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REVIEW

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Local and Systemic Peptide Therapies for Soft Tissue Regeneration: A Narrative Review

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Background: The musculoskeletal system, due to inherent structure and function, lends itself to contributing toward joint pain, whether from inflammatory disorders such as rheumatoid arthritis, degenerative diseases such as osteoarthritis, or trauma causing soft tissue injury. Administration of peptides for treatment of joint pain or inflammation is an emerging line of therapy that seeks to offer therapeutic benefits while remaining safe and relatively non-invasive. **Purpose**: The purpose of this study is to review the current literature on existing oral peptide agents, intra-articular peptide agents, and new developments in human trials to assess route of administration (RoA) for drug delivery in terms of soft tissue regeneration. **Study Design**: Narrative Review. **Methods**: A comprehensive literature search was conducted using the PubMed database. The search included medical subject headings (MeSH) terms related to peptide therapy, soft tissue regeneration, and RoA. Inclusion criteria comprised articles focusing on the mechanisms of action of peptides, clinical or biochemical outcomes, and review articles. Exclusion criteria included insufficient literature or studies not meeting the set evidence level. **Conclusion**: The review identified various peptides demonstrating efficacy in soft tissue repair. Oral and intra-articular peptides showed distinct advantages in soft tissue regeneration, with intra-articular routes providing localized effects and oral routes offering systemic benefits. However, both routes have limitations in bioavailability and absorption. Still in their infancy, further inquiries/research into the properties and efficacy of emerging peptides will be necessary before widespread use. As a viable alternative prior to surgical intervention, peptide treatments present as promising candidates for positive outcomes in soft tissue regeneration.

INTRODUCTION

Chronic joint pain is a debilitating condition affecting approximately 20.4% of adults in the United States [1]. The musculoskeletal system is one of the highest contributors of joint pain stemming from conditions such as rheumatoid arthritis [2], osteoarthritis [3], and musculoskeletal injury [4,5]. Administration of peptides for treatment of joint pain or inflammation is an emerging line of therapy that seeks to offer therapeutic benefits

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Abbreviations: RoA, Routes of Administration; HC, Hydrolyzed Collagen; BPC-157, Body Protection Compound-157; GHK-Cu, Glycyl-l-histidyl-l-lysine-Copper(II); Tβ4, Thymosin β4; VEGF, Vascular Endothelial Growth Factor; NO, Nitric Oxide; FAK, Focal Adhesion Kinase; eNOS, Endothelial Nitric Oxide Synthase; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; RCT, Randomized Controlled Trials; MMP, Matrix Metalloproteinase; OPG, Osteoprotegerin; RANKL, Receptor Activator of Nuclear Factor Kappa-B Ligand; dGEMRIC, Delayed Gadolinium-Enhanced Magnetic Resonance Imaging of Cartilage; ACLR, Anterior Cruciate Ligament Reconstruction.

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while remaining safe and relatively non-invasive [6-8].

Peptides are the building blocks of proteins consisting of a short string of 2 to 50 amino acids joined together through covalent bonds [9]. Although bioactive peptides were introduced in the first half of the 20th century, advances in recombinant therapeutics, structural biology, and new technologies have allowed for a more sophisticated *modus operandi* of peptide drug development in the 21st century [10]. Specifically, several potential therapies are undergoing animal trials focused on administration of growth factors implicated for soft tissue repair [11,12]. Whether utilized for recovery of cartilage [13], extracellular matrix (ECM) [8], muscle [14], tendons [15], ligaments [16], or even assistance in bone repair [17]; advances in peptide therapy show promise as a treatment mechanism to aid in joint regeneration and prevent future degeneration.

Selection of the most appropriate peptide for treatment is attributed to many factors, but this review specifically focuses on the routes of administration (RoA), comparing oral peptide formulations with intra-articular injections. Recently, the number of oral peptide formulations in clinical trials has been increasing due to oral drug delivery being a widely preferred method [18]. However, oral peptides have limited efficacy due to a relatively short half-life on top of being quickly broken down by peptidases and being poorly absorbed by mucosal membranes [19]. Furthermore, bioavailability is an important factor that must be considered when determining dosing regimens owing to the nature of how orally administered agents are absorbed [20]. In the case of osteoarthritis, oral peptides may also be unable to adequately perfuse into the necessary joint tissues because of the avascular nature of articular cartilage [21]. Intra-articular injections serve as an alternate RoA which offers faster pain relief, virtually no systemic effects or side effects, and increased persistence compared to limited oral bioavailability [22]. Issues with injections in general do exist however, such as risk of infection, accuracy, and convenience [23,24], which are also important to consider. Each of these routes may have advantages and disadvantages but a specific juxtaposition between the two in the scope of joint treatment with peptides has yet to be made.

The purpose of this study is to review the current literature on existing oral peptide agents, intra-articular peptide agents, and new developments in human trials to evaluate different RoA in the treatment of chronic joint pain. Understanding the benefits of each factor may allow for allocation of resources to promote and develop new therapeutics that maximize satisfaction for the generalizable public. Through such a robust review, this study will also expound on various treatment mechanisms of action, etiology of joint-related symptoms, and the multicomponent nature of peptide therapy.

