Exploring tissue engineering avenues with mesenchymal stem cells: Opportunities and challenges in advancing veterinary orthopaedics

Chapter · September 2024

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Advances in Veterinary & Animal Sciences

(Volume II: Veterinary Science)

(Compendium of Critical Insights: A Collection of Review Articles)

EDITORS : IRSHAD A. & NARAYANAN S. B.

9 789334 044287

ISBN 13 : 978-93-340-4428-7

ISBN No : 978-93-340-4428-7

Advances in Veterinary and Animal Sciences

(Volume II: Veterinary Science)

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First Edition

2024

9 789334 044287

Indian Veterinary Association, Kerala
Veterinarians Building, TC 25/2067(1), Dharmalayam Road,
Thiruvananthapuram – 695001, Kerala, India

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Published by
General Secretary
Indian Veterinary Association, Kerala,
Veterinarians Building, TC 25/2067(1), Dharmalayam Road,
Thiruvananthapuram – 695001, Kerala, India

Design and Layout: Dr. A. Irshad, CVAS Mannuthy, KVASU
Printing : **Educare, St. Thomas acrade, Thrissur**

ISBN No : 978-93-340-4428-7

Chapter 21

EXPLORING TISSUE ENGINEERING AVENUES WITH MESENCHYMAL STEM CELLS: OPPORTUNITIES AND CHALLENGES IN ADVANCING VETERINARY ORTHOPAEDICS

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Abstract

Tissue engineering with mesenchymal stem cells (MSCs) has emerged as a promising frontier in veterinary orthopaedics, offering

ISBN No : 978-93-340-4428-7

innovative solutions for musculoskeletal disorders in companion animals. MSCs, known for their multipotency and immunomodulatory potential that play a crucial role in promoting tissue repair and regeneration. The personalized nature of MSC therapy, its minimally invasive delivery methods, and its integration with traditional treatments highlight its versatility in addressing a spectrum of orthopaedic conditions. MSCs play a pivotal role in promoting tissue repair and regeneration in bone, cartilage, and tendon/ligament. Their immunomodulatory properties are crucial in reducing inflammation and creating a conducive environment for healing. This comprehensive review delves into the multifaceted landscape of MSC-based tissue engineering, exploring opportunities and confronting challenges in the realm of veterinary orthopaedics. The paper provides a thorough examination of recent advancements in MSC research, focusing on their potential applications in regenerative therapies for bone and cartilage disorders. By scrutinizing the current state of the field, we aim to shed light on the pivotal role MSCs play in tissue engineering strategies for veterinary orthopaedics, addressing key opportunities for translational success and confronting the inherent challenges that need to be overcome. The promising outcomes observed in preclinical studies set the stage for rigorous clinical trials, ensuring a comprehensive evaluation of the safety and efficacy of MSC-based interventions. This emphasis on clinical validation underscores the commitment to translating the potential of MSC-based tissue engineering into tangible benefits for veterinary patients. As the field progresses, the collaborative efforts of researchers, clinicians, and veterinarians are essential for navigating the complexities of MSC-based interventions, offering a beacon of hope for the advancement of veterinary orthopaedic care.

Keywords: *Mesenchymal stem cells; Stem cells; Regenerative medicine; Bone regeneration; Veterinary orthopaedics; Cartilage healing*

ISBN No : 978-93-340-4428-7

Introduction

Tissue engineering has emerged as a transformative field in the realm of veterinary orthopaedics, offering unprecedented opportunities to address musculoskeletal disorders and injuries in companion animals and livestock (Laurencin *et al.*, 1999; Li *et al.*, 2021). At the forefront of this revolutionary approach lies the remarkable potential of mesenchymal stem cells (MSCs), versatile and multipotent cells that have captured the attention of researchers and clinicians alike (Laurencin *et al.*, 1999; Li *et al.*, 2021). As our understanding of MSCs continues to deepen, so too does the exploration of tissue engineering avenues that harness their unique regenerative properties (Li *et al.*, 2021). MSCs are multipotent cells with the unique ability to differentiate into various cell types, including osteoblasts, chondrocytes, and adipocytes (Hu *et al.*, 2018). Their self-renewal capacity and immunomodulatory properties make them an ideal candidate for regenerative therapies (Law and Chaudhuri, 2013; Hu *et al.*, 2018).

In veterinary orthopaedics, these characteristics play a pivotal role in promoting tissue repair and regeneration. MSCs modulate the immune response, reducing inflammation and creating a conducive environment for tissue healing (Law and Chaudhuri, 2013). Their regenerative properties contribute to the repair of damaged bone, cartilage, and soft tissues (Han *et al.*, 2022). Scaffold-based and scaffold-free strategies are employed to create a conducive environment for cell proliferation and tissue regeneration (Alghuwainem *et al.*, 2019). Growth factors and biomaterials, when

ISBN No : 978-93-340-4428-7

combined with MSCs, enhance their regenerative potential, offering a holistic solution for complex orthopaedic challenges (Stamnitz *et al., 2021*). Whether addressing fractures, non-unions, or congenital skeletal abnormalities, MSC-based therapies stimulate osteogenesis, fostering the restoration of skeletal integrity in diverse scenarios (Hu *et al.*, 2018; Stamnitz *et al.*, 2021). From their unique characteristics to diverse tissue engineering approaches, MSCs open new vistas for addressing musculoskeletal disorders in a regenerative and personalized manner (Hu *et al.*, 2018). As research and clinical trials progress, the impact of MSC-based therapies in veterinary orthopaedics is poised to redefine standards of care, enhancing the quality of life for our animal companions (Diaz *et al.*, 2021).

