

TOXICITY AND BIOCOMPATIBILITY OF STEEL COATING SAMPLES

Jana FRANKOVÁ^a, Jitka ULRICHOVÁ^a, Marta HAJDUGA^b, Maciej HAJDUGA^c, Dariusz SOŁEK^d,
Dariusz JEDRZEJCZYK^c

^a Palacky University Olomouc, Faculty of Medicine and Dentistry, Department of Medical Chemistry and Biochemistry, Olomouc, Czech Republic, EU, frankova0@seznam.cz, JitkaUlrichova@seznam.cz,

^b Medical University of Silesia, Faculty of Medicine and Dentistry in Zabrze, Zabrze, Poland, EU, martahajduga@wp.pl

^c University of Bielsko-Biala, Bielsko-Biala, Poland, EU, mhajduga@ath.bielsko.pl, djedrzejczyk@ath.bielsko.pl,

^d Concept Sp. z o.o., Bielsko-Biala, Poland, EU, dar_80@tlen.pl

Abstract

To control the spread of pathogens in hospital environments, good hygienic routines based on cleaning and disinfection of surfaces contaminated with biological materials, are obligatory requirements. Ambulance transporting patients with infectious diseases may often be contaminated because of the special construction and the narrow space inside, ventilation system, and all the devices present. This problem is well discussed, but little is known about the car surfaces, toxicity and biocompatibility of ambulance materials.

In our study we focused on toxicity and biocompatibility of seven steel coating samples (antibacterial coating, galvanic zinc coating, zinc flake - lamella coating, polyester - epoxide coating, without antibacterial additives: brown, green and blue-green) that can be used as internal material of ambulance. For toxicity determination, accredited test of direct contact and test of extracts were used. We found that five samples were not toxic. For two toxic samples (galvanic zinc coating, zinc flake - lamella coating) we provided additional tests (tests of extract) to determine the toxicity level. The non-toxic samples will be used for the biocompatibility assay.

Keywords: Fibroblasts, toxicity, steel, zinc, ambulance

1. INTRODUCTION

Patient safety has been identified as a high-priority area for improvement in health care. Cabin surface of ambulance cars transporting hospitalized patients are at risk of contamination that can be caused via ambulance car surfaces, ambulance car staff and patients at risk [1]. The medical interior of transport vehicles has numerous surfaces that are easily contaminated and difficult to clean and disinfect effectively [2, 3]. These surface materials are still in innovation to found the materials to protect adhesion of bacterial strains and also are not toxic.

For the toxicity tests, several methods are recommended. Direct contact assay and test of extracts were evaluated according to ISO 10993-5, the guidelines for "Biological Evaluation of Medical Devices" [4]. The cells incubated in direct contact with sample are a valid method for estimating the cytotoxicity of new materials. It can indicate the potential problems in vivo. Test of extract evaluate potential adverse effects of materials or medical products or that any substance are released from tested materials. Criteria for cell selection are also required. International standard preferred the use of established cell lines such as Balb/3T3, L-929 etc. [5].

The aim of the study was to determine and compare the cytotoxicity and biocompatibility of seven coating steel samples.

2. MATERIALS AND METHODS

2.1 Sample preparation and characterization

Seven different steel coating/varnish samples were prepared in dimensions 100x100x1.5mm. The mentioned samples were covered with varnish/paint and zinc coatings on cold rolled steel base grade DC01 - fine grains. RAL (Reichsausschuss für Lieferbedingungen) is the system of colours marking according to comparison with standards. The surface before zinc coating was mechanically grinded - electrocorund with grain size 120 and polished mechanically with cotton. For the cell cultivation and extraction, the samples were sterilized in 96% ethanol and 15 min by UV irradiation.

2.2 Cell culture

Mouse fibroblasts cells (NIH/3T3) were obtained from American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% of fetal calf serum (FCS), 100µg/ml penicillin and 100µg/ml streptomycin at the standard cultivation condition 37 °C and 5% CO₂.

2.3 Direct Contact Assay

Samples toxicity tests were conducted using the cells NIH/3T3 according standards: ISO 10993 and ISO 7405 (2009).

The cell line NIH/3T3 divides, multiply and undergo adhesion to the substratum. If the base exhibits cytotoxic properties, then it will affect mentioned processes, leading to cells damage, their separation from the surface and reduce total cells numbers in the culture. Evaluation of cytotoxicity consists in cells cultures observation after introduction of purple dye and microscopic examination of cells morphology changes.

For comparison cell cultures were cultivated also on samples made of copper (positive control sample) and titanium (negative control sample).

2.4 Test of extracts

Samples toxicity tests were conducted using the mouse fibroblast cells NIH/3T3 according standards: ISO 10993 and ISO 7405 (2009).

