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Polydimethylsiloxanes biocompatibility in PC12 neuronal cell line

Edi Simoni[a,1], Erica Gentilin[a,1], Mariarita Candito[a], Alessandro Martini[a,b], Laura Astolfi[a,b,*]

[a] Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, 35129, Padua, Italy
[b] Foundation Onlus "Staminali e Vita", 35138, Padua, Italy

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ABSTRACT

Cochlear implants, the only way to recover from severe/profound hearing loss, may cause adverse effects, among which reactions to silicone materials coating implant electrodes, leading to apoptosis and necrosis of spiral ganglion cells. Our aim was to evaluate whether three polydimethylsiloxane (PDMS) compounds (hexadimethylsiloxane, octamethyltrisiloxane, decamethylcyclopentasiloxane) used in silicone rods could exert toxic effects on an *in vitro* neuronal cell model (PC12). Cell viability, morphology and mRNA expression levels of apoptotic markers were evaluated on PC12 cells at different PDMS dilutions up to 6 days of exposure. The results showed that at the highest concentrations tested cell viability was reduced by hexadimethylsiloxane and octamethyltrisiloxane at all times of exposure, but only from 72 h onwards by decamethylcyclopentasiloxane. The number of neurites per cell was not affected by hexadimethylsiloxane, but was significantly reduced from 24 h onwards by octamethyltrisiloxane and decamethylcyclopentasiloxane. Neurite length was reduced by hexadimethylsiloxane only at 24 h, and by octamethyltrisiloxane and decamethylcyclopentasiloxane at all exposure intervals. In controls exposed to silicone or glass rods cell viability was reduced only after 24 h, but neurite number and length was never reduced at any exposure interval. Biomolecular investigations showed that apoptotic markers did not change in any experimental condition, suggesting that PDMS are biocompatible. The reduction of cell viability and neurite number and length caused by exposure to these compounds was probably caused by a PDMS surface film formed over the cell medium, preventing air exchange, and not by the release of cytotoxic molecules.

1. Introduction

Hearing loss is defined as an impairment of the ability to hear sounds that may deeply impact the quality of life and, according to World Health Organization, affects 328 million adults and 32 million children all over the world [1]. Hearing loss consequences include trouble to understand speech sounds, often affecting the ability to communicate, a delay in development of language skills and several socio-economic difficulties. Adults and children with severe/profound sensorineural hearing loss (SNHL), the most common form of deafness occurring after damage of inner ear hair cells and/or spiral ganglion, routinely undergo cochlear implants (CI), surgically implanted electronic devices that directly stimulate the auditory nerve in the inner ear, allowing the recovery of auditory perception [2–4]. Although surgically and technologically advanced [3,5] these devices may benefit from further advancements aimed to prevent adverse effects associated to surgery, leading to delayed auditory recovery and loss of residual hearing [4,6,7]. Recent studies focus on improving CI efficiency and outcome by increasing biocompatibility and biostability of the materials used in their production.

Electrodes in CI are currently coated by the most common form of silicone, polydimethylsiloxane (PDMS) [8,9], but the use of this polymer is impaired by material aging processes [10–12] and intolerance or allergic reactions [13–15]. Aging degradation processes causes the release of low molecular weight siloxanes in the surrounding tissues [16–18] that may cause damages to cochlear neurons, which are unable to regenerate and whose loss is a permanent event [2,19,20]. Therefore, it is necessary to evaluate the toxicity of possible PDMS degradation products on the cochlear neuronal cells, whose activity and functionality is required for CI efficiency [8]. The *in vitro* biocompatibility of solid PDMS by adhesion assay and differentiating inhibition tests was previously shown on a neuronal cell model, the rat pheochromocytoma cell line PC12 [21–23], while biocompatibility data on PDMS degradation products are still lacking. We therefore tested the toxicity of

* Corresponding author at: Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, via G. Orus, 2b, 35129, Padua, Italy.
E-mail addresses: edi.simoni@unipd.it (E. Simoni), erica.gentilin@unipd.it (E. Gentilin), mariarita.candito@gmail.com (M. Candito), alessandromartini@unipd.it (A. Martini), laura.astolfi@unipd.it (L. Astolfi).
[1] These authors are co-first authors.

