

Research Article

Biologic Therapies for Intervertebral Degenerative Disc Disease: A Review of Novel Applications

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Abstract

Intervertebral disc disease is a common cause of low back pain affecting both the young and the elderly. Standard treatment options involve conservative treatments such as physical therapy and anti-inflammatory medications but also include more invasive techniques such as injections, thermal ablation, and surgery. Despite these treatments, chronic low back pain in many of these patients continues to persist limiting their function and quality of life. There has been a great interest in using biologic agents, such as Platelet Rich Plasma (PRP) and Mesenchymal Stem Cells (MSCs), to repair the disc degeneration and tears when traditional treatments fail to provide symptomatic relief. This comprehensive report reviews these new approaches including the use of platelet rich plasma injections, bone marrow aspirate injections, lipoaspirate injections, protein based therapy, 3D printing and scaffolds, gene therapy, predictive analytics, and functional imaging. The authors have also shared their vision of anticipated growth and customization of this rapidly growing field as it applies to intervertebral disc degeneration. Regenerative medicine has the potential to revolutionize the way we approach spine care in patients and further collaboration is needed among involved disciplines to advance this very exciting and important field.

Introduction

Intervertebral disc disease is one of the most common causes of chronic Low Back Pain (LBP) with Internal Disc Disruption (IDD) estimated to affect 42% of symptomatic adults [1]. Back or spine problems are the 2nd most common cause of disability among Americans over 18 years of age [2]. IDD is a cascade that begins with changes to the cellular microenvironment and progresses to the structural breakdown and functional impairment of the intervertebral disc. Due to the sparse blood supply and age related decrease in endplate vasculature, permeability and proteoglycan content, the disc has a limited capacity to restore structural integrity [3]. The end result is continued degeneration and escalating disruption.

Standard treatment options for discogenic low back pain include activity modification, medications, physical therapy, injection therapies, and surgery. Injection therapy options include epidural injections and less commonly, thermal annular procedures like IDET or biacuplasty [4]. The effectiveness of these injection therapies is limited [5-7]. Operative management includes percutaneous or open disc decompression, surgical fusion, and artificial disc replacement. Surgical treatments have variable results, sometimes with comparable outcomes to non surgical treatments [8]. Despite these treatments, chronic low back pain continues to persist in many individuals limiting their function and quality of life.

There has been a great deal of interest in using biologic agents such as Platelet Rich Plasma (PRP), Mesenchymal Stem Cells (MSCs), and Growth Factors (GF) to repair disc degeneration and tears. The goal of this narrative review is to outline the current and novel application of biologic agents in the treatment of intervertebral disc degeneration. The authors have also shared their vision of anticipated

growth and customization of this rapidly growing field as it applies to IDD.

Materials and Methods

Studies relevant to Biologics were extracted from Pubmed and Medline database within the dates ranging from 2005 through 2016. These studies included *in vitro* and *in vivo* animal and human experiments. Careful analysis of the study population, biologics type, and outcomes was made. The primary focus of the review is on new and novel applications of tissue engineering for discogenic pathology, and the studies were included in accordance with this theme.

Results

Platelet-rich Plasma

Platelet Rich Plasma (PRP) has been used clinically for many years with few adverse effects. Many studies have been published showing PRP's efficacy in osteoarthritis, cartilage damage, and ligament and tendon pathologies. A recent review article covering 7 studies with 722 subjects illustrated the safety of PRP and its effectiveness in knee osteoarthritis [9]. Recent studies have begun to establish a similar pattern of safety and effectiveness of biologics in the treatment of degenerative disc disease [10].

Paglia et al and Cho et al demonstrated PRP induced arrest in the degeneration of intervertebral disc tissue in rabbit and porcine models [11,12]. As noted in Paglia et al's study, infusion of platelet derived growth factor BB into a gel scaffold prevented the progression of disc degeneration in a rabbit model [11]. While in Cho et al's study, PRP was demonstrated to both decrease the expression of proteolytic matrix metalloproteinases that contribute to disc degeneration, and increase synthesis of the major components of the extracellular



Figure 1: Whole blood draw for PRP processing.

matrix in an *in vitro* porcine model[12].

Human trials have shown that the effects of PRP translate to notable improvements in pain and function. A prospective clinical trial followed 22 patients with discogenic low back pain up to 6 months, and showed promising results with 47% of patients showing a 30% improvement in their Oswestry Disability Index (ODI) score, and 63% of patients showing a Visual Analog Scale (VAS) improvement of at least 20 mm [13]. The authors explained that 6 months may not be enough time for the mechanism of action of PRP to fully take effect. A recent case series demonstrated structural improvement in the disc in addition to similar functional improvements noted in other studies [14]. A prospective, double-blind, randomized controlled clinical trial of 47 patients followed the subjects for a longer length of time and showed that patients were still continuing to show improvements up to 2 years both in pain and function with no adverse effects among the group [10,15].

