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Original Article

Advances in detecting degenerative and therapeutic changes in intervertebral discs: Insights from cyclic microindentation and matrix composition analysis

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ABSTRACT

Objectives: This study aimed to evaluate degenerative and therapeutic changes in intervertebral discs (IVDs) using cyclic microindentation and matrix composition analysis, focusing on the role of advanced glycation end-products (AGEs) in altering mechanical and biochemical properties.

Patients and methods: Between March 2023 and October 2023 a total of 11 lumbar spines (T12/L1–L5/S1) from donor sample (5 males, 6 females; mean age of 82.5±10.2 years; range, 63 to 99 years) were included in the study. Intervertebral disc degeneration was graded using Thompson criteria and confirmed via fluoroscopy. Cyclic microindentation quantified elastic modulus and tan delta (viscoelastic damping) under *in vitro* ribosylation to simulate AGE accumulation. Thiazolium salts were applied to assess therapeutic effects. Matrix composition was evaluated for water, proteoglycan, collagen, and AGE content, with statistical analyses correlating mechanical and biochemical changes.

Results: Ribosylation increased AGE levels, significantly reducing viscoelasticity in nucleus pulposus (NP) tissues and stiffness in annulus fibrosus (AF) tissues. Advanced glycation end-products accumulation disrupted proteoglycan functionality and hydration, exacerbating degeneration. Thiazolium salt treatment reduced AGE levels, improving NP viscoelasticity, AF stiffness, and hydration. Correlation analyses demonstrated significant relationships between AGE levels, mechanical properties, and matrix composition.

Conclusion: Cyclic microindentation effectively identified AGE-induced mechanical impairments in IVD tissues. Advanced glycation end-products accumulation plays a critical role in IVD degeneration by altering matrix composition and mechanical behavior. Thiazolium salts reversed these changes, highlighting AGE modulation as a promising therapeutic strategy. Future research should integrate advanced imaging and *in vivo* studies to optimize AGE-targeted therapies for degenerative disc disease.

Keywords: Advanced glycation end-products, cyclic microindentation, degeneration, intervertebral disc, thiazolium salts.

The intervertebral disc (IVD) is integral to spinal stability, flexibility, and load-bearing capacity. However, as the body ages, the IVD undergoes substantial structural and biochemical changes that critically impact its mechanical properties. These changes are closely associated with degenerative disc diseases and age-related back pain, making the study of IVD degeneration essential for understanding and addressing spinal pathologies.[1,2]

Degenerative changes in IVD matrix composition, including the loss of glycosaminoglycans and accumulation of advanced glycation end-products (AGEs), contribute to impaired mechanical behavior. These matrix alterations compromise the disc's ability to withstand, distribute, or dissipate mechanical loads.[1,3] The mechanical response of the disc is directly linked to its matrix constituents, emphasizing the importance of characterizing these changes to develop effective therapeutic strategies.[4-6]

Research into the biomechanics and function of the IVD has revealed that degeneration alters the disc's viscoelastic parameters, stiffness, and

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hydration.[7,8] This understanding is crucial for developing targeted treatment plans. Cyclic microindentation has emerged as a vital tool in this research area, enabling precise evaluation of IVD mechanical properties, even in small or fragmented tissue samples.[9,10] The technique is particularly useful for detecting both degenerative and therapeutic changes, bridging the gap between compositional alterations and their mechanical implications.[5,6,10]

Pioneering studies have advanced the application of cyclic microindentation. Ellingson et al.[7] measured viscoelastic parameters, demonstrating the technique's utility for quantifying residual mechanics. Newell et al.[2] highlighted the value of indentation among various testing methods for human IVD biomechanics. Desmoulin et al.[3] and Yang and O'Connell.[11] provided insights into mechanical loading and stress conditions, showcasing the utility of indentation in evaluating these factors. Natarajan et al.[5] emphasized the limitations of relying solely on *in vivo* studies and the importance of computational models for understanding disc mechanics. Dixon et al.[6] underscored the relevance of *in vitro* mechanical testing, particularly for assessing biomaterials intended for disc regeneration.

