Organosilicon polymer for the release of antimicrobial drugs

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Abstract: An organosilicon polymer was developed for the release of antimicrobial drugs. The influence of the drug itraconazole (Itrax) on polymer properties such as density, hardness, rebound resilience, tensile strength, accelerated aging, structure and antifungal activity was investigated. There were slight changes in physicochemical properties and significant deterioration of mechanical ones. The evaluation of the fungicidal activity showed time-limited antifungal properties.

Keywords: antifungal silicone, drug-polymer composite, itraconazole, *Candida albicans*, biofilm.

Polimer krzemoorganiczny do uwalniania leków przeciwdrobnoustrojowych

Streszczenie: Opracowano polimer krzemoorganiczny do uwalniania leków przeciwdrobnoustrojowych. Zbadano wpływ leku itrakonazolu (Itrax) na właściwości polimeru takie, jak: gęstość, twardość, odbojność, wytrzymałość na rozciąganie, przyspieszone starzenie, strukturę i aktywność przeciwgrzybiczą. Stwierdzono niewielkie zmiany właściwości fizykochemicznych oraz znaczne pogorszenie właściwości mechanicznych. Ocena aktywności grzybobójczej wykazała ograniczone w czasie właściwości przeciwgrzybicze.

Słowa kluczowe: silikon przeciwgrzybiczy, kompozyt lek-polimer, itrakonazol, *Candida albicans*, biofilm.

Due to their unique properties, silicones have been used in healthcare for over 70 years [1]. Properties such as biocompatibility, biodurability, low surface tension, low glass transition point, and chemical and thermal stability led to their widespread application in medicine [2]. Moreover, they have high permeability to drugs and gases, which can be advantageous for applications such as wound dressing [1]. Bond linkage similar to the repeating inorganic structure in silicate materials, as well as the absence of double bonds in the molecular chain, contribute to their chemical inertness [3]. Many types of silicone rubbers are used in the medical field depending on the intended application. They range from elastomers that cure at high temperature, *i.e.*, high temperature vulcanizing silicones (HTV) or liquid silicone rubber (LSR), to room temperature vulcanizing (RTV) ones being the simplest to prepare, when the process of crosslinking does not require heat or moisture [3].

RTV silicones are commonly used for dentures, soft lining materials, and maxillofacial prostheses. Even though these materials are highly biocompatible, they are prone to be colonized by different microorganisms. It has been estimated that more than 72% of denture wearers have stomatitis, of which 93% is associated with *Candida albicans* [4]. This type of yeast has a remarkable adhering potential to the surface of biomaterials through different chemical bonds [5]. Following adherence, *C. albicans* changes its morphology to hyphae while producing a matrix of polysaccharides, proteins, and carbohydrates. Lastly, through the constant contact between the infected biomaterial and the skin or mucous membrane, the yeast can enter the human host and spread to internal organs [6, 7]. Plenty of research proved the dependency between the fungal colonization and the surface roughness and high hydrophobicity [5, 6, 8, 9]. Over the years, a great effort has been made to avoid pathogenic adhesion to silicones by performing various surface and material modifications. For instance a study was conducted on medical-grade silicone coated with sophorolipids, and its efficiency against *C. albicans* biofilm was proved [10]. Other authors also provided evidence for biofilm reducing ability of different coatings, such as parylene [11], carboxymethyl chitosan [12], or lipopeptide AC7 in combination with farnesol [13]. On the other hand, numerous studies investigated the influence of different antimicrobial agents incorporated directly into the silicone matrix. A series of recent studies indicated that incorporating nanomaterials such

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as silver nanoparticles [14, 15], or nano-titanium dioxide [16] has significantly increased the antifungal properties of silicone polymers. In a different approach, a number of authors introduced antifungal pharmaceuticals into silicones and studied their impact on the antimicrobial and mechanical properties. Several studies suggested that the incorporation of different antifungals (nystatin, miconazole, ketoconazole, itraconazole, and chlorhexidine) into soft liners resulted in the expression of antifungal activity, as well as minimal roughness, hardness, and tensile strength alterations [17–19].

In the light of the reported studies, it is convincible that the introduction of antimicrobial drugs into silicones promotes the expression of antifungal activity without significantly influencing the mechanical properties. However, a number of questions regarding the long-term performance of such composites in the oral cavity are yet to be answered. With this in mind, in this paper we investigated the possibility of obtaining a drug releasing composite for soft lining applications. For this purpose, we analyzed the impact of introducing itraconazole (Itrax) into a silicone matrix by performing several physicochemical, mechanical, and biological tests (density, hardness, resilience, accelerated aging, tensile testing, and antifungal activity assessment). The aging of the composite was performed in an artificial saliva environment, and the degree of degradation was assessed based on the changes in the tensile strength.

