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Comparative Safety Testing (Acute Systemic Toxicity in Mice) of Two Materials Prepared from Polypropylene-Polyester (Codubix S) or Acrylate Resin (Mendec Cranio) Used for the Manufacturing of a Calvaria Prosthesis

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Abstract
The aim of the study was a comparison of the acute toxicity of two popular prostheses used in the reconstruction of the bones of the skull. For the tests, the following materials were used: a polypropylene-polyester knitted Codubix S cranial bone prosthesis, made by TRICOMED SA, and polymethyl methacrylate Mendec Cranio resin. The tests were carried out in accordance with the following standards – PN-EN ISO 10993-11:2009 Biological evaluation of medical devices – Part 11: Tests for systemic toxicity, and PN-EN ISO 10993-12:2012 Biological evaluation of medical devices – Part 12: Sample preparation and reference materials. During the evaluation, adult male and female Balb/c mice were used. The animals were injected intravenously using extracts of both materials in 0.9% NaCl and intraperitoneally with the same extracts in sesame oil. The tests lasted 7 days, during which the health of the animals and their behavior were assessed. Both in the control and test groups, there was no mortality of the animals, and the health and behaviour of mice were unchanged when compared with the normal. After 7 days the internal organs of the chest and abdominal cavity of the animals were subjected to macroscopic pathomorphological examination, during which no changes indicating the toxic action of Codubix S and Mednec Cranio resin were found. Before the acute systemic toxicity tests, the chemical purity of both implants was assessed. The chemical purity of a product is one of the factors determining its biological properties. A product which is characterised by a higher degree of chemical purity contains fewer substances which may have a negative impact on biological reactions. Both prostheses meet the requirements of purity for medical devices.

Key words: *cranial implant, cranioplasty, acute texicity, chemical analysis, polypropylene-polyeser, acrylate resin.*

Introduction

Surgical reconstruction of large cranial defects requires careful selection of reconstructive material as well as a reconstructive procedure to obtain a functional and aesthetic result. The discussion regarding the best way of reconstructing cranial defects is as ancient as patient care itself. However, until now no general guidelines regarding cranial reconstruction procedures have been published in available literature sources. Moreover, the associations of medical professionals or medical organisations also have not published the guidelines mentioned. In 1997, a review was published regarding the advantages and drawbacks of the materials and methods used in cranioplasty, also describing the properties of an ideal implant, which should be characterised by immediate protection of the cranium, be osteoconductive and osteo-inductive, and allow to achieve the intended aesthetic effect [1].

The issue of procedures of cranial reconstructions was raised again with attempts to develop standards regarding handling increased intracranial pressure, both spontaneous (e.g. after a stroke) and post-traumatic [2, 3]. Very often the therapeutic procedure encompasses making a cranial defect exceeding even ¼ of the calvaria [4]. Next, such a defect has to be reconstructed, and owing to its size, it is often an urgent case or at least one which should not be delayed. Such proceeding is advised due to both mechanical protection [5] and possible changes in the value of intracranial pressure [6]. According to literature data and available meta-analyses, the world's most commonly used cranioplastic synthetic material is acrylate resin based on polymethyl methacrylate (PMMA) [7]. Additionally, it is estimated that methyl methacrylate based resins are the material most widely used intra-operatively, having required properties including inertness, radiolucency and strength [8-11]. PMMA based materials also have some limitations resulting from the intra-operative plasticity, which can lead to unsatisfactory results in the case of large defects. Besides this, the intra-operative preparation process may cause some complications, such as an exothermic reaction – increased temperature may even cause the necrosis of surrounding tissues [12], and the release of toxic monomers. The main disadvantage of PMMA material is the exothermic reaction during in situ polymerisation.

Nevertheless, there are also other materials used in cranioplasty [13]. The most widely known are as follows: metallic prostheses based on noble, tantalum, stainless steel and titanium, composite materials [14-17], bio-ceramic prostheses based on aluminium oxide or hydroxyapatite [18], and polymer materials such as polyether ether ketone (PEEK) [19-21], polyethylene (PE) and polypropylene [22-24], and mineralised collagen [25].

On the other hand, according to Polish National Health Fund data, the most commonly used prosthesis for cranioplasty in Poland is Codubix S, manufactured out of polypropylene-polyester (PP/PE) knitted material.