As the demand for effective and sustainable solutions to orthopaedic conditions in animals grows, this exploration becomes increasingly vital (Dias *et al.*, 2021). The journey begins by elucidating the fundamental characteristics of MSCs, unravelling their diverse sources and highlighting their immunomodulatory capabilities (Voga *et al.*, 2020). In the quest for innovation, we dissect emerging trends and transformative technologies that hold the promise of reshaping the future of veterinary orthopaedics. Collaborative efforts and interdisciplinary approaches take center stage, reflecting the dynamic nature of this field and the collective commitment to advancing animal health (Voga *et al.*, 2020; Diaz *et al.*, 2021). However, amidst the promises and potentials, challenges and limitations loom large. Ethical considerations, translational

ISBN No : 978-93-340-4428-7

hurdles, and the need for robust clinical evidence are thoroughly examined.

This comprehensive review delves into the intricate landscape of tissue engineering with a specific focus on MSCs, examining the opportunities and challenges that shape their application in advancing veterinary orthopaedics. Through a systematic examination of tissue engineering strategies, including scaffold-based and scaffold-free approaches, we navigate the intricate interplay between MSCs and biomaterials, growth factors, and cutting-edge technologies. Real-world applications in veterinary orthopaedics are illuminated, showcasing the tangible impact of MSC-based tissue engineering on bone regeneration, cartilage repair, and ligament/tendon reconstruction. As we embark on this journey of exploration, the review aims to not only provide a panoramic view of the current landscape but also to chart a course for future research directions. By identifying opportunities for innovation and emphasizing the collaborative spirit that defines this field, we hope to inspire continued advancements in MSC-based tissue engineering for the betterment of veterinary orthopaedics.

Mesenchymal stem cells

MSCs represent a distinct and versatile subset of adult stem cells with unique characteristics that distinguish them from other cell types (Salem and Thiemermann, 2010). These multipotent cells are defined by their capacity to differentiate into various cell lineages, including osteoblasts, chondrocytes, and adipocytes (Guest *et al.*, 2022). MSCs are typically characterized by their adherence to plastic

ISBN No : 978-93-340-4428-7

surfaces, specific surface marker expression such as CD73, CD90, and CD105, and the absence of hematopoietic markers CD45, CD34, or CD14 (Guest *et al.*, 2022). Their ability to undergo self-renewal while maintaining their multilineage differentiation potential makes MSCs an attractive candidate for tissue engineering and regenerative medicine applications (Salem and Thiemermann, 2010; Guest *et al.*, 2022).

MSCs can be harvested from various sources, each possessing unique advantages and considerations for applications in veterinary orthopaedics (Berebichez-Fridman *et al.*, 2018). Common sources include bone marrow, adipose tissue, umbilical cord, dental pulp, and synovial fluid (Fig. 1) (Costela-Ruiz *et al.*, 2022). The choice of source often depends on factors such as accessibility, abundance, and the desired therapeutic outcome (Berebichez-Fridman *et al.*, 2018). Bone marrow-derived MSCs, for instance, are recognized for their multilineage differentiation potential, while adipose-derived MSCs offer relative ease of isolation and higher cell yields (Berebichez-Fridman *et al.*, 2018; Costela-Ruiz *et al.*, 2022). Understanding the distinctive properties of MSCs derived from different sources is crucial in tailoring therapeutic strategies to address specific orthopaedic conditions in veterinary patients. One of the hallmark features of MSCs is their remarkable immunomodulatory capacity (Law and Chaudhuri, 2013). These cells can modulate immune responses through the secretion of anti-inflammatory cytokines, interaction with immune cells, and suppression of excessive immune reactions (Kavianpour *et al.*,

ISBN No : 978-93-340-4428-7

2020). This immunomodulatory prowess has profound implications for treating inflammatory and autoimmune conditions, providing a promising avenue for veterinary orthopaedic interventions (Law and Chaudhuri, 2013; Kavianpour *et al.*, 2020).

Moreover, MSCs exhibit potent regenerative properties, fostering tissue repair and regeneration (Dias *et al.*, 2021). Their ability to differentiate into bone, cartilage, and other mesenchymal lineages positions MSCs as key players in regenerating damaged tissues (Hwang *et al.*, 2021). In veterinary orthopaedics, this regenerative potential holds immense promise for addressing conditions such as osteoarthritis, bone fractures, and ligament injuries. In this comprehensive exploration of MSCs, we delve into the intricacies of their definition, diverse sources, and pivotal immunomodulatory and regenerative properties (Kavianpour *et al.*, 2020). Recognizing the significance of these characteristics is fundamental in harnessing the full potential of MSCs for advancing therapeutic approaches in the field of veterinary orthopaedics (Dias *et al.*, 2021).

Mesenchymal stem cells in veterinary orthopaedics

MSCs have the ability to differentiate into various cell types, including bone, cartilage, and tendon cells (Dias *et al.*, 2021). This regenerative potential makes them invaluable in addressing issues such as fractures, osteoarthritis, and ligament injuries in veterinary patients (Ivanovska *et al.*, 2022).

