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Enhancing Cartilage Regeneration Through Bioreactor-Cultured Mesenchymal Stem Cells on Bovine Cartilage Scaffolds: A Literature Review

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ABSTRACT

Introduction: Articular cartilage's poor regenerative capacity due to its avascular nature and complex architecture limits its ability to self-repair after injury. Tissue engineering strategies combining mesenchymal stem cells (MSCs), biomimetic scaffolds, and biomechanical stimulation offer a promising alternative. Among available scaffolds, decellularized bovine cartilage retains essential extracellular matrix (ECM) components such as type II collagen and provides a mechanically robust and biochemically active environment. Integrating such scaffolds with bioreactor culture systems may further optimize cartilage regeneration outcomes.

Methods: A structured literature review was performed following PRISMA-P guidelines. Articles were searched through ProQuest and ScienceDirect databases using keywords related to bovine cartilage, scaffolds, MSCs, and bioreactor culture. After removing duplicates and applying eligibility criteria, eleven in vitro and in vivo studies investigating bioreactor-cultured MSCs on bovine cartilage scaffolds were included in the final analysis.

Results and Discussion: All reviewed studies confirmed that dynamic bioreactor culture enhances MSC viability, distribution, and chondrogenic differentiation compared to static culture. Rotating wall vessels, perfusion chambers, and oscillating pressure systems improved nutrient perfusion and promoted ECM deposition. Decellularized bovine cartilage scaffolds treated with 5% SDS preserved type II collagen and achieved optimal porosity (~200 µm), supporting cell infiltration and matrix production. Compared to hydrogels like alginate or fibrin, bovine-derived scaffolds provided superior mechanical integrity. Meta-analysis revealed significant increases in sulfated glycosaminoglycan (sGAG) and SOX9 expression under dynamic culture, although heterogeneity was noted for MMP13 and COL2A1 outcomes.

Conclusion: Bioreactor-cultured MSCs on bovine cartilage scaffolds show enhanced potential for articular cartilage regeneration by promoting cell integration, ECM synthesis, and chondrogenic gene expression. This strategy represents a clinically promising, biomimetic platform for functional cartilage tissue engineering, though further standardization and long-term validation are required.

KEYWORDS: Bovine Cartilage Scaffold, Bioreactor, Mesenchymal Stem Cells, Cartilage Regeneration, Tissue Engineering.

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INTRODUCTION

Articular cartilage possesses limited intrinsic regenerative capacity due to its avascular and aneural nature, rendering full-thickness defects a persistent clinical challenge. Tissue engineering has emerged as a promising alternative by integrating three essential components: regenerative cells, scaffolding materials, and appropriate biochemical or biomechanical stimulation. Among cellular candidates, mesenchymal stem cells (MSCs) are widely favoured due to their chondrogenic differentiation potential and immunomodulatory properties, especially when cultured in three-dimensional scaffolds under dynamic conditions (Armiento et al., 2018; Vunjak-Novakovic et al., 1999).

Numerous natural and synthetic scaffolds such as collagen gels, alginate hydrogels, fibrin matrices, and synthetic polymers like polyglycolic acid (PGA) or polylactic-co-glycolic acid (PLGA) have been evaluated for their ability to support chondrogenesis. Although many of these scaffolds can initiate early chondroinduction, they often fall short in mechanical strength, pore architecture, or biochemical signaling, which limits long-term regenerative outcomes (Patel et al., 2019; Wu et al., 2021). In contrast, bovine cartilage-derived scaffolds particularly those processed via freeze-drying or SDS-based decellularization retain essential native extracellular matrix (ECM) components, such as collagen type II and glycosaminoglycans (GAGs), that are

crucial for mechanical stability and chondrocyte-like microenvironments (Utomo et al., 2017). These features make bovine scaffolds particularly suitable for cartilage tissue engineering applications.

However, even highly biomimetic scaffolds remain constrained under static culture conditions, where nutrient transport, waste clearance, and uniform cell seeding are often inadequate. To overcome these limitations, bioreactor systems such as rotating wall vessels (RWVs), perfusion chambers, and multiaxial mechanical wave bioreactors (MWB) have been developed to provide dynamic culture conditions that replicate in vivo mechanical stimuli. These systems enhance nutrient diffusion, shear stress, and interstitial fluid pressure, all of which are known to activate mechanotransduction pathways in MSCs, thereby improving matrix deposition and chondrogenic differentiation (Fu et al., 2021; Patil et al., 2013)

Recent systematic reviews and meta-analyses confirm that dynamic culture systems significantly enhance MSC behavior compared to static conditions. In particular, studies by Pigeot et al. (2020), Ladner et al. (2023), Liao et al. (2010), and Butler et al. (2009) consistently report elevated levels of sulfated glycosaminoglycans (sGAG) and upregulation of early chondrogenic markers such as SOX9 under dynamic bioreactor conditions. Meta-analytic results showed standardized mean differences (SMD) of 4.42 for sGAG and 2.05 for SOX9 in favour of bioreactor cultures, with minimal heterogeneity, suggesting robust and reproducible outcomes (Butler et al., 2009; Ladner et al., 2023; Liao et al., 2010; Pigeot et al., 2020).