The polypropylene yarn included in the implant is characterised by a low specific gravity and low melting point, which en-

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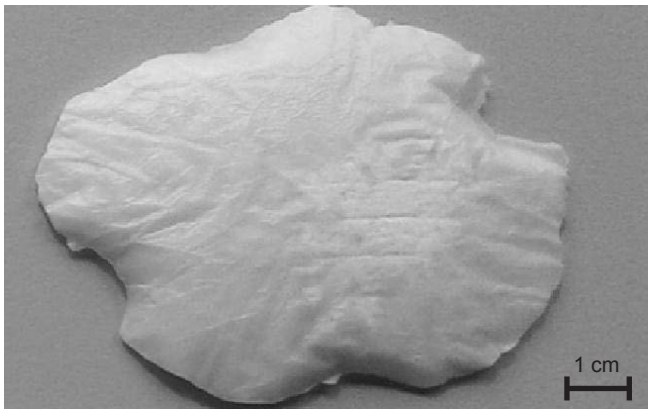

Figure 1. *Prosthetic material made of polyacrylate resin (PMMA).*

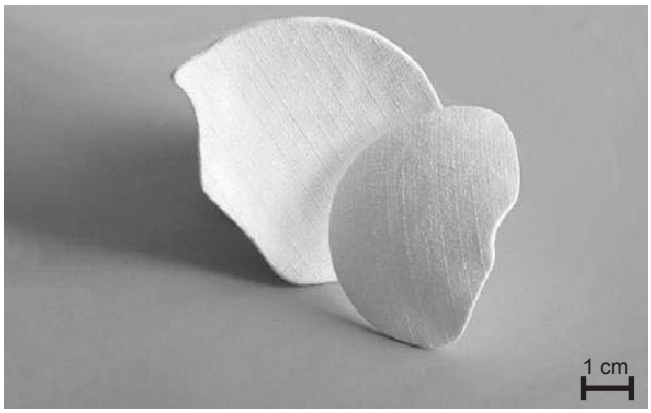

Figure 2. *PP/PE prosthetic material – Codubix/Codubix S cranial implant.*

ables to give the implant its appropriate stiffness and hardness. The second material used in the graft – polyester yarn – ensures adequate elasticity and porosity, enabling a relatively high surface in contact with the tissue, which contributes to quick overgrowth of the implant. The Codubix implant stands out from the crowd owing to its excellent immunologic characteristics, resistance to infections – despite its porosity, relatively easy intraoperative modelling, and no artifacts during further diagnostics. In cases where it is not possible to fit a standard Codubix implant into the existing cranial defect e.g. owing to a complicated head trauma, there is a possibility of modelling the defect with computed tomography CT [26]. The custom-made implant recreates the original shape of the missing bone [27, 28].

Undoubtedly, none of the currently available cranioplastic materials is a perfect one [29], however a comparison of whichever characteristic of the most popular materials in the world and in Poland may be of consequence in the discussion regarding the choice of a standard therapeutic procedure. The Codubix product family also encompasses rib prostheses used for the reconstruction of extensive chest wall defects and orbital wall implants used for reconstruction procedures of orbital wall defects [30].

Owing to this, the Authors decided to compare the acute toxicity of PMMA and PP/PE based prostheses. So far such a comparison has not appeared in literature sources, but the issue has often been raised by potential PP/PE users from outside Poland – those so far relying on PMMA or metal implants. The debate has also been raised during international neurosurgical conferences and conventions and named as one of the issues requiring both experimental confirmation and adequate description in the literature. The prosthetic material referred to as bone cement or bone glue (***Figure 1***) is prepared on the basis of polymethyl methacrylate (PMMA) [31], either pure or with additional materials influencing its density, polymerisation time, radiolucency or biological reactiveness (e.g. antibiotics). However, pure cements are used more often, both in neurosurgery or surgery and in dental prosthetics. Due to this fact, this type of material was used for comparison. Pure cement contains PMMA in a liquid fraction and PMMA with a radical polymerization initiator – benzoyl peroxide in a solid fraction. The material has good mechanical properties, polymerises in situ, and is to be used for bone reconstructions.