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three liquid PDMS compounds on PC12 cell line, following previous protocols employed to test the toxicity of the same PDMS compounds on an inner ear cell line [24]. We chose to test hexadimethylsiloxane (6-DMS), octamethyltrisiloxane (8-DMS) and decamethylcyclopentasiloxane (10-DMS) because they are typical compounds to produce silicone suitable to coat cochlear implant electrodes. Current information on PDMS degradation processes mainly derive from investigation of breast prostheses [25]. Relevant amounts of very low molecular weight compounds have been shown to be released from implants [25]. Accordingly, we selected these PDMS because of their very low molecular weight and because 6-DMS and 10-DMS are reported as PDMS degradation products [25].

2. Materials and methods

2.1. Cell culture

The rat pheochromocytoma cell line PC12 was obtained from Interlab Cell Line Collection (ICLC ATL98004, Genoa, Italy). The cells were cultured in RPMI-1640 medium containing 5% fetal bovine serum (FBS, Gibco, Gaithersburg, MD, USA), 10% horse serum (HS, Gibco), 2 mM L-glutamine (Gibco) and 1% penicillin-streptomycin (Pen-Strep, Gibco) (complete medium). Cells were induced to differentiate into neuron-like cells for 6 days using a differentiation medium composed of RPMI-1640 medium containing 0.5% FBS, 1% HS, 2 mM L-glutamine, 1% Pen-Strep and 50 ng/ml nerve growth factor β (NGF-β, Sigma-Aldrich, Milan, Italy). The PC12 cells were maintained at 37 °C in 5% CO_2. After 6 days the cells reached the differentiation plateau, showing at least one neurite per cell with a length equal to the cell body diameter [26].

2.2. Compounds

The liquid PDMS compounds hexadimethylsiloxane (6-DMS), octamethyltrisiloxane (8-DMS) and decamethylcyclopentasiloxane (10-DMS) were purchased from Sigma-Aldrich (Milan, Italy). Silicone rods, provided by Cochlear Research and Development Ltd (Addlestone, UK), were 20 mm long, 1.10 mm in diameter, flexible, transparent and with density lower than 1.00 g/ml. The glass rods were prepared by cutting 20-mm long pieces from Pasteur pipettes made of soda-lime glass with a diameter of about 1.10 mm; the ends of glass rods were melted to seal them. Glass rods were used as silicone rod controls to verify possible mechanical damages.

2.3. Cell viability assay

A total of 2×10^4 cells/well were seeded in 6-well plates containing 2 ml/well of RPMI medium supplemented as previously described (complete medium). The cells were incubated with the differentiation medium for 6 days and at the end of differentiation time were treated with liquid PDMS compounds for 24 h, 48 h, 72 h and 6 days, at dilutions 1:10 and 1:5 for 6-DMS, 1:50 and 1:10 for 8-DMS, 1:1000 and 1:100 for 10-DMS (Table 1), with matching untreated controls. The PDMS compounds were directly added to the well plates containing the complete culture medium. The dilutions were based on the behaviour of the compounds in the culture medium. The three PDMS compounds had different density and viscosity (10-DMS > 8-DMS > 6-DMS), thus their ability to dissolve in the culture medium was different according to the dilution. As controls, cells were also exposed to silicone and glass rods in the same conditions.

Table 1
Dilutions and concentrations (M) of the PDMS compounds tested.

Compound	Dilutions	
6-DMS	1:10 (0.471 M)	1:5 (0.942 M)
8-DMS	1:50 (0.068 M)	1:10 (0.340 M)
10-DMS	1:1000 (0.003 M)	1:100 (0.026 M)

Cell viability was assessed using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega, Milan, Italy) following the manufacturer's protocol. Cells grown in each well of the 6-well plate for all time intervals were collected, resuspended in 500 µl, divided into 100-µl aliquots and re-seeded in 96-well plates. To each well 20 µl of a solution 20:1 of 2 mg/ml 3- (4,5-dimethylthiazol-2-yl)-5- (3-carboxymethoxyphenyl)-2- (4-sulfophenyl)- 2H-tetrazolium (MTS) and 0.92 mg/ml phenazine methosulfate (PMS) were added and each plate was incubated for 3 h at 37 °C. Measures for optical density (OD) at 492 nm were performed by an Elisa microplate reader (SIRIO S, SEAC s.r.l, Florence, Italy). The OD values for each sample were normalized to the mean OD value of the respective untreated controls, considered as 100% viability rate. Data were expressed as the mean value ± standard error of the mean (SEM) of three independent experiments, each performed in triplicate.

