Biological And Chemical Properties of Cured Epoxy Resins

Anna Rudawska
Lublin University of Technology
Katarzyna Sarna-Boś (katarzynasarnabos@umlub.pl)
Medical University of Lublin
Adrianna Rudawska
National Veterinary Research Institute
Ingrid Różyło-Kalinowska
Medical University of Lublin
Renata Chałas
Medical University of Lublin

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Abstract

Introduction: Launched into production over 50 years ago, epoxy resins aroused enormous interest owing to their valuable properties that distinguish them from other polymeric materials. The investigation of biological and chemical effects of direct exposure to the materials under study on the human body may help in the organization of work when dealing with this type of materials.

Objectives: The objective of this study is to investigate selected biological and chemical properties of 3 cured epoxy compounds: Epidian 53 epoxy resin with polyaminoamide C curing agent, Epidian 53 epoxy resin with polyaminoamide C curing agent, and aluminum powder, and Epidian 53 epoxy resin with polyaminoamide C curing agent, and copper powder.

Methods: The experimental part of this paper describes the preparation and fabrication of adhesive compound samples, from a liquid state to cured plates. The study involved a cytotoxicity analysis (with an ELSA reader), pH measurements (with a pH meter), absorbance measurement over an entire reference wavelength range, and sterilization (on a specially designed test bench) along with microscopic examination of its effects.

Results: Cytotoxicity and absorbance analysis results demonstrate that extracts of all tested resin samples do not have cytotoxic effect on the cells of living organisms. The absorbance results over the wavelength range do not indicate the formation of aggregations, which proves that no toxic agents harmful to living organisms are extracted from the resin samples.

Conclusion: Results of this study demonstrate that cured epoxy resins are safe materials.

1. Introduction

Epoxy compounds for technical applications and the methods of their manufacturing were known already in the nineteenth century [1-4]. Over 85% of produced epoxy resins are created as a result of the reaction between bisphenol–A and epichlorohydrin (ECH) [4-6]. Launched into production over 50 years ago, epoxy resins aroused enormous interest owing to their valuable properties that distinguish them from other polymeric materials [7-9]. Due to their unique properties, epoxy resins have been applied in many fields of technology, including the aviation industry, boatbuilding, electrical engineering and electronics [5,10-12]. As a result of their polar nature, epoxy resin compounds have very high adhesion to many materials such as glass, ceramics, metal, concrete and polymers.

Over 55% of manufactured epoxy resins are used as coating materials. These are predominantly single- or two-component solvent-based lacquers or solvent-free powder paints that form considerably thicker anti-corrosion layers. Powder resins containing pigments or curing agents are applied to metals by fluidization or electrostatic method. The compound melts and pours over the metal surface, and then cures at high temperature, forming a thick, durable coating which is hard, elastic and resistant to chemical agents [13].

Epoxides are viscous liquids or brittle solids that change their structure during the curing process, and – as a result of crosslinking – become insoluble and infusible, acquiring high strength, good electrical insulating properties and considerable chemical resistance [14,15]. Resins are the main component of many compounds containing various additives such as curing agents and accelerators or modifying agents, including elasticizing

agents, reinforcing fibers, powder fillers, etc [16-22]. Using fillers and other additives we can produce materials with different parameters. The combination of resin and fiberglass yields construction materials that exhibit metal-like properties yet are several times lighter and corrosion resistant [23,24]. One of the factors constraining the widespread use of laminates is their fairly high price; however, it is possible to reduce this high cost by proper use of fillers and at the same time maintain all desired parameters.

The curing process is based on polyaddition reactions or polymerization where no by-products are generated, which means that the process does not have to proceed under high pressure conditions [25-29]. Curing through polymerization is initiated with the use of tertiary amines [6]. In the last 20 years, a photochemical (ultraviolet) curing process was developed [30,31], consisting in cationic polymerization initiated by onium salts ad also the other methods of curing (microwave [32,33], y-ray [34], electron beam [34,35] and others were also investigated. Along with the introduction of new compounds, research was carried out to investigate the mechanisms and kinetics of the curing process. The curing process is accompanied by a slight shrinkage of material, which leads to an accurate reproduction of the mold shape. In effect, internal stresses are low, and the introduction of elasticizing agents makes it possible to eliminate undesired stresses. Cured epoxides have high mechanical strength in combination with high resistance to weather conditions, water and chemicals [1,4,7].

Due to the above very numerous applications of epoxy resins, the purpose of many works is to determine the various properties of materials containing these resins (adhesives, composites, laminates, coats) - often modified resins and resins themselves. First of all, the structure, chemical properties of new structures and mechanical properties are tested, knowledge of which is necessary when designing structures containing materials based on epoxy resins. The characteristics of resins and curing agents in the data sheets show the necessary physical properties, including those related to the danger of using these chemicals. However, other properties exhibit epoxy compositions in the cured state. When making epoxy compositions, the safety of people preparing this material is important, but there is little information available on the subject. Toxicology examines the properties of toxic factors and the negative effects of their impact on the body.

The study of chemical compounds with potential medical applications requires many biochemical tests. One of the basic researches is to determine the cytotoxic activity of a given substance. Cytotoxicity is a term that means broadly understood the toxicity of various substances and different types of cells to the cells in a given organism. Cytotoxicity means the ability of a substance to destroy a particular type of cell [36].

The investigation of biological and chemical effects of direct exposure to the materials under study on the human body may help in the organization of work when dealing with this type of materials. Correct analysis of obtained results will help establish effective procedures for safe handling of such materials.

The objective of this work is to present selected biological and chemical properties of three different cured epoxy compounds, based on results obtained in experimental tests.

2. Methods

The study involved performing the following biological and chemical analyses: cytotoxicity analysis to identify toxic agents in cured resin compounds, absorbance measurement over an entire wavelength range, microscopic examination after autoclave sterilization and pH measurements. The following media were used in the research, available on the market forbiotechnological laboratory tests, FBS - serum, PBS - phosphate buffered saline, MTT

- 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazoliumbromide, DMSO - dimethylsulfoxide and MEM - Medium Eagle minimum essential medium. No animal or human cells collected by the researchers were used in the studies. The procedure for performing cytotoxicity tests is described in section 3.2.1.

2.1. Description of epoxy compounds under study

The study was performed on samples of 3 epoxy adhesives presented in Table 1. They were fabricated with the use of Epidian®53 epoxy resin, polyaminoamide C (PAC - trade name) curing agent, and two types of metal powder fillers (aluminum and copper).

Table 1
Types of epoxy compounds

Epidian® 53 content (g)	*PAC content (g)*	*Type of powder filler*	*Filler content (g)*	*Denotation of adhesive compound*
100	80	-	-	Epidian57/PAC/100:80
100	80	aluminum	2	Epidian57/PAC/Al/100:80:2
100	80	copper	2	Epidian57/PAC/Cu/100:80:2

2.1.1. Epoxy resin

Epidian® 53 epoxy resin is manufactured by CIECH Sarzyna S.A. [37]. It is primarily used for saturating, casting and air-tight sealing of electrical equipment, as an impregnating agent in glass fiber laminates and as a component of structural adhesives. This compound is a mixture of bisphenol A and epichlorohydrin with an average molecular weight less than 700 and with the addition of styrene. The mechanical properties of Epidian 53 epoxy resin are described in [38]. In this work, the focus was primarily put on biological and chemical properties of this resin, as they are essential for further analyzes undertaken in this study. Table 2 lists the toxic components of Epidian® 53.