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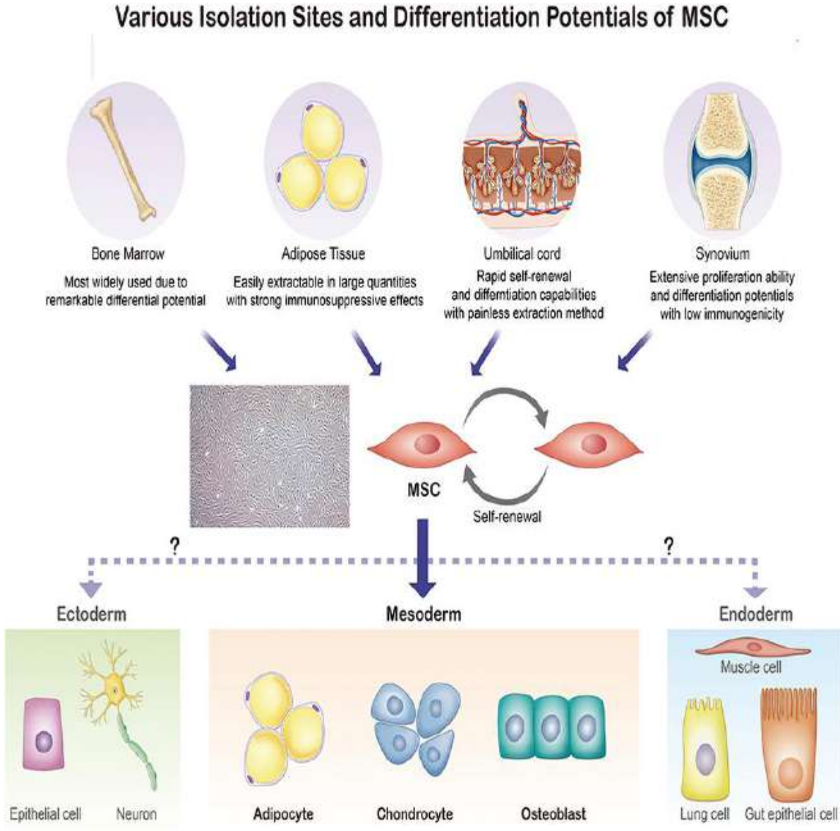

Fig. 1: MSCs can be sourced from diverse locations, each presenting distinct isolation sites and differentiation potentials. Common extraction sites for MSCs encompass bone marrow, adipose tissue, umbilical cord, and synovium. Following isolation, these MSCs exhibit the capability to undergo differentiation into various cell types, including adipocytes, chondrocytes, myocytes, and osteoblasts. Reproduced from Hwang et al. (2021) under Creative Commons Attribution 4.0 International (CC BY 4.0) license.

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They also possess potent immunomodulatory properties, enabling them to modulate the inflammatory response (Law and Chaudhuri, 2013). This is particularly relevant in orthopaedic conditions where inflammation plays a crucial role in disease progression (Merimi *et al.*, 2021). MSC therapy has the potential to mitigate inflammation, promoting a more favourable environment for tissue healing (Merimi *et al.*, 2021). In cases of osteoarthritis and cartilage defects, MSCs can contribute to cartilage repair and regeneration (Dias *et al.*, 2021; Zhu *et al.*, 2021). By promoting the synthesis of cartilage-specific extracellular matrix components, MSC therapy may help improve joint function and alleviate pain in veterinary patients (Zhu *et al.*, 2021).

MSCs are known for their osteogenic differentiation capacity, making them key players in bone regeneration (Shang *et al.*, 2020; Xue *et al.*, 2022). Veterinary orthopaedics often deals with fractures and bone defects, and MSC therapy offers a promising avenue for enhancing bone healing and restoring skeletal integrity (Dias *et al.*, 2021; Xue *et al.*, 2022). Ligament and tendon injuries are common in animals, particularly those engaged in activities such as agility training or working roles. MSCs can aid in the reconstruction of damaged ligaments and tendons by facilitating tissue regeneration and providing mechanical strength to the repaired structures (Dias *et al.*, 2021). MSCs can be delivered through minimally invasive procedures, such as intra-articular injections or targeted implantation, making the therapy feasible and less stressful for the

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animals (Prządka *et al.,* 2021). This is crucial for ensuring the widespread applicability of MSC therapy in veterinary practice.

Furthermore, MSC therapy allows for a personalized approach to treatment. Veterinary orthopaedic conditions can vary widely among different species and even within breeds. MSCs can be isolated and expanded from the patient's own tissues, enabling a tailored treatment approach for specific cases (Dias *et al.*, 2021; Prządka *et al.,* 2021). MSC therapy can be integrated with other orthopaedic interventions, such as surgical procedures or traditional treatments, to enhance overall outcomes (Maniar *et al.*, 2015). This flexibility in combining therapies provides a comprehensive approach to managing complex orthopaedic cases. The prospects of MSC therapy in veterinary orthopaedics are extensive, encompassing regenerative potential, anti-inflammatory effects, and versatile applicability. As research in this field progresses, the therapeutic benefits of MSCs are likely to play an increasingly pivotal role in advancing orthopaedic care for animals (Dias *et al.*, 2021).

Mesenchymal stem cells for bone regeneration

MSC therapy holds significant promise in the context of bone regeneration, offering a range of prospects for improving the treatment of bone-related conditions (Shang *et al.*, 2020; Xue *et al.*, 2022). MSCs are characterized by their ability to differentiate into osteoblasts, the cells responsible for bone formation. This osteogenic differentiation capacity makes MSCs a valuable tool for promoting bone regeneration and repair (Xue *et al.*, 2022). When MSCs are

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introduced into a damaged or fractured bone site, they can stimulate the local environment to undergo osteogenesis. This process involves the formation of new bone tissue, aiding in the healing of fractures or defects (Shang *et al.*, 2020; Xue *et al.*, 2022). In addition, MSCs secrete a variety of growth factors, cytokines, and extracellular vesicles that play a crucial role in promoting bone regeneration (Kangari *et al.*, 2020). These factors contribute to the recruitment of other cells involved in bone healing, such as endothelial cells and osteoprogenitor cells (Kangari *et al.*, 2020).

