Clinical trials of intervertebral disc regeneration: current status and future developments

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Abstracts:

Intervertebral disc (IVD) degeneration (IDD) is considered as one of the major causes for low back pain (LBP). However, conventional surgical approaches for treating LBP does not aim to counter the degeneration. Biological interventions have been investigated with an attempt to regenerate the IVD by restoring its matrices and cell activities. This review summarizes the current clinical trials that explore the efficacy of covering cell-, growth factor- and small molecule-based approaches. While investigations of growth factor- and small molecule-based therapies are still preliminary, intradiscal delivery of mesenchymal stromal cells has been more widely adopted and shown positive results in addressing the pain and the associated physical disability, albeit to a lower extent than observed in previous animal studies. Strategies that potentiate the endogenous disc progenitors may offer a valid alternative to the exogenous cell transplantation. Identification of the novel biologics to arrest IDD phenotype may potentiate disc repair in future. Large scale, high quality long-term trials should be conducted to clarify the safety and efficacy of these therapies.

Keywords:

Intervertebral disc degeneration, regeneration, clinical trial, mesenchymal stromal cells, growth factor, small molecule

Introduction

Intervertebral disc degeneration (IDD) is one of the most debilitating medical conditions and considered a major cause for low back pain in addition to radicular symptoms [1-3]. Intervertebral discs (IVD) are fibrocartilaginous tissues between vertebrae contributing to spinal mobility and shock absorption. The IVD is composed of an inner gelatinous nucleus pulposus (NP) core and an outer ring of annulus fibrosus (AF), sandwiched between the cartilaginous endplates (CEP) of the rostral and caudal vertebral bodies. The NP consists of primarily proteoglycans and collagen II in the extracellular matrix (ECM). This matrix meshwork is established collectively by a heterogenous NP cell population including notochordal and chondrocyte-like cells [4]. Loss of NP cells, particularly the resident progenitors, and consequently the disrupted balance of synthesis and degradation of ECM, especially proteoglycans which play an essential role in maintaining hydration of the IVD, is thought to be one of the leading causes of IDD [4]. Abnormal expression of growth factors (e.g. TGF β [5, 6], IGF [6, 7]) and pro-inflammatory cytokines (e.g. IL-1 [8], TNF α [9]) is also associated with the degenerative process. These changes ultimately compromise the anatomical and mechanical properties of IVD, resulting in a loss of disc height, disc deformation and spinal disability under load.

Surgical procedures, such as spinal fusion or disc arthroplasty, are the last resort for treating IDD if conservative therapies fail to relieve the symptoms [10, 11]. However, spinal fusion needs long recovery time and may cause adjacent level degeneration [12]. In addition to substantial healthcare related expenses, the surgeries may also result in adverse complications [13]. New biological approaches are therefore investigated aiming to control disc degeneration progression and preserve the spinal kinematics in a minimally invasive manner.

These approaches, often referred as regenerative medicine, include growth factor-, gene- and cell-based therapies [6, 14, 15]. They have been demonstrated to induce the repair of IVD, primarily via NP regeneration, in various in vitro and pre-clinical studies. The efficacy of some of these approaches in clinical settings have been demonstrated, therefore offering hope to low back pain patients [16]. This review aims to revisit the completed and undergoing clinical trials of the state-of-the-art biologics for treating IDD and attempt to discuss the direction of future regenerative strategies in light of the findings.

2. Cell-based therapy

2.1 Cell-based therapies in clinical trial.

Cell-based therapies, mostly by intradiscal delivery, have drawn considerable attention over past decades [15-18]. The major cell sources include notochordal [17] and chondrocyte-like NP cells [18], and mesenchymal stromal cells (MSCs) [15, 19]. In particular, initial clinical studies have utilized MSCs due to their advantages over the other cell sources in terms of production and possible auxiliary effects on suppressing inflammation [15, 16, 20-27].

