Elsevier Editorial System(tm) for Materials Science and Engineering C
Manuscript Draft

Manuscript Number:

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Article Type: Research Paper

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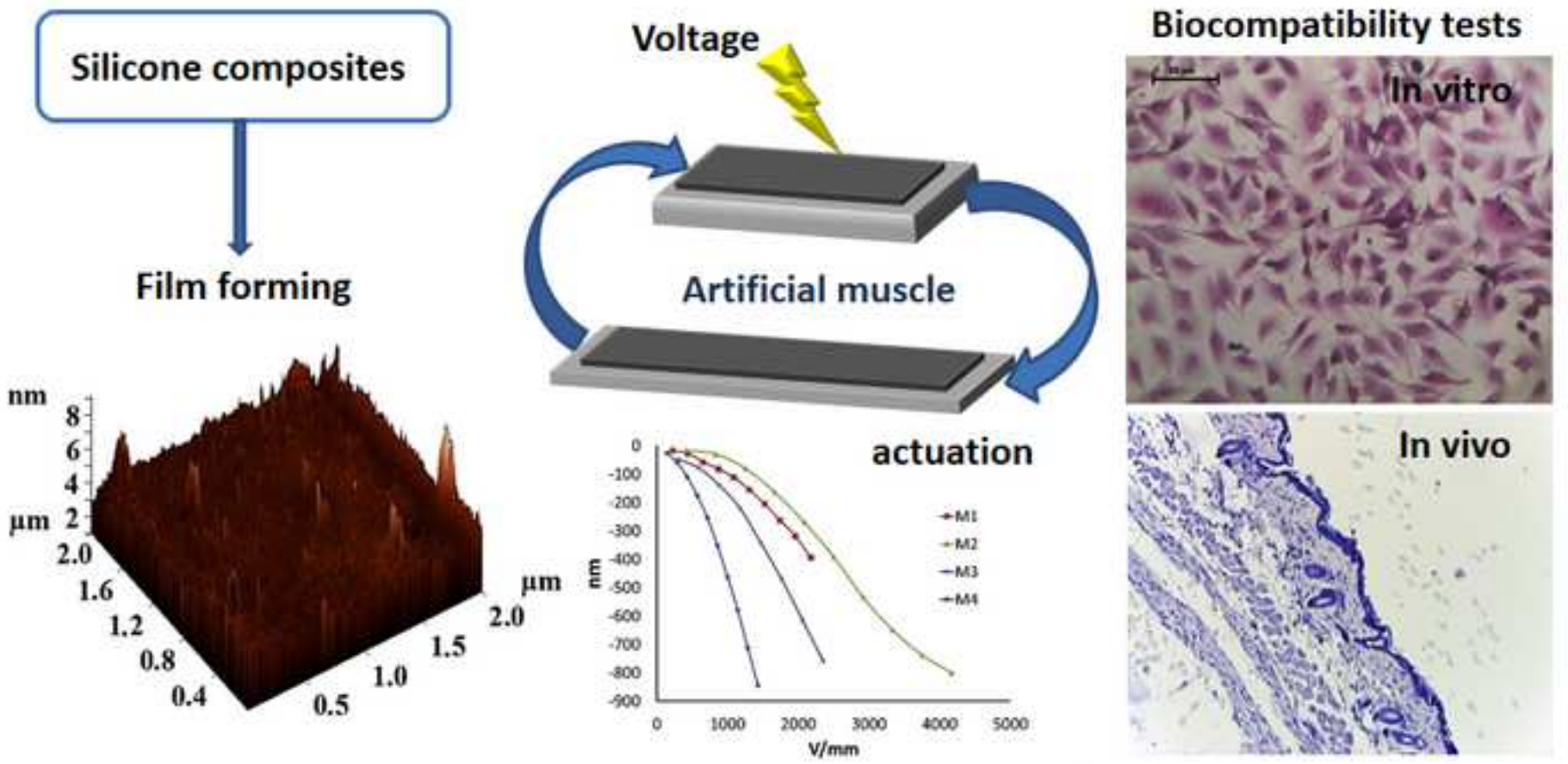
Graphical Abstract (for review)



Highlights

- Silicone composites differing by the filler and matrix characteristics were prepared.
- Stress-strain curves were registered in normal and cyclic mode for the composite films.
- The dielectric permittivity, dielectric loss, and conductivity were determined.
- Electromechanical response of the films was measured at an applied voltage.
- Some biocompatibility tests, both in vitro and in vivo, were performed.

Preparation of electromechanically active silicone composites and some evaluations of their suitability for biomedical applications

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Abstract

Some films based on electromechanically active polymer composites have been prepared. Polydimethylsiloxane-α,ω-diols (PDMSs) having different molecular masses (Mv = 60 700 and Mv = 44 200) were used as matrix in which two different active fillers were incorporated: titanium dioxide *in situ* generated from its titanium isopropoxide precursor and silica particles surface functionalized with polar aminopropyl groups. A reference sample based on simple crosslinked PDMS was also prepared. The composites processed as films were investigated to evaluate their ability to act as efficient electromechanical actuators for potential biomedical application. Thus, the surface morphology of interest for electrodes compliance was analysed by atomic force microscopy. Mechanical and dielectric characteristics were evaluated by tensile tests and dielectric spectroscopy, respectively. Electromechanical actuation responses were measured by interferometry. The biocompatibility of the obtained materials has been verified through tests in vitro and, for valuable films, *in vivo*. The experimental, clinical and anatomopathological evaluation of the *in vivo* tested samples did not reveal significant pathological modifications.

Keywords: silicone composites; fillers; dielectric elastomers; electromechanical response; artificial muscle; biocompatibility tests

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1. Introduction

Electromechanically Active Polymers (EAPs) with muscle-like properties such as high flexibility, strain, and energy density are a relatively new technology, which, depending on their fabrication and configuration, has numerous potential applications [1-8]. Compared to other smart materials, they have the advantage of light weight, are relative easy to be obtained and processed through scalable procedure, and in general with low cost [4-8]. The most attractive characteristic of EAPs is their ability to emulate the operation of biological muscle with large actuation strain and inherent vibration damping [9,10]. They are high compliant thus easily interfacing with human or other environmental sources of motion. Combined with their high energy output, these features make them attractive for a variety of energy harvesting applications. Among the candidates for artificial muscles, the dielectric elastomers, as a class of electroactive polymers, have also typical characteristics of light weight, flexibility, low cost, easy fabrication, etc. [2]. In addition, they have a versatile chemistry, and thus the performance of elastomer actuators can be tailored by choosing the appropriate elastomers, changing the cross-linking chemistry of polymer chains, adding functional entities, or modifying preparation techniques [11]. They have already found applications as artificial muscles and also mobile robots, micro-pumps, micro-valves, disk drives, flat panel speakers, intelligent endoscope, etc. [2]. A type of dielectric elastomer actuator consists of a soft elastomer sandwiched between two compliant electrodes generally made of carbon powder. When a voltage is applied between the electrodes, an electrostatic force is generated that compresses the elastomer thickness, expanding it in-plane [3]. Current artificial muscles are often limited due to the high power necessary to obtain reasonable displacement values [2]. Therefore, many studies are in progress now to create new materials and compositions suitable to act as performant artificial muscles.

Silicones and acrylic elastomers, both dielectric amorphous elastomers, have shown the best performance as artificial muscle actuator materials. Acrylic elastomer is a powerful material with regard to its strain response, but shows high viscoelastic losses, while silicone has a faster response time. Therefore, silicones can operate at frequencies greater than 1 kHz, while acrylic elasotmers currently have an upper limit of 100 Hz. Silicones have also better coupling efficiency, good temperature and humidity tolerance making it an attractive actuator material [9].

In a previous work [12], we have reported a series of silicone composites filled with mixtures of commercial SiO_2 and TiO_2 powders in different ratios. While the first was introduced as reinforcement filler, the second was added in order to increase the dielectric constant of the material. A high molecular mass (Mv = 346 000) polydimethylsiloxane-α,ω-diol (PDMS) was used as matrix, which after the filler incorporation was crosslinked by radicalic mechanism with organic peroxides when organic bridges are formed between chains somewhat restricting the sliding of

these one over each. Thus, composites with higher dielectric constant values were obtained but showing also high values for Young modulus. Traverse strain responses in the range 1.24 – 5.09 nm/V/mm thickness were obtained.

