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COMPARATIVE STUDY OF CYTOTOXICITY OF DIRECT METAL LASER SINTERED AND CAST Co-Cr-Mo DENTAL ALLOY

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The presented work investigated the cytotoxicity of direct metal laser sintered (DMLS) and cast Co-Cr-Mo (CCM) dental alloy. In vitro tests were done on human fibroblast cell line MRC-5. There was no statistically significant difference in the cytotoxic effects of DMLS and CCM alloy specimens. The results of this investigation show good potential of DMLS Co-Cr-Mo alloy for application in dentistry.

Key words: cast Co-Cr-Mo alloy, direct metal laser sintering (DMLS), cytotoxicity, dentistry

INTRODUCTION

At first Computer Aided (CA) systems in dentistry were based on milling procedure, whereby requested forms, such as dental devices, were fabricated by milling material from a block [1]. A new group of CA systems, known as additive manufacturing (AM), based on layer-by-layer fabrication of objects from different materials uses a layering additive technique to produce complex shapes and they could be used to fabricate complex shapes of dental devices such as removable partial denture (RPD) frameworks [2-4].

Currently, leading AM technologies include laser sintering techniques (Selective Laser Sintering – SLS, Selective Laser Melting – SLM, Direct Metal Laser Sintering – DMLS, etc.) In DMLS metal objects are produced directly in the building process on the basis of Computer Aided Design data [5]. A high power laser is used to melt a feedstock to form fully dense metalic objects of any complex shape [6].

Cast Co-Cr-Mo dental alloys are in use for many years for dental devices manufacturing and their biocompatibility is well documented. As DMLS can influence the microstructure of the alloy [7,8], the cytotoxicity and biocompatibility of DMLS Co-Cr-Mo dental alloy can be changed.

Materials for dental applications have unique requirements including nontoxicity, biocompatibility, and mechanical properties. Details of some mechanical properties and biocompatibility of AM Co-Cr-Mo alloys are accessible [9-14] but none relate to DMLS alloy cytotoxicity. Cell culture studies of cytotoxicity are the usual first step when evaluating biocompatibility of dental material or new technological procedure such as DMLS.

The aim of this study was to determine the cytotoxicity of the DMLS and cast Co–Cr-Mo dental alloy using human MRC-5 fibroblast cells (American Type Culture Collection CCL 171) and three test methods: dye exclusion test (DET), the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) assay and the agar diffusion test (ADT).

MATERIALS AND METHODS

Co-Cr-Mo alloy test samples (5 mm diameter, 1 mm thickness) were produced with two different technologies, conventional casting method (CCM samples) and by DMLS technology (DMLS samples). Five CCM samples were manufactured from a commercially available non-precious Co–Cr-Mo alloy containing 63,3 wt.% Co, 30,0 w.t% Cr, 5,0 wt.% Mo, 1,0 wt.% Si and trace quantities of Mn, N and C (Remanium 800+, Dentaurum, Ispringen, Germany). CCM samples were gained out of acrylic samples (Palavit G, Haraeus, Hanau, Germany), that were invested and cast according to the manufacturer's instructions. Rema dynamic investment was used (Dentaurum, Ispringen, Germany). Vacuum casting was performed using a Nautilus CC casting machine (Bego, Bremen, Germany). After casting, the discs were divested and blasted with 100 μm aluminium oxide particles, then polished with silicon carbide papers in the sequence 320, 400, 600, 1 200, 1 500, and 2 000. Final polishing was performed using oxide pastes.

Five DMLS samples were fabricated out of Co-Cr-Mo alloy containing 63,8 wt.% Co, 27,7 wt.% Cr, 5,1 wt.% Mo, 5,5 wt.% W and trace quantities of Si, Fe and Mn (EOS SP2, EOS, GmbH, Munich, Germany) using

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Figure 1 MTT eluate testing

EOSINT 270 (EOS, GmbH, Munich, Germany). DMLS samples were designed using SolidWorks 3D CAD software (Dassault Systemes SolidWorks Corp., Concord, MA, USA). CAD data were then sent to Renishaw for production (New Mills, Wotton-Under-Edge, Gloucester, UK). Polishing of DMLS samples was performed at the same way as CCM samples.

