Biomechanism Profile of Intervertebral Disc’s (IVD): Strategies to Successful Tissue Engineering for Spinal Healing by Reinforced Composite Structure

Kunal Singha* and Mrinal Singha**

1Department of Textile Technology, Panipat Institute of Engineering & Technology, Harayana, India
2Department of Pharmaceutical Chemistry, CU Shah College of Pharmacy & Research, Gujarat, India

Abstract

Complex multi-lamellar biomechanical structure of Intervertebral Disc (IVD) imparts flexibility between adjacent vertebrae, as well as allows transmission of loads from one vertebra to the next along the spine. The disc has a 15-25 concentric layered laminate structure; each layer is reinforced by collagen fibers which are aligned at approximately 30 degree angle in successive layers with respect to the transverse plane of the disc. This fibrous organization is critical to the proper biomechanical functioning of the disc, such as to convert compressive force to lateral force, to withstand extrinsic tensile stresses (circumferential, longitudinal and torsion). As a result spine becomes flexible to bend and twist. With the regular aging the disc gets dried up lost its flexibility and biomechanical elasticity. That's why we need tissue engineering of that degenerated tissue to make a proper alignment of that body part by the help of some textile fibers like silk- hydrogel, CMC, PVA- collagen, PGA – chitosan composites. The synthetic polymer has shown great promise for easiness of production, variability in properties and biodegradability and biocompatibility and non-immunogenic response inside the human spinal body for the novel cause of removal and restoration of degenerated human intervertebral disc.

Keywords: IVD disc; Nucleus pulposus; Biomechanical functioning; Tissue engineering; Silk- hydrogel; CMC; PVA- Collagen; PGA - Chitosan composites

Abbreviations: IVD: Intervertebral Disc; AF: Annulus Fibrosus; NP: Nucleus Pulpous; EP: End Plate; CMC: Carboxymethyl Cellulose; PVA: Polyvinyl Alcohol Fiber; PGA: Polyglycolic Alcohol Fiber; TE: Tissue Engineering; MMPs: Matrix Metalloproteinase’s; ADAMTS: A Disintegrin and Metalloproteinase with Thrombospondin Motifs; TIMP-1: Tissue Inhibitor of Metalloprotein; PCL: Polycaproactum Fiber; ECM: Extra-cellular Matrix

Introduction

Lower Back Pain is a common clinical complaint in these present days. In fact most of these symptoms are rises from the biomechanical sources, and the Intervertebral Disc (IVD) is the main culprit in that case. IVD rests in the spinal cavity with the help of huge body pressure, compression force due our body weight (BW) and normal body movement [1,2] and given their close proximity to the spinal cord and other peripheral nerves [3,4], it is no surprise that complications with the IVDs can lead to serious neurological effects and become detrimental to multiple areas of the body and the complex loading behaviour [5] of the cervical discs and their frequent involvement in pain and pathology, it is important to understand their mechanical properties. In human body we can three types of cartilage tissue (network of highly dense connective tissue) like (i) annulus fibrous tissue (AF tissue) – present in synovial bone joint [6] (ii) elastic cartilage – present in outer ear, larynx and epiglottis [7] (iii) fibro cartilage – present in IVD, meniscus, temporomandibular joint [8]. So IVD is basically a fibrocartilage type of body tissue, when the jelly like NP matrix prolapsed it forced out to rupture outward and thus creating a pressure on its surroundings nerve tissue or column and these may leads to symptoms of sciatica [9,10,11]. IVD or simply so called disc is consist of mainly three parts (i) NP (Nucleus Pulposus) the inner jellylike substance at the centre part of the disc which primarily contribute to the torsional or twisting movement of the body, (ii) AF (Anulus Fibrosus)- the outer soft biological tissue part relatively much stronger than NP that is the central part relatively easily deformable and that is the peripheral part [12,13], mainly distribute the stress on spine and degeneration of these part is mainly responsible for LBP (lower back pain). AF governs all the mechanical properties like viscoelasticity [14], hyperporoelastic mechanical profile [15], aggregate or elastic modulus, permeability or disc tissue porosity, anisotrohical or heterogenetical biomechanical characterization. AF part also governs four main biomechanical spinal disc manifestations like: stress-strain rate trend, hysteresis, creep [16] and stress relaxation from the mechanical deformation. (iii) EP (End Plate) is the peripheral subcutaneous bony part which surrounds the IVD or disc ring for protection helps in disc recovery. EP is generally the subchondral bone layer and maintains the contact between IVD and spinal cord (SC). It has no relation with LBP. The fluids flow inside the end plate play a main role for the recovery of the disc in vivo but in case of in vitro the role has limited (Figure 1). With ageing normally the AF layers gets dehydrated due to loss in hydration [17], so the disc bulging and finally gives enormous pressure to its surrounding symptomatic spinal nerves (C3-C4: cervical nerve roots) by the disc protrusion- which may cause chronic back pain [18,19,20]. So the main concern about successful disc repairmen

*Corresponding authors: Kunal Singh. Assistant professor, Department of Textile Technology, Panipat Institute of Engineering & Technology, Harayana, India, Tel: +091-9355029123; E-mail: kunalsingha28@gmail.com
Mrinal Singh. Assistant Professor, Department of Pharmaceutical Chemistry, CU Shah College of Pharmacy & Research, Gujarat, India, E-mail: mrinalsingha@gmail.com

