Research Article

Yipu Xu^{*#}, Zhifang Wu[#], Xiangzhe Zhao, Xiangchun Li

Two Cu(II)-based coordination polymers: Crystal structures and treatment activity on periodontitis

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Abstract: Two new Cu(II)-based coordination polymers (CPs) with the chemical formulae of $[\text{CuL}(\text{bimmb})_{0.5}]_n$ (**1**, bimmb is 4-bis(imidazole-1-ylmethyl)benzene) and $[\text{CuL}(\text{bbibp})]_n$ (**2**, bbibp is 4,4'-bis(benzoimidazo-1-yl)biphenyl) have been successfully prepared by the reaction of 3,3'-azodibenzoic acid (H_2L) ligand and metal salt Cu (NO_3) $_2$ ·3 H_2O in the presence of different nitrogen-donor coligands. In the bioactive evaluation experiments, the treatment activity of the prepared CPs against periodontitis was examined. Enzyme-linked immunosorbent assay detection was used to test interleukin-18 (IL-18) and IL-6 contents released into the gingival crevicular fluid after the treatment of above CPs, and the real-time reverse transcription polymerase chain reaction was subsequently performed to measure the relative expression level of *HmuY* gene in the *Porphyromonas gingivalis*.

Keywords: coordination polymers, periodontitis, *P. gingivalis*

1 Introduction

Periodontitis is a chronic infectious disease caused by the host's immune response induced by pathogenic microorganisms in the oral cavity [1]. The interaction between the

microorganisms and the host determines the course and progress of the disease. The mechanism of inflammation and destruction of periodontal tissue is still not very clear [2]. At present, it is believed that the oral pathogenic bacterial infection is the initiating factor of periodontitis, but most of the periodontal soft and hard tissue damage is mainly caused by the imbalance of the host's immune response to the infection [3]. During this process, proinflammatory cytokines such as interleukin-6 (IL-6) and IL-18 could lead to and further amplify the inflammatory response.

The synthesis along with the design of coordination polymers (CPs) has gained extensive attention due to their interesting structures as well as underlying applications in biomedicine, adsorption, and luminescence [4–8]. The final goal of coordination chemistry is to control the architectures of the target products and explore the correlations between performances and structures. Despite crystal engineering providing us with a useful tool for designing and establishing the desired coordination skeletons, the proper design together with the generation of CPs containing the expected performances and architectures are still a long-term challenge [9–11]. The architectural diversity of above materials is generally affected by many factors, such as properties of the organic ligands, metal and ligand ratio, metal ions, counter anion, and pH [12–14]. Among the above-mentioned factors, the choice of proper ligands is a very significant factor to create the required products. The coordination manners, stiffness, length, functional substituents, or groups of the organic ligands have an essential influence on the ultimate architecture of CPs. As is known to all, the mixed-ligand system constructed from two or more ligands gives more possibilities for the establishment of CPs with strange topologies [15–17]. One of the most effective aspects is the combination of a variety of carboxylates and auxiliary ligands involving neutral pyridine, in which carboxylate ligands balance the metal center's positive charge and produce secondary structural units, and the auxiliary ligands mediate and meet the metal center's coordination surroundings [18]. In the present study, by exploiting the mixed-ligand generation approach, two fresh CPs involving Cu(II), that is,

Authors have contributed equally to this study.

*** Corresponding author: Yipu Xu**, Department of General Dentistry and Emergency Dental Care, Capital Medical University School of Stomatology, Beijing 100050, China, e-mail: xuyipu86@126.com
Zhifang Wu: Department of General Dentistry and Emergency Dental Care, Capital Medical University School of Stomatology, Beijing 100050, China

Xiangzhe Zhao: Department of Oral and Maxillofacial Surgery, Stomatological Hospital of Lanzhou University, Lanzhou 730000, China

Xiangchun Li: Department of Oral and Maxillofacial Surgery, The First Hospital of Qinhuangdao, Qinhuangdao 066000, China

[CuL(bbibp)]_n (**2**, bbibp is 4,4'-bis(benzoimidazo-1-yl)biphenyl) and [CuL(bimmb)_{0.5}]_n (**1**, bimmb is 4-bis(imidazole-1-ylmethyl)benzene), have been generated with the reaction between 3,3'-azodibenzoic acid (H₂L) ligand and metal salt Cu(NO₃)₂·3H₂O in the presence of various nitrogen-donor coligands. The as-generated two CPS are characterized through the IR spectra, elemental analysis (EA), the diffraction of single-crystal X-ray, thermogravimetric analysis (TGA), and PXRD. In the biostudy, the treatment effect against periodontitis was assessed, and then the mechanism was explored.