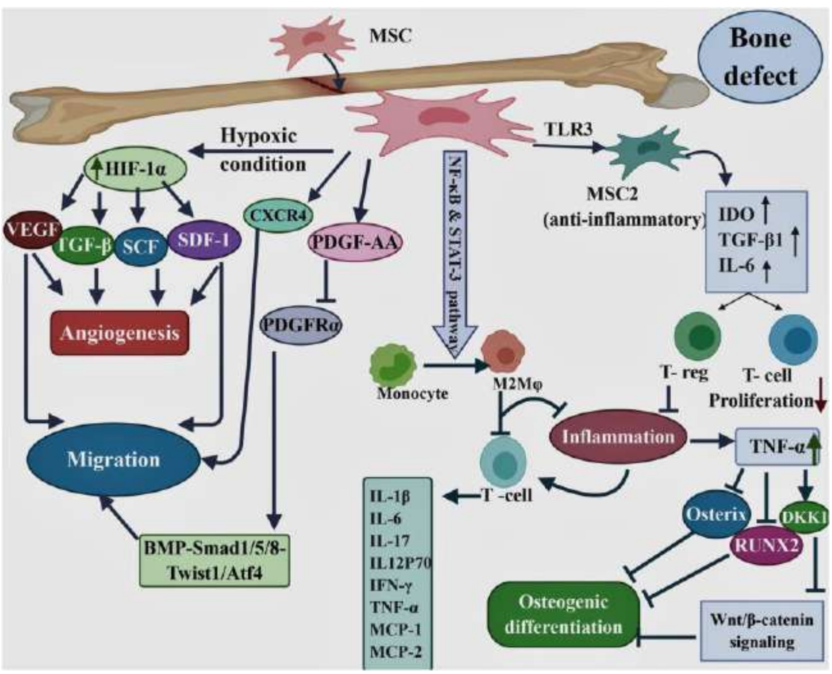

Fig. 2: Diagram illustrating the various mechanisms through which MSCs facilitate bone repair. MSCs play a role in bone regeneration by engaging in processes such as migration, angiogenesis,

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responsiveness to inflammatory conditions, and differentiation, achieved through the production of diverse mediators. Abbreviations: CXC chemokine receptor (CXCR) 4, Dickkopf 1 (DKK1), Hypoxia-inducible factor 1-α (HIF-1α), indoleamine 2,3-dioxygenase (IDO), interferon gamma (IFN-γ), macrophage inflammatory protein-1 (MIP-1), M2 type of macrophage (M2MQ), monocyte chemoattractant proteins-1 (MCP-1), nitric oxide (NO), nuclear factor kappa-B (NF-κB), platelet-derived growth factor (PDGF-AA), platelet-derived growth factor receptor-alpha (PDGFRα), regulatory T cell (T reg), runt-related transcription factor 2 (RUNX2), signal transducer and activator of transcription 3 (STAT-3), stem cell factor (SCF), stromal cell-derived factor (SDF)-1, Toll-like receptors (TLRs), transforming growth factor-beta (TGF-β), tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF). Reproduced from Kangari et al. (2020) under Creative Commons Attribution 4.0 International (CC BY 4.0) license.

The ability of MSCs to promote angiogenesis, the formation of new blood vessels, is essential for supplying the developing bone tissue with nutrients and oxygen (Fig. 2) (Kangari *et al.*, 2020). This vascularization is critical for the success of bone regeneration processes. MSCs possess immunomodulatory properties, which can help modulate the inflammatory response at the site of injury (Franco *et al.*, 2021; Kangari *et al.*, 2020). By reducing excessive inflammation, MSCs create a more favourable environment for bone regeneration to occur. Studies have suggested that MSC therapy not

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only promotes the quantity of bone formation but also enhances the quality of the regenerated bone (Knight and Hankenson, 2013). This is particularly important in cases where the structural integrity of the bone needs to be fully restored (Knight and Hankenson, 2013). MSC therapy has shown promise in the treatment of non-union fractures, where the normal bone healing process is impaired. By introducing MSCs into the non-healing fracture site, the regenerative properties of these cells can kickstart the healing process (Knight and Hankenson, 2013; Kangari *et al.*, 2020).

MSCs can be delivered through various methods, including direct injection into the bone defect, incorporation into scaffolds, or systemic administration (Fig. 3) (Franco *et al.*, 2021). This versatility allows for customized approaches based on the specific requirements of the bone regeneration scenario (Knight and Hankenson, 2013; Franco *et al.*, 2021). MSCs can be combined with various biomaterials, such as hydrogels or scaffolds, to create a supportive environment for bone regeneration (Shang *et al.*, 2020). This combination enhances the structural and mechanical properties of the regenerated bone. There is a growing body of preclinical and clinical evidence supporting the efficacy of MSC therapy in bone regeneration (Shang *et al.*, 2020; Xue *et al.*, 2022). Successful outcomes have been reported in animal models as well as in human clinical trials, highlighting the translational potential of this therapeutic approach. As research continues, the refinement of techniques and a deeper understanding of MSC behaviour in specific

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bone regeneration contexts will likely further enhance the therapeutic efficacy of MSCs in clinical applications.