PP/PE prosthetic material (***Figure 2***) is manufactured by the knitting technique from polypropylene yarn with the addition of polyester yarn (approx. 5%), which ensures combining of the characteristics of implants made of both polypropylene and polyester. The material is susceptible to bending, has shape memory and is characterised by high mechanical resistance. It also has low specific gravity, shows no toxic effect, is chemically inactive and has a good healing-in degree. It also allows for radiological diagnostics without radio-opacity.

Materials and methods

For comparison of two substances, PP/PE knitted material and one cranioplastic resin were used. The PP/PE knitted material, manufactured in Lodz, is the only one registered as a medical product. The choice of PMMA material was made arbitrarily due to the fact that there are several manufacturers offering it – the chemical substance is a generic one, and it is anticipated that it would be identical in terms of the parameters presented.

PP/PE material: samples of Codubix S implants, LOT 16.02.2016, size R-4

2 pieces of the Codubix S product, size R-4 ($75 \times 75 \times 3.3$ mm), LOT 16.02.2016, were manufactured according to the technological procedures. Before sterilization, the implants were cut into approx. 0.5×0.5 cm pieces, and each one of them was packed in a double paper-foil pouch dedicated for moist heat sterilisation. Manufacturing and packing were performed as in the manufacturing process of a standard series, i.e. in an environment controlled in terms of microbiological and particle contamination and in terms of temperature (clean room – ISO Class 7).

The samples were sterilised with moist heat in a validated process: temperature 121 °C for 16 minutes. The material thus prepared was taken for chemical and biological tests in order to assess the acute systemic toxicity of the Codubix S product.

Cranioplastic resin: samples of Mendec Cranio acrylate resin, LOT AA9924

As regards the acrylate resin intended for cranioplastic reconstructions, two sample pieces were prepared in such a shape and size that they would resemble Codubix S size R-4 material. The formation process was carried out according to the manufacturer's guidelines, i.e. the fluid was poured into a bowl, into which the powder was added, and then the in-

Table 1. *Codubix S and Mendec Cranio acrylate resin chemical test results.*

No.	Parameters tested	Unit	Test method	Test results			
				Codubix S		Acrylate resin	
				extract 1	extract 2	*extract 1*	*extract 2*
1.	organoleptic assessment of extract: transparency, colour	–	SOP-KJC.02	transparent		transparent	
		–		colourless		reddish (intense smell present)	
2.	pH of sample	pH unit	PN-EN ISO 3071:2007	7.26	7.23	3.06	3.10
3.	permanganate oxidation	mg O_2/g	PN-P-04896:1984	0.004	0.004	1.656	1.976
4.	max. UV absorbance at a given wavelength	Amax	SOP-KJC.05 PN-P-04990:1989	0.0104 (245 nm)	0.0202 (245 nm)	4.4063 (230 nm)	4.2885 (230 nm)
		nm					
5.	frothing agents	froth height (cm)	SOP-KJC.03 PN-P-04781-14:1989	none		none	
6.	electrical conductivity	µS/cm	SOP-KJC.04	4.4	2.6	78.0	68.9
7.	content of substances soluble in petroleum ether	%	SOP-KJC.09 PN-P-04607:1983 method A	0.884	1.057	–	–
8.	chloride ion content	mg Cl^-/g of the sample	PN-P-04895 method.2.1.	below 0.02		below 0.02	
9.	sulphate ion content	mg SO_4^{2-}/g of the sample	SOP-KJC.06 PN-P-04781-04	below 0.05		below 0.05	
10.	heavy metal ion content	mg Pb^{2+}/g of the sample	SOP-KJC.08 PN-P-04991	below 0.01		below 0.01	
11.	ammonium ion content	mg NH_4^+/g of the sample	SOP-KJC.07 PN-P-04992	below 0.01		above 0.01	

gredients were mixed with a spatula for a few seconds so that the powder could soak up the fluid. After approx. 15 seconds, the resin was divided into two parts from which cranial implants were modelled. Before hardening, on each implant small incisions were made in order to facilitate cutting the grafts after the polymerization into 0.5 × 0.5cm pieces. Next, similar to the Codubix S product, the cut samples were packed into a double paper-foil pouch and sterilised with moist heat. The processes of forming and packing were carried out in a clean room. The samples thus prepared were sent for chemical and biological tests.

