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Original Article

Molecular docking of polyether ether ketone and nano-hydroxyapatite as biomaterial candidates for orthodontic mini-implant fabrication

[Acoplamiento molecular de poliéter éter cetona y nano-hidroxiapatita como biomateriales candidatos para la fabricación de mini-implantes de ortodoncia]

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Abstract

Context: Modified polyether ether ketone (PEEK) by adding nano-hydroxyapatite (HA) material on its fixture for mini-implant fabrication may increase resistance force through osseointegration.

Aims: To analyze the binding molecular docking of PEEK incorporated with HA as a biomaterial candidate for orthodontic mini-implant fabrication through a bioinformatic approach, an *in silico* study.

Methods: 3D ligand structure consisting of HA, PEEK and target proteins consisting of osteopontin, osteocalcin, osteonectin, bone morphogenetic protein 4 (BMP4), bone morphogenetic protein 2 (BMP2), bone morphogenetic protein 7 (BMP7), alkaline phosphatase (ALP), runt-related transcription factor 2 (RUNX2), Insulin growth factor-1 (IGF-1), osterix, tartrate-resistant acid phosphatase (TRAP), collagen alpha-1 (COL1A1) obtained from RCSB-PDB. It was analyzed the binding affinity of a single HA, PEEK, and HA + PEEK complex to twelve target proteins related to osseointegration. The types of chemical interactions produced by the ligands in the target protein domain consisted of Van der Waals, hydrogen, hydrophobic, pi, and alkyl.

Results: The blind docking simulation succeeded in identifying the most negative binding affinity; it was found in the HA + PEEK molecular complex compared to HA and PEEK in the single condition**.** The type of chemical interaction formed consisted of hydrogen, van der Waals, pi, and alkyl. HA+PEEK showed the most negative binding affinity with ALP and IGF-1, as much as -8.7 binding affinity.

Conclusions: The molecular docking of PEEK with HA exhibited a prominent binding affinity with osteogenic markers like ALP and IGF-1 *in silico*, allowing it to have a higher potential than nano-HA or PEEK as a single biomaterial for osseointegration as the fabrication of mini-implants that may support orthodontic treatment.

Keywords: dentistry; good health and well-being; *in silico*; medicine; temporary anchorage device.

Resumen

Contexto: La poliéter éter cetona modificada (PEEK) puede aumentar la fuerza de resistencia a través de la osteointegración mediante la adición de material de nanohidroxiapatita (HA) para la fabricación de mini-implantes.

Objetivos: Analizar el acoplamiento molecular de PEEK incorporado con HA como candidato a biomaterial para la fabricación de miniimplantes de ortodoncia a través de un enfoque bioinformático, un estudio *in silico*.

Métodos: Estructura de ligando 3D que consiste en HA, PEEK y proteínas diana como osteopontina, osteocalcina, osteonectina, proteína morfogenética ósea 4 (BMP4), proteína morfogenética ósea 2 (BMP2), proteína morfogenética ósea 7 (BMP7), fosfatasa alcalina (ALP) , factor de transcripción relacionado con runt 2 (RUNX2), factor de crecimiento de insulina-1 (IGF-1), osterix, fosfatasa ácida tartrato resistente (TRAP), colágeno alfa-1 (COL1A1) obtenido de RCSB-PDB. Fue analizada la afinidad de unión del complejo único HA, PEEK y HA + PEEK a doce proteínas diana relacionadas con la osteointegración. Los tipos de interacciones químicas producidas por los ligandos en el dominio de la proteína objetivo consistieron en Van der Waals, hidrógeno, hidrofóbico, pi y alquilo.

Resultados: La simulación a ciegas de acoplamiento logró identificar la afinidad de unión más negativa. Esta se encontró en el complejo molecular HA + PEEK en comparación con HA y PEEK de forma individual. El tipo de interacción química formada consistió en hidrógeno, van der Waals, pi y alquilo. HA+PEEK mostró la afinidad de unión más negativa con ALP e IGF-1, con una afinidad de unión de -8,7.

Conclusiones: El acoplamiento molecular de PEEK con HA exhibió una afinidad de unión prominente con marcadores osteogénicos como ALP e IGF-1 *in silico*, lo que le permite tener un mayor potencial que HA o PEEK como biomaterial único para la osteointegración como la fabricación de mini-implantes que puedan soportar el tratamiento de ortodoncia.

Palabras Clave: buena salud y bienestar; dispositivo de anclaje temporal; *in silico*; odontología; medicina.

