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Potential of *Bombyx mori* fibroin peptide as an inhibitor of BMP2 and TGFB1 in the treatment of pulp tissue damage

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ABSTRACT

Background: *Silk from Bombyx mori, a species of silkworm, contains fibroin, which has good biocompatibility and is potentially suitable for medical applications, especially in the treatment of tissue damage.* ***Purpose:*** *This study evaluated the potential interaction of B. mori fibroin peptides with bone morphogenetic protein 2 (BMP2) and transforming growth factor beta 1 (TGFB1), which are protein markers for dentine reparative activity.* ***Methods:*** *The research was carried out in silico. The three-dimensional structure of the proteins was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB), and the antimicrobial potential of fibroin was evaluated using Antimicrobial Peptide Scanner v.1, the Collection of Anti-Microbial Peptides (CAMPR3), PeptideRanker, ToxinPred, AlgPred, and AllergenFP. Molecular modeling and analysis were performed with trRosetta, PrankWeb, the HDOCK server, and Discovery Studio.* ***Results:*** *The light chain 1 peptide (LC1), light chain 2 peptide (LC2), heavy chain 2 (HC2), and heavy chain 7 (HC7) showed high binding affinity to BMP2, while LC2, HC1, HC3, and HC6 showed high binding affinity to TGFB1 compared to silicic acid as a standard anti-inflammatory drug.* ***Conclusion:*** *These seven peptides can potentially interact with BMP2 or TGFB1 and might have anti-inflammatory capability.*

Keywords: *anti-inflammatory agent; bone morphogenetic protein 2; Bombyx mori; medicine; transforming growth factor beta 1*
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INTRODUCTION

In the ever-developing medical field, recent research has revealed the potential efficacy of natural ingredients for treating pulp tissue damage and reducing inflammation. Fibroin, the main protein in silk, has good biocompatibility and is highly suitable for various medical applications, especially in the treatment of tissue damage.[1] Fibroin, found in silk, has been a major focus of researchers because of its promising ability to accelerate healing and restore the normal function of dental pulp tissue.[2,3]

Furthermore, the importance of fibroin from the silkworm *Bombyx mori* in the treatment of tissue damage lies not only in its biocompatibility but also in its unique structure. Fibroin forms a series of fibers that are strong yet light, providing a solid base for new tissue formation.[4] It can also stimulate the growth of regenerative cells,[5] significantly speeding up the healing process[6] and potentially reducing the inflammatory response. Inflammation is often the cause of pain and can hinder healing. In a previous report, *B. mori* silk fibroin enzymatic hydrolysate demonstrated inflammation-reducing properties by decreasing interleukin-1β (IL-1β), IL-6, and tumor necrosis factor alpha cytokines.[6,7]

According to studies on dental pulp tissue, inflammation is one of the major challenges in overcoming pulp tissue damage. An important signaling network involved is the transforming growth factor beta (TGFB) and bone morphogenetic protein (BMP) pathways. These pathways play a crucial role in osteoblasts, which regulate embryonic skeletal growth and postnatal bone homeostasis.[8] The main proteins related to this pathway are bone morphogenetic protein 2 (BMP2) and transforming growth factor beta

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1 (TGFB1), which are receptor proteins upstream of the pathway and initiate the Smad-related cascade.[9] Both pathways are associated with the biological process of inflammation: the TGFB pathway functions as an immunomodulator that can act alternately as pro- or anti-inflammatory, while the BMP pathway tends to act as pro-inflammatory.[10,11] Based on these considerations, evaluating the potential inhibition of TGFB1 and BMP2 by fibroin may help close the screening gap for further anti-inflammatory drug development.

Moreover, bioinformatics analysis has been widely used to explore molecular pathways because it provides deep insights into the complexity of molecular interactions in cells and organisms. This analysis greatly accelerates exploration and research by generating baseline data.[12] Other advantages of this method include analyzing multi-omics data and global data on proteins and gene function,[13] as well as identifying biomarkers,[14] therapeutic targets,[15] bioactivity properties,[16] and differential gene expression.[17] Based on these advantages, the exploration of the potential applications of fibroin is still ongoing through various methods, including bioinformatics.[18]

Therefore, in this study, the potential inhibition of *B. mori* fragments on the two main proteins of these pathways, the TGFB and BMP receptors, will be evaluated using in silico methods, as these proteins are markers of dentine reparative activity.[19–21]

