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IDENTIFICATION OF ANTI-OSTEOARTHRITIS PEPTIDES FROM BOVINE CARTILAGE VIA VIRTUAL SCREENING, MOLECULAR DOCKING, AND CELL MODELS

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I. INTRODUCTION

Bovine cartilage, a valuable by-product accounting for approximately 5-10% of total bovine bone weight, represents a promising source for type II collagen (CII) extraction. As the predominant structural protein in articular cartilage, CII plays a vital role in maintaining chondrocyte function and preserving extracellular matrix integrity[1]. Efficient utilization of animal by-products such as bovine cartilage aligns with the growing interest in sustainable biomedical resource development. Osteoarthritis (OA), the most prevalent degenerative joint disease, has been recognized as the leading cause of disability worldwide[2]. According to the Global Burden of Disease (GBD) 2021 report, an estimated 595 million people are affected by OA globally, with incidence rates reaching as high as 85% among individuals over the age of 70[3]. Given its pathogenesis, effective OA management requires strategies that both suppress inflammation and promote cartilage repair. In our previous study, type II collagen hydrolysate was successfully prepared from bovine cartilage. The present work aims to further develop this by-product by screening its hydrolysates to identify type II collagen-derived peptides (CIIPs) with both anti-inflammatory activity and the ability to promote chondrocyte proliferation. This study provides new insight into the high-value utilization of bovine cartilage and proposes a novel peptide-based approach for the prevention and management of osteoarthritis.

II. MATERIALS AND METHODS

Virtual screening. Peptide bioactivity was predicted using the PeptideRanker online tool (http://distilldeep.ucd.ie/PeptideRanker/). Anti-inflammatory potential was further assessed using AIPpred (http://www.thegleelab.org/AIPpred/).

Molecular docking. Molecular docking was performed using AutoDock 4.2 with the epidermal growth factor receptor (EGFR, PDB ID: 1IVO) as the receptor. Water and ligands were removed, and docking was conducted at the original EGF binding site. Peptide structures were generated and minimized automatically before docking.

Cell Culture and Peptide Treatment. RAW264.7 cells were cultured in high-glucose DMEM with 10% FBS at 37 °C and 5% CO_2. Cells (5×10^5 cells/mL) were seeded in 96-well plates (100 µL/well) and allowed to adhere for 12 h. After removing the medium, control and model groups received 90 µL serum-free medium, while treatment groups received 90 µL of CIIP-containing medium (100 µg/mL) for 2 h. Lipopolysaccharide (LPS, 10 µL, final concentration 10 ng/mL) was added to the model and treatment groups with the control group receiving 100 µL serum-free medium. Each group was tested in quintuplicate. After 6 h, 50 µL of supernatant was collected and mixed with 50 µL of Griess Reagent I and II with absorbance being measured at 540 nm. Human articular chondrocytes (HACs) were seeded similarly and treated with diluted CIIP hydrolysate (100 µg/mL) for 24 h. Cell viability was assessed using the CCK-8 assay. Absorbance was measured at 450 nm.

III. RESULTS AND DISCUSSION

A total of 4,932 peptides were identified through polypeptidomic analysis, among which 177 high-confidence peptides were selected based on a De novo score > 95%. The bioactivity of these peptides was predicted using the PeptideRanker platform, and 18 peptides with predicted scores greater than 0.5 were retained (Table 1). To further assess their anti-inflammatory potential, AIPpred was used, with peptides scoring above 0.4 considered likely to possess anti-inflammatory activity (AIPs). Based on both confidence levels and predicted bioactivity, 7 candidate peptides were selected for molecular docking studies. Considering the critical role of EGFR in regulating cartilage matrix synthesis, chondrocyte proliferation, and cartilage regeneration via RAS-MAPK and PI3K/Akt pathways, EGFR was chosen as the target for docking analysis. Although EGFR has been recognized as a promising therapeutic target in cartilage repair, the involvement of collagen peptides in modulating EGFR signaling has not been previously explored. The 7 selected peptides were docked with the EGFR–EGF complex (PDB ID: 1IVO), and binding affinities were assessed based on docking energies (Figure 2). A binding energy below −8.0 kcal/mol was considered indicative of strong binding affinity[4]. Molecular docking results revealed that all peptides formed multiple hydrogen bonds with EGFR, suggesting high interaction potential. Subsequently, 6 peptides with favorable docking

scores were chemically synthesized for *in vitro* validation. Their anti-osteoarthritis activities were evaluated using the RAW264.7 macrophage inflammation model and a chondrocyte proliferation model. Among the tested peptides, GHRGF, SFYGR, and AAWLGDQM exhibited the most promising effects, significantly inhibiting inflammatory cytokine secretion while promoting chondrocyte viability.