METHODS

The authors conducted a comprehensive literature search using the PubMed database with MeSH terms related to peptide therapy, soft tissue regeneration, and RoA. Articles were excluded if they did not meet the set level of evidence or lacked sufficient substantive literature. An article's level of evidence necessary to be included in this study required either a controlled animal laboratory or randomized human clinical trial. The proteins included in this review were selected based on their documented efficacy in soft tissue repair, including both preclinical and clinical studies. The research on type II collagen, collagen hydrolysate, body protection compound-157, human peptide GHK (glycyl-l-histidyl-l-lysine), OP3-4, WP9QY, and thymosin β4 peptide therapies were all selected for this narrative review (Table 1).

Inclusion criteria consisted of articles focused on peptide agents that: (1) delved into the peptide's mechanism of action; (2) demonstrated clinical or biochemical outcomes of a treatment group compared to a control group; and (3) reviewed existing literature through meta-analyses or review. Peptide papers were excluded for review if: (1) there was not sufficient, substantive literature found; or (2) if the level of evidence set was not met.

Both animal studies and human randomized controlled trials (RCTs) were included in this review to provide a holistic understanding of peptide efficacy across different biological systems. While the biology of animals and humans differs significantly, the inclusion of both study types allows for a broader assessment of therapeutic potential, as findings from animal models often lay the groundwork for clinical applications. This approach differentiates this review from clinical papers focused solely on human trials by incorporating insights from basic science that can inform future clinical research.

PEPTIDE THERAPIES

Native Type II Collagen

Type II collagen or collagen-2, is a naturally occurring homotrimer peptide $[\alpha1(II)]_3$ that predominately contributes to the fibrillar matrix of articular cartilage, vitreous bodies, and the nucleus pulposus [25]. Similar to other types, its structure is comprised of rod-like triple-helical domains [26]. Collagen-2 has been thoroughly studied due to the initial hypothesis that supplementation would promote regeneration of soft tissues since collagen represents the main component [27]. Initial investigations evaluated collagen-2 supplementation for rheumatoid arthritis [28] and osteoarthritis [29], but further research has shown an immune-mediated response called oral tolerance separate from the mechanisms involved in hydrolyzed collagens. Oral tolerance posits that colla-

Table 1. List of Peptides for Soft Tissue Regeneration

Peptide	Derivation	Sequence	Route of Administration
Collagen-2	Bovine, Porcine, Poultry, Fish	Gly-Pro-X	Oral
Collagen Hydrolysate	Bovine, Porcine, Poultry, Fish	Gly-X-Hyp	Oral, Intra-articular
BPC-157	Human Gastric Juice	Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val	Oral, Intra-articular, Intra-peritoneal
GHK-Cu	Human Plasma, Saliva, Urine	Gly-His-Lys	Topical, Oral, Intra-articular, Intraperitoneal
OP3-4	Synthetic	Tyr-Cys-Glu-Ile-Glu-Phe-Cys-Tyr-Leu-Ile-Arg	Oral, Intra-articular
WP9QY	Synthetic	Tyr-Cys-Trp-Ser-Gln-Tyr-Leu-Cys-Tyr	Intra-articular
Thymosin-β4	All Tissue/Cell Types Except Red Blood Cells	Ser-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Gly-Glu-Ser	Intradermal, Intravenous

X represents any amino acid in the sequence

gen-2 would be effective at reducing autoimmune reactions against articular cartilage by diminishing immune responses to previously fed antigens [26]. Furthermore, collagen-2 is resistant to proteinases and is able to maintain triple helix conformation which aids in joint health by this oral tolerance mechanism [30].

Most collagens, including collagen-2, are obtained from animal-derived raw materials [31]. Traditional species chosen for collagen extraction are bovine and porcine; however, poultry and fish are becoming more popular as alternative sources to overcome concerns about transmission of zoonotic diseases [26]. Procurement of collagen-2 from a natural source may involve different techniques and ultimately defines the final product based on its main features and properties. Products such as "insoluble undenatured native collagen-2" and "soluble native collegen-2" have been extracted and differentiated. Thus, while insoluble undenatured native collagen-2 exhibits specific epitopes that reduce inflammation when taken orally, soluble native collagen-2 maintains its triple helical tertiary structure but has less crosslinking [26,32].

Treatment of articular soft tissue diseases has been shown to be effective with oral collagen-2. In a multicenter, double-anonymous, placebo-controlled trial, positive effects were observed in patients with rheumatoid arthritis when taking a low dose of collagen-2. The subjects that completed 24 weeks of treatment using 20μg/day had a significant response rate (p=0.035) compared to placebo [33]. Another randomized, single-anonymous, placebo-controlled investigation aimed to assess if such benefit was present for osteoarthritis. This study used the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) to assess pain, stiffness, and physical function with a score range of 0 (no symptoms) to 96 (worst symptoms). While the end results did not show any reduction in urine levels of Coll2-1, Coll2-1 NO2, and fibulin-3, all markers of cartilage destruction, there was a clear demonstration of symptomatic improvement when collagen-2 was taken concomitantly with acetaminophen. After 3 months of treatment with 1500mg/day of acetaminophen and 10mg/day of native collagen-2, individuals reported significant improvements compared to baseline in pain, function, and quality of life [34]. Furthermore, another double-anonymous study also emphasizes the safety profile not found in several other options [35]. While the biochemical benefit of oral undenatured collagen-2 may still be debated, it has shown a clinical benefit in pain reduction for those afflicted with osteoarthritis [36].

