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Regeneration Potential of Stem Cell in the Treatment of IVD

Riya Bhanushali[1], Siddhi Chugh[2], Arpita Adhikary[3], Ayushi Bhanushali[4], Mehvish Salman[5]
[1]*M.Sc. (H)Microbiology, Veer Narmad South Gujarat University, Gujarat, India*
[2]*M.Sc. (H) Stem cell & Tissue engineering, Panjab University, Chandigarh, India*
[3]*M.Sc. (H) Biotechnology, Visva-Bharti University, West Bengal, India*
[4]*B.Tech. Biotechnology, D.Y. Patil University, CBD Belapur, Maharashtra, India*
[5]*B. Sc. Life science, Integral University, Lucknow, India*

Abstract: ***Degenerative disc disease is a prevalent musculoskeletal disorder in which damaged spinal discs cause pain upon aging, accidental injuries. Spinal discs connect adjacent vertebrae and help in maintaining mobility, flexibility and rotation of spinal cord. Spinal discs also act as shock absorbers. Intervertebral disc (IVD) degeneration is often associated with low back and neck pain, which accounts for disability worldwide. Physical therapy, spinal fusion surgeries reduce severity and symptoms of degenerative disc disease but they are not complete cure for this disease. Current preclinical studies show that mesenchymal stem cells have the capacity to repair degenerative disks by differentiation to chondrocyte-like cells, which produce proteoglycans and type II collagen. Mesenchymal stem cells (MSCs) isolated from bone marrow (BM-MSCs), adipose tissue (AD-MSCs) and umbilical cord (UC-MSCs) show potential use in cartilage and intervertebral disc (IVD) repair. Regenerative medicine and stem cell therapy hold great promise for treatment of intervertebral disc (IVD) disease. This review discusses about progression of degenerative disc disease, various types of stem cells, potential use of mesenchymal stem cells (MSCs) and embryonic stem cells (ESCs) for the treatment of degenerative disc disease. This review also focuses upon challenges encountered by the application of stem cell therapy for treating degenerative disc disease as well as future perspectives.***
Keywords: IVD, Stem cell therapy, AF & NP cells, MSCs, Scaffolds, Cell therapy

I. INTRODUCTION

Intervertebral disc degeneration cover an annual worldwide socioeconomic impact as low back pain of over 70 billion. This disease has a high frequency over the working age class, which raises the impact bar over the years. A biochemical negative tendency of catabolic-anabolic balance gets triggered on acute physical trauma or prolonged intervertebral disc mistreatment, that progress to a chronic degeneration disease.

There is an urgent need of regenerative strategies in the treatment area. IVDs are fibro-cartilaginous tissues connecting the vertebral bodies, contributing about one-third of spinal length & play an important role in spinal functioning by providing stability while permitting motion between the vertebrae [1, 2]. Signs of disc degeneration detectable with MRI are decreased water content, a reduction of disc height, and bulging of the disc. The decrease in extracellular matrix produced by the cells in the IVD is the mechanism leading to these changes.

Among the biological disc repair therapies, cell therapy has gained more interest. Cell therapies approach in addressing disc inflammation by inhibiting aberrant cytokine production; disc rehydration and height restoration by initiating matrix anabolism, repopulating and stimulating the native cells [3]. Mesenchymal stem cells (MSCs) have immunomodulatory functions and ability to differentiate into cartilage, so, are considered as a potentially ideal cell source for IVD regeneration. Preclinical studies by using cell therapy have showed promising results in animal models [4, 5]. Transplanted MSCs have shown to restore normal disc environment by inducing production of extracellular matrix proteins, including aggrecan and other proteoglycans, and type I and II collagen [6, 7]. Pilot trial in humans with MSCs of autologous origin has indicated feasibility, safety, and improvement in clinical outcomes, including improved water content [8].

Freburger *et.al*. [9] found a 6.3% increase from 3.9% to 10.2% is reported chronic low back pain between 1992 and 2006. This increase is concerning and underscores the exploration of new treatment modalities with respect to costs and discomfort associated with chronic low back pain. Initially, cell therapy promises a greater potential for intervertebral disc regeneration, but there is a shortage of strong clinical evidence.

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II. DD/IVD DISEASE PROGRESSION

Numerous studies shows that the prevalence of LBP (Low Back Pain) increases with age, along with many other musculoskeletal disorders, including OA (Osteoarthritis) is likely increase due to a global aging population, changes in lifestyle & occupational stress. Also, genetic predisposition and environmental factors, including smoking, obesity and abnormal mechanical loading, have been implicated in the pathogenesis of LBP [10, 11, 12].

III. STRUCTURE OF IVDS

The IVD (Inter Vertebral Disc) that is localized between adjacent spinal vertebrae, plays vital role in the flexibility and mechanical integrity of the spine by virtue of the opposing forces generated by its two main components;

1) *Nucleus Pulposus (NP):* Gel like, central, hydrophilic, primarily consisting of aggrecan, proteoglycan and rich in extracellular matrix of type II collagen- that attract & bind to water. It includes small chondrocyte-like cells and a second population of cells termed notochordal cells. These unique cells are most likely to act as remnants of the embryonic notochord cells which guided the development of the spine and IVDs.
2) *Annulus Fibrosus (AF):* Tough, peripheral, fibrous, type I collagen-rich, contain nerves & absorb the small molecules and nutrients required for the disc cells.

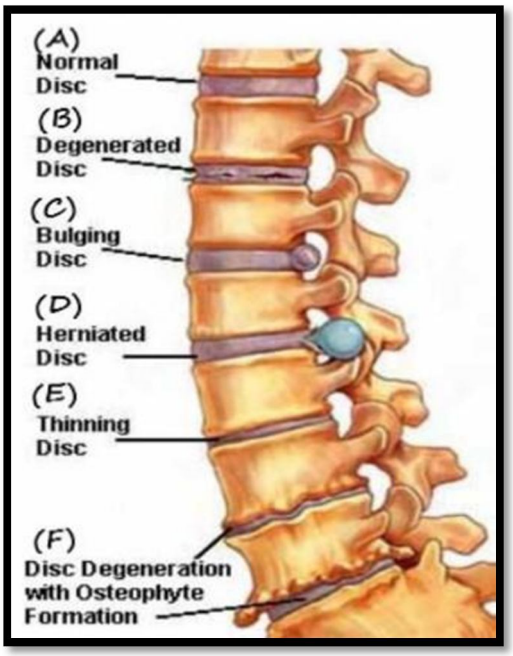

Figure 1: Examples of IVD problem
(https://images.app.goo.gl/vz9f22WT6pJTE6NMA)

Most of the AF cells arise from the mesenchyme and exhibit numerous characteristics of fibroblasts and chondrocytes, such as the ability to synthesize type I and II collagen and aggregating proteoglycans [13]. The AF may prevent the NP & its content from **herniating** or leaking out of the disc by hydraulically sealing the nucleus and by evenly distributing any pressure and force imposed on the IVD [14].