Determination of the purity of the materials

Evaluation of the chemical parameters of Codubix S and polyacrylate resin, according to the normative methodology presented in ***Table 1***, was made in the Quality Control Laboratory of Tricomed SA. Water extracts were prepared according to the PN-P-04894 standard, where the ratio between the mass of the sample (g) and the medium (cm^3) was equal to 1:10. The extraction was carried out at a temperature of 120 ± 1 °C for 1 hour.

Acute systemic toxicity tests

Acute systemic toxicity tests of Codubix S and Mendec Cranio acrylate resin were carried out at the Nofer Institute of Occupational Medicine, Department of Toxicology and Carcinogenesis, Toxicology Assessment Laboratory. All tests were done in accordance with the PN-EN ISO 10993-11:2009 standard [32]. 8-week old male and female Balb/c mice were subjected to tests during which extracts of the test products in polar solvent (0.9% NaCl) and non-polar solvent (sesame oil) were applied.

As a substrate, the following were used: 0.9% NaCl – B. Braun solution for washing, sterile, free of endotoxins, sesame oil (Sigma-Aldrich).

The mice were subjected to a 7-day acclimation in a living space for animals, in order to deaccustomise the animals of poorer conditions and habits. All mice used for the study were healthy. They were housed individually in ventilated cages, fed with standard rodent feed (Wytwórnia Pasz, Motycz, Poland), and given tap water to drink. Feed and water were given to the mice *ab libitum*. The animals were in a room with controlled temperature, air humidity and automatically regulated lighting (12h:12h light/no light).

After acclimation, the mice were randomly divided into 4 groups and injected intravenously, and into 4 more groups and subjected to intraperitoneal injection, according to the following schedule:

- for Codubix S product:
  - Group 1 – females – intravenous 0.9% NaCl injection – control group,
  - Group 2 – females – intravenous Codubix S in 0.9% NaCl extract injection,
  - Group 3 – males – intravenous 0.9% NaCl injection – control group,
  - Group 4 – males – intravenous Codubix S in 0.9% NaCl extract injection,
  - Group 5 – females – intraperitoneal sesame oil injection – control group,
  - Group 6 – females – intraperitoneal Codubix S in sesame oil extract injection,
  - Group 7 – males – intraperitoneal sesame oil injection – control group,
  - Group 8 – males – intraperitoneal Codubix S in sesame oil extract injection;
- for acrylate resin:
  - Group 1 – females – intravenous 0.9% NaCl injection – control group,
  - Group 2 – females – intravenous acrylate resin in 0.9% NaCl extract injection,
  - Group 3 – males – intravenous 0.9% NaCl injection – control group,
  - Group 4 – males – intravenous acrylate resin in 0.9% NaCl extract injection,
  - Group 5 – females – intraperitoneal sesame oil injection – control group,
  - Group 6 – females – intraperitoneal acrylate resin in sesame oil extract injection,
  - Group 7 – males – intraperitoneal sesame oil injection – control group,
  - Group 8 – males intraperitoneal acrylate resin in sesame oil extract injection.

There were 5 animals in each group.

The extracts were prepared in accordance with the PN-EN ISO 10993-12:2012 standard [33].

One of the extracts was prepared in a polar medium (0.9% NaCl solution) and the other in a hydrophobic medium (sesame oil). For the sterile tube type Falcon (F1 Sterile), the materials tested were weighed and the solutions added in a ratio of 0.2 g/ml. The samples were shaken (IKA VIBRAX, VWR) in a thermostat (Heraeus T-12) at temperature 37 ± 1 °C for 72 ± 2 h. 0.9% NaCl and sesame oil (control samples) were also incubated in the thermostat. The prepared extracts were administered intravenously or intraperitoneally to mice. The injection dosage was the same for all groups and amounted to 50 ml/kg of the body mass. During the tests, the following parameters were controlled: body mass of the animals, daily feed, water intake, and mortality. Every day the behaviour of the animals, their physical activity, respiratory functions, reactions, fur bristling, muscle tension, pain sensitivity and gastric symptoms were observed. After seven days, mice both from the control and test groups underwent section tests which consisted of macroscopic pathomorphological assessment of internal organs.

Statistical evaluation of the results of body mass measurement, weight gain, daily feed and water consumption were performed by variance analysis followed by Dunnet's test. Differences between the groups compared were considered statistically significant at $p < 0.05$. All data was analysed using the program GraphPad Prismv.6.01 for Windows (GraphPad PrismSoftware, Inc., USA).