In order to decrease the modulus value, a request for applications in actuation, in the present paper we have prepared composites based on home-made PDMS with moderate molecular masses, lower than those used in our previous work but higher than those reported in the literature [13,14], and cross-linked through the ends of the chains with a trifunctional silane, methyltriacetoxysilane, without any catalyst. Either *in situ* generating titania or silica particles functionalized with polar groups were used as filler to increase the dielectric constant of the composites, without addition of silica as hardener. The composites processed as films were further investigated from point of view of mechanical, dielectric and electromechanical behaviours which are of interest for actuation. As a result of the structural changes (chain length, crosslinking pattern, filler), electromechanical performances different from the previous ones were obtained. In addition, some evaluations (*in vitro* biocompatibility tests and *in vivo* clinical and anatomopathological evaluation) on the suitability of the obtained composites for biomedical applications were performed this time.

2. Experimental

2.1. Materials

Fumed silica, Aerosil 380 (Degussa), 100% purity, specific surface 380 m^2/g, particle diameter 0.003 – 0.015 μm was treated with 3-aminopropyltriethoxysilane (APTES) in vapour state (NH_2-SiO_2).

Polydimethylsiloxane-α,ω-diols, PDMSs, of different molecular masses, PDMS1 (Mv=44 200) and PDMS2 (Mv=60 700) were prepared according to the already described procedure [15]: cationic ring-opening polymerization of octamethylcyclotetrasiloxane in the presence of a cation exchanger as catalyst.

Methyltriacetoxysilane, MTAS, $(CH_3CO_2)_3SiCH_3$, assay-90%, bp94-95 °C/9 mmHg, density-1.20 g/mL at 20 °C (Aldrich)

Titanium(IV) isopropoxide, $Ti[OCH(CH_3)_2]_4$, TIP, assay ≥97.0%, bp=232 °C, density =0.96 g/mL at 20 °C (Aldrich)

Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol), Pluronic L 31, average M_n ~1,100, PEG - 10 wt. %, surface tension -47 dynes/cm, 25 °C, 0.1 wt. % in H_2O (Aldrich)

Materials and reagents for in vitro tests: DMEM (Dulbecco's Modified Eagle Medium, with 4500 mg/mL glucose, 110 mg/L sodium pyruvate and 0.584 mg/L L-glutamine); BFS (Bovine Fetal Serum, heat inactivated, non-USA origin, sterile-filtered, suitable for cell culture); P/S/N

(Penicillin/Streptomycin/Neomycin solution with 5,000 units penicillin, 5 mg streptomycin and 10 mg neomycin/mL, sterile-filtered, suitable for cell culture); PBS solution (Phosphate Buffered Saline solution, sterilised, suitable for cell culture); MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide); DMSO (dimethyl sulfoxide). All materials and reagents were purchased from Sigma-Aldrich unless otherwise mentioned.

2.2. Measurements

The surface images were obtained with a Solver PRO-M scanning probe microscope (NT-MDT, Russia), in atomic force microscopy (AFM) configuration. Rectangular silicon cantilevers NSG10 (NT-MDT, Russia) with tips of high aspect ratio were used. All images were acquired in air, at room temperature (23°C), in tapping mode, at scanning frequency of 1.56 Hz. The scan length ranged between 5 μm and 20 μm.

Stress–strain measurements were performed on dumbbell-shaped cut samples from thin films on a TIRA test 2161 apparatus, Maschinenbau GmbH Ravenstein, Germany. Measurements were run at an extension rate of 20 mm/min, at room temperature. All samples were measured three times and the averages of the obtained values were taken into consideration. To test the fatigue resistence, cyclic tests were performed. Five cycles were run with: stationary time at maximum strength – 5 s, stationary time at minimum strength – 5 s, maximum strain – 100 %, minimum strain – 2%, maximum strength - breaking strength at previous test.

Novoncontrol setup (Broadband dielectric spectrometer Concept 40, GmbH Germany), integrating an ALPHA frequency response analyzer and a Quatro temperature control system, was used to investigate the dielectric properties of the polymer composites over a broad frequencies window, 10^0-10^6 Hz, at room temperature. The bias voltage applied across the sample was 1.0 V. Samples having uniform thickness in the 0.15-0.38 mm range were placed between gold plated round electrodes, the upper electrode having a 20 mm diameter.

The electromechanical actuation measurements were made using an AGILENT5529A LASER interferometer with 10 nm resolution setup to measure linear displacement. The samples were placed on the reference surface between two copper electrical electrodes (20 mm x 20 mm), the sandwich being pressed by the retro-reflector of the interferometer which followed the deformation of the samples as various electrical voltages were applied to the electrodes.

2.3. Procedure

2.3.1. Preparation of series I (samples 1-3)

3.000 g (0.049 mmol) PDMS having $\overline{Mv}$ = 60 700 was mixed with various amounts of TTIP (0, 0.3, and 0.6 g corresponding to the samples **1**, **2**, and **3**, Table 1) by magnetically stirring for 60

min after that 0.42 g (1.909 mmol) MTS (8 %) was added and the stirring continued for another 5 min. Then, the mixture was poured as thick film on a Teflon substrate and left to crosslink under the influence of the atmospheric moisture.

2.3.2. Preparation of series II (sample 4)

0.21 g (3 wt%) surface treated SiO_2 was slowly incorporated under stirring in a solution containing 0.21 g Pluronic L 31 diluted with about 2 ml chloroform. The mixture was then added to 7 g (0.105 mmol) PDMS with $\overline{Mv}$ =44 200 and well-homogenized by mechanical mixing. Then, 0.56 ml (8%) MTS was added and quickly incorporated in the matrix, the mixture was degased by ultrasonication and poured as film on Teflon substrate allowing to crosslink under the influence of the atmospheric moisture.

2.3.3. *In vitro* biocompatibility tests

As *in vitro* biocompatibility tests, MTT cytotoxicity assay, cell morphology and cell adhesion analyses were performed.

Material cytotoxicity was evaluated using MG63 cell line and MTT protocol [16]. The cells were plated 24 hours prior the test on 24 well culture plate, using $1{,}5x10^4$ cells/well cell density in DMEM culture media supplemented with 10% BFS and 1% P/S/N. In the same day, the material samples were cut to obtain 6 mm Ø membranes which were sterilized by immersion in 70% ethanol solution for 15 min. Sterilized membranes were pre-equilibrated in culture media at 37 ^oC, humidity and 5% CO_2 by day of the test. By one of 6 mm Ø membranes was added to the each well of the pre-plated cells. The MTT test was performed at 24 hours and 48 hours of the material incubation with cells. Briefly, at each test point, the material samples from culture well were removed and culture media was replaced with 0.5 mL of MTT work solution (0.25 mg/mL of DMEM without BFS and P/S/N). The culture plates were incubated with MTT solution for 2.5 hours at 37 ^oC, humidity and dark condition. After 2.5 hours of incubation, the MTT solution was replaced with 1 mL/well of isopropanol, to solubilize the insoluble formazan crystals. The absorbance of the blue formazan solution was measured at 570 nm wave length, using UV-1700 Pharma Spec spectrophotometer. The cell viability was calculated by normalizing absorbance results for experimental wells to the negative control, in which cells without any sample were incubated. As negative control the cells incubated with 5% DMSO solution in culture media were considered.

For *cell adhesion ability*, the sterilized and pre-equilibrated samples were plated on the bottom of the culture wells and covered by cell suspension, using 2×10^4 cells/well. After 24 and 48 hours of culturing, material samples were washed in PBS several times and fixed in 10% formaldehyde

solution. Cell attached to the materials were stained by haematoxylin and eosin protocol (H&E) and analysed by light microscopy. The cell morphology of the cells attached to the material was compared to those attached to the polystyrene culture dish. SEM analysis of the experimental samples with attached cells was performed as well.