Out of 12 reports, 10 are related to MSCs, of which 7 have been completed with data disclosed, while the other two propose the use of NP cell derivatives (clinicaltrials.gov: NCT01640457 [20], NCT03347708). In contrast to adipose-derived MSCs (NCT02338271 [21], NCT02097862 [22], NCT03461458), bone marrow-derived (BM-) MSCs have been more widely investigated (NCT01290367 [23], NCT01860417 [24, 25], NCT03011398 [28], NCT03692221, NCT03340818, etc. [26, 27]. Although allogeneic MSCs might induce weak and transient immune response, indicated by anti-HLA antibodies at a detectable level in serum within 12 months post-intradiscal injection, the therapeutic efficacy appeared not dependent on HLA matching [24, 25]. This may be presumably due to the suppressed host immune responses by transplanted MSCs [29]. Indeed, allogenic BM-MSC may be of valid alternative as it allows one-step treatment for patients. BM-MSCs implantation was explored in 5 trials and generally reported to provide significant benefits in terms of pain relief for 12 and up to 36 months and increased IVD hydration [23, 25-28]. Interestingly, IVD height was usually not restored. Patient mobility and quality of life was improved for up to 6 years post-treatment [27]. It is also noteworthy that a higher dosage of MSCs appeared to result in better outcome, where fewer patients needed further surgical intervention [23].

However, cautions should be taken when interpreting the results due to the limited sample size and non-controlled or non-randomized design. Two phase II trials have been completed using allogenic BM-MSC with up to 36 months follow-up in 125 patients [23-25]. They are in blinded, randomized and controlled setup. However, the other 5 completed trials using autologous MSCs were open-label and single-arm with attempt to clarify the safety and tolerability of the treatment [21, 22, 26-28]. To date, there are two undergoing autologous MSCs trials in a total of 84 patients involving a more robust design based on a double-blinded, randomized and controlled setup (NCT03692221, NCT03340818).

2.2 Limitations and future developments.

Despite the encouraging findings from the clinical trials, the limitations should be carefully considered in treating human IDD via the cell-based therapy. For instance, inflammation and endplate destruction have been reported in a goat degeneration model after injection of adipose-derived stromal vascular fractions or purified stromal cells [30]. Although this could be species (goat)- and source (adipose)- dependent and no resembling observations were reported in current clinical trials, such outcomes deserve attentions in future long-term studies. Interestingly, platelet-rich plasma (PRP) supplemented with MSCs could induce disc repair in a rabbit IDD model without severe adverse effects [31]. Comella et al. also reported no adverse effect in the clinical trial of PRP supplemented with adipose-derived MSCs in a short-term (6 months) and small-scale (n=15) study [22]. This may suggest a synergic effect of PRP and adipose-derived MSCs in treating IDD.

Human IVDs have distinct cellular compositions, biomechanics, and nutritional supply

compared to various pre-clinical models such as rodents, rabbits and other large quadrupeds [32]. However, insights obtained from these pre-clinical studies may improve the therapies. Studies in animal models suggested that the efficacy of MSCs in IVD repair may be largely based on their intrinsic chondrogenic potential and inhibitory effect on inflammatory cascades or endogenous cell apoptosis [33-35]. However, IVD is an avascular tissue. Either the implanted cells or newly regenerated cells need to adapt to the low-glucose supply, hypoxia, acidic and hypertonic environment. Otherwise, they may suffer from suppressed metabolic activity or even cell death [36]. Consistent with the limited nutritional supply in the IVD, our previous study in rabbit lumbar discs indicated that delivering a large quantity of MSCs could be detrimental [34]. Studies have attempted to test other sources of stem cells for the reparative effect, such as umbilical cord-derived MSCs [37]. Moreover, priming of MSCs before implantation was also investigated, such as genetically modified hTIMP-expressing BM-MSCs which might elicit additional inhibitory effects on matrix degradation [38] and MSC preconditioned by pentosan polysulphate for better chondrogenic differentiation potentials [39]. However, their efficacy awaits to be compared to the unmodified MSCs.