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is to synthesise and simulate the biomechanical and kinematics properties [21,22] of that AF tissue layers. For that purpose goat IVD is used to carry out the biomechanical experiments to study the native disc biomechanics. Goat IVD is used because it has almost similar kinematics and dynamic profile under loading or stress like in case of human IVD [23]. Besides that the silk based novel scaffold (fibre-hydrogel ECM (extracellular matrix) composite) [24] has been also carried out to various mechanical testing to check out the proximity of their mechanical properties to the native goat AF tissue construction. AF is approximately a ring (some of the ring of complete and continuous and some of them are incomplete and discontinuous) like angle-ly structure where the collagen II fibers [25,26] are specially oriented +30°/-30° in above and in below the transverse plane of the body spinal axis [27,28,29]. The AF structure is more complex than NP structure because it consist of circumferentially discontinuous and traversed by fibrous assembly that runs radially outwards from the spinal axis [30,31,32,33]. Due to special arrangement of the fiber in the AF layers this layer can take more load bearing capacity (like mainly compression, torsion, shear, tensile etc.) [34,35,36] by readily resolving the uploaded body force on it. Also the Poisson’s ratio of NP is lower than the AF; so AF got higher degree of deformation [37,38] in it so the biomechanics of AF is our main concern than NP layers [39,40].

Scope rationale of this review

The review will address progress made in polymeric implants over the last decade, dealing primarily with spinal therapeutic devices and replacements. The review is mainly includes the followings;

a. Biomechanics of the spinal intervertebral disc or cartilage

b. Polymeric materials or scaffoldous substrates which are being used for replacement of spinal lower back pain.

In today’s world full of heavy work load the medical problems becomes stronger day by day in everyone’s life. Lower back pain (LBP) is most common type of medical disease which cause near about 7 million people in UK in every year. LBP generally produced due to the malnutrition of the cartilage inside our spine. This small cartilage discs are called intervertebral disc (IVD) which mainly bears 90% of our body load with the incorporation of our spinal body axis. With aging the disc gets dehydrated and loss in its height therefore as the result it collapsed toward the cervical nerves and produce severe back pain and sometime obesity also. The ways to remedies for that are taking regularly pain-killer tablets, anesthetic, fixing of unhealthy disc and fusion of the disc but in all this process the patient lose their natural flexibility and body movement due to the heavy weight of that non-biodegradable, non-biocompatible material. The current object of this review paper is to make use of Tissue Engineering (TE) with the help of making scaffold hydrogel by the help of some textile material like: silk, CMC, PVA, PGA, PCL-collagen composite material. This scaffold composite material can bear the same bio-mechanical properties with superb bio-compatibility and bio-degradability also, they are very light in weight and easy to replace inside the patient body. In this current paper we had also discuss about the different mechanical force can acts inside a disc matrix and the measurement techniques generally used those forces. The others alternatives options are also discuss which can be very handy in disc therapy besides the tissue engineering. The working principle involved for an ideal scaffold material has been also discussed which is very important for a successful disc replacement with the help of tissue engineering techniques.

Spinal therapy

The spinal therapeutic broadly cover both the herniation of interverbral disc and the damage of the articular ligament. This review specially limited and focuses on the herniation or degeneration and procurement of the intervertebral disc. We specially consider the biomechanics of the disc as the disc profile and behavior is fully controlled by the disc biomechanology and elasticity (loss of elasticity) and flexibility. The tensile, compressional, torsional, hyperflexion [41] of neural arch and shear force behavior is very important to predict the disc anatomy and body-load bearing capacity. This is the first and primarily most important focus of our discussion. In general, spinal disc herniation gives the patients symptomatic nerve pain which is amenable to treatment with removal of the herniated part of the disc or with the disc angioplasty [42] which may vary from patient to patient and totally artificial. Other spinal disc therapeutic conditions such as fixing the screw after removal of the degenerated disc or a ceramic-cement fixation over the spinal cord [43,44], but these techniques fails due to the reason that the patient loss natural movement and flexibility of own’s spinal cord and body weight seems get heavier. There is also a chilled feel due to the metallic screw uses inside the body during the change of the seasonal weather and the immune response and non-biocompatibility of this type of foreign substrate is become very detriment for the patient in the future. For all of those above constraints, this all later techniques are fall these falls outside the scope of this review.

Polymetric biomaterials are generally derived from three sources: natural polymers, including those of plant and animal origin; totally synthetic sources; and synthesis based on materials of natural origin. The first two categories are self-explanatory; the third is of relatively recent vintage. It encompasses materials synthesized to mimic a naturally occurring polymer, but not necessarily identical to it. The most important materials in this category are the man-made protein structures, which resemble natural proteins but differ from them in some details of the primary structure. This third class of polymers promises innovative materials that have the potential to functionally replace diseased or unavailable cell components, such as the extra-cellular matrix, which plays a structural role in many organs and tissues by the super ability of controlling the matrix stiffness (Figure 2) by the shock absorption capacity with the macro, micro or nano level inside the living tissue [40,41]. Within each application, we will highlight the
advances made in the development of each type of polymer, and the benefits they confer. This is the second focus of our discussion.