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INTRODUCTION

Malocclusion is the third most common dental and oral health problem in the world. Malocclusion is a fairly large dental and oral health issue in Indonesia, and its prevalence is still very high, around 80% of the population, and ranks third after dental caries and periodontal diseases (Ardani et al., 2020). The prevalence rate of malocclusion worldwide is reported to vary in number, ranging from 11 to 93% consisting of mild to severe malocclusion. The highest was 193%, which Silva carried out in 2001 in Latin America and the lowest was 8.8% found by Sridharan in India in 2011 (Herwanda et al., 2016). The prevalence of malocclusion and the need for orthodontic treatment in Indonesia are still quite high, around 80%. Based on the 2013 South Kalimantan Province Basic Health Research results, the prevalence of malocclusion cases at the age of 12-15 years was 15.6%. This age group is the age group with the highest malocclusion compared to other ages. Orthodontic treatment for men is only 0.3% and 1.7% for women (Syada et al., 2017).

Malocclusion is a form of maxillary and mandibular relationship that deviates from the standard form that is accepted as a normal form malocclusion can be caused due to lack of dentofacial balance. The severity of malocclusion and its effect on the aesthetics and function of the oral cavity need to be a major concern in the world of health, especially in the field of orthodontics (Loblobly et al., 2015). The etiology of malocclusion can be classified into general factors and local factors. General factors are factors that do not directly affect the teeth. Local factors are factors that directly affect the teeth. Bad habits are one of the common factors that play a role in the occurrence of malocclusion. Various bad habits include sucking fingers and thumb, pushing the tongue, biting lips and nails, wrong swallowing habits, breathing through the mouth, and bruxism (Gupitasari and Putri, 2018). The severity of the malocclusion varies from low to high, reflecting individual biological variations. These forms of deviation must be grouped into smaller categories so that malocclusion classification is needed. Malocclusion classification is a description of dentofacial deviation based on general characteristics. The Angle's malocclusion classification is the most common classification frequently used today (Simangunsong et al., 2018).

High prevalence rates of malocclusions make it became a worldwide public health problem. Oral health-related quality of life (OHRQoL) measures have long been used to complement assessments of dental care needs and priorities and outcomes of treatment strategies. According to WHO, quality of life is the perception of one's position in one's life in the context of the culture and values in which one lives, concerning goals, expectations, standards and concerns. Oral health is an integral part of general health, so OHRQoL is a multidimensional concept that addresses the effects of harmful oral disorders on psychosocial and functional well-being (Vieira-Andrade et al., 2015). Like other unfavorable oral conditions, malocclusions are profoundly common and can have outcomes that influence physical and monetary prosperity, in this manner applying an adverse consequence on personal satisfaction. It is normal for people with malocclusion to foster methodologies, like hiding their teeth and avoiding smiling and developing social anxiety, emotional insecurity, dread, and trouble regarding individual connections. These aspects increase the negative impact on quality of life. To be sure, ongoing examinations report that malocclusions stand apart among the principal issues that influence OHRQoL because of the effect on work, appearance, relational connections, socialization, confidence, and mental prosperity (Vieira-Andrade et al., 2015).

Dental implants, which have been well described in the prosthodontic literature, are also used in orthodontic practice. Recently, smaller implants were introduced as temporary skeletal anchor devices (TSADs). Orthodontic mini-implants are considered capable of being an absolute anchor because the reciprocal force from the anterior retraction will be transmitted to the alveolar bone without involving the posterior teeth and is not influenced by patient compliance factors (Antolis et al., 2021). In addition, mini implants in orthodontic can be used to treat intrusion/extrusion of teeth, close edentulous spaces, repositioning of a malposed tooth, reinforced anchorage, partial edentulism, correct undesired occlusion, and orthopedic movement (Nayak and Malviya, 2011; Sheoran et al., 2021).

In contrast to dental implants, which achieve stability through osseointegration, the stability of mini-implants is achieved by mechanical retention. Compared to dental implants, mini-implants are relatively less stable. Primary stability is important for clinical success, as temporary skeletal anchors do not require osseointegration (Mešić et al., 2021). The loaded mini-implant clearly shows minimal displacement, although it is stable, immediately after loading, mainly due to tissue movement or during periods of bone remodeling (Ntolou et al., 2018).

Titanium (Ti) and its alloys are often used as dental implant materials because of their high biocompatibility, corrosion resistance and mechanical properties. However, some clinicians have patients with hypersensitivity to medical or dental titanium im-

plants, as indicated by the temporal relationship between titanium exposure and the occurrence of proximal tissue reactions to titanium implants. Clinically, symptoms of oral hypersensitivity to dental materials and implants include mucosal erythema, swelling or purpuric patches on the hard and soft palate, oral ulcers, hyperplastic gingivitis, tongue depapillation, angular cheilitis, eczematous eruptions on the face, or lichenoid reactions (Alqahtani et al., 2021).