The cytotoxicity was measured in accordance with standards ISO 10993–5 and ISO 7405 [15, 16]. Three test methods for evaluation of the cytotoxicity were used: Dye exclusion test (DET), MTT assay and Agar diffusion test (Figure 1). (ADT) [17, 18].

Each product was tested in quadruplicate and the experiment was repeated twice.

Statistical analysis was realised using the SPSS ver. 20,0. The data were statistically evaluated by the Student's t-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results of the DET and MTT assays are presented in Figures 2 and 3, respectively and in Table 1.

The cell number steadily increased during the recovery period for both CCM and DMLS alloys (48 – 96 h), which indicated that no cytotoxic effects were registered in the several cell generations (Figure 2). There were no statistical significant differences between treatments regardless of the recovery period.

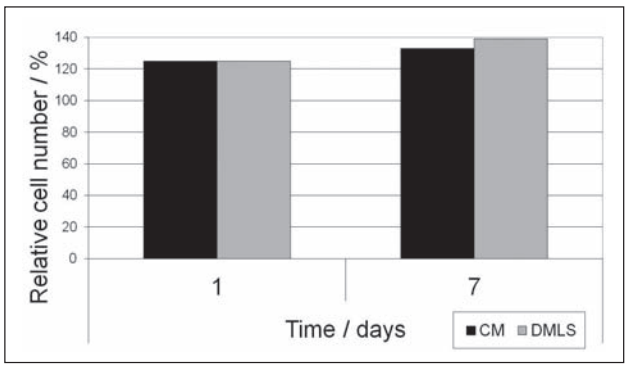

Figure 2 Dye exclusion test (DET). The results show the relative cell number obtained from two independent experiments completed in triplicate. Data are shown as the mean and the bar indicates the standard deviation (p > 0,05)

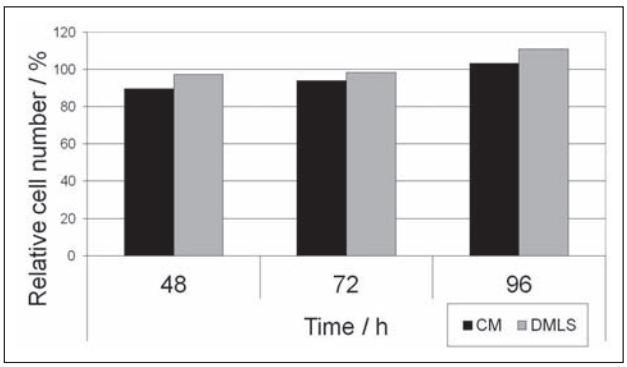

Figure 3 MTT Assay. Survival of MRC-5 cells pre-incubated with CCM and DMLS alloys for 1 and 7 days. The results represent the relative absorbance of the pre-treated cells obtained from two independent experiments, completed in quadruplicate. The data are shown as the mean and the bar indicates the standard deviation (p > 0,05)

The results of the MTT eluate testing after an extraction period of 1 and 7 days indicated no cytotoxic effects of the CCM or DMLS alloys against MRC-5 cells (Figure 3, Table 1). Optical microscope image of the human cell line MRC-5 after the testing period can be seen in Figure 4.

Differences between the growth inhibitory effects of CCM and DMLS alloys were found but the growth inhibition level was not statistically significant ($p > 0.05$). Therefore, both alloys can be rated as non-cytotoxic.

The results of two independent ADT with CCM and DMLS alloys showed no detectable discoloration neither around nor under the discs, or a detectable difference in the staining intensity. As the cell response to both the CCM and DMLS alloys was 0/0, the discs can be rated as non-cytotoxic.

Safe use, non-irritant and nontoxic effect on oral tissues and body as a whole, is a necessary feature of the dental materials. Fixed or mobile prostheses stay in the mouth for a long time, and the attribute of biocompatibility must be maintained for the duration of their use.