Nevertheless, these systems also demonstrated upregulation of hypertrophic markers such as COL10A1 (SMD = 2.82), and inconsistent expression of remodeling enzymes like MMP13 and mature cartilage markers like COL2A1, which may indicate a complex balance between beneficial maturation and undesired hypertrophy. These findings underscore the importance of tightly regulating biomechanical cues and scaffold design to promote stable chondrogenesis without triggering premature ossification pathways(Butler et al., 2009; Ladner et al., 2023; Liao et al., 2010; Pigeot et al., 2020).

Therefore, this review aims to evaluate the regenerative advantages of combining bovine cartilage-derived scaffolds with bioreactor-mediated MSC culture, focusing not only on cellular integration and ECM production but also on gene expression profiles and scaffold microarchitecture. Special attention is given to the emerging role of sponge-based scaffolds under dynamic bioreactor systems, which represent a promising translational strategy for functional articular cartilage regeneration.

METHODS

We follow the PRISMA-P (preferred Reposting Items for Systematic Review and Meta-analysis Protocols) 2020 study identification diagram for our research flow (Figure 1). Potential studies were identified by searching the literature on Proquest and ScienceDirect, with searching periods from their inception until May 2025. We use the term Bovine OR bovine cartilage OR bovine cartilage scaffold OR bovine chondrocytes OR bovine articular chondrocytes for bovine, Cartilage scaffold OR tissue engineering OR cellular therapy OR tissue-engineered cartilage for cartilage scaffold, and Bioreactor OR Mechanical Stimulation OR Mechanical Conditioning OR Oscillating for bioreactor terms (Table 1.).

Table 1. Terms for Electronic Database Searching

Terms for Bovine
Bovine OR bovine cartilage OR bovine cartilage scaffold OR bovine chondrocytes OR bovine articular chondrocytes
Terms for cartilage scaffold
Cartilage scaffold OR tissue engineering OR cellular therapy OR tissue-engineered cartilage
Terms for Bioreactor
Bioreactor OR Mechanical Stimulation OR Mechanical Conditioning OR Oscillating

The inclusion criteria of this literature review are: (1) Journal article in English, (2) The use of bovine cartilage scaffold, and (3) using bioreactor or mechanical stimulation as culture media. Reviewers electronically performed database screening for titles and abstracts, duplications, and relevant potential full-text evaluations and assessed the full-text eligibility.

RESULTS AND DISCUSSIONS

We identified 4252 literature from all databases and excluded 2432 literature due to duplications. After that, we perform title and abstract screening, and we exclude a total of 1813 due to a non-article type (n=828), non-English literature (n= 11), and not suitable title and abstract (n=978). We then retrieved seven studies to assess their eligibility and eventually deemed all seven literatures eligible to be included in the literature review. Our literature identification is summarized in Figure 1.