The integration of the implant with the surrounding bone can be referred to as osteointegration, where it is very important for long term success in the rehabilitation process using implants in dentistry, and the use of modified polyether ether ketone (PEEK) by adding hydroxyapatite material can also increase osteoinduction, which is defined as where primitive cells can differentiate and pluripotent to stimulate bone formation, osteoconduction is the growth of a bone on the surface that requires a remodeling process or requires the development of new bone, increasing the resistance force and these components can be made easily and cheap (Albrektsson and Johansson, 2001; Jubhari et al., 2020; Kazimierczak and Przekora, 2020).

PEEK is a polymer that is often used as a base material in orthopedic treatments such as spinal implants because the material is highly biocompatible with the human body. In recent years, many studies have used PEEK as a base material for implants in humans because the biological properties of the material are translucent to x-rays, and the mechanical properties of the material are very strong and can be reinforced by carbon fiber and other materials such as Hydroxyapatite (Barkarmo et al., 2013; Geng et al., 2020; Zhou et al., 2019). PEEK can also be applied in the manufacture of mini-implant materials in dentistry, where implants in dentistry usually use titanium-type materials but these materials still have many shortcomings, such as causing excessive bone resorption and inhibiting bone regeneration. Therefore, the use of PEEK In the manufacture of mini-implants, it is very useful to replace the implant material for the better because it tends to avoid allergic reactions to the implant and can reduce the pressure-holding effect well (Barkarmo et al., 2013; Jubhari et al., 2020; Zhou et al., 2019).

PEEK was offered as a mini-implant biomaterial since it is a non-toxic biopolymer with modulus elasticity similar to human bone. Furthermore, it has a greater stress shielding effect than titanium implants, is easier to produce, has color stability, and is close to the hue of teeth (Ma et al., 2020). Because of the scarcity of PEEK in *in silico, in vitro, in vivo*, and clinical trial results, a preliminary molecular docking analysis was performed in this study (Papathanasiou et al., 2020). Previous researchers have investigated surface modification methods to stimulate PEEK's osseointegration, such as cellular compatibility, osteogenic activity, and antibacterial activity to increase the biological capability, inertia, and connecting ability with surrounding bone tissue as implant biomaterial (Ma et al., 2020).

To improve implant attachment to surrounding tissue, the implant can be added to a material that is acceptable to human bone tissue and can avoid immune system rejection reactions, so the best material, in this case, is hydroxyapatite material, which is a ceramic material with a calcium and phosphorus ratio similar to the natural bone so that the reaction caused by the bone tissue can accept the existing implant as part of the bone (Guo et al., 2013; Jubhari et al., 2020; Samirah et al., 2021; Zhou et al., 2019).

Making changes to the surface, the physicochemical of dental implants was created using nanostructured hydroxyapatite (HA) generated by microarc oxidation (MAO), a synthetic material that could enable osteoconductivity osteoinductivity, and angiogenesis of the dental implant surface. MAO-HA-treated dental implant surfaces increase *in vitro* osteogenesis and angiogenesis and activate M2 macrophages to modulate the immunological milieu, which improves the interaction between osteogenesis and angiogenesis and, ultimately, accelerates the process of osseointegration *in vivo* (Wang et al., 2021). Previous research discovered that a thin nano-HA coating produced by an electrochemical technique has the potential to improve implant osseointegration in ovariectomized rats (Cheng et al., 2012). In addition, de Olivera et al. (2021) discovered that the level of osseointegration related bone markers such as runt-related transcription factor (RUNX2), alkaline phosphatase (ALP), osteocalcin, and osteopontin was increased in osteoblast proliferation in the early stage of osseointegration after nano-hydroxyapatite coated implant surface that promoting new bone formation in diabetic rats, *in vivo*, similar to Cheng et al. (2012).

There are twenty varieties of bone morphogenic proteins (BMPs), and some studies have indicated that BMP-2, 4, and 7 can improve the regeneration of faulty or injured bone tissue, such as in tooth extraction skeletal fractures, and critical-sized defects. BMPs promote the development of pre-osteoblasts from mesenchymal stem cells or stromal cells (Prahasanti et al., 2020). Insulin-like growth factor 1 boosted BMPs activity (IGF-1). Serum levels of IGF-1, a hormone thought to influence skeletal development. IGF-1 and BMPs have a synergistic impact on bone repair (Peng et al., 2019).

