Multidisciplinary Journal of Academic Publications
ISSN (Online): 3107-538X
Vol. 01, Issue 02, November – December 2025

PLANT-BASED BIOACTIVE SCAFFOLD CONSTRUCTS FOR ENHANCING REGENERATION IN OSTEOARTHRITIC TISSUES: AN EXPERIMENTAL ANALYSIS

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Abstract

Osteoarthritis (OA) represents a significant global health challenge, affecting millions of individuals worldwide with progressive cartilage degradation and joint dysfunction. This experimental study investigates the therapeutic potential of plant-based bioactive scaffold constructs in enhancing tissue regeneration within osteoarthritic environments. The primary objective was to evaluate the efficacy of scaffolds derived from natural polymers including chitosan, alginate, and cellulose, incorporated with phytochemical compounds such as curcumin, resveratrol, and quercetin. A randomized controlled experimental design was employed utilizing 150 Wistar rats divided into five groups: control, OA-induced, and three treatment groups receiving different scaffold formulations. The methodology encompassed scaffold fabrication, characterization, in vivo implantation, and comprehensive histological and biochemical analyses over 12 weeks. The hypothesis proposed that plant-based bioactive scaffolds would significantly enhance chondrocyte proliferation, reduce inflammatory markers, and promote extracellular matrix synthesis compared to conventional treatments. Results demonstrated substantial improvements in cartilage thickness ($p<0.001$), reduced inflammatory cytokines including IL-1β and TNF-α ($p<0.01$), and enhanced collagen type II expression in treatment groups. Statistical analyses revealed dose-dependent responses with curcumin-loaded scaffolds showing superior regenerative outcomes. The study concludes that plant-based bioactive scaffolds present a promising therapeutic strategy for osteoarthritis management, offering biocompatibility, controlled drug release, and enhanced tissue regeneration capabilities.

Keywords: *Plant-based scaffolds[1], osteoarthritis[2], tissue regeneration[3], bioactive compounds[4], cartilage repair[5].*

1. Introduction

Osteoarthritis constitutes one of the most prevalent degenerative joint diseases globally, affecting approximately 528 million people worldwide and representing a leading cause of disability among the elderly population (Hunter & Bierma-Zeinstra, 2019). The disease is characterized by progressive deterioration of articular cartilage, subchondral bone remodeling, osteophyte formation, and synovial inflammation, collectively resulting in chronic pain, stiffness, and functional impairment (Martel-Pelletier et al., 2016). The pathophysiology of OA involves complex interactions between mechanical stress, inflammatory mediators, and metabolic factors that

disrupt the delicate balance between cartilage synthesis and degradation. Current therapeutic approaches predominantly focus on symptomatic relief through pharmacological interventions including non-steroidal anti-inflammatory drugs, corticosteroid injections, and viscosupplementation, yet these strategies fail to address the underlying degenerative processes or promote substantial tissue regeneration (Bannuru et al., 2019). The limitations of conventional treatments have catalyzed extensive research into regenerative medicine approaches, particularly tissue engineering strategies employing bioactive scaffold constructs. Scaffolds serve as three-dimensional frameworks that provide structural support, facilitate cell attachment and proliferation, and guide tissue formation through controlled release of bioactive molecules (O'Brien, 2011). Plant-based biomaterials have emerged as particularly promising candidates for scaffold fabrication due to their inherent biocompatibility, biodegradability, non-immunogenic properties, and abundant availability. Natural polymers derived from plant sources including chitosan, alginate, cellulose, and hyaluronic acid possess structural similarities to native extracellular matrix components, making them ideal for cartilage tissue engineering applications (Roseti et al., 2017).

Recent advances in phytochemical research have identified numerous plant-derived bioactive compounds with potent anti-inflammatory, antioxidant, and chondrogenic properties. Curcumin, extracted from Curcuma longa, demonstrates significant anti-inflammatory effects by inhibiting NF-κB signaling pathways and reducing pro-inflammatory cytokine expression (Henrotin et al., 2013). Resveratrol, a polyphenolic compound found in grapes and berries, exhibits chondroprotective effects through SIRT1 activation and mitochondrial function preservation (Dave et al., 2013). Quercetin, a flavonoid abundant in various fruits and vegetables, promotes chondrocyte proliferation and extracellular matrix synthesis while suppressing matrix metalloproteinase activity (Zheng et al., 2017). The incorporation of these phytochemicals into plant-based scaffolds represents an innovative strategy for creating multifunctional constructs that simultaneously provide structural support and deliver therapeutic agents to osteoarthritic tissues. The integration of natural polymers with bioactive phytochemicals addresses several critical challenges in osteoarthritis treatment including limited intrinsic healing capacity of cartilage, chronic inflammatory environment, and inadequate delivery of therapeutic agents to target sites. This experimental investigation aims to comprehensively evaluate the regenerative potential of plant-based bioactive scaffold constructs in osteoarthritic tissue repair, examining their effects on cellular responses, inflammatory markers, and extracellular matrix composition through rigorous in vivo experimentation and multifaceted analytical approaches.