2.3.4. Research protocol for *in vivo* acute toxicity tests

Two of the film samples, **1** and **2**, were tested in the Laboratory of Experimental Medicine for Drugs, Materials and Medical Devices Biotolerance (LEBMAD) from University Apollonia of Iasi. Investigations were carried out in accordance with a research protocol for which was obtained the ethical approval of the speciality committee. The study of biocompatibility was performed according to the regulations of OECD420 (Guidelines for the Testing of Chemicals) – procedure with fixed dose – method of testing which recommends the use of moderately toxically doses.

To study the acute toxicity, we have used Wistar ICR mice (gnatobiotics – obtained by aseptic technology commended from the Cantacuzino Institute Bucharest, Baneasa Station), nulliparous females 8 weeks of age, with a medium weight of 25±0,20 g. The acclimatisation of mice was done in identical conditions of temperature (22±1°C), humidity (50 ± 10%) and the cycle light/night was of 12 hours. The animals had permanent access (ad libitum) to water (autoclavable bottles with dropping system) and food (standardized at Cantacuzino Institute, with the composition: 25% proteins, 12% lipids, 55% carbohydrates, 8% fibres and other micronutrients). Three groups of animals were gathered (with 5 mice/lot), a group for each type of investigated substance respectively: a lot for sample **1**, a second group for the sample **2**, and a control group.

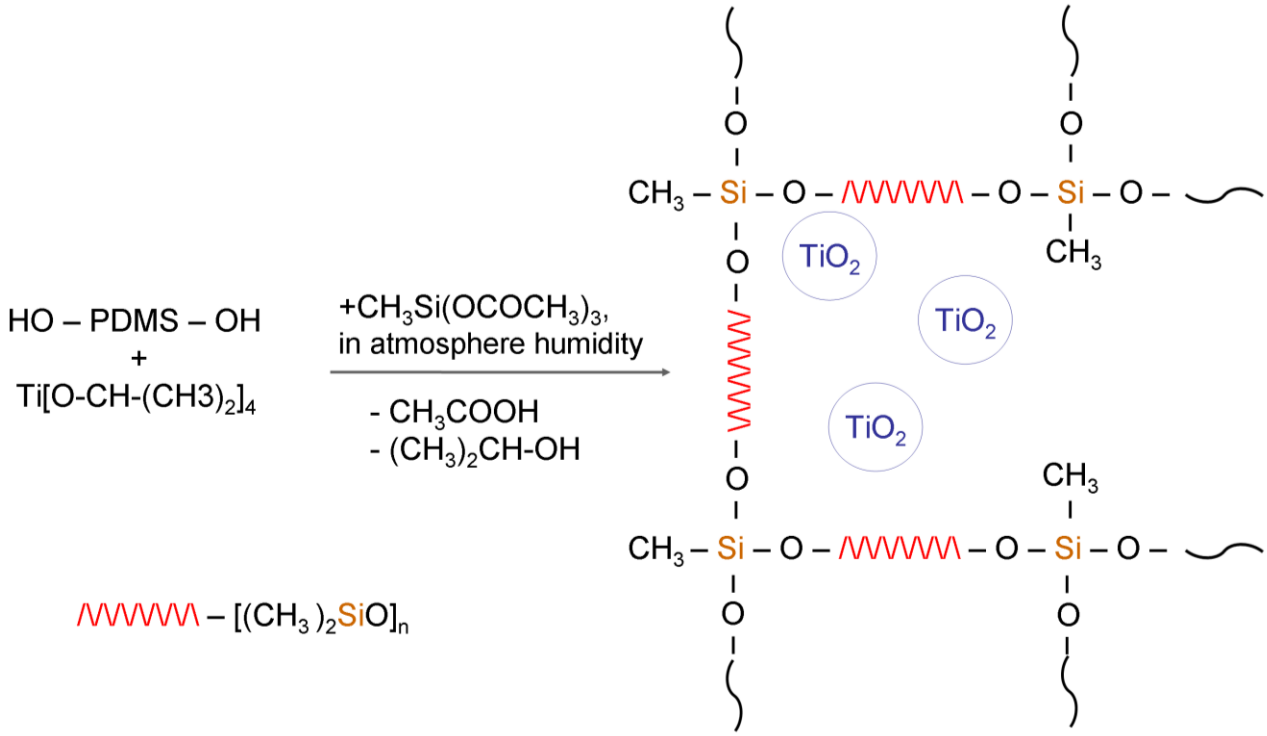
The monitored clinical parameters were: the weight determined in the days 1, 3 and 7 and was calculated in the presence of forage consumption; the behavioural modifications (decrease of motor activities, agitation, uncoordinated movements, etc.) and daily detailed observations which include toxicity signs. For the study of local toxicity were used probes sterilised in Safety Cabinet II (Bioquell, UK) and directly exposed on each side for 15 minutes to UV light λ=254 nm. The animals were anesthetised with thiofuran and were subcutaneously implanted with tested substance probes of 0.5x0.5 cm in three administrations: abdominal, lateral and thoracic, under controlled surgical asepsis. The mice were monitored locally and clinically to determine any modifications after inoculation. Probes were taken at 24, 72 hours and 7 days.

We also performed an irritability test on the conjunctiva mucosa in mice. In the view of macroscopically examination and harvest of probes for the histological examination, the mice were sacrificed after anaesthesia at equal intervals: 1, 3 and 7 days. After the macroscopically examination, from each animal were harvested probes of skin and muscle which were in contact with the studied materials in the view of histological examination. We have observed, in

comparison with the control lot, the macroscopically examination of organs and tissues, evaluating the size, the volume, shape, surface, colour and consistence. The morphological diagnostic was performed according to conventional methods. The probes were fixed in formalin 10% and included in paraffin and histologically examined. The histological examination was performed on the same organs and in the same conditions for all the examined mice.

3. Results and discussions

Due to the high flexibility of the Si-O bonds, silicones possess good elastomer behavior [17,18], which is useful for the electromechanical transduction properties. However, silicones have the disadvantages of low values for dielectric permittivity (ε'), requiring increased activation voltages to obtain reasonable actuation effects. Different methods were used to increase the dielectric permittivity of silicones. One of them, which also can led to better mechanical characteristics is based on the dispersion of a filler of high ε' into the elastomeric matrix before vulcanization [12,19-21]. The filler can be preformed or *in situ* generated within the polymeric matrix during crosslinking process. Titanium dioxide having a high dielectric constant (ε ~ 89) is of real interest for this purpose. Therefore, in a first approach, we prepared silicone composites by *in situ* generating titania from its proper precursor, titanium(IV) isopropoxide (TIP). The later was mixed with PDMS before crosslinking procedure to be initiated. The presumed pathway for the titania formation consists in the hydrolysis of the TIP catalyzed by acid medium created during crosslinking as a result of the hydrolysis of MTAS (Scheme 1).



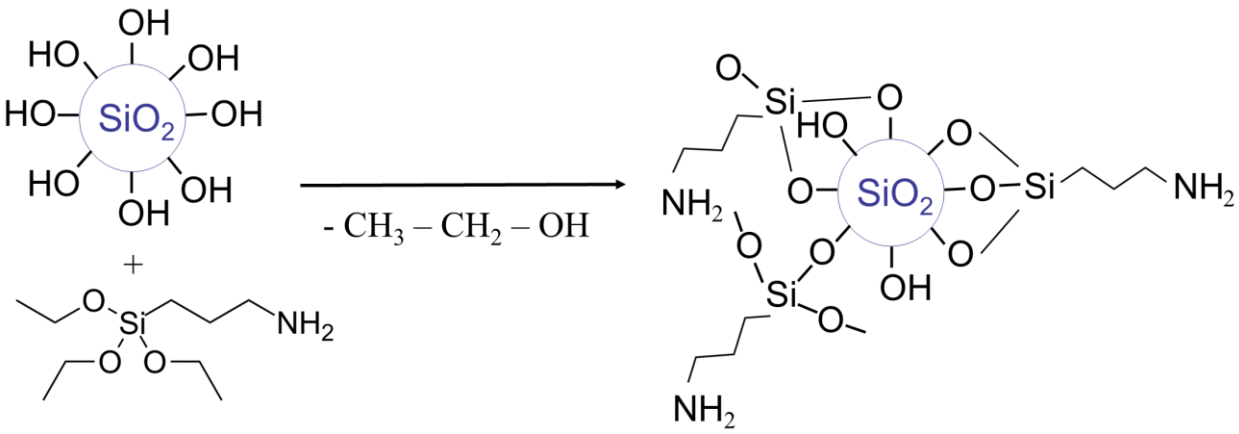
Scheme 1. Pathway leading to silicone-titania composites

Composites with two different quantities of TIP precursor added and as a result different amounts of titania formed, **2** and **3**, were prepared (Table 1). A blank sample, **1**, without filler precursor was prepared in the same conditions. A PDMS of the same molecular mass (Mv=60 700) was used in all three cases.