Types of implantable polymers

Synthetic polymer have been wide used as the implantable materials due to the reasons include ease of production; control over the properties of the polymer during spinning and over of its end products; ready availability and versatility of manipulation. Conversely the polymer from natural resources likes collagen being variable on its properties from source to source; possibility of bacterial and viral contamination and chances of antigenicity is not being very popular implantable materials. If these organic materials are of animal origin, there are added complication of harvesting the polymer or protein and purifying it. For these reasons, synthetic polymers have dominated the spinal implantable therapeutic landscape. For examples, the alginate/chitosan electrospun, poly-methylmethacrylate hybrid fibers provides the non-

Outline of the review

The discussion will address these aspects in each application:

a. The composition and SEM (scanning electron microscopy) image analysis of the intervertebral disc to get an idea about it structural parameter and components.

b. The biomechanology of the disc with the details of various mechanical behavioral characteristics.

c. Polymers that have been evaluated using in vitro methods

d. Outcome of animal studies and (if available) human performance data for the benchmarking comparison of experimental and actual mechanical modulus of those polymeric implants.

e. Commercial success.

f. Others methods for disc healing except the tissue engineering implants methods.

g. Future directions.

Compositions of IVD

The composition of IVD is shown in the Table 1. The 60-70% of the IVD is water [46,47]. The re mainder is mainly PG (Proteoglycan) and collagens. PG is mainly consists of cell ground substances GAGs (Glycosaminoglycan) [48] which is primarily CS (Chondroitin sulfate) and KS (Keratan sulfate) [49,50] with the high molecular weight complex proteins, disaccharides [51]. This PG acts as the backbone of the HA (Hyaluronic acid) to make a macromolecules weight ~ 200 millions [52,53] (Figure 3). Approximately 30% dry weight of IVD is PG and there is always a variation in gradient of PG and water across the depth of the disc, in the upper subchondral bone layer the concentration of PG is less but water is high and inside the tissue concentration of the PG is high and water is less [51]. HA acts as the binding sites for CS and KS or makes the aggregate thus makes the big brush like macromolecules inside the IVD cells [46,53].

SEM analysis of native annulus fibrosus tissue

Goat’s intervertebral discs (IVD) corresponding to L-1 to L-7 were dissected from the spinal column of 6-8 year old goat within 24 hours of slaughter from nearby slaughter house. Following dissection the discs were rinsed in PBS. Subsequently the disk tissues were finely cut with a sharp surgical blade ensuring uniform dimensions of (2 × 5 × 7) mm of annulus tissue sample (Figure 4). The specimens tested were cut to widths of 2.3 mm in accordance with American Society for Testing and Materials (ASTM) [56,57] standards maintaining a ratio

Figure 3: (A) A proteoglycan aggregate illustrating a collection of proteoglycans attach to a hyaluronic backbone. PGs are the bottlebrush-like structures comprising of a protein core with side chains of CS and KS [54] (B) Structure of hyaluronan [55].
of 0.5 (ASTM 1990). After collection of those samples with proper dimension measurements are proceeds for the different mechanical testing characteristics techniques for IVD (for the mechanical testing was set at (1 mm/min).

The thickness of each AF layer increases towards the centre and the peripheral region the thickness is around ~100 µm whereas near as near the centre the AF layer thickness is around ~150-175 µm [60,61] and the distance between two AF layers decrease towards centre (Figure 5).

SEM picture shows orientation of collagen fibers in AF layer +/- 30⁰ in the alternative layer. This opposite angular position with a preferred fiber direction gives the opportunities for easy force resolutions under a small loading over the annulus tissue. Thus the disc can withstand it structural support and compatibility inside the spinal cord of human body and bears the body weight normally [62,63].

Mechanical Force Exerted on the IVD in the Spinal Cord

The healthy disc can be degenerated or unhealthy one due to the combine effects of mechanical loading precipitation, genetic inheritance, irregular loading history which may cause the generation of ‘weak-link’ in the anterior of the disc by breaking the ‘shield-stress’ layer inside the posterior lamellar domain or region of the disc. As a results, the anterior part of the disc is gets collapsed and form the degenerated or unhealthy (herniated disc) due the formation of ‘wedge fracture’ [64,65] in the anterior-posterior interface region of the disc. The percentage of the stress or body load bearing capacity is different for the healthy and unhealthy disc is being totally different due to the reason of the profile and surface contour or aerial distribution of the collagen fiber, extracellular matrix over its structure [66]. Vertebral damage could cause back pain indirectly by generating high stress concentrations within the adjacent intervertebral discs (Figure 6) and subsequently could cause the annulus to collapse into the nucleus [67]. This mechanism is supported by a survey of adolescents, which confirmed that vertebral body damage is often followed by disc degeneration several years later [68].

Disc is tightly fitted in the spinal vertebral cavity under a huge compressive force [42]. So the main aspects to look for of these kind of tissue is study the compressive force or strength on it. The forces exerted on AF layer of the disc (Figure 7) are (i) compressive force (uniaxial (unconfined compression which is done normally), biaxial (confined compression - specially tested for soft biological tissue like AF, cartilage ), triaxial compression) (ii) tensile force (uniaxial, biaxial, triaxial tension) (iii) shearing force (iv) torsional or twisting force and the (v) water hydrostatic force [44].