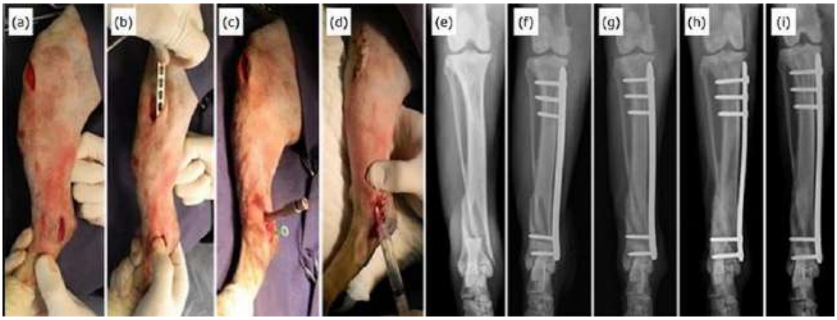

Fig. 3: Intraoperative images depict a minimally invasive osteosynthesis procedure involving a plate and the application of adipose tissue-derived mesenchymal stem cells (AD-MSCs) in a 15-year-old, 13 kg dog with a distal metaphyseal tibial fracture. The spiral fracture, accompanied by a fractured fibula, underwent indirect reduction through manual traction **(a)**. The plate was inserted through the proximal access, traversing the epiperiosteal tunnel over the tibia's medial surface **(b)**, with bone perforations for screw application made through distal and proximal accesses. A needle delineated the tibia in the joint space **(c)**. Three million allogeneic AD-MSCs diluted in 2 mL of saline were percutaneously applied to the fracture site **(d)**. Radiograph of fractured bone **(e)**. Treatment involved a 2.7 mm 12-hole locked plate with three proximal and two distal locked screws **(f)**. Radiographic follow-up at 15 **(g)**, 30 **(h)**, and 60 days **(i)** demonstrates successful bone healing. Reproduced from Franco *et al.* (2021) under Creative Commons Attribution 4.0 International (CC BY 4.0) license.

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Mesenchymal stem cells for ligament and tendon reconstruction

Ligaments and tendons are crucial components of the musculoskeletal system, providing stability, support, and facilitating movement (Dias *et al.*, 2021; Xue *et al.*, 2022). However, their structure and function make them susceptible to injuries, and their intrinsic healing capacity is often limited (Snedeker and Foolen, 2017). The repair process is characterized by the formation of scar tissue, which lacks the structural and functional properties of the original tissue (Dias *et al.*, 2021). This compromises the biomechanical integrity and increases the risk of re-injury. In veterinary orthopaedics, ligament and tendon injuries are prevalent, often stemming from traumatic incidents, overuse, or degenerative processes (Snedeker and Foolen, 2017). These injuries pose significant challenges due to the limited regenerative capacity of these connective tissues. Conventional treatment approaches, while offering symptomatic relief, often fall short in providing complete and durable solutions. This is where the regenerative potential of MSCs comes into play (Snedeker and Foolen, 2017; Dias *et al.*, 2021).

MSCs possess unique regenerative properties that make them ideal candidates for ligament and tendon reconstruction (Snedeker and Foolen, 2017). These multipotent cells have the ability to differentiate into tenocytes and fibroblasts, the cell types predominant in tendons and ligaments, respectively (Zou *et al.*, 2023). Moreover, MSCs secrete various growth factors, cytokines, and extracellular matrix components, creating a microenvironment

ISBN No : 978-93-340-4428-7

conducive to tissue repair (Snedeker and Foolen, 2017; Zou *et al.*, 2023). Collagen is a key structural component of ligaments and tendons. The regenerative potential of MSCs lies in their ability to stimulate collagen synthesis (Tang *et al.*, 2022). MSCs, when introduced into the site of injury, promote the production of organized and functional collagen fibers. This not only aids in tissue repair but also contributes to the restoration of the biomechanical properties essential for the proper functioning of ligaments and tendons (Tang *et al.*, 2022; Zou *et al.*, 2023).

Inflammation is a common response to ligament and tendon injuries. While a controlled inflammatory phase is necessary for the initiation of healing, excessive inflammation can impede the regenerative process (Zou *et al.*, 2023). MSCs exhibit immunomodulatory effects by suppressing inflammatory responses, thus creating an environment conducive to healing without chronic inflammation. In ligament and tendon reconstruction, scaffold-based approaches are often employed in conjunction with MSCs (Snedeker and Foolen, 2017; Zou *et al.*, 2023). Scaffolds provide structural support and mimic the natural extracellular matrix, guiding the organization of newly formed tissue. MSCs seeded onto these scaffolds enhance their regenerative potential, leading to the development of functional ligament and tendon tissue (Snedeker and Foolen, 2017; Zou *et al.*, 2023). Clinical studies and case reports in veterinary medicine have demonstrated the efficacy of MSC-based therapies in ligament and tendon reconstruction (Renzi *et al.*, 2013). From equine athletes with suspensory ligament injuries to dogs with

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cranial cruciate ligament ruptures, MSC-based treatments have shown promising outcomes (Renzi *et al.*, 2013). These success stories underscore the potential of MSCs in addressing a wide spectrum of ligament and tendon pathologies.

MSC-based therapeutics have emerged as transformative agents in ligament and tendon reconstruction in veterinary orthopaedics (Renzi *et al.*, 2013; Zou *et al.*, 2023). Their regenerative properties, coupled with advancements in scaffold-based strategies, offer a regenerative frontier for addressing the complexities of ligament and tendon injuries in animals (Renzi *et al.*, 2013). As research progresses, MSC-based therapies are poised to become integral components of veterinary orthopaedic practice, offering novel solutions for improved patient outcomes and enhanced quality of life.

