Evaluation of copper electrodes for biomedical monitoring systems

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ABSTRACT – REZUMAT

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This paper presents several aspects of in vivo biocompatibility tests using the animal model, considering conductive electrodes based on cotton fabric and a conductive paste-based polymeric matrix with copper microparticles that offer the potential to be used in medical devices. Additionally, this work presents a potential application of the electrodes for temperature sensors using the Arduino development board. In addition, after performing in vivo tests, analysis of the primary irritation scores, animal irritation scores, and cumulative irritation index showed that the electrodes resulted in a negligible measurement of perceived skin irritation.

Keywords: *textile, electrodes, conductive, biocompatibility testing, in vivo*

Evaluarea electrozilor din cupru pentru sisteme de monitorizare biomedicală

Această lucrare prezintă aspecte ale testelor de biocompatibilitate în vivo utilizând modelul animal, luând în considerare electrozii conductivi din țesătură din bumbac și pastă conductivă pe bază de matrice polimerică cu microparticule de cupru cu potențial de a fi utilizați în dispozitive medicale. În plus, această lucrare prezintă o aplicație a electrozilor pentru senzorii de temperatură utilizând o placă de dezvoltare Arduino. În continuare, după efectuarea testelor în vivo, analiza scorurilor de Iritație Primară, Iritaţie de Animal și Indexului Cumulat de Iritaţie a arătat că electrozii au provocat un răspuns iritativ neglijabil la nivelul pielii.

Cuvinte-cheie: *textil, electrozi, conductiv, teste de biocompatibilitate, in vivo*

INTRODUCTION

Biomaterials and medical devices represent an extraordinarily diverse and heterogeneous group [1]. The use of these products entails direct or indirect contact with the body; commercial availability requires testing of their safety. The safety assessment of medical devices establishes the risk of adverse health reactions in case of their normal or abnormal use [2, 3]. Since these adverse reactions may occur following exposure to these materials, preclinical testing of the toxic potential of the materials or their components is required [4]. Since January 1995, medical devices used in practice in the EU must have been evaluated in terms of safety according to Directive 93/42 EEC [5, 6]. This directive aims to establish a single European market that ensures that both patients and users are protected against exposure to additional risks.

Currently, the safety of medical devices is established through toxicological studies [7] and other studies recommended in SR EN ISO 10993-1:2021 – Biological evaluation of medical devices [8], SR EN ISO 10993-10:2014 [9] and SR EN ISO 10993-23: 2021 [10].

Recent research has investigated the biocompatibility of copper in combination with other metals. For example, Cu–Au core-shell nanowire electrodes for electrophysiological monitoring (electromyogram, electrocardiogram sensors) [11] were tested using artificial perspiration and cell culture. However, copper nanowires have the disadvantage of oxidation and lack biological compatibility. In addition, electrode-based Cu–Au core-shell nanowires [11], polypyrrole and Cu(II) metal-organic nanocomposites [12], copper–ruthenium composites [13], and CuO electrodes [14] present desirable biocompatibility (*in vitro* and *in vivo*) without generating inflammatory responses [12]. According to some studies, the limitations of flexible sensors include inadequate biocompatibility, even for noninvasive products that only come into contact with human skin [15]. Even if conductive inks based on metal or carbon nanotubes (CNTs) were to be intensively studied, these inks have raised concerns regarding their possibly harmful effect on health and their manufacture is expensive. Thus, metallic reactive inks (e.g., Ag) are more attractive due to their excellent electrical conductivity and biocompatibility [16].

EXPERIMENTAL PART

Biocompatibility testing of conductive/insulating materials used to make medical monitoring devices was carried through *in vivo* tests because these products (namely, electrode materials) involve direct or indirect contact with the body and require safety testing before becoming available for commercial use. The safety assessment of medical devices establishes the risk of adverse health reactions in case of their

normal or abnormal use. As these adverse reactions may occur following exposure to such electrode materials, preclinical testing of the toxic potential of the materials or their components is required according to directive 93/42 EEC.