MATERIALS AND METHODS

This research was conducted from January to July 2024 in the Laboratory of Technology Pharmacy, Universitas Muhammadiyah Yogyakarta, and the Laboratory of Pharmacology and Clinical Pharmacy, Padjadjaran University. The amino acid sequence of the *B. mori* fibroin protein was obtained from the UniProt database (https://www.uniprot.org/). The fibroin protein from *B. mori* consists of two chains: the light chain (LC) (ID: P21828) and the heavy chain (HC) (ID: P05790). Bioactive peptide segmentation, with a length limitation of 20 amino acids, was performed using the Antimicrobial Peptide Scanner v.1 server (https://www.dveltri.com/ascan/v1/)[22]

The CAMPR3 database (http://www.camp3.bicnirrh.res.in/) was used to predict amino acid sequence regions with potential as antimicrobial peptides using three prediction methods: Support Vector Machines (SVMs), Random Forest, and discriminant.[23] The PeptideRanker database (http://distilldeep.ucd.ie/PeptideRanker/) was used to predict the To-Be-Active score of each mined peptide.[24] The ToxinPred database (https://webs.iiitd.edu.in/raghava/toxinpred/ index.html) was used to predict toxic peptides.[25] The AlgPred database (https://webs.iiitd.edu.in/raghava/algpred/submission.html) was used to predict the potential of proteins as allergens using hybrid methods (Immunoglobulin E [IgE] epitope+ allergen representative peptides [ARPs], The Basic Local Alignment Search Tool [BLAST+] motif alignment and search tools [MAST], and SVM classifier).[26] The AllergenFP database v.1.0 (http://www.ddg-pharmfac.net/ AllergenFP/) was used to predict the potential of proteins as allergens using the auto-cross covariance (ACC) method.[27]

The three-dimensional (3D) construction of the peptide structure was built using the trRosetta webserver (https://yanglab.nankai.edu.cn/trRosetta/), which applies a deep neural network (NN) approach.[28] The 3D structures of the selected target proteins were obtained from the RCSB PDB database (https://www.rcsb.org/): BMP2 (PDB ID: 1REW)[29] and TGFB1 (PDB ID: 5FFO).[30] The proteins were prepared by removing water molecules in the Discovery Studio 2021 software. Docking was performed using the HDOCK server (http://hdock.phys.hust.edu.cn/).[31] Docking was carried out between the fibroin peptide and salicylic acid as ligands, and BMP2 and TGFB1 as receptors, using the targeted docking method. The grid box size was adjusted to the position of amino acid residues based on literature and predictions from the PrankWeb server (https://prankweb.cz/). The docking results included docking scores and interacting residues. The interactions between the compounds and the docked proteins were then visualized using Biovia Discovery Studio 2021 software.

RESULTS

Peptide segmentation of the LC proteins produced 243 bioactive peptides with a sequence length of 20 aa. The optimal peptide length ranges from 10 to 51 aa.[32] Peptide segmentation of the HC proteins produced 5,244 bioactive peptides with a sequence length of 20 aa. Most peptides derived from both the light and HC segments belong to the non-antimicrobial peptide group. The data are provided in the supplementary file.

The results of evaluating the bioactivity of fibroin peptides are presented below. The selection and screening of bioactive peptides began with the prediction of the peptide bioactivity score using the PeptideRanker server, with a minimum score threshold of 0.7. The first screening successfully selected 15 bioactive peptides from the LC and 333 bioactive peptides from the HC. Each amino acid sequence was then predicted for its potential as a toxin or allergen. The analysis showed that almost all peptides had no toxic potential, except for one peptide sequence from the HC, which was predicted to be toxic. Additionally, six of the 15 LC peptides and all HC peptides were predicted to have allergenic potential using the SVMs machine learning method. Consequently, the bioactivity score threshold was increased to 0.8, selecting three LC bioactive peptides and nine HC-derived peptide sequences (Table 1). The bioactivity probability score ranges from 0 to 1, with values closer to 1 indicating stronger predicted bioactivity. The server's standard threshold ranges from 0.5 in general to 0.8 for short amino acid sequences.[24] A validation of allergen predictions using another machine learning method, ACC,

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Table 1. Selected bioactive peptides from the light chain and heavy chain of *Bombyx mori* fibroin protein