There may be three types of compression test can be done on AF tissue – uniaxial (only in Y- direction), bidirectional (both in X-Y directions) and tridirectional (X-Y-Z directions) [44,45]. The unidirectional test is called as unconfined test and the bidirectional or tridirectional test is called confined test of AF tissue. In case of unconfined mechanical test we consider the amount of water and its hydraulic pressure contribution to the mechanical testing [70]. Normally under the impulsive compressive force or loads on the AF tissue experiences a large lateral deformation due to its high Poisson’s ratio of about 0.5 [7]. This expansion is restrained by comparatively stiffer underlying subchondral bone which produced a higher shear stress (Figure 8) at the cartilage bone interface (cartilage- bone boundary)[71].

The Poisson’s ratio (υ) of the native AF layer for goat IVD with by the using of following formula [56] by considering it as an uncompressible, poroviscoelastic material like the AF tissue by the following formula (1,2); (E/H) = (1-υ) / (1+υ) (1-2υ) (1)

Now by simplifying the above formula; we can get:

\[ \Rightarrow 2\nu\gamma^2 + \gamma a - a = 0 \]  

(2)

The positive root of these quadric equation will give us the Poisson’s ratio of the material, Where H = aggregate modulus i.e. compressive modulus or strength (force), E = elastic modulus, \( \nu \) = Poisson’s ratio of the material respectively and Es (MPa) = force at break of the material in compressive test / 1000 x % of elongation.

Compressive test

Compressive strength test has been carried out for large numbers of AF tissue with Hounsfield load cell force accuracy ~ 0.5% applied force [72,73]. Two types of - confined and unconfined compression test has been carried out and the compressive modulus is produced the higher value (Figure. 9(A)). The longitudinal and radial pressure on annulus tissue is proportionally increased with the magnitude of the compressive stress (load) Figure 9 (B), (C).

On loading upon in a typical displacement of annulus tissue in a confined test gives us a curve between displacement and time (Figure. 10(A)). Initially the deformation is rapid, as relatively large amounts of fluid (water) being going out from the annulus tissue. Then reaching at a constant value the displacement slows down after a certain time as the fluid flow slows to zero [74,75]. The material properties of annulus tissue are determined from this test. The typical compressive stress-strain behavior under uni-axial force and bi-axial force is shown as in the Figure. 10(B),(C).

The total compressive stress on the disc matrix is further carried out by different parts of the disc:

\[ W_{total} = W_{matrix} + W_{flow} + W_{shear int.} + W_{normal int.} \]  

(1)
The distribution of vertically acting compressive "stress" was investigated using a precision surgery blade. Figure 4 presents schematic representations of an intervertebral disc and annulus fibrosus sample, with posterior elements removed, showing the three regions of disc from which samples were obtained and the cutting planes used to create square cross-section samples [55] (B). A single annulus fibrosus sample, demonstrating that for a sample cross-sectional dimension of 2 mm x 2 mm (C) Collagen fibre inclination in each sample [58,59].

A stress-free reference configuration was ensured by enforcing $W = 0$ for $I = 0$ for Matrix, Fibers, Shear Int, Normal Int. The sum of the structural terms, $W$, was required to be greater than zero: $W(C) ≥ 0$. The Mooney–Rivlin model [65] for AF and NP tissue like anisotropic, structural terms, $W$, was required to be greater than zero: $W(C) ≥ 0$.

Figure 5: (A) SEM of native tissue (goat) (B) SEM picture shows orientation of collagen fibers in AF layer +/- 30° in the alternative layer (C) Orientation in AF layers [58,59].

Figure 6: (A) The distribution of vertically acting compressive "stress" measured along the sagittal mid-plane of a 46-year-old cadaveric lumbar intervertebral disc (anterior on right). Compressive damage to the vertebral body (lower) reduces the pressure in the nucleus, and generates high stress peaks in the annulus. This disc was subjected to a compressive force of 2 kN during the "stress" measurements. (C) Load sharing in the lumbar spine is affected by intervertebral disc degeneration. The disc is normal (left), the neural arch resists only 8% of the applied compressive force, and the remainder is distributed fairly evenly between the anterior and posterior halves of the vertebral body. However, severe disc degeneration (right) causes the neural arch to resist 40% of the applied compressive force, whereas the anterior vertebral body resists only 19%. Data from cadaveric lumbar motion samples [35] (B) A single anulus fibrosus sample, demonstrating that for a sample cross sectional dimension of 2 mm x 2 mm (C) Collagen fibre inclination in each sample [58,59].

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1. $I_1 = tr C, I_2 = 1/2 [(tr C)^2 - tr C^2]$ (4)
2. $I_3 = det. C, I_4 = a_3,C,a_3$ (5)
3. $I_5 = a_4 C^2, I_6 = b_4 C, b_4$ (6)
4. $I_7 = b_6 C, b_6 I_8 = \cos (2\Phi) a_6 C, b_6$ (7)
5. $J = det. F$ (8)

Where; $I_1 = Lagrangian deformation$ [82,83] strain vectors working inside the AF tissue ($a_1, 1, 2, 3,...,8), C = Right Cauchy-Green$
deformation tensor \([82,84]\), \(E = \text{Stress tensor}, 2\Theta = \text{Angle between the fiber populations inside the AF material matrix} [85,86]\). So from those above equation we get: \(I_1 = I_2\) and \(I_1 = I_3 = I_4\), as \(a_3 = b_3 = d = 0\) (for soft biotissue like AF tissue) [87].