2. Literature Review

The application of plant-based biomaterials in tissue engineering has gained substantial momentum over the past decade, with numerous studies demonstrating their efficacy in various regenerative medicine applications. Chitosan, a deacetylated derivative of chitin obtained from crustacean shells and fungal cell walls, exhibits excellent biocompatibility, biodegradability, and mucoadhesive properties that make it particularly suitable for cartilage regeneration scaffolds (Croisier & Jérôme, 2013). Research by Radhakrishnan et al. (2018) demonstrated that chitosan-based scaffolds promote chondrocyte adhesion and proliferation while maintaining cellular phenotype, essential characteristics for successful cartilage tissue engineering. The cationic nature of chitosan facilitates electrostatic interactions with negatively charged glycosaminoglycans in cartilage matrix, enhancing scaffold integration with native tissue. Alginate, a naturally occurring anionic polysaccharide extracted from brown seaweed, has been extensively investigated for cartilage tissue engineering due to its gel-forming properties, biocompatibility, and similarity to cartilage extracellular matrix (Bidarra et al., 2014). Studies have shown that alginate hydrogels support chondrocyte encapsulation and maintain cellular viability while allowing nutrient diffusion and waste removal. The ability to control alginate gelation through calcium cross-linking provides versatility in scaffold fabrication and mechanical property modulation (Oliveira et al.,

2017). Combination approaches utilizing chitosan-alginate hybrid scaffolds have demonstrated synergistic effects, combining the advantages of both polymers to create superior constructs for cartilage regeneration.

Cellulose nanocrystals and bacterial cellulose represent another category of plant-derived biomaterials showing promise in osteoarthritis treatment. Research by Martínez Ávila et al. (2014) revealed that bacterial cellulose scaffolds possess excellent mechanical properties, high water retention capacity, and nanofibrous structure mimicking natural extracellular matrix. These characteristics promote chondrocyte attachment, proliferation, and differentiation, essential processes for cartilage regeneration. The incorporation of growth factors and bioactive molecules into cellulose-based scaffolds enhances their therapeutic potential through controlled release mechanisms. The therapeutic application of curcumin in osteoarthritis management has been substantiated through numerous preclinical and clinical investigations. Belcaro et al. (2010) conducted clinical trials demonstrating that curcumin supplementation significantly reduces pain scores and improves functional outcomes in osteoarthritis patients, with effects comparable to ibuprofen but with superior safety profiles. The anti-inflammatory mechanisms of curcumin involve inhibition of cyclooxygenase-2, lipoxygenase, and inducible nitric oxide synthase, key enzymes involved in inflammatory cascade activation (Shakibaei et al., 2011). Furthermore, curcumin exhibits direct chondroprotective effects by suppressing matrix metalloproteinase expression and enhancing collagen type II synthesis. Resveratrol has emerged as a potent chondroprotective agent through multiple molecular mechanisms. Research by Wang et al. (2017) demonstrated that resveratrol activates SIRT1, a NAD-dependent deacetylase that regulates cellular senescence, apoptosis, and inflammation in chondrocytes. This activation results in suppression of catabolic pathways, reduced oxidative stress, and enhanced autophagy, collectively contributing to cartilage preservation. Additionally, resveratrol inhibits NF-κB signaling and reduces production of inflammatory mediators including interleukin-1β, tumor necrosis factor-α, and prostaglandin E2, which are key contributors to osteoarthritis progression (Csaki et al., 2009). Quercetin demonstrates multifaceted beneficial effects on cartilage metabolism and inflammation control. Studies by Li et al. (2015) showed that quercetin promotes chondrocyte proliferation and differentiation through activation of Wnt/β-catenin signaling pathway while simultaneously suppressing inflammatory responses. The compound exhibits strong antioxidant properties, neutralizing reactive oxygen species that contribute to chondrocyte damage and matrix degradation. Quercetin also inhibits advanced glycation end-product formation, which accelerates cartilage aging and degradation in osteoarthritic joints (Ohashi et al., 2012).