Table 1. Recipes for preparing composites

Sample	Mv of PDMS	PDMS amount, g	Surfactant, g	Filler		MTAS, g
				Added precursor, g (type)	Calculated filler, wt %	
Series I						
1	60700	3.0	-	-		0.42
2	60700	3.0	-	0.3 (TIP)	2.7 (TiO_2)	0.42
3	60700	3.0	-	0.6 (TIP)	5.4 (TiO_2)	0.42
Series II						
4	44200	7.0	0.21	0.21 (NH_2-SiO_2)	2.9 (NH_2-SiO_2)	0.67

In another approach, commercial silica particles were treated with aminopropyltriethoxysilane to attach polar groups on their surface through a well-known mechanism (Scheme 2). The alkoxysilane groups hydrolyze and condense between them but also with Si-OH groups on the silica surface. In this way, the silica particles will be coated with a shell having aminopropyl groups (NH_2-SiO_2). Being polar one, it is expected as the presence of the aminopropyl groups on the silica surface to lead to increasing in dielectric permittivity values of the composite in which this was incorporated (Table 1, sample **4**).



Scheme 2. Illustration of attaching amine groups on the silica surface

In order to prevent the incompatibility between the nonpolar silicone matrix and polar silica particles, these were previously treated with a surfactant, Pluronic-31. A PDMS with average viscosity molecular weight of 44 200 was used as a matrix this time.

The obtained composite films look like in Figure 1.

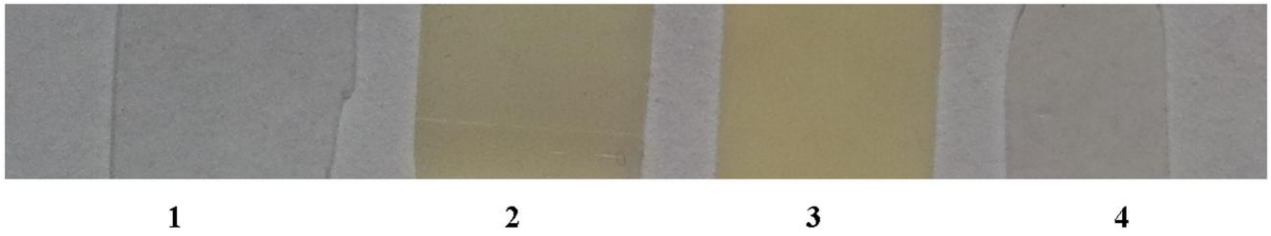


Figure 1. The aspect of the prepared composite films

The surface morphology of the film is very important in the using of the materials in electromechanical devices. A smooth surface will ensure proper compliance with the electrode. Therefore, the films were investigated by AFM, the obtained images providing also information about the distribution of the filler within the polymeric film surface. It can be seen, by the fillers incorporation, the roughness of the surface increases as compared with reference sample **1** (Table 2, Figure 2). In the case of the samples in which TIP was incorporated, uniform distributed TiO_2 nanoparticles of about 100 nm were formed (Table 1, sample **2**). By doubling the TIP amount, nanoparticles of about the same size resulted but agglomerated in spherical aggregated, this time (Table 1, sample **3**). In the case of the sample **4**, rare particles with diameter of about 200 nm dispersed on the film surface are emphasized by AFM images.

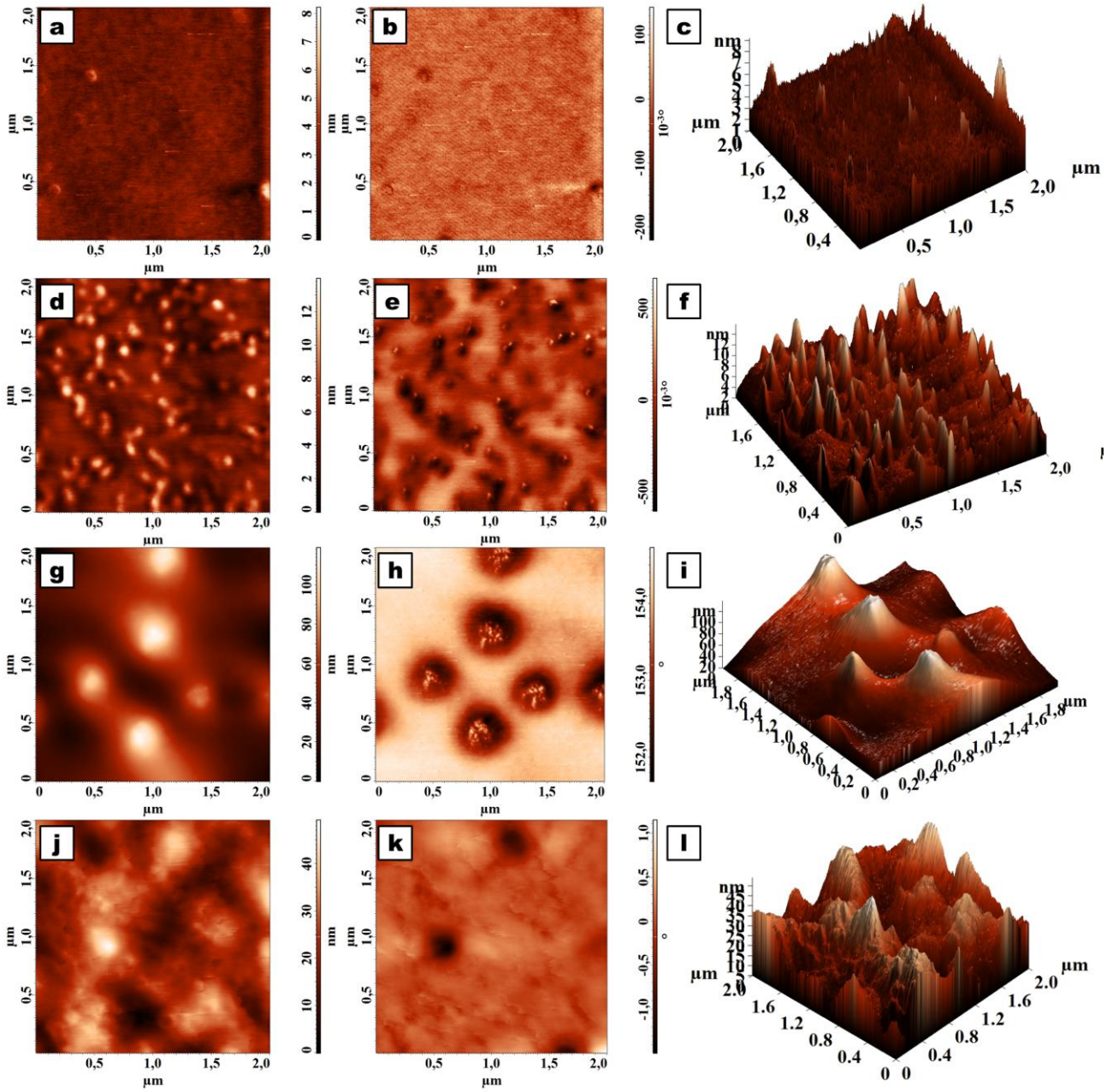


Figure 2. 2D (left), phase (middle), and 3D (right) surface images for the different samples: a, b, c – **1**; d, e, f – **2**; g, h, i – **3**; j, k, l - **4**

Table 2. Roots mean square for films based on samples **1**, **2**, **3**, and **4**

Sample	Root Mean Square, Sq (nm)
1	0.50247
2	1.69557
3	28.92800
4	7.41539

Because the characteristics of interest for electromechanically active materials are mainly mechanical and dielectric ones, the samples were investigated under these aspects. The tensile tests permitted to register stress-strain curves (Figure 3a). It can be seen the samples **2** and **3** have the highest values for stress (1.01 MPa and 1.13 MPa, respectively) and strain (358 % and 370 %, respectively). It can be concluded that the addition of the titanium isopropoxide has a better effect on the mechanical properties of the films in which it is incorporated. Surprisingly, the sample with silica has weaker mechanical characteristics than others. The explanation might be the presence of the polar aminopropyl groups attached to the silica surface, which reduce their compatibility with nonpolar silicone matrix, the reinforcement effect of silica being thus greatly diminished. It seems that the treatment with surfactant did not help in this direction.