Due to the large peptide size of natural collagens, intra-articular injections of collagen-2 are not available. Unlike hydrolyzed collagen which can be given orally or locally, collagen-2 is poorly absorbed and digested thus making local and oral applications inefficient [37]. Therefore, continued studies of collagen-2 should focus on better understanding the biochemical pathways that would allow for an improvement in symptomatology [33-35,38]. Additionally, other native collagens such as type I and III also play an important structural role in forming different soft tissues such as ligaments and tendons

[39]. Recent efforts have been made to understand how collagen supplementation may affect tendon/ligament properties [40], but any regenerative effect of oral native collagen on ligament/tendon soft tissues has yet to be investigated. Such findings would increase the versatility of native collagen therapy and expand its target population beyond just rheumatoid arthritis or osteoarthritis.

Collagen Hydrolysate

Hydrolyzed collagen (HC), also known as collagen hydrolysate, is formed by denaturing native collagen resulting in three separate alpha chains that assemble in random coiled form [41]. Once separated, these chains are hydrolyzed by proteolytic enzymes into small peptides with a molecular weight typically between 3-6 KDa [42]. Alternatively, this protein can receive thermal treatment or be exposed to hot temperatures (between 100-374°C) and pressure (less than 22MPa) to separate the chains [43-45]. The resulting peptide's solubility and functional activity, as well as antioxidant or antimicrobial properties, are related to the enzymes used and the degree to which collagen was hydrolyzed [46,47]. Smaller size also confers a benefit in contrast to native collagen, which needs multiple enzymes in order to incorporate the triple helix structure into the extracellular matrix [48]. Additionally, HC has higher therapeutic loading and cost-effectiveness; does not require a multistep extraction procedure, is highly digestible, and also is easily absorbed and distributed throughout the human body [49,50].

Extraction of HC can be done from different tissues such as bovine Achilles tendon, lung, and nuchal ligament [41]. Through hydrothermal processes and membrane fractionation, HC can also be obtained from porcine skin [51]. However, traditional methods not only raise health limitations, ie, risks of infection, such as swine flu [52], but can also present religious challenges. Therefore, new developments have been made to allow for safer extraction using marine sources such as fish, jellyfish, or sponges [53,54].

HC's greater bioavailability compared to native collagen makes it a good candidate for oral administration. HC is absorbed by the gastrointestinal tract after being consumed and then incorporated into joint cartilage [55]. In one double-anonymous, randomized, placebo-controlled monocentric study, researchers found that individuals between the ages of 51-70 had significantly reduced joint pain indexes (12.4 ± 3.7; $p < 0.05$) when taking 4000mg oral HC compared to baseline (29 ± 5.6; $p < 0.05$) [56]. In patients specifically with osteoarthritis, two meta-analyses have shown benefits on joint cartilage, synovial fluid, and overall clinical outcomes with the first analyzing nine studies between 2005-2008, and the second analyzing four randomized controlled studies with specifically HC as an intervention [57,58]. One group found that oral supplementation with 10g of HC structurally improved thickness of articular cartilage in osteoarthritic joints via delayed gadolinium-enhanced MRI of cartilage (dGEMRIC). These subjects had significant changes to mean (± standard deviation) dGEMRIC scores at the medial tibia (29.6 ± 70.5; $p=0.03$) and the lateral tibia (25.5 ± 60.2; $p=0.02$) after 24 weeks [59]. Additionally, evidence shows that consumption of oral HC decreased the number of additional treatments necessary to reduce activity-related knee pain in young adults [60]. HC may also play a role in integumentary health by exerting beneficial effects in skin hydration, wrinkling, and elasticity, giving it an auxiliary function [61].

Local injections of HC is possible, but clinical trials are limited, with one using polymerized collagen to some success by showing statistically significant improvements using the visual analogue scale to estimate pain at the site of injection [62]. One promising medical device (ChondroGrid, Biotek, Arcugnano, Italy) consisting of bovine hydrolyzed <3 kDa type I collagen(4mg/ 2mL) has been developed and is currently available on the market. ChondroGrid is a novel HC injectable that has been tested and shown to have significant improvements in median (interquartile range) WOMAC pain (4.5(3.25) baseline, 2(3) after 15 days, 1(2.25) after 30 days; $p=0.03$), stiffness (2(2.25), baseline, 2(2.25) after 15 days, 1(2.25) after 30 days; $p=0.04$), functional (18(13) baseline, 13.5(12.5) after 15 days, 5(10.25) after 30 days; $p=0.04$), and total (23.5(14.5) baseline, 17(15.75) after 15 days, 8(13.75) after 30 days; $p=0.03$) scores with respect to baseline. However, ChondroGrid does not act on a biochemical level to alter any signaling pathways nor disrupt the progression of osteoarthritis. Rather, it may promote the formation of articular cartilage from chondrocytes to off-balance fibrous tissue [63]. The positive effects of ChondroGrid have been replicated in another study which reported no side-effects, but due to it being a relatively newer injectable, it is possible that potential side-effects have not been discovered yet [64].