Results and discussion

In the first stage of the research, the chemical purity of both materials used in the manufacture of prostheses was examined (***Table 1***).

It was found that Codubix S (PP/PE knitted prosthesis) has a slightly higher degree of chemical purity than Mendec Cranio acrylate resin (***Table 1***). The extract from Codubix S contains less contamination, mainly organic and in a smaller degree non-organic, which was proved by the permanganate oxidation and UV absorbance tests. Both materials tested do not contain chloride, sulphate, or heavy metal ions. In the case of Mendec Cranio acrylate resin, a small amount of ammonium ions was

Table 2. *Body weight and weight gain in female and male mice (N = 5/group) exposed intravenously to 0.9% NaCl or the extract of CODUBIX S in 0.9% NaCl and intraperitoneally to sesame oil or the extract of CODUBIX S in sesame oil. N = number of animals per group.* ***Note: *** *– difference between the weight on the day of injection and on that of the autopsy.*

	Body weight, g on subsequent days								Weight gain, g*
	0	1	2	3	4	5	6	7	
Females	Control 0.9% NaCl (i.v.)								
Avg	24.7	24.3	24.2	24.6	24.7	24	24.1	24.2	+0.1
SD	1.2	1.1	1.6	1.3	2.1	0.91	0.9	0.9	1.3
Extract of CODUBIX S in 0.9% NaCl (i.v.)									
Avg	25.3	24.9	25.3	25.5	25.6	25.5	25.6	25.6	-0.2
SD	1	1.3	1.4	1.3	1.2	1.4	1.4	1.4	1.0
Males	Control 0.9% NaCl (i.v.)								
Avg	30.2	28.7	29.3	29.5	30.3	30.6	30.7	30.8	+1.0
SD	2.7	2.2	2.4	2.3	2.9	2.9	2.9	3.0	1.2
Extract of CODUBIX S in 0.9% NaCl (i.v.)									
Avg	28.8	28.6	29	29.4	29.2	29.3	29.5	29.6	+1.8
SD	1.2	1.9	2	1.8	2.6	2.6	2.7	2.6	0.4
Females	Control sesame oil (i.p.)								
Avg	25.5	25.0	25.6	26.0	26.2	26.5	26.9	26.8	+1.3
SD	0.6	0.6	0.6	1.0	1.0	1.2	1.4	1.1	1.3
Extract of CODUBIX S in sesame oil (i.p.)									
Avg	25.0	24.7	24.9	25.6	25.7	25.8	25.9	26.0	+1.0
SD	1.5	1.6	1.7	1.2	1.3	1.2	1.3	1.1	0.9
Males	Control sesame oil (i.p.)								
Avg	30.9	30.6	30.2	30.7	31.2	31.3	31.8	31.8	+0.9
SD	0.7	1.2	2.2	1.6	1.4	1.4	1.4	1.5	1.2
Extract of CODUBIX S in sesame oil (i.p.)									
Avg	31.5	31.1	31.1	31.7	32.3	32.6	32.8	33.1	+1.6
SD	1.0	1.8	1.7	1.9	1.7	1.9	2.0	1.9	1.2

Table 3. *Body weight and weight gain in female and male mice (N = 5/group) exposed intravenously to 0.9% NaCl or the extract of MENDEC CRANIO resin in 0.9% NaCl and intraperitoneally to sesame oil or the extract of MENDEC CRANIO resin in sesame oil. N = number of animals per group.* ***Note: *** *– difference between the weight on the day of injection and on that of the autopsy.*