Here, the biocompatibility of electrode materials (namely, electroconductive textile materials with a surface resistance of 10^3 Ω) was evaluated using two reference materials (P_1 – textile material treated by alkaline boiling-bleaching, and P_2 – raw textile material treated in plasma with O_2 to obtain a hydrophilic surface). In addition, the textile electrode material was functionalized by depositing conductive pastes comprising a polymeric matrix (polyvinyl alcohol) and copper (Cu) microparticles after treatment in RF oxygen plasma (P_3). Table 1 shows images of the electrode materials. The biocompatibility assessment was carried out by testing skin irritation according to the SR EN ISO 10993-10:2014/SR EN ISO 10993-23:2021 standard (Biological evaluation of medical devices. Part 23: Tests for irritation).

Figure 1 shows electron microscopy images (magnitude 60x) of the fabric surface for samples P_1 (untreated – figure 1, *a*), P_2 (raw fabric → O_2 plasma RF treatment, using an RF generator in kHz and power P = 100 W – figure 1, *b*), P_3 (treatment by alkaline boiling-bleaching and deposition of conductive paste based on PVA and Cu – figure 1, *c*).

For biocompatibility testing, three rabbits (male New Zealand albino rabbits) weighing between 3400 and 4600 g were used for each test sample, as provided by the Bio base of the Cantacuzino Institute, Băneasa Station.

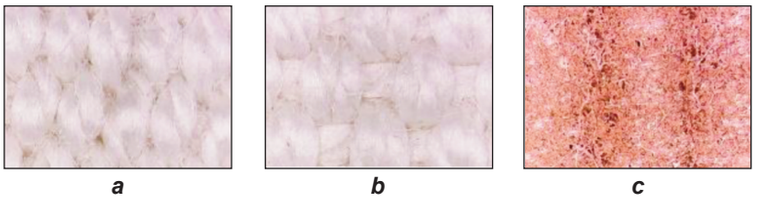
The samples were cut into 2×3 cm samples and were used after impregnation with physiological serum. They were then covered with an additional dressing and immobilized with semiocclusive adhesive tape for a minimum of 4 hours. Over the control areas, the electrode samples were applied and impregnated with physiological serum, with control over the exact dimensions and duration of exposure. After 4 hours, the test sample (and blank) were removed, and the exposed areas were washed with distilled water.

Two examiners observed the animals to monitor the reactions of their exposed skin, in an environment with natural lighting. The exposed areas of the test sample and control were examined at 1, 24, 48 and 72 hours after the first exposure. The experimental room temperature was 20±1°C, and the relative humidity was 50–60%. Lighting was artificial, with alternating 12-hour spans of light/darkness.

Due to the possibility of irritation reactions, the study was initiated by exposing each test sample to a single animal (A_i). For this test, the samples (electrode material) were cut into 2×3 cm samples and used after impregnation with physiological serum. To fix a sample on animal skin, it was covered with an additional dressing and immobilized with semiocclusive adhesive tape for a minimum of 4 hours (figure 2). Initially, the control samples P_1 and P_2 impregnated with physiological serum were applied for 4 hours, and after 4 hours, the test sample and control were removed. Then, the exposed areas were washed with distilled water. Examination of the exposed areas, test sample and control was carried out 1, 24, 48, and 72 hours after the first exposure.

Since no reactions indicated that the test sample induced irritation, the study continued with two more exposures performed as follows. After the 72-hour observation interval following the first exposure, two additional 4-hour exposures were made in two successive applications. The above procedure was repeated on two more animals. Three skin exposures were made for the test/control sample on three animals each. Approximately one hour after removing the semiocclusive bandages, skin reactions were recorded. After exposure and removal of the dressing, the observations were repeated at intervals of 24, 48, and 72 hours. Skin observations were made before the second additional exposure and after 24, 48, and 72 hours. After three exposures and monitoring of the skin reactions, the Primary Irritation Score was determined according to the types of skin reactions and their applicable quantification (table 1, table 2). The Irritation Score per Animal (SIA) is obtained by summing the Primary Irritation Score (SIP) of each moment of observation divided by the number of observations. The cumulative irritation index (CII) was obtained by dividing the sum of irritation scores per animal by the number of animals (table 3).