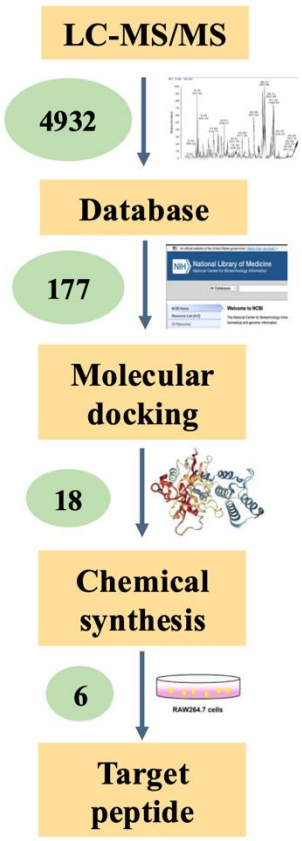


Figure 1 – Screening process of anti-osteoarthritis peptides.

Table 1 – Peptide activity prediction.

Peptide	PeptideRanker Response	AI score	AIP
GHRGF	0.876459	0.4047	AIP
SFYGR	0.85893	0.4093	AIP
LLKMF	0.827769	0.5628	AIP
WTPAPR	0.820973	0.4209	AIP
LDDAAKKAKDLGFKM	0.787257	0.3163	Non-AIP
YLDDAAKKAKDLGFKM	0.762895	0.5302	AIP
FSKAAFG	0.728707	0.3698	Non-AIP
FDSQWHK	0.69745	0.3791	Non-AIP
FYQTFKR	0.696817	0.3837	Non-AIP
AAWLGDQM	0.650426	0.5279	AIP
VTASHFFR	0.620758	0.5116	AIP
AAPHLRK	0.599814	0.3651	Non-AIP
APLRAP	0.587367	0.4605	AIP
YDMLH	0.562948	0.3814	Non-AIP
FYQTFKRN	0.560219	0.4047	AIP
LYGKDF	0.539602	0.3767	Non-AIP
PSSSFKDAK	0.513817	0.4395	AIP
VSPRPR	0.511448	0.507	AIP

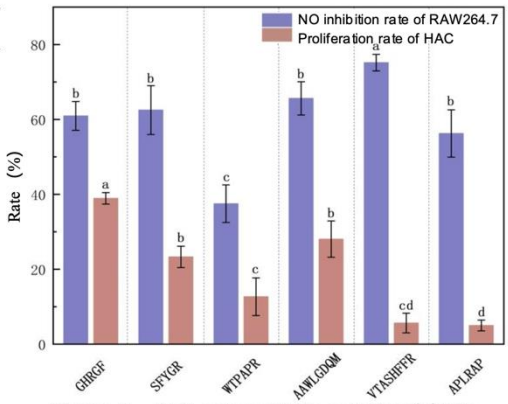
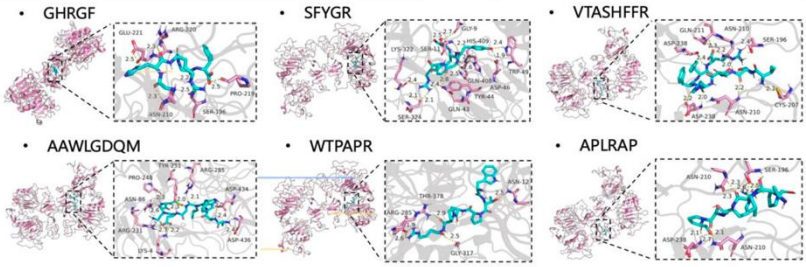


Figure 3 – Anti-osteoarthritis activity of CIIPs.



Peptide	Binding energy	Hydrogen bonds formed site
GHRGF	-8.5	GLU-221, ARG-220, ASN-210, PRO-219, SER-196
SFYGR	-8.8	LYS-322, SER-11, GLY-9, HIS-409, TRP-49, ASP-46, GLN-408, TYR-44, GLN-43, SER-324
AAWLGDQM	-9.6	PRO-248, TYR-251, ARG-285, ASP-434, ASN-86, ARG-231, ASP-436, LYS-4
WTPAPR	-8.6	ARG-285, THR-378, ASN-12, GLY-317
APLRAP	-8.0	ASN-210, SER-196, SAP-238, ASN-210
VTASHFFR	-8.8	GLN-211, ASN-210, SER-196, ASP-238, ASN-210, GYS-207

Figure 2 – Prediction of docking binding sites and minimum binding energy.

IV. CONCLUSION

In this study, 6 type II collagen peptides (CIIPs) derived from bovine cartilage were identified with potential therapeutic effects against osteoarthritis, which effectively inhibited inflammatory cytokine secretion and promoted chondrocyte proliferation. Notably, GHRGF, SFYGR, and AAWLGDQM demonstrated the most favorable bioactivities. These findings highlight the value of animal by-product-derived bioactive peptides and suggest a novel strategy for osteoarthritis management targeting both inflammation and cartilage regeneration.

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