	Body weight, g on subsequent days								Weight gain, g*
	0	1	2	3	4	5	6	7	
Females	Control 0.9% NaCl (i.v.)								
Avg	24.7	24.3	24.2	24.6	24.7	24.0	24.1	24.2	+0.1
SD	1.2	1.1	1.6	1.3	2.1	0.9	0.9	0.9	1.3
Extract of MENDEC CRANIO in 0.9% NaCl (i.v.)									
Avg	24.6	24.3	24.2	24.5	24.0	24.1	24.3	24.4	+0.3
SD	2.2	2.1	2.1	2.2	1.6	1.6	1.6	1.6	1.2
Males	Control 0.9% NaCl (i.v.)								
Avg	30.2	28.7	29.3	29.5	30.3	30.6	30.7	30.8	+0.6
SD	2.7	2.2	2.4	2.3	2.9	2.7	2.9	3.0	1.2
Extract of MENDEC CRANIO in 0.9% NaCl (i.v.)									
Avg	28.4	29.4	29.5	30.2	30.2	30.5	30.6	30.6	+0.5
SD	1.4	1.5	1.6	1.8	1.8	1.6	1.6	1.6	1.1
Females	Control sesame oil (i.p.)								
Avg	25.5	25.0	25.6	26.0	26.2	26.5	26.9	26.8	+1.3
SD	0.6	0.6	0.6	1.0	1.0	1.2	1.4	1.1	1.3
Extract of MENDEC CRANIO in sesame oil (i.p.)									
Avg	25.0	26.4	24.8	25.1	25.5	25.6	25.9	26.3	+1.3
SD	0.8	1.0	1.0	1.2	1.3	1.3	1.5	1.8	1.0
Males	Control sesame oil (i.p.)								
Avg	30.9	30.6	30.2	30.7	31.2	31.3	31.8	31.8	+0.9
SD	0.7	1.2	2.2	1.6	1.4	1.4	1.4	1.5	1.2
Extract of MENDEC CRANIO in sesame oil (i.p.)									
Avg	30.9	30.9	31.1	30.9	31.4	31.8	32.1	32.4	+1.5
SD	2.9	3.3	3.4	3.0	3.0	3.0	3.1	2.4	1.3

Table 4. *Average daily feed and water consumption by the mice (N = 5/group) used in systemic acute toxicity testing of Codubix S.*

Sex	0.9% NaCl			Extract of Codubix S in 0.9% NaCl intravenously		
	Average daily feed consumption (g/mouse) on selected days of the study					
	day 1	day 3	day 7	day 1	day 3	day 7
Females	4.1 ± 1.8	5.2 ± 1.3	4.9 ± 0.5	4.9 ± 1*.7	5.3 ± 0.5	4.7 ± 1.1
Males	5.3 ± 1.7	5.3 ± 0.6	6.1 ± 1.1	6.4 ± 2.2	6.8 ± 1.9	5.9 ± 1.0
Average daily water consumption (ml/mouse) on selected days of the study						
Females	10.4 ± 2.3	9.8 ± 1.7	9.0 ± 1.6	10.6 ± 1.1	9.2 ± 1.5	8.6 ± 0.9
Males	11.4 ± 2.7	10.0 ± 1.2	9.6 ± 0.9	13.6 ± 1.3	9.4 ±0.5	10.6 ±1.1
Sex	**Sesame oil**			**Extract of Codubix S in sesame oil intraperitoneally**		
	Average daily feed consumption (g/mouse) on selected days of the study					
Females	2.4 ± 0.6	4.8 ± 0.5	4.9 ± 0.8	2.0 ± 0.4	4.9 ± 0.4	4.9 ± 0.7
Males	3.0 ± 0.8	4.9 ± 0.7	5.1 ± 0.8	2.3 ± 0.5	5.6 ± 0.8	5.9 ± 0.7
Average daily water consumption (ml/mouse) on selected days of the study						
Females	7.6 ± 1.0	9.2 ± 1.1	10.4 ± 0.6	8.0 ± 0.7	10.0 ± 0.71	9.8 ± 0.5
Males	10.0 ± 1.2	10.0 ± 1.6	10.0 ± 1.2	8.8 ± 0.8	12.0 ± 1.00	11.6 ± 1.1

Table 5. *Average daily feed and water consumption by the mice (N = 5/group) used in systemic acute toxicity testing of Mendec Cranio resin.*

