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Bovine hydroxyapatite for biomedical applications: A review of the effects of crosslinker and Secretome

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Abstract: Bovine Hydroxyapatite (BHA), a naturally derived material with a composition similar to human bone, has emerged as a promising candidate for biomedical applications, particularly in bone tissue engineering and regeneration. This review systematically explores the role of crosslinkers and secretomes in enhancing the properties of BHA scaffolds. Crosslinkers, such as glutaraldehyde, improve the mechanical stability and structural integrity of BHA, while secretomes from Mesenchymal Stem Cells (MSCs) contribute to cellular differentiation, angiogenesis, and tissue regeneration. In vitro and in vivo studies were analyzed to assess the osteoconductivity, biocompatibility, and regenerative potential of BHA scaffolds, particularly in combination with crosslinkers and MSC-derived secretomes. Findings suggest that crosslinkers significantly enhance the mechanical performance of BHA, although their concentration must be carefully optimized to prevent cytotoxicity. Secretomes, on the other hand, amplify the biological activity of BHA scaffolds by promoting angiogenesis and immune modulation, which are essential for tissue integration and healing. The synergistic application of crosslinkers and secretomes offers a promising strategy to optimize BHA scaffolds for bone regeneration. This review highlights the current advancements in the field and identifies future research directions to further refine these approaches for clinical applications in bone and dental tissue engineering.

Keywords: *Bovine hydroxyapatite, Biomedical applications, Bone tissue engineering, Crosslinkers, Secretome.*

1. Introduction

Bovine Hydroxyapatite (BHA) has become a key material in biomedical applications due to its structural and chemical similarities to human bone. Hydroxyapatite (HA) is a naturally occurring mineral, primarily composed of calcium and phosphate, which forms the inorganic matrix of bones and teeth. Bovine-derived hydroxyapatite retains many of these beneficial characteristics, such as biocompatibility, osteoconductivity, and bioactivity, making it an ideal candidate for applications in bone tissue engineering, orthopedic implants, and dental repair. The growing demand for biocompatible and effective materials in regenerative medicine has prompted significant interest in optimizing BHA for a range of clinical uses [1].

The mechanical and biological properties of BHA can be significantly enhanced by the use of crosslinkers, which are agents that chemically bond the material's polymer chains. Crosslinking is known to improve the structural integrity, stability, and longevity of BHA-based biomaterials. The ability to fine-tune the degree of crosslinking allows researchers to control the material's biodegradability and bioactivity, both of which are crucial for ensuring the functional integration of BHA in vivo [2]. As such, the selection and application of appropriate crosslinkers are fundamental in enhancing the overall performance of BHA in various biomedical contexts, particularly in long-term implantation [1, 2].

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Another key factor influencing the effectiveness of BHA in biomedical applications is the secretome—the array of bioactive molecules secreted by cells, including growth factors, cytokines, and extracellular matrix components. The secretome plays a critical role in modulating cellular behavior, promoting tissue regeneration, and facilitating the repair of damaged tissues. When incorporated with BHA scaffolds, the secretome can augment osteoblast differentiation, support angiogenesis, and enhance tissue integration. The synergistic effects of BHA and the secretome have the potential to accelerate bone healing and improve the efficacy of bone grafts, making it a promising area of study for regenerative medicine [3, 4].

Optimizing the properties of BHA for biomedical applications requires a thorough understanding of its interactions with both crosslinkers and the secretome. These interactions govern not only the material's mechanical properties but also its ability to support cellular activity and tissue regeneration [2, 5]. Effective BHA-based scaffolds must promote osteogenesis, encourage vascularization, and integrate with the surrounding tissues. Recent advancements in the combination of BHA with specific crosslinkers and secretome components have shown promise in enhancing these properties, offering a more robust solution for bone repair and regeneration [6].

This review aims to systematically examine the effects of crosslinkers and the secretome on the properties of BHA, focusing on their role in improving the material's performance in bone tissue engineering. By synthesizing recent findings, this paper will provide a comprehensive overview of the current state of research on BHA, highlighting the challenges, opportunities, and future directions in optimizing BHA for clinical use. The ultimate goal is to offer insights into the potential of BHA as an advanced biomaterial for regenerative therapies in bone and dental applications.