BPC-157

Bepecin, known as Body Protection Compound-157 (BPC-157), is a stable gastric pentadecapeptide that is synthetically derived from another protein for pharmaceutical use [65]. BPC-157 is currently being investigated for its regenerative capabilities in small animal models using oral, topical, and intraperitoneal RoA, and has shown great promise in healing an array of tissues including tendons, ligaments, skeletal muscle, and bone [66].

For Achilles tendon repair in rats following surgical transection, systemic delivery through intraperitoneal injections of BPC-157 improved recovery measures and revealed formation of granulation tissue with active

angiogenesis throughout the recently severed ends [67]. This effect protected the endothelium and promoted nitric oxide (NO) synthesis [67,68]. Interestingly, mononuclear inflammatory cell migration to the site of injury was down regulated, fibroblast counts were increased, and production of highly vascularized reticulin and collagen fibers occurred [67]. In various other tissue types, fibrosis was mitigated by preventing the overexpression of endothelin [69]. A similar study was conducted with the transected medial collateral ligament (MCL) and yielded similar results of tissue regeneration in rats [70]. Treatment using this stable gastric pentadecapeptide was administered in three different routes, orally, topically, and intraperitonially, suggesting that the peptide has an array of potential therapeutic delivery mechanisms [66,70,71].

In muscle injury models, BPC-157 has also proven itself to be an effective therapy. In rats, a transected quadricep muscle treated with BPC-157 intraperitoneal injections promoted healing. BPC-157 releases a high amount of specific growth factors that contribute to the injured myofibrils, stimulating myogenesis *in vivo*. With reduced inflammatory markers, increased collagen synthesis, angiogenesis, endothelium protection, and NO modulation in healing, all of which comprise a multifaceted process of induction by which BPC-157 promotes proliferation of many new connective tissues [72]. In a similar fashion, Novinsack carried out research on a damaged gastrocnemius muscle. This study investigated the effectiveness of intraperitoneal injections and topical cream in promoting the healing of muscle contusions. In this study, BPC-157 more rapidly and effectively stimulated early collagen organization, expression of the egr-1 gene, and the repressor nerve growth factor 1-A binding protein-2 (nab2). BPC-157 exerts a protective measure by preventing activation of the transcription factor (erg-1). This regulatory signal from nab2, a negative transcriptional cofactor, interrupts the release of potent proinflammatory and prothrombic mediators. Hence, BPC-157 serves as a great therapeutic means for accelerated post-injury healing in external and internal wounds as well as restoring complete function of injured muscles [73].

In addition to soft tissue regeneration, BPC-157 has been closely implicated in the recovery of blood flow to rat ischemic muscle by stimulating angiogenesis. This was proven by peptide induced expression of vascular endothelial growth factor receptor 2 (VEGFR2) and activation of the VEGFR2-Akt-eNOS signaling pathway [74].

Currently, researchers are trying to elucidate the potential mechanism by which BPC-157 stimulates outgrowth and proliferation of fibroblasts. Chang et al. suggests that BPC-157, in a dose dependent manner, induced migration and new growth of tendon fibroblasts from tendon explants mediated by activation of the Focal Adhesion Kinase (FAK)-paxillin pathway. Along with migration, an actin dependent process, BPC-157 has been shown to stimulate F-actin microfilament. Under oxidative stress induction via hydrogen peroxide, cells treated with BPC-157 had markedly increased survival. However, BPC-157 did not exert direct effects on the proliferation of fibroblasts derived from rat Achilles tendon. This was in part due to the *in vitro* experimental condition not being replicated within the *in vivo* environment of the tendon. It was concluded that BPC-157 interacts with other cells *in vivo*, such as leukocytes and stems cells, to communicate with each other and employ an alternative method for promoting tendon fibroblast reproduction [75].

In a later study Chang et al., found that BPC-157 induced the expression of the growth hormone receptor and was notably one of the most abundantly up-regulated genes in tendon fibroblasts isolated from the rat Achilles tendon. These researchers theorized that upregulation of growth hormone receptor from BPC-157 contributes to healing of the injured tendon [76]. Alternatively, Sikiric conducted a study comparing soft tissues induced by BPC-157, investigating the natural healing response of the NO system to injury. In various models with compromised vascular integrity, BPC-157 exhibited a specific role in either reducing thrombosis or mitigating bleeding induced by thrombocytopenia. BPC-157 consistently demonstrated the ability to enhance healing in severe injuries where various soft tissue types were inherently unable to heal on their own [77].

BPC-157 is presently in its initial phases of clinical development, and most studies have been carried out using small animal models. Additionally, studies have not documented adverse associations with this drug, possibly arising from a limited comprehension of its biochemical mechanisms. Another limitation is that only a small number of researchers have investigated this peptide's effects. Although the gastric pentadecapeptide's mechanism is not clearly understood, potential actions have been indicated involving NO generation, the FAK-paxillin pathway, Vascular Endothelial growth factor (VEGF), and upregulation of the growth hormone receptor [74-77]. Additional research is necessary to elucidate the exact mechanism and efficacy of this drug, along with the successful completion of human trials before clinical translation can occur [66]. In contrast to the tumor-promoting effects observed with numerous growth factors and other peptides, BPC-157 has demonstrated the ability to inhibit and counteract the elevated expression of VEGF and its associated signaling pathways, effectively preventing VEGF induced tumorigenesis [78,79]. Investigations into this medication have unveiled promising regenerative effects on diverse soft tissue injuries. These studies strongly indicate that this drug might serve as a minimally invasive method for regenerative therapy in

hypovascular and hypocellular soft tissues, including tendons and ligaments [66].