Mesenchymal stem cells for cartilage repair

Cartilage injuries and degenerative conditions are common in veterinary orthopaedics, posing significant challenges due to the limited regenerative capacity of cartilage (Kangari *et al.*, 2020). Traditional treatments often fall short in providing lasting solutions. The emergence of MSCs has revolutionized the landscape of cartilage repair in veterinary medicine, offering a promising avenue for regenerative therapy (Sasaki *et al.*, 2019). Cartilage, a specialized connective tissue, lacks a direct blood supply and has limited intrinsic regenerative potential (Zhang *et al.*, 2009). Injuries or degenerative conditions, whether caused by trauma or age-related wear and tear, can lead to cartilage defects (Nurul *et al.*, 2021). These

ISBN No : 978-93-340-4428-7

defects often progress, causing pain, inflammation, and reduced joint function. Traditional treatments focus on symptom management, but the repair of damaged cartilage remains a formidable challenge (Sasaki *et al.*, 2019; Nurul *et al.*, 2021).

MSCs, with their ability to differentiate into chondrocyte-like cells, play a pivotal role in cartilage repair (Kangari *et al.*, 2020). Chondrogenesis, the process of cartilage formation, involves the differentiation of MSCs into chondrocytes, the cells responsible for producing cartilage matrix components (Zhang *et al.*, 2009). Beyond their direct involvement in chondrogenesis, MSCs exert profound paracrine signaling and trophic effects. The secretion of growth factors, such as transforming growth factor-beta (TGF-β) and insulin-like growth factor (IGF-1), enhances the microenvironment conducive to cartilage repair (Fig. 4) (Nurul *et al.*, 2021). These trophic effects contribute to the recruitment of endogenous cells, further amplifying the regenerative response (Sasaki *et al.*, 2019; Nurul *et al.*, 2021). This capability positions MSCs as prime candidates for regenerating cartilage tissue. The extracellular matrix (ECM) is essential for cartilage structure and function (Zhang *et al.*, 2009). MSCs contribute to cartilage repair by secreting growth factors, cytokines, and extracellular matrix molecules. These bioactive factors create a conducive microenvironment for chondrogenesis, stimulating the production of cartilage-specific components like collagen and proteoglycans. To enhance the efficacy of MSCs in cartilage repair, scaffold-based approaches are commonly employed (Han *et al.*, 2022). Scaffolds provide structural

ISBN No : 978-93-340-4428-7

support, mimic the natural cartilage environment, and facilitate the integration of newly formed tissue. MSCs, when seeded onto these scaffolds, adhere, proliferate, and differentiate, promoting the regeneration of functional cartilage (Zhang *et al.*, 2009).

Inflammation is a common feature of cartilage injuries. Excessive inflammation can impede the regenerative process and contribute to cartilage degradation (Sasaki *et al.*, 2019; Nurul *et al.*, 2021). MSCs exhibit immunomodulatory effects, suppressing inflammatory responses and creating an anti-inflammatory environment. This immunomodulation aids in reducing inflammation, preserving existing cartilage, and supporting the regeneration of new tissue (Nurul *et al.*, 2021). Clinical applications of MSCs in cartilage repair have shown promising outcomes in veterinary patients. From osteoarthritis in dogs to joint injuries in horses, MSC-based therapies have demonstrated improvements in lameness, pain reduction, and enhanced joint function. These success stories underscore the potential of MSCs in addressing a diverse range of cartilage pathologies (Sasaki *et al.*, 2019; Nurul *et al.*, 2021).

While scaffold-based approaches have shown success, scaffold-free strategies are gaining attention (Alghuwainem *et al.*, 2019). In these approaches, MSCs are coaxed into forming three-dimensional aggregates, known as spheroids (Yoon et al., 2012). These spheroids mimic the natural cell-cell interactions within cartilage and enhance the chondrogenic differentiation of MSCs (Alghuwainem *et al.*, 2019; Han *et al.*, 2022). Scaffold-free

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strategies offer advantages such as simplified application and reduced risk of adverse reactions to scaffold materials (Alghuwainem *et al.*, 2019; Han *et al.*, 2022). Translating MSC-based cartilage repair strategies from in vitro studies to in vivo applications requires rigorous evaluation in relevant animal models. Veterinary orthopaedics often relies on naturally occurring joint pathologies in companion animals as suitable models (Alghuwainem *et al.*, 2019). These models provide insights into the safety, efficacy, and long-term outcomes of MSC-based interventions, bridging the gap between preclinical research and clinical application (Nurul *et al.*, 2021).