Bone marrow mesenchymal stem cells (BMSC) play an important role in osteoblast destiny and development, which is associated with bone rebuilding (Purnama et al., 2018). MicroRNAs are emerging as important post-transcriptional modulators in bone remodeling, influencing osteoblast and osteoclast activities. Intercellular interaction between osteoblasts and osteoclasts is mediated by miR-21, which regulates the bone homeostasis response and provides prospective targets for osteoblast function maintenance. Previous research using immunohistochemistry discovered that in miR-21KO mice, ALP, RUNX2, and osterix expression was reduced in bone tissue (Oka et al., 2020).

Bone comprises 10% water, 30% organic matter, and 60% inorganic matter. The organic component is 85 to 90% collagen (mainly type 1 (Coll1a1, -resisting tensile forces), proteoglycans (resisting compressive stresses), non-collagenous proteins (osteocalcin and osteonectin), and glycoproteins (osteopontin). The inorganic component, or mineralized matrix, is made up of hydroxyapatite crystals [Ca10(PO4)6(OH)2], which offer protection and support while also acting as the body's calcium and phosphate store. Osteoblasts indirectly govern osteoclast production and bone remodeling by cell-cell contact, paracrine signaling, and cell-bone matrix interaction (Henry and Bordoni, 2021). However, an interaction between osteoblast and osteoclast modulates osteoblast activity for bone apposition. Tartrate-resisting acid phosphatase (TRAP). TRAP, which is expressed by osteoclasts, is important in skeletal development, collagen synthesis and breakdown, bone mineralization, cytokine generation by macrophages and dendritic cells, and macrophage recruitment. To sustain bone tissue remodeling, TRAP can destroy skeletal phosphoproteins such as osteopontin (OPN), which is similar to the T-cell cytokine Eta-1 (Hayman, 2008). The addition of nano-hydroxyapatite (HA) material to a modified PEEK surface as a mini-implant fixture for tiny implant production may boost resistance force through osseointegration. Thus, this study aims to analyze the molecular docking of PEEK incorporated with nano-HA to osteogenic markers related to osseointegration as a biomaterial candidate for orthodontic mini-implant fabrication through a bioinformatic approach, an *in silico* study.

MATERIAL AND METHODS

Sample retrieval

This study used a 3D ligand structure consisting of hydroxyapatite (Symbol: HA, SID: 14781, Formula: $Ca_5HO_{13}P_3$) and polyetheretherketone (Symbol: PEEK, SID: 135076451, Formula: $C_{19}H_{12}O_3$) obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Pubchem is a specific database for storing information such as compound ID, Canonical SMILE, mechanism, formula, molecular weight, and toxicity of a chemical compound (Kim et al., 2016). Then, for target proteins consisting of osteopontin, osteocalcin, osteonectin, bone morphogenetic protein 4 (BMP4), bone morphogenetic protein 2 (BMP2), bone morphogenetic protein 7 (BMP7), alkaline phosphatase (ALP), runt-related transcription factor 2 (RUNX2), Insulin growth factor-1 (IGF-1), osterix, tartrate-resistant acid phosphatase (TRAP), collagen alpha-1 (COL1A1) obtained from RCSB PDB (https://www.rcsb.org/). RCSB PDB is a specific database used to store protein information such as experimental method (X-ray or NMR), number of constituent atoms, sequence length, expression system, 3D structure, and an organism of origin (Rose et al., 2017).

Screening docking

The simulation of the binding mechanism in the ligand with the protein, which aims to determine the level of activity and the interaction pattern, is called molecular docking. Screening docking plays a role in identifying the binding affinity ratio of the ligand in influencing the activity of the target protein, AutoGrid is set to fill the entire protein surface for the screening process (Kharisma et al., 2020). This study used a screening docking method through the Vina Wizard plug-in using PyRx 0.8 versions software to compare the binding affinity of a single HA, PEEK, and HA + PEEK complex to twelve target proteins.

Ligand-protein interaction

The positions and chemical interactions of the docked molecular complexes were identified through the Discovery Studio software 2016 version. The types of chemical interactions produced by the ligands in the target protein domain consist of Van der Waals, hydrogen, hydrophobic, pi, and alkyl. Weak interactions in the ligand-protein complex play a role in triggering the formation of binding affinity and specific biological responses to the target protein (Luqman et al., 2020).

Molecular visualization

The 3D structure of the protein-ligand complex was displayed using the PyMol 2.5 version software. Proteins were displayed through the structure of cartoons and transparent surfaces, while sticks for ligands were selected for staining on both molecules (Prahasanti et al., 2021).