2 Experimental

2.1 Chemicals and measurements

All of the reagents and chemicals utilized in this research were purchased from market source, which could be used with no modification. For exploring the elements of carbon, nitrogen, as well as hydrogen, the EA1110 CHNS was used. Varian 800 FT-IR (USA, Varian Company) spectrophotometer was used for recording IR spectra utilizing potassium bromide (KBr) pellets between 400 and 4,000 cm⁻¹. The PANalytical X'Pert PRO MPD system (Netherlands, PANalytical Company) (PW3040/60) was utilized to investigate the PXRD. The thermal analysis was implemented using the Netzsch STA-409 PC (Germany, NETZSCH-Gerätebau GmbH) thermoanalyzer with 10°C min⁻¹ increasing rate and 30 cm³ min⁻¹ flow rate of (N₂). JASCO FP-8600 (Japan, JASCO Company) fluorescence spectrophotometer was used for recording the luminescent spectra.

2.2 Preparation and characterization for [CuL(bimmb)_{0.5}]_n (**1**, bimmb = 4-bis(imidazole-1-ylmethyl)benzene) and [CuL(bbibp)]_n (**2**)

The mixture synthesized from 0.024 g and 0.1 mmol Cu(NO₃)₂·3H₂O, 0.1 mmol and 0.027 g H₂L, 0.019 g and 0.1 mmol bimmb, 6 mL of acetonitrile, and 3 mL N,N-dimethylformamide (DMF) was stirred under the ultrasonic conditions for 30 min. The solution was subsequently transformed into the stainless steel container lining by Teflon (15 mL), placed, and heated to a temperature of 130°C for 72 h. Afterward, cooling the container to room temperature (RT) at 5°C h⁻¹. The blue massive crystals were gathered at 61.2% yield (according to Cu(NO₃)₂·3H₂O). The calculated elemental analysis result for C₂₁H₁₅CuN₄O₄: N, 12.43, C, 55.94, and H, 3.35%. Found: N, 12.87, C, 55.96, and H, 3.52%. IR (KBr disk, cm⁻¹): 656 (w),

690 (s), 771 (s), 800 (m), 1,108 (m), 1,400 (s), 1,430 (m), 1,522 (m), 1,575 (m), 1,634 (s), 2,331 (w), and 2,361 (w).

The mixture formed by 0.024 g and 0.1 mmol Cu(NO₃)₂·3H₂O, 0.1 mmol and 0.027 g H₂L, 0.038 g and 0.1 mmol bbibp, 4 mL of H₂O, and 4 mL of DMF was stirred under the ultrasonic conditions for 30 min. The solution was subsequently transformed into the stainless steel container lining by Teflon (15 mL), placed, and heated to a temperature of 130°C for 72 h. Afterward, cooling the container to RT at 5°C h⁻¹. The dark blue crystal was harvested at 54.2% yield (in accordance with Cu(NO₃)₂·3H₂O). The calculated elemental analysis result for C₄₀H₂₆CuN₆O₄: N, 11.70, C, 66.89, and H, 3.65%. Found: N, 11.77, C, 66.33, and H, 3.64%. IR (KBr disk, cm⁻¹): 676 (w), 687 (m), 783 (s), 829 (m), 1,239 (s), 1,295 (m), 1,336 (m), 1,403 (s), 1,456 (m), 1,508 (s), 1,548 (s), 1,596 (s), 3,270 (w), and 3,520 (w).