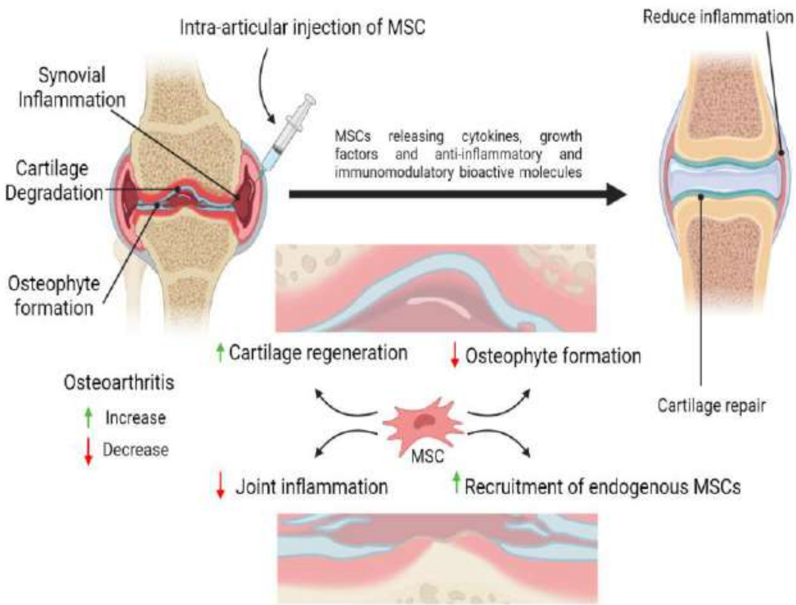

Fig. 4: Diagram illustrating potential therapeutic approaches employing mesenchymal stem cell (MSC) therapy for cartilage

ISBN No : 978-93-340-4428-7

repair and regeneration. MSCs exhibit anti-inflammatory and immunomodulatory characteristics, potentially mitigating joint inflammation. These cells may contribute to the recovery process by differentiating into chondrocytes or stimulating the growth and differentiation of existing healthy chondroprogenitors into mature chondrocytes, or both. Through the release of trophic factors and interactions between cells, MSCs have the potential to augment cartilage regeneration and alleviate synovial inflammation in osteoarthritic joints. Reproduced from Nurul et al. (2021) under Creative Commons Attribution 4.0 International (CC BY 4.0) license.

Advancements in genetic engineering have opened avenues for tailoring MSCs to specific applications. Genetic modification can enhance the chondrogenic potential of MSCs, augment their anti-inflammatory properties, or improve their survival and integration within the joint (Yoon *et al.*, 2012). These personalized approaches hold promise in addressing the heterogeneity of cartilage pathologies encountered in veterinary patients. Standardizing protocols, optimizing dosages, and understanding the long-term safety profile are critical considerations (Alghuwainem *et al.*, 2019; Han *et al.*, 2022). Additionally, investigating the interplay between MSCs and the immune system in the joint microenvironment will provide insights into the durability of regenerative effects. MSCs have emerged as transformative agents in cartilage repair in veterinary orthopaedics (Zhang *et al.*, 2009). Their ability to stimulate

ISBN No : 978-93-340-4428-7

chondrogenesis, modulate inflammation, and integrate with scaffold-based strategies offers a paradigm shift in addressing the complexities of cartilage injuries and degenerative conditions in animals (Nurul *et al.*, 2021).

Tissue engineering strategies

Bone tissue engineering strategies involving MSCs are at the forefront of regenerative medicine, offering promising avenues for bone repair and regeneration (Xue *et al.*, 2022). These approaches leverage the unique properties of MSCs to enhance the healing process and restore the structural and functional integrity of damaged or diseased bone tissue (Shang *et al.*, 2020; Xue *et al.*, 2022). Utilizing biocompatible scaffolds is a common approach in bone tissue engineering (Shang *et al.*, 2020). These scaffolds provide structural support and mimic the natural extracellular matrix, creating a conducive environment for MSC attachment, proliferation, and differentiation (Shang *et al.*, 2020; Xue *et al.*, 2022). Various materials, including synthetic polymers, ceramics, and natural polymers, are employed to fabricate scaffolds with tunable properties (Krishani *et al.*, 2023).

In scaffold-free strategies, MSCs are encouraged to form three-dimensional structures without external support. Aggregates, spheroids, or cell sheets are examples of scaffold-free approaches (Lee *et al.*, 2017). These methods aim to enhance cell-cell interactions and promote a more native-like tissue formation. Growth factors, such as bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), and transforming

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growth factor-beta (TGF-β), play a crucial role in regulating MSC behaviour (Poniatowski *et al.*, 2015). Combining these factors with biomaterials further enhances the efficacy of tissue engineering strategies, promoting osteogenic differentiation and angiogenesis (Shang *et al.*, 2020; Xue *et al.*, 2022). Furthermore, pre-conditioning MSCs through exposure to specific mechanical, biochemical, or environmental cues before implantation has shown promising results (Hu and Li, 2018). This approach primes MSCs to exhibit enhanced therapeutic properties, ensuring better integration and functionality within the host tissue (Shang *et al.*, 2020; Xue *et al.*, 2022).

Combining MSC-based therapies with other approaches, such as gene therapy or 3D bioprinting, holds significant potential (Shang *et al.*, 2020; Xue *et al.*, 2022). These synergistic strategies aim to address multiple aspects of bone regeneration simultaneously. Once prepared, MSCs or MSC-laden constructs are implanted into the defect site (Shang *et al.*, 2020; Xue *et al.*, 2022; Krishani *et al.*, 2023). The in vivo environment stimulates further differentiation of MSCs into osteoblasts, promoting bone tissue formation. Monitoring and assessing the integration and remodelling processes are crucial for evaluating the success of the tissue engineering strategy (Hu and Li, 2018). Despite significant progress, challenges such as immune response, vascularization, and long-term stability still need to be addressed (Yeo *et al.*, 2023). Ongoing research focuses on refining existing strategies and exploring novel approaches, including the use of advanced biomaterials and biofabrication techniques (Hu and Li, 2018; Yeo *et al.*, 2023). Therefore, bone tissue engineering strategies

ISBN No : 978-93-340-4428-7

employing MSCs offer a multifaceted approach to address the complexities of bone regeneration. These strategies continue to evolve, bringing us closer to clinical applications that can revolutionize the treatment of bone defects and disorders.