The combination of plant-based scaffolds with phytochemical compounds represents a synergistic approach that addresses multiple pathological aspects of osteoarthritis simultaneously. Research by Venkatesan et al. (2015) demonstrated that chitosan scaffolds loaded with curcumin exhibited enhanced anti-inflammatory effects and superior chondroprotection compared to either component alone. The scaffold provides sustained release of bioactive compounds while serving as a structural framework for tissue regeneration. Similar synergistic effects have been observed with other combinations including alginate-resveratrol and cellulose-quercetin constructs (Gupta et al., 2016). Mechanical properties of scaffolds play crucial roles in cartilage tissue engineering success. Native articular cartilage exhibits complex mechanical behavior including viscoelasticity, compressive stiffness, and load-bearing capacity that must be replicated in engineered scaffolds (Mow et al., 1992). Studies by Guo et al. (2018) demonstrated that incorporation of nanocellulose into chitosan-alginate scaffolds significantly enhanced mechanical strength and elastic modulus, approaching values observed in native cartilage. The mechanical environment influences chondrocyte behavior, gene expression, and matrix synthesis, making mechanical property optimization essential for successful scaffold design.

Scaffold architecture and porosity critically influence cellular infiltration, nutrient transport, and tissue integration. Research by Murphy et al. (2010) established that optimal pore sizes ranging from 100-300 micrometers facilitate chondrocyte migration and matrix deposition while maintaining structural integrity. Interconnected porous networks enable efficient nutrient diffusion and metabolic waste removal, essential for

cell survival in three-dimensional constructs. Advanced fabrication techniques including freeze-drying, electrospinning, and 3D printing enable precise control over scaffold architecture and porosity distribution. Biodegradation kinetics of plant-based scaffolds must be carefully controlled to match tissue regeneration rates. Scaffolds should maintain structural integrity during initial healing phases while gradually degrading as newly formed tissue provides mechanical support (Chen & Liu, 2016). Studies have shown that cross-linking methods, polymer molecular weight, and composition ratios can be modulated to control degradation rates of chitosan, alginate, and cellulose scaffolds. The degradation products should be non-toxic and easily metabolized or excreted to avoid local or systemic adverse effects.

3. Objectives

1. To synthesize and characterize plant-based bioactive scaffolds composed of chitosan, alginate, and cellulose incorporated with phytochemicals (curcumin, resveratrol, and quercetin) for osteoarthritis therapy.
2. To assess the in vivo regenerative efficacy of the developed scaffolds in osteoarthritic animal models by evaluating cartilage repair, inflammation reduction, and extracellular matrix synthesis using histological, biochemical, and immunohistochemical methods.
3. To compare therapeutic performance among different scaffold formulations and phytochemical loadings based on chondrocyte proliferation, inflammatory marker modulation, collagen type II expression, and joint functional recovery.
4. To optimize scaffold design parameters, including composition, architecture, and bioactive loading, to achieve maximal regeneration with acceptable biocompatibility, mechanical strength, and controlled degradation for clinical applicability.

4. Methodology

The study adopted a randomized controlled experimental design to evaluate the therapeutic potential of plant-based bioactive scaffolds for osteoarthritic cartilage regeneration. The investigation was conducted in a certified biotechnology and pharmaceutical sciences facility, with ethical approval obtained from the Institutional Animal Ethics Committee in accordance with CPCSEA guidelines. A total of 150 male Wistar rats (12–14 weeks; 250–300 g) were housed under standardized environmental conditions with ad libitum access to food and water following acclimatization. Animals were randomly assigned into five groups: normal control, disease control, curcumin-loaded scaffold, resveratrol-loaded scaffold, and combined phytochemical scaffold groups. Osteoarthritis was induced via intra-articular injection of monosodium iodoacetate into the knee joint under anesthesia. Scaffolds were fabricated using chitosan, alginate, and cellulose polymers incorporated with curcumin, resveratrol, and quercetin, cross-linked through ionic gelation, freeze-dried to obtain porous structures, and sterilized prior to implantation. Comprehensive scaffold characterization included morphological, chemical, mechanical, swelling, and release kinetic analyses. Scaffold implantation was performed two weeks post-induction into standardized cartilage defects, followed by postoperative care and monitoring. Animals were sacrificed at 4, 8, and 12 weeks for evaluation. Outcome measures included histological, immunohistochemical, and biochemical assessments of cartilage regeneration, extracellular matrix synthesis, and inflammatory markers. Data were analyzed using appropriate statistical tests, with significance set at $p < 0.05$.

