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SUPPLEMENTARY MATERIAL TO

In vitro biocompatibility assessment of Co–Cr–Mo dental cast alloy

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IN VITRO CYTOTOXICITY TESTS

Colorimetric methyl-thiazol-tetrazolium (MTT) test.

The MTT test is based on the ability of mitochondrial succinate dehydrogenase (SDH) to convert yellow 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) into the insoluble, dark purple formazan product in metabolically active cells. The procedure was described in detail previously.[1,2] Briefly, viable cells (2×10^5 cells mL^{-1}) were sown in Petri dishes (50 mm, Centre well, Falcon) which contained disc-shaped Co–30Cr–5Mo alloy. Control samples did not contain the examined metallic material. The Petri dishes with sown cells were thermostated at 37 °C with 5 % CO_2 for 48 h and then the cells were resown in fresh medium. Viable cells (5×10^3 cells 100 μL^{-1}) were sown in 96-well microtiter plates and incubated at 37 °C with 5 % CO_2 for 48 h, 72 h and 96 h. MTT solution (10 μL) was added to each well of the plate and the incubation was continued for a further 3 h. Afterwards, 100 μL of 0.04 M HCl in 2-propanol was added to each well. The absorbance readings were performed immediately after incubation period using a microtiter plate reader (Multiscan, MCC/ /340) at a wavelength of 540 nm with reference to 690 nm. The wells that contained only medium and MTT solution without cells were used as blanks. The fraction of surviving cells (%*K*) was expressed as:

$$\%K = \frac{100N_s}{N_k} \quad (1)$$

where N_s is the number of surviving cells with the examined material and N_k is the number of surviving cells in the control sample.

Dye exclusion test (DET)

The Petri dishes which contained Co–30Cr–5Mo alloy with sown cells were incubated at 37 °C in 5 % CO_2 for 48 h. At the end of the incubation period, the cells were counted in the

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counting chambers after 48 h, 72 h and 96 h using an inverted microscope Reichert–Jung, Biostar, model 1820E. After that, 100 μL of cells was taken and added to 100 μL of 0.1 % trypan blue. After intensive shaking, a few drops were placed on the counting fields of the Neubauer chamber in order to determine the number of cells. Trypan blue painted dead cells but not living ones. The fraction of surviving cells (%*K*) was obtained using Eq. (1).

Agar diffusion test (ADT)

For the purpose of the ADT testing, cells (2×10^5 cells mL^{-1}) were sown in Petri dishes and 10 mL of the suspension was incubated at 37 °C in 5 % CO_2 for 24 h. Sterile agar was heated and a nutrient medium was added. The cells were combined with the agar-nutrient mixture and allowed to solidify over 30 min. The cells were stained with a neutral red solution and kept in the dark for 15–20 min. The Co–30Cr–5Mo alloy discs were placed in Petri dishes and were incubated at 37 °C in 5 % CO_2 for 24 h. Any interaction between Co–30Cr–5Mo alloy and the cells, causing the cells death, was recorded using an inverted microscope. It is well known that living cells retain the red dye. Thus, the decolorized zones of dead cells were measured using a ruler and analyzed according to the ISO 7405 standard.[3] The results were evaluated according to decolourization index and lysis index:

$$\text{Cell response} = \text{decolourization index/lysis index} \quad (2)$$

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