**Simple shear test**

Pure shear (no hydrostatic stress) [83] is a difficult stress state to achieve so simple shear test had been carried out by using a simple shear stress. Then the equivalent pure shear state (stress and strain) was calculated with some mathematical formulae between pure shear strain energy density (U) and simple shear stress. The schematic experimental set-up has Figure 11 (A) and the stress-strain behavior of the AF Figure 11 (B) under a small shear force has been shown.

The value of shear force modulus [86] value is always less than compression modulus value because shear force is a biaxial phenomenon (X-Y plane velocity) unlike the compression or tensile which is an uniaxial velocity only. The value of the shear force modulus actually determines the interlaminar slippage between the fiber and the differential surface velocity between the fibers inside the annulus tissue matrix. The noted value of the shear modulus value of bovine annulus fibrosus is 21.3 ± 2.3% MPa [87] which is higher due to the hyperviscoelastic [88,89], porous and interlocked nature of the collagenous fibers and proteins inside the AF material’s matrix. The value of shear force is poor in IVD because due to lack of sectional movements (sliding, gliding phenomenon inside the IVD matrix etc.) for highly porous and viscoelastic structure of AF tissue.

The interlamellar shear strain is due to the joint results of skewing and stretching or slipping of the ply oriented AF tissue material at its peripheral or circumferential areas. The collagen and elastic fibres located along the ply boundaries are radially oriented and the localized concentration of radially oriented collagen fibres [90] are divided in multiple plies in minutely distributed cross bridge architecture. The AF tissue is a perfect example of composite material where the micro-failure does not normally occur in a single loading and instantly because this composite laminated structure can effectively resist crack propagation and requires multiple cracks and micro-failure to occur prior to final failure of the laminate [91], while more homogeneous structure needs a single crack to failure. Typically annulus tissue needs damage initiation by various modes like fibres pullout from the matrix, matrix deterioration by cracking, excess longitudinal tension and then damage accumulation by fibres buckling which leads to final failure of the tissue [92]. Failure chances due to cracking increases with aging as the numbers of degenerated circumferential plies of cartilage increases and thickness of each layer increases. For this reason the potential of interlaminar shear stress increases due to over delimitation probability of the relative weaker fibrous part of annulus tissue [93] and to know more better about the micro-mechanics of the annulus tissue Cartesian coordinate system had been already applied to a particular rectangular region of the specimen called region of interest by the various researchers. They had used particle image velocimetry (PIV) technique to quantify the shear factors but somehow were not able to determine the values of various shear factors [94]. Generally the polar co-ordinates (X, Y, Z, θ) are widely used to find out the amplitude of the average angle, angle of inter-annulus layer shear orientation during this whole study and analysed the micro-structural assumption in longitudinal and transverse direction inside the specimen by building up a lamination theory in axial, circumferential and radial mode [95]. This idea needs an assumption that ±θ angular deformation is always held in consecutive plied layers in the AF tissue and only the boundary layer having the highest degree of free movement freedom with the appropriate co-ordinate shear mechanics [96]. The inter-shear force production is strictly dependent on the angle of dynamics created at the time of shear test and the length of the specimen tested, which is determined by the following formula (9,10):

\[
\Theta^* = 2 |\tan^{-1}(1/\tan\Theta) + \tan\gamma|^{-1} - \Theta_0 \\
\theta^* = 2 \left[\cos\Theta + \sin\Theta \tan(\gamma)^2 + \sin^2(\Theta_0) \right]^{-1} - \theta_1
\]

Where \(\theta^* = \text{simple shear stretch}, \Theta^* = \text{angle of rotation or shear angle}, \gamma = \text{strain amplitude (DL/L at sample initial length}, L = 7 \text{~mm}, \Delta L = \text{deformation} \) depending upon average angle of orientation of collagen bundle at \(\Theta_0 = 30^\circ\).

**Tensile test**

Tensile test was done by loading for circumferential loading for axial loading. Each annulus tissue was loaded five times to a maximum strain of 55-60% and the specimens were permitted to relax for 5 minutes in between the load application. In all those above experiments sheep disc (AF tissue) has been used because sheep (goat) disc follows almost same kinematic and biochemical properties to human discs [97]. The time dependent response of the annulus tissue is very difficult to establish under in vitro environment due to lack of time-dependent transient equilibrium state, so we used the near linear region after the non-linear “tie-in” region [98,99] to estimate the Young’s modulus where all the collagen bundle are straightened out [100] due to the tensile loading and stretching. Depending upon the stress-strain and rate of loading the stress-deformation curves obtained may be linear or non-linear [101,102] which shows that the modulus is a function of the rate of loading (stress or strain range) [tensile modulus of L3-L4 = 0.88±0.38 [103]. As the tensile force increased the pore in the annulus tissue matrix got diminished in sizes [104], resulting in increased diffusional drag force [105] which occurs due to the increase in the Donann’s osmotic pressure [106] (according to the Darcy’s law interlaminar planes [107,108] in the annulus tissue matrix makes the
sample very difficult to extent and finally it breaks at a yield modulus/force).