Table 2
Safety data sheet of Epidian 53 [38]

	CAS Number	*WE Number*	*Symbol*	*R-phrases*	*% wt.*
Epoxy Resin (average molecular weight ≤ 700)	25068-38-6	500-033-5	Xi, N	36/38-43-51/53	>50%
styrene	100-42-5	202-851-5	Xn, Xi	10-20-36/38	<12.5%
Legend: Xi – irritant Xn – harmful N- dangerous for the environment R 36/38 – irritating to eyes and skin R 43 – may cause sensitization in skin contact R 51/53 – toxic to aquatic organisms					

Epidian® 53 is irritating to skin and eyes. It may cause sensitization in direct skin contact. This epoxy resin is also dangerous for the natural environment; it is toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment. This product must be stored with particular care. The mixture is flammable and contains styrene which may form an explosive mixture with air. Since styrene vapors are heavier than air, they tend to accumulate in the lower parts of rooms such as basements, canals and hollows. It should be stored in original, tightly-sealed packaging in a dry, well-ventilated place, away from direct sunlight at a temperature below 25^{o}C. Under the recommended conditions, the product does not pose any hazards; it does not decompose, and remains stable. Amines and amides that promote the curing process should be avoided.

The basic physical and chemical properties of Epidian 53 are listed in Table 3.

Table 3
Physical and chemical properties of Epidian 53 [38]

Property	*Characteristic/Value*
Form	Pale yellow, highly viscous liquid
Odor	Weakly perceptible
pH value	7 pH
Density at 20°C	1,11-1,15 g/cm^3
Viscosity at 25°C	900-1500 mPa
Water solubility	Insoluble

The product is classified as a hazardous chemical mixture, irritating to the eyes and skin. It may cause sensitization. Table 4 lists the resin values considered acutely toxic. Product waste must be handled with special care, in accordance with provisions of the Waste Act, environmental protection requirements and waste management plans.

Table 4
Toxic properties of Epidian® [38]

Inhalation	*Epoxy resin LC_{50}*	*No data available*
	Styrene LC_{50} (rat) 4h	24 000 mg/m^3
	TCL_0 (human)	2600 mg/m^3
	LCL_0 (human)	43 000 mg/m^3
Skin	Epoxy resin LD_{50} (rat)	> 2000 mg/kg
	Styrene LD_{50}	No data available
Ingestion	Epoxy resin LD_{50} (rat)	> 2000 mg/kg
	Styrene LD_{50} (rat)	5000 mg/kg

Other waste products must be properly stored and disposed. The price of the product includes the container deposit, which means that the retailer is obliged to accept empty containers from the user. Disposal to soil, sewage systems and water reservoirs is strictly prohibited [30].

2.1.2. Polyaminoamide C curing agent

The polyaminoamide C (PAC - trade name) curing agent, is a polyamide hardener, and its characteristics are given in [38]. This curing agent is used for curing liquid, low molecular weight epoxy resins and compounds based on such resins. The addition of polyaminoamide C leads to increased elasticity and impact strength of the compound, therefore this hardener is used for making high-strength joints, e.g. in adhesive-bonding of thin sheets or bonding rubber with metal; it is also used in household adhesives or casting elements in electronics and electrical engineering. This curing agent is a brown-color polyaminoamide with low reactivity. The weight ratio of curing agent to epoxy resin can be varied in a wide range to adjust the reaction rate and properties of the cured material. When compared to the compound with a higher epoxy resin content, the compound with a higher curing agent content is more elastic and more impact resistant but has lower hardness and lower resistance to elevated temperatures. The addition of polyaminoamide C in a quantity above 70 % wt leads to increased elasticity of the material. On adding the curing agent to the epoxy compound, the curing process is initiated and the compound's pot life begins. The pot life of a compound, also known as open time, is the gel time during which the compound should be processed. This time depends on several factors, such as temperature and compound amount, and will vary depending on individual conditions. After this time, the curing process takes place. Polyaminoamide C is one of the slow curing agents. Under standard conditions, i.e. at room temperature and relative humidity of 50%, the gel time is 180 minutes. After this time, initial curing takes place within the next 6-8 hours, and after 72 hours the curing reaches 80-90%. Total curing occurs after 7-14 days. The curing process can be accelerated by the application of a higher temperature after the first curing stage [38].

When handling with this curing agent, one must follow the provisions of the Product Safety Data Sheet along with health and safety requirements and fire regulations. The product is caustic, skin irritating and may cause sensitization. It also causes long-term adverse effects in the aquatic environment and is toxic to aquatic organisms [38].

2.1.3. Powder fillers – aluminum Al and copper Cu metal powders

Epoxy compounds were prepared with an admixture of fillers in the form of aluminum and copper metal powders. The characteristics of these materials are given below.

Electrolytic copper powder is predominantly used in the powder metallurgy industry for producing sintered machine parts and structural components, and in the chemical industry. It may contain inclusions of lead (max 0.05%), iron (max 0.02%), oxygen (max 0.3%) and residues insoluble in HNO_3 (max 0.05%). Under Directive 67/548 / EEC and Regulation 1272/2008 / EC, it is not classified as harmful or hazardous. However, prolonged exposition to the product in powdered form may cause irritation of the eyes, skin and respiratory system. The product is non-flammable and flame retardant. Larger amounts of the product mustn't be freely released or spread in the natural environment, particularly in the vicinity of ground and surface waters, wells and water intakes. The product must be stored in a dry and well-ventilated room, and kept away from humid environments and oxidants. When properly stored and used, the product is stable and poses no risk to the user. Table 5 gives the toxicological information about copper powder, while its physical and chemical properties are described in Table 6.

Table 5
Copper powder safety data sheet [39]

Derived Non-Effect Levels:	
DNEL (long-term, oral, dermal)	0.041 mg/kg bodyweight/day
DNEL (acute, oral, dermal)	0.082 mg/kg bodyweight/day
Predicted No-Effect Concentrations:	
PNEC aqua (freshwater)	
PNEC aqua (marine water)	
PNEC sediment (freshwater)	87 mg/kg dry matter
PNEC sediment (marine water)	676 mg/kg dry matter
PNEC soil	65.5 mg/kg dry matter
PNEC sewage treatment plant	

Table 6
Physical and chemical properties of copper powder [39]

Physical state / Appearance	*Solid / Powder*
Color	Copper
Odor	Odorless
Melting point/Freezing point	1083^{o}C
Flammability (solid, gas)	Non-flammable
Upper/Lower flammability or explosive limits	No data available
Water solubility	Insoluble
Explosive properties	Risk of dust explosion
Oxidizing properties	Not applicable
Bulk Density	1.2-3.5 g/cm^3
Particle size	0.063 mm

Aluminum powder, chemically known as stabilized aluminum powder, is mainly used as a substitute in the production of autoclaved aerated concrete, and as a pigment for paints and varnishes in pyrotechnics. According to the classification under Directive 67/548 /EEC and Regulation 1272/2008/EC, this substance is highly flammable and releases flammable gases in contact with water. It presents no significant hazards by skin contact. Adverse effects are possible, i.e. prolonged exposure to dust or fume concentrations will produce irritation of the eyes, skin and respiratory tract. Dust may cause mechanical irritation of eyes. Physical and chemical properties of aluminum powder are listed in Table 7.