2. Methodology

This literature review analyzed in vitro and in vivo studies on Bovine Hydroxyapatite (BHA) in biomedical applications, focusing on the effects of crosslinkers and secretomes. A systematic search was conducted in PubMed, Scopus, and Google Scholar using keywords like "Bovine Hydroxyapatite", "crosslinker", "secretome", and "bone regeneration", including only studies published from 2000 onward. The review focused on experimental studies using cell culture or animal models that investigated BHA's osteoconductivity, biocompatibility, and regenerative potential. Only English-language articles were considered. Data from the selected studies were extracted and analyzed to assess the impact of crosslinkers and secretomes on BHA's biological performance.

3. Results

The systematic review identified ten studies investigating the use of BHA scaffolds in bone tissue engineering. These studies focused on several important aspects, including the application of crosslinking agents, the impact of secretomes derived from MSCs, and in vivo biocompatibility. Each of these factors played a critical role in enhancing the mechanical properties, biological performance, and regenerative potential of BHA scaffolds.

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Table 1.
Characteristic of studies included.

Authors	Study type	Research objective	Methodology	Crosslinker used	Secretome used	Key findings
Azami, et al. [7]	In vitro	Investigating the effect of GA as a crosslinker in HAp/gelatin scaffolds for bone tissue repair	Scaffold fabrication, compression tests, degradation tests	GA	None	GA concentration optimized at 1 w/v%, resulting in enhanced compressive strength and reduced fracture work.
Kikuchi, et al. [8]	In vivo	Assessing the effect of GA crosslinking on HAp/collagen composites	Animal tests (rabbit model), material testing (bending strength, swelling ratio)	GA	None	GA crosslinking enhanced mechanical properties and reduced resorption rate without compromising biocompatibility.
Putra, et al. [9]	In vitro	Developing a bone filler using HA-gelatin composite with glutaraldehyde for osteoporosis treatment	FTIR, morphology tests, compressive strength tests, cytotoxicity tests	GA	None	The addition of GA improved the bone filler characteristics, maintaining structure longer and increasing cell viability.
Gough, et al. [10]	In vitro	Evaluating glutaraldehyde-induced toxicity in collagen/PVA composite films	Apoptosis assay, cell attachment and spreading tests	GA	None	Glutaraldehyde crosslinking caused apoptosis in osteoblasts, which could be prevented by glutamic acid or IGF-1.
Goh, et al. [11]	In vitro	Evaluating TPP-crosslinked CHA scaffolds for bone tissue engineering	SEM, porosity analysis, biodegradability, cell viability assays	TPP	None	0.1 M TPP-CHA scaffolds exhibited the highest porosity and biocompatibility, promoting cell viability, adhesion, and early differentiation.
Budiatin, et al. [12]	In vivo	To determine the effect of BHA-GEL-GEN bone implants on bone defect regeneration in vivo.	Wistar rats divided into three groups: negative control, BHA-GEL implant, and BHA-GEL-GEN implant. Bone defects were created using burr hole model. Analysis included radiography, HE staining, and immunohistochemistry for VEGF and ALP expression	Gelatin	None	BHA-GEL-GEN implants accelerated bone defect repair by enhancing tissue vascularization (higher VEGF expression) without disrupting bone remodeling. No significant differences in ALP expression were observed.
Ogata, et al. [13]	In Vivo	To assess MSC-CM's role in early bone regeneration.	Rat calvarial defects (5 mm) treated with MSC-CM or phosphate buffered saline (PBS); analyses included cytokine arrays, imaging, and histology.	None	Atelocollagen suspended in MSC-CM	MSC-CM enhanced stem cell migration within 24 h and accelerated early bone regeneration.

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Shanbhag, et al. [14]	In Vivo	To compare MSC-CM and platelet-rich fibrin (PRF-CM) efficacy in guided bone regeneration (GBR).	Rat calvarial defects treated with collagen membranes functionalized with MSC-CM or PRF-CM; analyses included proteomics, micro-CT, and histology.	None	MSC-CM, PRF-CM	MSC-CM contained more extracellular matrix proteins and showed greater bone coverage via micro-CT after 2 and 4 weeks compared to PRF-CM.
Liu, et al. [15]	In vivo	To evaluate MSC-exosome (MSC-exo)-based therapy and its osteoinductive potential for bone regeneration	Compared osteoinductivity of MSC-exos (from rBMSCs/rASCs) under different culture conditions; used miRNA analysis, pathway verification, and rat cranial defect model with MBG scaffolds for delivery.	None	MSC-EXO	BMSC-OI-exos regulated Smad1/5/9 signaling via miRNA and enhanced bone regeneration in a rat defect model.
Qiu, et al. [16]	In vivo	To compare gingival mesenchymal stem cells (GMSC-CM) and periodontal ligament stem cells (PDLSC-CM) effects on periodontal regeneration.	Rat periodontal defects treated with collagen + CM; analyzed histology, inflammation, and osteogenesis.	None	GMSC-CM, PDLSC-CM	Both GMSC-CM and PDLSC-CM improved regeneration and reduced inflammation, with GMSC-CM showing higher IL-10 expression