Formica et al reviewed studies from 2007-2012 about the preclinical evidence on the use of PRP in intervertebral disc degeneration [16]. They found all included studies lead to positive preclinical results, however due to the lack of standardization of methodology, they were unable to reach a conclusion regarding the consistency and replication of PRP induced effects in intervertebral disc degeneration. This lack of standardization of methodology and analysis is an issue that is pervasive in the study of autologous biologics. Since the factors at play in healing and regeneration are still unknown, clinicians continue to test new combinations, contributing to the heterogeneity in composition, techniques and outcomes.

Bone marrow aspirate

Bone marrow is an important and commonly used source of Mesenchymal Stem Cells (MSCs), and though more technically demanding as compared to PRP, is still relatively easy to collect via a well-established process (Figure 2). Post-collection processing is simple, lending to its popularity as a treatment option for degenerative conditions.

In a novel approach addressing both mechanical and physiological aspects of IVD, Pirvu and colleagues seeded Bone Marrow-Derived Mesenchymal Stem Cells (bMSCs) into a scaffold, and sutured a



Figure 2: Technique of extracting bone marrow from iliac crest using sterile technique.

membrane to an annulus fibrosus lesion in a bovine model. Their technique showed a restoration of disc height, an improvement in the extracellular matrix environment with an upregulation of anabolic gene expression, and downregulation of catabolic gene expression [17].

BMC has shown to be effective in human trials in decreasing pain, improving function, and stopping structural deterioration. A small pilot study of 10 patients showed a rapid improvement in pain and function after injection of BMC into the Nucleus Pulposus (NP), showing the validity of bMSCs as a safe treatment option for degenerative disc disease as compared to conventional treatments such as spinal fusion or disc replacement [18]. In a 2015 study, Pettine and colleagues performed intradiscal autologous BMC injections to treat 26 patients for discogenic back pain. All 26 patients had a reduction in pain with ODI and VAS score improvements of 71% and >64% respectively and 21 patients avoided surgery at two-year follow-up. Additionally, 20 of 20 patients rescanned by MRI at one year showed halted progression of degenerative disc disease [19]. The study also showed a positive correlation between higher MSC concentration and pain relief. Another randomized controlled trial followed 22 patients up to one year after treating half with allogeneic bMSCs which were isolated and expanded before being injected intradiscally [20]. Efficacy was only 28%, but showed a bimodal distribution of responders and nonresponders, suggesting an unknown factor at play in the injectate. At one year follow-up, the responders showed statistically significant improvements in pain, function, and imaging.

BMC shows great promise as a simple, effective, single step intradiscal injection in treating degenerative disc disease and

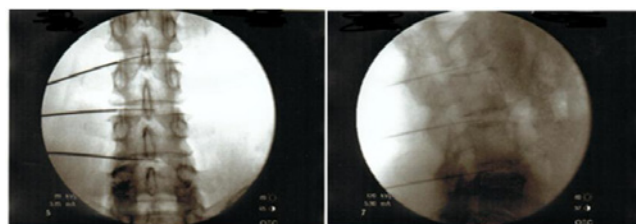


Figure 3a

Figure 3b

Figure 3a & 3b: AP (3a) and lateral (3b) Fluoroscopic images of Intradiscal biologic injection.

researchers are planning on expanding research (Figure 3a & 3b). After completing a phase 2 study [21], a phase 3 clinical trial called the CASCADE trial was started in March 2015 that aims to test the effectiveness of bMSC intradiscal injections with and without hyaluronic acid in 360 human subjects in centers across the United States [22]. The trial is expected to take about four years to complete data collection.

Lipoaspirate

Lipoaspirate has been the focus of recent studies in autologous biologic research due to a number of promising characteristics. Adipose Tissue-Derived Mesenchymal Stem Cells (aMSCs) are easier to harvest as compared to bone marrow aspirate. aMSCs contain a higher frequency of MSCs versus bone marrow-derived MSCs [23]. aMSCs have also been shown to be more potent immunomodulators compared to bone marrow-derived MSCs (bMSCs) at equal cell numbers [24]. These characteristics as well as the aMSCs' ability to differentiate into a nucleus pulposus-like phenotype make aMSCs an attractive single-step treatment option for degenerative disc disease [25].