In order to appreciate the behavior of the films to the repeating tensile stress, as it would happen in the case of actuator or artificial muscle function, cyclic tests were performed with strains up to 100 % of initial length (Figure 3b). A hysteresis is clearly visible in the first cycle for all samples. In the next cycle, it disappears in the case of blank sample (**1**) and that filled with silica (**4**), when tensile curve coincides with the return. Instead, in the case of samples filled with titania, a hysteresis slightly diminished in comparison with that in the first cycle is kept in the following.

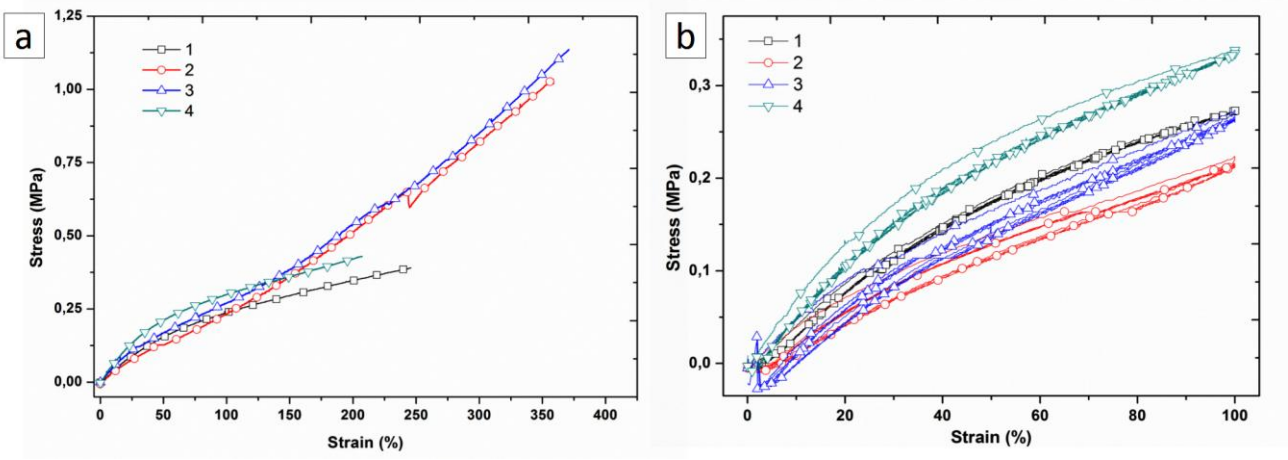


Figure 3. Normal (a) and cyclic (b) stress-strain curves for the prepared films

Table 3. The main parameters of the mechanical and dielectric tests

Sample	Young's modulus, MPa[a]	Tensile strength, MPa	Elongation at break, %	Dielectric permittivity, ε` (at 10 Hz)	Dielectric loss, ε``(at 10 Hz)
1	0.36	0.39	245	3.08	0.20
2	0.32	1.01	358	3.67	0.44
3	0.47	1.13	370	3.85	0.17
4	0.58	0.42	207	3.96	0.45

[a] Young's modulus was calculated as a ratio between the stress and strain when the latter has 10%. Linear stress-strain dependence is considered on this region of the curve.

The dielectric measurements (Figure 4, Table 3) revealed an increase in the permittivity value of the silicone matrix as a result of the filler`s incorporation. Thus, the dielectric permittivity at 10 Hz increases, from 3.08 for reference sample **1**, to 3.85 for sample **3**, the highest value registering for the sample **4** - 3.96. As the frequency increases, these values decrease stabilizing at frequencies above 1000 Hz, as follows: **1** - 3.0, **2** - 3.5, **3** - 3.6, **4** - 3.3. The dielectric loss values decrease when the frequency rises up to about 10^4 Hz (Figure 4b), while the conductivity increases within entire studied frequency range (Figure 1ESI)

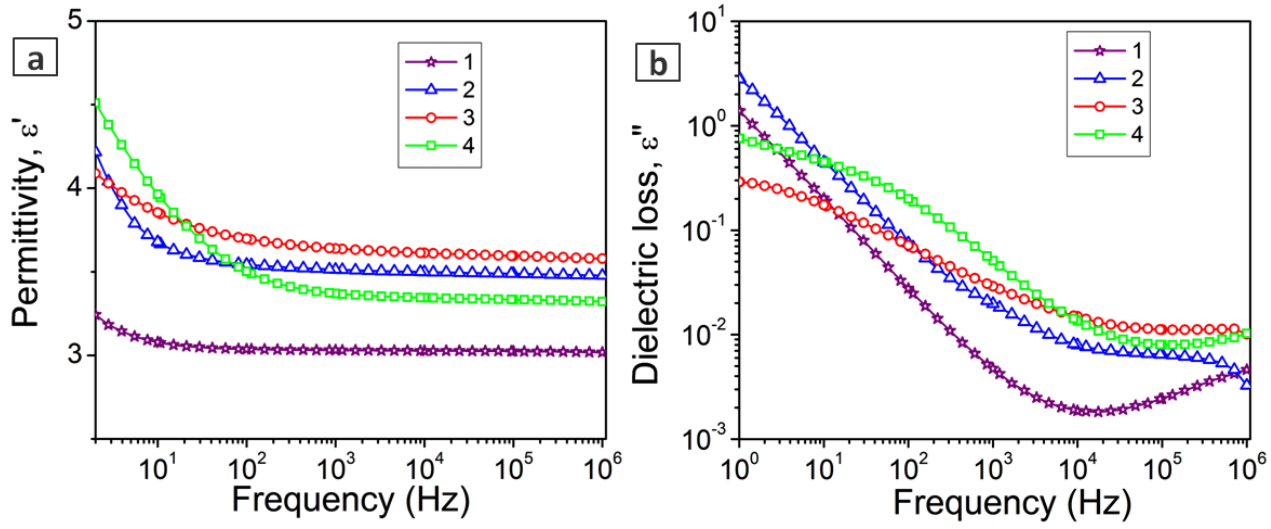


Figure 4. Dielectric spectra for composite films **2 - 4** as compared with reference sample **1**: a- dielectric permittivity; b – dielectric loss.

The increased stress, strain and dielectric permittivity values constitute premises for good electromechanical response.

The electromechanical actuation behaviour was evaluated by measuring the traverse strain when the film sample was placed between two copper electrodes and an increasing field up to 500 V was applied. The interpolated values of the electrostriction displacement, measured by interferometry, in dependence on the electrical field relative to thickness of the studied films are presented in Figure 5. To be able to better compare the electromechanical behaviour of the films having different thickness, the normalized displacement values, expressed as nm per mm thickness have been considered (Table 4). It can be seen that the best response is given by sample **4** containing silica particles functionalized with polar amine groups with a displacement of 2408 nm per mm thickness of the film, while the reference sample **1** showed 795 nm displacement per mm film thickness. The samples **2** and **3** filled with increasing amount of *in situ* generated titania showed displacements of 967 and 1905 nm on each mm film thickness, respectively. The limit voltage of our supply was 500 V and thus the last measured points do not represent the breakdown. Larger displacements are expected at higher electric fields excepting sample **3** which was pierced to 450 V. By considering for all samples the displacements read at the same voltage of 1428 V/mm resulted from the voltage of 500 V related to the thicker film, i.e. sample **4**, the order of the electromechanical responses is the same: **1**<**2**<**3**<**4**. Traverse actuation strain percent calculated at the same value of the electric field ranges between 0.08 and 0.24 %. These values are comparable to those found in the literature for commercial silicones functionalized with polar groups [22] at an applied electric field of the same order.