Sex	0.9% NaCl			Extract of Mendec Cranio in 0.9% NaCl intravenously		
	Average daily feed consumption (g/mouse) on selected days of the study					
	day 1	day 3	day 7	day 1	day 3	day 7
Females	4.1 ±1.8	5.2 ±1.3	4.9 ±0.5	3.8 ±1.0	5.1 ±0.6	4.6 ±0.5
Males	5.3 ±1.7	5.3 ±0.6	6.1 ±1.1	4.9 ±0.7	5.2 ±1.3	5.2 ±0.8
Average daily water consumption (ml/mouse) on selected days of the study						
Females	10.4 ±2.3	9.8 ±1.7	9.0 ±1.6	10.4 ±1.7	8.8 ±0.8	9.0 ±1.4
Males	11.4 ±2.7	10.0 ±1.2	9.6 ±0.9	13.2 ±1.3	9.2 ±0.8	10.0 ±1.2
Sex	**Sesame oil**			**Extract of Mendec Cranio in sesame oil intraperitoneally**		
	Average daily feed consumption (g/mouse) on selected days of the study					
Females	2.4 ±0.6	4.8 ±0.5	4.9 ±0.8	2.9 ±0.7	4.3 ±0.5	4.7 ±1.4
Males	3.0 ±0.8	4.9 ±0.7	5.1 ±0.8	2.4 ±0.7	5.0 ±1.1	5.7 ±0.9
Average daily water consumption (ml/mouse) on selected days of the study						
Females	7.6 ±1.0	9.2 ±1.1	10.4 ±0.6	9.1 ±0.9	10.8 ±1.3	10.0 ±0.7
Males	10.0 ±1.2	10.0 ±1.6	10.0 ±1.2	9.4 ±0.6	10.6 ±1.1	12.2 ±1.1

observed. This result may translate into a slightly higher biological compatibility of Codubix S implants.

During the tests of Codubix S and Mendec Cranio acrylate resin, neither in the groups with intravenous injections of 0.9% NaCl and extracts from both prostheses tested in 0.9% NaCl, nor in the groups with intraperitoneal injections of sesame oil and extracts from the products in sesame oil, was the death of mice observed. The behaviour and appearance of the animals were normal. Between the mice in the control and test groups, there were no significant differences in the body mass (***Tables 2*** and ***3***) or in the mean daily feed and water consumption (***Tables 4*** and ***5***). As part of daily clinical observations, parameters such as animal behaviour, motor activity, respiratory activities, muscle tension, pain sensitivity and gastrointestinal symptoms were also evaluated.

There were no statistically significant differences in body weight between the control group mice and those with an intravenous injection of Codubix S extract in 0.9% NaCl (***Table 2***). Similarly, the average daily intake of feed and water in the control groups did not differ significantly from the parameters in the groups of animals after intravenous injections of Codubix S extracts at 0.9% NaCl (***Table 4***). Statistical analysis of body mass, weight gain on the day of the injection and the mass on the day of the section, and of the daily feed and water consumption did not reveal statistically significant differences between the parameters evaluated for animals in the control group and those receiving intraperitoneal injections of Codubix S extracts in sesame oil (***Tables 2*** and ***4***). The average daily feed intake of female mice ranged from 2.5 to 5.5 g/mouse, and by males from 3.0 to 6.0 g/mouse, which was similar in the control groups and in those receiving injections of extracts from Codubix S. The daily water intake was from 8 to 12 ml/mouse and from 10 to 15 ml/mouse in females and males, respectively. There were no statistically significant differences between groups of mice of the same sex.

Also, in the case of the Mendec Cranio resin extract in 0.9% NaCl in intravenous injections, no statistically significant differences in the body weight of the mice were found compared to the control group injected with 0.9% NaCl solution (***Table 3***). Similarly, the average daily intake of feed and water in the control groups did not differ significantly from these parameters in the groups of animals after the intravenous injection of extracts from Mendec Cranio resin in 0.9% NaCl (***Table 5***).

For intraperitoneal injections of sesame oil and Mendec Cranio resin extract in sesame oil, statistical analysis of body mass, weight gain between the mass on the day of the injection and weight on the day of the section, and daily feed and water intake did not reveal statistically significant differences between the parameters assessed in animals of the control group and those treated with a resin extract (***Tables 3*** and ***5)***.

The average daily feed intake of female mice ranged from 2.5 to 5.5 g/mouse, and by males from 3.3 to 6.3 g/mouse, which was similar in the control groups and those receiving injections of extracts from Mendec Cranio resin. The daily water intake was from 7 to 12 ml/mouse and from 8 to 16 ml/mouse in females and males, respectively. There were no statistically significant differences between groups of mice of the same sex.

During the 7 days of the test, in none of the mice was there observed toxic action caused by single intravenous or intraperitoneal injection of 0.9% NaCl, sesame oil, PP/PE and acrylate resin extracts in the above-mentioned solvents at a dosage of 50 ml/kg.

