BIOMEDICAL MEASUREMENTS AND DEVICES

ESTABLISHMENT OF PERMISSIBLE LIMITS FOR LEACHING SUBSTANCES FROM BONE SUBSTITUTES CONTAINING HYDROXYAPATITE AND BETA-TRICALCIUM PHOSPHATE

Vadym Chernobrovchenko, PhD Student; Kostiantyn Dyadyura,
Dr.Sc., Prof.; Mark Balynskyi, Student, e-mail: vadim-golik@ukr.net
Sumy State University, Ukraine

Abstract. It has been proven that the main factor in the uncertainty of laboratory results is biological variation, that is, a change in the composition of human biomaterials, reflecting the course of life processes in the body and is characterized by a combination of the constancy of the internal environment and dynamic fluctuations around the homeostasis point. The paper offers objectively substantiated recommendations for the accuracy of laboratory tests, established maximum allowable values of analytical errors of quantitative research methods (measurements) of physical quantities (composition and properties of components of biological materials, analytes) in samples of biological materials. The interpretation of LOD and LOQ for detecting the concentration of leaching micro-impurities in the bioliquid. The identified patterns indicate that the elements of micro-impurities have different dissolution rates. The ratio of hydroxyapatite/tricalcium phosphate affects the dissolution rate of the material: the higher the content of β-tricalcium phosphate, the higher the dissolution rate. The results of the research allow establishing recommendations for reducing inaccuracies in determining the composition of bone substitutes based on hydroxyapatite/β-tricalcium phosphate, which is associated with manifestations of biological variation, reflecting the body's response to various environmental factors and subject to statistical laws.

Key words: Standardization, Validation method, Limit of detection, Limit of quantification, Bone substitutes.

1. Introduction

The creation of biomaterials, components of medical devices, finished medical devices, as well as three-dimensional printed and regenerative medicines are regulated by various international and national standards and recommendations. The regulatory framework regulates, first of all, the safe application of new-synthesized materials that are part of medical devices and contact with biological fluids and tissue structures of the human. Bone substitutes, which contain nanostructured materials based on hydroxyapatite and β-tricalcium phosphate, are popular in orthopedics and dentistry and deserve special attention in studies related to the study of biodegradation processes. The defining characteristics, which are the basis of practical recommendations for the use of the bone substitutes for their intended purpose, include leaching of impurities from the material.

Searching for opportunities to control reparative processes, studying the features of newly formed bone tissue under the conditions of plastic defects with different materials, reducing the time of reconstruction of the latter by modifying the physicochemical properties are important tasks for orthopedists-traumatologists and materials scientists [1]. The bone substitutes used must fit into the anatomical defect, have appropriate mechanical properties that can withstand the loads arising in vivo, promote the formation of new blood vessels, and the products of their degradation should not be toxic to the human body [2-3]. The unique structure of natural bone cannot be reproduced by conventional methods of synthesis. Therefore, there are risks of biocompatibility when using scaffolds from different biomaterials for their intended purpose [4-5]. The risks of leaching from medical devices of substances that can be dangerous to human health are also taken into account [6-7]. Risk management is also applied to medical devices that contain nanomaterials and release nanoobjects as a result of degradation, wear, or machining of medical devices (eg, grinding, polishing in situ) [8].

ISO 10993 series standards [9-10] are guiding documents for forecasting and research of biological action of medical devices at the stage of selection of materials intended for the manufacture of medical devices, and research of ready-made medical devices. According to ISO 10993-9 [11], special methods are used to obtain, identify and (or) quantify the products of destruction of bone implants based on hydroxyapatite and beta-tricalcium phosphate.