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3.1. Characterization of Bovine Hydroxyapatite (BHA)

Bovine Hydroxyapatite is a naturally derived material that mimics the composition and structure of human bone, making it an ideal candidate for various biomedical applications, particularly in bone tissue engineering and regeneration. Characterization of BHA typically begins with its structural analysis, where methods such as X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) are used to confirm the crystallinity and chemical composition of the material. These analyses reveal that BHA has a highly crystalline structure similar to human bone hydroxyapatite, consisting primarily of calcium and phosphate ions. Additionally, the porosity and surface area of BHA are assessed using techniques like scanning electron microscopy (SEM) and Brunauer-Emmett-Teller (BET) analysis, which provide insight into its suitability for cell attachment and nutrient exchange in tissue engineering [17, 18].

The mechanical properties of BHA are another critical aspect of its characterization. BHA's compressive strength, elasticity, and brittleness are evaluated to ensure that the material can withstand the mechanical loads typically encountered in bone structures. Various crosslinking agents or surface treatments are often applied to improve these properties, making the material more robust while maintaining its osteoconductivity [17]. Moreover, BHA's biodegradability is assessed to determine how it integrates into the body over time, particularly its rate of resorption as new bone forms. These properties are crucial in determining the long-term effectiveness and safety of BHA in clinical applications [17, 19].

In addition to its structural and mechanical properties, BHA's biological behavior is thoroughly characterized, particularly in relation to cell interactions. In vitro tests involving osteoblasts or other bone-forming cells are performed to assess the material's ability to support cell proliferation, differentiation, and mineralization [1, 19]. The surface characteristics of BHA, including roughness and surface charge, significantly influence cell adhesion and growth. Research has shown that BHA promotes the attachment of osteogenic cells, making it an excellent scaffold for bone tissue engineering. Furthermore, in vivo studies in animal models provide insight into how BHA interacts with surrounding tissues, its biocompatibility, and its role in promoting bone regeneration and healing after injury or surgery [2, 20, 21].

3.2. Crosslinking Agents and Their Impact on Scaffold Properties

Crosslinking agents significantly impact scaffold properties, particularly in mechanical strength, porosity, degradation, and biological activity. Azami, et al. [7] showed that GEL/HAp nanocomposites with glutaraldehyde (GA) achieved a porosity of 85% with interconnected pore sizes of 300–500 μm, and compressive strength peaked at 1 w/v% GA, followed by a decline at higher concentrations. Similarly, Kikuchi, et al. [8] found that HAp/collagen scaffolds crosslinked with GA achieved maximal 3-point bending strength at 1.35 mmol/g(coll), while higher concentrations reduced biodegradability but maintained high osteoconductivity Azami, et al. [7]. Putra, et al. [9] noted that hydroxyapatite-gelatin scaffolds crosslinked with GA (0.25%, 0.5%, and 0.75%) exhibited increased pore sizes and prolonged degradation, although higher GA levels reduced compressive strength [9]. Using sodium tripolyphosphate (TPP) as a crosslinker, Goh, et al. [11] reported that 0.1 M TPP scaffolds had the highest porosity (58.6%) and superior biocompatibility, while 0.4 M TPP resulted in compact, less porous structures with reduced cellular activity Goh, et al. [11]. Budiatin, et al. [12] investigated bovine hydroxyapatite-gelatin (BHA-GEL) scaffolds incorporating gentamicin (BHA-GEL-GEN) and demonstrated accelerated bone regeneration in vivo. The BHA-GEL-GEN scaffolds promoted osteoclast, osteoblast, and osteocyte migration, increased vascular endothelial growth factor (VEGF) expression, and maintained bone remodeling without significant differences in alkaline phosphatase (ALP) expression compared to controls [12]. However, Gough, et al. [10] highlighted glutaraldehyde's cytotoxicity, showing that it induced apoptosis in osteoblasts, leading to poor cell attachment and growth. The study suggested that post-treatment with L-glutamic acid or insulin-like

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growth factor-1 could mitigate these effects, offering strategies to improve the biocompatibility of GA-crosslinked scaffolds [10].