2.4. Cell morphology

A total of 5×10^3 cells/well were seeded in 6-well plates containing 2 ml/well of RPMI medium supplemented as previously described (complete medium). The cells were incubated with the differentiation medium for 6 days and at the end of differentiation time were treated with the highest dilutions of liquid PDMS compounds for 24 h, 48 h, 72 h and 6 days. Changes in cell morphology were examined by phase contrast microscopy (Nikon Eclipse TE2000-U, Nikon, Florence, Italy). Images were acquired with Nis Elements D 3.2 software (Nikon). Thirty different fields of view (10 fields/well) were analyzed for the number of neurites and their length using the ImageJ software (https://imagej.nih.gov/ij/). The number of neurites was calculated with data normalized to the related number of neurons, the neurite length was calculated with data normalized to the related number of neurites. Values calculated for each sample were normalized to the mean value of the respective untreated controls, considered as 100% rate. Data were expressed as the mean value ± SEM of three independent experiments, each performed in triplicate.

2.5. Real time PCR

A total of 2×10^4 cells/well were seeded in 6-wells plates containing 2 ml/well of complete medium. The cells were incubated with the differentiation medium for 6 days and at the end of differentiation time were treated with 6-DMS, 8-DMS and 10-DMS for 24 h and 6 days, at the dilutions shown in Table 1, with matching untreated controls. Cells were also exposed to silicone rods and to glass rods in the same conditions. At the end of the incubation time, total RNA was extracted with Trizol ® (Thermo Fisher Scientific, Milan, Italy) and treated with DNase I (New England Biolabs, Hitchin, UK) following manufacturer's protocols. The samples were reverse transcribed using iScript™ Reverse Transcription Supermix (Bio-Rad, Milan, Italy). The quantitative Real Time PCR (RTqPCR) for the genes of interest (*Bax*, *Bcl2, Bcl2l11*, *Bid*, *Casp3*, *Casp8*, *Casp9*, *Tp53*) was performed in a Chromo-4 System thermocycler (Bio-Rad) with Sso-fast™ EvaGreen® Supermix (Bio-Rad). Gene data were analysed with the comparative cycle threshold method, using GenEx 6.1 software (bioMCC, http://www.biomcc.com/genex-software.html) with three housekeeping genes (*Actb*, *B2m* and *Gapdh*) as reference. Primer sequences of investigated genes are reported in (Table 2. The results were represented as mean value ± SEM *versus* untreated cells of at least three independent experiments performed in duplicate. The *Bax/Bcl2* ratio was calculated with data normalized to controls as previously reported [24].

Table 2
Primer sequences and amplicon length of investigated genes.

Gene	Forward primer	Reverse primer	Amplicon length
Actb	CGTTGACATCCGTAAAGACC	GATAGAGCCACCAATCCACAC	178
B2m	CGAGACCGATGTATATGCTTGC	GTCCAGATGATTCAGAGCTCCA	114
Bax	CTAGCAAACTGGTGCTCAAGG	CTCAGCCCATCTTCTTCCAG	231
Bcl2	GCCTTCTTTGAGTTCGGTG	AGTTCCACAAAGGCATCCC	158
Bcl2l11	CGGCACCCATGAGTTGTGACAA	TGCAAACGCCCTCCTCGTGTA	199
Bid	TCTGAGGTCAGCAATGGCTCAGG	TTCTTCCATGATAGAAGGAGCGGCT	210
Casp3	GAACCAGATCAGAAGCTCCT	CTTTCCAAGTCCCGTGTG	191
Casp8	CCAAATGAAGAGCAAACCTCG	GATACTAGAACCTCATGGATTTGAC	216
Casp9	GAGGGAAGCCCAAGCTGTTC	GCCACCTCAAAGCCATGGT	70
Gapdh	GACTTCAACAGCAACTCCCA	GCCATATTCATTGTCATACCAGGA	104
Tp53	ATGTGCACGTACTCAATTTCC	TGTGTTGTGACTTCTTGTAGATGG	139