RESULTS

Protein samples consisting of osteopontin, osteocalcin, osteonectin, BMP4, BMP2, BMP7, AP, RUNX2, IGF1, Osterix, TRAP, and COL1A1 were obtained from the RCSB database. PDB was obtained with information on ID, visualization method, PDB ID, resolution, weight, sequence length, and chains (Table 1). This study uses a screening docking simulation to identify the comparative activity of HA, PEEK, and HA + PEEK against twelve target proteins. The docking simulation results show that the most negative binding affinity is found in the HA + PEEK molecular complex compared to HA and PEEK in the single condition (Table 2). The results showed that the type of chemical interaction formed consisted of hydrogen, van der Waals, pi, and alkyl (Fig. 1). It was predicted that the HA + PEEK complex could affect the activity of the target protein.

Table 1. Results of target protein preparation from RCSB PDB.

Name	Visualization method	PDB ID	Resolution	Weight (kDa)	Sequence length	Chain
Osteopontin	X-ray	1MOY	1.55	13.80	130	A
Osteocalcin	X-ray	1QSH	2.00	5.85	49	A
Osteonectin	X-ray	1BMO	3.10	55.23	233	A/B
BMP4	X-ray	1REU	2.65	11.73	103	A
BMP2	X-ray	4UI1	2.35	51.34	114	A/B
BMP7	X-ray	1LX5	3.30	29.23	139	A
AP	X-ray	2GLQ	1.60	53.57	484	A
RUNX2	X-ray	6VGE	4.25	62.53	117	D
IGF1	X-ray	1IGR	2.60	57.07	478	A
Osterix	NMR	6X46	-	14.35	121	A
TRAP	X-ray	1WAR	2.22	35.48	310	A
COL1A1	NMR	2LLP	-	4.97	18	A/B/C

DISCUSSION

Molecular docking simulation was used to identify the level of interaction activity between the ligand and the target protein with reference to the resulting binding affinity. Binding energy or binding affinity is formed when the ligand interacts with a specific domain of the target protein. If the docked molecular complex has the most negative values, it can trigger a specific biological response (Luqman et al., 2020). The screening docking method is used to determine the activity of the ligand against the target protein by referring to the binding affinity produced (Ramadhani et al., 2022). Weak binding interactions are formed when the ligand binds to the target protein domain to contribute to triggering a biological response (Prahasanti et al., 2021). The results showed that the type of chemical interaction formed consisted of hydrogen, van der Waals, pi, and alkyl. It was predicted that the HA + PEEK complex could affect the activity of the target protein. So, the possibility of PEEK in a single condition does not trigger an increase in osteogenic markers compared to HA single. However, the combination of PEEK with HA can trigger an increase in osteogenic markers allowing it to have a higher potential than HA single for the fabrication of mini-implants to support orthodontic treatment.

Osseointegration is the biological fixation of the implant in direct contact with the bone on the implant or Bone Implant Contact (BIC) without the intervention of connective tissue. Bone Implant Contact is considered a key indicator for successful osseointegration, which governs the success and viability of the dental implant as a whole. Osseointegration is influenced by many factors, including anatomical location, implant size and design, surgical procedure, load effects, biological environment, age and sex, and specifically implant surface characteristics, including

Table 2. Results of docking screening simulation.

Protein	AutoGrid		Binding affinity (kcal/mol)		
	Center (Å)	Dimensions (Å)	HA	PEEK	HA + PEEK
Osteopontin	X: -13.074	X: 38.151	-7.0	-6.6	-7.2*
	Y: 8.026	Y: 42.830			
	Z: -26.463	Z: 73.905			
Osteocalcin	X: 8.069	X: 24.568	-6.2	-6.0	-7.3*
	Y: 25.299	Y: 21.085			
	Z: 22.859	Z: 16.889			
Osteonectin	X: 38.335	X: 88.373	-7.7	-7.5	-8.0*
	Y: 17.716	Y: 72.496			
	Z: 19.753	Z: 93.243			
BMP4	X: -18.049	X: 35.286	-7.4	-7.1	-7.6*
	Y: -21.892	Y: 56.805			
	Z: -40.965	Z: 38.239			
BMP2	X: 21.772	X: 39.081	-6.8	-6.3	-7.1*
	Y: 9.256	Y: 45.617			
	Z: 9.271	Z: 55.099			
BMP7	X: 79.198	X: 54.126	-7.0	-6.9	-7.8*
	Y: 35.787	Y: 36.155			
	Z: 45.639	Z: 43.191			
AP	X: 43.872	X: 64.911	-7.5	-7.2	-8.7*
	Y: 23.205	Y: 70.704			
	Z: 9.204	Z: 66.173			
RUNX2	X: -50.959	X: 50.452	-6.3	-6.2	-6.8*
	Y: 39.758	Y: 29.127			
	Z: -15.615	Z: 38.471			
IGF1	X: 40.018	X: 54.253	-8.1	-7.5	-8.7*
	Y: 29.476	Y: 99.095			
	Z: 52.605	Z: 80.499			
Osterix	X: 3.937	X: 66.285	-6.0	-5.9	-6.7*
	Y: 3.965	Y: 36.739			
	Z: -46.055	Z: 27.656			
TRAP	X: 68.304	X: 25.885	-6.4	-6.2	-6.6*
	Y: -24.336	Y: 27.644			
	Z: 17.176	Z: 39.737			
COL1A1	X: 0.568	X: 49.934	-5.2	-5.0	-5.3*
	Y: 0.032	Y: 17.523			
	Z: -0.246	Z: 22.589			