3.3. Secretomes from Mesenchymal Stem Cells (MSCs)

Several studies have demonstrated the promising role of secretomes in enhancing scaffold biological activity and promoting bone regeneration. Ogata, et al. [13] showed that secretomes derived from human bone marrow mesenchymal stem cells (MSC-CM) significantly accelerated early bone regeneration in rat calvarial defects. MSC-CM enhanced osteoprogenitor cell migration to bone defects within the first 24 hours, as confirmed by in vivo imaging and immunohistochemistry. Cytokine analysis revealed that MSC-CM contained numerous factors involved in cell migration and tissue regeneration [13]. Similarly, Shanbhag, et al. [14] reported that MSC-CM functionalized collagen membranes led to greater total bone coverage in critical-size rat calvarial defects compared to platelet-rich fibrin-conditioned media (PRF-CM). Proteomic analysis revealed higher extracellular matrix-related protein levels in MSC-CM, contributing to greater hybrid bone formation and early bone regeneration Shanbhag, et al. [14]. Liu, et al. [15] demonstrated that exosomes derived from bone marrow stem cells (BMSC-OI-exo) significantly enhanced osteogenic differentiation and bone formation when loaded onto mesoporous bioactive glass (MBG) scaffolds. The sustained release of osteoinductive exosomes facilitated Smad1/5/9 phosphorylation, promoting rapid bone formation Liu, et al. [15]. Qiu, et al. [16] further highlighted the potential of gingival MSC-conditioned media (GMSC-CM) in periodontal regeneration. GMSC-CM significantly improved inflammatory profiles and osteogenic differentiation markers, achieving comparable results to periodontal ligament stem cell-conditioned media (PDLSC-CM) [16].

4. Discussion

This section discusses the roles of these two factors individually and explores their combined effects, linking the findings to existing theories and prior studies in the field.

4.1. Role of Crosslinking Agents in BHA Scaffold Performance

The integration of crosslinking agents such as GA and TPP in scaffold fabrication plays a pivotal role in modulating mechanical, structural, and biological properties, which are critical for bone tissue engineering. Azami, et al. [7] and Kikuchi, et al. [8] demonstrated that GA effectively enhances mechanical strength and controls biodegradability, with optimal concentrations (1 w/v% and 1.35 mmol/g(coll), respectively) balancing compressive strength and flexibility. However, higher concentrations may disrupt the structural integrity and reduce the natural alignment of HAp crystals with collagen, compromising mechanical and biological compatibility [22]. This indicates the necessity of fine-tuning GA levels to avoid over-crosslinking, which can impair scaffold functionality [7, 8].

The results from Putra, et al. [9] further highlighted the role of GA in controlling degradation rates, as GA-crosslinked hydroxyapatite-gelatin scaffolds exhibited prolonged stability suitable for drug delivery applications [9]. Nonetheless, higher GA concentrations led to increased pore sizes and reduced compressive strength, emphasizing the importance of optimizing crosslinking density to meet specific clinical requirements [23, 24]. TPP-crosslinked scaffolds, as explored by Goh, et al. [11] offered an alternative with superior porosity and cellular activity at lower concentrations (0.1 M TPP), supporting their use in applications prioritizing biocompatibility and osteoconductivity. However, higher TPP levels (0.4 M) resulted in compact structures that diminished cellular interactions, underscoring the need for precise concentration control [11, 25].

Budiatin, et al. [12] extended this understanding by demonstrating the synergistic effect of incorporating antibiotics such as gentamicin in BHA-GEL scaffolds. The BHA-GEL-GEN scaffolds promoted vascularization and cellular migration, as evidenced by increased VEGF expression and accelerated bone regeneration in vivo [26, 27]. These findings suggest that crosslinking agents can enhance scaffold functionality when combined with bioactive components, such as antibiotics or growth

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factors, for targeted therapeutic applications. Importantly, the BHA-GEL-GEN scaffolds maintained bone remodeling processes without compromising ALP activity, indicating compatibility with natural bone healing mechanisms [12, 27].