Source	Position	Sequence	CAMPR3	Bioactivity Probability Score	Toxicity Prediction	Allergen		
						IgE Epitope + ARPs BLAST + MAST	SVMc	ACC
LC1	154-173	VGPALGCAGGGRIYDFEAAW	AMP	0.860693	nontoxic	nonallergenic	nonallergenic	allergenic
LC2	124-143	QSLGPFFGHVGQNLNLINQL	NAMP	0.824962	nontoxic	nonallergenic	allergenic	nonallergenic
LC3	155-174	GPALGCAGGGRIYDFEAAWD	NAMP	0.816902	nontoxic	nonallergenic	nonallergenic	allergenic
HC1	5244-5263	CGIPRRQLVVKFRALPCVNC	AMP	0.853223	nontoxic	nonallergenic	allergenic	nonallergenic
HC2	5242-5261	KNCGIPRRQLVVKFRALPCV	AMP	0.840736	nontoxic	nonallergenic	allergenic	nonallergenic
HC3	2403-2422	SGAAFGAGAGAGAGSGAGAG	NAMP	0.827947	nontoxic	nonallergenic	allergenic	allergenic
HC4	2401-2420	AGSGAAFGAGAGAGAGSGAG	NAMP	0.822907	nontoxic	nonallergenic	allergenic	allergenic
HC5	5185-5204	GSGAGAGSGAGAGSGAGAGG	NAMP	0.820077	nontoxic	nonallergenic	allergenic	allergenic
HC6	2402-2421	GSGAAFGAGAGAGAGSGAGA	NAMP	0.81605	nontoxic	nonallergenic	allergenic	allergenic
HC7	5243-5262	NCGIPRRQLVVKFRALPCVN	AMP	0.811737	nontoxic	nonallergenic	allergenic	allergenic
HC8	1065-1084	SGAGAGSGAGAGAGSGAGAG	NAMP	0.805344	nontoxic	nonallergenic	allergenic	allergenic
HC9	5082-5101	SGAGAGSGAGAGAGAGAGAG	NAMP	0.80488	nontoxic	nonallergenic	allergenic	allergenic

Abbreviation: allergen representative peptides (ARP), motif alignment and search tools (MAST), support vector machine classifier (SVMc), and auto-cross covariance (ACC)

Table 2. Physicochemical properties of bioactive peptides from the light chain and heavy chain of *Bombyx mori* fibroin proteins

Source	Hydro-phobicity	Steric Hindrance	Side-bulk	Hydro-pathicity	Amphi-pathicity	Hydro-philicity	Net Hydrogen	Charge	pI	Mol. Weight
LC1	0.07	0.62	0.62	0.38	0.19	−0.36	0.4	−1	4.38	2010.54
LC2	0.02	0.61	0.61	0.08	0.26	−0.73	0.7	0.5	7.1	2196.83
LC3	0.01	0.62	0.62	0	0.19	−0.14	0.45	−2	4.03	2026.49
HC1	−0.16	0.62	0.62	0.44	0.61	−0.17	0.9	4	9.83	2273.11
HC2	−0.22	0.63	0.63	0.12	0.8	0.03	1	5	10.94	2298.15
HC3	0.18	0.6	0.6	0.6	0	−0.3	0.1	0	5.88	1421.72
HC4	0.18	0.6	0.6	0.6	0	−0.3	0.1	0	5.88	1421.72
HC5	0.12	0.61	0.61	0.2	0	−0.1	0.15	0	5.88	1333.58
HC6	0.18	0.6	0.6	0.6	0	−0.3	0.1	0	5.88	1421.72
HC7	−0.2	0.63	0.63	0.14	0.61	−0.11	1	4	10.79	2284.08
HC8	0.13	0.6	0.6	0.31	0	−0.13	0.15	0	5.88	1347.6
HC9	0.15	0.6	0.6	0.44	0	−0.17	0.1	0	5.88	1331.6

Table 3. Quality of three-dimensional structure modeling of fibroin bioactive peptides