The diffractometer of SuperNova was used with the aim of gaining the X-ray data. CrysAlisPro was used for the exploration of the strength data, which was subsequently converted to the HKL files. The direct mean-based SHELXS together with the least-squares strategy based SHELXL-2014 software (Germany, Goettingen University) were, respectively, used for the synthesis and modification of original architectural modes. The anisotropic parameters were mixed after using the whole non-H atoms. Eventually, the entire H-atoms could be fixed on the C atoms that they are connected to in geometry with AFIX commands. The complexes' optimization details together with their parameters of crystallography were listed in Table 1.

2.3 Enzyme-linked immunosorbent assay (ELISA)

ELISA test was used for detecting the IL-18 content together with IL-6 content released into the gingival crevicular fluid after treating via compounds. This study was performed completely following the instructions after minor modifications. Shortly, 50 Sprague-Dawley rats (from 6 to 8 week old and between 200 and 220 g) were used in the current research; they were all provided by Experimental Animal Research Center of Nanjing University. All of the rats were stored under a temperature between 20 and 25°C, with 45% humidity with free water and free food prior to conducting the treatment of complexes. All of the rats were classified as four groups, namely model group (with *n* of 10), control group (with *n* of 10), complex **1** treatment group (with *n* of 10), and complex **2** treatment group (with *n* of 10). In complex treatment and model groups, the *Porphyromonas gingivalis* was injected to rats to produce a periodontitis model. Complexes were subsequently injected

Table 1: The complexes' optimization details together with their parameters of crystallography

Identification code	1	2
Empirical formula	C ₂₁ H ₁₅ CuN ₄ O ₄	C ₄₀ H ₂₆ CuN ₆ O ₄
Formula weight	450.91	718.21
Temperature (K)	173.0	173.0
Crystal system	Monoclinic	Monoclinic
Space group	P2 ₁ /c	C2/c
a (Å)	10.9632(13)	16.624(2)
b (Å)	19.852(2)	18.716(3)
c (Å)	10.6923(15)	13.143(4)
α (°)	90	90
β (°)	109.231(2)	127.675(11)
γ (°)	90	90
Volume (Å ³)	2197.2(5)	3236.3(13)
Z	4	4
ρ _{calc} (g cm ⁻³)	1.363	1.474
μ (mm ⁻¹)	1.027	0.730
Data/restraints/parameters	4,161/12/271	2,815/0/231
Goodness-of-fit on F ²	1.042	1.066
Final R indexes (I ≥ 2σ(I))	R ₁ = 0.0573 and ωR ₂ = 0.1506	R ₁ = 0.0491 and ωR ₂ = 0.1351
Final R indexes (all data)	R ₁ = 0.0909 and ωR ₂ = 0.1741	R ₁ = 0.0563 and ωR ₂ = 0.1403
Largest diff. peak/hole (e/Å ⁻³)	0.59/−0.77	0.41/−0.80
Cambridge crystallographic data centre	2127148	2127149

intraperitoneally to implement treatment at 5 mg kg⁻¹ concentration. All of the implementation in the study were authorized through the Ethics Committee of the Experimental Animal Research Center of Nanjing University. After accomplishing the specific treatment, the gingival crevicular fluid in various groups was gathered, and the ELISA kit was used for the detection of the IL-18 and IL-6 contents in the gingival crevicular fluid.

2.4 Real-time reverse transcription polymerase chain reaction (RT-PCR) assay

In this study, a real-time RT-PCR assay was performed to measure the relative expression level of *HmuY* gene in the *P. gingivalis* after treating through the as-prepared compound. This research was conducted totally based on instructions after slight change. In short, *P. gingivalis* were cultivated in a medium of tryptic soy broth (TSB), the bacterial cells in the medium of TSB were harvested and inoculated into the culture plates of cell. The wells were added with compound with a variety of concentrations (namely 10, 20, and 50 ng mL⁻¹). After finishing the specific treatment, the *P. gingivalis* were gathered, cleaned,

the TRIzol Reagent was used for the extraction of overall RNA in *P. gingivalis*. Moreover, the overall RNA concentration was detected after determining the OD260/OD280 ratio, and it was reverse transcribed subsequently into the cDNA through utilizing the high-capacity cDNA reverse transcription kit. Eventually, the relative expression of *HmuY* gene in *P. gingivalis* was detected with Synergy Brands Synergy Brands, Inc Green Master Mix after treating via the complex. A 2^{-ΔΔCt} approach was utilized to perform relative quantification for three times. All the results were described with mean ± standard deviation.