Permeability test

In addition to the confined test we can get information from the same experiment called permeability which simply indicates the resistance of fluid flow through the IVD matrix. The average fluid velocity \(V_{avg}\) is proportional to the pressure gradient or pressure head \(\Delta p\) which is called the Darcy’s law [108] as shown in the equation (12);

\[ V_{avg} = k \Delta p \]  

\( k \) is the constant of proportionality called the permeability (k), which determines the fluid (various nutrients, hormone, growth factors or gases like oxygen, carbon dioxide) flow characteristic inside the cartilage matrix [7]. The experimental set-up has been shown as (Figure 12) and the pressure head is calculated by dividing the fluid pressure difference \((p_2 - p_1)\) between inside and outside of the matrix by the matrix height \(h\) as shown in the equation (13);

\[ \Delta p = \frac{p_2 - p_1}{h} \]  

Indentation test

This test has been carried out to find out the aggregate modulus, Poisson’s ratio, permeability by the fitting of the experimental data in biphasic model [109]. Indentation test is basically a confined compression test alternative for very shorter sample length about 0.8mm (Figure 13).

Tearing or fracture test

The tearing test or fracture test soft biological tissue like AF tissue is carried out by tensile testing machine. By this we can test the J-integral value [110] which indicates the crack propagation energy needed or fracture energy dissipated for per unit of crack extension [111]. As the soft tissue is not readily gives the crack so the tear is tested in that case by making a V-notch of say (1-3) μm at one end of the material Figure 14 (A). The other end is pulled by a tensile force by tensile tester to study the crack propagation [112] through the material which yields a similar parameter like in J-integral; similar to the tensile stress-strain failure criteria for a material. The value of J integral is calculated by the equation (14);

\[ G_{Pc} = \frac{K_{Pc}^2}{E} \]  

\( G_{Pc} \) is the J-integral value indicating the surface roughness, \( K_{Pc} \) is a fracture parameter and \( E \) is elastic modulus of the material respectively [113]. Sample shape and load application for the modified single-edge notch and trouser tear tests. Each test yields a specific measure of fracture, the energy required to propagate a crack in the material [114]. The crack initiation/critical opening stress were estimated from the fracture toughness expression;

\[ K_{Pc} = \sigma_{op} \sqrt{IC} \]  

\( J \)-value \((\text{kN/m}) = G_{Pc} = \frac{K_{Pc}^2}{E} \)  

 KPIC define where, \( \sigma_{op} \) is the critical opening stress of the collagen fibre where all the collagen bundle fibers start to open and becomes straight. This can be calculated from the toe-region of the stress-deformation graph. The viscoelasticity and hyperflexion [111], torsion and shear mobility of the collagen fiber makes the main contribution to initiate the strain-produced crack in the sample. The critical opening stress was calculated from the maximum load on the annulus tissue divided by cross-sectional area of the sample [115], which is the threshold stress where collagen bundle are about to straighten along the tear force direction. The \( J \)-value or \( G_{Pc} \) is the main factor which determines the concentration of PG and other matrix substances inside the matrix of annulus tissue.

Lap or peeling testing

This test is done to measure the interfibriler layers frictional force in between the AF tissue or simply interlayer frictional force by the help of nanoindentation through the help of AFM (Atomic force microscopy). This interlayer frictional value help us to gain an idea about the force required to peel off [116] the each of the AF layers from another layer i.e. matrix adhesion rigidity Figure 14 (B).

AFM (Atomic Force Microscopy) test

In this experiment with the help of nano indentation probe [117] rod the surface attribute profile or structure of the AF tissue can be studied and the matrix stiffness or roughness (roughness is calculated by Nano scope IIIA software) can be measured very accurately by this.
method according to the Hertz model equation as shown by equation (17,18) which is a modified form of Young's modulus.

\[
E = 3F \left(1 - \frac{v^2}{R^2}\right) \quad (18)
\]

\[
F = kd \quad (19)
\]

Variable are; \( F \) = force, \( k \) = spring constant of the nanotip used in probing, \( E \) = elastic modulus, \( R \) = radius of curvature of the tip, \( v = \) Poisson’s ratio or indentation ratio, \( d = \) indentation of the sample \[118\]. So overall we can summarize all kind of biomechanical test that can be proceed with AF tissue (Figure 15).

**Confined torsion test analysis**

The confined torsion modulus is much lesser than other values like compression, tensile or shear modulus due to the restricted rotational movement of the AF tissue material along its axis at a short range \[118\] of angle \((14.5\text{°})\). This angle which is called the absolute rotating angle (ARA) comes due to the particular bow-like bended structure of the human spinal axis. ARA provides the flexibility \[119\] and the ease of body movement by the releasing of pressure due to external loading on the spinal body \[120\]. The annulus fibrosus tissue also takes the rotation to this special amount of angle. ARA to maintain its continuity on the spinal body \[120\]. The annulus fibrosus tissue also takes the factor which decides twisting forces \[122\] and rotating properties under small load on the disc.

The mechanical properties of AF depend not only on fiber strength, alignment and matrix composition but also on fiber–matrix interactions at the interfaces \[123\]. Collagen I and collagen II and proteoglycans produced by the cells play a crucial role in imbiding water, which would in turn make the AF matrix more resilient to compressive force and increase its global stiffness \[80\].