T a b l e 1. RTV22F silicone properties [20]

Property	Unit	Value
Density	g/cm^3	1.1
Viscosity	mPa · s	3250
Hardness	ShA	22
Tensile strength	MPa	3
Elongation at break	%	350

EXPERIMENTAL PART

Materials

This research used an addition of crosslinking silicone RTV22F as the drug releasing composite matrix (Table 1).

Due to the proposed field of application, Itrax (itraconazole, ITZ) was selected as a filler. Each capsule contained 100 mg of itraconazole and excipients (hydroxypropyl methylcellulose E5, eudragit, and saccharose) [21]. For the preparation of the materials, base and catalyst were mixed following the manufacturer's instructions (mass ratio of 1:1). The active substance (granules) was taken out of the hard starch capsules and incorporated into the polymeric matrix in the amount of 20 wt% (4.5 wt% of itraconazole). The composite and the reference material were prepared by gravity casting into forms sized 150 × 200 × 4 mm and cured at room temperature for 24 h

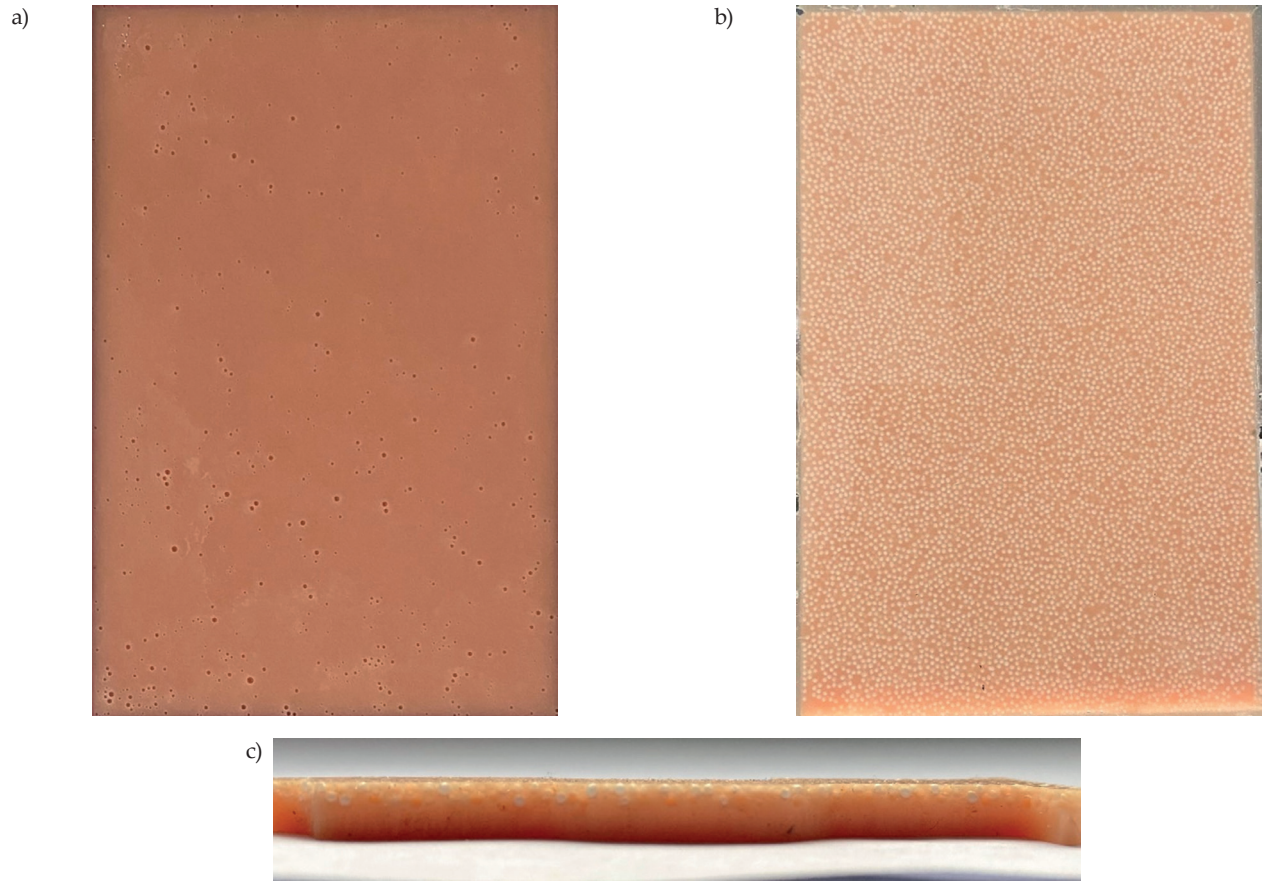


Fig. 1. Reference material (a) and composite (b, c)

(Figure 1). This resulted in the sedimentation of granules and the obtained variability of drug distribution on the cross-section gives specific application possibilities. It should be noted that the materials did not undergo a vacuum deaeration process. Dosing of components was carried out using a lab balance (precision: ±0.001 g). Before submitting to further tests, the prepared materials underwent conditioning at 23±2°C and 50% humidity.