Overall, these studies emphasize that crosslinking agents serve as a crucial parameter in scaffold design, influencing porosity, degradation, mechanical stability, and biological interactions. While GA offers robust mechanical and degradation control, alternatives like TPP provide enhanced biocompatibility for certain applications. The addition of bioactive components, expands the versatility of these scaffolds, making them promising candidates for clinical applications such as osteomyelitis therapy and complex bone defect repair [9, 12].

4.2. Role of Secretomes in Enhancing BHA Scaffold Biological Activity

The emerging role of secretomes in enhancing scaffold biological activity represents a paradigm shift in tissue engineering. Ogata, et al. [13] demonstrated that MSC-CM facilitates early osteoprogenitor cell recruitment, a key prerequisite for successful bone regeneration [13]. The rapid migration and recruitment of endogenous cells observed in their study can be attributed to the cytokine-rich environment provided by MSC-CM, which enhances chemotaxis, adhesion, and proliferation. This aligns with broader findings in regenerative medicine that underscore the importance of paracrine signaling in activating host repair mechanisms [28].

Shanbhag, et al. [14] extended these findings by demonstrating that MSC-CM not only facilitates cellular recruitment but also modulates extracellular matrix remodeling and hybrid bone formation. The superior efficacy of MSC-CM over PRF-CM highlights the importance of protein composition, with MSC-CM offering a more robust repertoire of bioactive molecules essential for scaffold integration and hybrid bone synthesis. This suggests that functionalization of biomaterials with MSC-derived secretomes could address key limitations in current scaffold designs, such as delayed mineralization and poor matrix organization [14].

Exosomes, as shown in Liu, et al. [15] provide a more targeted and sustained therapeutic effect due to their miRNA content. The study revealed that exosomal miRNAs regulate the Acvr2b/Bmpr2 signaling axis, shifting the balance towards Smad-driven osteogenesis. This mechanistic insight not only underscores the precision of exosome-based therapy but also demonstrates how scaffold modifications, such as MBG surface entrapment, can optimize bioavailability and therapeutic outcomes. The use of lyophilized delivery systems further addresses the stability challenges associated with exosome therapy, making this approach highly translatable to clinical applications [15, 29].

The work by Qiu, et al. [16] underscores the role of secretomes in modulating the inflammatory microenvironment during bone healing. The reduction in pro-inflammatory cytokines (TNF-α, IL-1β) observed with GMSC-CM and PDLSC-CM is critical for creating a conducive environment for osteogenic differentiation. Additionally, the upregulation of anti-inflammatory cytokines (e.g., IL-10) and osteogenic markers (BSP-II, Runx2) suggests that secretomes not only accelerate tissue repair but also promote long-term stability of the regenerated tissue. This dual regulation of inflammation and osteogenesis highlights the multifaceted role of secretomes in tissue regeneration [16, 30].

Collectively, these findings emphasize that secretomes and exosomes represent a significant advancement in the development of biologically active scaffolds. By addressing critical challenges such as poor cellular recruitment, inadequate vascularization, and unresolved inflammation, secretome-functionalized BHA scaffolds offer a versatile and effective solution for complex bone regeneration scenarios. Future research should explore combinatory approaches, integrating secretome-based strategies with advanced scaffold designs, to further optimize their regenerative potential and translational applicability [30].

4.3. Synergistic Effect of Crosslinkers and Secretomes

The synergistic application of crosslinkers and secretomes presents a promising strategy for optimizing the mechanical and biological performance of BHA scaffolds. Crosslinking agents play a

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pivotal role in modifying the structural and functional characteristics of BHA scaffolds by facilitating molecular interactions that result in the formation of crosslinked networks. These networks enhance scaffold stability, rigidity, and resistance to dehydration, which are crucial for maintaining the integrity of the scaffold in physiological environments [17]. However, excessive crosslinking can detrimentally impact the mechanical properties and biological functionality of the scaffold. This is due to an overabundance of intrafibril bonds that restrict flexibility, thereby compromising the scaffold's ability to undergo deformation and absorb mechanical stress, which may ultimately reduce its performance [8, 31].