Table 4. Normalized values of the electromechanical response at the applied electric field

Sample	**1**	**2**	**3**	**4**
Film thickness, mm	0.23	0.12	0.17	0.35
Electric field (V/mm)	2173	4166	2353	1428
Total displacement, nm	393	799	759	843
Displacement in nm/mm film thickness	795	967	1905	2408
Displacement in nm at 1430 V/mm	183	116	324	843
% strain at 1430 V/mm	0.08	0.1	0,19	0.24
Resolution, nm/V/mm	0.18	0.19	0.32	0.59

The reference sample **1** consisting in pure crosslinked PDMS, without any filler, has an electromechanical resolution, expressed as nm/V/mm, with 180% higher than reference sample

from our previous paper showed [12] due to the different crosslinking pattern and lower molecular mass that lead to a decrease of nearly twice of the Young modulus value (0.36 as compared with 0.63 MPa in [12]). Instead, all derived composites show lower resolution values (0.19 – 0.59 nm/V/mm as compared with 1.06 – 5.09 nm/V/mm in [12]). This is due to the lower amounts of filler and smaller elongations of the current samples.

In general, it is known that the dielectric elastomers actuate at voltages ranging between few hundred and 10 kV [1] and most of the studies reported in literature were performed at such fields [22,23]. However, for case of contact with living organisms, the voltage used for our tests were high enough. We were forced to use this because of the thickness of our hand-made films. The safety of using such high voltages for medical applications is dependent on the degree of electrical insulation and on other aspects related to electrical induction in human body. In other words, to be safe, high voltage supply should present a very good electric insulation coupled with a current limiting system within a few mA. Our supply has a limitation at 10 mA, this being known as limit of supportability for the human body. However, it is possible to reduce the voltage necessary for getting the reasonable actuations by increasing the dielectric permittivity, reducing the film thickness, or making, for example, a stack actuator (alternated electrode/dielectric elastomer/electrode).

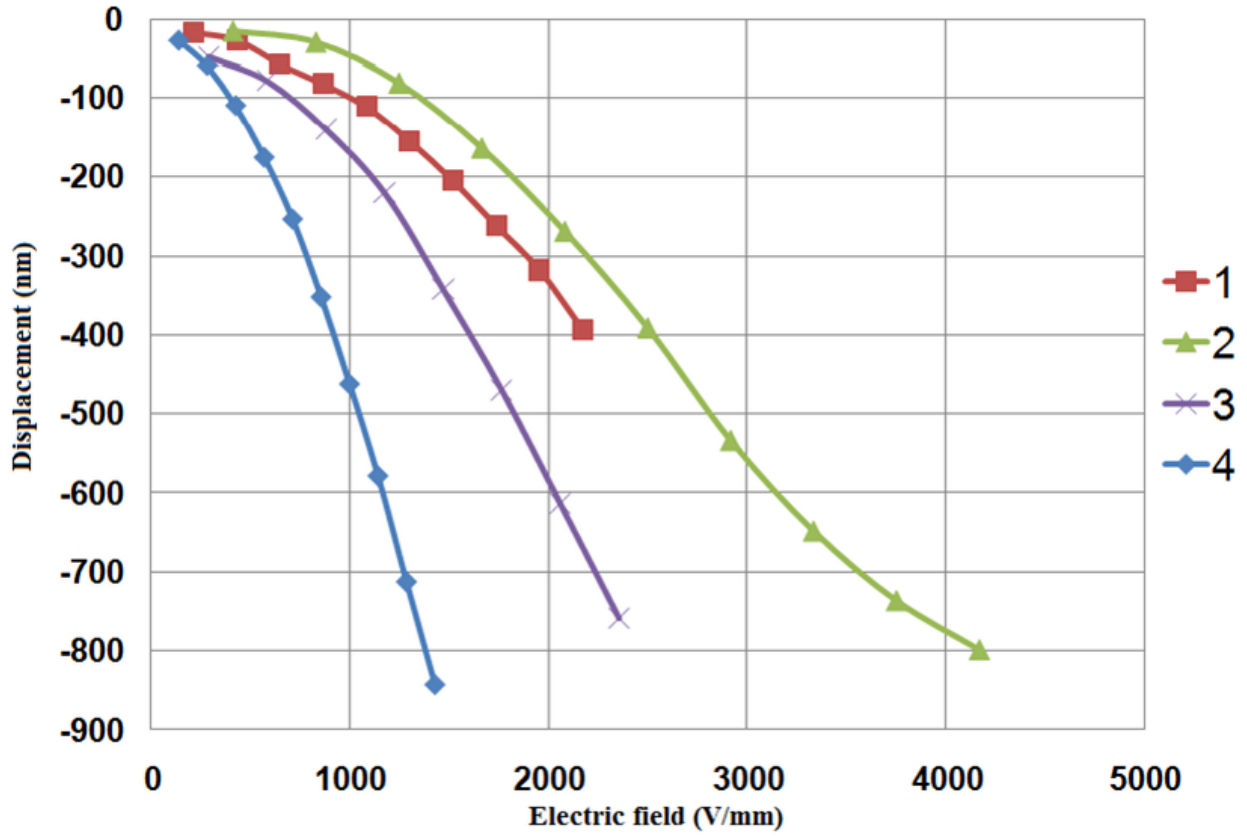


Figure 5. The comparative displacement (electrostriction) values (nm) as a function of electric field related to the thickness (V/mm) of the composite films.

Due to their muscle-like behavior, these materials may have biomedical applications. For this, compatibility testing is a mandatory step.

In vitro biocompatibility tests

As *in vitro* biocompatibility tests, the cytotoxicity and ability to allow cell adhesion were performed. Samples **1**, **2**, and **4** were selected for this study. The sample **3** showing low breakdown field value is inadequately for electromechanical actuation.

The results of MTT assays for assessing cell viability in response to a cytotoxic challenge by samples **1**, **2** and **4** were presented in the Table 4. The dilution factor for the results presented in the table is 4.

Table 4. MTT test results for samples **1**, **2** and **4**

Sample	24h	48h
Negative control	1.47±0.02	2.54±0.2
Positive control	0.13±0.01	0.008±0.003
1	1.52±0.07	2.5±0.07
2	1.59±0.05	2.57±0.05
4	1.46±0.03	2.49±0.06

Based on the MTT results, the normalized cell viability was calculated for each sample for 24 and 48 hours experiment. The results are presented in Figure 6. In our experiments, the culture wells in which cells are cultured without any materials (cell grow control) we considered as negative control (named "control" in the figures). All experimental results were normalized to this negative control that always has been considered as 100%. As positive control we used DMSO, added in the culture media to obtain a concentration of 5%. Although the original experiments include the positive control, the low cell viability, less than 10% at 24 hours of culture (see Table 4), has led us to simplify the images, giving only negative controls. Only samples with a cell viability greater than 90% after 48 hours of culture have been considered being non-cytotoxic.