Methods

Density

Density was determined in accordance with the standard ISO 1183-1 using a lab balance (Ohaus Adventurer Pro, OHAUS Europe GmbH, Nänikon, Greifensee, Switzerland) equipped with a hydrostatic density measurement kit. The samples sized 5 × 5 × 4 mm were cut using a knife. The test consisted of measuring the sample's weight in air, then in distilled water of known density ($\varrho = 0.998$ g/cm^3). From the obtained results, the density ϱ (g/cm^3) of the samples was determined according to Eq. (1):

$$\varrho = \varrho_w \frac{m_1}{m_1 - m_2} \quad (1)$$

where:
m_1 – mass of the sample in air (g)
m_2 – mass of the sample in distilled water (g)
ϱ_w – density of distilled water (g/cm^3)
The measurements were repeated five times for each material.

Hardness

The hardness measurements were performed in accordance to the standard ISO 7619-1 using a Shore type A durometer (Zorn Stendal, Saxony-Anhalt, Germany). The sample measured 30 × 30 × 4 mm and the test was repeated five times while maintaining a distance of at least 10 mm from the edges.

Rebound resilience

To evaluate the elasticity of the prepared materials, rebound resilience test was carried out according to the standard ISO 4662, using the Schob machine (Heckert, Chemnitz, Germany). Before conducting the tests, samples were mechanically conditioned with two impacts. The measurements consisted of hitting the specimens with a weight placed on a pendulum and reading off the value indicated by the pointer (%). The samples size was 30 × 30 × 4 mm. The arithmetic mean of the three measurements was taken as the result.

Tensile properties

The static tensile properties were performed according to the standard ISO 527-1 (specimen type 5-B) (Figure 2) using an MTS Insight 10 testing machine (MTS Systems Corporation, Eden Prairie, Minn., USA). The specimens were 60 mm long, 4 mm wide along the measuring length, and had a thickness of 4 mm. The crosshead speed was 50 mm/min. The samples were installed using pneumatic grips supplied with a pressure of 0.5 atmosphere, ensuring secure fixation. The extension of the materials was carried out until the destruction of the sample, indicated by the separation of the material. The arithmetic mean of the five measurements was taken as the result.

Accelerated degradation

A group of samples of the obtained composite was subjected to an accelerated degradation process by immersing in artificial saliva and kept for 60 days at 70°C. The samples had the shape adopted for tensile testing. The test was performed in accordance with the standard ISO 10993-13. The chemical composition of artificial saliva is presented in Table 2. The degradation degree was assessed by comparing the changes in tensile strength characteristics after aging with respect to the unaged samples.

a) b)

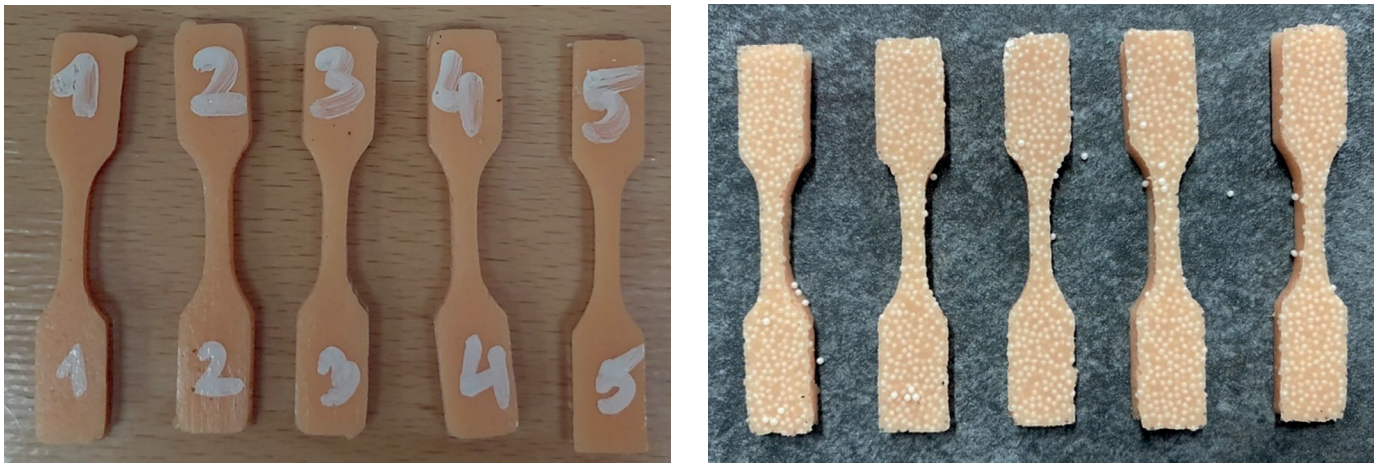


Fig. 2. Tensile testing samples: reference material (a) and drug-silicone composite (b)