In contrast, secretomes—biologically active factors secreted by stem cells—play a critical role in enhancing the biological activity of scaffolds. These secretomes are rich in growth factors, cytokines, and extracellular matrix proteins that regulate various cellular processes essential for tissue regeneration, such as cell proliferation, migration, angiogenesis, and immune modulation [11]. When incorporated into BHA scaffolds, secretomes promote cellular colonization, facilitate neovascularization, and enhance the integration of the scaffold within the host tissue [10, 32]. The presence of angiogenic factors in the secretomes is particularly beneficial in promoting vascularization within the scaffold, thereby improving nutrient and oxygen supply to support tissue formation and regeneration [33].

The interplay between crosslinkers and secretomes is essential for achieving optimal scaffold performance. Crosslinkers influence the porosity and mechanical properties of the scaffold, which directly impacts its ability to support cellular activities. Porosity is crucial for nutrient diffusion, cellular migration, and vascular ingrowth, but excessive porosity can compromise the scaffold's mechanical integrity [34]. Thus, a precise balance must be struck between achieving sufficient porosity for biological functions and maintaining the structural strength necessary for mechanical support. In this context, crosslinkers help regulate scaffold porosity while simultaneously enhancing the scaffold's resistance to mechanical deformation [34, 35].

The biological benefits of secretomes significantly complement the structural enhancements provided by crosslinkers, creating a highly effective environment for tissue regeneration. Pro-angiogenic factors present in secretomes, such as VEGF and FGF, stimulate the formation of new blood vessels within the scaffold, improving oxygen and nutrient supply to the regenerating tissue while facilitating waste removal. This angiogenesis is crucial for sustaining the growth of larger tissue constructs. Additionally, secretomes exert immunomodulatory effects by regulating the inflammatory response, ensuring that the acute inflammation necessary for tissue repair is properly controlled, while preventing excessive or chronic inflammation that could impede healing. By promoting a favorable balance between immune activation and resolution, secretomes reduce the risk of adverse immune reactions and graft rejection, further enhancing scaffold integration. Together, the mechanical stability provided by crosslinkers and the biological activity driven by secretomes optimize scaffold performance, fostering tissue regeneration through improved vascularization, immune regulation, and cellular integration [33]. This immune modulation, in conjunction with the structural stability provided by crosslinkers, supports a more favorable environment for tissue regeneration and integration of the scaffold [36, 37].

Together, the synergistic combination of crosslinkers and secretomes offers a comprehensive approach to the design of BHA scaffolds with enhanced mechanical and biological properties. By optimizing the concentration and application of crosslinking agents while integrating the regenerative potential of secretomes, scaffolds can be engineered to provide both the structural integrity and biological cues necessary for effective tissue regeneration. This strategy holds significant promise for advancing scaffold-based therapies in regenerative medicine, particularly for applications in bone tissue engineering and repair [38].

4.4. Future Directions and Research Needs

While the findings reviewed in this discussion present significant advancements in the design of BHA scaffolds, there is still a need for more longitudinal studies to assess the long-term efficacy of

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crosslinked BHA scaffolds in clinical settings. Future research should focus on in vivo models that simulate human bone defects more accurately to better predict the clinical outcomes of these scaffolds. Additionally, optimizing the crosslinking process to minimize cytotoxicity and improve biocompatibility will be critical in advancing the clinical translation of these materials. The interplay between crosslinkers and secretomes requires more detailed investigation to ensure that these factors can be harmonized for maximal therapeutic effect. By addressing these gaps, future studies will contribute to the development of more efficient, biocompatible, and mechanically robust scaffolds that will significantly improve outcomes in bone tissue engineering and regenerative medicine [4, 17].

5. Conclusion

In conclusion, this review underscores the significant role of crosslinkers and secretomes in enhancing the functionality of BHA scaffolds for bone tissue engineering. Crosslinkers like glutaraldehyde improve the mechanical properties of BHA scaffolds, though careful optimization is necessary to avoid negative effects on biocompatibility. On the other hand, the incorporation of secretomes from MSCs boosts the biological activity of the scaffolds, promoting osteogenesis and angiogenesis, which are critical for tissue regeneration. Combining both approaches appears to offer a balanced solution to improving both the structural integrity and biological function of BHA scaffolds. Further studies are needed to refine these strategies, focusing on optimal concentrations and combinations of crosslinkers and secretomes, as well as in vivo validation to ensure their long-term efficacy in clinical applications.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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