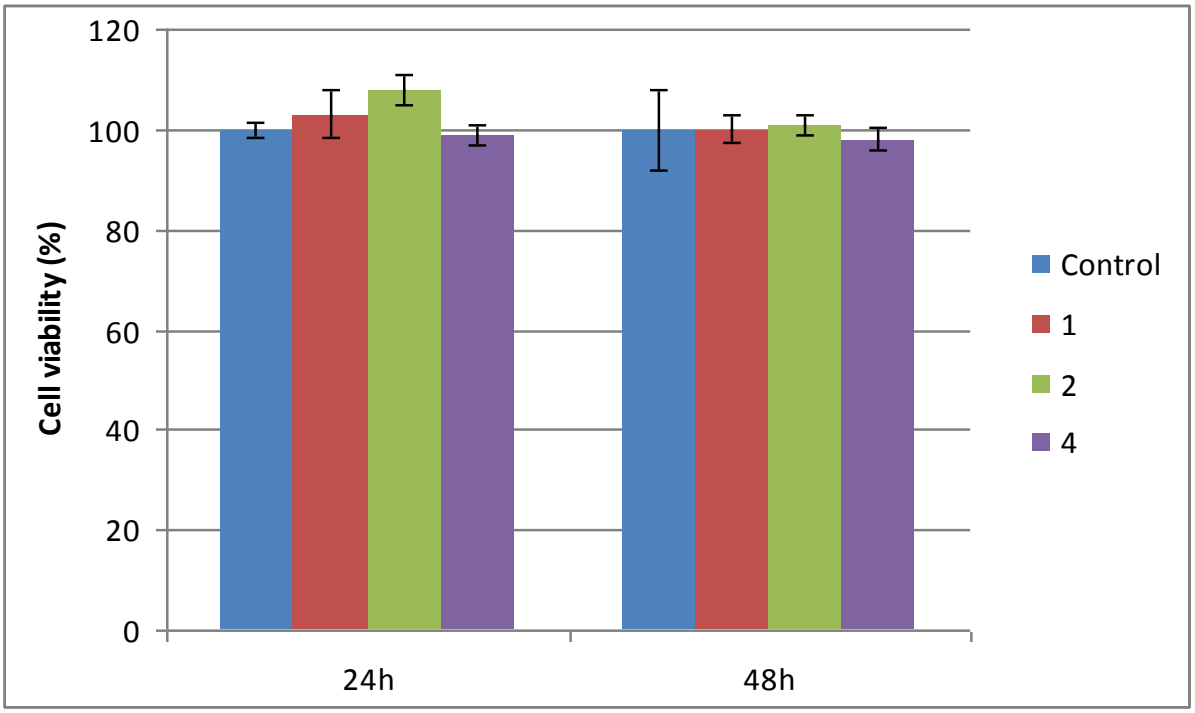


Figure 6. Cell viability based on MTT results for samples **1**, **2** and **4**.

From Figure 6 one could conclude that the studied samples **1**, **2** and **4** are not cytotoxic as other studies have also demonstrated for the similar composites [24]. The cell viability of the experimental cultures has no significant differences compared to the control culture for both, 24 and 48 hours of incubation.

Cell adhesion experiments for these samples were also performed. In Figure 7, the light microscopy images for stained cells in experimental culture compared to the control at 48 hours are presented.

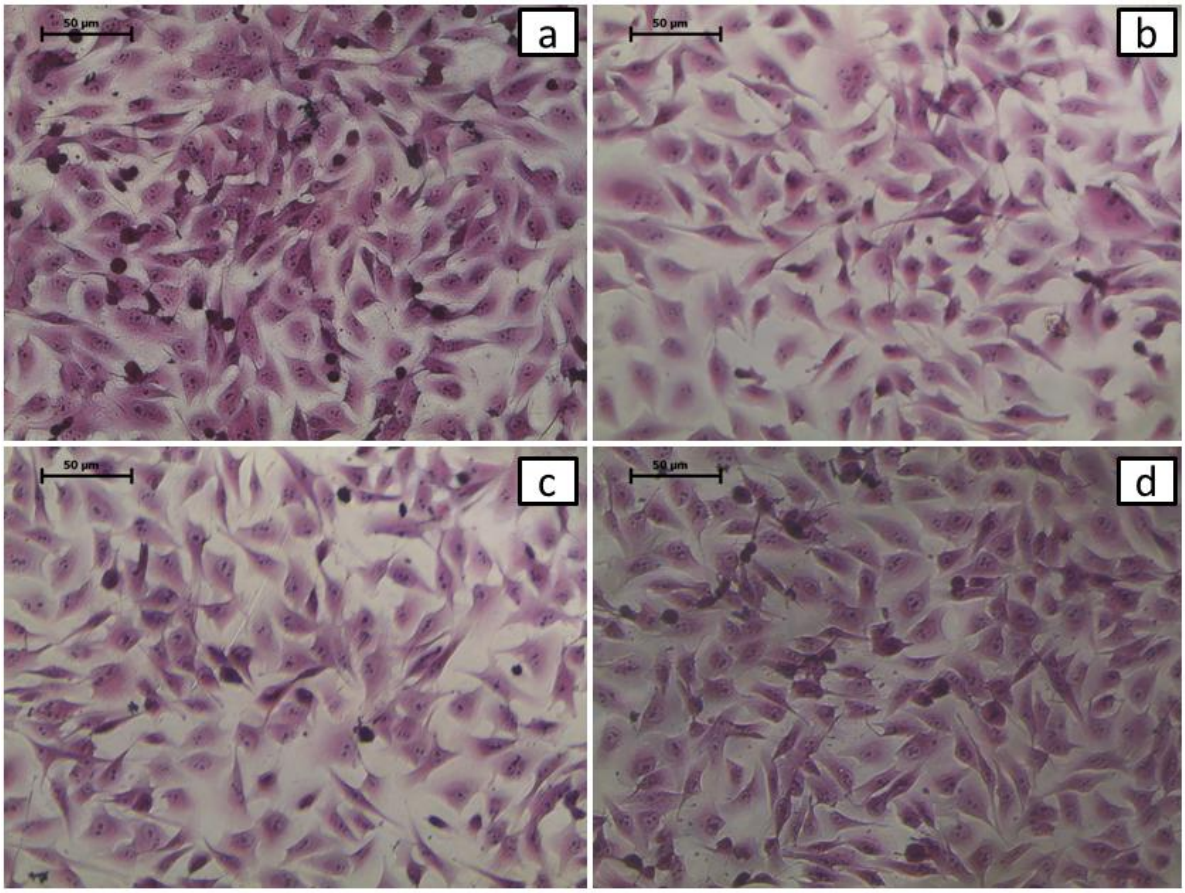


Figure 7. Light microscopy images of the cell culture stained H&E at 48 hours: control (a), **1** (b), **2** (c) and **4** (d)

From Figure 7 it could be conclude that the samples **1**, **2** and **4** did not affect the cell morphology. The cells in the experimental cultures express the same characteristics as the control. The selected SEM image for **1** and **2** samples with attached cells are presented in Figure 8 where it could be observed a slight increased density of the cell population on the sample **2** compared to sample **1**, for both 24 and 48 hours of culture.