Source	Sequence	3D Structure	
		Quality	Score
LC1	VGPALGCAGGGRIYDFEAAW	Very Low	0.167
LC2	QSLGPFFGHVGQNLNLINQL	High	0.537
LC3	GPALGCAGGGRIYDFEAAWD	Very Low	0.177
HC1	CGIPRRQLVVKFRALPCVNC	High	0.6
HC2	KNCGIPRRQLVVKFRALPCV	Medium	0.414
HC3	SGAAFGAGAGAGAGSGAGAG	Medium	0.442
HC4	AGSGAAFGAGAGAGAGSGAG	Low	0.212
HC5	GSGAGAGSGAGAGSGAGAGG	Low	0.306
HC6	GSGAAFGAGAGAGAGSGAGA	Low	0.342
HC7	NCGIPRRQLVVKFRALPCVN	Low	0.372
HC8	SGAGAGSGAGAGAGSGAGAG	Medium	0.406
HC9	SGAGAGSGAGAGAGAGAGAG	Low	0.289

Table 4. Results of molecular docking analysis

Code	Ligand	BMP2			TGFB1		
		Docking Score	Confidence Score	Ligand RMSD (Å)	Docking Score	Confidence Score	Ligand RMSD (Å)
SA	Silicic acid (Control)	−45.29	0.1097	72.71	−38.69	0.0974	88.86
LC1	VGPALGCAGGGRIYDFEAAW	−161.08	0.5552	55.03	−0.05	0.0475	87.04
LC2	QSLGPFFGHVGQNLNLINQL	−180.24	0.6468	52.58	−132.84	0.415	116.14
LC3	GPALGCAGGGRIYDFEAAWD	−114.33	0.3288	66.98	−81.58	0.2029	98.64
HC1	CGIPRRQLVVKFRALPCVNC	−121.12	0.3595	69.11	−153.82	0.5191	104.15
HC2	KNCGIPRRQLVVKFRALPCV	−149.55	0.4978	56.79	13.58	0.0366	93.55
HC3	SGAAFGAGAGAGAGSGAGAG	−131.49	0.4085	57.27	−127.7	0.3903	94.46
HC4	AGSGAAFGAGAGAGAGSGAG	−122.2	0.3645	64.65	−99.32	0.2663	101.03
HC5	GSGAGAGSGAGAGSGAGAGG	−86.89	0.2206	52.43	21.21	0.0315	79.36
HC6	GSGAAFGAGAGAGAGSGAGA	−111.18	0.3151	58.74	−124.15	0.3736	96
HC7	NCGIPRRQLVVKFRALPCVN	−168.96	0.5937	60.21	−103.27	0.282	86.41
HC8	SGAGAGSGAGAGAGSGAGAG	−105.7	0.2919	72.86	12.06	0.0376	74.46
HC9	SGAGAGSGAGAGAGAGAGAG	−91.51	0.2369	49.26	−99.47	0.2669	108.14

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was also performed. Furthermore, 12 bioactive peptides were analyzed for their physicochemical characteristics, particularly to assess their stability during the absorption, distribution, metabolism, and excretion process. Peptides with dominant hydrophobic characteristics include LC3, HC1, and HC2 (Table 2).

Ab initio 3D model construction using a deep NN approach produced 3D structures of varying quality, ranging from high to very low.[28] The results showed that only five peptides out of a total of 12 had a quality score above 0.4, corresponding to medium or high quality (Table 3). After evaluating the bioactivity of fibroin and obtaining its models, molecular docking was performed between the 12 peptides and a control compound, silicic acid (SA) (colors as shown in the table and figure), which is the main component of the gold standard anti-inflammatory drug, despite its side effects.[33,34] The docking results indicated that all 12 peptides had better affinity than SA. Inter-peptide analysis identified LC1, LC2, HC1, HC2, HC3, and HC7 as the candidate peptides with the highest affinity for the two marker proteins (Table 4). These six peptides were assessed as potential candidates based on docking score, confidence score, the Root Mean Square Deviation (RMSD) score, and amino acid interactions, which were comparable to the control (Figure 1).

The active amino acids that must be bound by the peptide in the chains A and B of the BMP2 protein are Arg114:A, Cys43:A, His44:A, Gly45:A, Glu46:A, Gln64:A, Lys76:A, Ala77:A, Cys78:A, Cys79:A, Pro81:A, Arg114:B, Cys43:B, His44:B, Gly45:B, Glu46:B, Cys47:B, Gln64:B, Pro75:B, Lys76:B, Ala77:B, Cys78:B, Cys79:B, and Pro81:B. The active amino acids that must be bound by the peptide in chain G of the TGFB1 protein are Ile102:G, Leu128:G, Leu129:G, Arg130:G, Gln137:G, Val139:G, Leu158:G, Leu167:G, Leu190:G, Phe210:G, Leu218:G, Ala219:G, Ile221:G, and Pro227:G. The peptide with the highest affinity was identified as a sequence containing essential amino acids from fibroin that belong to the branched-chain amino acid category: valine (V), isoleucine (I), and leucine (L), which have anti-inflammatory effects.[35,36]

