BIOCOMPATIBILITY OF METALS AND THEIR SURFACES

HOMOLKOVÁ Monika, BŘEZINA Vítězslav

University of South Bohemia in České Budějovice, FFPW, CENAKVA, České Budějovice, Czech Republic, EU
Institute of Complex Systems, Nove Hrady, Czech Republic, EU
homolkova@frov.jcu.cz, Vitezslav.brezina@gmail.com

Abstract

Metals and their alloys are often used in the production of implants or stents, and are applied to a living organism. For this application it is necessary to provide for biocompatibility, i.e. determine the likely response of the organism to the embedded metal material. Reactions of cell populations in vitro or ex vivo are primary biocompatibility tests. Determination of cytotoxicity is inefficient for these purposes; it is necessary to find out the degree of tolerance of the cell population to the implanted metallic material before the subsequent tests are conducted on experimental animals. Special test trials in patients with allergy to some of the components of the selected alloy are the peak of the pyramid. In the statement we deal with reaction of animal cell lines to certain metals and alloys used in medicine that are examined in the system of time lapse cinephotomicrography of live cell populations in vitro. Cell lines are scanned in the direct contact with a metal, possibly metal alloy for 72 hours, and subsequently the direct reaction of biocompatibility is evaluated.

Keywords: Metals, metal alloys, cell line, biocompatibility

1. INTRODUCTION

Metals, their alloys, or composites of various materials, as well as a number of other materials are significant in health care. They are used for production of implants, either dental or orthopedic, and also for production of stents. In dentistry are also used as an alloy for making dentures. Their use in health care is extensive. Biocompatibility is their key feature. Naturally, materials for health care are carefully monitored by state authorities [1]. On the other hand, it is necessary to point out that even though the material for dental implants is recognized as biocompatible and non-toxic, it does not say anything about tolerance of the cells of a particular tissue to the material. Cell tolerance of material is a property that significantly determines convenience of e.g. osseointegration of such implant. The content of the present communication is the way the cell tolerance towards material can be expressed by testing it in on living cells in vitro, or ex vivo. The term "bio-tolerance" was used by professor Půža in the seventies [2]. As it turned out, this term is a much better replacement of the term "toxicity" or "biocompatibility" for materials intended for implantation into the body that must be non-toxic. This applies mainly to scaffolds when the material is listed as non-toxic by previous tests and is also tolerated by cells. In this case, there is an additional parameter, namely the way the cells behave on the accepted material in the three-dimensional shape. In any case, it is inevitable to evaluate several basic parameters of cells and materials interaction. It is the growth potential and its reduction or acceleration, then the ability of the cell population to adhere and reconstruct the cytoskeleton; determination of mitotic activity and mitotic atypia, and the capacity to colonize the surface of the material. To achieve this, a number of methods can be used. We are going to present these in response to the results based on them. It should be noted that the techniques of the extracts are not only used for materials in health care, but are useful in general.

2. METHOD DESCRIPTION

The basic method is "live cell imaging", which with the help of collecting cinephotomicrography, enables faster and more precise analysis of the ongoing action. Additionally, it leaves pictorial record of the action, which can be re-analyzed. The video recording is captured by a camera, which is connected to the optical output of a

microscope. The camera is controlled by a timer. This allows to make images at selected intervals. The microscope itself is equipped with one of the optical contrast [3]. An object, which is the material sample and the cell, is placed in a way that enables us to ensure cultivation conditions of the used cell line or the explanted tissue. Sophisticated microscopes include all these features, moreover it is possible to record several fields of vision from a single dish. It facilitates the quantification assessment of cell behavior. The temperature in the culture device of the microscope is 37 ° C with humidity of 90% with admixture of 5% CO 2, which are necessary conditions for culturing cell lines.

2.1. Extract Method

The first method is a method for displaying the behavior of cells in the environment of an extract of the tested material. It has two variants. Either the cell suspension is inoculated with a mixture of the extract; or the extract is added to the grown cell population. We prefer the first option because the adherence of the cells to the bottom of the culture dish can be deducted. This is the basis for evaluation of cell expansion (spreading) [2] thus also for assessment of the speed at which restitution of the cell cytoskeleton and full function of the cell population continues.

2.2. Method of Direct Contact

The second method allows the cell reaction at the current exposure of the sample material. It again has two variants. The first inoculates the cells to the sample material, and the time response of the cell population in the vicinity of the material is recorded. The second one places material sample on the grown cell layer. Again, we prefer the first variant, which reveals the dynamics of cell adhesion, followed by either toxic or stimulatory reactions.