5. Results

Table 1: Physical and Mechanical Characterization of Plant-Based Scaffold Constructs

Parameter	Group III (Curcumin Scaffold)	Group IV (Resveratrol Scaffold)	Group V (Combined Scaffold)
Porosity (%)	78.5 ± 3.2	76.3 ± 2.8	79.8 ± 3.5
Average Pore Size (μm)	185.4 ± 12.6	178.2 ± 11.3	192.7 ± 14.2
Compressive Strength (MPa)	2.45 ± 0.18	2.28 ± 0.16	2.67 ± 0.21
Elastic Modulus (MPa)	8.32 ± 0.54	7.86 ± 0.48	9.15 ± 0.62
Swelling Ratio	12.8 ± 0.9	11.6 ± 0.8	13.4 ± 1.1
Degradation Rate (% in 12 weeks)	64.2 ± 4.3	62.8 ± 3.9	66.5 ± 4.7

Table 1 presents the comprehensive physical and mechanical characterization data of the fabricated plant-based bioactive scaffold constructs across the three treatment groups. The porosity values ranged from 76.3% to 79.8%, indicating highly porous structures essential for cellular infiltration and nutrient transport. The combined scaffold (Group V) demonstrated the highest porosity at 79.8 ± 3.5%, followed closely by the curcumin scaffold at 78.5 ± 3.2%. Average pore sizes fell within the optimal range of 178.2 to 192.7 micrometers, facilitating chondrocyte migration and matrix deposition. Mechanical properties showed that the combined scaffold exhibited superior compressive strength at 2.67 ± 0.21 MPa and elastic modulus at 9.15 ± 0.62 MPa, significantly higher than individual component scaffolds ($p<0.05$). The swelling ratios indicated excellent water retention capacity, with the combined scaffold showing the highest value at 13.4 ± 1.1, suggesting favorable conditions for cellular activities. Degradation rates demonstrated controlled biodegradation over the 12-week experimental period, with all groups showing approximately 60-67% degradation, matching tissue regeneration timelines.

Table 2: Bioactive Compound Release Kinetics from Scaffold Constructs (Cumulative Release %)

Time Point	Group III Curcumin	Group IV Resveratrol	Group V Curcumin	Group V Resveratrol	Group V Quercetin
24 hours	18.4 ± 1.2	22.6 ± 1.5	16.8 ± 1.1	20.3 ± 1.4	19.5 ± 1.3
7 days	42.7 ± 2.3	48.2 ± 2.6	38.5 ± 2.1	44.6 ± 2.4	41.2 ± 2.2
14 days	61.3 ± 3.1	67.8 ± 3.4	56.7 ± 2.9	63.2 ± 3.2	59.4 ± 3.0
28 days	78.5 ± 3.8	83.6 ± 4.1	74.2 ± 3.6	79.8 ± 3.9	76.5 ± 3.7
56 days	89.2 ± 4.2	93.4 ± 4.5	85.7 ± 4.0	90.6 ± 4.3	87.9 ± 4.1
84 days	94.6 ± 4.6	97.8 ± 4.8	92.3 ± 4.4	95.4 ± 4.6	93.7 ± 4.5

Table 2 illustrates the in vitro release kinetics of bioactive compounds from the scaffold constructs over the 84-day experimental period. The release profiles demonstrated characteristic biphasic patterns with initial burst release within the first 24 hours, followed by sustained gradual release throughout the remaining period. Resveratrol exhibited slightly faster release kinetics compared to curcumin and quercetin, with Group IV showing 22.6 ± 1.5% release at 24 hours compared to 18.4 ± 1.2% for curcumin in Group III. The combined scaffold (Group V) demonstrated modulated release profiles for all three compounds, with cumulative releases reaching 92.3%, 95.4%, and 93.7% for curcumin, resveratrol, and quercetin respectively by day 84. Statistical analysis revealed significant differences between time points ($p<0.001$), indicating progressive compound

release. The sustained release pattern over 12 weeks ensures continuous therapeutic concentrations at the implantation site, avoiding initial toxic concentrations while maintaining efficacy throughout the regeneration period. The release kinetics correlated well with scaffold degradation rates observed in Table 1, suggesting that compound release was primarily governed by polymer matrix degradation rather than simple diffusion mechanisms.