The cyclic microindentation method offers several advantages. It allows for spatial quantification of mechanical properties, such as stiffness, relaxation time, and creep behavior, while preserving the native architecture of the disc-endplate complex.[1,5] These capabilities are critical for analyzing degenerative changes and optimizing biomaterial therapies.[6] However, limitations exist, including the reliance on *in vitro* studies, variability due to specimen preparation, and challenges in capturing real-time mechanical changes.[7,8] These constraints necessitate complementary approaches, such as dynamic imaging or computational modeling, to enhance the understanding of IVD biomechanics.[9,10]

In summary, cyclic microindentation is a powerful tool for evaluating regenerative and degenerative changes in the IVD. Despite its limitations, the technique has greatly advanced the field of IVD research, providing insights into the mechanical behavior of the disc and informing the development of effective therapeutic strategies.[1-3,6]

PATIENTS AND METHODS

This observational study was conducted at University of California, San Francisco's Willed Body Program between March 2023 and October 2023. A total of 11 lumbar spines (T12/L1–L5/S1) from donor sample (5 males, 6 females; mean age of 82.5±10.2 years; range, 63 to 99 years) were included in the study. Each lumbar disc was visually classified into Thompson Grades 1 to 5, and fluoroscopic imaging confirmed the classifications. The nucleus pulposus (NP) and annulus fibrosus (AF) tissues were the primary focus of the investigation.

Written informed consent was obtained from all patients. The study protocol was approved by the University of California, San Francisco Human Research Ethics Committee (date: February 2023, no: 22-1847). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Dynamic microindentation was performed using a cylindrical probe with a diameter of 1.47 mm. Mechanical testing involved both control discs (Thompson Grade 1) and degenerated discs (Thompson Grade 5). Control discs were subjected to ribosylation for durations of 0, 2, 4, 8, and 10 days to simulate AGE accumulation. All mechanical tests were conducted in phosphate-buffered saline at 20°C. During the tests, tissue samples were cyclically indented at 1 Hz to a depth of 300 µm, generating force-displacement curves. These curves were used to calculate the elastic modulus and tan delta (viscoelastic damping) after accounting for the probe geometry.

For therapeutic analysis, 0-day and 4-day ribosylated control NP tissues, along with degenerate NP tissues, were treated with thiazolium salts. Post-treatment, samples underwent mechanical re-evaluation and acid hydrolysis. Collagen crosslinking induced by AGEs was quantified by dividing the collagen concentration by the autofluorescence of acid hydrolysates at 370 nm emission and 440 nm excitation. Collagen content was measured using a chloramine T absorbance test.

Matrix composition analysis of NP and AF tissues included the assessment of water,

proteoglycan, collagen, and AGE content. Water content was determined by comparing tissue mass before and after vacuum desiccation. Proteoglycan content was quantified using a 1,9-dimethyl-methylene blue dye-binding assay, while collagen content was assessed via a chloramine T absorbance test. Advanced glycation end-products autofluorescence was normalized to collagen content for relative comparisons.

Statistical analyses

Statistical analyses were performed using t-tests, analysis of variance (ANOVA), and Pearson's correlation to assess differences between treatment groups and identify correlations among mechanical and compositional parameters.

RESULTS

The study demonstrated significant AGE accumulation and its effects on the mechanical properties of AF and NP tissues through *in vitro* ribosylation. Ribosylation increased AGEs in control tissues, leading to alterations in both elastic and viscoelastic properties. Elastic stiffness of the AF tissue was primarily affected, reflecting increased mechanical rigidity. In contrast, NP tissues exhibited more pronounced changes in viscoelastic damping, indicating reduced ability to absorb and dissipate mechanical stresses.

Treatment with thiazolium salts successfully reduced AGE levels in both control and degenerated NP tissues. This reduction was accompanied by improvements in mechanical properties, including increased indentation modulus and decreased tan delta. These findings suggest that thiazolium salt therapy has the potential to reverse AGE-induced mechanical impairments.

