A novel coping metal material CoCrCu alloy fabricated by selective laser melting with antimicrobial and antibiofilm properties

Ling Ren[1, a], Kaveh Memarzadeh[1, b], Shuyuan Zhang [a], Ziqing Sun [a], Chunguang Yang [a], Guogang Ren[c], Robert P. Allaker[*, b], Ke Yang[*, a]

a. Institute of Metal Research, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang, China 110016

b. Institute of Dentistry, Barts and The London School of Medicine & Dentistry, Queen Mary, University of London, Newark Street, London E1 2AT UK

c. University of Hertfordshire, Hatfield AL10 9AB, UK

[1] These authors contributed equally to this work.

[*]: Corresponding authors：Fax:+86 24 23891320, E-mail: kyang@imr.ac.cn; Tel: + 44 20 7882 2388, E-mail: r.p.allaker@qmul.ac.uk

Abstract：

Objective: The aim of this study was to fabricate a novel coping metal CoCrCu alloy with superior antimicrobial ability and biofilm inhibitory effects by using a selective laser melting (SLM) technique, and to investigate its antimicrobial ability, antibiofilm effects and other performance indicators including microstructure, mechanical properties, corrosion resistance and biocompatibility.

Methods: Novel CoCrCu alloy was fabricated using SLM from a mixture of commercial CoCr based alloy and elemental Cu powder. Samples without Cu served as a control. Antibacterial and antibiofilm abilities were assessed by using standard antimicrobial tests. And the analysis of microbial colonisation were conducted by confocal laser scanning microscope (CLSM). Cu distribution and microstructure in CoCrCu were examined using scanning electron microscope (SEM), optical microscopy and X-ray diffraction. Corrosion resistance was evaluated by potential dynamic polarization. Additionally, biocompatibility was measured by MTT assay.

Results: Antibacterial and antibiofilm tests demonstrated that SLM CoCrCu alloys are cidal to bacteria and therefore, in turn, it can inhibit biofilm formation. Other physical properties such as microstructure, mechanical characteristics, corrosion resistance and biocompatibility were close to those maintained by SLM CoCr alloys.

Significance: The addition of appropriate amounts of Cu not only maintains normal beneficial properties of CoCr alloys, but also offers the novel SLM CoCrCu alloys with excellent antibacterial and antibiofilm abilities, and confers potential to be used as coping materials for dental implants.

1. Introduction

The Selective laser melting (SLM) technique has recently been introduced for fabricating metal coping for dental applications. In comparison with the traditional casting method, SLM offers several advantages, such as a higher definitive product density provided, reduced manufacturing time and costs, minimized human errors, and the prevention of casting defects [1-4]. The SLM technique is attracting particular interest in prosthetic dentistry because Bio- functionality and biocompatibility are two key factors for the selection of dental alloy materials [5]. Currently, CoCr based alloys are widely used in prosthodontics due to its combination of favorable properties, and was recently introduced into the dental application for producing the base metal coping using SLM [6, 7], benefiting from its superior mechanical strength, ductility, corrosion resistance, and biocompatibility.

In the oral cavity, it has been suggested that the diversity of bacteria in an individual oral cavity is around 500 species, which can readily colonise the different types of surfaces including teeth, prosthetic devices and dental implants [8-10]. The formation and maturation of biofilms on dental biomaterial surfaces may then have pathogenic implications in the development of peri-implant diseases, such as peri-implant mucositis or peri-implantitis, influencing the long-term implant success [11-13]. With respect to such complications， oral biofilms comprise complex three-dimensional structures consisting of diverse microbial multispecies communities as formed on oral tissues and prosthetic devices [14, 15]. Although treatments can provide immediate benefits with respect to elimination of the biofilm or prevention of secondary infection, the prospect of long-term clinical success relies more so on the antimicrobial properties of the dental materials used [16]. Hence, a dental biomaterial that creates a sustained antimicrobial environment around the restoration will be able to discourage the biofilm formation with considerable clinical benefit [17]. To this end, several antimicrobials have been incorporated within restorative materials and bonding systems [16].

Amongst the different antimicrobials used, such as chlorhexidine (CHX) and 12-metacryloyloxydodecylpyridinium bromide (MDPB), the copper (Cu) element is completely unique [18, 19]. Cu is not only an essential trace element required to maintain healthy cellular functions in the human body, but also is an alloying element in many alloys used in biomaterials, and human daily life for a long time [20, 21]. This provides the impetus to immobilize Cu into the current CoCr alloy to formulate a new CoCrCu alloy which could be tailoring processed/fabricated by SLM technology. In addition, not like nickel (Ni) giving an allergic and toxic influence upon human health,

CoCrCu based alloy is safe to use in terms of toxicology studies (Ref).