Together, the NP and AF provide both fluid and viscoelastic properties uniquely suited to the role of the IVD of the spine [15]. The human spine is composed of 23 IVD that separate the vertebrae which provide flexibility. Discogenic low back pain (**DLBP**) is defined as having one or more intervertebral discs that is identified as the root cause of the pain.

IV. BIOLOGY OF INTERVERTEBRAL DISC DEGENERATION

1) Early disc degeneration is characterized by the loss of notochordal cells and their replacement by fibroblast-like cells has also been implicated in the later stages of IVD degeneration [16]. The normal IVD is a relatively acellular tissue with the average cell density of 5.8×10^3 cells/mm^3 (NP is 4×10^3cells/ mm^3 and AF is 9×10^3cells/ mm^3) that decreases significantly.
2) *Cell Death:* Over the past few years, increasing evidence has indicated that cell death contributes to degenerative disc disease [17], spinal degenerative disease, and intervertebral disc (IVD) degeneration. In IVD degeneration, the rate of matrix anabolism and catabolism decreases & increases, respectively. Because of nutrients and oxygen tension within the disc, cells become partially anaerobic, which leads to high lactic acid concentrations, low pH conditions & also produces nitric oxide (NO), reactive oxygen species (ROS) [18]. As a result proteoglycan content as well as recollecting water ability of the ECM drops & reduces the nutritional supply to the disc [19]. Consequently, changes to the microenvironment, pH, nutrient depletion (especially glucose) and stress result in cell death during IVD degeneration.

3) *Signaling Pathway Regulation:* A broad range of molecular factors, including cytokines, growth factors, inflammatory mediators, proteinases and their inhibitors, soluble or insoluble adhesion molecules, are present within the IVD microenvironment [20]. Elevated level of inflammatory mediators like Interleukins (IL-4, IL-6, IL-8 & IL-12), tumor necrosis factor (TNF-α), Interferon (IFN-γ), cytokines, cathepsins, aggrecanases and matrix metalloproteinases (MMPs) plays important role in the biology of the internally disrupted disc [21, 22]. A deficiency in anabolic factors, such as transforming growth factor-β (TGF-β) and insulin-like growth factor-1 (IGF-1), may cause further reduction in cellular viability and ECM synthesis [23, 24].
4) *Apoptosis:* Initially apoptosis was identified by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (**TUNEL**) assay; the IVDs obtained from the patients were having more TUNEL positive cells as compared to IVDs of healthy one [25]. Studies shows that numerous AF & NP cells undergo apoptosis in degenerative discs through the process of extrinsic and intrinsic pathway. (**Figure 2)**
5) *Autophagy:* Autophagy maintains the homeostasis, inhibits the apoptosis and prevents the senescence of nucleus pulposus cells (NPCs) [26]. Autophagy is upregulated under conditions of starvation, glucose limitation, growth factor withdrawal, high bio-energetic demands, oxidative stress, infection, or protein aggregate accumulation [27]. Autophagy are highly regulated by Atg proteins, LC 3, tumor necrosis factor-α (TNF-α) and IL-1β, which leads to cell degeneration [28, 29].

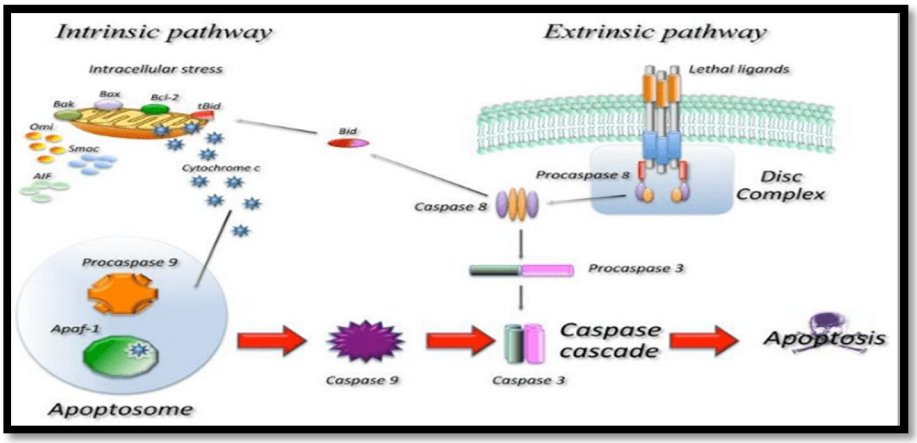

Figure 2: Intrinsic and Extrinsic pathway involved in the apoptosis of NP & AF cells of IVD.

a) *Functioning of IVD:* It is a cushion of fibrocartilage and act as the principal joint between two vertebrae in the spinal column. There are 23 discs in the human spine: cervical region, thoracic region, and lumbar region contains 6, 12 & 5, respectively. It allow the spine to be flexible without sacrificing a great deal of strength by provide a shock-absorbing effect within the spine and also prevent the vertebrae from grinding together.
b) *Sign & Symptoms:* IVD disease or DDD can cause periodic or chronic pain in the back or neck, depending on the location of the affected disc or discs. Which is often worse when sitting, bending, twisting, or lifting objects. In herniation, the protruding disc can press against one of the spinal nerves that run through the spinal cord to other body parts & can causes pain, weakness, and numbness in the back and legs. It often cause nerve pain called sciatica that travels along the sciatic nerve, which runs from the lower back down the length of each leg.
c) *Diagnosis:* The diagnosis of DDD disease is made by combination of physical and neurologic examinations, radiographs and other (advanced) imaging of the spine. To get a greater look at the discs and bony structures imaging tests like X-rays, magnetic resonance imaging scan (MRI) or computed tomography (CT) scan and a special test called electromyography (EMG) can be used.