Table 7
Physical and chemical properties of aluminum powder [39]

Physical state / Appearance	*Solid / Powder*
Color	silver
Odor	odorless
Melting point/Freezing point	660 ^{o}C
Initial boiling point	2327^{o}C
Flash point	460^{o}C
Flammability (solid, gas)	Flammable
Solubility (20^{o}C)	Water insoluble, in contact with water releases flammable gas; insoluble in organic solvents
Explosive properties	Risk of dust explosion
Particle size	0.063 mm

2.2. Shape, dimensions and preparation method of test samples

The samples of epoxy compositions were prepared in the form of rigid solids cast in a silicone form. Fig. 1 shows the dimensions of cured adhesive compound samples.

The preparation of epoxy resin compound samples proceeded as follows:

- casting molds were prepared and cleansed, and then coated with an anti-adhesion agent for polymeric materials, Polsilform® (manufactured by Polish Silicone [40]),
- 100 g of epoxy resin was metered,
- 2% wt. (2 g) of a metal filler was added to the resin,
- the components were mixed with a high-speed mixer for about 120 s at 460 rev/min,
- the compound was left to degas for 120 s,
- 80 g of polyaminoamide C curing agent was added to the compound,
- the compound was re-mixed for about 120 s,
- the compound was left to degas for 120 s.
- samples were fabricated in the prepared molds.

Compounds without metal fillers were fabricated in an analogous manner, without adding modifier particles. These epoxy compounds were fabricated at an ambient temperature of 27±3 ° C and cured for about 7 days. Fig. 2 shows the epoxy resin samples with different compounds.

2.3. Experimental procedures

2.3.1. Cytotoxicity analysis

A cytotoxicity analysis was conducted to determine harmful components in the cured epoxy resin. This method is used to assess the toxicity level of a substance relative to adjacent cells. It is based on enzymatic reactions that produce a colored product, identified spectrophotometrically with the ELSA reader. The cytotoxicity analysis of material particles is a perfect introduction to toxicological tests that are necessary to determine the correct operation and safety of the material being tested. In the case of cell cultures, test substances and their precursors or metabolites are added to the medium in order to determine their activity at the cellular level, and often at the molecular level too.

The toxicity analysis was performed with the use of the following laboratory equipment:

- incubator with humid environment, 37^{o}C, 5% CO_2, (Binder C150);
- laminar flow cabinet (biological hazard standard) with HEPA filters: a HEPA H-14 filter capturing 99.999% of particles with a diameter < 0.3 μm, and a HEPA filter capturing 99.999% of particles with a diameter > 0.3 μm;
- water bath, 37^{o}C;
- inverted phase contrast microscope;
- centrifuge;
- scales;
- plate photometer provided with 570 nm filter and shaking mode;
- Bürker hemocytometer;
- automatic pipette controller with serological pipettes;
- automatic pipettes, multi-channel automatic pipettes;
- vessels for cell cultivation.

The tests were conducted using the following chemical reagents, media and serum: MEM (Minimum Essential Medium Eagle, without phenol red, glutamine and serum), FBS (serum), penicillin/streptomycin, glutamine, Trypsin-EDTA solution, PBS (phosphate buffered saline), MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and isopropanol: analytically pure.

To ensure proper testing conditions, all solutions, media, vessels for cell cultivation and tools must be sterile. All operations were carried out in the laminar flow cabinet under sterile conditions. The MTT solution was dissolved in a clean and fresh MEM medium without phenol red at a concentration of 1 mg / ml. The compound was sterilized with the use of a syringe filter and used on the same day. Material samples were extracted from the cured samples of adhesive compounds. Prior to use and contact with cells, all fluids were heated in the water bath to 37^{o}C. On the first day of testing, cells were collected by enzymatic digestion and centrifuged for 3 minutes. The prepared pallet was suspended in a cell culture medium and diluted to achieve a concentration of $1x10^5$ cells / ml. 100 μl of PBS was loaded into the peripheral wells on the 96-well microplate (Fig. 3), and 100 μl of the prepared cell suspension was loaded into the other wells. The microplate was incubated for 24 hours under the following conditions: 5% CO_2, 37°C, humidity > 90%.

On the next day, the microplate was examined under a phase-contrast microscope to check for even cell growth. Diluents of the extracts of the tested cured epoxy compounds were prepared, as specified in Table 8.

Table 8
Extract concentrations of cured epoxy compounds

Concentration	*Extract Volume*	*MH Volume*
1x	500 µl	0 µl
2x	250 µl	250 µl
3x	170 µl	330 µl
1x	500 µl	0 µl
MH – cell culture medium		

Next, the cell culture media were removed from the microplate, and 100 µl of a relevant solution was added to the wells with the cells, as specified in Table 9.

Table 9
Schematic design of a 96-well microplate used for examination of extracts of three types of cured epoxy compounds

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
A	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
B	PBS	B	A1x	A1x	A1x	A1x	A2x	A2x	A2x	A2x	B	PBS
C	PBS	B	A3x	A3x	A3x	A3x	A4x	A4x	A4x	A4x	B	PBS
D	PBS	B	B1x	B1x	B1x	B1x	B2x	B2x	B2x	B2x	B	PBS
E	PBS	K-	B3x	B3x	B3x	B3x	B4x	B4x	B4x	B4x	K+	PBS
F	PBS	K-	C1x	C1x	C1x	C1x	C2x	C2x	C2x	C2x	K+	PBS
G	PBS	K-	C3x	C3x	C3x	C3x	C4x	C4x	C4x	C4x	K+	PBS
H	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS

K- – negative control

K+ – positive control

A1x – extract of medical product A, undiluted

A2x – extract of medical product A, 2-fold diluted

A3x – extract of medical product A, 3-fold diluted

A4x – extract of medical product A, 4-fold diluted

B1x – extract of medical product B, undiluted

B2x – extract of medical product B, 2-fold diluted

B3x – extract of medical product B, 3-fold diluted

B4x – extract of medical product B, 4-fold diluted

C1x – extract of medical product C, undiluted

C2x – extract of medical product C, 2-fold diluted

C3x – extract of medical product C, 3-fold diluted

C4x – extract of medical product C, 4-fold diluted

B – blank (bottled medium cells)

The microplate was re-incubated for 24 hours at 5% CO_2, 37°C, humidity > 90%. On the next day, the microplate was examined under a phase contrast microscope for verification of cell culture growth.

2.3.2. Absorbance assay

Aimed at identification of toxic agents in the cured compounds, the cytotoxicity analysis also involved absorbance measurement in an entire wavelength range. The absorbance assay technique is based on the Lambert-Beer law, which describes the relationship between absorbance and the concentration of an absorbing material and the width of a cuvette affecting the path length (distance that light travels through the material). Material concentration is one of the key factors affecting the amount of absorbed light. In the experiments, the absorbance assay was performed with the µQuant microplate spectrophotometer from Bio-Tek Instruments. The µQuant is a single-channel analyzer used for research and development and in-vitro diagnostics, designed to automatically perform endpoint analysis. The wavelength range is from 200 nm to 999 nm.