Table 3: Cartilage Thickness and Chondrocyte Density at 12 Weeks Post-Treatment

Group	Cartilage Thickness (µm)	Chondrocyte Density (cells/mm²)	Safranin-O Staining Intensity (AU)	OARSI Score
Group I (Normal Control)	485.7 ± 22.3	2840 ± 142	245.8 ± 12.4	0.5 ± 0.2
Group II (Disease Control)	156.3 ± 18.6**	980 ± 128**	78.4 ± 9.6**	18.7 ± 2.1**
Group III (Curcumin Scaffold)	382.4 ± 25.7*#	2245 ± 156*#	198.6 ± 15.3*#	4.8 ± 0.9*#
Group IV (Resveratrol Scaffold)	358.6 ± 23.4*#	2108 ± 148*#	185.2 ± 14.1*#	5.6 ± 1.1*#
Group V (Combined Scaffold)	428.5 ± 26.8*#†	2612 ± 168*#†	223.7 ± 16.2*#†	2.3 ± 0.6*#†

**p<0.01 vs. Group I; *p<0.01 vs. Group II; #p<0.05 vs. Group I; †p<0.05 vs. Groups III and IV

Table 3 presents the histomorphometric analysis results evaluating cartilage regeneration at 12 weeks post-treatment, demonstrating significant therapeutic effects of plant-based bioactive scaffolds. Cartilage thickness in the disease control group (Group II) showed severe reduction to 156.3 ± 18.6 micrometers compared to 485.7 ± 22.3 micrometers in normal controls ($p<0.01$), confirming successful osteoarthritis induction. Treatment with bioactive scaffolds resulted in substantial cartilage thickness restoration, with the combined scaffold group (Group V) achieving 428.5 ± 26.8 micrometers, representing 88.2% recovery relative to normal controls and significantly superior to individual component scaffolds ($p<0.05$). Chondrocyte density analysis revealed similar patterns, with Group V achieving 2612 ± 168 cells per square millimeter compared to only 980 ± 128 in disease controls ($p<0.01$). Safranin-O staining intensity, reflecting proteoglycan content crucial for cartilage function, demonstrated marked improvements in all treatment groups, with the combined scaffold showing 223.7 ± 16.2 arbitrary units versus 78.4 ± 9.6 in disease controls. OARSI histological scores, where lower values indicate better cartilage integrity, showed dramatic improvements from 18.7 ± 2.1 in disease controls to 2.3 ± 0.6 in the combined treatment group, approaching normal control values of 0.5 ± 0.2.

Table 4: Inflammatory Cytokine Levels in Serum at 12 Weeks Post-Treatment (pg/mL)

Group	IL-1β	TNF-α	IL-6	PGE_2	COX-2 Activity (U/L)
Group I (Normal Control)	24.6 ± 2.8	32.4 ± 3.2	48.5 ± 4.6	156.3 ± 15.2	8.4 ± 1.2
Group II (Disease Control)	186.4 ± 18.7**	245.8 ± 22.4**	312.7 ± 28.6**	648.9 ± 54.3**	42.8 ± 4.6**

Group III (Curcumin Scaffold)	58.3 ± 6.4*#	72.6 ± 7.8*#	98.4 ± 9.2*#	248.7 ± 24.1*#	15.7 ± 2.1*#
Group IV (Resveratrol Scaffold)	64.7 ± 7.1*#	81.5 ± 8.6*#	108.2 ± 10.4*#	276.4 ± 26.8*#	17.4 ± 2.4*#
Group V (Combined Scaffold)	38.5 ± 4.2*#†	51.8 ± 5.6*#†	72.6 ± 7.8*#†	198.3 ± 19.2*#†	11.2 ± 1.6*#†

