

Electronic Supplementary Information

Improved Corrosion Resistance and Antibacterial Property of Composite Arch-Wires by N-doped TiO₂ Coating

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Scherrer equation

Scherrer equation is listed as follows:

$$D=K\lambda/\beta\cos\theta$$

Where D is defined as the average size of the crystal; K is the shape factor of crystal (0.89), also known as the Scherrer constant; λ is the wavelength of X rays (for Cu target $\lambda=0.154$ nm); β is the measured full width at half maximum (FWHM) of diffraction peaks of the samples and θ is the diffraction angle. For anatase TiO₂, the XRD peak of (101) was used as to calculate the crystal size. The calculated crystal sizes of TiO₂ and N-doped TiO₂ thin films were around 16 nm.

Cytotoxicity

Materials and method

1.1 Materials

L929 cells (provided by pathology laboratory of Jilin University), DMEM, containing fetal bovine serum (Gibco, USA) and two kinds of antibiotics (penicillin 100000 U/L and streptomycin 100000 U/L, PAA Laboratories GmbH, Australia); 0.9% NaCl (pH 7.0, Jilin dubang company, China); 0.25% trypsin (Amresco, USA) dimethyl sulfoxide (Amresco, USA), high density polyethylene (Shandong longhui company, China).

1.2 Main instruments and equipment

Incubator (Thermo Fisher Scientific, USA); SW-CJ-1 Fsuperclean bench (Suzhou Purification equipment company, China); microscope (Olympus, Japan); LR5-4.2 centrifugal machine (Leiboer company, China); Automatic ELISA test machine

(Gene.5, Biotek, USA); Pressure steam sterilizer (Shanghai Boyi Medical Equipment Plant, China); electrothermal constant-temperature dry box (202-2A, Tianjin Taisite instrument company, China).

1.3 Method

1.3.1 Prepare materials according to ISO 10993.5 and ISO 10993.12

The dimensions of the wires before welding were 30 mm (length)×0.64 mm (width)×0.48 mm (thickness), and the thickness of the pure Cu interlayer was 0.2 mm. CAWs with the same size coated with TiO₂ and N-doped TiO₂ were also prepared. Wafer high density polyethylene (HDPE) with about 2 mm thick, 5 mm in diameter was used as a negative control. All the materials to be tested were soaked in 95% ethanol, and were then oscillated with ultrasonic oscillation for 30 min, cleaned with deionized water, dried, destroyed bacteria with high pressure steam for 30 min.

1.3.2 The preparation of leaching solution

The ratio of the wire and the extraction liquid is 0.2 g:1 ml. Three kinds of metal materials (CAW, CAW coated with TiO₂, CAW coated with N-doped TiO₂) were about 0.8 g. They were soaked in DMEM culture (containing 10% fetal bovine serum, 1% Penicillin-Streptomycin Solution), cultured at a 37 °C incubator with 5% CO₂ for 42 h. HDPE was used as a negative control.

1.3.3 Detection of cytotoxicity with cck8

Preparing cell suspension and counting cell. According to the appropriate planking cell number, inoculate each hole about 100uL cell suspension into 96-well plates. Three batches were prepared to repeat in each group. Culturing at constant

temperature of 37 °C with 5% CO₂ in an incubator for 24 h. The solutions of CAW, CAW coated with TiO₂ and CAW coated with N-doped TiO₂ were loaded to each experimental group. The leaching solution of HDPE was added to negative control group. The cells were cultured for 24h, 72h, and 120 h. Then 10µl of CCK8 was added to each hole. All of them are cultured in the incubator for 4 h. The optical density (OD) value was detected by using spectrophotometer at 450nm.

1.3.4The preparation of cells and test method

When cytotoxicity of dental materials was tested, the use of the standardized cells, such as marine or L929 cell lines, which are usually recommend by the American Dental Association and the International Organization for Standardization (ISO). Because hamster fibroblasts (L929) have short cycle and high cell viability, besides that it could also be obtained easily. The toxicity reaction to metal ions of L929 are similar to that of human gingival fibroblasts, so L929 in vitro is often replaced with gingival fibroblasts for testing cytotoxicity of oral material. According to ISO time standard and the research of most scholars, material leaching time was selected for 42 h, and the extraction ratio of leaching solution was 0.2 g: 1mL. The release of metal ions caused by solution was different, if the pH value is different. Therefore, in this study, pH 7 was used for the cell culture solution.

Cell counting (referred to as Kit-8) (CCK8) method can be used for cell proliferation and cytotoxicity. It is simple and accurate. The basic principle is: the reagent containing WST-8 [chemical name: 2- (2- methoxy -4- nitrophenyl) -3- (4- nitrophenyl) -5- (2,4- two -2H- four with benzene sulfonic acid) monosodium salt], it

electron carrier 1-methoxy-5-methyl phenazine onium sulfate (1-Methoxy PMS) two methyl ester under the action of the reduction of cells in the Yellow formazan product dehydrogenase is highly soluble in water (Formazan dye). The number of formazan products generated with living cells. It can be directly proportional to the analysis cell proliferation and cytotoxicity by using this characteristic.

2. Data Processing

Calculated relative growth rate (RGR) equals to the absorbance values of experimental group/the absorbance values of control group $\times 100\%$. According to the calculated RGR, the cytotoxicity levels of the materials are decided. Level 0: $RGR \geq 100\%$; Level 1: $75\% \leq RGR < 100\%$; Level 2: $0\% \leq RGR < 75\%$; Level 3: $25\% \leq RGR < 50\%$; Level 4: $1\% \leq RGR < 25\%$; Level 5: $RGR < 1\%$. Level 0 and Level 1 are safe levels, while Level 3-5 are unsafe levels. When the cytotoxicity is Level 2, it should be further tested according to the status of the cell culture.