Thus, for sample P_3, the exposure areas after the first and second applications are presented in table 4. Table 5 shows the images obtained after the first application upon examining the exposed areas at 1, 24, and 48 hours after the first application. Table 6 shows the images obtained after the second application. According to the Primary Irritation scores, the Animal Irritation Scores and the Cumulative Irritation Index for samples P_1, P_2, and P_3 all were determined to be in the Negligible response category.



a ***b*** ***c***

Fig. 1. Analysis of the fabric surface topography by digital electron microscopy: *a* – sample P_1; *b* – sample P_2; *c* – sample P_3

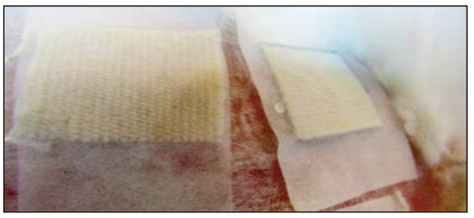


Fig. 2. Application of samples for 4 hours

Table 1

TYPES OF SKIN REACTIONS AND THEIR QUANTIFICATION	
Reactions	**Primary irritation score**
Erythema and eschar	-
No erythema	0
Weak erythema (barely perceptible)	1
Perceptible erythema	2
Moderate erythema	3
Intense erythema (with a tendency to eschar formation)	4
Edema	-
No edema	0
Slight edema (barely perceptible)	1
Perceptible edema	2
Moderate edema	3
Intense edema (more than 1 mm – extended outside the contact area)	4
Maximum possible irritation score	8

Table 2

IRRITANT RESPONSE CATEGORIES	
Average	**Response category**
0 to 0.4	Negligible
0.5 to 1.9	Weak
2 to 4.9	Moderate
5 to 8	Severe
0 to 0.4	Response category

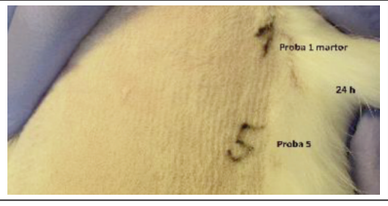
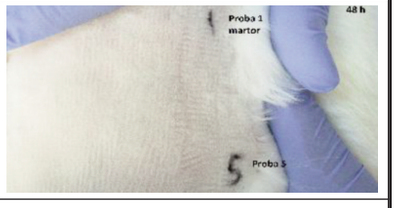
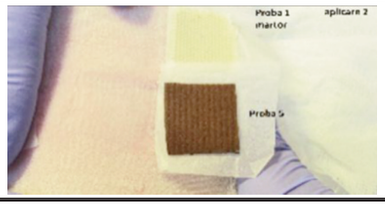
RESULTS AND DISCUSSION

For all samples (P_1–P_3), the Animal Irritation Score (SIA) and Primary Irritation Score (SIP) were 0. In addition, the cumulative irritation index for test samples P_1 (control sample obtained through classical methods for hydrophilization (boiling-bleaching)) and P_2 (control sample obtained by hydrophilization using RF plasma oxygen) was 0 (table 5). Thus, according to table 2, the scores reflect a Negligible response. Following this biocompatibility evaluation, we concluded

Table 3

SCORES OBTAINED FOR SINGLE AND REPEATED APPLICATION OF ELECTRODE MATERIALS											
	Single application				**Repeated application**						
SIP	1	2	3	4	5	6	7	8	9	10	Total
Exposure	-	I			II			III			-
Hours	1	24	48	72	24	48	72	24	48	72	-
Samples P_1 and P_2											
A1	0	0	0	0	0	0	0	0	0	0	0
A2	0	0	0	0	0	0	0	0	0	0	0
A3	0	0	0	0	0	0	0	0	0	0	0
Sample P_3											
A1	0	0	0	0	0	0	0	0	0	0	0
A2	0	0	0	0	0	0	0	0	0	0	0
A3	0	0	0	0	0	0	0	0	0	0	0

Table 4

EXAMINATION OF EXPOSED AREAS AFTER THE FIRST AND SECOND APPLICATION			
Examination of exposed areas after the first application			
	1 h after the first application	24 h after the first application	48 h after the first application
P_3			
Examination of exposed areas after the second application			
P_3			