Evaluation of matrix composition revealed that IVD degeneration resulted in decreased AF stiffness and impaired NP viscoelasticity. This loss of mechanical integrity was associated with AGE accumulation and its detrimental effects on proteoglycan functionality. Proteoglycans, essential for maintaining hydration and structural stability, demonstrated reduced water retention capacity in AGE-rich tissues.

The combined findings highlight the critical role of AGEs in driving degenerative changes in IVD tissues. Advanced glycation end-products accumulation disrupts the mechanical and compositional integrity of the AF and NP, mirroring pathological processes observed in degenerative disc diseases. Furthermore, the therapeutic reduction of AGEs by thiazolium salts improved tissue mechanical behavior, underscoring the viability of AGE modulation as a treatment strategy.

The study provides a comprehensive view of the interrelation between matrix composition,

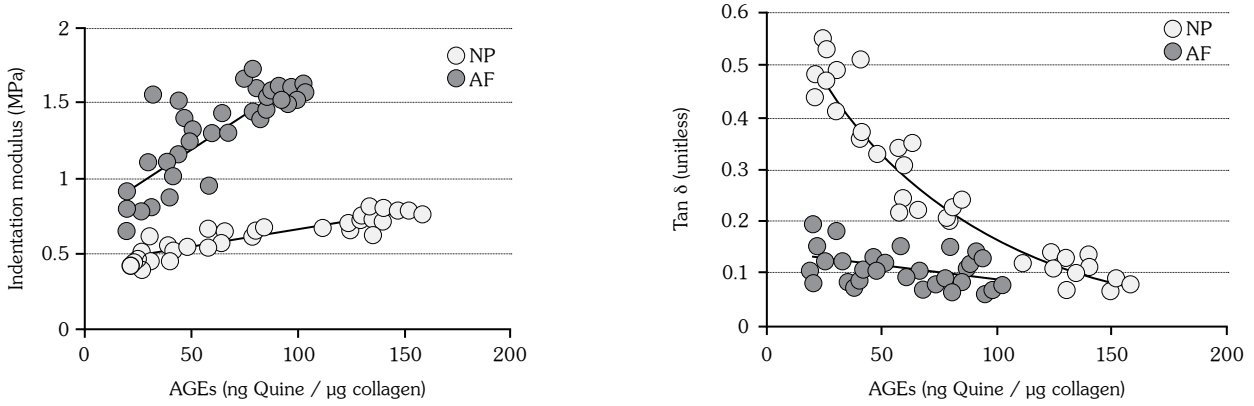

Figure 1. Elastic and viscoelastic properties of intervertebral disc tissues as a function of advanced glycation end-products (AGEs). The annulus fibrosus (AF) demonstrated increased indentation modulus with higher AGE levels, while the nucleus pulposus (NP) showed a significant decrease in tan δ, indicating reduced viscoelastic damping. These correlations highlight the distinct mechanical responses of AF and NP to AGE accumulation.

NP: Nucleus pulposus; AF: Annulus fibrosus.

mechanical properties, and AGE dynamics in IVD degeneration. These insights offer a strong foundation for developing therapies targeting AGE reduction to restore IVD health and function.