On the other hand, preventing cell loss after implantation is also a key concern. A study indicated that cell loss could reach up to 90% after implantation due to the annulus failure [40]. However, injection at high doses appeared to avoid the issue, and that injection at early stage might minimize cell loss [41]. Various cell carriers have been developed to facilitate the MSCs implantation as well as chondrogenic differentiation [42-44]. For example, Zhou et al. generated a genepin cross-linked type II collagen/chondroitin sulfate composite hydrogel for MSCs delivery in a mouse IDD model [42]. Other examples were self-assembly peptide nanofibers [43] and collagen-low MW (150-300KDa) hyaluronic acid hydrogel [44]. An alternative is to develop deliver approaches that minimize damage to the annulus, such as intravenous injection [45] and transpedicular approach [46].

Altogether intradiscal injection of MSCs seems to be safe and able to relieve the IDD symptoms in initial human trials. However, long-term safety and efficacy are awaited to be clarified by larger-scale and well-controlled studies. Pre-clinical findings supported the use of cell sources alternative to MSCs or disc cells, benefits of pre-conditioned or functionally enhanced MSCs, and strategies that maximize cell engraftment. Their safety and efficacy warrant further investigation in humans.

2.3 Endogenous progenitors-based therapy

Strategies that can activate endogenous progenitors may be an alternative approach to exogenous cell-based therapies. Accumulative evidences have suggested the existence of disc progenitors in NP, AF and EP regions and their reduced activity in aging and IDD [47, 48]. Cells clusters observed in NP and AF lesions in different animal models and clinical lumbar degenerative discs are indicative of an attempted self-repair by resident stem cells [49]. These progenitors could offer an opportunity to overcome the

practical and regulatory hurdles related to cell implantation mentioned above. For example, NP progenitors were in vitro differentiated and transplanted for sciatic regeneration [50]. While Ishii etl al. showed that, in contrast to the MSCs, these disc progenitors presented a lower proliferative capacity and differentiation potential [51], further study is required to understand their metabolic activities in vivo and how these progenitors may be activated and migrate to injury sites. The progenitor function and regulation in IVDs has been reviewed by Clouet et al [15]. In particular, studies have highlighted the role of SDF1 in stem cell migration and GDF5/6 in progenitor differentiation, providing potential implications for harnessing disc progenitor activity.

3. Growth factor-based therapy

Several growth factors have been reported to restore the balance of anabolic and catabolic activities in both in vitro and animal studies (reviewed by Kennon et al. [6]). In particular, studies have focused on the use of TGF β family members or their modifiers [52, 53].

Kwon et al. evaluated a total of 50 subjects with symptomatic lumbar disc degeneration (Thompson grade 2-3) and VAS>40mm and ODI >30% at first visit [53]. YH14618 is biglycan fragment that binds to TGF β1 and arrest IDD in a rabbit model [53]. YH14618 were intradiscally injected into the IVDs at three different dosages of 1, 3 and 6 mg/disc. Adverse effects were reported by 27 patients in this study. 50% subjects were responsive to YH14618 treatment and VAS scored -2.18 from baseline after 6 months. ODI was reduced by 12.38 while placebo group by 6.67. Two patients (6mg/disc) with MRI improvement were noted albeit no statistical significance. Peniel 2000 is another biglycan-derived peptide that regulates TGF signaling and reported to attenuate IDD in a rabbit model [54]. These suggest that moderating TGF signaling may have a role in modifying IDD.

Three on-going phase II trials (NCT01158924, NCT01124006, NCT01182337) investigating the efficacy of GDF5 are documented. The outcomes are yet to be released.

4. Small molecule-based therapy

Compared to cell- or growth factor-based therapy, small molecules are barely degradable in vivo and commonly considered as a relatively economic approach.

Abaloparatide is parathyroid hormone (PTH)-related protein analog drug for treating osteoporosis (NCT03708926). PTH has been shown to effectively attenuates disc degeneration in aged mice [55]. A phase II clinical trial is being conducted to investigate its effect in improving pain and physical function in lumbar disc degeneration patients.