Thus, the aim of this work is to evaluate the CoCrCu alloy performance in antimicrobial, biofilm inhibition,, corrosion resistance, while some basic mechanical properties and general biocompatibility are also initially investigated. The results of this work will provide a preliminary platform for further clinical studies and potential applications for a novel antimicrobial metal coping material.

2. Materials and methods

2.1 Materials

The new CoCrCu alloy was formulated from a mixture of commercial CoCr based alloy and the elemental Cu powder, and fabricated by the SLM technology. The CoCr alloy powder particles sizes 25 μm and elemental Cu powder particles were sized 45, μm, which were obtained from a Laser Particle Sizer Analyser (Mastersizer 2000, Malvern, UK). The fully densed CoCrCu alloys (Φ10 cm × 1 cm, and Φ5 cm × 1 cm) were formed by mixing powders together in a Turbula T2F mixer (Glen Mills Inc, New Jersey, USA) for 30 min. The subsequent SLM process performed on a Realizer SLM100 (Borchen, Germany) according to the standard SLM strategies for fully densed materials. The chemical composition of CoCrCu alloy is listed in Table 1. After fabrication, the alloy was processed by solution treatment (1200℃, 1 h, with fast cooling), and subsequently followed an aging treatment (900 ℃, 2 h, slow cooling). As a control group, the same SLM strategic fabrication of CoCr alloy (without Cu) was used.. All SLM fabricated alloys were mechanically polished, followed by further chemically etching treatments..

Table1. Chemical composition (wt.%) of SLM CoCrCu alloy

Co	Cr	Cu	W	Si	Fe	Ni	Mn	Cd	Be	Nb
58.2	28.21	2.8	8.98	1.49	0.82	<0.1	<0.3	<0.0001	<0.01	<0.05

2.2 Elemental distribution and Microstructure investigation

Scanning electron microscopy (SEM, 1555 VP-FESEM, Zeiss, Germany) with an analysing energy dispersive spectrometer (EDS) was used to determine the elemental distribution within the SLM CoCrCu alloy. Imaging and EDS mapping were performed at an accelerating voltage of 15 kV and working distance of 12 mm. Mapping was performed on a 1024x768 pixel grid, with a dwell time of 1ms. Metallurgical structures of a SLM CoCrCu alloy was observed under the optical microscope (Axiovert 200 MAT). The microstructure analysis of the alloys was performed by X-ray diffraction (XRD) on a Rigaku D/max 2500 pc type X-ray diffraction. The experimental conditions for XRD

were recorded as CuKa X ray, tube voltage 50 kV, tube current 300 mA, and scan velocity 1.2/min.

2.3 Tensile test

The tensile test was carried out at room temperature on a universal testing machine (Instron, 1251, USA), with a tensile cross-head rate of 0.5 mm/min according to the ISO 6892-1: (2009, MOD) using standard specimens (SLM CoCr alloy and SML CoCrCu alloy) of M10×Φ5 mm. Ultimate tensile strength, yield strength and elongation values were obtained through the experimental tests. An average value of five samples was taken for each material for its Standard-Dilation calculations. The surface fracture morphologies of both SLM alloys were observed by using an optical microscope (Axiovert 200 MAT).

2.4 Potential dynamic polarization

To evaluate the corrosion resistance of the SLM CoCrCu alloys, potential dynamic polarization curves were plotted by an EG&G 273A type Integrated Electrochemical Analyzer using a three-electrode system, in which the experimental SLM sample was taken as the working electrode, a platinum foil as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. The scan velocity was 0.5 mV/s; test solution was a physical saline (0.9 wt.% NaCl solution), and a constant temperature of 37℃ was maintained in a water bath. The ratio of the sample area over the volume of physical saline was 1 cm^2/350 mL.

2.5 Antimicrobial testing

Gram-negative bacteria, *Escherichia coli* ATCC25922 (*E. coli*), and Gram-positive bacteria, *Staphylococcus aureus* ATCC25923 *(S. aureus*), used in this study were obtained from a preserving center for bacterial species. Each SLM sample was placed in individual wells of a 24-multiwell culture plate. 50 μL of bacterial suspension with a concentration of 1×10^6 CFU/mL was dropped directly onto the sample, and then incubated at 37 ℃ for 24 h. After contact with the samples, the bacterial suspension was removed using agitation and diluted. 0.1 mL of this diluted bacterial suspension at a range of dilution points was then inoculated onto agar plates and spread evenly; these were then incubated at 37 ℃ for 24 h. Finally the number of bacteria colonies on the plate was counted to evaluate the antimicrobial ability of SLM samples by means of bacteria survival rate, which is equivalent to the colonies on SLM samples (CoCr or CoCrCu)/colonies on control culture medium.