DISCUSSION

This research identified nine nonallergenic peptides derived from the LC and HC regions of fibroin, which demonstrate potential interactions with BMP2 and TGFB1 as peptide inhibitors. The findings suggest that fibroin-derived peptides may influence inflammatory pathways. However, the analysis could not identify specific residues essential for peptide interactions. Furthermore, no corroborative in vitro or in vivo studies have been found to substantiate the findings of this study.

Prior reports have identified specific fibroin-derived residue compositions with established bioactivity, including Gly-Ala-Gly-Ala-Gly-Tyr (GAGAGY), Gly-Val-Gly-Tyr (GVGY), and Gly-Val-Gly-Ala-Gly-Tyr (GVGAGY).[37,38] The GAGAGY sequence is known to augment phosphoinositide 3-kinase signalling,[37] mitigating the persistent insulin-induced reduction of insulin-stimulated glucose uptake, whereas GVGY and GVGAGY demonstrate the angiotensin-converting enzyme (ACE)-inhibitory properties.[38] However, none of these bioactive sequences were identified among the peptides in this study, despite their established anti-inflammatory properties. This underscores the need for additional research to determine whether the identified peptides exhibit analogous or divergent mechanisms of action in inflammation modulation.

Furthermore, several applications of fibroin in the medical field have been developed, such as fibroin-based plasters, tissues, and artificial organs.[39,40] The development of fibroin in dental medicine is still ongoing, and this study explores the antigenicity and interaction potential of fibroin peptides. Toxicity prediction analysis was carried out using the BLAST search method on ARPs,[26] IgE epitopes,

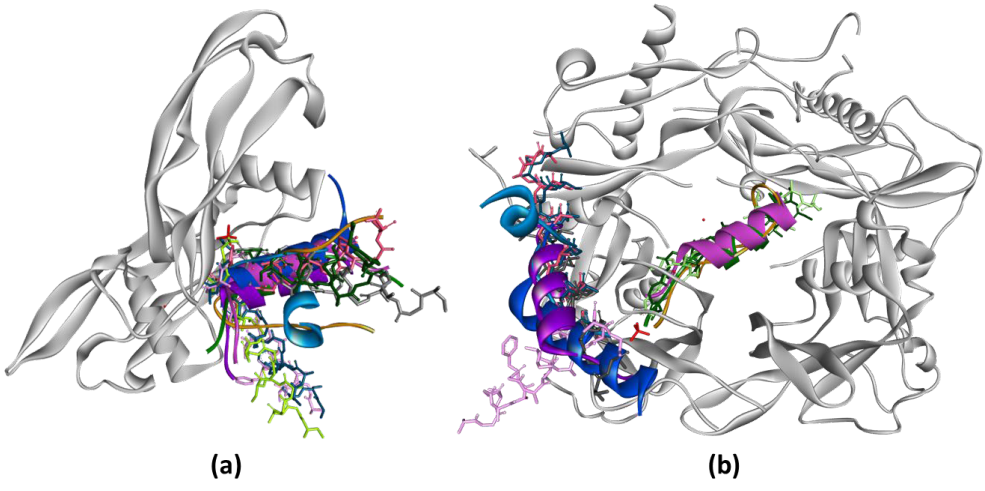

Figure 1. Binding sites for interaction between bioactive peptides with the receptors: (a) Bone morphogenetic protein 2 (BMP2) and (b) Transforming growth factor beta 1 (TGFB1).

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MAST, and SVMs,[26] which showed complementary or even conflicting results regarding allergenic versus nonallergenic peptides.[27] These discrepancies are due to the different thresholds used in the algorithms of the four analysis methods. Based on the docking results, several peptides were found to have higher binding affinity for BMP2 and TGFB1 than the control compound.