Bone marrow and adipose-derived MSCs have recently been therapeutically characterized according to their differentiation ability [26]. While aMSCs and bMSCs were found to have the same colony forming ability, aMSCs have greater proliferative potential than bMSCs. More pragmatic, however, were specific applications they found for each cell type based on differentiation ability. bMSCs were found to have a higher capacity for osteogenesis and chondrogenesis. aMSCs were better for immunomodulatory applications [24,26]. Both bMSCs and aMSCs were found to be equivocal at adipogenesis.

Despite a lesser ability to differentiate into chondrocytes compared to bMSCs, aMSCs are still multipotent cells and thus have potential in degenerative disc disease. A group of researchers recently published two separate studies regarding the differentiating characteristics of aMSCs. With the knowledge that type I collagen exists in degenerative Nucleus Pulposus (NP), and type II collagen exists in normal NP, it was found that a culture medium of type II collagen promotes differentiation of aMSCs into a NP-like phenotype [27]. Adipose-derived stromal cells (ADSCs) may also have a protective ability on degenerating NP cells. *In vitro* experiments show that ADSCs may provide mechanical protection, subsequently decreasing degradation enzymes and inflammatory factors and increasing expression of genes and proteins involved in maintaining Extra Cellular Matrix (ECM) integrity [28]. These are important and essential characteristics for MSCs given the harsh microenvironment of the discs.

Several recent studies have shown the beneficial effect of combining lipoaspirate with plasma or PRP by keeping the adipose cells viable and stimulating the proliferation of stem cells. The addition of PRP or plasma to lipoaspirate has been proposed to be more effective [29-31].

Protein based therapy

Proteins have been an area of interest for researchers studying IDD for years, as well as recent studies, as the LMP-1, Fox C2, MMP-3 and TIMP-1 proteins have been discovered to be involved in disc

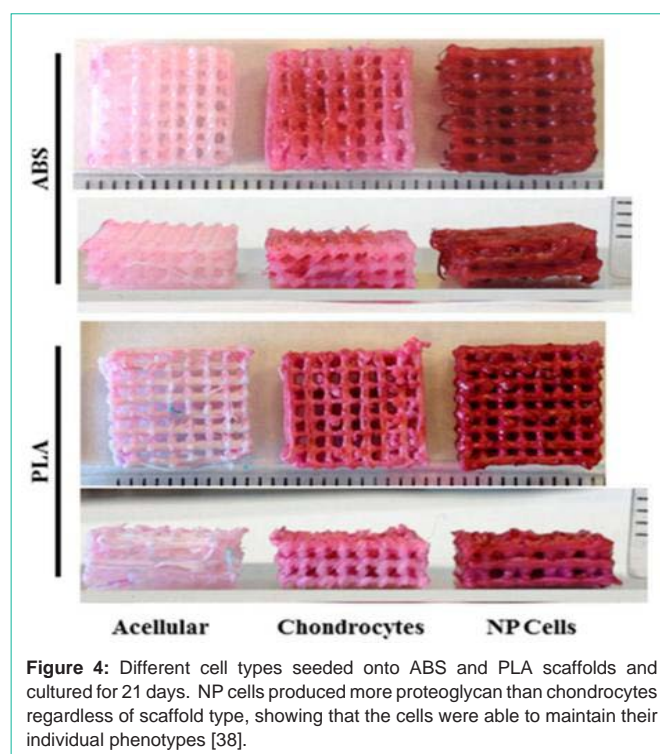


Figure 4: Different cell types seeded onto ABS and PLA scaffolds and cultured for 21 days. NP cells produced more proteoglycan than chondrocytes regardless of scaffold type, showing that the cells were able to maintain their individual phenotypes [38].

degeneration and repair.

A study by Liu et al showed that LIM Mineralization Protein-1 (LMP-1) suppresses TNF- α induced intervertebral disc degeneration by maintaining nucleus pulposus extracellular matrix production and inhibiting matrix metalloproteinases expression [32]. This effect was via up-regulation of matrix genes expression at least partially through ERK1/2 activation, and down-regulation of MMPs expression through NF- κ B inhibition.

FoxC2 is a gene responsible for cell proliferation and differentiation, and is correlated with increased expression in disc degeneration. BMP-7 is a growth factor known to promote anabolism of intervertebral disc ECM. FoxC2 has a strong synergistic effect on BMP7-mediated anabolism, and combination therapy with the two together shows promise in degenerative disc disease [33]. Wang et al opined that future studies are warranted to elucidate the relationship between FoxC2 and other signaling pathways and crosstalk between them.