GHK-Cu

The human peptide GHK (glycyl-l-histidyl-l-lysine) is a naturally occurring peptide found in human serum, saliva, and urine implicated in the acceleration of wound healing and skin repair [80]. Combined with its Cu^{2+} chelate, GHK-Cu naturally stimulates blood vessel and nerve outgrowth, increases collagen synthesis, and supports the function of fibroblasts [81]. By using intra-articular injections, these properties may contribute to the maintenance and repair of joint tissues.

GHK-Cu tripeptide is released naturally from human tissues in response to injury, with average serum levels of 200ng/mL at age 20, decreasing to 80ng/mL by the age of 60 [82]. With this decline in GHK-Cu level comes the noticeable decrease in the regenerative capacity of an organism. To date, a series of animal experiments have established this peptide's wound healing activity, anti-inflammatory, and tissue remodeling properties [83,84]. Emerging research also suggests that this peptide possesses powerful cell protective actions, such as synthesis of collagen and systemic wound healing in rats, mice, and pigs [80]. Thus, this review considers GHK's role and mechanism of action in protection with regenerative properties in humans, and employs the use of current clinical and animal studies to evaluate the safety and efficacy of GHK-Cu usage via intra-articular injections.

In early experiments in the 1970s, addition of plasma from younger individuals to liver tissue obtained from older individuals resulted in production of proteins from aged liver tissue that more closely resembled those typically found in younger individuals [81,85,86]. Pickart et al. traced this effect to a growth modulating peptide present in human plasma—GHK [85]. Pickart et al. further demonstrated that GHK-Cu accelerated the process of wound healing and contraction, enhanced the acceptance of transplanted tissue, and exhibited anti-inflammatory effects [87-89]. Later studies conducted by Borel and Maquart et al. also showed that GHK-Cu stimulated synthesis of collagen and glycosaminoglycans [90]. The mechanism of action thought to be behind these protective and regenerative effects was linked to the ability of the GHK peptide to chelate copper.

Pronounced wound healing properties of GHK-Cu were confirmed in multiple animal studies. Gul and Cangul et al. found that topical administration of GHK to experimental full thickness rabbit wounds in the dorsal midline facilitated improved wound contraction and formation of granulation tissue [83,84]. Filling of the open wound with granulation tissue, as well as time to first observed granulation was faster in the group treated with this tripeptide [83]. Treatment with GHK-Cu also resulted in a significantly smaller mean unhealed wound area [84]. Histopathologically, this tripeptide treatment also resulted in decreased neutrophil counts and an increase in neovascularization [83]. The results of these experiments in tandem suggest an increased activity of antioxidant enzymes, stimulation of angiogenesis, and improved wound healing and granulation in the context of GHK-Cu treatment.

GHK-Cu also promotes a systemic improvement in healing across rats, mice, and pig models. Specifically, when this peptide was administered in one region of the body, such as the thigh muscles, it also resulted in enhanced tissue healing at distant areas. Additionally, intraperitoneal injections of GHK-Cu healed tubular bone fractures. These interventions notably elevated key healing indicators, including collagen production, angiogenesis, and wound closure, observed in both wound chambers and full thickness wounds [91]. In the study by Canapp Jr. et al., when full-thickness wounds created in an ischemic skin flap on the backs of rats were treated daily with topical GHK-Cu, these researchers found that the initial wound area had decreased significantly compared to an untreated control group. Furthermore, wounds treated with this tripeptide exhibited reduced levels of TNF-α, MMP-2, and MMP-9 [92]. These findings suggest that GHK-Cu treatment fosters an accelerated wound healing environment and exhibited anti-inflammatory effects in ischemic open wounds.

While the aforementioned studies underscore the tissue remodeling stimulating effects of topical treatment of GHK-Cu, there exist wound healing studies in mice that investigate the intra-articular injection route for administration of this tripeptide. Sai-Chuen Fu et al. explored the biological healing role of GHK-Cu intra-articular injections after anterior cruciate ligament reconstruction (ACLR) in mice. They found that intra-articular supplementation with 0.3 mg/mL of GHK-Cu improved graft healing and enhanced outcomes following ACLR. In post-ACLR mice, GHK-Cu injections promoted a significant decrease in knee laxity and higher graft complex stiffness as compared to saline injections. However, these beneficial effects were transient and did not persist following the discontinuation of GHK-Cu injection treatments. Additionally, GHK-Cu treatment did not significantly improve the ultimate load to failure or gait parameters, suggesting possible limitations to the intra-articular use of this tripeptide treatment [93].