2.6 Live/Dead BacLight bacterial viability staining

In addition, biofilms were observed by using the LIVE/DEAD® BacLight TM Bacterial Viability Kit L7012 (Invitrogen, Molecular Probes, Inc, Eugene, OR, USA) according to the manufacturer's instructions. The kit consists of two stains, propidum iodide (PI) and SYTO-9, which both stain nucleic acids. When used alone, green fluorescent SYTO-9 generally labels all bacteria in a population, whereas red fluorescent PI only penetrates bacteria with damage membranes, causing a reduction in the green SYTO-9 stain fluorescence. Thus, with an appropriate mixture of the SYTO-9 and PI, bacteria with intact membranes stain fluorescent green, while bacteria with damaged membranes stain fluorescent red. 1 mL of bacterial suspension was dropped onto the tested SLM samples in each well of the 24-multiwell culture plate，which then was incubated at 37 ℃ for 24 h. After incubation, the bacteria adhere to the surface of the sample to form a biofilm. Before staining, the sample surface was washed gently for three times by using 1 mL phosphate buffer saline (PBS) to remove any significant traces of culture medium, planktonic and loosely bound bacteria. 200 μl of the staiming solution comprised of 1.5 μl SYTO-9, 1.5 μl PI and 1 mL sterilized distilled water were applied onto the sample for 15 minutes in dark at room temperature. After that, the dyed sample was observed on a CLSM (Confocal Laser Scanning Microscope MTC-600 from America) at 514/488 nm in argon laser.

2.7 Cytotoxicity assay

The cytotoxicity of the new SLM CoCrCu alloys was determined using the MTT assay. A total of 2×10^4 cells/cm^2 rat marrow mesenchymal stem cells (rMSCs) were seeded on all the SLM samples. The cells were cultured in the DMEM culture medium supplemented with 10% (v/v) fetal bovine serum (Biowest, France), antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin), and 2m$_{ML}$-glutamine, and incubated at 37 ℃ in an atmosphere of 5% CO_2 and 95% air for 1, 3 and 7 days. The culture medium was changed every 3 days. The MTT solution was prepared by adding thiazolyl blue tetrazolium bromide powder into the phosphate buffered saline (PBS, OXOID Limited, England), and 10 mL of 5 mg/mL MTT solution was added on the first day. The culture dish was then incubated at 37 ℃ under 5% CO_2 and 95% air for 1 day. 100 μL of 10% sodium dodecyl sulphate (SDS, Sigma, USA) in 0.01 M hydrochloric acid was added to each well and re-incubated at 37 ℃ in an atmosphere of 5% CO_2 and 95% air for overnight. Lastly, the absorbance was recorded by a multi-mode detector on the Beckman Coulter DTX 880 at a wavelength of 570 nm with a reference wavelength of 640 nm. The cell viability was determined by the absorbance readings.

2.8 Statistics

All the experiments in this work were conducted in quintuplicate, and data were analyzed by one-way ANOVA. The values were expressed as means ± standard deviations and statistical significance was considered when the p value < 0.05.

3. Results:

3.1 Elemental distribution and Microstructure analysis

Since the antimicrobial ability of CoCrCu alloy would be attributed from the Cu in the alloy, the distribution of Cu within the alloy's microstructure could directly influence its antibacterial performance. A homogenous microstructure is desirable for this new alloy with Cu as a new ingredient. To investigate whether or not the SLM process can produce a homogenous Cu in CoCr alloy, EDS was performed on a polished Cu-CoCr alloy section. Fig.1a illustrated that the Cu (along with all the other elements) is in fact, homogeneously distributed in the hosting CoCr alloy while Fig.1b confirms the presence of Cu in the CoCrCu alloy.