2. Limitations

Validation of methods is an essential and necessary part of the work of the analytical laboratory. The calculation of the maximum amount, ie the maximum dose to the patient, which is set as the mass of the substance leached from the medical device, should be correlated with the threshold value (permissible level) of ISO for the substance being leached. There are thresholds for systemic exposure, which include acceptable levels for some or all three categories of the application according to ISO 10993-1, namely, short-term contact, long-term contract, and permanent contact. Within each of these categories of use, acceptable levels maybe for certain groups of patients in addition to the acceptable level for adults. Different levels may exist for specific

routes of administration. It is known [3] that dissolution in the human body is a complex physicochemical process that includes diffusion, chemical, and electrochemical stages. The risks associated with exposure to hazardous leachable substances can be managed by defining these substances, setting acceptable limits for leachates, and limiting exposure to acceptable levels. It is important to know the lowest analyte concentration that can be detected with a given confidence level using the method. That is, at what true concentration the critical value described above will most likely be exceeded. Terms such as "limit of detection" (LOD), "minimum detectable value" or, in EU directives, CCβ are used for this concept [8]. It is also important to set the lowest level at which the characteristic values are acceptable for a typical application. This level is commonly referred to as the "quantification limit" (LOQ).

3. The goal of the work

The current work aims to create an integrated system for evaluating the limit values of substances that are leached from bone substitutes based on hydroxyapatite and β-tricalcium phosphate after implantation.

4. Determination of limit values for leaching micro-impurities

The scheme of a systematic approach to the assessment of the biological action of medical devices is shown in Figure1.

The process of setting limit values for identified leaching substances from medical devices consists of the following steps:

a) assessment of the biological risk associated with the leachable substance, by:

- data collection and determination of health-critical results;
- determination of tolerance dose (TI) for a specific protocol of the substance into the human body and the duration of exposure;
- determination of the contact action carried by the substance that is washed away as a result of contact with the medical device (PEF), if irritation is an acceptable result;

b) determining the effect of transferred (TE) on the patient from the substance, which is washed out by:

- determination of body weight of the corresponding patient (m_B);