T a b l e 2. Artificial saliva composition according to the standard ISO 10993-15

Chemical name	Chemical formula	Concentration, g/L
Sodium hydrogen phosphate	Na_2HPO_4	0.260
Sodium chloride	NaCl	0.700
Potassium thiocyanate	KSCN	0.330
Potassium dihydrogen phosphate	KH_2PO_4	0.200
Sodium hydrogen carbonate	$NaHCO_3$	1.500
Potassium chloride	KCl	1.200

Morphology

The composite morphology was analyzed using Zeiss Supra 35 scanning electron microscope (Carl Zeiss AG, Oberkochen, Germany). Prior to the test, each sample was sputtered with gold powder for 90 s. The electron accelerating voltage of 5 kV was applied. Magnifications of 100×, 250×, and 1500× were selected.

Fungicidal activity

The antifungal activity evaluation test was performed using the disk diffusion method. The employed strain was *Candida albicans* ATCC 10231 incubated at 37°C for 48 h until the turbidity of 1.5 McFarland standard units per ml was achieved. A total of seven specimens with a diameter of 15±1 mm were submitted to the test – six drug-containing samples and one control sample. Prior to testing, the specimens were sterilized by autoclaving at 121°C for 15 minutes. The samples were placed on the surface of the inoculated Sabouraud dextrose agar plates and subsequently incubated at 37±1°C. The inhibition zone of fungal growth, if any, was measured after 18, 22, and 46 h.

RESULTS

Density, hardness, and rebound resilience

The average values of the tested properties are graphically illustrated in Figure 3.

The unmodified silicone showed an average density of 1.07 g/cm^3 being lower than the value given by the producer – 2.72%. That could probably be caused by air bubbles enclosed in the structure, since the materials were not deaerated after casting. The addition of drug slightly increased the density of the material; nevertheless, the increase was of little importance. A 3% decrease in hardness was observed for composite samples comparing with the control group. This may be because the filler granules were encountered during the measurement. Similar behavior was observed for the composite's rebound resilience value, which decreased by 4.2% comparing with the native material.

Tensile properties

The static tensile strength was performed using native samples as well as aged composite. Most of samples tended to shear breaking during the static tensile test of both aged and unaged composites. The fracture occurred from the sediment drug side, whereas the other side underwent further tension (Figure 4). Graphical representation of the obtained results is illustrated in Figure 5 for tensile strength and Figure 6 for elongation at break.

The silicone's tensile strength ranged from 0.77 MPa to 1.75 MPa showing a significant scattering of results compared to the remaining materials. Furthermore, the reference samples were characterized by the highest elongation at break among the tested materials. Both tensile strength and elongation at break exhibited a decreasing tendency (by 35% and 12%, respectively, comparing with the reference material) for unaged composite samples. This could be due to the separation of drug granules during tension, which caused the formation of micro-

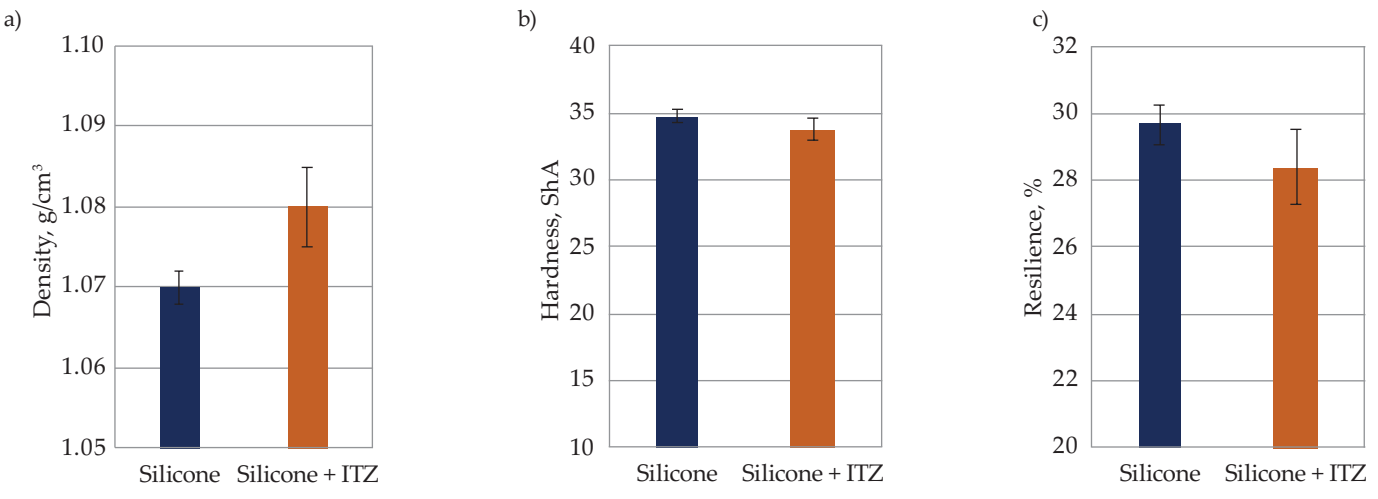
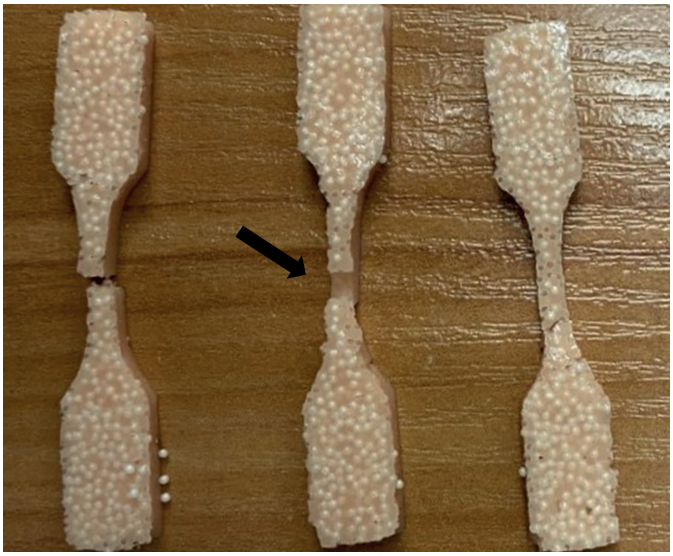