*The combination of PEEK + HA has binding affinity on related variables than HA only or PEEK only.

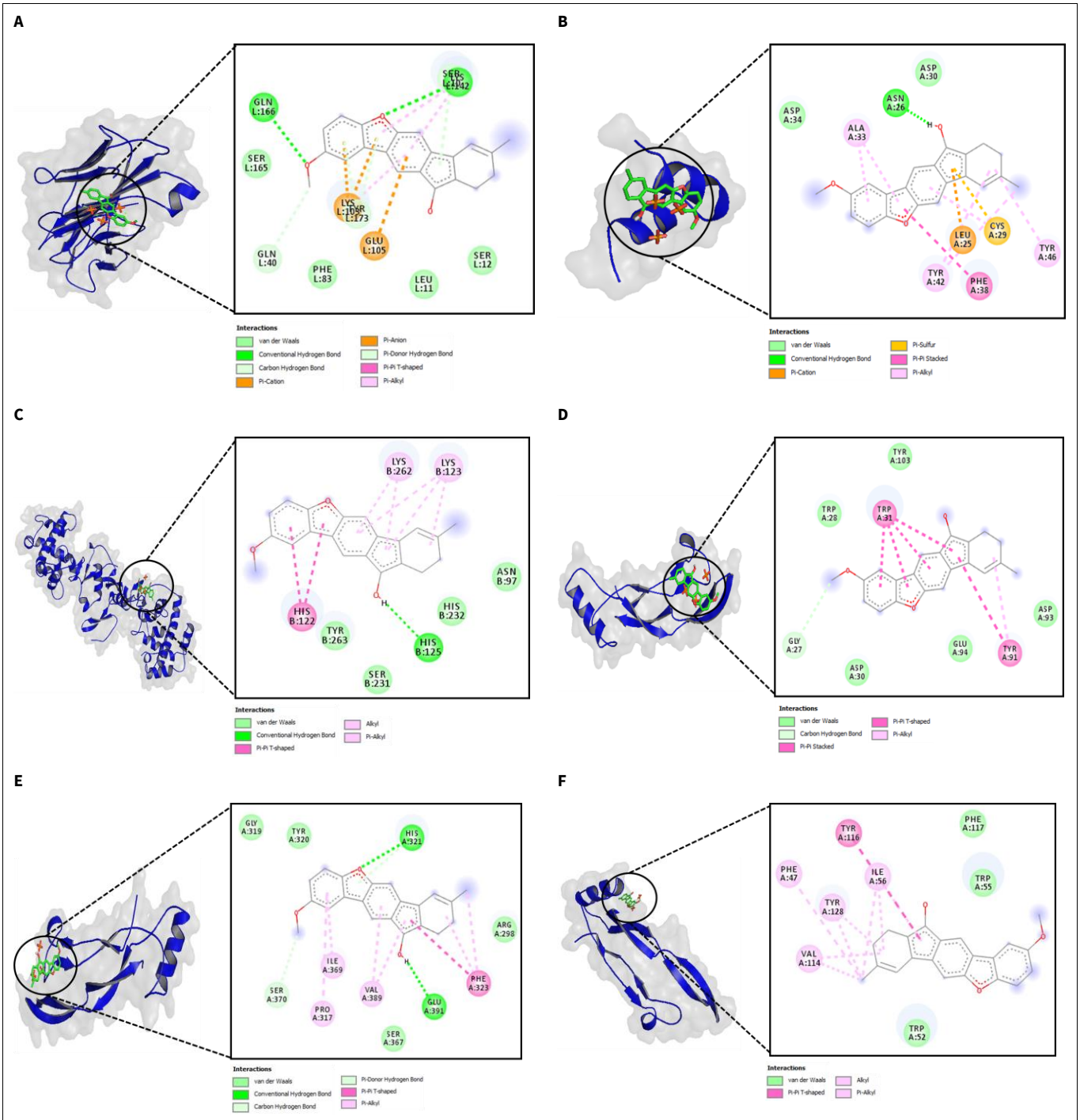

Figure 1. Visualization of the 3D structure of PEEK + HA docking complex with twelve target proteins.