50 µl of the MTT solution was added to the wells with cells and incubated at 37°C for 2 hours. After that, the MTT solution was removed from over the cells, and 100 µl of isopropanol was added to the wells with the cells. The microplate was incubated at 37°C for 10 minutes, and then shaken for 1 minute, and the absorbance was measured at 570 nm. Analysis of the results was performed for the OD_{570} of negative control greater than 0.2. The difference between the mean OD_{570} of negative control on the left and right of the microplate (B2; C2; D2 versus B11; C11; D11) should not exceed 15%. Cell viability was calculated based on Eq. (1):

viability % = 100x *OD570e/ OD570b* (1)

where: OD570e – mean OD570 of the samples containing 100% extract

OD570b – mean OD570 of negative controls

If cell viability drops below 70%, this means that the tested material has cytotoxic properties. The cell viability of samples containing diluted extracts should be similar or higher than that of the samples containing 100% extract. Otherwise, the test should be repeated. The absorbance was measured for 200 µl solutions of each extract (repeated 3 times) in the wavelength range between 200 nm and 800 nm. Water was used as a blank.

2.3.3. pH measurement

The pH measurement of the analyzed samples was made with the VWR pHenomenal® 1100L bench pH-meter. The process consisted in measuring an electric potential by measuring electromotive force of the cell recorded by two electrodes: an indicator electrode immersed in the test solution and a reference electrode immersed in the reference solution of known pH. The electrodes are connected to a highly sensitive voltmeter which automatically converts the read-out value into a relevant pH value. Simple pH-meters are calibrated for standard testing conditions at about 25°C. The pH-meter used in the experiments has an articulated electrode stand and IP 43-rated housing, which provides high resolution and accuracy for precise measurements. It has a large LCD graphic display with continuous LED backlight showing both pH/mV and temperature value simultaneously. It is provided with a data logging function selectable between 1 min and 1 h, and has a memory capacity of 5000 data sets. The instrument has automatic power shutoff, adjustable from 10 min to 24 h, as well as automatic buffer recognition of DIN and NIST buffers (1.68/4.00/6.86/9.18/12.54). It has three additional technical buffers at 25°C (4.00/7.00/10.00) in memory. The pH meter has a certificate confirming the compliance with the provisions of EN 61010-1: 2010 and EN 61326-1: 2013.

2.3.4. Sterilization

The main objective of sterilization is to remove all microorganisms on the surface of the material under study. The object is considered sterile if the probability of microorganism occurrence is less than 1:1000000. The most popular and reliable form of sterilization is steam sterilization. Moist heat acts here as a disinfectant which destroys microorganisms by denaturing their proteins in the cells. Closed in the sealed chamber, saturated vapor reaches a temperature higher than 100°C and thus acts as a sterilizing agent. A device for performing this type of process is known as autoclave. The device is equipped with temperature and pressure regulators.

Two variants of the sterilization process were performed:

- 121^{o}C for 15 minutes;
- 134^{o}C for 3 or 5 minutes.

The sterilization process consisted of the following stages:

- heating-up time: time required for heat to penetrate the material;
- equalization time: time during which the heat accumulated in the material is carried away, between reaching operating temperature inside the chamber and reaching the sterilizing temperature in all parts of the material load to be sterilized;
- sterilization time: time during which the operating temperature is maintained in the chamber;
- cooling-down time: time between switch-off of heating at the end of the sterilization time and reduction of internal pressure in the chamber to the level of atmospheric pressure.

The method is employed to sterilize materials that will not decompose at the temperature of the process. When sterilizing sealed containers, negative pressure is generated inside the container; therefore special care must be taken not to fill the container more than 85% of its volume. Water solutions can also be sterilized with saturated water vapor.

The experiments were performed with the use of the Medotti 22L PRO autoclave sterilizer. This instrument has a capacity of 22 l and an operating temperature range of 5-140°C. The autoclave has 5 operating modes enabling

sterilization of various types of material loads.

2.3.5. Microscopic examination

Microscopic examination involved sampling the test material with adequately prepared surface and its magnified observation. The aim of the examination was to determine the structure of the material and identify defects invisible to the unaided eye. The examination was performed with the VWR VisiScope IT 404 inverted microscope equipped with an optical system for high-magnification imaging and a high-resolution photo camera. This microscope is also provided with microscopic image analysis features such as assay, count, measurement, automatic or manual recording mode, and lens calibration. The source of light is a light white-emitting 8W P-LED system, with adjustable intensity and a color temperature of 6300 K.

3. Results

3.1. Cytotoxicity results

The quality control of the test yielded the following results:

1. The mean OD570 of the blank > 0.2 – the criterion is met and equal to 0.545;
2. The difference between the mean values of OD570 of the blank on the left and right side of the microplate <15% – the criterion is met and equal to 1.48%;
3. Cytotoxic positive control – the criterion is met; cell viability equals 45.9%

Table 10 lists the absorbance values of the tested material extracts, measured with the use of a photometer located on the prepared microplate.

Table 10
Absorbance values of microplate wells in extract analysis

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
A	0,266	0,264	0,259	0,262	0,251	0,266	0,262	0,263	0,262	0,261	0,265	0,271
B	0,252	0,516	0,499	0,532	0,594	0,536	0,568	0,576	0,573	0,543	0,535	0,26
C	0,248	0,535	0,556	0,598	0,644	0,625	0,599	0,607	0,588	0,576	0,546	0,266
D	0,258	0,572	0,594	0,606	0,644	0,632	0,632	0,623	0,612	0,573	0,566	0,268
E	0,253	0,550	0,59	0,616	0,665	0,646	0,631	0,641	0,614	0,590	0,248	0,269
F	0,259	0,555	0,583	0,611	0,621	0,637	0,635	0,635	0,611	0,583	0,249	0,271
G	0,263	0,538	0,566	0,577	0,669	0,612	0,582	0,576	0,586	0,560	0,254	0,283
H	0,278	0,275	0,276	0,275	0,269	0,279	0,279	0,282	0,279	0,279	0,280	0,284

To facilitate the execution of subsequent calculation procedures, the values listed in Table 11 were averaged for the tested epoxy compound samples. They are denoted by sample number and extract dilution value, i.e. x 1x denotes the base extract of Sample x, x 2x denotes the twofold diluted extract of Sample x, etc.

Table 11
Mean cytotoxicity values

Cytotoxicity											
1 1x	1 2x	1 3x	1 4x	14 1x	14 2x	14 3x	14 4x	23 1x	23 2x	23 3x	23 4x
99.1	**103.7**	**111.1**	**108.7**	**113.6**	**111.9**	**115.5**	**113.6**	**112.5**	**113.0**	**111.2**	**105.7**

Table 12 gives the cell viability results of the cells treated with extracts of the tested materials, obtained for each microplate well.

Table 12
Cell viability results

91.6	*97.6*	*109.0*	*98.3*	*104.2*	*105.7*	*105.1*	*99.6*
102.0	109.7	118.2	114.7	109.9	111.4	107.9	105.7
109.0	111.2	118.2	116.0	116.0	114.3	112.3	105.1
108.3	113.0	122.0	118.5	115.8	117.6	112.7	108.3
107.0	112.1	113.9	116.9	116.5	116.5	112.1	107.0
103.9	105.9	122.8	112.3	106.8	105.7	107.5	102.8

Final experimental values are given in Table 13. The cytotoxicity results take account of sample dilution and maximum possible standard deviation.