**p<0.01 vs. Group I; *p<0.01 vs. Group II; #p<0.05 vs. Group I; †p<0.05 vs. Groups III and IV

Table 4 presents comprehensive inflammatory marker analysis demonstrating the anti-inflammatory efficacy of plant-based bioactive scaffolds. Interleukin-1 beta levels in disease controls reached 186.4 ± 18.7 picograms per milliliter, representing a 658% increase compared to normal controls at 24.6 ± 2.8 pg/mL (p<0.01). Treatment groups showed substantial reductions, with the combined scaffold group achieving 38.5 ± 4.2 pg/mL, representing 79.4% reduction relative to disease controls and significantly better than individual scaffolds (p<0.05). Tumor necrosis factor-alpha demonstrated similar patterns, with Group V reducing levels to 51.8 ± 5.6 pg/mL from 245.8 ± 22.4 pg/mL in disease controls, indicating 78.9% reduction. Interleukin-6 concentrations decreased from 312.7 ± 28.6 pg/mL in disease controls to 72.6 ± 7.8 pg/mL in combined scaffold-treated animals. Prostaglandin E2 levels and cyclooxygenase-2 activity, key inflammatory mediators in osteoarthritis pathogenesis, showed significant reductions in all treatment groups, with combined scaffolds demonstrating superior efficacy. These results confirm that bioactive compound-loaded scaffolds effectively suppress inflammatory responses in osteoarthritic joints, with synergistic effects observed when multiple phytochemicals are combined, likely through complementary mechanisms targeting different inflammatory pathways.

Table 5: Extracellular Matrix Component Expression at 12 Weeks Post-Treatment

Group	Collagen Type II (% Positive Cells)	Aggrecan Expression (AU)	GAG Content (µg/mg Tissue)	MMP-13 Expression (% Positive Cells)	COMP Levels (ng/mL, Serum)
Group I (Normal Control)	82.4 ± 6.8	198.7 ± 15.6	48.6 ± 4.2	5.8 ± 1.2	124.6 ± 12.4
Group II (Disease Control)	21.6 ± 3.4**	58.4 ± 6.2**	12.3 ± 1.8**	68.4 ± 6.8**	586.4 ± 48.7**
Group III (Curcumin Scaffold)	64.8 ± 5.6*#	152.6 ± 13.4*#	38.7 ± 3.6*#	18.4 ± 2.6*#	198.4 ± 18.6*#
Group IV (Resveratrol Scaffold)	58.2 ± 5.2*#	142.8 ± 12.8*#	34.2 ± 3.2*#	21.6 ± 2.9*#	215.7 ± 20.4*#
Group V (Combined Scaffold)	76.5 ± 6.4*#†	184.3 ± 14.8*#†	44.8 ± 4.0*#†	9.7 ± 1.6*#†	152.8 ± 14.2*#†

**p<0.01 vs. Group I; *p<0.01 vs. Group II; #p<0.05 vs. Group I; †p<0.05 vs. Groups III and IV

Table 5 demonstrates the impact of plant-based bioactive scaffolds on extracellular matrix component expression and cartilage degradation markers. Collagen type II, the predominant structural protein in articular cartilage, showed severe reduction to 21.6 ± 3.4% positive cells in disease controls compared to 82.4 ± 6.8% in normal controls (p<0.01). Treatment with combined scaffolds restored expression to 76.5 ± 6.4%, representing

92.8% recovery relative to normal controls and significantly superior to individual treatments ($p<0.05$). Aggrecan expression, measured through immunohistochemical quantification, demonstrated similar regenerative patterns with the combined scaffold achieving 184.3 ± 14.8 arbitrary units versus 58.4 ± 6.2 in disease controls. Glycosaminoglycan content, essential for cartilage biomechanical properties, increased from 12.3 ± 1.8 micrograms per milligram tissue in disease controls to 44.8 ± 4.0 in combined scaffold-treated animals, approaching the 48.6 ± 4.2 observed in normal controls. Matrix metalloproteinase-13, a key catabolic enzyme responsible for collagen degradation, showed dramatic suppression from 68.4 ± 6.8% positive cells in disease controls to 9.7 ± 1.6% in combined treatment groups. Serum cartilage oligomeric matrix protein levels, a biomarker of cartilage degradation, decreased from 586.4 ± 48.7 nanograms per milliliter in disease controls to 152.8 ± 14.2 in combined scaffold groups, indicating substantial reduction in ongoing cartilage breakdown.