2.6. Statistical analyses

Data were analyzed by Student's *t*-test or one-way ANOVA for parametric data, and by Mann–Whitney U test or Kruskall-Wallis for non parametric data, using the STATISTICA 7.1 software (Stat Soft Italia S.r.l, Milan, Italy). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effects on PC12 cell viability

The effects of the liquid PDMS compounds hexadimethylsiloxane (6-DMS), octamethyltrisiloxane (8-DMS) and decamethylcyclopentasiloxane (10-DMS) and silicone and glass rods were first investigated on viability of differentiated PC12 cells. The results are shown in Fig. 1. The higher dilutions of the compounds (6-DMS 1:10, 8-DMS 1:50 and 10-DMS 1:1000) did not affect cell viability at all exposure intervals (Fig. 1A–C). However, the lower dilutions (6-DMS 1:5, -DMS 1:10 and 10-DMS 1:100) significantly reduced viability: for 6-DMS, the viability reduction was about 60% at all intervals (Fig. 1A); for 8-DMS, the viability reduction was 43% at 24 h ($p < 0.01$), 37% at 48 h ($p < 0.01$), 25% at 72 h ($p < 0.01$) and 17% at 6 days ($p < 0.01$) (Fig. 1B); for 10-DMS, the viability reduction was 28% at 72 h ($p < 0.05$) and 42% at 6 days ($p < 0.05$) (Fig. 1C). The exposure to silicone and glass rods significantly reduced cell viability only after 24 h (8%, $p < 0.05$; 24%, $p < 0.01$, respectively) (Fig. 1D).

3.2. Effects on PC12 cell morphology

The effects of 6-DMS, 8-DMS, 10-DMS, silicone and glass rods on PC12 cell morphology were analyzed by exposing the cells at lowest dilutions for 24 h, 48 h, 72 h and 6 days (Figs. 2 and 3). The exposure to 6-DMS, silicone and glass rods did not significantly affect the number of neurites per cell as compared to untreated cells at all intervals tested (Figs. 2 and 3A), while 8-DMS and 10-DMS significantly reduced this number after 24 h (44%, $p < 0.01$; 33%, $p < 0.01$, respectively) and 48 h (18%, $p < 0.01$; 13%, $p < 0.05$, respectively). The total neurite length was significantly reduced by exposure to 6-DMS, 8-DMS and 10-DMS: for 6-DMS, the reduction was 36% after 24 h ($p < 0.01$); for 8-DMS, the reduction was about 50% at all intervals tested; for 10-DMS, the reduction was 27% after 24 h ($p < 0.05$), about 34% after 48 h ($p < 0.01$), 53% after 72 h ($p < 0.01$), 29% after 6 days ($p < 0.05$) (Figs. 2 and 3B). Silicone and glass rods did not affect the total neurite