A recent study suggested that MMP-3/TIMP-1 imbalance is involved in IVD herniation [34]. They showed that MMP-3/TIMP-1 ratio was higher in cell supernatant from disc herniation cultures than from cell supernatant of degenerated IVDs. Accurate localization of MMP-3 and TIMP-1 was suggested, as well as investigating its relationship with macrophages which are likely involved in IVD degeneration through MMP-3 secretion.

Scaffolds

A natural target for degenerative disc lesions is to address the macro level damage and repair the annulus fibrosus itself. The annulus fibrosus injury needs additional support and structure for satisfactory long-term results due to the mechanical stresses placed upon the structure. At the micro level, injectable scaffolds and/or *in*



Figure 5: BioScaffolder 3D bioprinter by GeSiM [43].

situ forming scaffolds can provide structural support to the MSCs injected into the intervertebral space (Figure 4). The scaffold should be as similar as possible to the natural ECM in both composition, as well as matching physical properties to maximize regenerative potential [35]. Several suitable injectable scaffold have been found that show potential. Nowotny et al proposed a composite scaffold of 20% fibrinogen and 9% thrombin that is compatible with bMSCs and PRP [36]. Numerous biocompatible natural and synthetic injectable scaffolds have been studied including natural proteins of chitosan, alginate, collagen, and synthetic polymers like polyethylene glycol, poly N-isopropylacrylamide, pHEMA-co-APMA grafted with polyamidamine. These natural and synthetic polymers can also be cross-linked with HA, aggrecan, elastin-like polypeptide, or chondroitin sulfate [37,38]. These scaffolds assist in prevention of injectate migration, enhance adhesive strength, and help cellular survival by providing a healthier ECM microenvironment to combat the harsh environment of a surrounding degenerating disc.

Li et al reviewed both biocompatible and synthetic materials and combinations of the two and found varying degrees of success in cell differentiation, expansion, and gene and protein expression. The most promising prospect was an alginate/chitosan scaffold which produced good cell growth and ECM deposition [39]. Pirvu et al used a poly(ester-urethane) membrane as well as a scaffold seeded with bMSCs to address the mechanical compressive and shear forces in the spinal column to prevent mechanical herniation of the nucleus pulposus to maintain disc height after implantation of the scaffold in a bovine model [17].

In the avascular, nutrient-poor environment of the intervertebral disc, it may be beneficial to supplement the supply of nutrients via injected or implanted materials. One method recently studied involves infusing a construct with the helper agent of choice. *In vitro* experimentation with a polymer scaffold embedded with electrospun nanofibers of strontium showed enhanced mineralization and osteogenesis of MSCs via slow elution of strontium nanoparticles [40]. Another experiment involving a polyethanol glycol hydrogel designed to slowly release BMP-2 and VEGF showed a sharp increase in the differentiation of MSCs into osteoblasts and endothelial cells [41]. A proof-of-concept experiment used an additive 3D printing technique to create discs of Polylactic Acid (PLA) loaded with variable levels of nitrofurantoin and hydroxyapatite [42,43]. The discs were able to

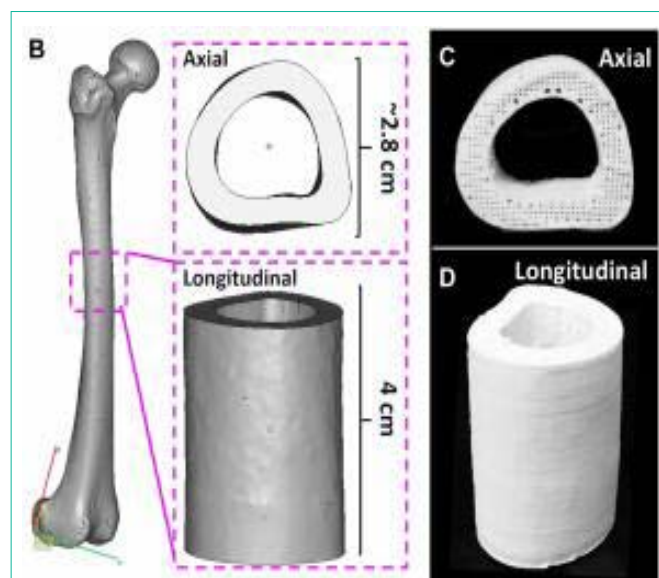


Figure 6: Printed portion of 3D model of adult femur [46].