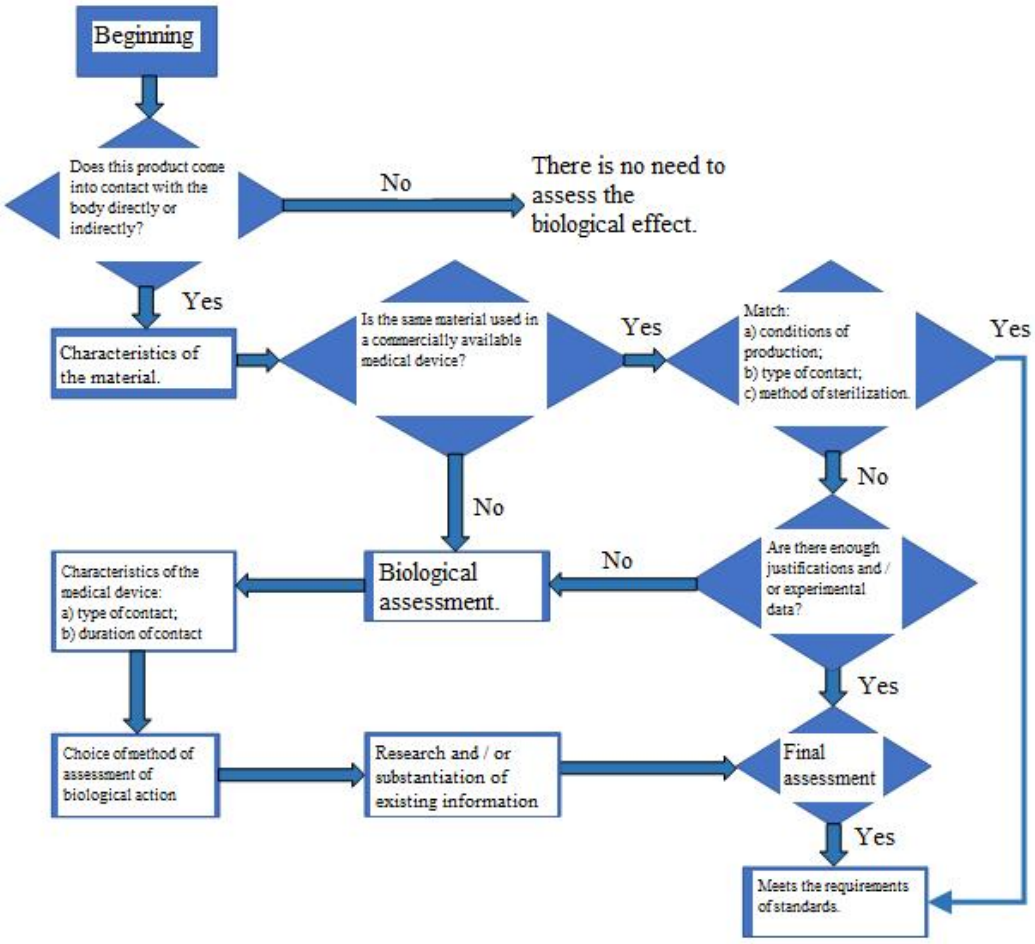

Fig. 1. Evaluation of the biological action of medical devices

- modifications of the product of consumption and body weight based on the utilization factor (UTF);

c) determining the feasibility and utility use, if necessary.

A review of toxicological data provides the information needed to establish the "no-observed-adverse-effect level" (NOAEL) [12].

4.1 Establishment of allowable limits for leachable substance

The formula for calculating the values of TI in milligrams per kilogram of body weight per day, using the modifying factor approach, is shown in equation (1):

$$TI = \frac{\text{NOAEL}}{\text{MF}}, \quad (1)$$

where NOAEL is the level of absence of the observed adverse effects; MF is a modifying factor.

The modifying factor is calculated as the product of the uncertainties

$$MF = UF_1 \cdot UF_2 \cdot UF_3, \quad (2)$$

where UF1, • UF2, • UF3 are the uncertainty factors.

An approximate estimate of uncertainties has many different aspects. These factors take into account the uncertainty inherent in assessing the potential effects of chemicals on the human body based on results obtained in populations or surrogate species. When selecting such factors, some aspects (Table 1) include human-to-human variations, species extrapolations, and other uncertainties.

The scheme of routine use of a medical device, including its use in systemic therapy, is determined for this population group. The calculation of the utilization factor, if possible, takes into account the expected scheme of use of medical devices. This involves calculating the concomitant factor (CEF) and the proportional contact factor (PEF). They are multiplied to obtain the utilization factor (UTF), as shown in the equation

$$UTF = CEF \cdot PEF \quad (3)$$

According to formulas (4) and (5) determine the CEF values between 0.2 and 1.0:

$$CEF = \frac{TI \cdot m_B}{m_{proc}}, \quad (4)$$

where m_B is the body weight, in kilograms; m_{proc} is the total mass of the substance that is washed away (released during the procedure, in milligrams per day).

$$CEF = \frac{TI \cdot m_B}{\sum_{25000\ days} m_{LIFE}}, \quad (5)$$

where m_{Life} is the mass of the substance that is washed away (released during life), which is expressed as the average daily exposure in milligrams.

The utilization factor (UTF) can be adjusted upwards to analyze the situation in which the product/device is not used for the entire duration of the exposure category. Therefore, the PEF is calculated. As shown in equation (6), PEF is equal to the number of days in the exposure category divided by the number of days the product is used before disposal:

$$PEF = \frac{n_{EXP}}{n_{USE}}, \quad (6)$$

where n_{EXP} is the number of days in the impact category; n_{USE} is the number of days of product application. If the number of days varies, it is necessary to apply restrictions ща a reasonable upper limit. If the latter cannot be determined, the PEF value is 1.

Permissible exposure (TE) is determined by the formula:

$$TE = TI \cdot m_B \cdot UTF \quad (7)$$

In the absence of accurate information, use m_B = 70 kg.

The degree of safety assurance considered appropriate for medical devices recognizes the fact that the application of medical devices benefits health. Only then a factor TE can become useful for the correction of acceptable exposure for estimating the health benefits. It should be borne in mind that the toxicity of leaching substances from a medical device is considered acceptable when compared with certain expected health benefits from therapy. In this case, the leaching substances were reduced to the lowest possible minimum following the protection, maintenance, and improvement of human health in general.