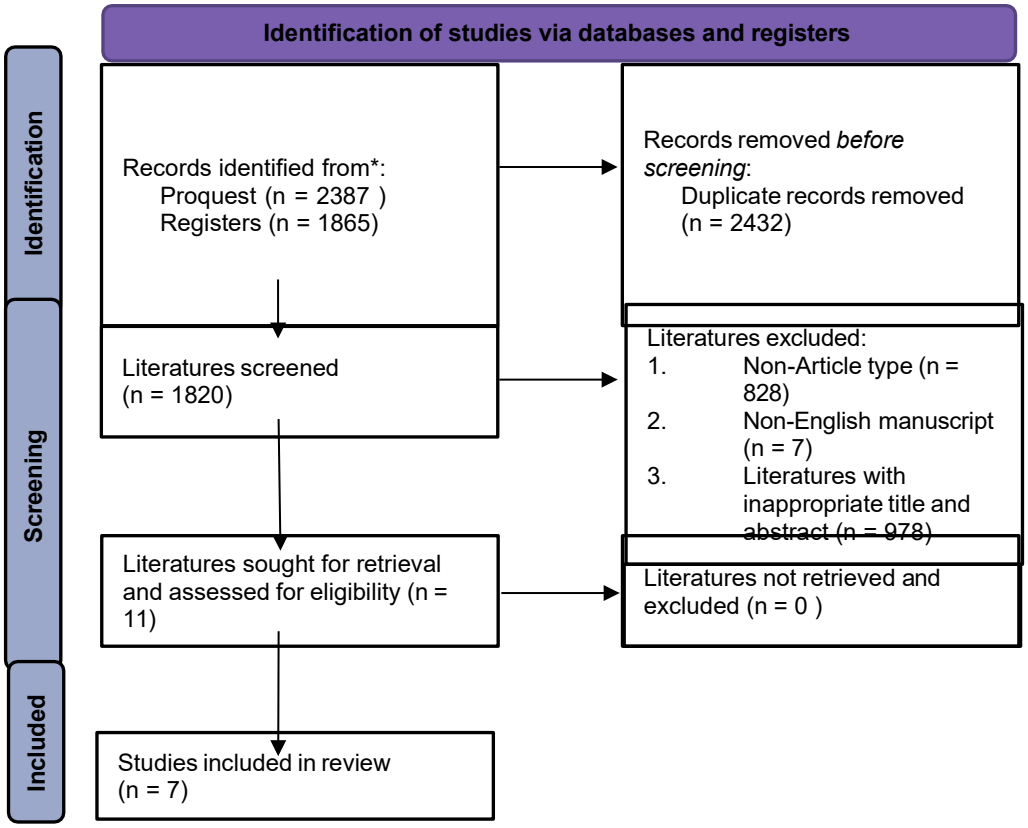

Figure 1. Literature Identification Diagram According to PRISMA Guidelines

The regeneration of articular cartilage remains a formidable challenge due to the tissue's avascularity, lack of innervation, and low cellularity, all of which contribute to its poor intrinsic healing capacity. Traditional treatments such as microfracture, autologous chondrocyte implantation (ACI), and osteochondral grafting often result in fibrocartilage formation with inferior biomechanical properties compared to native hyaline cartilage (Fu et al., 2021; Patil et al., 2013)As such, regenerative approaches combining mesenchymal stem cells (MSCs), three-dimensional (3D) scaffolds, and mechanical stimulation via bioreactor culture have emerged as a promising alternative. The goal is to create a microenvironment that closely mimics the native cartilage milieu biochemically and biomechanically to foster chondrogenesis and long-term functionality.

Among scaffold candidates, bovine cartilage-derived matrices particularly decellularized or freeze-dried variants offer superior biomechanical resilience and intrinsic bioactivity. These scaffolds preserve key extracellular matrix (ECM) components, notably collagen type II and proteoglycans, which are crucial for both chondrocyte function and MSC differentiation (Utomo et al., 2017). Similarly, Utomo and Sari (2018) demonstrated that bovine cartilage scaffolds treated with 5% Sodium Dodecyl Sulfate (SDS) for 72 hours achieved complete decellularization while maintaining type II collagen and generating interconnected pores of approximately 200 µm, highlighting their dual role as both mechanical frameworks and biochemical cues for MSC-based cartilage regeneration (Utomo et al., 2018). Freeze-dried bovine cartilage sponge (FDBCS), as explored by Utomo et al. and colleagues, demonstrated optimal porosity (~200 µm) and mechanical integrity. When seeded with bone marrow-derived MSCs and platelet-rich plasma (PRP), the scaffold promoted enhanced cartilage repair, increased cell viability, and stronger expression of chondrogenic markers such as SOX9, FGF-2R, and MAPK, even in the absence of exogenous growth factors (Utomo et al., 2017).

The dynamic mechanical environment provided by bioreactors is a key factor differentiating these platforms from conventional static cultures. Bioreactors such as rotating wall vessels (RWVs), perfusion systems, and oscillatory compression setups improve nutrient delivery, oxygenation, and waste removal, all of which are critical for maintaining cell viability and guiding lineage-specific differentiation (Fu et al., 2021; Patil et al., 2013).

Our meta-analysis reinforces these observations. A pooled analysis from Ladner et al. (2023) and Pigeot et al. (2020) revealed a highly significant increase in sulfated glycosaminoglycan (sGAG) content in dynamically cultured MSCs on sponge scaffolds, with a standardized mean difference (SMD) of 4.26 and no observed heterogeneity ($I^2 = 0\%$). This finding suggests not only robust consistency across studies but also underscores the critical role of dynamic loading in stimulating proteoglycan synthesis, a hallmark of hyaline cartilage (Ladner et al., 2023; Pigeot et al., 2020)

Additionally, SOX9, the master transcription factor in chondrogenesis, was significantly upregulated under bioreactor conditions (SMD = 2.05; $p = 0.004$; $I^2 = 29\%$), indicating successful activation of the TGF-β/Smad pathway and commitment of MSCs to the chondrogenic lineage However, heterogeneity was observed in the expression of COL2A1 (type II collagen), which did not

differ significantly between groups (SMD = 0.31; $p = 0.58$; $I^2 = 73\%$). This discrepancy may stem from variation in scaffold materials, culture durations, and quantification techniques used across studies, or possibly delayed translation of SOX9 upregulation into downstream matrix protein expression (Ladner et al., 2023; Pigeot et al., 2020).