From Jakus *et al.* Hyperelastic "bone": A highly versatile, growth factor-free, osteoregenerative, scalable, and surgically friendly biomaterial. *Science Translational Medicine*. 2016; 8(358). Reprinted with permission from AAAS.

inhibit growth of *S. aureus* in a bacterial suspension, and exhibited a drug release rate that correlated with increased drug content of the feedstock material. These methods hold promise and as technology and techniques improve, it may be possible to miniaturize these constructs into an injectable form for minimally invasive techniques.

3D printing

3D printing has existed for decades, and recent industry efforts aiming to place a 3D printer in every home has made the burgeoning industry part of everyday jargon. The intrinsic precision of 3D printing methods and versatility of materials used for printing lend itself to many biologic research applications where the cellular level scale matches well with the precision of the printers (Figure 5). Many of these new materials are finding their way into regenerative medicine applications, and have been the target of several current research projects.

Biologic applications of 3D printing may use different approaches such as a feedstock material made of a combination of both synthetic and natural materials, a printed product made of single-substance feedstock material combined with complementary natural materials after printing, or several printing techniques combined together [44,45]. A 3D bioprinted scaffold of alginate and gelatin infused with aMSCs successfully demonstrated bone matrix formation in mouse models [42]. Another 3D printed elastic scaffold of synthetic hydroxyapatite and polycaprolactone or poly(lactic-co-glycolic acid) have shown rapid tissue integration with an intrinsic ability to stimulate osteogenesis by simply surgically implanting the scaffold in mouse and rhesus monkey models, without requiring any post-printing additions or processing other than trimming and shaping (Figure 6). This scaffold has also shown to mediate MSC adhesion and proliferation and show promise for use in bone reconstruction [46]. In another example, 3D printed collagen scaffolds post-processed by a cross-linking technique and infused with aMSCs have shown to

Lesion Type	Pt Age	Lesion Age in Months	Activity Level	Centrifuge Type	Platelet count (x million/ μ L)	ml Injected	Leucocyte Content	RBC content	Platelet Activation	# of Injections	Injection Interval in Weeks	Adjunct Therapies Used	ODI Score Baseline	ODI Score @ 6mo	ODI Score @ 1yr	ODI Score @ 3yr
1 Knee OA	65	130	8 Harvest	1.00	4.0 High	3.5			3	4			50	48	35	32
2 Supraspinatus tendon tear	35	4	8 Mitek	1.70	0.6 Low	2.6			1	0			42	43	38	24
3 Hip OA	70	96	6 Autospin	1.35	5.0 High	2.0			3	5			62	59	54	37
4 Meniscal tear	55	6	11 Arthrex Ange	2.20	5.0 High	4.0			2	3			19	19	16	12
5 Hamstring tear	25	1	18 Rotofix	2.00	2.0 High	3.5			1	0			73	72	56	48
6 ACL tear	47	10	10 Celling Biosc	1.85	4.5 High	2.0 Heat			1	0			25	26	20	17
7 Lateral Epicondylitis	34	5	14 Generic	1.75	0.5 High	1.5			1	0			53	47	34	28
8 ACL tear	36	26	13 Generic	2.20	4.0 High	3.0			3	3			23	25	15	13
9 Annular tear	56	7	8 Harvest	2.50	1.5 High	3.5			1	0			53	50	34	28
10 Disc protrusion	45	3	13 Mitek	2.15	1.5 High	1.5 Heat			1	0			52	50	32	26
11 Degenerative disc	62	18	3 Arthrex Ange	1.95	1.0 High	2.5			3	3			57	55	34	28

Figure 7: Sample database table with simulated PRP data.

mediate differentiation of the aMSCs into NP-like phenotypal cells [46,47].

Gene therapy

Gene therapy has been used clinically for several years. There have been continual advances in gene mapping and editing including manipulation of nucleic acids and in some cases, entire genomes, leading to expanding applications of gene therapy in human diseases. In its natural course, gene therapy has found its way into the challenging IDD world. There have now been numerous studies demonstrating the efficacy of gene therapy via viral vectors in the treatment of IDD. *In vitro* and *in vivo* studies of various factors delivered with adenovirus or lentivirus vectors, including BMP-7, SOX9, GDF-5, TGF- β 3, CTGF, and TIMP1 have shown to improve the intervertebral disc extracellular environment with increased synthesis of type II collagen, glycosaminoglycan, and aggrecan [48-51]. However, no clinical studies were found for viral associated gene therapy in the treatment of IDD in our literature search. The unavailability of viral vector induced gene therapy for clinical use stems from the inherent risks with its use including immunogenicity, toxicity, and possibility of insertional mutagenesis [52].

We anticipate this field to rapidly advance over the next few years as health care progresses towards precision medicine with demands of tailoring the biologic therapy to the disease process and the individual.