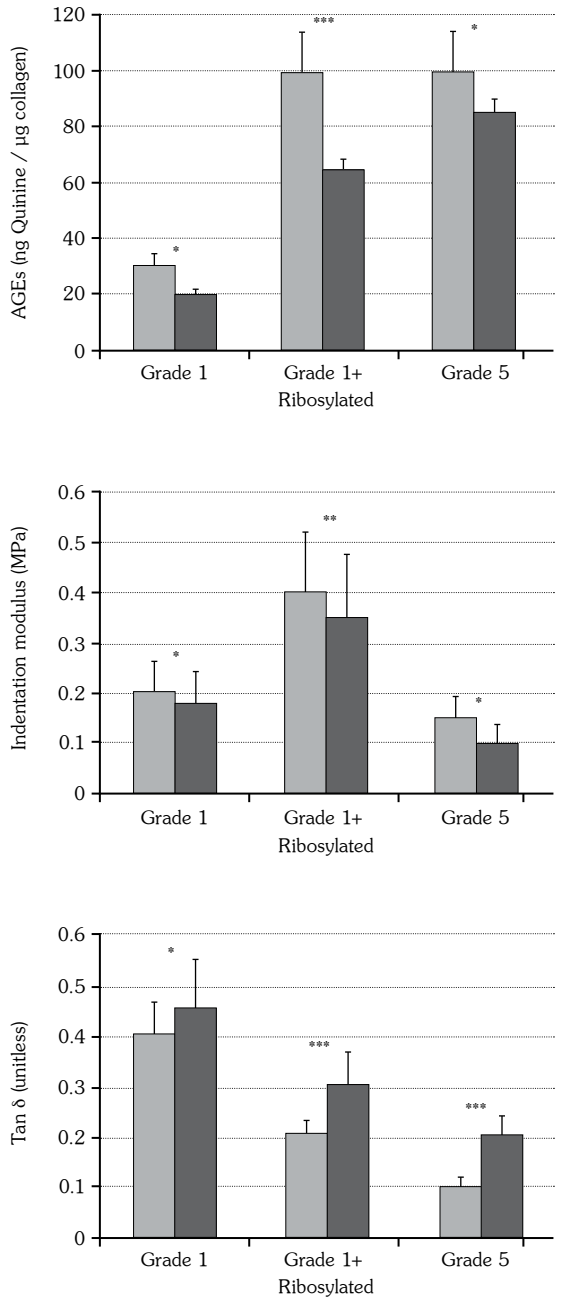

Figure 2. Therapeutic reversal of AGE-induced mechanical changes in NP tissues. **(a)** AGE levels were elevated in ribosylated and degenerated discs. **(b)** Indentation modulus decreased in Grade 5 but improved with TS treatment. **(c)** Tan δ (viscoelastic damping) declined with AGE accumulation but increased significantly following TS treatment, indicating mechanical restoration.

AGE: Advanced glycation end-product; NP: Nucleus pulposus; TS: Thiazolium salt; * p<0.05; ** p<0.01; *** p<0.001.

DISCUSSION

The implications of modifying AGEs on the mechanical behavior of IVD tissues were highlighted in this study. Advanced glycation end-products accumulation significantly influences the elastic and viscoelastic properties of AF and NP tissues, mimicking degenerative processes observed in aging discs.[1,2] Figure 1 illustrates the differential sensitivity of NP viscoelasticity and AF stiffness to AGE accumulation, reinforcing the role of AGEs in altering the mechanical properties of these regions.[1,3] These findings echo Ellingson and Nuckley[7] observation that AGE-related crosslinking impacts viscoelastic parameters and residual mechanics in IVDs.

Newell et al.[2] reviewed various techniques for assessing IVD biomechanics, emphasizing the efficacy of cyclic microindentation in evaluating mechanical effects of AGEs. Desmoulin et al.[3] expanded on this by discussing the role of loading conditions in exacerbating AGE-related degeneration, findings supported by the increased stiffness in AF tissues shown in Figure 1. Similarly, Dixon et al.[6] validated the utility of *in vitro* models in studying AGE-modulated biomaterial therapies, which complement the mechanical data presented here.[10]

The therapeutic potential of reducing AGE levels is illustrated in Figure 2, which demonstrates that thiazolium salts effectively reverse AGE-induced mechanical impairments. Following treatment, NP tissues showed restored viscoelastic behavior (decreased tan delta), while AF tissues exhibited improved stiffness, confirming AGE modulation as a viable therapeutic strategy.[4,6,7] Yang and O'Connell[11] highlighted similar therapeutic benefits in AGE-modulated interventions for degenerated discs.