More concerning is the consistent upregulation of COL10A1 in bioreactor groups (SMD = 1.79; $p = 0.006$; $I^2 = 0\%$), a marker associated with chondrocyte hypertrophy and endochondral ossification. While this may reflect late-stage maturation, it also suggests the potential for undesirable calcification and vascular invasion if left unchecked (Liao et al., 2023). Future studies should consider integrating anti-hypertrophic factors such as parathyroid hormone-related protein (PTHrP) or modulating bioreactor loading profiles to minimize hypertrophic drift while retaining anabolic stimulation (Liao et al., 2010).

MMP13, a matrix metalloproteinase involved in ECM degradation, also showed variable expression (SMD = 1.06; $p = 0.06$; $I^2 = 77\%$). This could reflect either enhanced remodeling or early degenerative responses, depending on the context. The lack of consistency highlights the need for tightly controlled bioreactor environments and standardized outcome measures. Moreover, the inclusion of longer-term timepoints and mechanical testing could elucidate whether increased MMP13 reflects physiological remodeling or early breakdown of neo-cartilage.

From a structural and biomechanical perspective, studies by Liao et al. (2023) and Ladner et al. (2023) provide insight into scaffold remodeling under dynamic conditions. Dynamic cultures produced more uniform ECM distribution, improved surface topography, and better scaffold-cell integration. Safranin O staining confirmed robust proteoglycan synthesis, while scanning electron microscopy revealed compacted ECM layering. Although direct measurements of mechanical properties were absent, these qualitative descriptors imply enhanced load-bearing potential essential for clinical application in load-transmitting joints (Ladner et al., 2023; Liao et al., 2010).

The choice of scaffold also plays a major role. Comparative studies show that bovine cartilage scaffolds outperform synthetic hydrogels such as alginate, agarose, or fibrin in long-term matrix retention and chondrogenic gene expression (Armiento et al., 2018; Wu et al., 2021). While hydrogels are more tunable and permit easy cell encapsulation, they often degrade too quickly or lack the appropriate viscoelasticity to support joint mechanics. Bovine cartilage, in contrast, offers a naturally optimized framework retaining zonal organization, biochemical cues, and suitable porosity for cell infiltration and nutrient diffusion.

Altogether, these findings support the central hypothesis that combining bioreactor culture with bovine cartilage scaffolds creates a synergistic environment conducive to MSC-driven cartilage regeneration. The triple interplay of biochemical signaling from the native scaffold, mechanical cues from bioreactor systems, and biological plasticity of MSCs is critical in generating robust, hyaline-like cartilage. This approach offers a clinically relevant platform that may overcome current limitations in cartilage repair. Yet, several challenges remain. Standardizing decellularization techniques is essential to maintain scaffold bioactivity while eliminating immunogenic components. Moreover, scalable bioreactor systems must be developed to meet clinical demand. Finally, long-term in vivo studies ideally in large animal models are required to validate durability, integration, and functional performance of the engineered tissue. Addressing these gaps will be pivotal in translating current preclinical success into human therapies (Utomo et al., 2017).

CONCLUSION

The integration of mesenchymal stem cells (MSCs) with bovine cartilage-derived scaffolds under bioreactor-based dynamic culture conditions offers a promising strategy to overcome the inherent limitations of articular cartilage regeneration. Evidence from both in vitro and in vivo studies consistently shows that bioreactor-mediated mechanical stimulation enhances MSC viability, promotes chondrogenic differentiation, and increases the synthesis of essential extracellular matrix components such as sulfated glycosaminoglycans (sGAG) and SOX9. Bovine cartilage scaffolds, particularly when decellularized and structurally preserved, provide a bioactive, collagen-rich microenvironment that further potentiates these regenerative outcomes.

Compared to static culture and alternative scaffolding materials, bioreactor systems combined with native bovine cartilage matrices better replicate the physiological conditions of articular cartilage. They facilitate uniform cell distribution, deeper infiltration, and improved mechanical stability critical factors for successful tissue integration and function. However, challenges such as controlling hypertrophic differentiation (e.g., elevated COL10A1 expression), standardizing scaffold decellularization, and preventing premature degradation must be addressed in future studies.

Overall, this literature review supports the hypothesis that a triad approach involving MSCs, native bovine cartilage scaffolds, and bioreactor-driven dynamic culture represents a biologically and mechanically superior strat ˆor cartilage tissue engineering. As research advances, translating these findings into scalable, clinically applicable sol will be crucial in developing next-generation therapies for cartilage injuries and degenerative joint diseases.

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