In none of the animals put to death were there observed macroscopic pathological changes in the internal organs taken from the chest or abdominal cavity

which could suggest toxic activity of either Codubix S (***Figure 3***) or Mendec Cranio.

Thus, the risk of occurrence of an acute systemic toxic reaction caused by both substances is infinitesimal.

Modern implants used in cranioplasty have to fulfill a whole range of requirements, e.g. they must be biocompatible, plastic, with the ability to heal into the surrounding bone tissue without scarring the connective tissue; they must possess high mechanical endurance, must have low thermal and electrical conductivity, and they must be characterised by high chemical purity. Some of the elements taken into consideration while choosing a substance used as reconstruction material are also, apart from its availability, price or ease of implantation, its direct and late post-implantation properties – such as acute toxicity.

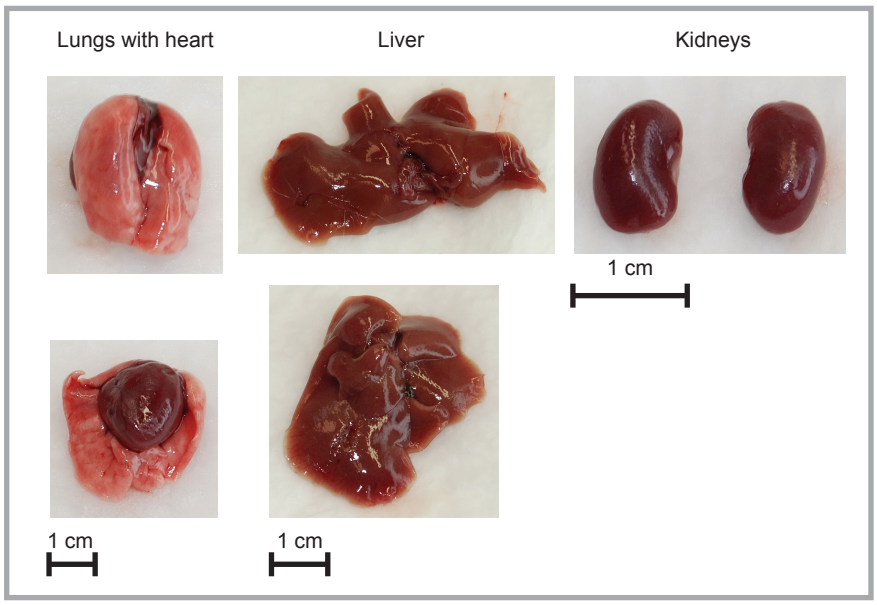

Figure 3. *Selected organs isolated from animals exposed to Codubix S extracts in 0.9% NaCl. The images present normal organs.*

Conclusions

For both prostheses tested, it was found that single intravenous or intraperitoneal administration of extracts from Codubix S (size R4) and Mendec Cranio resin in 0.9% NaCl or sesame oil at 50 ml/kg body weight does not cause toxicity in female or male Balb/c mice during the 7 days after injection. Moreover, it also does not reduce the body weight by more than 10% in relation to the animals' body weight before the injection.

The results of the tests carried out with Codubix S and Mendec Cranio resin, whose toxicity was tested in accordance with PN EN ISO 10993-11, allow to state that these medical devices do not cause an acute systemic reaction in mice.

Control tests were also performed to indicate that a single intravenous or intraperitoneal administration of 0.9% NaCl or sesame oil at a dose of 50 ml/kg body weight does not cause toxic effects in female or male Balb/c mice during 7 days after the injection, nor does it lower the body weight by more than 10% in relation to that of the animals before injection.

Studies on mice indicate that both commercially available prostheses can be considered products that do not exert an acute systemic effect on humans.

Comparing the tested characteristics of the two substances used for manufacturing medical implants: Mendec Cranio resin based on PMMA and knitted Codubix prosthesis based on PP/PE, it can be stated that there are no counter-indications for using both of them in experimental animals. Such results mean that making a choice between the two substances mentioned in clinical practice requires further tests in terms of other physical, chemical and medical properties. However, it seems prudent to propose new procedures for cranial reconstruction in which for both materials tested a new solution combining the advantages of both prostheses would be established.

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