- Bone scan is use to detect spinal problems such as osteoarthritis, fractures, or infections related to DDD. In this a very small amount of radioactive material injected into a blood vessel. That will travel through bloodstream and absorbed by bones. More radioactive material will be absorbed where abnormal activity observed, such as an inflammation. The areas with more radioactive material called “hot spots” which are detected by scanner.

- *Discogram or Discography:* It confirms or denies the disc(s) as the source of pain. Here, a harmless dye injected into one of discs. If there's any problem with disc—like it's herniated—the dye will leak out of the disc. Which can be identified through x-ray.
- *Myelogram:* In this test, a special dye injected into the area around spinal cord and nerves. (Before that happens, the area will be numbed.) Then have an x-ray or a CT scan. The image will provide a detailed anatomic picture of spine, especially of the bones, which will help to identify any abnormalities [30].

V. SOURCE OF CELLS FOR DISC REGENERATION

It is necessary to select a specific cell source for DDD regenerative therapy for the effective transfer of current preclinical research to trials towards humans. The cells are chosen based on their abundance, ease of acquisition, the capacity to differentiate into cells similar to chondrocytes, viable in the hypoxic, and the hypoglycemic environment, with limited or no immune response. They should have a low to no chance of tumor development [31]. Potential use in IVD regeneration has been described for some types of adult stem cells, including mesenchymal stem cells (MSCs) [32], Induced pluripotent cells [33], NP cells [34], embryonic stem cells [35].

1) *Mesenchymal Stem Cells:* MSCs are non-differentiated cells found in several adult tissues. The multipotent existence of individual MSCs was first demonstrated by Pittenger et al. [36], and since then it has been found to be pluripotent, giving rise to endoderm, ectoderm, and mesoderm cells [37]. MSCs are well suited for therapeutic application because they can be easily cultivated and have a high *ex vivo* expansive potential, robust, persistently engrafted, and do not involve ethical arguments.[38, 39, 40, 41] MSCs derived from different tissues are generally referred to by their organ of origin. Bone marrow stromal cells and adipose-derived MSCs are sources of MSCs in this respect [42].
2) *Induced Pluripotent Stem (iPS) Cells:* Tissue culture of immortal strains of diseased patients is an invaluable resource for medical study but is primarily confined to tumor cell lines. Here the identification of the generation of induced pluripotent stem cells from patients with a variety of genetic diseases takes place. The disease-specific stem cells give an unparalleled opportunity to recapitulate both normal and pathological human tissue formation in vitro, thus enabling disease investigation and drug production [43]. iPS cells are phenotypically similar to ES cells, the potential for differentiation, and the capability of differentiating like chondrocyte cells [44].
3) *Embryonic Stem Cells:* Eugene J. Koay identified how to chondrogenically distinguish human embryonic stem cells by using chondrogenic medium alone (CM) or CM with two growth factor regimens: transforming growth factor (TGF)-β3 followed by TGF-β1 plus insulin-like growth factor (IGF)-I or TGF-β3 followed by bone morphogenic protein (BMP)-2. The results of this study suggest that it is possible to modify the characteristics of hESC-generated tissue for specific musculoskeletal cartilage applications [45].
4) *NP Cells:* As NP cells; chondrocyte-like cells, extracellular matrix output capacity is retained after ex vivo expansion and re-implantation. The dog model demonstrated that ex vivo expanded NP cells were viable and capable of proliferating after autologous re-implantation, with evidence of proteoglycan and type II collagen production in the disc [44, 45, 46]. Secretoma analysis of Notochordal cells rich in nucleus pulposus will lead to the identification of key proteins that delay the onset of DDD. Ingenuity Pathway Analysis revealed that in vitro treatment with TGFβ1 and CTGF promoted the synthesis of healthy extracellular matrix proteins, increased cell proliferation, and reduced cell death in human degenerative disc NP cells [47].

VI. POTENTIAL RISKS ASSOCIATED WITH STEM CELL THERAPY

Stem cell therapy for IVD regeneration can be potentially associated with several risks. One experimental study showed that even after transplantation there is no sign of regeneration. X-ray and gross anatomy examination revealed large anterolateral osteophytes. Histological analysis showed that osteophytes were made up of mineralized tissue surrounded by *chondrocytes*, with MSCs classified as osteophyte-forming cells. The labelled MSCs were not present in the nucleus. Unintended differentiation and tumorigenesis is another possible danger that stem cell therapy will normally face [48]. If MSCs needed long cell expansion, chromosome stability could be impaired and the risk for tumorigenesis increased, specifically because immune surveillance is not possible [49]. In conclusion, although animal studies indicate possible disc regeneration through HSC injections [50]. The analysis states that the number of adverse effects associated with rhBMP-2 use in spine fusion ranges from 10% to 50% depending on the approach. Previous cervical fusion with rhBMP has an estimated 40% higher risk of adverse effects with rhBMP-2 in the early postoperative period, including life-threatening events. After anterior interbody lumbar fusion, the rate of implant displacement, dissidence, infection, urogenital events, and retrograde ejaculation was higher than the control when rhBMP-2 was used [51].

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VII. TREATMENT

A. Current Treatment

Current treatments for inter vertebral disc degeneration can be divided into two categories. Conservative, rehabilitation programs, nonsurgical management entails analgesics and lifestyle adjustments like weight loss. Conservative treatment includes physiotherapy and pain management. It has been the treatment option for patients with chronic low back pain. The conservative management is the most accurate method of treatment for many cases of IVD degeneration. Patients not gaining from this management can get to know about benefits from surgical fusion [52]. The rationale for pain relief with spinal fusion is not known, but it is believed that degeneration of the discs and facet joints leads to pain from the involved motion segments and that surgical stabilization leads to clinically significant improvement in pain. Furthermore, fusion surgery has significant downsides. Further the loss of flexibility between fused vertebrae, the stress and strain can also be increased by fusion on abutting discs and thus increases the rate of degeneration which facilitates surgical intervention [53-55].

Pharmacological treatments with NSAIDs, lidocaine patches, analgesics and muscle relaxants are limited in efficiency, while epidurally administered steroids and local anesthetics and percutaneous heat treatments might improve symptoms [56, 57]. Nontraditional treatment including massage and acupuncture are also commonly utilized and exercise therapy by the McKenzie method is popular amongst physical therapists [58]. IVD prosthesis replacement has been introduced as an alternative treatment modality in the cervical and lumbar regions as an option for late-stage disease [59, 60].