Fig. 3. Selected properties for reference material and drug-silicone composite

a)

b)



c)

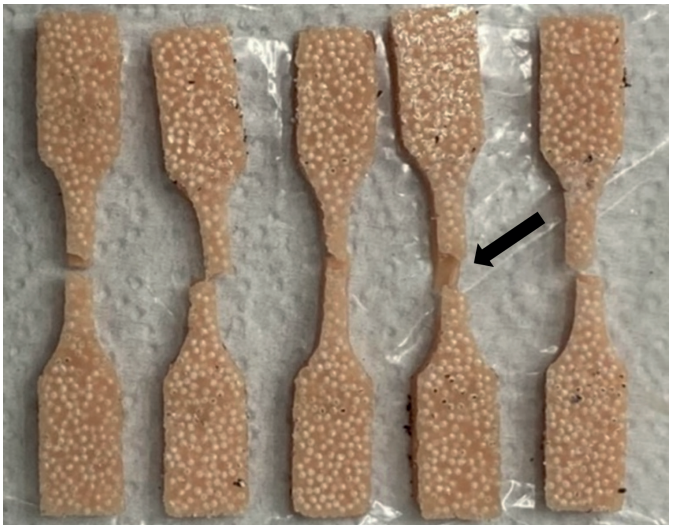


Fig. 4. Tensile testing samples: reference material (a), unaged (b) and aged (c) composites – the arrows indicate the samples' fracture

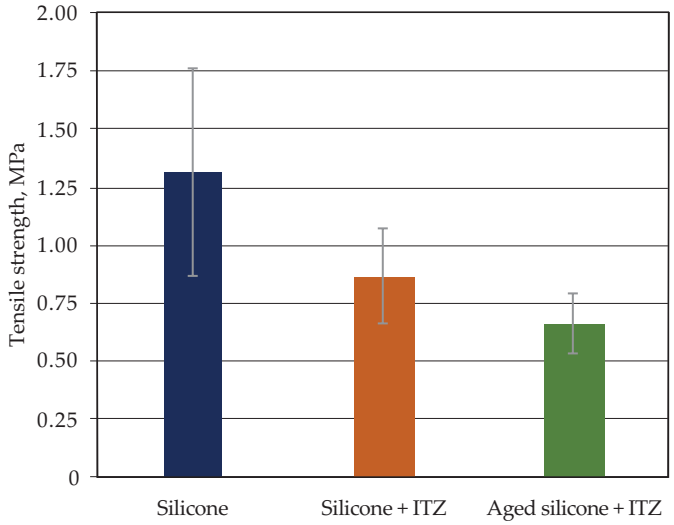


Fig. 5. Tensile strength for reference material, the unaged and aged drug-silicone composite