Predictive analytics

The use of machine learning algorithms holds great promise in this rapidly advancing field, and the authors recognize the need for a methodology that aims at adapting the dynamic interaction of various human and biologic factors in providing an accurate outcome estimate for an individual.

Its great potential may be illustrated in a recent experiment where Wigley et al used machine learning algorithms to read sensors and control input parameters to produce Bose Einstein Condensates (BECs) [53]. The algorithm not only eliminated variables that it found to be nonessential, but produced higher quality BECs in larger quantities, and produced them faster with each iteration. The algorithm also found pathways to producing BECs that humans

had not considered in their decades of speculation and research. Navani et al recently applied statistical analysis and machine learning algorithms to predict modified chronic pain and disability scores based on a subject's engagement in health behaviors [54].

Through analysis of global registries of variables such as age, sex, type and extent of pathology, characteristic and quality of biologic and outcomes, machine learning algorithms applied to regenerative biologics could help leapfrog past years of trial-and-error experimentation, and establish a standard of care regarding application of each regenerative biologic formulation to specific disorders and patient populations. Several supervised and unsupervised statistical algorithms along with artificial intelligence decision trees are particularly well suited to distill a large number of variables down to the select few that are critical to reaching better outcomes. Also, the algorithms are not burdened with needing to understand the physiology, and thus are likely to generate correlations that humans may not have considered, and may not initially understand.

The authors propose development of a central database to report and track method and outcomes of all biologic treatments and implementation of comprehensive, intelligent, and predictive analytic models to allow consumers and healthcare providers to make evidence based choices about the biologics and treatment methods for forthcoming precision medicine. A sample database is illustrated for PRP injections in Figure 7.

Functional imaging

Humans are inherently visual beings with the ability to gather and process large amounts of information at a glance of the eye. By using specialized cellular probes, functional imaging enables scientists to see the cellular physiology that is hidden to traditional imaging techniques. Functional imaging has been a powerful tool in regenerative medicine, allowing visualization of gene expression, cell viability, cell differentiation, cell concentration, cell migration and engraftment, and other valuable parameters. There are currently several stem cell tracking probe types and several imaging modalities with their own advantages and disadvantages that make each combination ideal for specific applications. Leahy et al reviewed many of these imaging combinations and predict that advancement in this field will come from development of new labels and multimodality

Table 1:

Year of Publication	Author	Journal	Conclusion	Reference
PRP				
2016	Sadabad	Electronic Physician	Meta-analysis of HA vs. PRP of 722 pts 2 years after treatment showing PRP is better than HA	[9]
2016	Monfett	International Orthopaedics	Intradiscal PRP injections show continued safety and improvements in pain and function at 2 years post-procedure	[10]
2016	Paglia	Spine	Primary component of PRP, PDGF-BB halts degeneration of IVD in a rabbit model	[11]
2016	Cho	Artificial Organs	PRP decreases expression of degradatory enzymes and increases synthesis of ECM proteins in a porcine model	[12]
2015	Levi	Pain Medicine	Prospective trial of 22 patients show improvement of pain and function at 6 months after PRP intradiscal injection	[13]
2015	Navani	Techniques in Regional Anesthesia and Pain Management	Intradiscal PRP injections are safe, improve function, and may improve structure on imaging at 6 months follow-up	[14]
2016	Tuakli-Wosornu	American Academy of PM&R	Intradiscal PRP injection show safety and improvements in pain and function at 1 year post-procedure	[15]
2015	Formica	European Spine Journal	Lack of standardization in PRP use leads to inconclusive results treatment of DDD	[16]
BMC				
2014	Pirvu	Biomaterials	Multi-factor approach using bMSCs, scaffold, and annulus fibrosus repair improves ECM environment and restores disc height	[17]
2011	Orozco	Clinical and Translational Research	bMSCs are a viable alternative to traditional DDD treatments but are simpler and more conservative	[18]
2016	Pettine	International Orthopaedics	bMSCintradiscal injections are safe, and improve pain, function, and structure	[19]
2016	Noriega	(Epub ahead of print)	Prospective randomized trial of 22 pts shows poor efficacy, but improvements in pain, function, imaging in those who respond to allogeneic bMSCintradiscal injection	[20]
2011	Mesoblast, Ltd.	ClinicalTrials.gov	Phase 2 trial with 100 pts, comparing intradiscal injection of 6 million vs. 18 million cells in HA	[21]
2015	Mesoblast, Ltd.	ClinicalTrials.gov	Phase 3 trial with 360 pts, evaluating efficacy of intradiscal injection of 6 million cells with/out HA	[22]
Lipoaspirate				
2013	Murphy	Experimental & Molecular Medicine	Adipose tissue produces higher frequency of MSCs vs. bone marrow	[23]
2012	Melief	Stem Cells Translational Medicine	aMSCs are more potent immunomodulators vs. bMSCs	[24]
2008	Hoogendoorn	Journal of Cellular and Molecular Medicine	High concentration of MSCs in adipose tissue makes it an attractive treatment option for DDD	[25]
2015	Li C	Stem Cell Research & Therapy	aMSCs and bMSCs have biological advantages and applications for each type	[26]
2016	Tao	BioFactors	Higher proportion of collagen type II promotes hADMSCs differentiation into NP cells	[27]
2015	Sun	International Journal of Biological Sciences	<i>In vitro</i> experiment shows adipose stromal cells provide mechanical protection for NP cells and subsequent changes in gene and protein expression	[28]
2016	McClish	TOBI 2016	PRP allows expansion of MSCs in lipoaspirate. Mechanical lipoaspirate processing method.	[29]
2015	Stillaert	International Journal of Surgical Reconstruction	Autologous plasma prevents fat graft resorption	[30]
2016	Pak	BioResearch Open Access	aHSCs, homogenized ECM, HA, plus PRP can regenerate cartilage-like tissue in knee OA	[31]
Protein Based Therapy				
2015	Liu	Journal of Orthopaedic Research	Lentivirus encoded LMP-1 maintained extracellular matrix production under TNF-alpha induced inflammation	[32]
2016	Wang Z	PLOS One	FOXC2 works synergistically and potentiates BMP7-mediated ECM anabolism in NP cells	[33]
2012	Baillet	Joint Bone Spine	A MMP-3/TIMP-1 imbalance is involved in IVD herniation	[34]
Scaffold				
2014	Godwin	International Journal of Biochemistry and Cell Biology	The ideal ECM for regeneration is as similar as possible to natural ECM	[35]
2016	Nowotny	Journal of Applied Biomaterials & Functional Materials	BMC and PRP can be combined with 10-20% fibrinogen and 5-25% thrombin to form a injectable scaffold	[36]
2015	Wang F	Stem Cells and Development	Injectable scaffolds provide both survival and mechanical support to injected stem cells	[37]
2016	Li X	College of Physicians and Surgeons	Methods using biocompatible, synthetic, and combinations of both have benefits and disadvantages for each	[39]
2016	Meka	Colloids and Surfaces B: Biointerfaces	Nanofibers can elute strontium in a predictable manner for augmentation of osteogenesis	[40]
2016	Barati	Journal of Controlled Release	Experimental hydrogel slowly releases BMP-2 and VEGF and sharply increases differentiation of MSCs into osteoblasts and endothelial cells	[41]

2016	Wang XF	PLOS one	3D bioprinted constructs consisting of hASCs may be useful for applications in bone tissue engineering	[42]
3D Printing				
2015	Do	Advanced Healthcare Materials	A multitude of 3D printing technologies are currently available and are finding novel applications in biologic research	[44]
2015	Water	Journal of Pharmaceutical Sciences	3D printed constructs infused with drugs shows promise for controlled release applications	[45]
2016	Jakus	Science Translational Medicine	Novel biocompatible compound for 3D printing with intrinsic osteogenic inducing characteristics	[46]
2016	Zhou	Society for Biomaterials	Collagen type II scaffold cross-linked with EDAC/NHS provides the best skeleton for ADSCs to grow on	[47]
Gene Therapy				
2015	Ren	Genetics and Molecular Research	Adeno-associated virus mediated BMP-7 and SOX9 co-transfection had a synergistic therapeutic effect on intervertebral disc degeneration (<i>in vitro</i>)	[48]
2015	Luo	Journal of Zhejiang University Science B	Adenovirus mediated GDF-5 promoted the production of the extracellular matrix in human nucleus pulposus cells	[49]
2016	Liu	Experimental and Therapeutic Medicine	Lentivirus mediated TGFβ3-P2A-CTGF-T2A-TIMP1 promoted synthesis of aggrecan and type II collagen	[50]
2016	Yue	Spine	Lentivirus mediated survivin, TGFβ3, TIMP1 injection had a weak effect on slowing degeneration in an intervertebral disc degeneration rabbit model (<i>in vivo</i>)	[51]
2015	Priyadarshani	Osteoarthritis and Cartilage	Review article studying stem cells, gene therapy, and scaffolds.	[52]
Predictive Analytics				
2016	Wigley	Scientific Reports	Machine learning algorithms can achieve a goal faster, simpler, and of better quality using techniques not considered by humans	[53]
2016	Navani	Journal on Recent Advances in Pain	Changes in chronic pain can be accurately predicted by a machine learning algorithm based on exercise, nutrition, and depression	[54]
Functional Imaging				
2016	Leahy	Stem Cell Research and Therapy	Review of functional imaging modalities	[55]

aMSC: Adipose tissue-derived MSCs; bMSC: Bone Marrow-derived MSCs.