B. Gene Therapy

Gene therapy is an exciting technology that directs a target cell to synthesize a desired protein (growth factor) through delivery of the corresponding genetic sequence via a viral or non-viral vector [61]. Adenovirus, adeno-associated virus (AAV) and Lentivirus are the most commonly used viral vectors for gene therapy. The use of adenovirus vectors has slowly decreased and has been substituted by priority for lentivirus and AAV vectors. Recent technologies, like RNAi and CRISPR, have further raised the favor of viral vectors. The classic non-viral vectors are liposomes and their descendents are polyplex micelles and exosomes. They have higher ability for application in gene therapy for IVD degeneration.

Most of the current biological therapies aspire to restore proteoglycan level or synthesis within the degenerated IVD. For instance, the use of TGFbeta1, TIMP1, SOX9, and BMP2 has been shown to save the architecture of disc tissue and/or build up collagen and proteoglycan synthesis by adenovirus-mediated gene therapy [62, 63, 64].

C. Artificial Disc Replacement

An alternative to surgical fusion is artificial disc replacement, though long-term follow-up research needs to be conducted, as complications may occur years after the procedure. Arthroplasty involves substituting native discs with artificial discs. Arthroplasty might be dominant to bony fusion when compared to maintaining basic mobility and spine mechanics. Prevalence of adjacent disc segment degeneration (ASD). A recent systematic review, Jacobs et al. concluded there was no solid proof of superiority between disc replacement and fusion surgery [65, 66]. There are various risks such as spinal cord damage, hardware infection and inflammation of tissues which further causes degeneration of hardware [67, 68].

D. Growth Factors

The efficacy of stem cells in promoting fusion of spine is very much dependent on osteoinductive factors, especially growth factors which will improve the osteogenic ability of osteoprogenitors like Mesenchymal Stem Cells [69].

Growth factors used with mesenchymal stem cells for spine fusion [70]:

Growth Factor	MSC Type	Model
BMP-2	Adipose, BM	Rat, Rabbit
BMP-6	Adipose, BM	Mouse, Rabbit
BMP-7	BM	Rat
BMP-9	BM	Rat
GDF-5	Adipose	In vitro
FDG	BM	Rabbit

BM, bone marrow; BMP, bone morphogenic protein; FGF, fibroblast growth factor; GDF, growth and differentiation factor; MSC, mesenchymal stem cell

Platelet-rich plasma contains growth factors and has been shown to induce AF cell proliferation and matrix production [71]. Furthermore injection of platelet-rich plasma into rat and rabbit IVD has demonstrated that it may delay progression [72]. Anabolic growth factors (including TGF-b, IGF-1, OP-1, GDF5 and GDF6) promote matrix synthesis in vivo [73, 74], and a clinical trial injection of recombinant BMP-7 is currently underway whereas IL-1 receptor antagonist (IL-1RA) has shown to decrease cytokine and proteolytic enzyme production by NP cells [75, 76].

Studies show that disc cells have increased proliferation and activate matrix synthesis. The viability of disc cell might originate from the elevated growth factors in the co-culture system, which includes but is not limited to TGF-β, IGF-1, endothelial growth factor (EGF), basic fibroblast growth factor (bFGF), and various bone morphogenetic proteins (BMPs) [77, 78]. Most of these growth factors are anabolic to disc cells [79] as well as anti-catabolic (TGF-β, BMP-7), and anti-inflammatory (TGF-β, BMP-7) [80]. Treating disc cells or direct intradiscal injection with growth factors OP-1(BMP-7), GDF-5, recombinant GDF5 (rhGDF5) or intradiscal delivery of adenovirus coupled LMP-1 expression vector can elevate the proteoglycan level [81, 82].

VIII. SCAFFOLDS

Cells which are transplanted recently are subjected to tremendous mechanical loads, which might be harmful to function or growth; moreover, such loads can potentially be decreased by placing a screw or a rod construct that bridges the intervertebral disc for a limited time period. A long-term approach that can increase the survival of MSC and differentiation post-implantation is cell incorporation into a biomaterial scaffold. For example, the chemoattractant SDF-1 to recruit resident progenitor cells or MSCs to the disc [83]. Several ex vivo studies have shown that some scaffolds could potentially meet those requirements, including atelocollagen gel, hydrogel, and hyaluronan gel, in which MSCs remain viable expressing a chondrocyte-like phenotype [84, 85, 86].

A. Characteristics for an Ideal Scaffold

A scaffold for stem cells shall increase the osteogenic and osteoinductive effects of cells sent to the region of interest, preserve growth factors at that region for optimal time of release, used as an osteoconductive scaffold, not compete with or limit formation of bone, and limit inflammatory response through biocompatibility [87]. Essential scaffold properties to consider include material, geometry, pore size and porosity. Type I collagen is a natural polymer and represents the simplest of scaffolds meeting all described criteria. It has been discovered that both human bone marrow–derived and adipose–derived mesenchymal stem cells on a type I collagen sponge activated spinal fusion. Other natural polymers including matrigel [88] and hydrogels [89], synthetic polymers including polylactic-co-glycolic acid (PLGA), its derivatives, chitosan, and others have been investigated in preclinical studies as well.

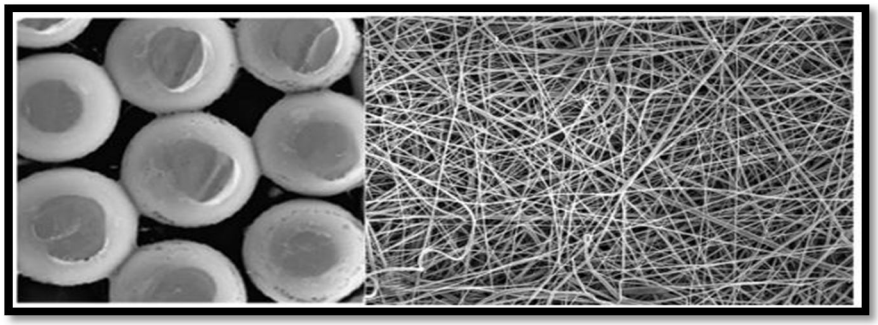

Figure 3: Scaffolds are an integral element in the development of cell-based strategies for spine fusion and disc regeneration. Two examples used in spine applications are: Polylactic-co-glycolic acid (PLGA) microspheres (Left) and a PLGA nanofiber scaffold (Right)

Biomaterials such as calcium phosphate derivatives including hydroxyapatite (HA) and beta tricalcium phosphate (b-TCP) are most popular. Researchers discovered that cell-loaded implants were powerful than cell-free implants with remarkable bone formation. More recently, Minamide *et al*. [90] and Seo *et al*. [91] both reported that bone marrow MSCs on HA scaffolds with and without growth factors induced posterolateral fusion in rat and rabbit models. Beta tricalcium phosphate is another calcium phosphate biomaterial that serves as a purely osteoconductive scaffold. It has higher solubility than HA and is quickly resorbed. b-TCP is an ideal choice for stem cell based scaffolds because the resorption rate of b-TCP matches the course of bone remodeling.