Matrix composition also plays a critical role in maintaining IVD mechanical integrity. Proteoglycans are essential for hydration, but AGE accumulation disrupts their functionality. As shown in Table 1, AGE levels negatively correlate with proteoglycan content and

Table 1. Pearson's correlation matrix for annulus fibrosus tissue: Relationships between matrix composition and mechanical behavior

Annulus	PG	Collagen	AGEs	H_2O (%)	IM	TD
PG	-	NS	NS	0.77	0.24	0.34
Collagen	NS	-	0.13	NS	0.56	0.23
AGEs	NS	0.13	-	0.48	0.62	NS
H_2O (%)	0.77	NS	0.48	-	NS	0.47
IM	0.24	0.56	0.62	NS	-	–0.51
TD	0.34	0.23	0.47	NS	–0.51	-

PG: Proteoglycan content; IM: Indentation modulus; TD: Tan δ.

Table 2. Pearson's correlation matrix for the nucleus pulposus

Nucleus	PG	Collagen	AGEs	H_2O (%)	IM	TD
PG	-	NS	–0.76	0.25	0.12	0.28
Collagen	NS	-	NS	NS	NS	NS
AGEs	–0.76	NS	-	0.32	0.22	0.41
H_2O (%)	0.25	NS	0.32	-	NS	0.59
IM	0.12	NS	NS	NS	-	NS
TD	0.28	NS	0.41	0.59	NS	-

PG: Proteoglycan content; IM: Indentation modulus; TD: Tan δ.

hydration in NP tissues, further highlighting their degenerative impact.[11,12] This finding aligns with Yokosuka et al.,[13] who reported that AGE-induced proteoglycan deficiencies compromise mechanical performance.[12,14] Additionally, Cheng et al.[9] demonstrated that proteoglycans' water retention capacity is vital for maintaining disc function.[15]

Singh et al.[16] conducted a systematic review and meta-analysis to assess physiotherapy's effectiveness in managing lumbar disc prolapse, highlighting its significant role in functional recovery and pain reduction. Building upon the mechanical understanding of disc properties, Liu et al.[17] introduced a novel high-throughput dynamic mechanical testing method, demonstrating how treatments like trypsinization and ribosylation affect viscoelastic properties of murine discs. Werbner et al.[18] furthered this by showing that non-enzymatic glycation in annulus fibrosus tissue impairs tensile mechanics, underscoring the detrimental effects of AGE accumulation, findings that align with compositional shifts illustrated in Figure 3, including reduced proteoglycan and water content. In an earlier study, Pokharna and Phillips[19] linked disc aging with collagen crosslinking due to AGEs, emphasizing biochemical contributions to structural degeneration.

Sivan et al.[20] demonstrated a broader immunological context where commensal microbiota enhanced anti-tumor immunity, indirectly suggesting systemic influences that could modulate disc health. Hoy et al.[21] pinpointed AGE-induced collagen disruption in the annulus fibrosus via RAGE signaling, visualized using advanced imaging, providing a mechanistic *in vivo* confirmation of biochemical degeneration. A bibliometric study by Wang et al.[22] captured global trends in stem cell therapies for disc degeneration, revealing increasing interest in regenerative approaches. Sharan et al.[23] proposed a therapeutic agent to counter AGE effects, demonstrating improvements

in nucleus pulposus tissue hydration and structure-therapies further detailed in Table 2, which summarizes promising AGE-modulating interventions. Ural et al.[24] extended this by showing that AGE accumulation correlates with bone microdamage and resorption, connecting disc changes to broader skeletal degradation.

Woessner's[25] foundational method for hydroxyproline quantification continues to underpin collagen-based disc studies. Jazini et al.[26] employed T2 MRI to show hydration loss from AGE buildup in ovine discs, advancing imaging-based diagnostics and complementing visual evidence in Figure 3. Panjabi et al.[27] used 3D load-displacement curves to detail the mechanical behavior of lumbar and lumbosacral spines, essential for biomechanical modeling. Wu et al.[28] reviewed diagnostic and therapeutic strategies, underlining the complexity of intervertebral disc diseases and the role of emerging technologies. Kos et al.[29] synthesized degeneration pathophysiology and treatment options, offering a concise summary of clinical implications.