SM04690 is a Wnt pathway inhibitor capable to induce chondrogenic differentiation of both MSCs and disc cells and reported to regenerate disc structure in a rat IDD model [56]. Samumed initiated this clinical trial to test its therapeutic potential in alleviating

pain and improving disc health (NCT03246399). Three different dosages (0.03mg, 0.07mg and 0.15mg per disc) were intradiscally injected and the subjects monitored up to 6 months.

Small molecule therapy is broadly applied to treat various pathological conditions, including osteoarthritis [56, 57] and IDD [58-61]. Several small molecules with their molecular targets known or unknown have been proposed, including IL17A inhibitor [58], epigallocatechin 3-gallate [60], resveratrol [59], nicotinamide phosphoribosyltransferase inhibitor (APO866) [61] to inhibit matrix degradation in animal models. Resveratrol could also inhibit NP cells apoptosis [62]. Polyphenol epigallocatechin 3-gallat was shown with anti-inflammatory and anti-catabolic activities and could reduce radiating pain [60]. Urolithin A could inhibit inflammatory responses of NP cells and alleviate IDD in rat [63]. However, as the key pathological events/molecules for IDD and the target of these small molecules are yet to be elucidated, their application in humans is debatable. Identification of effective small molecules with defined molecular target is not only highly desirable for management of IDD, but also the understanding of IDD etiology.

5. Conclusion:

In summary, this review collects and summarizes the clinical evidences for IVDs regeneration using cell-based, growth factor-based and small molecule-based therapies. MSCs-based therapy has been more widely investigated in clinical trials. Encouraging results have been obtained albeit at a lower extent of efficacy than expected. Therapies that rely on eliciting self-repair mechanism, such as endogenous progenitor activation may be a valid alternative strategy. Small molecule-based therapy is an area relatively underdeveloped presumably due to the limited understanding of the degenerative mechanism and hence difficulty in pinpointing the regulatory targets. A combination of above regeneration strategies as well as identifying degeneration stage-specific therapeutic windows may be one of the ways to enhance disc repair efficacy.

IDD is a chronic disorder with predeposition from aging [64], genetic [65] and environmental risk factors including smoking [66], obesity [67], physical loading [68] etc. As the regulatory target/pathway is still largely unknown [69], the strategies likely require repeated administration to effectively control the progression. Moreover, not all IDD patients have LBP [1]. Whether IVD regeneration may effectively prevent LBP and other associated IDD symptoms in long term needs to be addressed by large scale randomized controlled studies.

Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Table 1. Summary of cell-based IDD therapy under clinical trials. BM: bone marrow; MSCs: mesenchymal stromal cells; ODI: Oswestry Disability Index; VAS: visual analog scale; NPS: numeric pain score; SANE: single assessment numeric evaluation; FRI: functional rating index; PPI: present pain intensity; BDI: Beck Depression Inventory; PROMs: Patient Reported Outcome Measures.

Authors	Clinical trial ID	Type of study	Year	Cells details	Number of patients	Observation duration	Analysis variables	Deliverables
Bae et al. [23]	NCT01290367	Prospective, randomized, double-blinded, controlled phase II clinical trial	2014	Immunoselected, nucleated allogenic BM-MSC (two dosages: 6M and 18M cells) and hyaluronic acid (HA)	100	36 months	Physical examination and lab tests (inflammation and immunology); ODI; VAS; MRI	No adverse effects; After 12 months, significant improvement in ODI and VAS; 10% (HA) VS 3.3% (18M+HA) needed surgery
Pettine et al. [26]		Open-label, single-arm	2015-2017 3 years follow-up	Autologous BM concentrate; percutaneously injection	26	Up to 36 months	ODI; VAS; Pfirrmann grade (MRI)	2 (2015), 5 (2016) and 6 (2017) patients needed surgical interventions; Reduction of ODI (56.7 to 17.5) and VAS (82.1 to 21.9) and 1 grade improvement (40% patients); No adverse effect
Elabd et al. [27]		Open-label, single-arm	2016	Hypoxia cultured BM-MSC	5	48-72 months	Physical examination; low back MRI; quality of life questionnaire	No neoplasms in treated region; no adverse effect report; self-reported improvement in strength and mobility
Noriega et al. [25]	NCT01860417	Randomized, blinded, controlled phase I-II trial	2017	Allogenic BM-MSC	25	12 months	VAS, short form-12; Pfirrmann grade (MRI)	Improvement
Garcia-Sancho et al. [24]							HLA typing; algogunctional indexes	Weak and transient immune responses
Centeno, et al. [28]	NCT03011398	Open-label, single-arm	2017	Autologous BM-MSC	33	72 months	NPS; SANE; FRI; disc posterior dimensions (MRI)	3 reported pain; no serious adverse effect; NPS and SANE improvement; 17/20