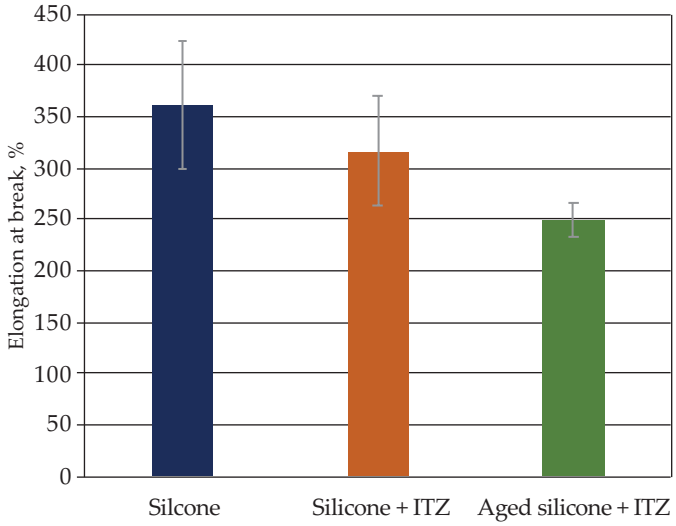


Fig. 6. Elongation at break for reference material, the unaged and aged drug-silicone composite

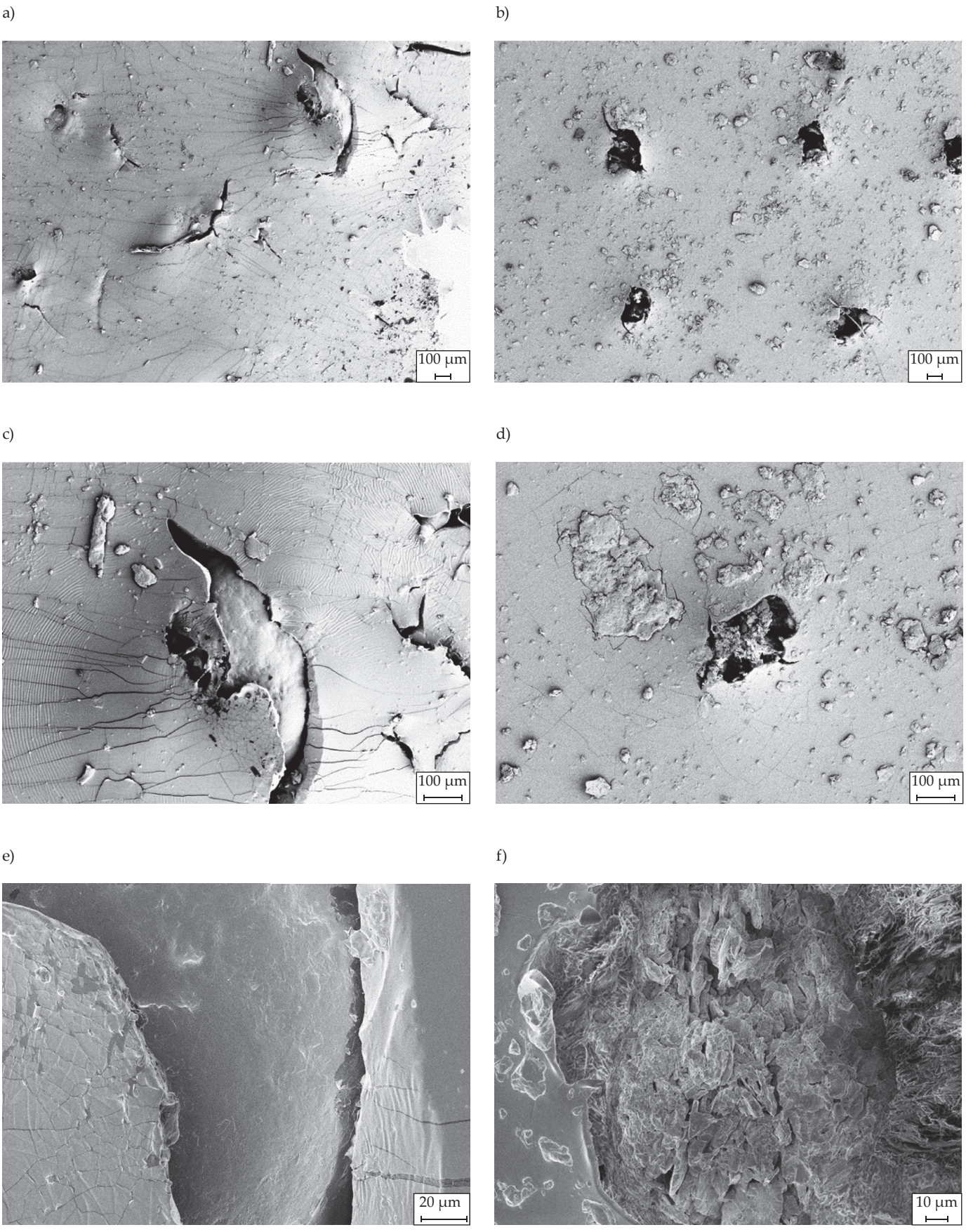


Fig. 7. SEM micrographs of native (a, c, e) and aged samples (b, d, f)

scopic cracks simultaneously weakening the sample and accelerating tearing of rubber. Moreover, elongation at break decreased by 21% for aged composite comparing with the native composite samples, which could be caused by further crosslinking reaction during accelerated aging [22]. Furthermore, the tendency of the composite samples to shear breaking could be due to the difference in Young's modulus of the sediment drug layer and that of the silicone (Figure 1c). Several authors have previously reported similar behavior [23–25].