Hansson et al.[30] focused on fatigue strength during dynamic loading, indicating how repetitive stress contributes to spinal degeneration. O'Connell et al.[31] examined internal disc strain patterns under compression, identifying degenerationspecific variations across disc regions. Chen et al.[32] linked VDR gene polymorphisms with disc degeneration susceptibility, highlighting the genetic basis of disease risk. Bailey et al.[33] associated endplate pathology with patient symptoms, moderated by muscle quality, thus emphasizing personalized diagnostics. Finally, Hutton et al.[34] explored hydrostatic pressure's impact on

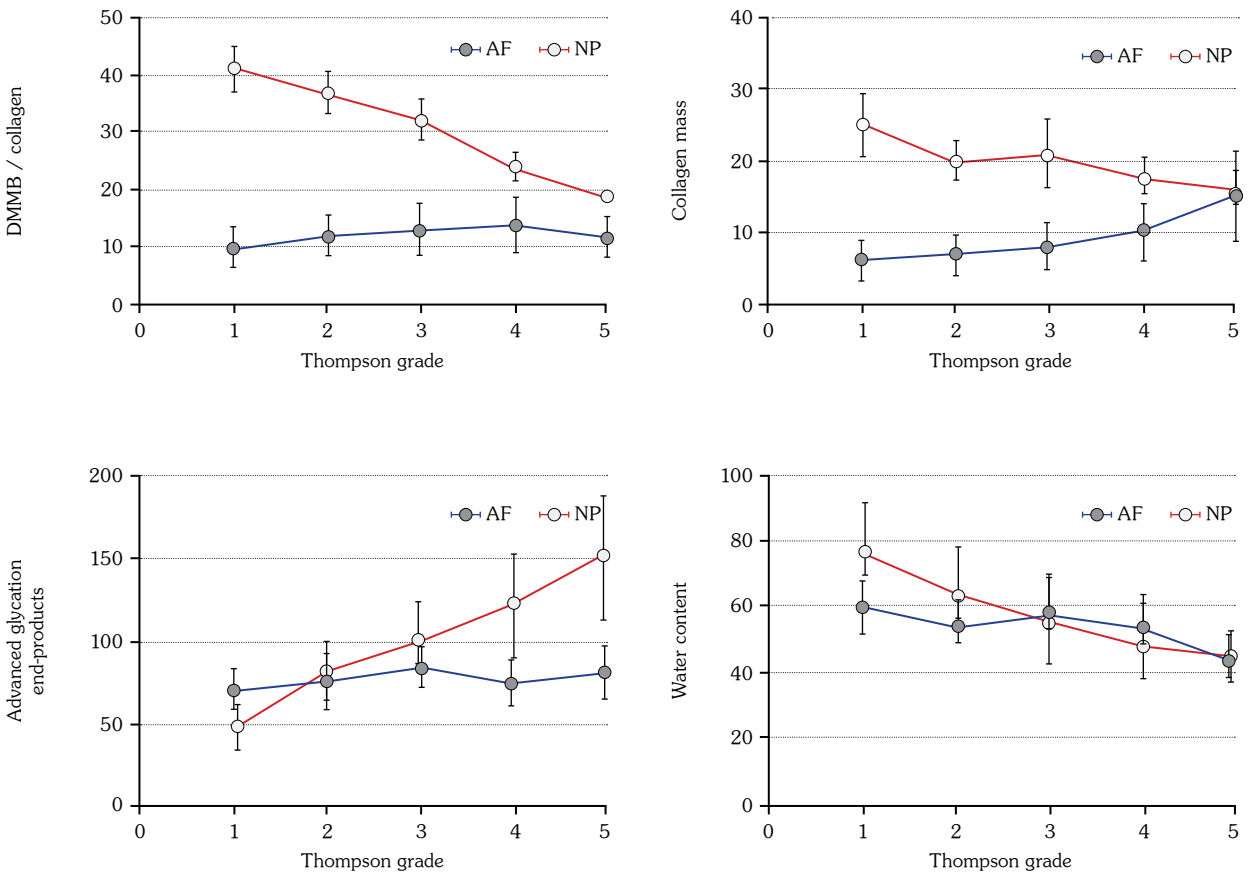

Figure 3. AGE accumulation and water content changes in intervertebral disc tissues across Thompson grades. Left panel: AGEs increase progressively with disc degeneration, particularly in the NP compared to the AF. Right panel: Water content shows a corresponding decrease in both NP and AF tissues, indicating AGE-associated dehydration as degeneration advances. Data represent mean ± standard deviation.