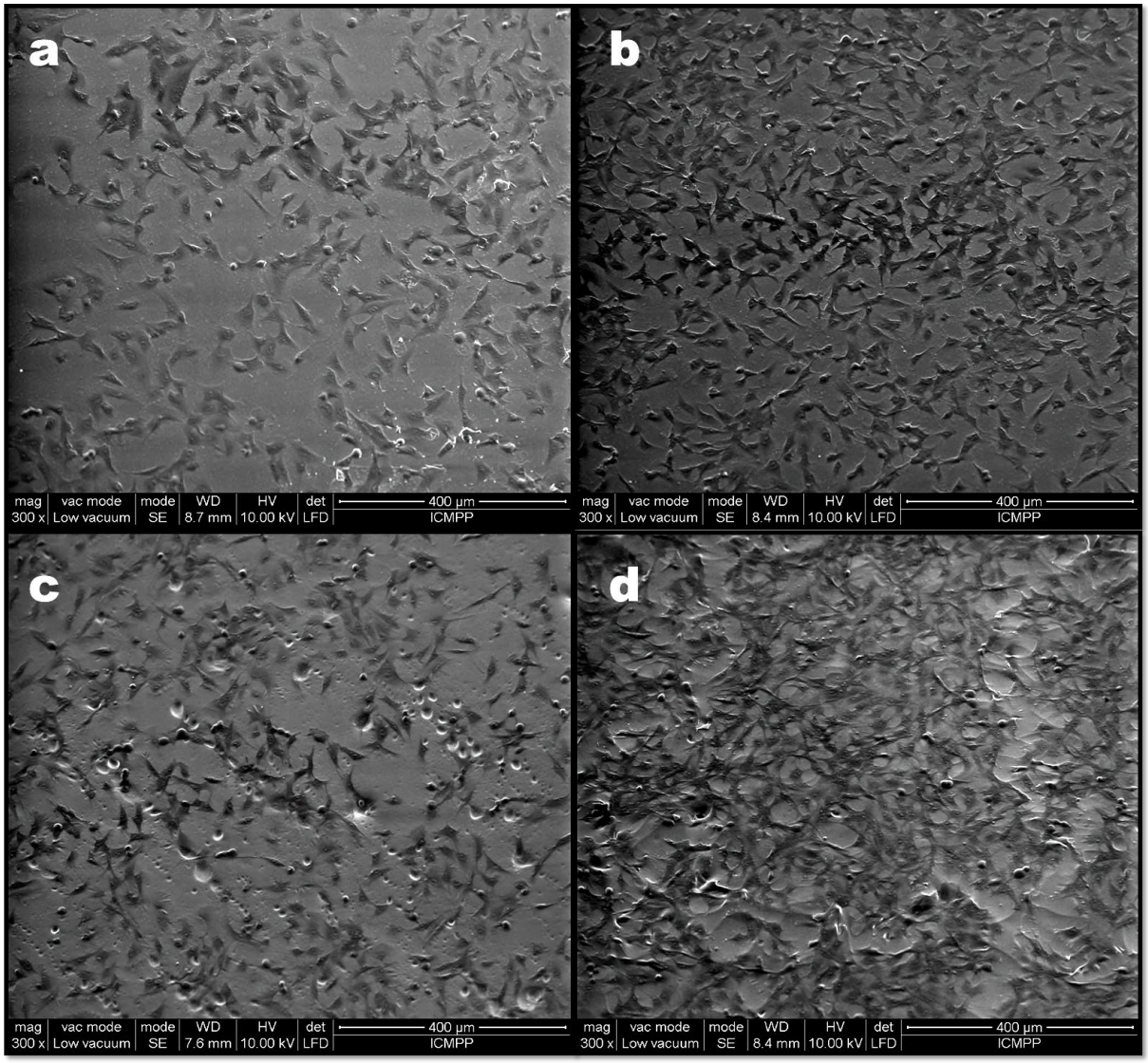


Figure 8. SEM images of the **1** and **2** samples populated with cells: **1** after 24 hours of culture (a), **1** after 48 hours of culture (b), **2** after 24 hours of culture (c) and **2** after 48 hours of culture (d)

In vivo acute toxicity tests

The principal element of investigation, in the interval 1 – 3 days, was clinical observation. Modifications of tegument color, turgescence and integrity were not significant, fact which proves a good tolerance and the absence of acute toxicity. The cutaneous and muscular fragments harvested accordingly to the protocol, which were in contact with the two studied samples, and also from the controls, presented no modifications at the macroscopically and microscopically evaluation. Histological data obtained in day 1 are not relevant due to inflammation produced by surgical trauma (for muscle implant) that leads to a false negative result. At 3 and 7 days, the absence of clinical signs as reddening, flaccid skinfold, piloerection or eruptions, was confirmed by the histopatological examination (gross necropsies, see Figure 9) that leads to the conclusion of an excellent compatibility of the artificial muscle with biological structures.

On the images one can observe the integrity of derma and epidermis at the implanted area level with sample **1** without the presence of any contact inflammation (Figure 9a,b).

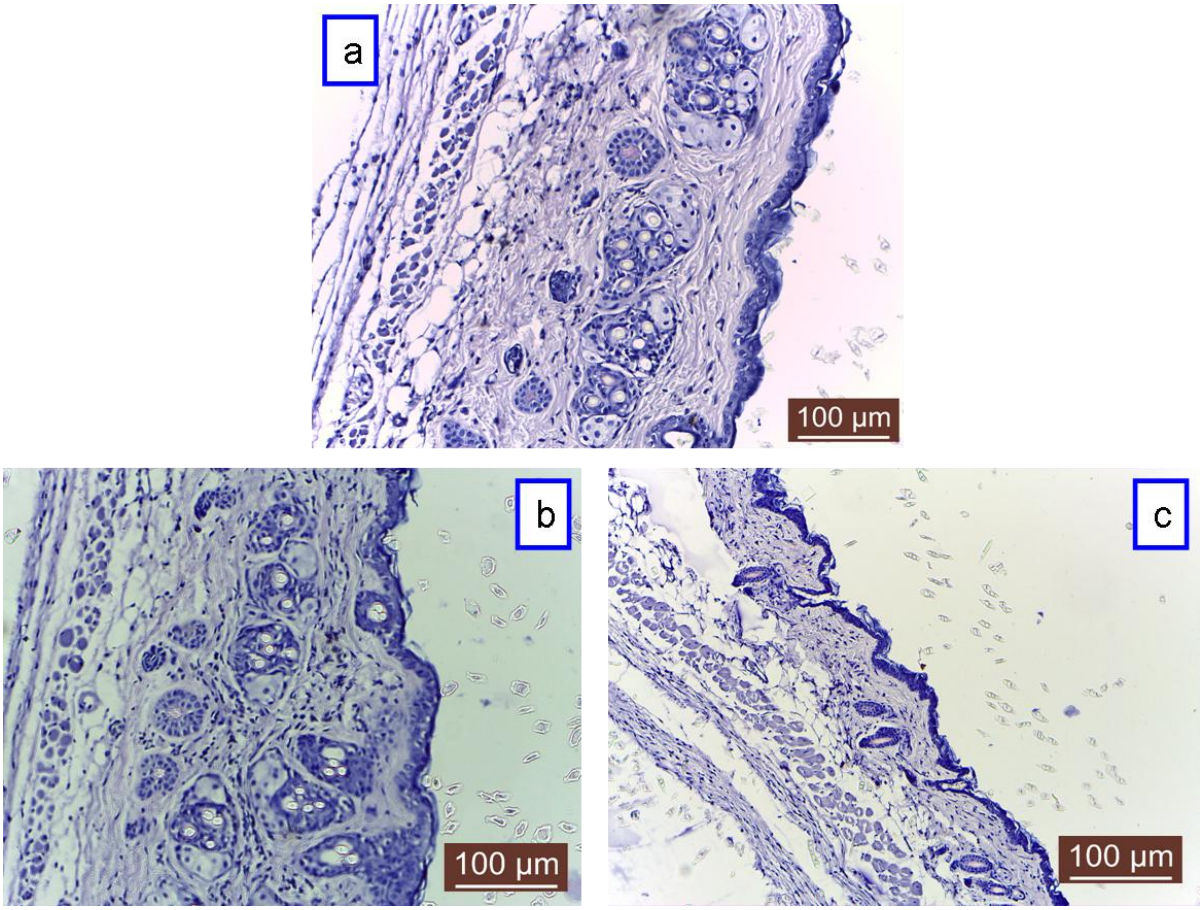


Figure 9. In vivo testing results for the sample **1**: a - image on derma and epidermis at the implanted area level with sample **1** at 3 days (Col. HEA, x200); b - image on derma and epidermis at the implanted area level at 7 days; c - zone of granulation tissue in contact with the implanted film at 7 days.

We noted the integrity of the subcutaneous tissue and a zone of granulation tissue which seems to isolate the implanted film at 7 days (Figure 9c). The conjunctiva irritability test was negative at the clinical evaluation.

4. Conclusion

Three crosslinked silicone composites were prepared by adding different amounts of titanium dioxide precursor or silica particles functionalized with polar aminopropyl groups for potential muscle-like bioapplications and were investigated from point of view of the characteristics of interest for such aim. The AFM study of composite film surface emphasized the differences between the dispersability of the fillers within the polymer matrix revealing an increasing in the

surface roughness, expressed as root mean square, from about 0.5 nm in the case of pure crosslinked PDMS film up to 28.9 on the composite film with highest content of titania; the surface-treated silica incorporation also induces increased roughness. However, due to adequate choice of the electrodes and to the presence of the active fillers, the composite films showed better actuation as compared with smooth reference sample. Thus, all samples containing filler (titania or surface functionalized silica) showed better electromechanical responses (**2** – 967, **3** – 1905, **4** – 2408 nm displacement/mm film thickness) as compared with sample **1** taken as reference (795 nm/mm). The response intensity is higher for the sample filled with surface functionalized silica as compared with films containing titania but the displacement increases as the amount of filler rises. These results are influenced by the improved mechanical and dielectric permittivity values as a result of the incorporation of active fillers (titania or silica having attached polar amino groups). *In vitro* tests proved that the samples are not cytotoxic and their presence did not affect the cell morphology. *In vivo* compatibility tests did not reveal modifications at the macroscopically and microscopically evaluation.

Acknowledgments

The work presented in this paper is developed in the context of the project PolyWEC (www.polywec.org, prj.ref. 309139), a FET-Energy project that is partially funded by the 7th Framework Programme of European Community. Support from COST (European Cooperation in Science and Technology) in the framework of ESNAM (European Scientific Network for Artificial Muscles) - COST Action MP1003 – is also gratefully acknowledged.

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