The ligand structure of the PEEK + HA complex is shown in the form of green and orange sticks, and then the target protein consists of **(A)** Osteopontin; **(B)** Osteocalcin; **(C)** Osteonectin; **(D)** BMP4; **(E)** BMP2; **(F)** BMP7; **(G)** AP; **(H)** RUNX2; **(I)** IGF1; **(J)** Osterix; **(K)** TRAPase; **(L)** COL1A1 with transparent surfaces and cartoon in gray and blue.

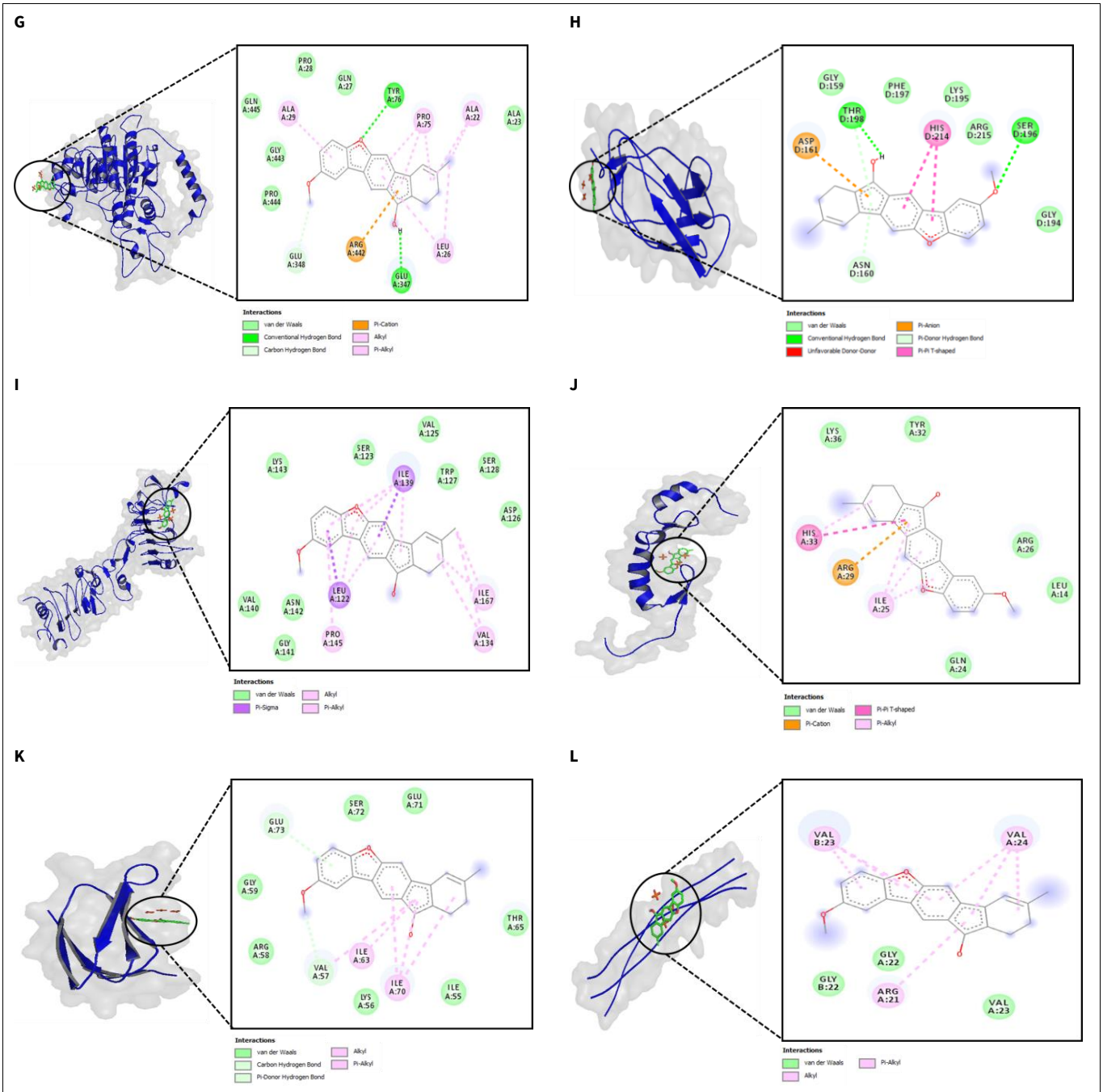

Figure 1. Visualization of the 3D structure of PEEK + HA docking complex with twelve target proteins (continued...)