B. Types of Scaffolds

1) *Tissue-derived bioscaffolds:* A number of natural tissue-derived bioscaffolds have been developed from decellularized porcine NP ECM [92], allogeneic IVD [93], small intestinal mucosa [94], decellularized cartilage with in situ biomimetic ECM components still attached [95]. All of these found functions in MSC delivery strategies and as supportive scaffolds for growth of MSCs to help IVD repair.
2) *Self-assembling Bioscaffolds:* Injectable, functionalized, self-assembling peptide scaffolds are used in the regulation of resident and therapeutically administered cells. Matricyptins are bioactive fragments of ECM proteins and GAG side chains of tissue proteoglycans that demonstrate a different functional property to that of the native molecule it emerges from [96]. Many of these fragments have antiangiogenic activities and can influence cell signaling through integrins, and growth factor receptors (VEGFR1, VEGFR2) to regulate physiological processes [129,130]. LinkN [97, 98] and P2K [99] are peptides derived from link protein and biglycan.
3) *Bioscaffolds Mimicking IVD Structure:* Scaffolds displaying oriented fibrous lamellar structure based on silk scaffolds have attempted to mimic AF structural form and have proved useful in repair strategies on the AF [100].
4) *Natural Bioscaffolds:* A number of scaffolds and hydrogels assembled from natural polymers such as photo cross-linked alginate [101], collagen silk–fibroin [102], silk–tropoelastin [103], silk–fibrin–HA composite, albumin–HA [104], collagen–gelatin–chitosan composites [105] have all been developed. Type II collagen-HA-C6S tri-copolymer [106], collagen-GAG biomimetic scaffold, type II collagen-HA [107], dextran-chitosan-teleostean, alginate-chitosan [108] have all been examined for their ability to promote IVD repair by providing a protective matrix for resident and administered therapeutic cells.
5) *Bioactive Scaffolds:* Self-assembling KLD peptide-TGF-β1 scaffolds have been developed to promote differentiation of MSCs into NP cells [109]. An injectable fibrin–gelatin TGF-β1 scaffold has been used to deliver MSCs into degenerated IVDs, these slowed the decrease in disc height evident in non-MSC treated IVDs [110]. Porous alginate and alginate–collagen memory scaffolds containing TGF-β3 have been developed in an attempt to repair AF defects and the NP of degenerated IVDs [111]. These scaffolds promoted cell migration and proliferation.
6) *Synthetic Scaffolds:* They have been used extensively in tissue engineering applications for the past two decades. These polymers are used to produce injectable nanoparticle cell delivery systems or to utilize 3D printing. Amine functionalized, injectable, porous microcarriers of polylactic coglycolic acid generated admirable results in terms of attachment of cell and generation of ECM components co-related with non-functionalized or nonporous microcarriers [112]. Recently, allograft is the most frequently used osteoconductive scaffold and is usually combined with autograft to give the mandatory osteogenic, osteoinductive and osteogenic factors and cells. Thus, the application of scaffolding materials has been firmly suggested to reduce the risk of leakage and to act as a retainer of the cells that are transplanted [113].

IX. CELL THERAPY

In research, three approaches are typically used to describe the degenerative process called modulating catabolic processes, stimulating anabolic processes and providing new cells. The 'providing new cells' approach appears to be very appealing, provided that one essential feature of IVD degeneration has a decline in functional cell numbers, and a considerable proportion of the cells are in a senescent state.

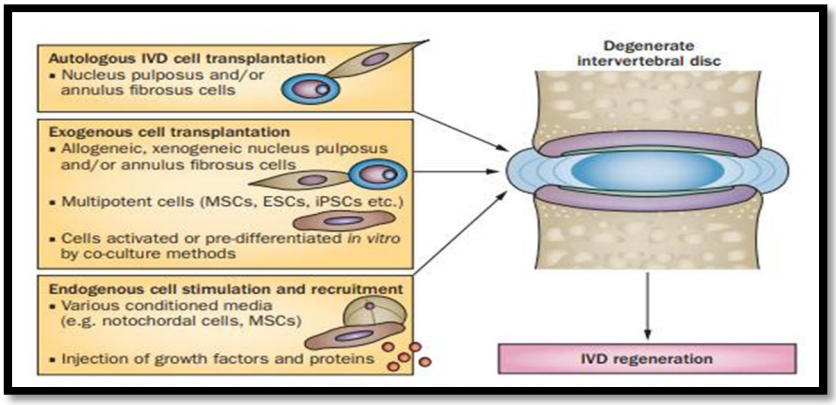

Figure 4: Treatment options for IVD degeneration using cell therapy
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The aim of cell therapy is to escalate ECM synthesis by multiplying cells to the degenerate NP. To achieve this, certain cells are directly inserted into the NP.