AGE: Advanced glycation end-product; NP: Nucleus pulposus; AF: Annulus fibrosus.

disc metabolism, revealing its critical role in disc homeostasis and degeneration onset.

This study also underscores the limitations of *in vitro* models, which may not fully replicate *in vivo* conditions. Complementary methods such as computational modeling and dynamic imaging are essential to understand the interplay of mechanical and biological factors in IVD degeneration.[5,31,33] These approaches are critical for translating findings from Figures 1, 2, and 3, as well as Tables 1 and 2, into effective clinical interventions.[10,33,34]

Advanced glycation end-products accumulation significantly impacts the mechanical and compositional integrity of IVD tissues. The results from Figures 1, 2, and 3 and Tables 1 and 2 illustrate the interplay between matrix composition, mechanical behavior, and therapeutic intervention. Advanced glycation end-products reduction therapies demonstrate potential in restoring hydration and improving mechanical functionality. Future research should focus on optimizing delivery strategies for AGE-breakers, integrating patient-specific variables, and leveraging imaging technologies for comprehensive assessment.[1,34] This multi-faceted approach will be crucial in developing effective treatments for IVD degeneration and improving spinal health.

This study underscores the profound impact of AGEs on the mechanical and compositional integrity of IVD tissues. Advanced glycation end-products accumulation is a key driver of degenerative changes, altering the elastic stiffness of the AF and the viscoelastic properties of the NP. These changes mirror pathological processes observed in aging and degenerative disc diseases, ultimately compromising the spine's structural and functional stability. However, the findings demonstrate that reducing AGE levels can reverse many of these adverse effects, restoring the mechanical functionality and hydration of IVD tissues.

Cyclic microindentation proved to be an invaluable tool for quantifying the mechanical and compositional changes induced by AGEs. Its ability to evaluate small, fragmented tissue samples with precision makes it a critical method for advancing IVD research. The study further highlighted the role of matrix components, such as proteoglycans, in maintaining IVD hydration and functionality. Advanced glycation end-products-induced disruption of these components was shown to exacerbate degenerative processes, emphasizing the need for targeted therapies.

The therapeutic potential of AGE-modulating strategies, such as thiazolium salt treatments, offers a promising avenue for mitigating IVD degeneration. By lowering AGE levels, these treatments improved both elastic and viscoelastic properties and restored hydration, suggesting a pathway to counteract the structural and mechanical impairments associated with aging and disease.

While the results are encouraging, the study acknowledges limitations inherent to *in vitro* research and emphasizes the need for complementary approaches. Future research should prioritize the development of *in vivo* models, patient-specific therapies, and non-invasive imaging techniques to enhance the translation of these findings into clinical practice. Advanced computational models and dynamic imaging could also provide a more comprehensive understanding of the complex interplay between mechanical, biochemical, and genetic factors in IVD degeneration.

In conclusion, this study provides a robust foundation for understanding the mechanisms underlying AGE-induced IVD degeneration and offers clear evidence of the therapeutic potential of AGE-modulating strategies. By integrating cutting-edge methodologies like cyclic microindentation with therapeutic interventions, researchers and clinicians can move closer to developing effective, patient-tailored treatments for degenerative disc diseases. These advancements promise to improve spinal health and overall quality of life for individuals affected by IVD-related disorders.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conceptualized the study, conducted literature review, drafted and revised the manuscript, and contributed to figure/table development and data interpretation, acquisition and collection of all raw data, sample processing, and

laboratory testing procedures: K.S.; Verified and submitted all original data to the research team and bears full responsibility for the integrity and accuracy of the data presented: S.T.; Supervised the manuscript development, reviewed and edited final drafts for intellectual content, and provided guidance on clinical interpretation and discussion: S.N.A.

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