The incorporation of biotynlated GHK-Cu into collagen biomaterial is another viable method of administration. In diabetic rats treated with GHK-Cu incorporated collagen, the soft tissue healing process was accelerated with an increased rate of wound contraction. Treatment with GHK-Cu promoted elevated levels of glutathione (GSH) and ascorbic acid, improved epithelialization, and

enhanced synthesis of collagen. Additionally, there was activation of fibroblasts and mast cells in these treated wounds. In healthy non-diabetic rats, the application of GHK-Cu incorporated collagen into wounds resulted in a nine-fold increase in collagen production [94]. However, one limitation of GHK-Cu use as a peptide therapy is its high susceptibility to degradation by carboxypeptidase enzymes. Wounds such as diabetic skin ulcers or pressure sores often produce a "wound serum," believed to be created by airborne bacteria settling on the wound [81]. This serum has been implicated in the breakdown of GHK-Cu, and is an important factor to consider when discussing its viability as a peptide therapy for wound healing.

While extensive research spanning nearly 4 decades has been conducted on the tissue remodeling and wound healing properties of GHK-Cu in animal models, there exists very few large-scale clinical studies regarding the tripeptide's effects in humans. Currently, GHK-Cu is included in topical formulations designed for skin protection, advisable post-procedures such as plastic surgery or similar interventions, and was submitted with sufficient supporting information to the FDA for approval in September 2023 [81]. Recently however, injectable GHK-Cu was placed on a list of "Bulk Drug Substances that Raise Significant Safety Risks" by the FDA because of a high risk for immune reactions and impurities during the compounding process, stalling its approval process [95]. Yet, the product remains available in topical skincare products.

Despite this setback, the potential of GHK-Cu to accelerate wound healing and regeneration, while exhibiting significant antioxidant and anti-inflammatory effects both *in vitro* and *in vivo*, warrants the continued investigation of its use in interventional routes of administration, such as intra-articularly or through peptide-incorporated collagen. This tripeptide has profound effects in tissue regeneration, angiogenesis, and graft healing, ie, the ability of GHK-Cu therapy to accelerate these protective effects has the potential to greatly improve clinical outcomes in soft tissue regeneration.

OP3-4 and WP9QY

A set of novel receptor activator of nuclear factor kappa-B ligand (RANKL) binding peptides, OP3-4 and W9, have recently been implicated for both soft and hard tissue regeneration, namely the prevention of degeneration of cartilage and the acceleration of bone formation in osteoarthritis [96-98]. RANKL is an important cytokine for bone resorption and an inhibitor of osteoclastogenesis. The binding of RANKL to its receptor RANK leads to increased osteoclast differentiation and maturation, promoting bone resorption [99]. The catabolic effects of RANKL are prevented by osteoprotegerin (OPG), an inhibitory factor that binds to RANKL and prevents activation of its cognate receptor [100]. Current therapies to increase bone mineral density (BMD) in osteopenic patients involve the administration of anti-RANKL antibodies; however, this treatment can result in the reduction of bone formation, owing to their powerful inhibition of bone resorption [96]. Thus, other treatment avenues or methods of inhibiting RANKL may pose more beneficial health outcomes.

In 2015, Kato et al. designed and crafted the peptide OP3-4 (YCEIEFCYLIR), a cyclic peptide that structurally mimics osteoprotegerin (OPG). These researchers demonstrated that OP3-4 binds to RANKL with high affinity, resulting in inhibition of osteoclastogenesis and bone resorption. While these effects are well-noticed in bone regeneration, soft tissue regenerative effects have also been studied in OP3-4. This peptide has been linked to cartilage preservation and inhibition of cartilage degradation [96]. Similarly, the WP9QY peptide (W9) upon binding to RANKL, also blocks RANKL-induced increases in bone resorption and osteoclastogenesis. W9 is thought to have its unique effect on the coupling mechanism between osteoclasts and osteoblasts, resulting in the promotion of bone formation while simultaneously suppressing bone resorption [101]. This portion of the current review seeks to evaluate the effects of the administration of these two novel peptides, OP3-4 and W9 on cartilage and bone regeneration by the promotion of healing in the context of inflammatory osteoarthritis or fracture treatment.

Denosumab is a monoclonal antibody designed to broadly inhibit RANKL and prevent osteoclast-mediated bone resorption [102]. OP3-4 and WP9QY, unlike Denosumab, are synthetic peptides that mimic the natural function of osteoprotegerin, providing a more targeted inhibition of RANKL and offering additional therapeutic benefits [96]. Specifically, these peptides not only inhibit bone resorption but also promote cartilage preservation and bone formation, particularly in osteoarthritis models [96]. This dual action differentiates them from Denosumab, which primarily focuses on reducing bone loss [96,102].

The use of OP3-4 in mouse models has demonstrated an ability to prevent articular cartilage deterioration and subchondral bone loss and destruction. Oral treatment with the peptide OP3-4 significantly inhibited cartilage degradation in a dose-dependent manner in mice with collagen induced arthritis, resulting in proliferation of cartilage cell lines *in vitro* [96]. In subsequent studies, OP3-4 was also found to promote vascularization, thereby accelerating fracture healing. In a rat femoral defect model, this cyclic peptide treatment inhibited osteoclast activation, promoted vascularization, and increased deposition of type I collagen [103].

Concurrent OP3-4 use with W9 peptide in the form

of injections into the intra-articular space of the knee joints of mice with monosodium-iodoacetate (MIA)-induced osteoarthritis has also implicated these two RANKL-binding peptides in the prevention of cartilage degeneration.