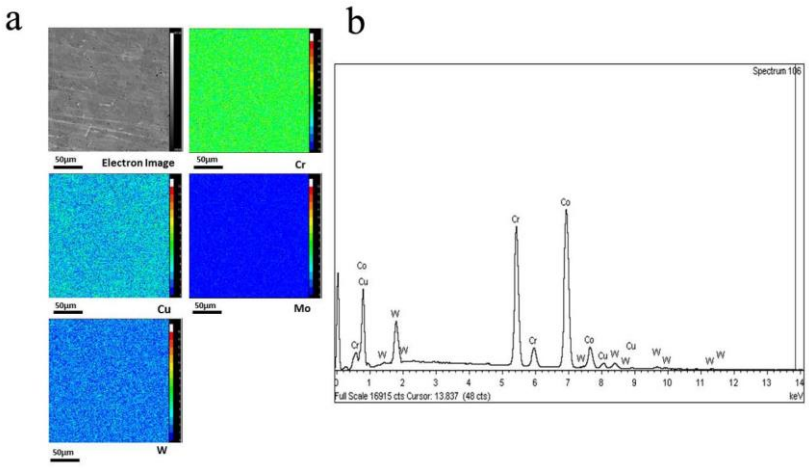

Fig.1 a. EDS maps of metal elements confirming the uniform distribution of Cu and all other elements.

b. Full spectra from the SLM CoCrCu alloy showing the presence of Cu in the alloy

The metallography of etched SLM CoCrCu samples were observed and shown in Fig. 2, consisting of three magnifications done by optical microscopy (OM). On a macro-level, the accumulative rapid solidification of adjacent melt pools revealed a network appearance of stacked melting pools as reflected in Fig. 2a. From magnification images of Fig. 2b and Fig. 2c, some fine grains were clearly visible. The metallography of the SLM CoCrCu alloy is similar to the original SLM CoCr alloy, which has been described elsewhere (Ref?).. Fig.3 demonstrated the XRD patterns of SLM CoCr and CoCrCu alloys. The two profiles were similar and revealed diffraction grams with peaks at 43.9° (2θ), 50.4° and 74.4°, where indicated that the addition of Cu to this alloy did not change the microstructure of the CoCr based alloy.

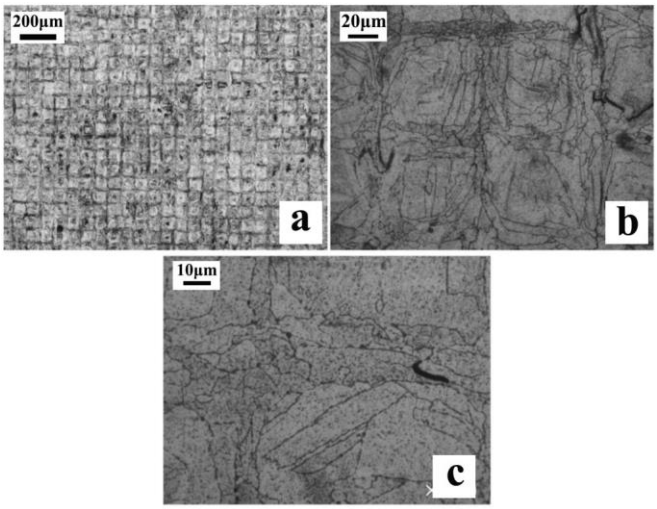

Fig.2 The metallographic images of etched SLM CoCrCu samples consisted of three magnifications: a. 200μm; b. 20μm; c. 10μm

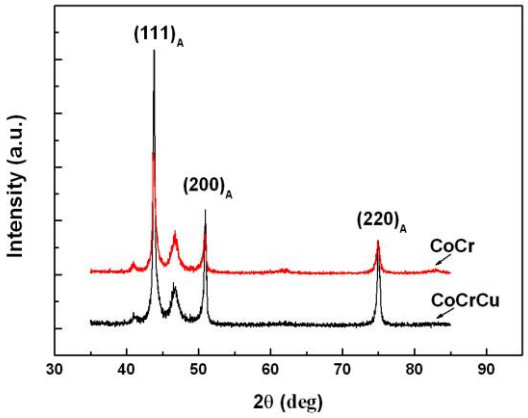

Fig.3 X-ray diffraction patterns of the SLM CoCr and CoCrCu alloys

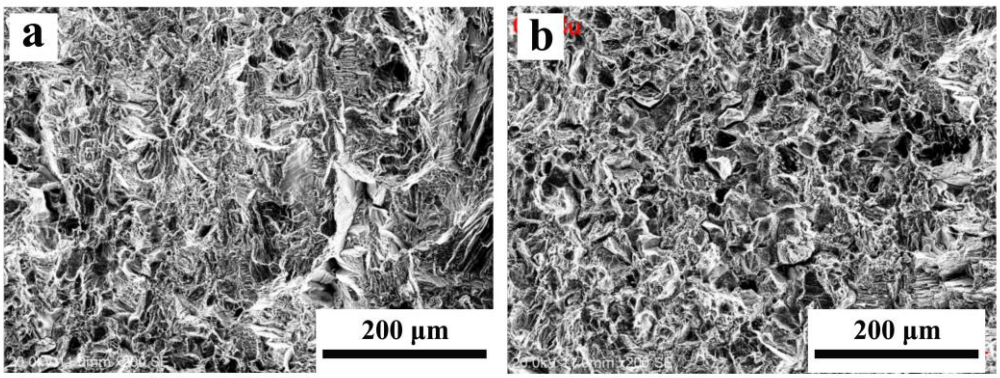

Fig.4 Fractographical images of the SLM CoCr (a) and CoCrCu (b) alloy surfaces after tensile tests.