2.3. Method of cell colonization of substrates

The third method is a display of the cell population on the surface of the tested sample material. There are again two options. The first is stationary, it does not use collection cinephotomicrography, but offers insight into the variability of the sample surface colonization. [4] It consists of culturing the cells on the sample surface usually for three days, or rather for three consecutive cell divisions of the cell. Then, the culture is interrupted, cells are fixed, stained and the area occupied by cells is determined. The second option is based on the cinematographic cell scanning on the surface of the sample. This method is more suitable for qualitative image analysis - that is, to assess how the cell reacts to the surface of the structured surface. It is suitable for research on cell action on scaffolds or reaction of the tissue ex vivo.

Quantification of records allows both parametric statistical tests, as well as non-parametric.

3. METHOD RESULTS AND DISCUSSION

Here are examples of the results of these methods, carried out in an accredited laboratory.

3.1. Exctract Method

The method is based on culturing the cell line in the obtained extracts, which results in relationship between the dose and effect. Extracts for this method were made of four metal alloys **Fig. 1.** The used cell line was L929 cultured in DMEM medium (with Earle's salts, without L-glutamine, GE Healthcare company, with the addition of 10% fetal bovine serum, PAA company). The cell line was inoculated into samples of the extracts and recorded at given intervals for 72 hours. Simultaneously with extracts, a control was prepared, which was the L929 cell line cultured in DMEM medium with its appendages. Dilatation ability in the given extracts **Fig. 1** was observed for a determined period of time.

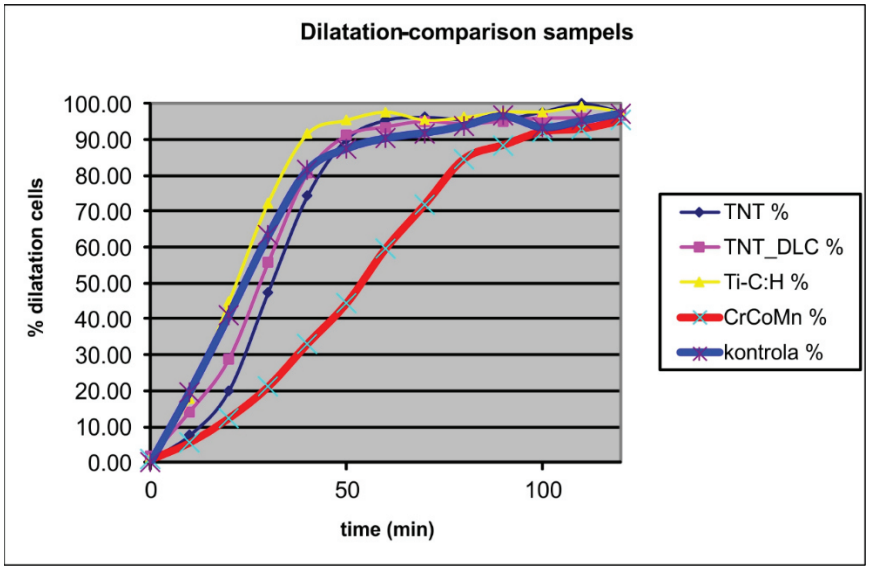

Fig. 1 Graph of expansion curves - comparison of extract samples following monitoring period

Dilation of L929 cell line in three of given extract samples was almost identical with the control extract an hour after inoculation. These observed values demonstrated a quick reconstruction of the cytoskeleton. CrCoMn sample extract revealed lower values at the same observed time.

Another indicator of this method was the ability of mitotic activity expressed as specific growth rate observed for 72 hours **Fig. 2**. This indicator shows us the link with the timeline and increase in the cell population. Thirteen extracts from samples of alloys have been made, while the last fourteenth one was control. Extracts from samples manifested very different values of mitotic activity and thus different specific growth rate.