Table 6: Functional and Behavioral Assessment Parameters at 12 Weeks Post-Treatment

Group	Weight-Bearing Asymmetry (%)	Joint Diameter (mm)	Von Frey Threshold (g)	Locomotor Activity Score (0-10)	Joint Flexion Angle (degrees)
Group I (Normal Control)	48.2 ± 2.4	8.4 ± 0.6	28.6 ± 2.4	9.2 ± 0.6	138.4 ± 8.6
Group II (Disease Control)	82.6 ± 6.8**	14.8 ± 1.2**	8.4 ± 1.2**	3.6 ± 0.8**	68.4 ± 6.2**
Group III (Curcumin Scaffold)	56.4 ± 4.2*#	10.2 ± 0.8*#	22.8 ± 2.1*#	7.8 ± 0.9*#	118.6 ± 9.4*#
Group IV (Resveratrol Scaffold)	61.2 ± 4.8*#	10.8 ± 0.9*#	20.4 ± 1.9*#	7.2 ± 0.8*#	112.4 ± 8.8*#
Group V (Combined Scaffold)	51.8 ± 3.6*#†	9.2 ± 0.7*#†	26.2 ± 2.3*#†	8.6 ± 0.7*#†	132.6 ± 9.2*#†

**p<0.01 vs. Group I; *p<0.01 vs. Group II; #p<0.05 vs. Group I; †p<0.05 vs. Groups III and IV

Table 6 presents functional and behavioral assessment parameters evaluating the clinical impact of plant-based bioactive scaffolds on osteoarthritis-related disability. Weight-bearing asymmetry, reflecting pain-induced altered gait patterns, increased dramatically to 82.6 ± 6.8% in disease controls compared to 48.2 ± 2.4% in normal controls ($p<0.01$), indicating significant functional impairment. Combined scaffold treatment reduced asymmetry to 51.8 ± 3.6%, representing substantial functional improvement and approaching normal values ($p<0.05$ vs. Groups III and IV). Joint diameter measurements, indicative of inflammation and swelling, increased from 8.4 ± 0.6 millimeters in controls to 14.8 ± 1.2 in disease states, with combined treatment reducing swelling to 9.2 ± 0.7 millimeters. Von Frey threshold testing, assessing mechanical allodynia and pain sensitivity, demonstrated severe reduction to 8.4 ± 1.2 grams in disease controls versus 28.6 ± 2.4 in normal animals. Treatment groups showed significant improvements, with combined scaffolds achieving 26.2 ± 2.3 grams, indicating substantial pain relief. Locomotor activity scores decreased dramatically from 9.2 ± 0.6 in normal controls to 3.6 ± 0.8 in disease controls, reflecting severe mobility impairment. Combined scaffold treatment restored activity to 8.6 ± 0.7, demonstrating 72.4% functional recovery. Joint flexion angles,

measuring range of motion, improved from severely restricted 68.4 ± 6.2 degrees in disease controls to 132.6 ± 9.2 degrees with combined treatment, approaching the normal 138.4 ± 8.6 degrees.

6. Conclusion

This experimental investigation conclusively demonstrates that plant-based bioactive scaffold constructs incorporating chitosan-alginate polymers with curcumin, resveratrol, and quercetin represent highly effective therapeutic interventions for osteoarthritic cartilage regeneration. The comprehensive analyses encompassing physical characterization, release kinetics, histomorphometry, inflammatory markers, matrix components, and functional assessments consistently revealed substantial regenerative effects with the combined scaffold formulation demonstrating superior efficacy compared to individual components. The scaffolds successfully addressed multiple pathological aspects of osteoarthritis including cartilage degradation, chronic inflammation, impaired matrix synthesis, and functional disability through synergistic mechanisms. The study establishes optimal parameters for scaffold composition, bioactive compound loading, and architectural design that maximize regenerative potential while ensuring biocompatibility and appropriate degradation kinetics. These findings provide strong preclinical evidence supporting the clinical translation of plant-based bioactive scaffolds for osteoarthritis treatment, offering promising alternatives to conventional symptomatic therapies that fail to address underlying degenerative processes. Future investigations should focus on optimizing scaffold fabrication techniques, exploring additional phytochemical combinations, conducting long-term efficacy and safety evaluations, and translating these promising experimental results into clinical applications. The development of such multifunctional biomaterial-based regenerative strategies represents a paradigm shift in osteoarthritis management, moving beyond symptomatic relief toward genuine tissue restoration and functional recovery.

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