Table 2 Means (with Standard Deviations) of mechanical properties of SLM CoCr and CoCrCu alloys

Variables	Yield Strength (MPa)	Tensile Strength (MPa)	Elongation (%)
SLM CoCr alloy	565±5.68	1084±12.56	26.5±1.2
SLM CoCrCu alloy	636.5±8.12**	991±13.98	13.5±0.45**

**represent significant differences (p<0.05) between groups

As shown in Table 2, the tensile strength and yield strength of SLM CoCrCu alloy are similar to SLM CoCr alloy. Elongations of the SLM CoCrCu alloys were significantly decreased with the addition of Cu, 50% lower than those of the CoCr alloy. However, the absolute value is still 10% higher than the standards (ISO 22674:2006, Dentistry-Metallic materials for fixed and removable restorations and appliances). Meanwhile, there was no difference from the fractograph or fractured images between SLM CoCrCu and CoCr alloys as demonstrated of the typical metal surface morphologies of the ductal failures in Fig. 4. Or on another words, both of the fractographs exhibited typical ductile dimple fracture pattern, which further proved that the addition of Cu did not affect the mechanical properties of the CoCr based alloys.

3.3 Corrosion resistance

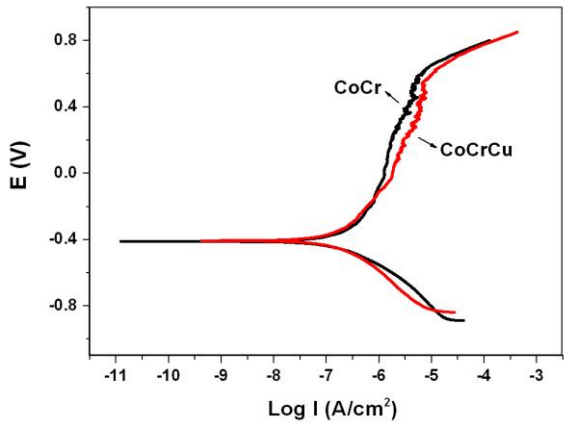

Fig.5 Potential dynamic polarization curves of SLM CoCr and CoCrCu alloys in 0.9wt.% NaCl (or 0.9% mole?) solution

Table 3 Means (Standard Deviation) kinetic parameters associated with corrosion process of SLM CoCr and CoCrCu alloys in 0.9 wt.% NaCl solution

Variables	E_{corr}, mV	I_{corr}, nA	E_{pit}, mV
SLM CoCr alloy	-411.0±22.15	211.8±10.0	582.8±25.0
SLM CoCrCu alloy	-406.2±16.73	217.2±9.45	562.1±22.34

Potential dynamic polarization curves of the SLM CoCr and CoCrCu alloys in 0.9wt.% NaCl solution were plotted in Fig. 5. Cathodic polarizations of alloys were used in combination with the anodic polarization to determine the corrosion current density (I_{corr}), i.e., corrosion rate, free corrosion potential (E_{corr}) and the pitting corrosion potential (E_{pit}). I_{corr}, E_{corr} and E_{pit} of each experimental sample were listed in Table 3. As described from Fig. 5, it revealed that all the polarization curves of the experimental samples, either SLM CoCr or SLM CoCrCu alloy were similar to each other in shape, and the value: The I_{corr}, E_{corr} and E_{pit} were very close, indicating additional Cu in the SLM CoCrCu alloy did not affect the level of performance in corrosion resistance for the alloys.

3.4 Antibacterial result

E.coli and *S. aureus*, are pathogens causing implant-related infection diseases and commonly used to preliminary evaluate the antibacterial property of materials. The SLM CoCrCu alloy was mixed with bacterial suspensions. As shown in Fig. 6, a clear inhibitory effects of SLM CoCrCu alloy were presented on bacterial growth after direct contact. In comparison with the SLM CoCr, the CoCrCu alloy, killed 99.99% *E.coli* and *S. aureus* bacteria, respectively. In contrast, 98% of *E.coli* and 92% of *S. aureus* survived on the surface of SLM CoCr alloy. Obviously, addition of the Cu provided SLM CoCrCu alloy an excellent antimicrobial ability.