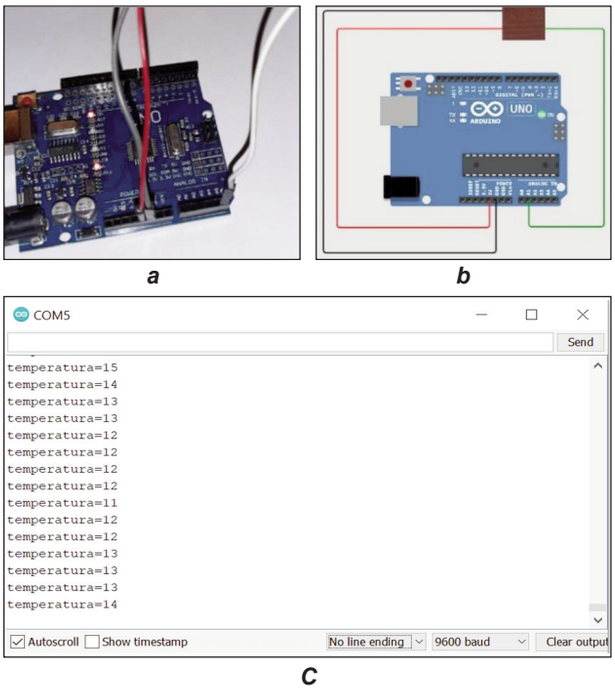


Fig. 3. Demonstrative model based on flexible Cu electrode (P_3) for temperature monitoring: *a* – development board Arduino connection to analogue pins; *b* – simulation of the temperature monitoring using P_3 electrode; *c* – reading data from analogue pins on the Arduino (sample P_3)

that material P_3 can be used as an electrode material to realize physiological monitoring systems. The P3 material was implemented in a demonstrative model using an Arduino development board (figure 3, *a*) to highlight the change in electrical resistance depending on the temperature and verify the potential application of sample P_3 in temperature monitoring systems. Conductive connectors (figure 3, *a*), flexible textile electrodes made of electrode materials (P_3 – figure 3, *b*) and support materials (P_1 or P_2) were used as shown.

The discrete values for temperature were read each 0.8 seconds, corresponding to a sampling frequency of 1.25 Hz:

$$f = \frac{1}{T} \quad (1)$$

where f in Hz represents the frequency and T in s represents the time.

CONCLUSIONS

In conclusion, our electrode material based on a polymer matrix and copper microparticles can be used in sensor-based systems as electrical resistance monitors to measure the variation temperature-dependent electrical resistance of a surface and thus determine the temperature of that body. Biocompatibility tests to evaluate skin irritation were carried out *in vivo* using an animal model, and analysis of the primary irritation, animal irritation and cumulative irritation index scores for samples P_1, P_2 and P_3 indicated a negligible irritant response. In the future, the commercial use of these materials in biomedical monitoring systems will involve supplementary investigations, such as preclinical testing of the toxic potential of the materials or their components, which is required according to the MDD 93/42 EEC (Medical Device Directive) because biomedical monitoring devices fall into class I devices for noninvasive measurement. Our demonstrative model based on the Arduino development board highlighted that it is possible to record temperature variation using our electrode materials based on textile materials covered with a conductive paste based on Cu microparticles.

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Table 5

SCORES OBTAINED FOR SINGLE AND REPEATED APPLICATION OF ELECTRODE MATERIALS

	Primary Irritation Score (SIP)										**Animal Irritation Score (SIA)** $SIA = \sum_{i=1}^{10} SIP$	**Cumulative Irritation Index (CII)** $CII = \sum_{i=1}^{3} \frac{SIA}{3}$
	Single application				Repeat application							
SIP	1	2	3	4	5	6	7	8	9	10	-	
Exposure		I			II			III			-	
hours	1	24	48	72	24	48	72	24	48	72	-	
	Samples P_1 and P_2											
A1	0	0	0	0	0	0	0	0	0	0	0	0
A2	0	0	0	0	0	0	0	0	0	0	0	
A3	0	0	0	0	0	0	0	0	0	0	0	
	Sample P_3											
A1	0	0	0	0	0	0	0	0	0	0	0	0
A2	0	0	0	0	0	0	0	0	0	0	0	
A3	0	0	0	0	0	0	0	0	0	0	0	

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