Table 13
Cytotoxicity results

Sample	*Cytox*	*Standard Deviation*
1 1x	99.1	7.2
1 2x	103.7	2.8
1 3x	111.1	7.0
1 4x	108.7	2.5
14 1x	113.6	4.2
14 2x	111.9	4.8
14 3x	115.5	6.1
14 4x	113.6	4.1
23 1x	112.5	4.2
23 2x	113.0	4.5
23 3x	111.2	8.5
23 4x	105.7	2.1
Positive control	45.9	0.589825735

Obtained cytotoxicity results are summarized in given in Table 14.

Table 14
Summary of cytotoxicity results

Material	*Cell Viability*	*Criteria*
Positive control	45.9	Satisfied
Negative control	100.5	Satisfied
1 1x	99.1	No Cytotoxicity
1 2x	103.7	No Cytotoxicity
1 3x	111.1	No Cytotoxicity
1 4x	108.7	No Cytotoxicity
14 1x	113.6	No Cytotoxicity
14 2x	111.9	No Cytotoxicity
14 3x	115.5	No Cytotoxicity
14 4x	113.6	No Cytotoxicity
23 1x	112.5	No Cytotoxicity
23 2x	113.0	No Cytotoxicity
23 3x	111.2	No Cytotoxicity
23 4x	105.7	No Cytotoxicity
OD570	0.545	Satisfied
Differences in cell growth	1.48%	Satisfied

The test was carried out correctly as indicated by the cytotoxicity assay positive control. Absorbance values obtained over the entire wavelength range shown in Fig.4 prove that there is no extraction of toxic agents from the resin samples harmful to the living organism.

3.2. pH results

Results of the pH measurement are listed in Table 15.

Table 15
pH values

Samples	*Test I*	*Test II*	*Test III*	*Mean*	*Standard Deviation*	*pH value*	*P*
Sample no. 1	6.115	6.104	6.086	6.102	0.015	6	0.039
Sample no. 14	6.087	6.015	6.077	6.060	0.039	6	0.045
Sample no. 23	5.750	5.896	5.942	5.863	0.100	6	0.733
Water	5.803	5.910	5.956	5.890	0.079	6	1.000

All samples have acid reaction. The p-value is calculated probability describing the critical level of significance that corresponds to an acceptable risk of error. Since the significance level used in practice is α = 0.05, this value was also taken in this study.

The pH values of Samples 1 and 14 are statistically higher than the benchmark for water because P <0.05. For Sample 23 P> 0.05, this means that the pH value is statistically insignificant with respect to the benchmark for water.

3.3. Sterilization results

The cured epoxy compounds were subjected to autoclave sterilization at 134°C for 5 minutes. The sterilization was aimed at investigating the effect of a specified environment, i.e. an environment with high temperature, humidity and pressure, on the structure of material. The figures given below (Figs. 5-7) show the samples before and after sterilization, in 4-fold magnification.

3.4. Microscopic results

Microscopic examination allows analyzing the structure of cured epoxy resins. Figs. 8-10 show the images captured with a bright-field microscope. The samples are shown in the magnifications of 4x and 10x, respectively.

4. Discussion

Bisphenols (BPs) are produced all over the world in large quantities. They are frequently used in the manufacture of polycarbonate plastics and epoxy resins, which are used in a variety of consumer products such as baby feeding bottles, toys and epoxy liners for food cans [41]. Epoxy resins are also widely used as dental materials, medical adhesive or coatings, bone material, etc [42,43,44,45,46].

At present, most epoxy resins are industrially manufactured from bisphenol A (BPA). Independent studies of vom Saal et al. and Okada et al. [47,48] have shown that BPA is a hormonally active agent it exhibits oestrogen-like behaviour and causes human endocrine disorders, such as precocious puberty. BPA has been acknowledged to be an estrogenic chemical able to interact with human estrogen receptors (ER). This is particularly important because ERR-γ is expressed in a tissue-restricted manner for example, it is expressed very strongly in the mammalian fetal brain and placenta at sites that could have important outcomes for newborns. Ikhlas et al. [49] also found that bisphenols are capable of inducing cytotoxicity through oxidative stress and genotoxicity. Therefore, the harmful effects of BPA on human health and the environment force us to find a BPA substitute.

O'Boyle et al. [36] investigated analogues of bisphenol F (DGEBF), which is widely used as components in epoxy resin thermosetting products, with regard to contact allergy and cytotoxicity. They are known to cause occupational and nonoccupational allergic contact dermatitis. It was found that the allergenic effects of DGEBF were dependent on its terminal epoxide groups. Lee et al. [21] evaluated the physical properties and cytotoxicity of a novel root-end filling material (EPC) which is made from epoxy resin and Portland cement as a mineral trioxide aggregate (MTA) substitute.

On the other hand Kostoryz, et al. [50] investigated the effect of adding spiroorthocarbonate (SOC) or polyol on the cytotoxicity of epoxy-based dental resins, and found that the addition of SOC and polyols to epoxy-based

resin formulations could contribute to the development of biocompatible dental composites. The SOC and polyols reduced the cytotoxicity of some epoxy-based dental resins suggesting that improved polymerization may have taken place in these epoxy-based formulations.

A complete knowledge of the structural features that contribute to the allergenic and cytotoxic effects of DGEBF will guide the development of future novel epoxy resin systems with reduced health hazards for contact with them.

Cytotoxicity and absorbance analysis of 3 cured epoxy compounds performed in this study demonstrate that the extracts of all tested resin samples have no cytotoxic effect on the cells of living organisms. Correctness of the test was verified by cytotoxicity assay positive control. The absorbance values obtained over the entire wavelength range do not point to the formation of aggregations, which proves that no toxic agents harmful to living organisms are extracted from the resin samples. The pH measurement revealed that all tested samples are acidic. The pH values of Samples 1 and 14 are statistically higher than the benchmark value of water because $P<0.05$. Sample 23 has $P>0.05$, which means that the pH values are statistically insignificant with respect to the water sample. Both the microscopic and naked-eye examination of obtained autoclave sterilization results showed no changes in the structure, form or shape of the tested cured resin compound samples.

5. Conclusions

1. Cured epoxy compounds are homogeneous, monolithic structures. Additives such as metal powders do not cause deviations in compound curing. The material is coherent and exhibits typical mechanical strength properties.

2. Results of this experimental study demonstrate that cured epoxy resins are safe materials, even in contact with the human cells.