3.5 Biofilm inhibition by SLM CoCrCu alloy

Bacterial stain assay was conducted in order to check the inhibitory effect of SLM CoCrCu alloy on biofilms. Representative CLSM surface micrographs of both *S. aureus* and *E. coli* after direct contact incubation at 37 ℃ for 24 h are depicted in Fig. 7. The images showed in general that the lower density of bacterial colonization was formed on SLM CoCrCu samples in comparison to the control ones. Dense biofilms containing high proportions of living bacteria were visible on the control specimens (Fig. 7). After 24 h, only scattered bacterial colonies or isolated bacteria were presented on the SLM CoCrCu samples. In addition, multiple microbial aggregations and multilayer biofilms (thickness is about 22 μm) were visible on the SLM CoCr samples. In opposite, only scattered and damaged bacteria were left, and there was no biofilm formation that could be detectable on the surfaces of the SLM CoCrCu alloy as demonstrated from the images of 3D reconstruction. These results indicated that the SLM CoCrCu alloy is able to inhibit the proliferation of bacteria and biofilm formation.

3.6 Cytotoxicity

The biocompatibility of a novel biomaterial should be properly considered before clinic applications. Therefore, SLM CoCr and CoCrCu alloys were tested with the cell viability test, i.e., cytotoxicity assay, in order to be preliminarily evaluated of the effect of Cu addition on the biocompatibility of the SLM CoCr alloy. Cell viabilities of the experimental samples at different time points are shown in Fig. 8. The test results of SLM CoCrCu alloy in culture medium indicated no significantly differences with SLM CoCr alloy during the test periods of 1, 4 and 7 days, reflecting that the SLM CoCrCu alloy had no cytotoxic effect. Consequently, the addition of Cu not only offered the alloy antibacterial ability but also possessed a satisfactory biocompatibility.

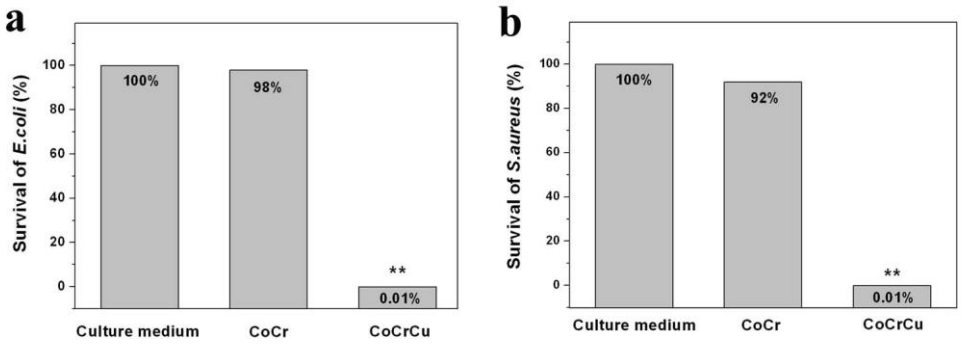

Fig.6 Bacteria survival rates of *E.coli* (a) and *S.aureus* (b) after direct contacting with different groups for 24 h at 37 ℃ to evaluate the antimicrobial ability

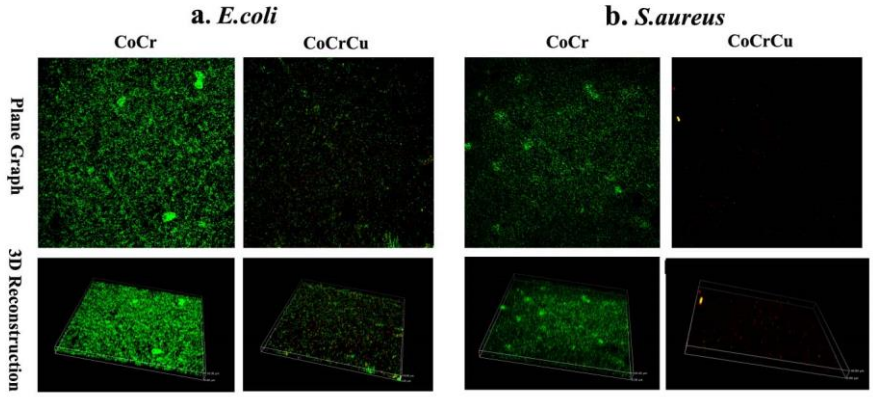

Fig.7 CLSM images (including plane and 3D reconstruction graphs) of *E.coli* (a) and *S.aureus* (b) after direct contacting with SLM CoCr and CoCrCu alloys for 24 h at 37℃ to evaluate the antibiofilm properties