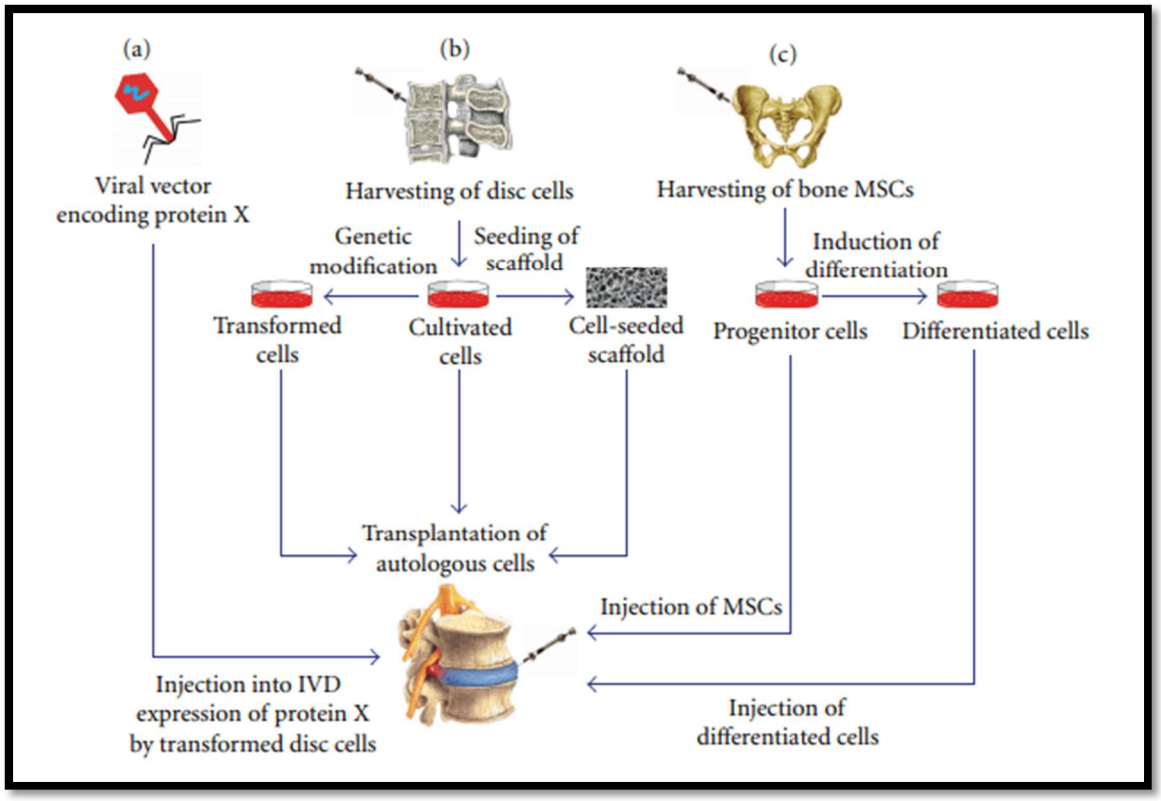


Figure 5: Different treatments for IVD degeneration are illustrated: (a) Injecting a viral vector into the IVD causes expression of the coded protein by the transformed disc cells, (b) Cells from the NP are harvested and then can be cultivated, genetically modified, or seeded into a scaffold before being transplanted into the IVD, (c) Bone MSCs are harvested and injected into the IVD as MSCs or as differentiated cells

The concept of cell therapy includes the procurement, processing, and transplantation of cells. There may be a further need to combine cell therapies with a supportive matrix, and bioactive molecules (growth factors, gene therapy) [114].

MSCs show noticeable promise for use in cartilage and IVD repair and are being clinically probed as a new therapeutic for treating a wide category of other immune mediated diseases. MSCs have promising applications in tissue engineering and regenerative medicine and can represent an attractive choice for repairing focal lesions in cartilage and IVD degeneration [115]. Furthermore, MSCs have been identified in a variety of tissues including bone marrow (BM-MSCs), umbilical cord (UC-MSCs), muscle, periosteum, and adipose tissue (AD-MSCs). It has been proved that MSCs are an ideal cell source for IVD regeneration, with a high rate of research showing that of both AD-MSCs and BM-MSCs differ into NP-like phenotype [116, 117]. Research has showed the implanted MSCs have the ability to improve matrix production, specifically GAG synthesis, and escalate the disc height and hydration [118-123].

Research on discogenic differentiation of MSCs depended on the fact that NP cells are 'like-chondrocyte' and deliver chondrogenic markers such as type II collagen, SOX-9 and aggrecan [124]. The in vitro expansion of MSCs needs extreme care to avoid contamination and is also time consuming. The MSCs were directly injected into the IVD (with or without carrier/scaffold) and revealed promising results, whereas the injected cells were viable, differentiated into a "NP-like phenotype", builds matrix synthesis and restored height of disc in rabbit, rat, goat and canine models and also in xenogeneic transplant of human MSCs into a porcine model [125 - 129].

Carriers such as hyaluronic acid, Atelocollagen®, and injectable hydrogels are frequently used to retain the cells that are transplanted at injection site, mimic the IVD environment to provide the survival of the transplanted cells and/or facilitate matrix production [130, 131].

Embryonic stem cell transplantation may require immunosuppression and raise ethical concerns that vary across national boundaries. The source of the stem cells could be autologous (from the bone marrow) or embryologic. The embryonic origin of the AF cells is the mesenchyme. The cells are elongated in the outer layers and more chondrocyte-like in the inner parts of the annulus. The cells in the nucleus pulposus (NP) in adult humans are chondrocyte-like cells that, compared with chondrocytes from articular cartilage and annulus fibrosus cells, produce more extracellular matrix [132].

Transplantation of mature disc cells after increasing the number of cells in cell culture has been performed in animal models with either NP cells and/or AF cells [133]. Nomura *et. al.* [134] have shown that injecting intact nucleus pulposus was more effective in retarding disc degeneration than injection of nucleus pulposus cells alone.

A. MSCs from Adipose Tissue

1) The adipose tissue is a good source of MSCs for various applications in case of spine. Adipose tissue is easily procured from patients and large numbers of MSCs can be obtained from relatively small amounts of adipose tissue, in contrast to bone marrow [135].