The ligand structure of the PEEK + HA complex is shown in the form of green and orange sticks, and then the target protein consists of **(A)** Osteopontin; **(B)** Osteocalcin; **(C)** Osteonectin; **(D)** BMP4; **(E)** BMP2; **(F)** BMP7; **(G)** AP; **(H)** RUNX2; **(I)** IGF1; **(J)** Osterix; **(K)** TRAPase; **(L)** COL1A1 with transparent surfaces and cartoon in gray and blue.

chemical composition, wettability, presence of crystalline and amorphous phases, roughness and porosity. The application of HA aims to improve the osteoconductive properties of the HA layer (Khotib et al., 2019; Naini et al., 2020). Coating with HA can also accelerate the adaptation of bone to the implant surface. As a surface coating material for implants, the required properties of HA include crystallinity and purity. The higher the percentage of crystallinity of HA as a coating, the lower the biodegradation and resorbability that occurs (Levingstone et al., 2015). Osseointegration of implant relies upon various variables. Bone volume, implant geometry and surface topography, systemic health of the patient, and local factors are all critical for the success of implant therapy (Najeeb et al., 2016).

The gene expression of the early osteogenic differentiation markers such as BMP-2, BMP-4, BMP-7, RUNX-2, osterix, Coll1a1, and late osteogenic markers such as osteocalcin and osteopontin (Gromolak et al., 2020). BMP is an important signaling pathway for osteogenesis. The BMP pathway starts by activating

SMAD intracellular proteins, which, in turn, control the expression of the master gene RUNX2 (Valenti et al., 2016). RUNX2 is a master transcription factor for osteoblast differentiation, matrix production, and mineralization during bone formation. RUNX2 regulates osterix (Sitasari et al., 2020). Osterix is a zinc-finger-containing transcription factor expressed in osteoblasts that is essential for osteoblast differentiation and bone formation, and ALP, which is translated into a ubiquitous cellular protein that is an early indicator of cellular activity and differentiation (Megat Badarul Hisham et al., 2019). RUNX2 also regulates major osteoblast-specific downstream genes, such as COL1A1, osteopontin and osteocalcin, which determine the osteoblast phenotype and function in skeletogenesis and are translated into matrix proteins. In other words, RUNX2 controls osterix, which may regulate COL1A1, osteopontin and osteocalcin (Choi et al., 2017).

A comparison of metal and non-metal materials used as the main material for mini-implants has their respective advantages and disadvantages, such as the metal material that is often used is titanium, which has the advantages of good mechanical properties and low density (4.5 g/cm^3), biocompatibility to tissue. Around, in particular, the bones are very good. There are also aluminum and vanadium materials used for mini-implants, but there are still many shortcomings, such as aluminum, which can interfere with bone mineralization. In contrast, vanadium can be cytotoxic to the human body and can cause type 4 allergic reactions determined so that its biocompatibility can be achieved properly and is not harmful. The use of non-metallic materials in mini-implants is very dependent on the strength of these materials and their biocompatibility. Usually, metal materials, if the levels in these materials are too high, they will be rejected by the body, but if the basic ingredients are non-metal, the same and by the elements of the bone or human body such as PEEK. PEEK, which is coated with hydroxyapatite material, will cause beneficial physiological effects and can occur a very strong attachment to the surrounding tissue, especially bone tissue, such as better osteointegration so that the anchorage obtained is very maximal (Barkarmo et al., 2013; Elias et al., 2012; Geng et al., 2020; Zogheib et al., 2021).

CONCLUSION

Based on molecular docking investigation, it was found that the combination of PEEK with nano-HA has a higher binding affinity to osteogenic markers related to osseointegration like ALP and IGF-1, allowing it to have a higher potential than nano-HA or PEEK as a single biomaterial for the fabrication of mini-implants that may support orthodontic treatment. Further study is still needed to investigate PEEK and HA combinations' mechanical, biological, and chemical properties, *in vitro*, *in vivo,* and in clinical study settings.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Concepts or ideas	x	x	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x	x	x	x	x	x
Literature search	x	x	x	x	x	x	x	x	x	x	x
Experimental studies	x			x	x	x					
Data acquisition	x			x	x	x					
Data analysis	x			x	x	x					
Statistical analysis	x	x	x	x	x	x	x	x	x	x	x
Manuscript preparation	x	x	x	x	x		x	x	x	x	x
Manuscript editing	x	x	x	x	x		x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x

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