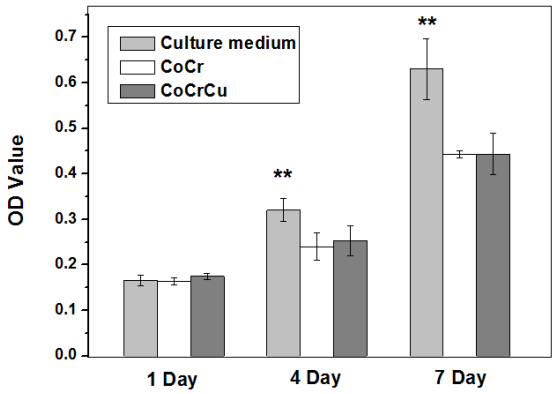

Fig.8 OD values of rat marrow mesenchymal stem cells (rMSCs) for different groups at different time points by using MTT assay

4. Discussion

Within the oral environment, dental materials are preferentially colonized by bacteria with subsequent biofilm formation [22, 23]. Among the bio-active functions proposed for dental materials antibacterial activity seems the most promising [17, 24]. The use of CoCr based alloys has become quite popular in recent years as a coping material for replacement of missing teeth. As the widely used coping material, CoCr based alloy is bio-inert which does not possess antibacterial ability by itself, and therefore easily colonized by bacteria with subsequent development of a biofilm. Although efforts have been devoted to the use of antibiotic treatment, dental implant material associated infection remains an urgent problem that need to be dealt with.

Elemental Cu is not only an alloying element being incorporated into metal in order to enhance mechanical properties, wear resistance and corrosion resistance, but also is well known for its antimicrobial ability since 19th century [20, 21]. Accordingly, appropriated amount of Cu has been immobilized into the bio-inert metallic biomaterials to provide antibacterial ability as shown in previous studies. For example, Cu was added into the medical grade stainless steel (316L, 304 type stainless steel) and titanium (Ti-6Al-4V) during material making process. Results suggested that these Cu-bearing metallic materials killed bacteria and inhibited biofilm formation using *in vitro* and *in vivo* tests, which show promise to address the problem of implant associated infection. These studies revealed the significant inhibitory influence of released Cu ions from the material upon bacterial survival and biofilm formation. Part of the released Cu ion accumulated at the surface, and part diffused to the surrounding liquid environment [25-30]. Based on the above Cu strategy, the previous study immobilized Cu into the cast CoCr alloy during alloy making process. It was found that this cast CoCrWCuNi alloy possessed obvious antibacterial features against both *Escherichia coli* and *Staphylococcus aureus*, and showed biofilm inhibitory effect as well [31].

Based on the above principle and research results, a novel kind of SLM CoCrCu alloy was fabricated in this present work. This SLM CoCrCu has three benefits: first, it has antibacterial ability and biofilm inhibitory effects by itself rather than via surface modification, which could maintain the antimicrobial role for longer ; second, the SLM technology offers the novel chemical design of CoCrCu alloy definitive product density, reduced manufacturing time and costs, minimization of human errors, and the prevention of casting defects; third, the removing of Ni element from the chemical composition of the alloy make it more biocompatible. Thus, the aim of this work was to focus on the antimicrobial ability of this novel dental SLM CoCrCu alloy.

The formation of biofilms on a material usually occurs by two sequential steps. The first is the initial attachment of bacteria to the material surface through van der Waals force using fimbriae or pili. When they adhered on the material, bacteria start to release extra-cellular matrix (ECM), which is beneficial for attachment to the material surface and gives protection from the surrounding environmental conditions. The second step is the proliferation and accumulation of bacteria in multi-layers. This proto-biofilm grows through the initial attached bacteria or bacteria adhesion from the surrounding environment. When the bio-films are large enough, areas of the ECM are degraded with enzymes, which lead to dispersal of a portion of the bio-film, allowing cells to disperse and establish more bio-films [32].