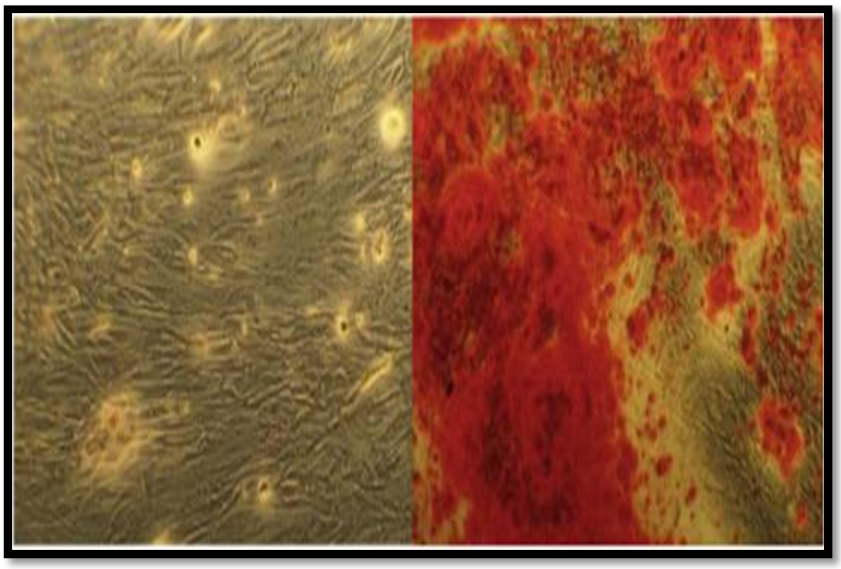


Figure 6: Human adipose derived stem cells (Left) undergo effective osteogenic differentiation (Right) as demonstrated by alizarin red staining in this in vitro experiment.

2) Research says that MSCs from adipose tissue are a potential cell source. Adipose tissue is considered an expendable, abundant and easily accessible source of MSCs. The use of these cells may diminish the need of in vitro expansion that raises the chances of a 1-step treatment method. Hoogendoorn and colleagues [136] reported that adipose-derived MSCs may be beneficial for cell therapy for IVD disease as they can be isolated easily. The disks which received the cells derived from adipose showed similarity to the healthy controls, as proved by the translucency of matrix, AF compartmentalization and density of cell in the NP.

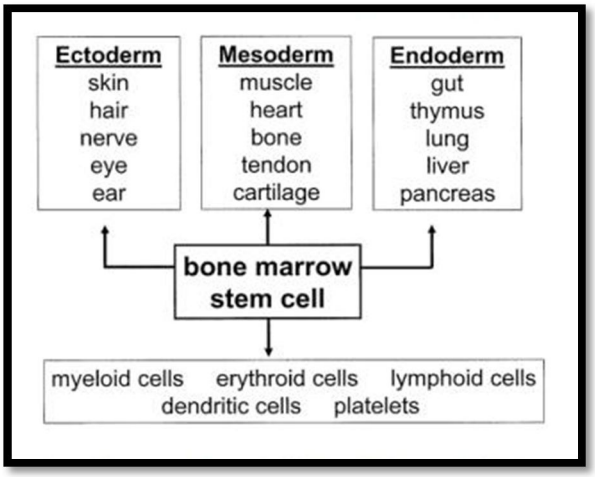
B. MSCs from Bone Marrow

1) It has been reported that when mesenchymal stem cells taken from adult bone marrow were grown in microaggregates in the presence of appropriate growth factors, including transforming growth factor-b, and under low oxygen tension, they formed cartilage-like structures with a multilayer matrix-rich structure, chondrocyte-like lacunae, and hypertrophic phenotype [137, 138]. Expression of various key cartilage matrix glycans, matrix proteins, and type II collagen was demonstrated by immunotyping, Western blot, and RNA assays [139, 140]. The tissue filling the cartilage defects was investigated with mechanical testing, histologic [141, 142], and RT-PCR for collagen type II [143].

2) Bone marrow stem cells produce not only hemopoietic cells, but are able to transdifferentiate into other cell lineages.

Embryonic Stem Cells or other multipotent stem cell types, including induced pluripotent stem cells (iPSCs), might be better sources of functional donor cells owing to their ability to differentiate into various IVD cells, such as notochordal cells. An attempt to induce notochordal NP cells using iPSCs was reported in 2013 [144].

Type of cells except adult IVD cells, like notochordal cells, might provide preferred regenerative results, but unfortunately don't able to find any good results in humans.



C. Inducing Stem Cells Towards the Inter Vertebral Disk Cell Phenotype

1) The author tried to show differentiation of MSC with NP/AF cells mixed co-culture system. The AF and NP cells when co-cultured with MSC with a 50:50 ratio showed density of 30,000/bead. MSCs co-cultured with NP cells show greater average cell size, when compared to AF cells. MSCs co-cultured with NP cells express type II collagen and keratin sulfate, whereas AF cells show type I collagen. After the analysis through reverse transcription-PCR (RT-PCR), it was confirmed that with different IVD cells (in same co-culture) resulted in MSC differentiation.

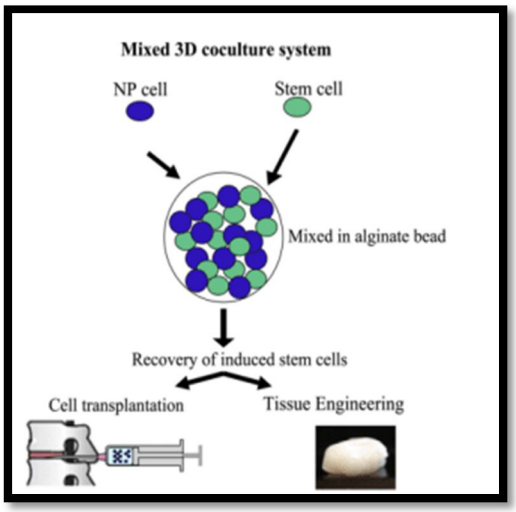


Figure 7: Use of stem cells for direct induction toward NP phenotype.

2) In 2003, Sakai and colleagues [145] first reported on transplantation of MSCs into a rabbit disk degeneration model. In the following study, the transplanted autologous MSCs were tagged with GFP, transplanted into a rabbit disk degeneration model, and followed for 48 weeks [146, 147]. Immunohistochemistry was performed to assess the expression of chondroitin sulfate; keratin sulfate; types I, II, and IV collagen; HIF-1a and HIF1b and HIF-2a and HIF-2b; glucose transporter (GLUT)-1 and GLUT-3; and matrix metalloproteinase (MMP)-2. They also applied RT-PCR to quantify the expression levels of the genes for aggrecan, versican, types I and II collagen, interleukin (IL)-1b, IL-6, tumor necrosis factor (TNF)-a, MMP-9, and MMP-13.

3) GFP positive cells were detected in the nucleus. The percentage of positive cells increased which proved that the MSCs survived and proliferated.