Histomorphometric analyses showed that both peptides effectively prevented degradation of cartilage. This suggests that these two peptides, upon binding to RANKL may stimulate mesenchymal cell proliferation, potentially leading to the prevention of cartilage degeneration [97].

While still in its infancy, the use of the RANKL-binding peptides OP3-4 and W9 presents a promising form of therapy for increased bone formation at periarticular sites, decreased bone resorption, and providing protective effects against cartilage destruction in animal models. These two peptides, which are designed analogs to the body's natural OPG, could present as lead peptide therapy candidates for treatment of joint conditions such as rheumatoid arthritis, while contributing to the inhibition of disease progression in osteoarthritis [97]. These findings necessitate further studies involving OP3-4 and W9, which could serve as a useful template for the development of peptide therapy against inflammatory or mechanical soft tissue degeneration.

Thymosin β4

Thymosin β4 (Tβ4) is a ubiquitously distributed peptide, found in all body fluids and cells except red blood cells [104]. Its highest concentration is in platelets, the initial cells to arrive at an injury site, and subsequently in white blood cells, elucidating its presence in wound fluids [104,105]. Comprised of 43 amino acids, its low molecular weight enables rapid migration to the body's healing sites [106]. Its biological activity is determined by encoded gene fragments which overall regulate activities via anti-inflammatory and antifibrotic properties, toxicity reduction, along with promoting angiogenesis [104]. As such, Tβ4 has shown promise for soft tissue repair and regeneration [104,105].

Tβ4, a member of the actin monomer-sequestering protein forms complexes with monomeric actin and exhibits the capacity to bind to F-actin at increased concentrations. This peptide plays a role in maintaining the cytoplasmic pool of actin monomers essential for rapid filament elongation. Tβ4 is predominantly present in actively dividing cells and embryonic skeletal muscle [107].

Tβ4's interaction with actin and its role in enhancing cell migration, including the facilitation of mobilization, migration, and differentiation of stem progenitor cells, contributes to the formation of new blood vessels for tissue regeneration. Tβ4 exerts its angiogenic properties by recruitment of stem cells and increasing the expression of VEGF [106].

In different stages of wound repair in various tissues, Tβ4 plays an important part in recruitment and differentiation of progenitor stem cells into mature cells, downregulation of inflammatory cytokines, decreasing the number of fibroblasts in wounds, which ultimately results in reduced scar formation and fibrosis [108-118]. Similarly, Tβ4 has shown therapeutic benefit in preventing scar formation in various animal models [105]. The mechanism of Tβ4 seems to be related to a reduction in the inflammatory response, including a decrease in macrophage infiltration, which reduces swelling and thus allow for normal repair cells to be more effective [104,106]. The amino N terminal end of Tβ4 appears to contain the majority of antifibrotic activity and has shown success in many animal models of fibrosis [104]. In addition, Tβ4 organizes the alignment and distribution of collagen, as well [106].

In another study, Tβ4 was used to alter articular cartilage chondrocyte matrix constituents and metalloproteinase (MMP) expression. Studies have demonstrated that Tβ4 can modulate the mechanoregulation of MMPs. The ability of this actin-sequestering peptide to reorganize and disassemble skeletal F-actin suggests its involvement in promoting mechanically induced MMP synthesis in cartilage. In one of the many complex pathways for collagen matrix turnover, it was shown that Tβ4 influences MMP expression, likely playing a role in collagen replacement [119].

Tβ4 has many regenerative properties but research involving tendon, ligament, or cartilage restoration is limited. Tβ4 acts on many cell types and has numerous different biological effects [106]. In most studies performed, researchers are using direct implantation of Tβ4 locally into various tissues [104]. Moreover, Tβ4's mechanism has not been definitively elucidated and its role in cancer cannot be determined [120], with some studies indicating this peptide possibly exerting a tumor suppressive effect, induced apoptotic activity of malignant cells, and activation of inhibitory signaling pathways [120-123]. Other researchers have found elevated Tβ4 levels in cancer cell lines [124]. Tβ4 has demonstrated considerable potential with its diverse tissue regenerative effects. In the context of this paper, further studies are necessary to investigate this peptide's influence on tissue regeneration and remodeling in cartilage, tendon, and ligament, as well as its route of entry besides localized injections.

DISCUSSION

Soft tissue engineering is an expanding field within regenerative biomedicine. Peptides, over the last decade, have found diverse applications in medicine and biotechnology, presenting numerous opportunities for research and clinical therapeutics. Table 2 shows the breakdown of peptide characteristics discussed in this review (Table 2).