imaging approaches that can combine the strength of each modality for a comprehensive detection [55]. They also anticipate the importance of developing advanced label-free imaging techniques and techniques which employ non-toxic cellular contrasting agents.

Discussion

Intervertebral disc degeneration is a multifactorial process involving changes in disc composition, structure, and function. Such changes may include progressive loss of proteoglycans and water content in the nucleus pulposus, filling of the nucleus pulposus space with fibrocartilage, disruption of the annulus fibrosus, and osteophyte formation in adjacent vertebral bone. Following disc disruptions, there is very limited capacity of the disc to heal or restore structural integrity [56]. Changes in the IVD cell population have been implicated as the cause of IVD degeneration. The repairing capacity of the IVD has been debated extensively over the years. The disc Notochordal Cells [NC], which are primarily responsible for producing cells of the nucleus pulposus of the intervertebral disc, are noted to diminish in time thus resulting in disc degeneration [57]. Degeneration seems to be initiated with an increase in matrix degradatory factors like TNF alpha, IL-1, MMPs etc. [58]. The biochemical environmental changes occurring inside the discs leading to IVD are unknown. Some researchers speculate a complex interactive phenomenon between growth factors, genes and proteinases as the cause [59]. Due to the unpredictable rate of the chemical and structural changes, the outcomes from the biologic therapy are not always reliable. The efficacy of intradiscal PRP seems to rely on the presence of viable and functioning resident disc cells which have been shown to decrease during the progression of the IVD degeneration. Adult mesenchymal stem cells, capable of differentiating down the discogenic lineage have been considered by some as a suitable source for IVD tissue

engineering. However, several questions such as ensuring correct lineage, providing adequate environment for cell sustainability and optimal functioning in a harsh disc environment are still open for debate.

Intradiscal biologics have shown promise in restoring the progression of disc degeneration. Studies on Intradiscal PRP have demonstrated the potential of improving pain and functional outcomes [15]. Bone marrow MSCs shows promise in the treatment of DDD by improving disc height and ECM in animal studies, and significantly improving pain and function in human trials [19]. Lipoaspirate, through its higher content of MSCs, better immunomodulating properties and its capacity to differentiate into a nucleus pulposus-like phenotype, may seem a more attractive option for intradiscal degenerative disc treatments. Studies on specific proteins like LMP-1, Fox C2, MMP-3, TIMP-1 and BMP 7 show their role in supporting anabolism and halting catabolic process involved in disc degeneration. Using a scaffold, whether biocompatible, synthetic materials, or any combination of the two, adds structural support and increases chances of cellular survival. The use of 3D bioprinting in generating a more precise scaffold, and infusing them with MSCs and important growth factors for slow release makes for a potentially powerful treatment for disc degeneration.

There is a current lack of standardization of methodologies, protocols and reporting systems which makes analysis and evidenced based recommendations difficult. Although there have been some efforts in the past including PRP classification systems [60], there is no current consensus on the different biologic preparations. The authors foresee a need for a global registry with central database system to measure outcomes, predict trends, and develop protocols using statistical algorithms and machine learning tools.

Despite the challenges, positive outcomes have been noted with repair and restoration of discs in *in vitro* and *in vivo* animal studies and improved pain and functional clinical outcomes in human studies. The quest for more specific and effective therapies will continue as we gain more knowledge about the pathophysiology of the discs and the role of biologics therein. With the acquisition of more knowledge in this field, regenerative medicine may help beyond our own Earth, and assist us in understanding the effects of gravity on our mechanisms for repair or renewal in space [61].

We anticipate future research in this field to answer questions about the optimal nature and concentration of growth factors and cells for a specific discogenic condition, customize the ideal solution for a particular individual or pathology, and incorporate complementary and adjuvant therapies for the ideal outcome. With the advent of precision medicine, we foresee integration of biologics and technology via artificial intelligence machine learning algorithms for the best clinical outcomes. Regenerative medicine has the potential to revolutionize the way we approach spine care in patients and we propose collaboration among all stakeholders in order to make an impactful move into this new era.

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