Declarations

Conflict of Interest

The authors have declared no conflict of interest

Corresponding author:

Katarzyna Sarna-Boś*

ORCID: 0000-0002-1899-0300

Department of Dental Prosthetics, Medical University of Lublin, Chodźki 6 , 20-093, Lublin, Poland, mobile: 501148262, katarzynasarnabos@umlub.pl

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Figures

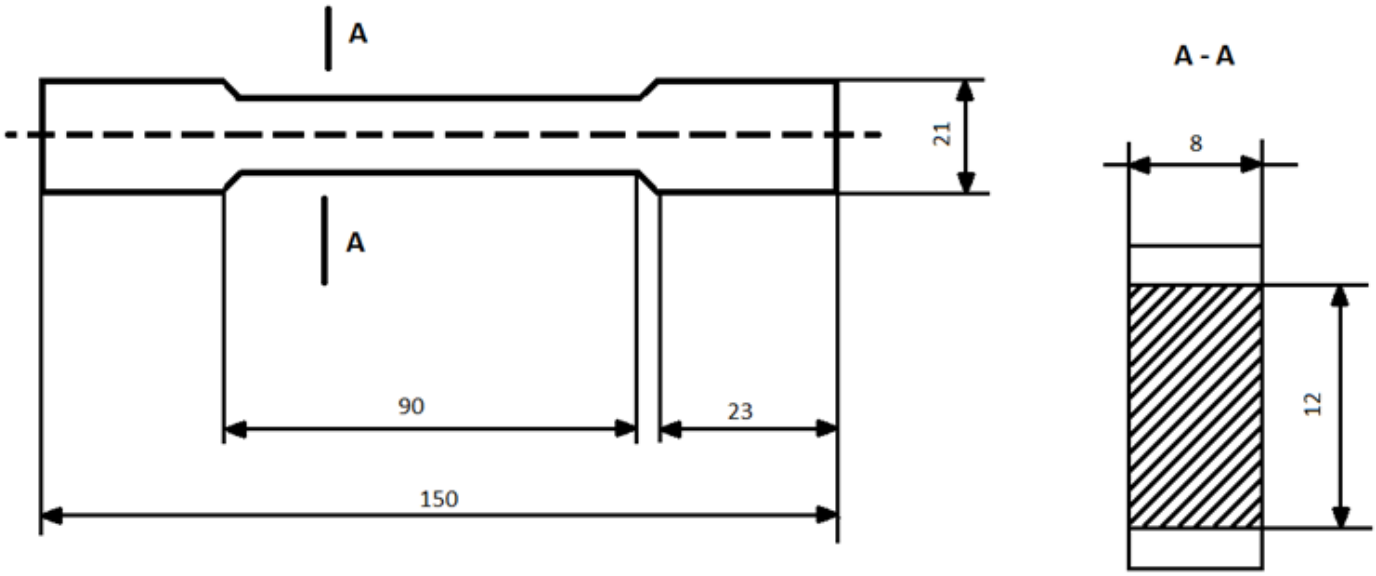

Figure 1

Dimensions of cured adhesive compound samples

Figure 2

Epoxy resins mixed with PAC curing agent: a) pure resin, b) resin with aluminum powder, c) resin with copper powder

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS
B	PBS	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	PBS
C	PBS	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	PBS
D	PBS	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	PBS
E	PBS	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	PBS
F	PBS	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	PBS
G	PBS	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	ZK	PBS
H	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS	PBS