Table 2. Peptide Characteristics

Peptide	Animal vs Human Studies	Proposed Effect	Currently in Market	Possible Adverse Effects
Collagen-2	Human	Cartilage protection	Yes (Oral)	Nausea, heartburn, diarrhea, constipation, drowsiness, skin reactions, and headache.
Collagen Hydrolysate	Human	Cartilage protection, increases cartilage thickness, and antioxidant properties	Yes (Oral and Injectable)	Nausea, heartburn, diarrhea, constipation, drowsiness, skin reactions, and headache.
BPC-157	Animal	Activation of FAK-paxillin pathway, growth hormone receptor expression, and promotes NO generation	No	None reported
GHK-Cu	Animal and Human	Wound healing, blood vessel and nerve growth, and supports fibroblasts	Yes (Topical)	Immune reactions
OP3-4	Animal	Binds RANKL	No	None reported
WP9QY	Animal	Blocks RANKL	No	None reported
Thymosin-β4	Human	Angiogenesis through VEGF, stem cell maturation, and MMP modulation	Yes (Not FDA approved for musculoskeletal repair)	Redness/pain at site of injection

FAK, focal adhesion kinase; NO, nitric oxide; RANKL, receptor activator of nuclear factor kappa-B ligand; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase

Although collagen-2 has been extensively researched, it can only be administered orally due to its large size. Despite not having a local option for delivery, clinical improvements have been repeatedly shown in conditions such as osteoarthritis and rheumatoid arthritis after supplementation with oral collagen-2 [33-35,38]. A shift in focus has been made towards newly developed peptides; however, recent efforts by scientists have tried to explore the effect that collagen-2 supplementation may have on tendon and ligament health [40]. Regardless, oral administration of collagen-2 has been an effective method of systemic soft tissue repair for years. Conversely, HC is a much smaller peptide that can be more effectively absorbed [42]. Offering both an oral and intra-articular RoA, this peptide confers the same clinical benefit to its natural counterpart [56,64]. Collagen-2 and HC share similar mechanisms of being incorporated into soft tissues, but a difference in size and absorption allows for greater utility with HC.

BPC-157 is a stable gastric pentadecapeptide that is synthetically derived [65,66]. It can be administered orally, topically, and via intraperitoneal routes, demonstrating significant potential for the healing of various tissues, including tendons, ligaments, skeletal muscle, and bone [66]. While the precise mechanism of this gastric pentadecapeptide remains unclear, potential actions have been suggested, including NO generation, involvement in the FAK-paxillin pathway, modulation of VEGF, and upregulation of the growth hormone receptor [74-77]. Due to the necessity for additional clinical trials and the predominant use of small animal models in existing studies, the FDA has not granted approval for medical treatment currently [67,68,70,73,125]. This drug presents itself with promising potential for minimally invasive regenerative therapy in a diverse range of soft tissues.

GHK-Cu, a natural peptide found in human tissues, shows promise for wounds and soft tissue repair, with animal studies suggesting its potential for intra-articular treatment of joint tissues and locally for wound healing [80]. Despite natural decline in the body with age, GHK-Cu has demonstrated anti-inflammatory effects and collagen synthesis stimulation in animal studies. This peptide is versatile since it has various RoA. Its topical use accelerates wound healing, intra-articular injections improve graft healing post-surgery in mice, and incorporating GHK-Cu into collagen enhances wound contraction in diabetic rats [83,84]. Limited clinical studies exist, but GHK-Cu is currently primarily used in topical formulations for skin protection and post-procedure wound care, with FDA submission for approval [81]. Further research into this peptide's mechanisms and clinical applications, including intra-articular and collagen-based delivery, is

warranted for soft tissue repair and regeneration.

The RANKL-binding peptides OP3-4 and W9 show promise for both soft and hard tissue regeneration, offering potential in preventing cartilage degeneration and promoting bone formation in osteoarthritis [96-98]. OP3-4, resembling naturally existing OPG, effectively binds to RANKL, inhibiting osteoclastogenesis and preserving cartilage in animal models [97]. Similarly, W9 peptide also blocks RANKL-induced bone resorption while promoting bone formation [96]. Scientific studies have demonstrated the efficacy of OP3-4 and W9 in preventing cartilage degradation and promoting vascularization in animal models, suggesting therapeutic avenues for rheumatoid arthritis and osteoarthritis [98]. Further clinical research into these two peptides holds promise for peptide therapy to counteract soft tissue degeneration.

Tβ4 is a peptide that exhibits versatility and widespread distribution throughout the body with the highest concentration observed in platelets [104,105]. Ongoing research primarily focuses on experimental or clinical trials related to conditions such as myocardial infarction, myocardial-ischemia-reperfusion injury, xerophthalmia, liver or renal fibrosis, ulcerative colitis, colon cancer, and skin trauma [104]. In many conducted studies, this peptide is administered through direct implantation of Tβ4 into various tissues and has displayed significant potential due to its diverse effects on tissue regeneration. This peptide is FDA approved for other clinical uses, but not for musculoskeletal repair and remodeling in cartilage, tendons, and ligaments. In the context of this paper, additional research on Tβ4 is required to explore alternative RoA beyond localized injections to specifically target the subset of soft tissues under consideration.

CONCLUSION

Peptide therapeutics is a promising facet of regenerative medicine that seeks to promote a minimally invasive method for treating soft tissue degeneration. This review delineates different peptide therapies with an array of effects for repair and regeneration of soft tissues on both local and systemic levels. Further high-quality *in vitro* and *in vivo* research, including randomized clinical trials is needed to gain deeper insights into the safety and efficacy of these agents. The findings of such scientific studies will be crucial for equipping physicians with effective therapeutics to optimize regenerative medicine in the context of minimally invasive healthcare.

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