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium salt) method was used to analyse the viability and proliferation of the cells. MTT is taken up through endocytosis, reduced by mitochondrial enzymes and transported to cell surface to form formazans. Coloured product was detected by photometer at 540nm.

The samples were extracted 24 hours in serum free cultivation medium at 37 °C. Extract was diluted in ratio 1:1 (50%), 1:3 (25%), 1:9 (5%) and 1:99 (1%). Triton X-100 at the final concentration 1% in serum free medium was used as a positive control sample and polystyrene as a negative control sample.

3. RESULTS AND DISCUSSION

Seven steel (DC01) samples with different varnished and zinc coating were evaluated to determine the level of toxicity. For description of the samples see **Table 1**. During surface investigation the roughness Ra and 3D topography was determined by application of the profile measurement gauge - Perthometer Concept (MAHR) - **Fig. 1**.

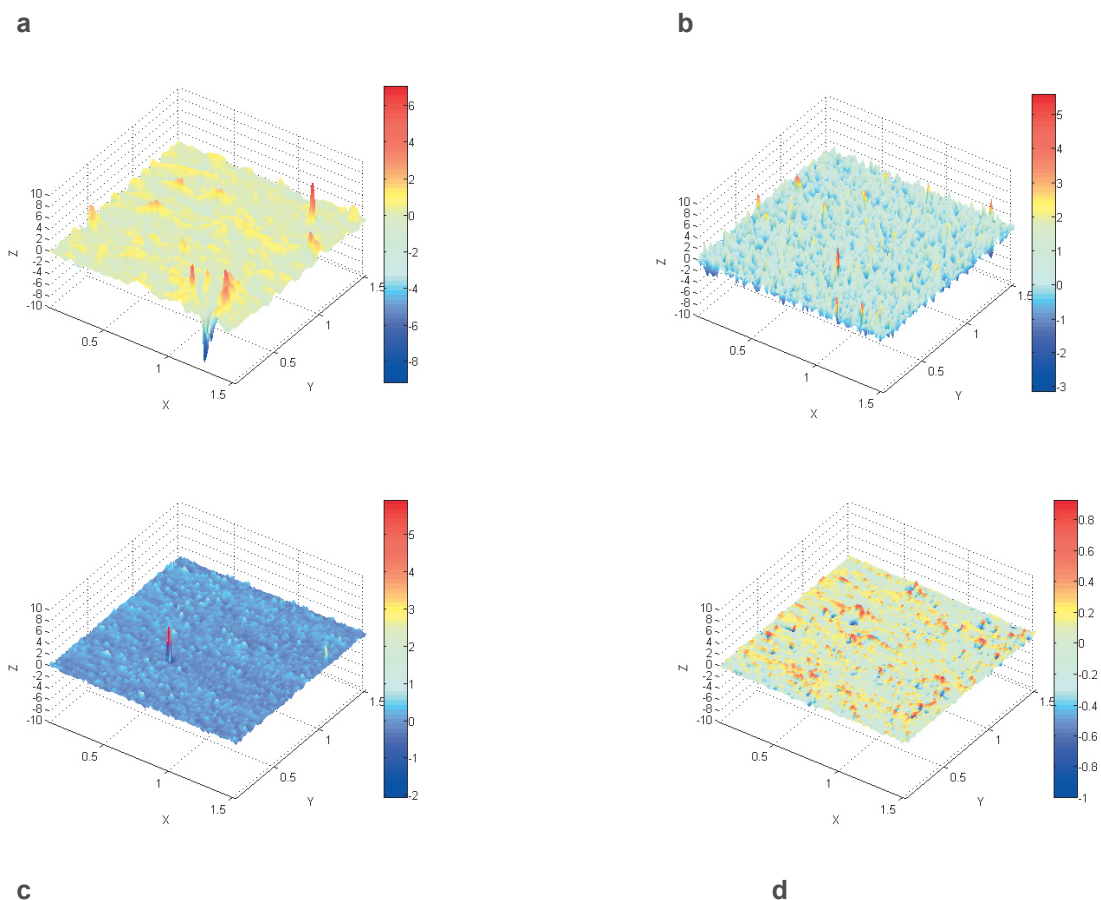


Fig. 1 The 3D topography of investigated surfaces: a - with galvanic Zn coating; b - with lamellar Zn coating; c - with antibacterial paint; d - painted with RAL 9002

Table 1 Description of the samples

| Sample | Type of coating | Colour | Description | Ra [μm] |
|--------|-----------------------|-----------------------|---|---------|
| 1 | antibacterial | light yellow | Bis (2,3-epoxypropyl) terephthalate; Tris (oxiranylmethyl) benzene-1,2,3-tricarboxylate | 0.15 |
| 2 | zinc | gold | galvanic zinc | 0.42 |
| 3 | zinc | silver | slice (lamellar) zinc | 0.56 |
| 4 | Without-antibacterial | white (RAL 9002) | polyester-epoxide varnish with white colour | 0.16 |
| 5 | Without-antibacterial | brown (RAL 8028) | polyester-epoxide varnish with brown colour | 0.16 |
| 6 | Without-antibacterial | green (RAL 6024) | polyester-epoxide varnish with green colour | 0.16 |
| 7 | Without-antibacterial | blue-green (RAL 5018) | polyester-epoxide varnish with blue-green colour | 0.16 |

We found that five antibacterial coating samples were not toxic after direct contact test (**Fig. 2**).

