrane Database of Systematic Reviews, and Scopus, from each database's inception to Week 3, 2015. Controlled vocabulary supplemented with keywords was used to search for studies of intervertebral disc height after stem cell transplantation. Search terms were broad and without language or country restrictions. The detailed strategy is available in Appendix 1.

### 2.2. Inclusion and exclusion criteria

We included pre-clinical controlled trials (randomized controlled trials (RCTs), and non-randomized controlled trials (N-RCTs)) that evaluated stem cell transplantation on experimental regeneration of the intervertebral disc in animals. We focused on the outcomes that were pertinent to the effect and mechanism in IVD regeneration (disc height index, MRI T2 signal intensity, Type II collagen expression, and histologic disc degeneration grade). Animals with any type of model in IVD degeneration secondary to IVD trauma by changing mechanical loading, puncture incision or gamma irradiation, or chemical assault with chemonucleolysis by chondroitinase ABC, chymopapain or fibronectin fragments were included regardless of species/breeds of animal. We did not restrict the type of intervention in control groups. Studies were excluded if they combined multiple treatments (e.g., stem cells and Rho-GTPase inhibitory agents) or if models of nontraumatic spinal cord injury were used. We also excluded studies without original data (e.g., clinical reviews, editorials, letters, or erratum) or without the outcomes of interest.

### 2.3. Data extraction

Two independent reviewers reviewed the abstracts and full texts of potentially relevant studies. Discrepancies between the reviewers were resolved through discussion and consensus. The same two reviewers extracted study details from the full text studies using a standardized pilot-tested form. The following data were extracted: the author, year of publication, animal species, disc degeneration model (traumatic or chemical), cell type, interventions in control groups, and outcomes of interest. When outcomes of interest were assessed serially, we extracted data for the final time point. Where multiple arms were included in the study, the control group and the stem cell transplantation group were selected.

### 2.4. Quality assessment

We applied the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) checklist to assess the methodological quality of the included studies (Sena et al., 2007). A 9-point-item check list was used to assess the risk for bias, including: (1) published in a peer-reviewed journal; (2) control of animals' temperature; (3) randomized treatment allocation; (4) treatment allocation concealment; (5) blinded assessment of outcome; (6) use of anesthetics other than ketamine; (7) reporting of a sample size calculation; (8) statement of compliance with regulatory requirements; and (9) statement of potential conflict of interest.

### 2.5. Statistical analysis

We calculated standardized mean difference (SMD) and related 95% confidence interval (CI) for each study using Cohen's d method to normalize for the different animal species. We then combined outcomes of interest across the included studies using the DerSimonian and Laird random effect methods. The heterogeneity was estimated using the Mantel–Haenszel model. We conducted subgroup analyses based on animal species (rabbit, dog, rat, pig, and sheep), study designs, and cell types (bone marrow stromal cells (BMSCs), and adipose-derived stem cells (ADMSCs)) to investigate potential sources of heterogeneity and the robustness of our findings. Heterogeneity across individual studies was assessed using the $I^2$ index and Cochran's Q statistical test, where $I^2 > 50\%$ and/or $p < 0.10$ suggest high heterogeneity. All meta-analyses were conducted using STATA version 13.1 (StataCorp, College Station, TX).