Morphology

SEM micrographs of the tested specimens are presented in Figure 7, which show the surface of native and aged composite samples after performing tensile testing. In case of native samples, the drug granules remained intact (Figures 7c, 7e), while in the aged samples, the granules started dissolving, losing their round shape (Figures 7d, 7f). The silicone matrix in unaged samples turned out to have stretching lines on its surface and weak integration with the drug granules, which resulted in the separation of drug granules during tensile testing. After the accelerated aging treatment, the surface of aged sample exhibited a deposition of an unknown substance that simultaneously altered the surface roughness. In order to identify the adhered substance morphology, Energy Dispersive Spectroscopy (EDS) was performed.

EDS was performed using an accelerating voltage of 15 kV and magnification of 253×. The analysis was performed on two areas, the first was the unidentified adhered substance, and the second was the silicone matrix (Figure 8). The results are presented in Figure 9.

EDS analysis proved the presence of several elements in the deposited substance (Na – 1.5%, Cl – 3.8%, K – 1.3%) absent in the silicone matrix area. The adhered substances have been identified as compounds precipitated from the artificial saliva solution (Table 2).

T a b l e 3. Fungicidal activity

Incubation period	Average inhibition zone, mm	
	Silicone	Silicone + ITZ
18 h	0	5.2
22 h		4.25
46 h		0

Antifungal activity

The test substrate and the unmodified material constituted the positive test control, while the substrate without inoculation served as the negative control. All controls were correct. The results are presented in Table 3 and Figure 10.

The tested silicone did not show an inhibition zone during the incubation period. On the other hand, the composite exhibited a time-limited fungicidal and fungistatic activity against the *Candida albicans* ATCC 10231 strain despite the optimal conditions, *i.e.*, 37±1°C and the presence of nutrients and humidity provided by the substrate. After 18 h of incubation, the samples displayed a clear inhibition zone with the presence of single colonies, which during further incubation (after 22 h) formed larger colonies and reduced the zone. After 46 h, all samples exhibited a gradual disappearance of the inhibition zones. This proves that treatment using itraconazole requires a longer continuous administration of a specific dose [21, 26].

CONCLUSIONS

Within the limitations of this work it can be deduced that the addition of itraconazole (Itrax) to the silicone matrix and the accelerated degradation carried out in

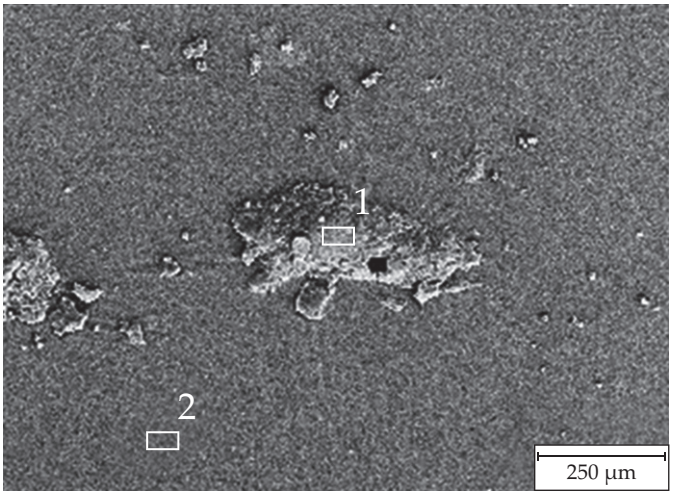


Fig. 8. EDS analysis areas: adhered substance (1) and silicone matrix (2)

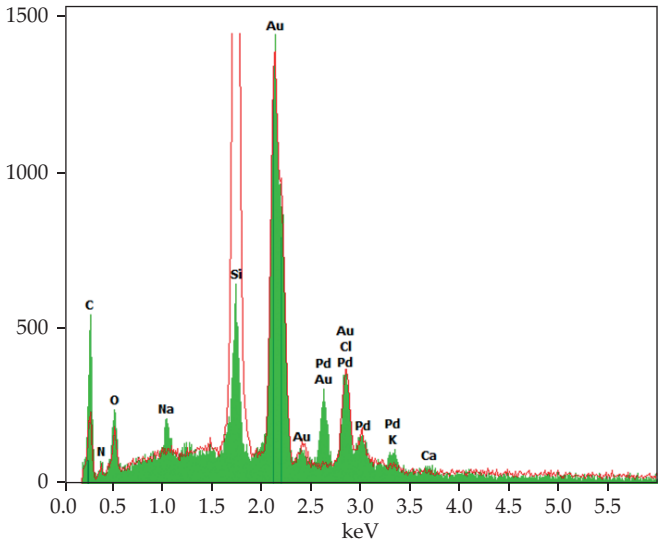


Fig. 9. EDS of adhered substance (green graph) and silicone matrix (red graph)