ZK – 100 µl of cell suspension ($1x10^5$ cells/ml) in cell culture medium

Figure 3

Schematic design of a 96-well microplate

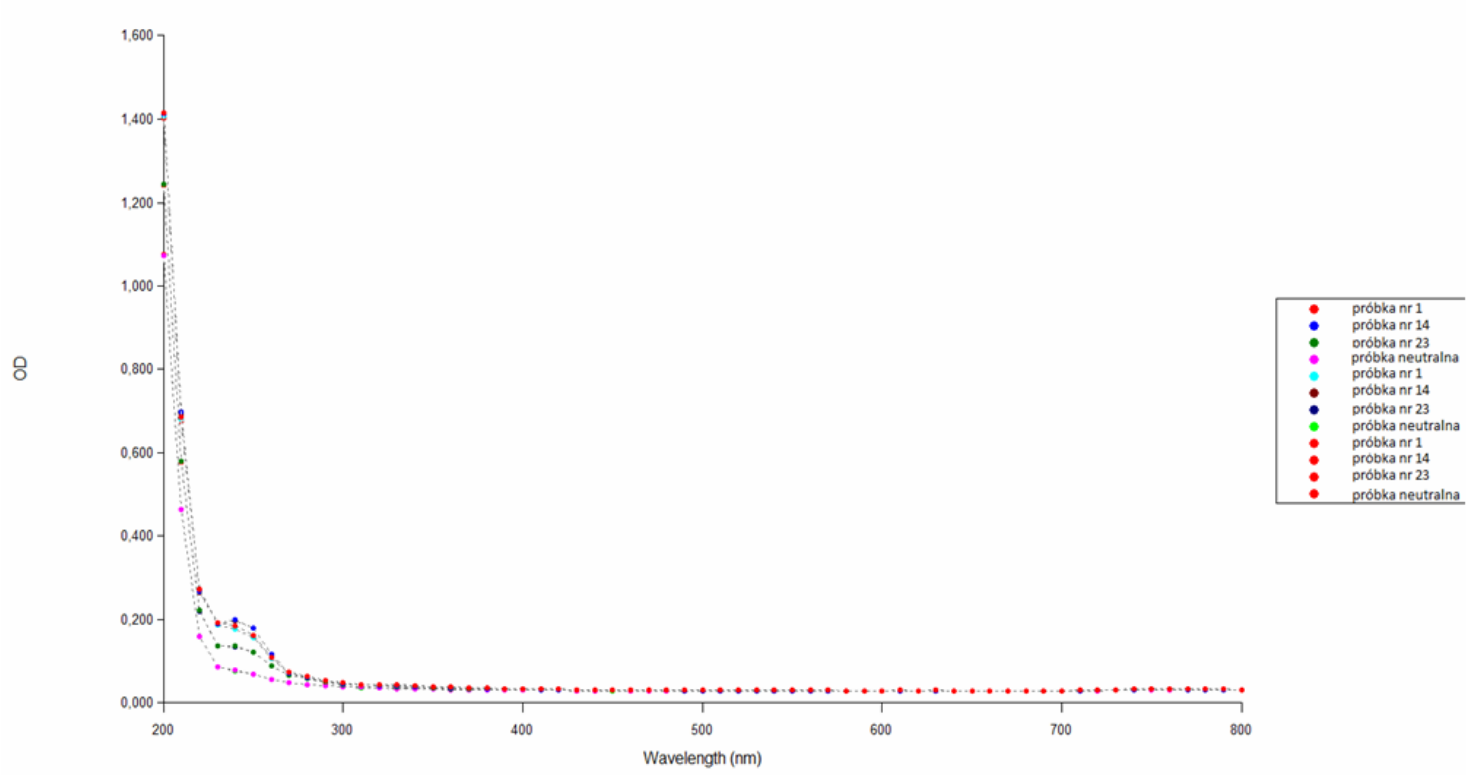

Figure 4

Absorbance values of the extracts of epoxy compound sample

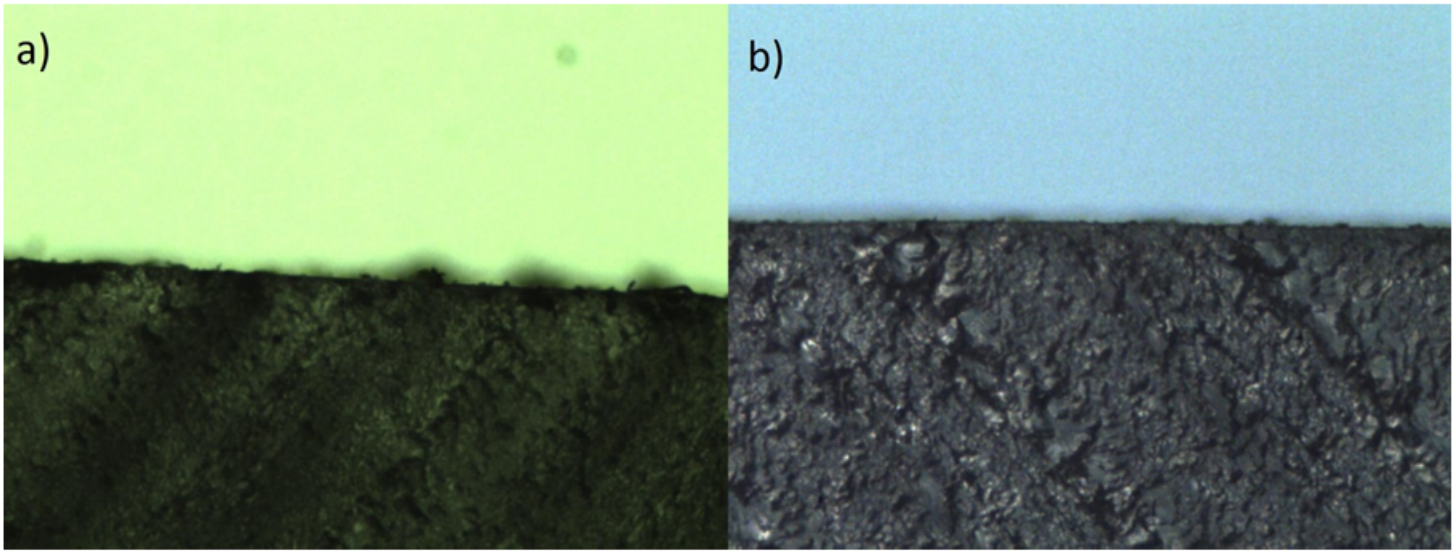

Figure 5

Epidian®53 epoxy resin: a) after curing, b) after sterilization

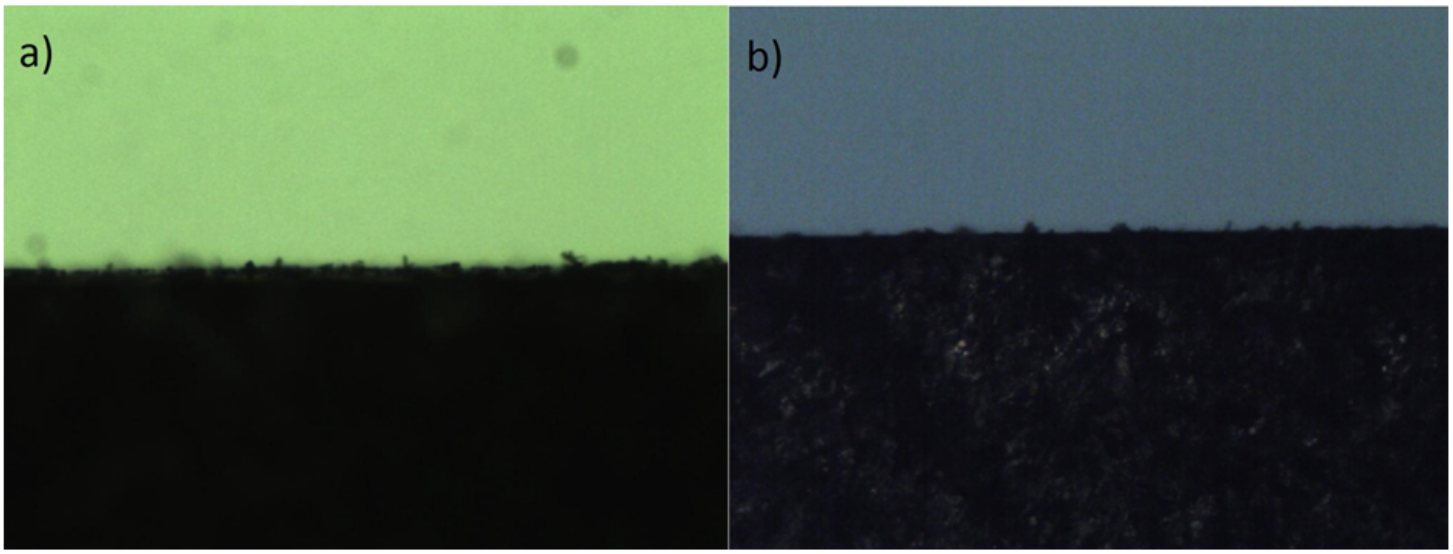

Figure 6

Cured compound of Epidian® 53 epoxy resin with PAC curing agent and aluminum powder: a) after curing, b) after sterilization

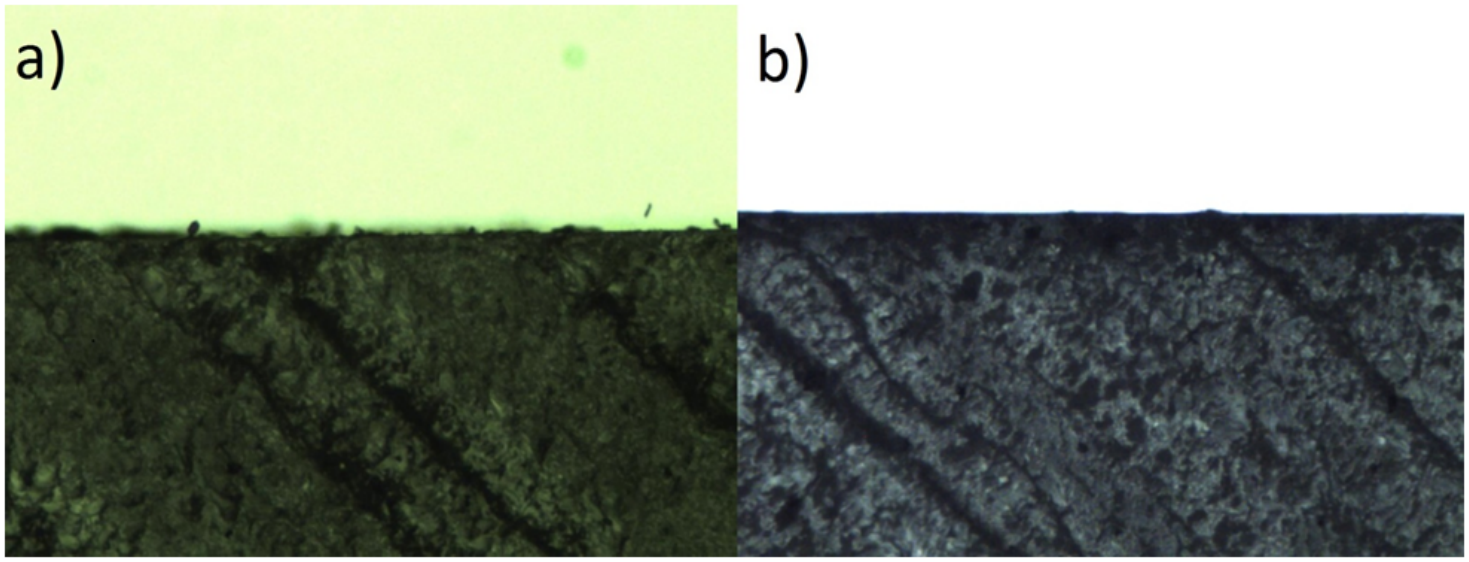

Figure 7

Cured compound of Epidian® 53 epoxy resin with PAC curing agent and copper powder: a) after curing, b) after sterilization