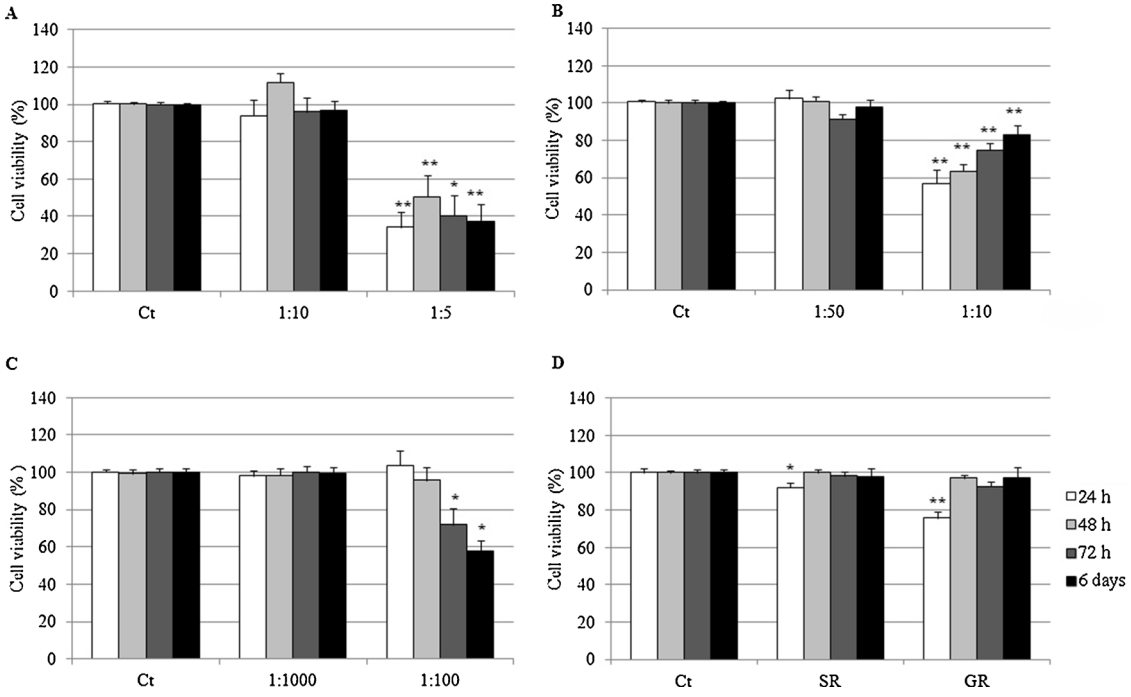

Fig. 1. Effects of PDMS compounds on viability of differentiated PC12 cells. Cells were incubated for 24 h, 48 h, 72 h and 6 days in culture medium supplemented or not with different dilutions of 6-DMS (A), 8-DMS (B), 10-DMS (C) and exposed to silicone and glass rods (D). Cell viability was expressed as mean value percent ± SEM *vs* control cells (Ct). SR, silicone rods; GR, glass rods. Asterisks indicate significant differences in comparison to Ct.* = $p < 0.05$; ** = $p < 0.01$.

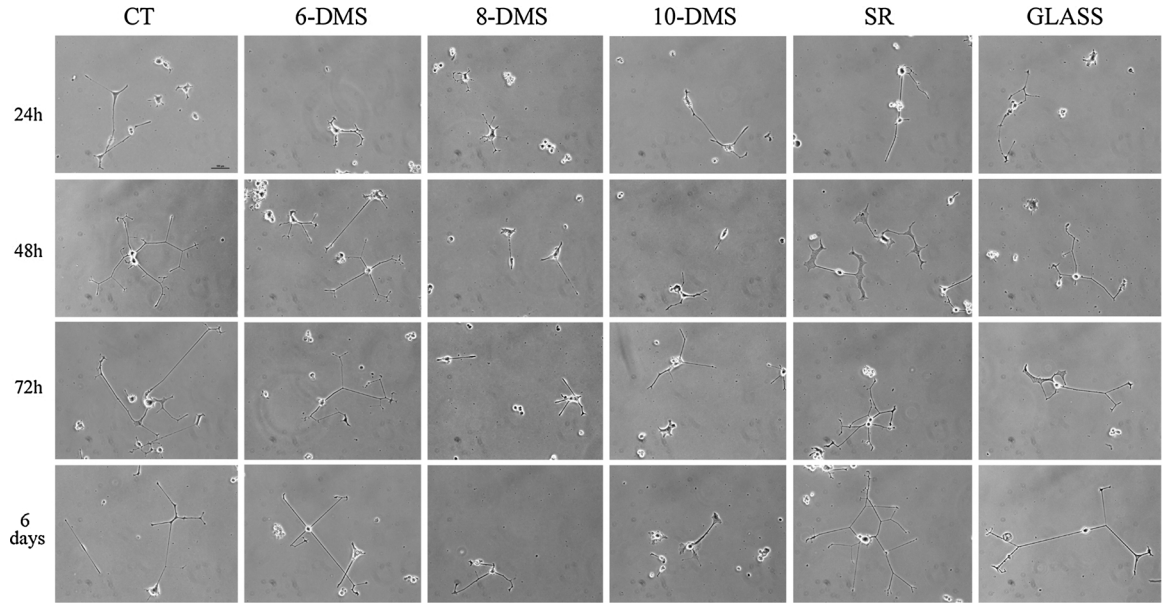

Fig. 2. Effects of PDMS compounds on morphology of differentiated PC12 cells. Cells were incubated for 24 h, 48 h, 72 h and 6 days in culture medium supplemented or not with 6-DMS 1:5, 8-DMS 1:10, 10-DMS 1:100 and exposed to silicone (SR) and glass rods (GR). Ct, control cells. Magnification 15×.