For SLM CoCrCu as presented here, excellent antibacterial ability was shown . When the bacteria were in direct contact with the SLM CoCrCu alloy for 24h, the majority of bacteria were killed, with only 0.01% of *E.coli* and *S.aureus* surviving on the surface. This result suggested that the alloy killed early colonizing bacteria directly and might influence the viability of bacteria in the earlier biofilm. Thus, the initial attachment of bacteria to the material surface is significantly reduced and less bacteria adhered on the material releasing ECM, which could block the subsequent formation of bacterial biofilm as from the initial step as mentioned above. Significantly, the results of CLSM are consistent with the above analysis and demonstrated that SLM CoCrCu alloy strongly inhibited biofilms *in situ*. In contrast to this, bacteria aggregates and multi-layered biofilms were visible on the surface of SLM CoCr alloys. These current qualitative and quantitative assays suggested that this SLM CoCrCu could kill early colonizing bacteria directly and influence the development of biofilm indirectly. In addition to this, the homogenous distribution of Cu and other elements in the alloy by means of SLM technology promote the Cu ions release promoting antibacterial ability from another aspect. Some studies regarding dental materials with strong antimicrobial ability also focus on inhibiting the biofilm in order to reduce related infection.

Christian Apel *et al* developed composite materials containing the biomolecule carolacton, which could inhibit the biofilm growth of *Streptococcus mutans* UA159 and is therefore potentially able to prevent secondary caries formation [23]. Jin Feng *et al* recently developed glass ionomer cement (GIC) containing dimethylaminododecyl methacrylate (DMADDM), which have antibiofilm effects by observing bacterial morphology and biofilm accumulation under oral conditions [17]. Significantly, dental materials related infection should be addressed from the point of biofilm inhibitory effect.

The excellent antibacterial ability of SLM CoCrCu is likely to be attributed to the Cu element. Some studies concerning Cu-bearing biomaterial suggest that it is the Cu that possesses the antimicrobial ability due to the release of Cu ions from the surface of materials. Wang *et al* revealed that the Cu-rich phases on the surface of the alloy promoted the release of Cu ions in the cast CoCrWCuNi alloy which is the reason behind bacterial killing and antibiofilm activity [31]. Chengtie Wu *et al* fabricated copper-containing mesoporous bioactive glass scaffolds with satisfactory antibacterial ability, in which Cu ions contribute to the antibacterial property of this scaffold as used as a bone filling device [33]. Another previous study depicted that the formation of voltaic cells caused the release of Cu ions from the surface of Cu-bearing stainless steel to the surrounding media, playing the role of antimicrobial ability [34]. Therefore, we hypothesized that the release of Cu ions from the surface should contribute to the antibacterial ability for SLM CoCrCu based on the above studies.

Other properties such as mechanical stability, corrosion resistance and biocompatibility are also critical to the novel functional SLM CoCrCu alloy, especially for its future applications in the clinic. For mechanical property, the strengths of SLM CoCrCu alloy are almost identical to that of SLM CoCr alloy. Although the elongation of SLM CoCrCu alloy was lower than that of CoCr alloy, it meets the requirements of dental materials, and there was no difference depicted from the fractographs of the two alloys. The oral environment is an ideal place for corrosion assessment because of the presence of saliva, acid producing bacterial plaque, changes in pH and temperature related to food or beverage intake, and the actions of different medications. Once the corrosion occurs, the corrosion products released from dental restorations can penetrate the enamel, dentin, and gingiva, and cause local symptoms, such as mucositis or even carcinogenicity and mutagenicity [35-39]. Thus, corrosion behavior is an important consideration of biomaterials. The results of potential dynamic polarization curves (Fig. 5) and table 3 demonstrated that the corrosion resistance of the SLM CoCrCu alloy was almost equivalent to SLM CoCr alloy, for both of them meeting the requirement of the corrosion standard for dental restoration materials. Apart from these properties,

the result of XRD also indicated that the microstructure of the SLM CoCrCu alloy was not altered by addition of Cu additive in comparison with the SLM CoCr alloy. Thus, immobilizing an appropriate amount of Cu did not change the original properties of CoCr alloys. What's more important as a novel biomaterial, is the SLM CoCrCu alloy's biocompatibility that should be considered seriously before clinic applications, The cell viability result showed the excellent satisfied cytocompatibility of SLM CoCrCu alloy.

Based on this preliminary study, we could confirm that the additional amount of Cu can not only offer the SLM CoCrCu alloy with excellent antibacterial and biofilm inhibitory ability, but also maintain other beneficial properties of CoCr alloy, which are promising to be used as coping metal materials in the dental clinic. In order to provide a more substantial database for its clinical application, however, some more tests should be conducted thoroughly in the future work. These tests will include the analysis of the Cu ion release level, the *in- vivo* anti-infective evaluation, antimicrobial ability against particular oral bacteria.

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