Table 1

Uncertainties for calculating TI

Assignment of the uncertainty factor	Range	Standard UF value	Description
UF1, individual variability of the human population	From 1 to 10	10	To take into account the variability of the response between the mean of a healthy population and the response of a certain proportion of the sensitive subpopulation
UF2, interspecific extrapolation	From 1 to 10	1	To take into account the possibility that humans are more sensitive to the negative effects of the compound than experimental animals
UF3, quality, and relevance of research data	From 1 to 100	1	To take into account the limitations of available toxicological data for the calculation of TI, including the absence of NOAEL values, the absence of NOAEL long-term studies, and the lack of data on the clinically relevant route of exposure

Each permissible level of AL is calculated by the formula:

$$AL = TE \cdot BF, \quad (8)$$

where AL is the maximum amount of leaching substances that are recognized as acceptable when entering the human body daily by exposure to a medical device (mg/day); BF is a utility factor.

When leaching agents are toxic compounds and their impact cannot be easily avoided by the application of alternative materials or treatment methods, the significance of the benefits of these agents is considered. Justification of the need and significance of the utility factor in the calculation of thresholds is documented. The maximum dose of leaching substances from a medical device in the category of long-term use is calculated by:

$$m_{dev,prol} = AL_{prol} \cdot 30, \quad (9)$$

where $m_{dev,prol}$ is the maximum dose for the patient in milligrams; AL_{prol} is the permissible level for the category of long-term use, in milligrams per day.

4.2. Studies of the mass transfer processes

Bioliquids are complex multi-component solutions. To model the mass transfer processes in the human body requires knowledge of the physical laws. The dissolution rate of the substance depends on the total resistance of all successive stages of the process: diffusion supply of the solvent to the interaction surface, the transition of the substance from solid to dissolved state, and diffusion removal of the dissolved component from the surface to the bulk solvent (solution) [13]. Analysis of dissolution concerns the law of conservation, which underlies the compilation of material balance. Mass balance seems to be the major in the study of the mass transfer process since its composition determines the flows of components. The obtained experimental data are considered from the standpoint of both the constant volume of leaching substances and their variability as a result of changes in the density of the solution. The kinetic equation that characterizes the dissolution process can be represented as

$$-\frac{dM}{d\tau} = k \cdot F \cdot (C_S - C), \quad (10)$$

where M is the mass of the components of the bone substitute, mg; F is the bone substitute surface, mm^2; C_s is the saturation concentration, mg/m^3; C is the concentration of the substance in the bioliquid, mg/kg; t is the time, s; k is the mass transfer factor, m/s.

Inductively coupled plasma atomic emission spectrometry (ICP / AES), inductively coupled plasma mass spectroscopy (ICP / MS), and atomic absorption spectroscopy (AAS) are applied for quantification.

4.3. Calcium phosphates. Hydroxyapatite and beta-tricalcium phosphate bone substitutes

The material samples of bone substitutes, which were studied in this work can be divided into three types: 1) synthetic monophasic hydroxyapatite; 2) synthetic monophasic β-tricalcium phosphate; 3) synthetic biphasic hydroxyapatite / β-tricalcium phosphate.

Following the standard [14] the maximum content of micro-impurities for bone substitutes based on hydroxyapatite and β-tricalcium phosphate are shown in Table 2.

4.4. Test methods. Criteria for analyzing research results

The rate of dissolution of bone substitute in vitro is used to compare the ability of different bone substitutes to absorb in vivo, even if in vivo will be mechanisms other than dissolution. Changing the pH level after implantation may weaken osteoconduction in the surface of the bone substitute.

The purpose of such tests is to measure the rate of dissolution of bone substitutes in vitro and to change the pH level of the dissolution medium. The solubility of the product studied. Three samples of bone substitute were placed in three flasks with Tris-buffer solution with a pH of 7.3 (± 0,1) at a temperature of (37 ± 1) C. The solutions were stirred at 200 rpm for 24, 48, and 72 hours, respectively. The dissolution rate was measured under a constant ratio of the mass of material to the total volume of solvent. The ratio of the mass of material to the volume of solvent should be between 0.1 and 4.0 mg/ml. The pH level was measured after 0, 24, 48, and 72 hours of immersion. The pH level should not change by more than 0.3 from the start of the test. The validation study was performed based on the analysis of the blank.