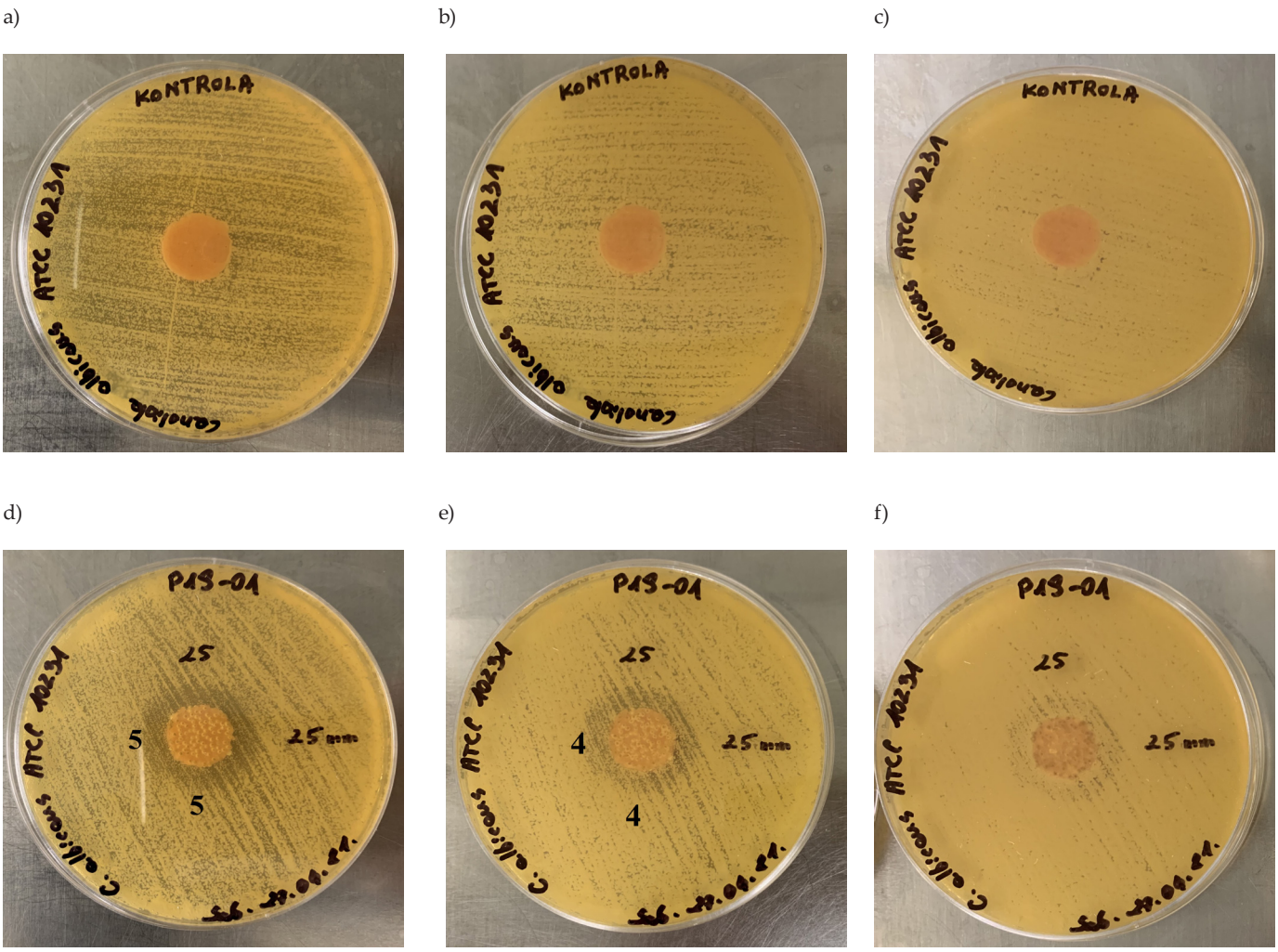


Fig. 10. Antifungal activity: reference material after 18 h (a), 22 h (b), and 46 h (c) and composite after 18 h (d), 22 h (e), and 46 h (f)

an artificial saliva environment affected the material's mechanical strength to a varying degree. At the same time, the physicochemical properties remained with no significant alteration. Furthermore, the composite exhibited a time-limited antifungal activity against *Candida albicans* strain. The fungistatic activity of the drug-silicone composite is associated with the lack of colony growth and is highly dosage-dependent. In conclusion, due to its adequate mechanical and biological properties, the obtained drug-silicone composite could be employed in short-term applications, including temporary soft liners, silicone wound dressings, or single-use menstrual cups. Future research should be devoted to improving the mechanical properties of the drug-silicone composite, which may extend the application period.

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