								disc bulge size reduced.
Kumar et al. [21]	NCT02338271	Open-label, single-arm phase I	2017	Adipose tissue-derived MSC; two dosages single injection	10	12 months	ODI; VAS; Short Form-36; X-ray and MRI	No adverse effects; ODI VAS and SF-36 improved; 3 patients increased water content
Comella et al. [22]	NCT02097862	Open-lable, single-arm	2017	Stromal vascular fractions (adipose-derived stem cells) and platelet rich plasma (PRP)	15	6 months	Range of motion; ODI; VAS; PPI; BDI; Dallas pain questionnaire; Short Form-12	No adverse effect for up to 12 months; improvement in flexion, VAS, PPI and SF-12; ODI and BDI trends positive.
	NCT03347708	Randomized, controlled, phase I	2021 (expected)	Discogenic cells and hyaluronate	60	24 months	ODI; VAS; MRI	N/A
	NCT03461458	Open-label, perspective phase I	2022 (expected)	Autologous adipose-derived MSCs	16	24 months	PROMs; MRI	N/A
Tschugg et al. [20]	NCT01640457	Open-label, randomized, controlled, phase I/II	2022 (expected); 2017 (short report)	Novocart Disc plus (autologous disc chondrocyte)	120 (enrolled); 24 (in short report)	60 month; 7 months (in short report)	ODI; VAS; SF-36; MRI	1 reherniation reported; no obvious adverse effect
	NCT03692221	Open-label, randomized, controlled, phase I	2022 (expected)	Autologous BM-MSCs	24	12 months	VAS; ODI; SF-36; MRI	N/A
	NCT03340818	Randomized, double-blinded, controlled	2021 (expected)	BM-concentrate	60	12 months	VAS; ODI	N/A

Table 2. Summary of the growth factor-based therapy under clinical trials. ODI: Oswestry Disability Index; VAS: visual analog scale; DHI: disc height index; GDF-5: Growth and differentiation factor 5.

Authors	Clinical trial ID	Type of study	Year	Therapeutics	Number of patients	Observation duration	Analysis variables	Deliverables
Kwon et al. [53]	NCT02320019	Randomized parallel phase I/II	2015	YH14618	50	6 months	VAS; ODI; DHI (X-ray); MRI	27 patients adverse effects; Improvement in ODI and VAS; no change in DHI and MRI grading
	NCT01158924	Randomized controlled phase II	2014	rhGDF-5	40	12 months	Neurological Assessment for Motor Function and Reflexes/Sensory; ODI; VAS; SF-36	Unknown
	NCT01124006				24			
	NCT01182337				31			

Table 3. Summary of small molecule-based therapy under clinical trials. ECG: electrocardiogram; ODI: Oswestry Disability Index; VAS: visual analog scale; PGA: physician global assessment.

Authors	Clinical trial ID	Type of study	Year	Therapeutics	Number of patients	Observation duration	Analysis variables	Deliverables
	NCT03708926	Randomzied controlled phase II	2022 (expected)	Abaloparatide; Intradiscal injection	109	12 months	Physical exam; health questionnaires; MRI	N/A
Samumed	NCT03246399	Open-label phase I	2018	SM04690 intradiscal injection	18	6 months	ECG; physical exam; VAS; ODI; PGA; MRI and X-ray	N/A