Table 2

The maximum content of micro-impurities

Element	Maximum content, mg/kg	The permissible daily dose for a person, mg/kg
Arsenic (As)	3.0	0.01 - 0.015
Cadmium (Cd)	5.0	0.01 - 0.02
Mercury (Hg)	5.0	0.0003
Lead (Pb)	30.0	0.0035
Zinc (Zn)	50.0	0.3
Strontium (Sr)		0.02 – 0.07

The analytical sensitivity of the method was investigated. The analytical sensitivity of the test method is the ability to detect the smallest difference between the two concentrations of the analyzed component. Terms such as "limit of detection" (LOD) and "limit of quantification" (LOQ) are used by authors [15]. LOD is calculated by the formula [16]:

$$LOD \approx 3.3 \cdot s_0 , \quad (11)$$

where s_0 is the standard deviation when performing (n) independent studies of the blank. LOQ is calculated by the formula [15]:

$$LOQ \approx 10 \cdot s_0 \quad (12)$$

The standard deviation s_0 is calculated by:

$$s_0 = \sqrt{\frac{1}{n-1} \cdot \sum_{i=1}^{n} (x_i - \bar{x})^2}, \quad (13)$$

where x is the value of the parameter determined during the research; $\bar{x}$ is an arithmetic mean value; n is the number of observations. If one real sample has N_b of blank samples, the variance of the estimate of the mass of the real sample was computed by the equation:

$$s^2_w = s^2[1 + (1/N_b)]. \quad (14)$$

For a given confidence probability (1 – g) (95%) the limit of the one-way confidence interval for s_w was calculated:

$$\sigma_{1-\gamma} = \sqrt{\frac{n}{c^2_{gn}}} \cdot s_0, \quad (15)$$

where n is the number of degrees of freedom in assessing the accuracy of the method (c^2_{gn} determined from the tables c^2 - distribution) and implicitly defined as the value for which the conditions are met:

$$prob\left[c^2 > c^2_{gn}\right] = 1 - g. \quad (16)$$

The convergence limit (r) is calculated by the formula [9]

$$r = 2.8 \cdot s_0 \quad (17)$$

5. Studies of the concentration of leaching micro-impurities

5.1 Calculation of permissible limit values for leaching micro-impurities in bone substitutes based on hydroxyapatite and β–tricalcium phosphates

The results of studies of the limit values for leaching micro-impurities when using bone substitutes based on hydroxyapatite and β-tricalcium phosphates are presented in Table 3.

Permissible limits for leaching micro-impurities in bone substitutes based on hydroxyapatite and β-tricalcium phosphate make it possible to establish for further researching the content of micro-impurities. The latter takes into account the duration of human contact with bone substitutes and his body weight.

5.2 The results of studies of the concentration of leaching micro-impurities in the bioliquid

The temporal t (24, 48, and 72 hours) studies of the dissolution of bone substitutes and the interpretation of experimental data (C is the concentration of leaching micro-impurities in the bioliquid at the number of revolutions n (200 rpm), respectively) are given in Table 4.

Table 3

Establishment of limit values for leaching micro-impurities

Element	NOAEL. mg/kg/d	MF	TI. mg/kg/d	CEF	PEF	UTF	TE mg/d	AL. mg/d	$m_{dev.prol}$ mg
As	0.015	50	0.0003	0.2	1	0.2	0.0042	0.0042	0.126
Cd	0.02	50	0.0004	0.2	1	0.2	0.0056	0.0056	0.168
Hg	0.0003	50	0.00007	0.2	1	0.2	0.00098	0.00098	0.0294
Pb	0.0035	50	0.00007	0.2	1	0.2	0.00098	0.00098	0.0294
Zn	0.3	50	0.006	0.2	1	0.2	0.084	0.084	2.52
Sr	0.02	50	0.0004	0.2	1	0.2	0.0056	0.0056	0.168

Table 4

Experimental study of the dissolution process

Element	C_{24}, mg/kg	C_{48}, mg/kg	C_{72}, mg/kg
As	0.00006	0.00026	0.001
Cd	0.000001	0.000068	0.00015
Hg	0.00002	0.00009	0.0003
Pb	0.00004	0.00004	0.0003
Zn	0.0005	0.0002	0.005
Sr	0.00001	0.168	0.0007

The ratio of hydroxyapatite / β-tricalcium phosphate affects the dissolution of the material, namely: the higher the content of β-tricalcium phosphate, the faster the dissolution. The implantation of bone substitutes depends not only on the osteoconductivity of the material but also on the porosity of the structure. For bone ingrowth throughout the implant, macroporosity must be large and interconnected. Porosity affects the rate of the resorption: the greater the number of micropores, the faster the dissolution. The sizes of macropores are in the range from 100 to 500 μm, which ensures the optimal formation of bone tissue [18, 19]. The micropores are less than 10 μm in size.