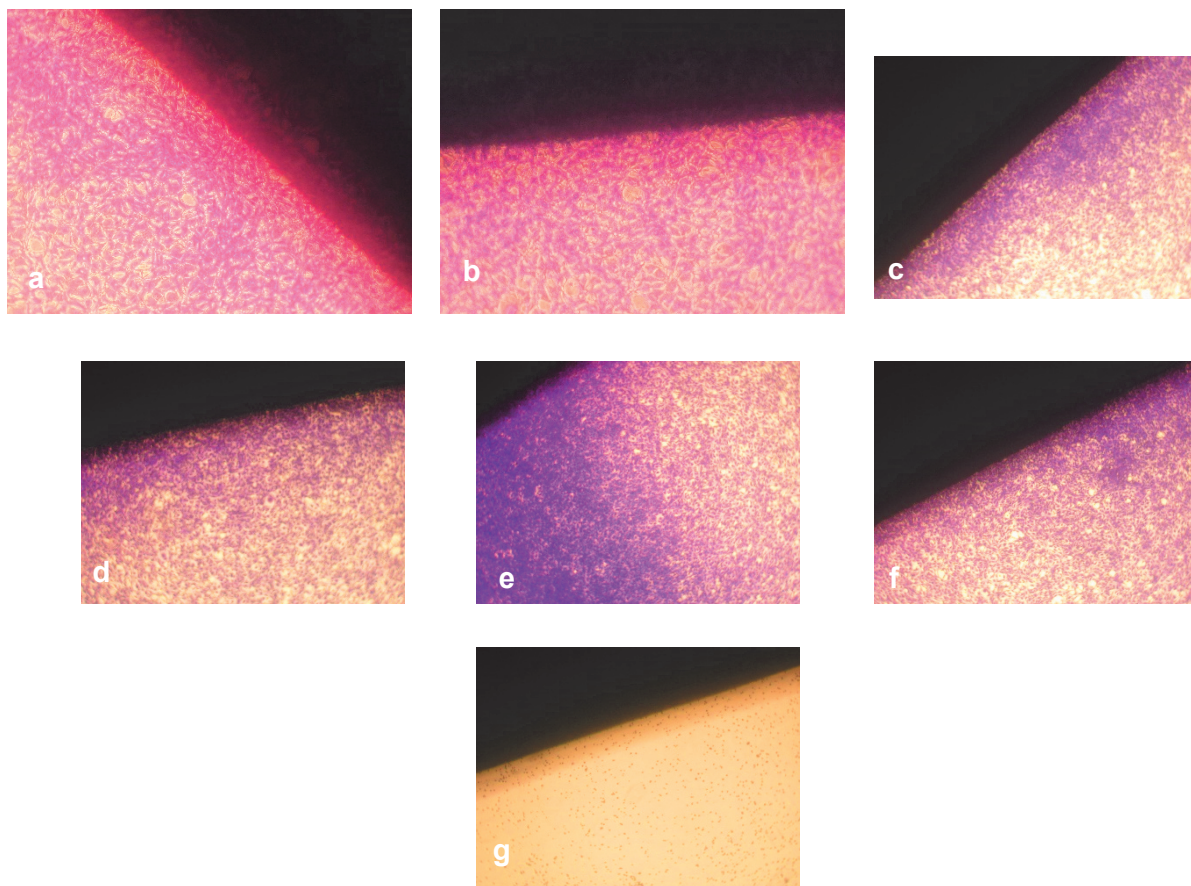
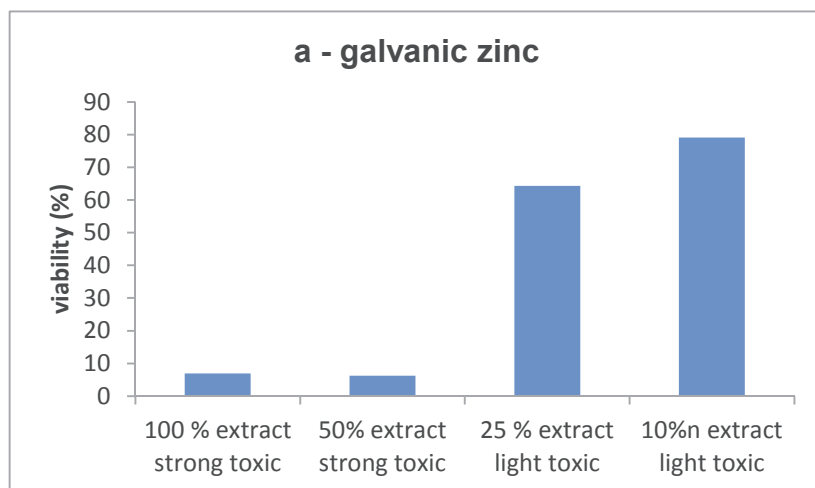