D. Use of autogenic MSCs in Retarding disc Degeneration

Ongoing studies about MSCs in rats and rabbits focus mainly on the outcome of the stem cells in normal discs. The use of MSCs o regenerate IVD was explained by Sakai et al. A rabbit model was proposed to study the degeneration of nucleus; the MSCs were inserted in an atelocollagen matrix. The cells lasted for 4-week period and enhancement of proteoglycan content in the implanted discs was observed [148]. Upon this, the research recommended that the MSCs which were present in the re-established nucleus had differentiated into a chondrocyte-like/nucleus pulposus cell phenotype expressing collagen II, keratin sulfate, and chondroitin-4-sulfate [149]. The result was, even though regeneration of disc was not fully achieved by autogenic MSC implantation, it helped in overcoming and countering the process of degeneration till a certain limit.

Ongoing clinical trials of cell therapy for IVD degeneration

Location	Mode	Cell type	Indication	ClinicalTrials.gov Identifier	Status
USA[111]	Allogeneic	Juvenile chondrocytes	Degenerative disc disease with low back pain	NCT01771471	Active, but not recruiting
USA[112]	Allogeneic	Adult mesenchymal precursor cells	Degenerative disc disease with low back pain	NCT01290367	Active, but not recruiting
Spain[113]	Allogeneic	Bone marrow MSCs	Degenerative disc disease with low back pain	NCT01860417	Active, but not recruiting
Austria, Germany[114]	Autologous	IVD cells	IVD herniation with back and/or leg pain	NCT01640457	Recruiting
Korea[115]	Autologous	Adipose MSCs	Degenerative disc disease with low back pain	NCT01643681	Recruiting
USA[116]	Autologous	Adipose MSCs	Degenerative disc disease with low back pain	NCT02097862	Recruiting

Abbreviations: IVD, intervertebral disc; MSC, mesenchymal stem cell.

The problem of immune rejection is likely to be even less for allogenic MSCs, since MSCs are capable of escaping alloantigen recognition [150, 151].

Disc degeneration can pave the way to secondary degenerative spinal diseases, such as degenerative spondylolisthesis, spinal canal stenosis, facet joint osteoarthritis etc. It might be favorable to perform cell therapy in patients with progressive disc degeneration before advancement of these diseases.

X. CONCLUSION

There has been extensive research on treatment of the IVD using Stem Cells, which overcomes the problems which are generally faced in the artificial treatments and implants. Moreover, there are many things which need to be focused upon and look out the way to empower the out comings of usage of stem cells which may include scaffold requirements and cell extraction sources. Stem cell treatment provides a unique way of treatment by differentiating themselves. Stem cells approach is not only promising in IVD treatment but can also be applied to cure a number of diseases. MSCs are already clinically approved for medical usage while many other cells are under approval and may hit the medical field in the next couple of years.

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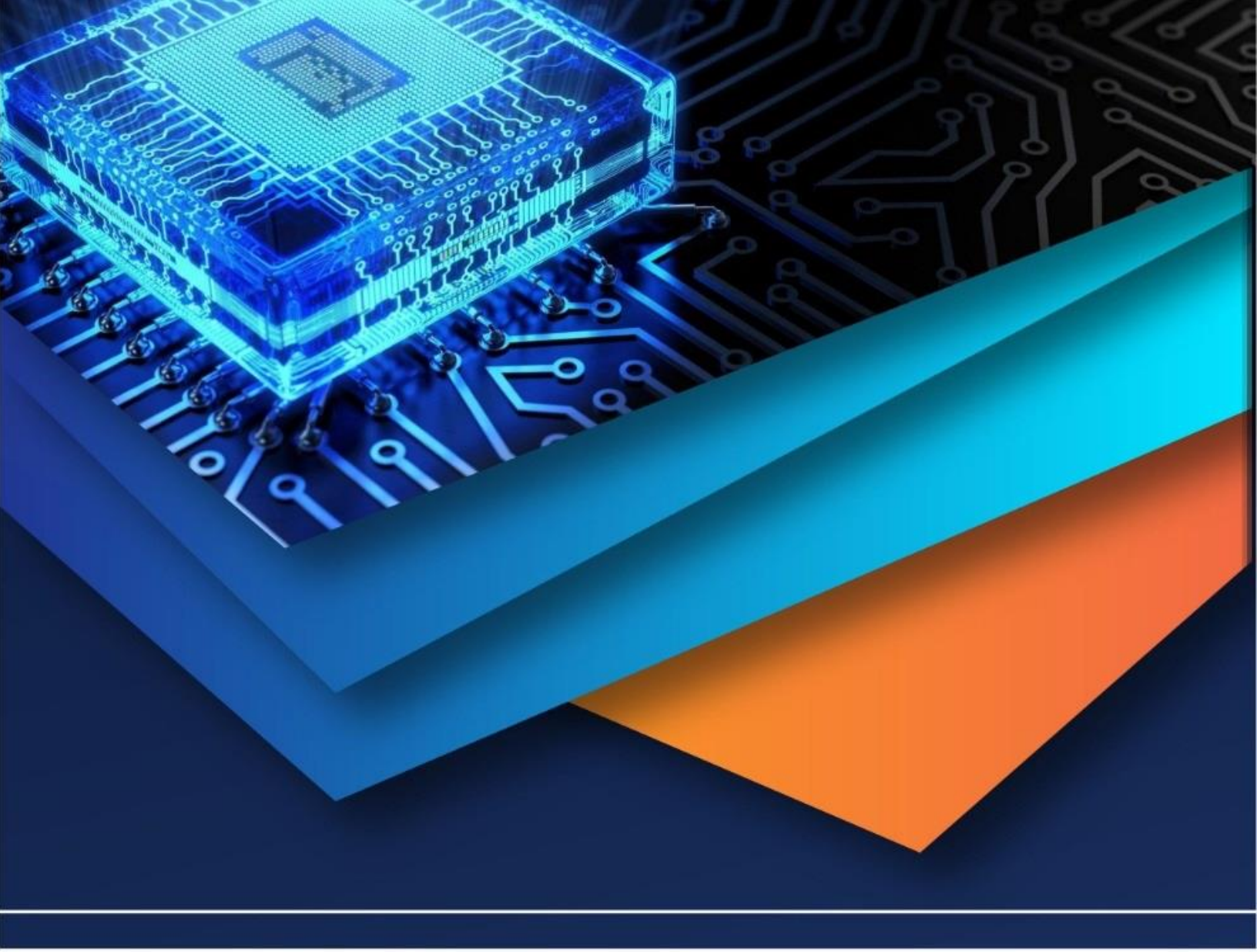

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