3 Results and discussion

3.1 Crystal structures

The diffraction of single-crystal X-ray suggested that **1** belongs to the monoclinic space group of P2₁/c displaying a three-fold interpenetrated topological skeleton of **pcu**. Based on Figure 1a, its structural unit is composed of a separated site of Cu(II) in crystallography, 0.5 bimb, and a H₂L. The distance of Cu–N bond is 2.055(2) Å, and the separation of Cu–O bond is varying from 2.031(2) to 2.082(2) Å. Each of the Cu(II) atom is a five-coordination manner, which is coordinated via four O atoms provided by four diverse H₂L and a N atom offered via a separated bimb, revealing a pyramidal coordination structure of {CuNO₄}. The H₂L is fully protonated and reveals a μ₄-(κ1–κ1)-(κ1–κ1) coordination manner. The Cu1 connects to the symmetry-related Cu1 via four carboxylic acid groups of H₂L to produce the famous paddle-wheel {Cu₂(O₂C)₄} cluster. In terms of topology, each subunit of {Cu₂(O₂C)₄} is linked with six identical subunits {Cu₂(O₂C)₄} through four ligands of H₂L together with two bimb, generating a six-join net (Figure 1b). This linkage in directions *a*, *b*, and *c* produces a three-dimensional (3D) structure, and H-bonds exist inside the 3D skeleton to make the skeleton more stable. In meantime, along axis *c*, and there exist a large gap. According to Figure 1c, there also is a three-fold interpenetration. In the above interspersed architecture, there exist a weak force. The topology as the whole architecture can be exhibited in Figure 1d.

The complex **2** reveals a two-fold interpenetrated topological skeleton of **sql**, and it belongs to the monoclinic C2/c space group, and its architecture is revealed next in Figure 2a. The fundamental unit of **2** is constructed from a center of Cu(II), a H₂L carboxylate ligand, and a bbbbp ligand containing nitrogen. The bond

Figure 1: (a) The 1's structural unit. (b) The six-linked node of compound 1. (c) The compound 1's three-fold interpenetration. (d) The **pcu** network of complex 1.

Figure 2: (a) The 2's fundamental unit. (b) The 1D Cu-L chain of compound 2. (c) The 2's 2D net. (d) The p-p stacking interactions between consecutive layers.

distance of Cu–N is 2.075(3) Å, and the separation of Cu–O bond is between 2.051(17) and 2.322(18) Å. Each of the Cu(II) center is six-coordinated through a coordination structure of octahedron, composed of four O atoms provided by two separated H₂L and two nitrogen atoms belong to two separated bbibp. Each of the H₂L ligand is bridged to a neighboring H₂L via two Cu(II) ions, and they produce a jagged one-dimensional (1D) [Cu–L]_n chain (Figure 2b), and the bbibp is connected to a consecutive ligand of bbibp through two Cu(II) ions. Neighboring [Cu–L] 1D chain is stitched via single [Cu–bbibp]_n chain to generate a fundamental two-dimensional (2D) planar architecture (Figure 2c). Two such 2D planar architectures interspersed with each other, there exist a weak force. The compound **2** creates a 2D + 2D → 2D architecture by a weak interaction among the layers (Figure 2d). In terms of topology, the **2**'s skeleton can be rationalized to a four-linked **sql** network with (4⁴.6²) point symbol.