Fig. 2 Direct Contact Assay: a - antibacterial; b - RAL 9002; c - RAL 8028; d - RAL 6024; e - RAL 5018; f - negative control; g - positive control

Two zinc coating samples (sample number 2 and 3) were found toxic and due to the high mobility of the samples on the Petri dishes we cannot detect the level of toxicity by direct contact assay. For the evaluation of toxicity we used the test of extract. Prepared extract was incubated with the cell 24 hours, MTT mixture was added and after incubation period the absorbance of colour product was measured at 540nm. The toxicity level was calculated from absorbance (**Fig. 3**).



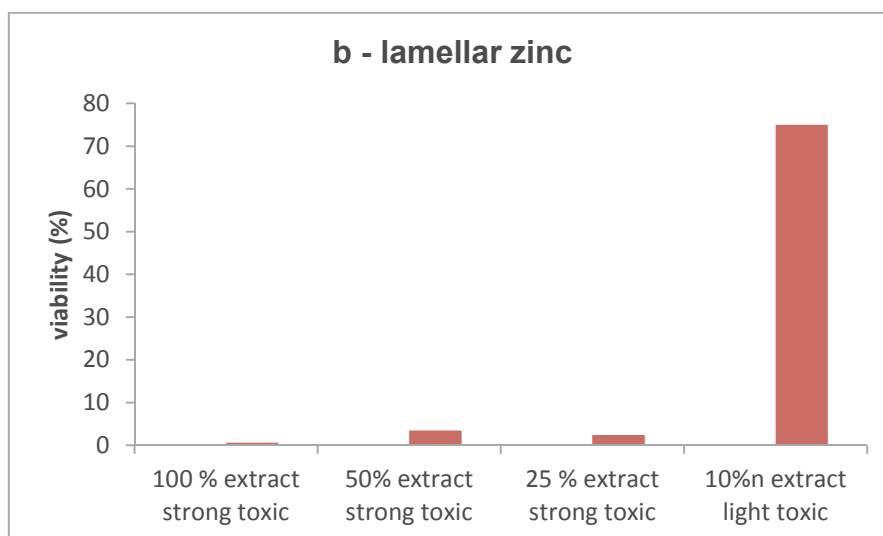


Fig. 3 Test of extract: a- galvanic zinc; b - lamellar zinc

CONCLUSION

Cytotoxicity of seven steel coating samples was evaluated by direct contact assay and by test of extract. We concluded that five samples were not toxic. Two zinc coating samples have from light to strong toxicity depending on dilution of stock extract solution. To study biocompatibility and in long term cultivation assessments, only five no toxic samples will be used.

ACKNOWLEDGEMENTS

This project was supported by grant MOBILITY (74MB13PL034) from the Ministry of Education, Youth and Sports.

REFERENCES

- [1] ANDERSEN, B. M., RASCH M., HOCHLIN K., JENSEN F. H., WISMAR P., FREDRIKSEN J. E. Decontamination of rooms, medical equipment and ambulances using an aerosol of hydrogen peroxide disinfectant. *Journal of Hospital Infection.* 62, 2006, 149-155.
- [2] GALTELLI, M., DESCHAMP, C., ROGERS, J. An Assessment of the Prevalence of Pathogenic Microorganism in the Rotor Wing Air Ambulance: One Program's Findings. *Air Medical Journal.* 25 (2), 2006, 81-84.
- [3] EIBICHT, S. J., VOGEL U. Methicillin-resistant *Staphylococcus aureus* (MRSA) contamination of ambulance cars after short term transport of MRSA-colonised patients is restricted to the stretcher. *Journal of Hospital infection.* 78, 2011, 221-225.
- [4] SUN, L., HUANG, X., SUO, J., FAN, B., CHEN, Z., ZANG, W., LI, J. Biological evaluation of a novel copper-containing composite for contraception. *Fertility and Sterility.* 95 (4), 2011, 1416-1420.
- [5] SAW, T.Y., CAO, T., YAP, A.J., NG, M.M.L. Tooth slice organ culture and established cell line culture models for cytotoxicity assessment of dental materials. *Toxicology in vitro.* 19, 2005, 145-154.