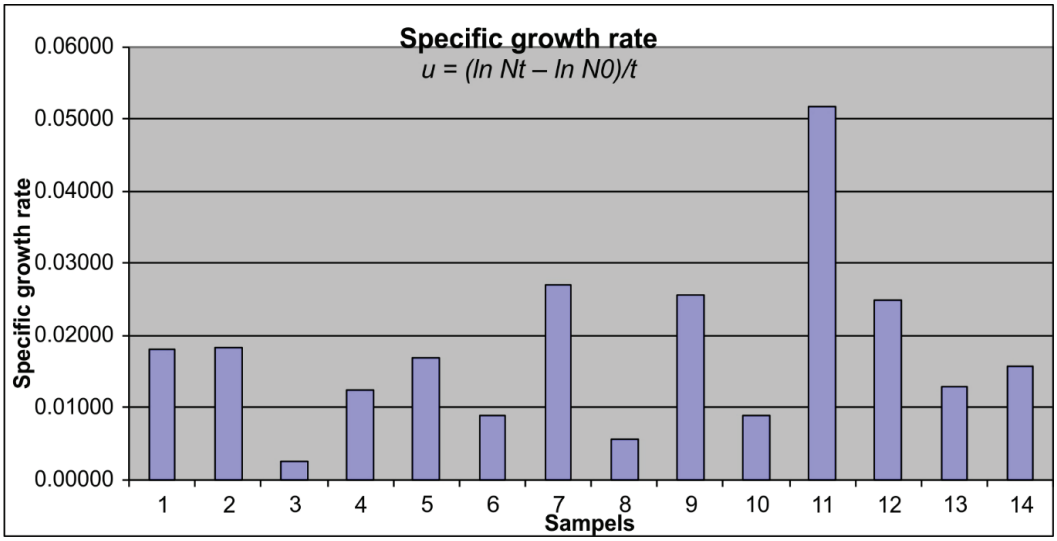

Fig. 2 Graph of specific growth rate for individual extract samples including control.

3.2. The Direct Contact Method

This method is based on culturing cell lines together with the sample material so that the cell reactions are photographed sequentially and are evaluated from an image record by determining the dead zone or otherwise by contacting the cells with the test material in the specified time interval. For results comparison copper (Cu) **Fig. 3**, alloy TiGr2 (pure titanium) with a polished surface **Fig. 4** and aluminum sample (Al) **Fig. 5** were used

as samples. The samples were placed in a petri dish and subsequently the cell line in the culture medium was inoculated. In this case heteroploid cell line MG63 was chosen (originated from the human osteosarcoma) cultured in MEM (minimum essential medium with Eare salts, without L-glutamine, GE Healthcare company, with the addition of 10% fetal bovine serum, PAA company). MEM culture medium is still most frequently used, even for the specific cell types, differentiating in culture conditions [5]. Samples were read in a special culture microscope, with a supply of 90% air humidity with addition of 5% CO2 and temperature-controlled at 37 DEG C., at a size of 10 x objective lens for the period of 24 and 72 hours. From the following records the level of cytotoxicity could be determined. In Cu sample dead zone of the cell line was found for the recorded scanning tome of 24 hours, which confirmed the cytotoxicity of the sample. By contrast, evaluated sample TiGr 2 showed a direct contact of live cells and therefore it was classified as a non-cytotoxic. For Al sample, zone of dead cells in close contact with the material was observed, wherein with increasing distance zone of live cells was recorded.

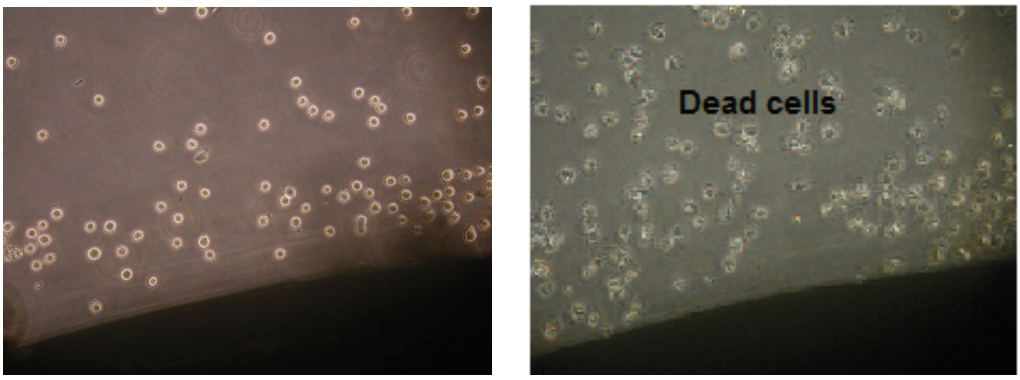

Fig. 3 Cu sample after the inoculation of the cell lines and subsequent cytotoxic reaction after 24 hours

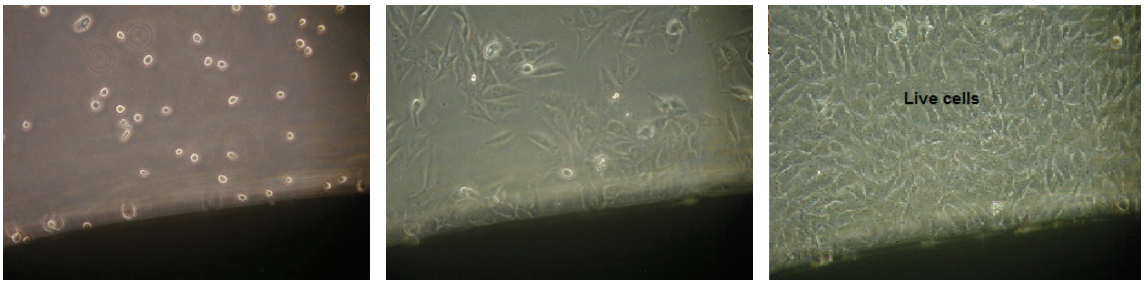

Fig. 4 TiGr2 sample after the cell line inoculation, state after 24 and 72 hours of culturing