**Reasons for disc degeneration:** With the aging the disc gets dehydrated and AF tissue collapsed and put the pressure towards the surrounding nerves in the cervical area of the body and produces lower back pain to the patient \[124\]. The healthy (hydrated) disc and unhealthy (dehydrated) disc has been shown (Figure 14) and degenerated disc show a distinct border between the AF and NP is still evident \[125\].

The AF has retained a lamellar structure \[128\] however the NP is composed of mostly fibrous tissue (arrow head) \( (6) \). There may be many reasons for the disc hernia (disc radially outward bulging) which ultimately produce damage and unhealthy disc like (i) since IVD is the largest avascular tissue \[129\] in the human body so any change in osmotic pressure \[130\] in the IVD leads to its degeneration. This is happen due to the unequal force or stress distribution inside the IVD which change the porosity-dependent permeability \[131\] of the disc and ultimately result in loss of disc hydration and disc degeneration \[130\].

(ii) Production of collagen X fiber which has been delocalized in degenerated disc associated in chondrocyte clusters which lead to cleft formation and disc abnormal activities \[131\].

(iii) Decrease in degree of cross-linking of pyridinoline and replacing of this kind of crosslinking by the penosidene cross-linking \[125\] which makes the tissue more prone to failure and increase the susceptibility of annular tear. (iv) Change in PGs synthesis: decrease of aggrecan and increase of versican, biglycan, decorin \[131\], KS, CS in proportional amount gives loss in hydration. So the disc will quickly degenerate. Also in this type of case the content of fibronectin will increased leads to faster disc degeneration \[132,133\].

(v) Increase in MMPs (matrix metalloproteinases- a large family extracellular zinc based proteinases broadly devided into four subfamilies like collagenases, stromelysins, gelatinase and MT-MMPs i.e. membrane-type MMPs, examples: MMP 1,2,7,9,13) \[134,135\] and ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) \[136,137\] \( (6) \).

(vi) During the life cycle the disc produced a huge amount of different MMPs in in extracellular matrix but these MMPs \[138\] degrade the many main cell components with it at the time of its own degeration. MMP 7,13 are more prone to disc damage by decreasing the aggrecan, collagen II particularly in NP regions \( (36) \). (vii) Production of TIMPs \[139,140\] (tissue inhibitors of metalloproteinases, irreversible non-covalent complexes to active MMPs in a 1:1 stoichiometric fashion \[139\], TIMP-1 and -2) are increased the rate of disc degeneration by triggering the activity of MMPs (MMP 7) by its proteolytic action \[141-146\].


Low back pain affects nearly 80% the population at least once in their lifetime \[147\]. Degeneration of the intervertebral disc (IVD) is responsible for most cases of back pain, resulting - spinal stenosis \[148\], instability, disc herniation \[149\], radiculopathy \[150\] and myelopathy \[151\]. Intervertebral disc (IVD) degeneration is thought to play an important role in producing the onset of lower back pain \[51\]. The core part of the disc, the nucleus pulposus (NP), which supports high compressive loads daily, shows early signs of degeneration, long before the outer part of the disc, the annulus fibrosus (AF) degenerates. So people are started to think about the physical therapy, medication or some surgical approach by the FEM (Finite Element Model) with the computational help \[152\] in this path TE is really a helpful substitute treatment for disc ailment. For any TE approach, we must consider four things, those are: cells, scaffolds, bioreactors and regulators \[153,154\]. Scaffold acts as a framework or matrix where the culture cell can grow by time and can adhere on it to regulate cell culture process. The bioreactor \[155,156\] acts as a server or assembly to maintain the particular condition where the scaffolds being put on with some

![Figure 15: Schematic representations of various mechanical testing for goat AF tissue, along with typical stress–strain profiles associated with each (right).](Image)
regulator which providing a wide choices to control the different parameters for a successful tissue engineering. So a proper ECM scaffold material should be chosen for a successful TE for a body organ.

**Scaffold material used in tissue engineering for IVD replacement**

There are many natural and synthetic materials which can be used as the matrix supporting material in the IVD/AF/NP tissue engineering as scaffolds construct, some of them are summarized (Table 2).

There may some others materials which can be used in TE foe a successful IVD implantation like- atelocollagen honeycomb [159], photocrosslinked CMC (carboxymethylcellulose) for encapsulated nucleus pulposus cells [154], electrosputn PVA / PVP hydrogel [160] for nucleus pulposus, the density (important for scaffold matrix stiffness characterization and workability) of the synthesized hydrogel material [161,162] is calculated by using n-heptane with the help of Denver Instruments M-120 balance by the following equation [79].

\[
p_{\text{hydrogel}} = p_{\text{hydrogel}} \times m_{\text{air}} / (m_{\text{air}} - m_{\text{heptane}})
\]  

(19)