For the sake of examining the products' phase purity, the investigation of powder X-ray diffraction (PXRD) for the as-prepared complexes was finished (Figure 3a). Between the PXRD patterns peak positions of the simulation and experiment, there exist a well accordance, and this result suggests that the crystal architectures is a real representation of massive crystal products. The strength differences may be resulted from crystal samples preferred selection. Simultaneously, for exploring the compounds' thermal stabilities, TGA curves for the four compounds were exhibited in Figure 3b. In the nitrogen flow, the samples were heated with 10°C min^{−1} increasing rate. For the **1**, when the temperature reaches 400°C, the sample was stable. The **1** quickly appears weightlessness between 400 and 440°C, and this is probably due to the architectural collapse; from 450 to

800°C, there is a weightlessness. There is a difference between 68.9% of total mass loss and the removal of the organic matter (with a calculated value of 83.2%), and this may be on account of the sample is not decomposed fully. The compound **2** naturally loses weight in a temperature between 50 and 380°C. A rapid weightlessness was subsequently detected when the temperature up to 400°C, and this is due to the architectural collapse; between 400 and 800°C, it gradually lost weight. An 89.1% of total mass loss is associated with the removal of organic species (with a calculated value of 89.5%).

3.2 Significantly reduced IL-6 and IL-18 contents released into the gingival crevicular fluid after compound exposure

During periodontitis, *P. gingivalis* usually cause an over-inflammatory response, exhibited as the upregulated IL-18 and IL-6 levels in the gingival crevicular fluid. Hence, in the study, the IL-18 and IL-6 contents released into the gingival crevicular fluid were detected through ELSA detection. According to Figure 4, the model group has a much higher IL-18 and IL-6 contents in the gingival crevicular fluid compared with the control group. There exist a marked difference between the above groups, where the *P* value is less than 0.005. After complex **1** treatment, the IL-18 and IL-6 levels released into the gingival crevicular fluid were remarkably suppressed. In comparison with **1**, the **2** revealed nearly no effect on the IL-18 and IL-6 contents released in the gingival crevicular fluid.

Figure 3: (a) The complexes' PXRD manners and their (b) TGA curves.

Figure 4: Compound markedly downregulate the releasing of IL-18 together with IL-6 into the gingival crevicular fluid. The *P. gingivalis* was injected into animals to create a periodontitis model. The treatment was performed after the addition of compounds at 5 mg/kg concentration. The gingival crevicular fluid in various groups was gathered, and the ELISA kit was used to test the IL-18 and IL-6 contents in the gingival crevicular fluid. Control refers to the normal animal without treatment.

3.3 Strongly inhibited relative expression of the *HmuY* gene in *P. gingivalis*

In the previous research, we demonstrated that **1** was superior to complex **2** on suppressing the releasing of IL-18 together with IL-6 into the gingival crevicular fluid. As the *HmuY* gene was significant for the *P. gingivalis* survival, the real-time RT-PCR was deeply implemented

in the study to test the relative expression of *HmuY* gene in *P. gingivalis*. Figure 5 shows that, in contrast to control group, **1** could downregulate the relative expression of *HmuY* gene in *P. gingivalis* in a dose-dependent manner. However, the **2** exhibited nearly no effect on the relative expression of *HmuY* gene in *P. gingivalis*. In accordance with the earlier outcomes, **1** revealed much stronger bioactivity than the complex **2**.

Figure 5: Compound strongly inhibited the relative expression of *HmuY* gene in *P. gingivalis*. The *P. gingivalis* was cultivated in TSB medium, and the treatment was carried out after the adding compounds at 10, 20, and 50 ng mL concentration. The relative expression of *HmuY* gene in the *P. gingivalis* was tested using the RT-PCR detection.

4 Conclusion

On the whole, we have produced two fresh CPs involving Cu(II) with the reaction between the corresponding metal salts with the 3,3'-azodibenzoic acid (H_2L) ligand in the existence of various nitrogen-donor coligands. The as-generated two CPS are characterized through the EA and the diffraction of single crystal X-ray. The diffraction of single crystal X-ray suggests that **1** exhibits a uninodal six-linked 3D topology skeleton of **pcu** with $(4^{12}.6^3)$ point symbol, but **2** has an uninodal four-linked topology skeleton of **sql**. With ELISA detection, the **1** could markedly decrease the releasing of IL-18 and IL-6 into the gingival crevicular fluid. Afterward, the relative expression level of *HmuY* gene in the *P. gingivalis* was also suppressed via complex **1**, instead of **2**. Eventually, complex **1** was superior to **2** on the treatment of periodontitis through suppressing inflammatory cytokines releasing and suppressing the relative expression level of *HmuY* gene in *P. gingivalis*.

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