Table 1 **Results of statistical analysis of MTT eluate testing of CCM and DMLS disc samples**

	Period of cell incubation /day	1		7	
Descriptive statistic	Technology	CCM	DMLS	CCM	DMLS
	Count	15	15	15	15
	Average	0,547	0,547	0,854	0,893
	Standard deviation	0,024	0,057	0,221	0,120
	St. error mean	0,006	0,015	0,057	0,031
	Minimum	0,500	0,440	0,110	0,700
	Maximum	0,590	0,710	0,990	0,990
Comparison of means	t- test	0,114		0,599	
	P value	0,997		0,554	

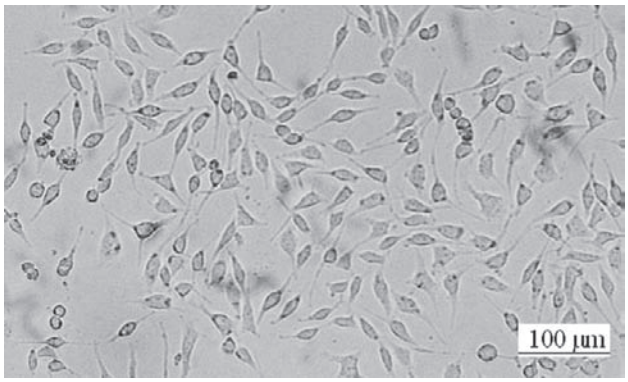

Figure 4 MRC-5 cells, (OM)

Generally, new materials should be evaluated using in vitro, then in vivo testing to ensure their safety before commercial use. In this study, cytotoxicity was examined by three test methods: viability was determined by ADT and DET assays, and cell survival by DET and MTT assays. The DET and ADT methods are based on the breakdown of membrane integrity, the MTT method focuses on the mitochondrial function [17]. The MTT assay showed a similar cellular proliferation after 1 day, and slightly better outcome for the DMLS alloy, which showed no significant damage to the cell function. ADT and DET assay showed no membrane lyses. The obtained results suggested that both alloys did not release harmful material that could cause cytotoxicity effects against experimental cell line.

A few studies concerning the ion release from the cast and AM samples, revealed the more suitable behaviour of the AM specimens [7,19]. The AM specimens showed lower emissions of ions than the cast specimens, probably because the laser-melted material is more homogeneous, contains less pores and has a finer microstructure [8,20]. Also, the use of alloys that is not in accordance with the manufacturer's recommendations, as well as recasting, can change its mechanical characteristics, structure and reduce its biocompatibility [21,22]. It is well known that the presence of nickel in the alloy is not desirable [21-24]. In addition to nickel, beryllium is also responsible for the poor biocompatibility of the alloy [25]. In this study, experimental alloys contain no Ni or Be that can be one of the reasons for good biocompatibility. It is documented that AM manufactured Co-Cr-Mo alloys have fine microstructure [8]. Homogeneity and fine microstructure can also have a positive impact on biocompatible properties of the alloy. Finally, better and modern technological processes of purification, processing and polishing of the alloys are resulting in reduced cytotoxicity.

CONCLUSIONS

AM technologies are advancing rapidly in dentistry. These technologies are making manufacturing of dental devices easier, faster, cheaper and more predictable.

The technological procedure (conventional casting or direct metal laser sintering) can influence the properties of Co-Cr-Mo dental alloy and may have a significant impact on the stability of dental devices in the complex environment such as oral cavity.

In vitro tests on human cell line MRC-5 employed in this study showed that both alloys conventionaly cast and DMLS Co–Cr-Mo alloy exhibited no cytotoxic effect. Data also indicated that there was no statistically significant difference in the cytotoxic effects of conventional casted samples and samples fabricated by DMLS. The results indicate that DMLS of Co-Cr-Mo alloy didn't have negative effect on in vitro citotoxic behavior of the samples.

The results of this investigation show good potential of DMLS Co-Cr-Mo alloy for application in dentistry.

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Note: The responsible translator for English language is Tanja Todorović, Faculty of Technical Sciences, University of Novi Sad