The uncertainty of results, which differs in nature from the analytical variation, is the biological variation (changes in the composition of human biomaterials that reflect the course of life processes in the body and are characterized by a combination of stability within certain stability of the internal environment and dynamic fluctuations around the homeostasis point). The study of the dissolution process makes it possible to adjust the allowable limits for leaching micro-impurities in bone substitutes based on hydroxyapatite and β-tricalcium phosphate for further research.

5.3. Estimation of measurement error in determining the limits of detection and quantification

Table 5 shows the changes in the mass of micro-impurities in the bioliquid for different elements.

Assuming that three blank samples were used for each batch, by formula (14) we obtain an estimate of the uncertainty of the mass measurements. This gives the following values of the limits of LOD detection and quantification of LOQ for the elements (Table 6).

Table 5

The results of measurements of weight gain of micro-impurities in the bioliquid

Element	Weight gain of micro-impurities in the bioliquid in 24 hours, μg						Standard deviation s_0
	Study number						
	1	2	3	4	5	6	
As	0.00002	0.000021	0.000015	0.000018	0.000014	0.000018	1.95448E-05
Cd	0.000004	0.0000011	0.000002	0.000002	0.000006	0.000002	3.61137E-06
Hg	0.00009	0.000022	0.000012	0	0.000012	0.000012	4.24641E-05
Pb	0.00002	0.00006	0.00002	0.00006	0.00008	0.00006	0.00006
Zn	0.00011	0.00011	0.0004	0.00045	0	0.00041	0.000333107
Sr	0.00001	0.000031	0.000014	0.000019	0.000027	0.00003	2.54833E-05

Table 6

The results of determining the limits of detection and quantitative determination of the mass of micro-impurities in the bioliquid

Element	S_{95}	LOD	LOQ	r
As	2.54884E-05	8.82944E-05	0.00038374	2.42539E-09
Cd	4.70959E-06	1.63145E-05	5.43817E-05	8.28063E-11
Hg	5.53775E-05	0.000191833	0.000639444	1.14489E-08
Pb	7.82461E-05	0.000271052	0.000903508	2.28571E-08
Zn	7.82461E-05	0.001504824	0.00501608	7.0451E-07
Sr	3.32328E-05	0.000115122	0.00038374	4.12317E-09

The LOD determines the lowest concentration of analyte present in the sample that can be detected using this measurement technique with a given confidence level (95%). The operating range of the measurement is limited from below by the limit of quantification of LOQ.

8. Conclusions

The defining characteristics, which are the application of bone substitutes, include leaching of impurities from the material. The study of the diffusion rate of molecules with the subsequent prediction of accumulation in tissues should belong to the first stage of assessing the biological safety of the material.

The paper develops objectively substantiated recommendations for the accuracy of laboratory tests in determining the concentration of leaching micro-impurities in the washing liquid. As a basis for the accuracy assessment, the maximum allowable values of errors of the quantitative method of studies of the liquid composition in the samples after exposure with bone substitutes based on hydroxyapatite and b-tricalcium phosphate, were defined. The identified patterns indicate that the elements of micro-impurities are inherent in the different dissolution rates. The ratio of hydroxyapatite/β-tricalcium phosphate affects the dissolution rate of the material: the higher the content of β-tricalcium phosphate, the higher the dissolution rate.

The results of the research establish recommendations for reducing uncertainty in determining the composition of bone substitutes based on hydroxyapatite and beta-tricalcium phosphate, which is associated with manifestations of biological variation, reflecting the body's response to various environmental factors and is subject to statistical laws.

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10. Conflict of interest

There are no financial or other potential conflicts concerning the work.

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