According to our research, the molecular mechanism of the anti-inflammatory peptide fibroin may involve inhibition of BMP2 and TGFB1. The potential interaction of several peptides with these two proteins provides new insights, given that current information is still limited. Moreover, the anti-inflammatory mechanisms of BMP2 and TGFB1 inhibitors involve modulation of various signaling pathways and cytokine production. Bone morphogenetic protein 2 has been shown to activate non-canonical inflammatory pathways, such as the p38 mitogen-activated protein kinases/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, leading to upregulation of pro-inflammatory cytokines, including the vascular endothelial growth factor (VEGF), and disruption of endothelial cells.[41] Additionally, BMP2 can induce an increase in proinflammatory cytokines, suggesting a direct role in promoting inflammation,[42] and it may contribute to inflammation in diabetic retinopathy by enhancing leukocyte adhesion to retinal endothelial cells and increasing the production of adhesion molecules and cytokines.[43] Bone morphogenetic protein 2 has also been shown to induce local inflammation, including polymorphonuclear cell infiltration and increased expression of pro-inflammatory cytokines.[44]

Furthermore, the role of TGFB1 in inflammation is multifaceted, involving complex interactions with various signaling pathways and immune cells. Transforming growth factor beta 1 has been implicated in regulating the production of anti-inflammatory cytokines, such as IL-10,[45] modulating the activity of transcription factors such as the signal transducer and activator of transcription 3 (STAT3),[46] and inhibiting the activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome.[47,48] Inhibition of genes and proteins related to this pathway can reduce its possible anti-inflammatory activity. As an inflammatory regulator, TGFB1 also exerts anti-inflammatory effects through several mechanisms, including modulation of macrophage polarization, particularly promoting the anti-inflammatory M2 phenotype,[49,50] and inhibition of the MAPK signaling pathway, which plays a pivotal role in regulating inflammatory responses and immune cell activation.[51]

Bone morphogenetic protein 2 (BMP2) and TGFB1 play pivotal roles in promoting odontoblastic differentiation and reparative dentin formation. These proteins activate signaling pathways essential for dentin repair, highlighting their significance as protein markers for dentine reparative activity. Several proteins are involved in the regulation of dental pulp, including BMP, dentin sialophosphoprotein (DSPP), and dentin matrix protein 1.[52] However, several studies have highlighted the significance of BMP2 and TGFB1 in promoting odontoblastic differentiation and reparative dentinogenesis. Bone morphogenetic protein 2 has been shown to enhance the mineralization and differentiation of dental pulp cells, promoting the formation of reparative dentin.[53] Additionally, BMP2 induces the expression of dentin matrix proteins and matrix mineralization, leading to the differentiation of pulp stem cells into odontoblast-like cells,[54] and the addition of BMP2 to culture media has been found to promote odontogenic differentiation, emphasizing its role in dentin formation and repair.[55] Moreover, the canonical BMP signaling pathway has been shown to stimulate the expression of DSPP in response to BMP2, underscoring its crucial role in dentin formation and repair.[56]

Transforming growth factor beta 1 has been implicated in promoting odontoblastic differentiation and reparative dentin formation. It is suggested that TGFB1 contributes to odontoblastic differentiation through the Wnt/β-catenin signaling pathway, which is essential for odontoblast differentiation and reparative dentin formation.[57] Moreover, TGFB1 has been linked to modulating healing during tertiary dentin production, indicating its involvement in the regulation of developmental processes and dentin repair.[58] The Wnt/β-catenin signaling pathway plays a key role in promoting reparative dentin formation by activating pulp stem cells and promoting an anti-inflammatory macrophage response, further highlighting its significance in dentin repair.[58]

This study has several limitations. The results are estimations and present conclusions in terms of potential effects, which are expected to approximate biological reality, given the complexity of the underlying systems. Fibroin peptides may be either nonallergenic or allergenic; however, none were categorized as toxic. The 3D structures of fibroin peptides were effectively modeled, exhibiting a quality range from very low to high, indicating structural resemblance to their actual 3D conformations. Light chain 2, LC3, HC1, HC3, HC4, HC6, HC7, and HC9 demonstrated potential binding affinity to BMP2 and TGFB1 relative to silicic acid, suggesting their potential function as peptide inhibitors. Our results highlight the novelty of the potential of fibroin peptides to interact with BMP2 and TGFB1, which may influence biological functions, including anti-inflammatory activity related to these signaling pathways. However, the limitations of this study are related to its computational nature. Additionally, key residues were not analyzed in this research; thus, these results are intended to serve as baseline data for subsequent in vitro and in vivo experimental validation.

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