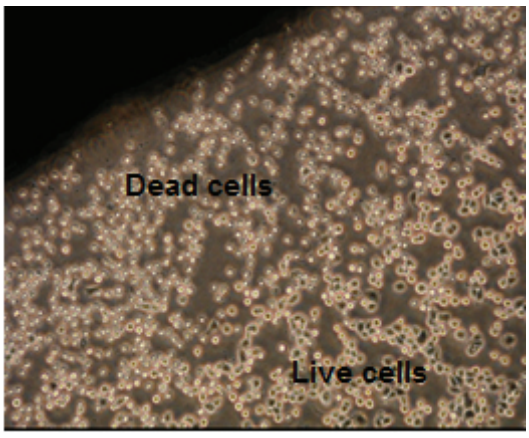

Fig. 5 Dead cells zone in direct contact with the Al specimen together with living cells zone further from the sample itself

3.3. Method of Cell Substrates Colonization

This method is applicable for testing the tolerance of the cell lines to the material surface in direct contact with the sample. The structure of the material may be of metal and non-metal nature; composites with shiny surfaces and different structure. The method is based on culturing cell lines together with the sample material. (6) The method determines a quantitative assessment of cell tolerance to a given material. To evaluate cell tolerance metal alloys with shiny surface TiGr 2 (pure titanium), TiGr 5 (Ti6Al4V) and TNT (Ti36Nb4Ta), TNT further sample of 120 with a rough surface were selected for this method. As a control sample TiGr 2 (pure titanium) was used in this case. The cell line used for this experiment was MG63 (human osteosarcoma). Cell line with a density of 3500 cells / cm2 in culture medium [6] was inoculated to the horizontal surface of the sample. Exposure time was 48 hours in sterile culture device with 90% air humidity blended with 5% CO2 at 37 ° C. After exposure, the cell line was fixed, stained with appropriate conventional fluorescent staining method, and the cell response was photographed [7]. 32 image records of the surface of each sample material were acquired. The values obtained were processed statistically [8] and occupation of the material surface by the cells, expressed as a percentage of colonization, which gives us the quantity of permissiveness, was assessed from the photo records. **Fig. 6**. On the chart below a higher percentage of colonization in metal alloys TNT with a shiny surface compared to the same metal alloy TNT 120 with a rough surface is visible. The latter showed a similar percentage of colonization as a sample TiGr2. The lowest percentage of colonization was found in the sample TiGr 5 with a shiny surface.

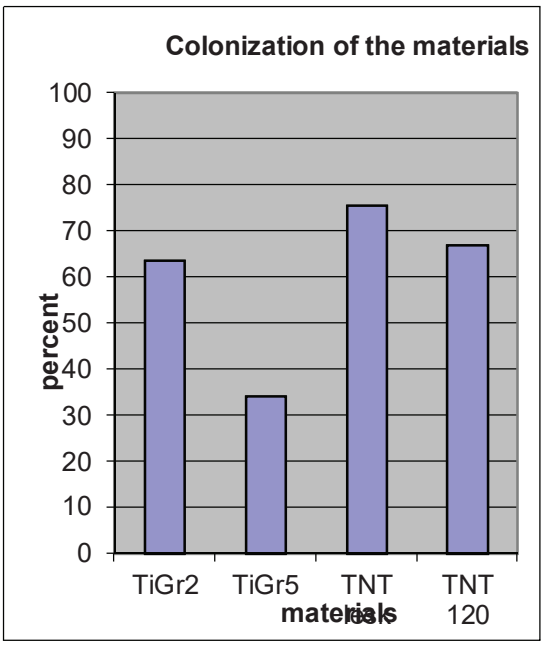

Fig. 6 Colonization of the surface of the material by the cell line on individual material samples stated in percentage

4. CONCLUSION

Live cell imaging is a method, which reveals cell reaction to the material under examination in a way that enables us to know the dynamic responses of the cells to the material as well as the dynamic behavior of the cells or tissues. Dynamics is quantifiable and in conjunction with the ex vivo procedure it is possible to evaluate responses of a specific entity prior to clinical intervention.

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