Conclusion and prospects

In the realm of veterinary orthopaedics, the exploration of tissue engineering strategies employing MSCs presents both exciting opportunities and complex challenges. As we delve into the multifaceted landscape of regenerative medicine for veterinary musculoskeletal disorders, the synthesis of our findings reveals a burgeoning field with tremendous potential. The journey through tissue engineering with MSCs has illuminated the remarkable regenerative capabilities of these versatile cells. From defining MSCs and understanding their characteristics to unravelling their immunomodulatory and regenerative properties, our exploration has traversed diverse landscapes. The applications in veterinary orthopaedics, including bone regeneration, cartilage repair, and ligament/tendon reconstruction, have showcased the transformative impact of MSC-based tissue engineering. MSCs, derived from various sources including adipose tissue, bone marrow, and umbilical cord, offer a rich repertoire of options for clinicians and researchers alike. The immunomodulatory properties of MSCs hold promise not only in mitigating inflammatory responses but also in fostering an environment conducive to tissue regeneration. Successful case studies have underscored the efficacy of MSC-based

ISBN No : 978-93-340-4428-7

tissue engineering in veterinary patients, providing a foundation for further advancements.

While scaffolds provide structural support and mimic the extracellular matrix, scaffold-free methods harness the inherent capabilities of MSCs to self-organize and differentiate. The synergy between MSCs and biomaterials, coupled with the controlled release of growth factors, exemplifies the sophistication of current strategies. However, the path forward is not without challenges. The optimization of delivery methods, ensuring proper cell retention and viability, remains a critical hurdle. Standardization of protocols, from isolation techniques to culture conditions, is imperative for fostering reproducibility and comparability across studies. Moreover, the intricate interplay of MSCs within the complex microenvironment of injured tissues demands a nuanced understanding to harness their full potential.

Looking to the future, innovative approaches will drive the field forward. Advances in biofabrication technologies, such as 3D printing, offer new avenues for creating tailored scaffolds that precisely mimic the native tissue architecture. Engineering the secretome of MSCs to enhance their regenerative paracrine signaling represents a frontier for bioengineering. Combining MSCs with gene therapy opens new dimensions, allowing for targeted modulation of cellular behaviours. As veterinary medicine increasingly intersects with regenerative therapies, the knowledge gained from tissue engineering with MSCs transcends the laboratory bench to offer tangible benefits to animal patients. The successes witnessed in

ISBN No : 978-93-340-4428-7

preclinical studies must now pave the way for rigorous clinical trials, ensuring the safety and efficacy of MSC-based interventions in diverse veterinary orthopaedic scenarios.

In parallel, ethical considerations surrounding the use of MSCs in veterinary patients merit careful attention. Establishing robust regulatory frameworks that balance innovation with patient safety is crucial. The harmonization of ethical standards across jurisdictions will facilitate the responsible and equitable advancement of veterinary regenerative medicine. Interdisciplinary collaborations between veterinarians, bioengineers, and cellular biologists are essential for unravelling the complexities of MSC-based interventions. Cross-species insights, drawing parallels between human and veterinary applications, will further enrich our understanding. The prospects are tantalizing, with the potential not only to alleviate the suffering of animal patients but also to inform and inspire advancements in human medicine. As we navigate the evolving landscape of regenerative therapies, the shared goal is clear: to enhance the quality of life for our animal companions and, in doing so, contribute to the broader tapestry of biomedical innovation. The road ahead beckons with exciting possibilities, and the collaborative spirit of scientific inquiry will undoubtedly illuminate new horizons in the ongoing saga of MSC-based tissue engineering in veterinary orthopaedics.

ISBN No : 978-93-340-4428-7

Acknowledgments

All the listed authors are thankful to their representative universities/institutes for providing the related support to compile this work

Declaration of Interest:

All authors declare that there exist no commercial or financial relationships that could, in any way, lead to a potential conflict of interest.

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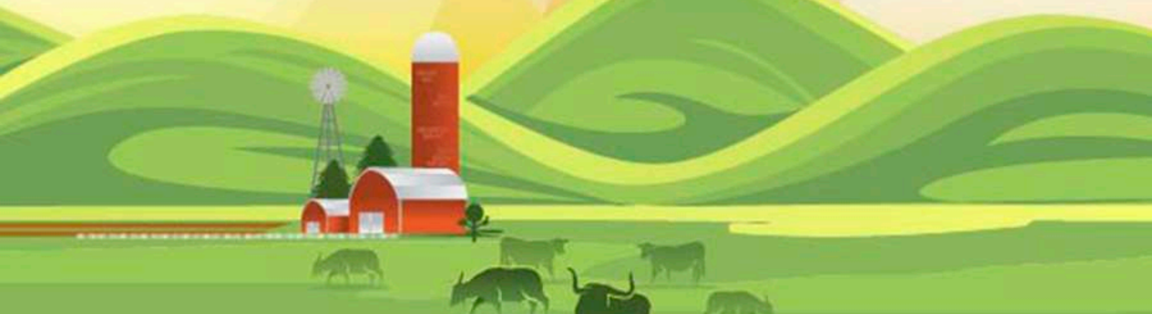

Published by

Indian Veterinary Association, Kerala

Veterinarians Building, TC 25/2067(1), Dharmalayam Road,
Thiruvananthapuram – 695001, Kerala, India