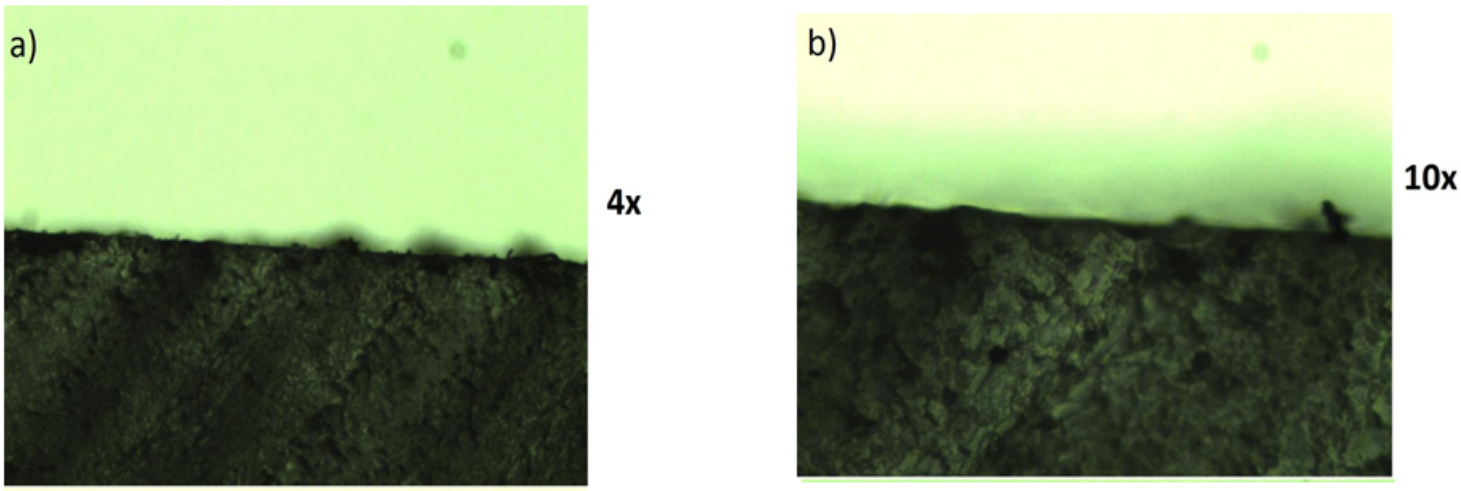

Figure 8

Pure epoxy resin: a) 4x magnification; b) 10x magnification

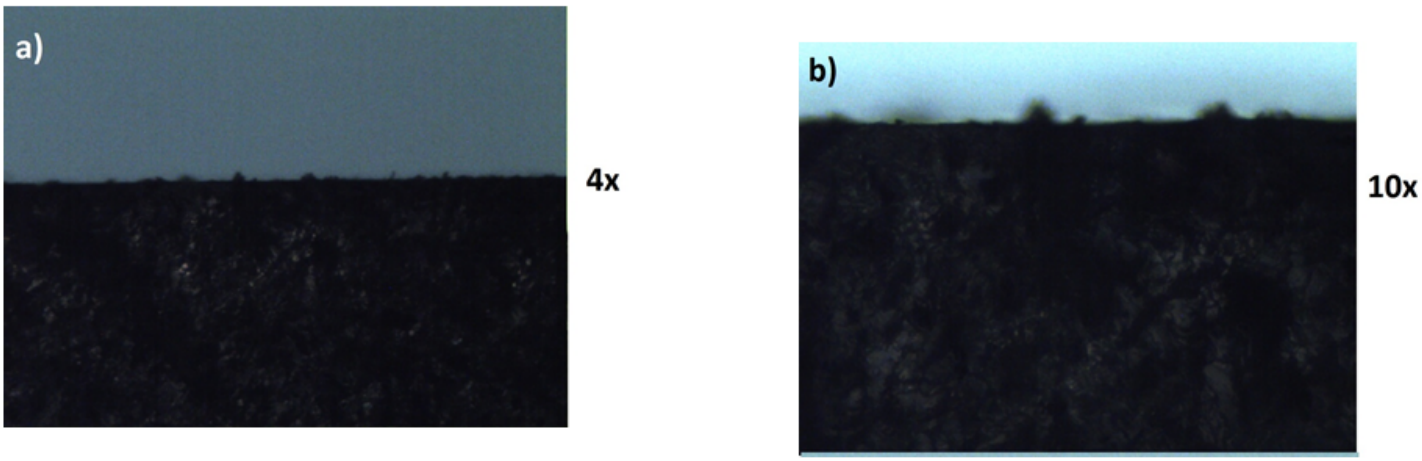

Figure 9

Cured compound of Epidian® 53 epoxy resin with PAC curing agent and aluminum powder: a) 4x magnification; b) 10x magnification

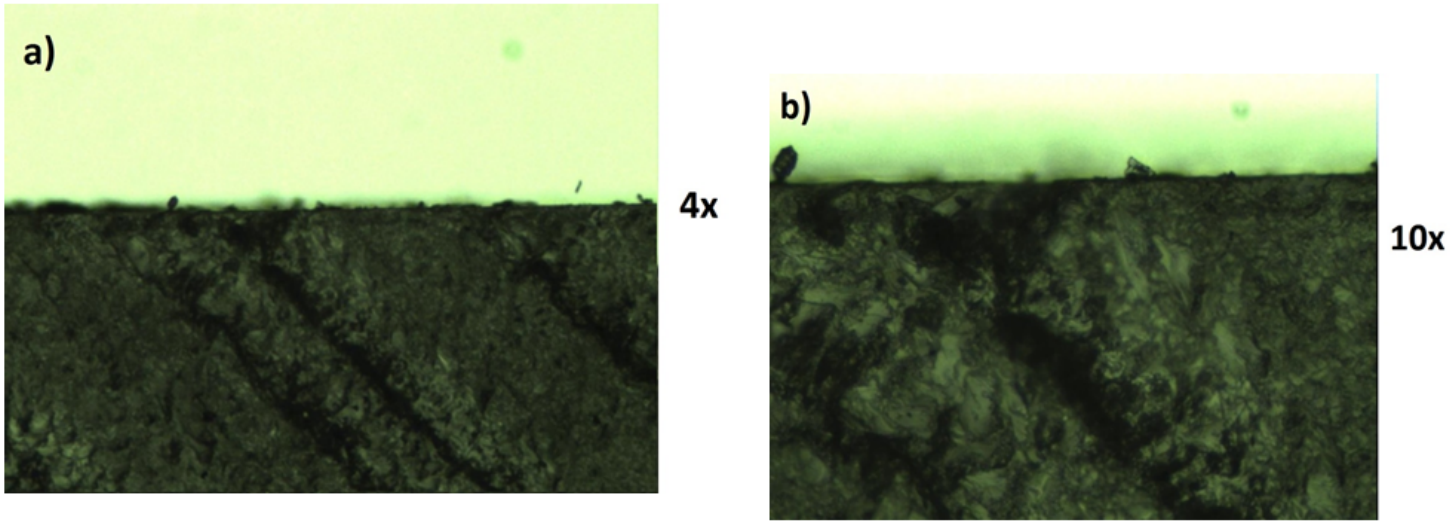

Figure 10

Cured compound of Epidian® 53 epoxy resin with PAC curing agent and copper powder: a) 4x magnification; b) 10x magnification