**Importance of the proper selection of scaffold structural material: obtain proper cell mechano-signal**

In every cell there are certain receptors: thermoreceptors, pressure receptors or mechanoreceptors. These receptors like mechanoreceptor receive the mechanical signals and send to the brain of the body via nerve impulse, on receiving these biomechanical signals from this receptor these biosignals [163] are transformed to the necessary catastrophic [164,165] biological activities. Now how these mechanoreceptor acting as a microtransducer and able to change the biosignal to various threshold bioprocess potential to initiate the process is largely remain unknown [166]. Now using a stiffer scaffold material the mechanorecetors of targeted organ cell could not able to get proper signal from the stem cell due to changes in protein folding as forces are exerted to expose binding sites (Figure 17) and the cells on soft matrix with weak intracellular forces cannot sufficiently alter the conformation of a mechanically-sensitive protein of interest to expose a cryptic binding site [167,168] by making it non-functional. On the other hand, cells on stiff matrix generate high tension causes the protein to unfold to a state that the binding site is hindered non-functional. However, cells on matrix with optimal elastic properties may put the appropriate amount of forces such that the cell can change the conformation of the protein, making the cryptic binding site accessible [169].

**Others Methods for Disc Repair**

Besides the tissue engineering there are some others techniques are also available in the market which has shown some promises for a good disc replacement as listed as (i) disc fusion - but that restricts the normalized disc movement by using of the screws. By the analysis of FEM (Finite element method) the proper IVD elemental analysis, screw optimized position, stress (Vont Mise’s stress), density, volume etc [162]. The software mainly used for this purpose is ABAQUS Version 6.6 (Simulia, Providence, RI, USA) [170] by considering the kangaroo biomechanics model [152] and initial human clinical trial have indicated that an elastomeric nucleus replacement may be able to overcome these limitations. However, there is a lack of understanding of how such a device will behave in a spinal segment under large compressive loads. Furthermore, an FEA model has not been used to study the ideal characteristics of an in situ curing elastomeric device implanted from the posterolateral corner of the IVD. (ii) Gene therapy [164,166,167] – By the up and down regulation of DNA inside the gene we can repair the unhealthy disc with the help of modern gene therapy technique. (iii) Full or partial nucleotomy (85% at an angle 20° or 72% at an angle 3°) with the help of finite element mechanics of the IVD models including the physiologic, nucleotomy and implant model. Nucleotomy is simply cut out the damage central part (NP) of the already degenerated disc for disc repairmen [166], (iv) By taking some clever strategies using the concepts that chondrocytes cell moves inside the bone tissue in-vivo during the growth of the bone organ of the body [171].

**Outlook of polymeric spinal implants**

In the current study we try to analyze the mechanical properties of AF, NP and IVD in more details and also for the scaffolds materials that had been synthesized from the different composite materials synthesized and cell cultured for variable period from textile fibers like silk, PVA, PGA [172]. The reason to choose this textile fiber is that they are very bio-compatible and also biodegradable inside the human body. For example: the silk has been selected as scaffold material because of the following reasons: For simulating lamellae like fibrous structure of AF, the materials have to be chosen which may be used for scaffold preparation. For this project, silk fiber has been chosen to from the fibrous structure. The reason behind choosing silk as a scaffold material is that, silk offers: unique mechanical property in different material formats (about 2-3 GPa) [173] with the excellent biocompatibility, controlled degradability with the versatile process ability which thus gives a variable potential for tissue engineering applications. Moreover, the ability to process silk into different structural formats using all-

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Scaffold material</th>
<th>Major finding</th>
<th>Mechanics measured</th>
<th>Experimental values</th>
<th>Native benchmark</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF/</td>
<td>Alginate / chitosan hybrid fibers</td>
<td>AF cells proliferate and expressed collagen II, construct were nonimmunogenic upon subcutaneous implantation</td>
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<tr>
<td>AF cells</td>
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<tr>
<td>AF cells (canine)</td>
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<td>AF cells (bovine)</td>
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<td>MSCa (bovine)</td>
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<td>Electrosputn PCL</td>
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<td>Electrosputn PCL</td>
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<tr>
<td>NP</td>
<td>Collagen I</td>
<td>Gel formation was tailored to replicate mechanical function, dynamic shear</td>
<td>Torsional shear</td>
<td>0°: 6.5-8.5° 23-30°</td>
<td></td>
</tr>
<tr>
<td>IVD: AF and NP cells (bovine)</td>
<td>PGA (AF) and alginate (NP)</td>
<td>Formed AF-NP composite ECM and increased compressive properties after implantation</td>
<td>Unconfined compression</td>
<td>H= 50kPa, k = 5x 10⁻¹⁰ m²/(Pa s)</td>
<td>3-10 MPa</td>
</tr>
</tbody>
</table>

**Table 2:** Materials (Scaffolds) used in IVD tissue engineering [19,157,158,161].
aqueous process render it useful for the delivery of the bioactive components via this biomaterial matrix, as well as avoiding concerns for residual organic solvents in the devices Sometime they are cross-linked with chondroitin sulfate (CS) to make it a highly bio-compatible composite tissue engineered architected structure [171]. MSC human nasal chondroside cell is used to culture this synthesized scaffolds. Using the silk fibers, the aim would be to fabricate a structure similar to that of collagen structure of native AF. In that case, the orientation angle of fibers, diameter of fibers and fiber content in the scaffold may need to be varied [174]. So the objective of current work to simulate and experimental evaluation of the biomechanical properties of the scaffolds with the native benchmark value of IVD for a successful tissue engineering. J Orthop Res 27: 620–626. 