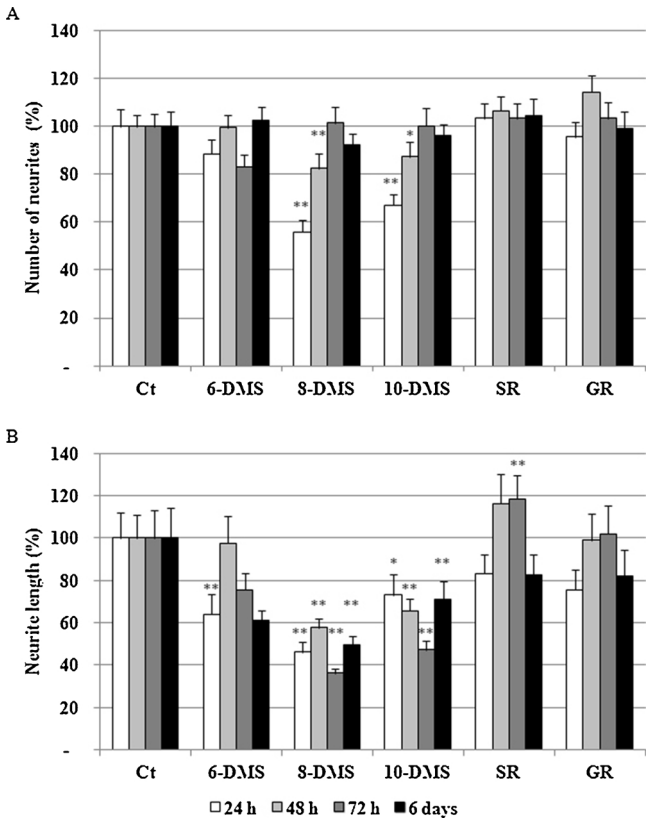

Fig. 3. Effects of PDMS compounds on morphology of differentiated PC12 cells. Cells were incubated for 24 h, 48 h, 72 h and 6 days in culture medium supplemented or not with 6-DMS 1:5, 8-DMS 1:10, 10-DMS 1:100 and exposed to silicone (SR) and glass rods (GR). Ct, control cells. The number of neurites (A) and neurite length (B) were expressed as mean value percent ± SEM *vs* control cells (Ct). Asterisks indicate significant differences in comparison to Ct. * = $p < 0.05$; ** = $p < 0.01$.

length in any experimental condition (Figs. 2 and 3B).

3.3. Effects on expression of apoptosis related markers

The effects of 6-DMS, 8-DMS, 10-DMS, silicone and glass rods on the levels of mRNA expression of pro-apoptotic and anti-apoptotic markers (*Bax*, *Bcl2*, *Bcl2l11*, *Bid*, *Casp3*, *Casp8*, *Casp9* and *Tp53*) were tested on differentiated PC12 cells exposed to liquid PDMS compounds at the dilutions indicated in Table 1 for 24 h and 6 days. No liquid PDMS compound nor glass rods affected mRNA expression profiles at any dilution and time tested (data not shown). The exposure to silicone rods induced a slight increase of mRNA expression profiles of *Casp3*, *Casp9* and *Tp53* after 24 h, and a slight increase of the profiles of all markers after 6 days. None of these variations of mRNA expression profiles attained a statistical significance (Fig. 4). At all dilutions and times tested, none of the liquid PDMS compounds nor silicone and glass rods showed any effect on the *Bax/Bcl2* ratio (Table 3).

4. Discussion

The compound 6-DMS at dilution 1:10 did not affect the viability of differentiated PC12 cells, while at dilution 1:5 it significantly reduced viability at all time intervals tested. These results agree with those previously obtained in an *in vitro* model of inner ear sensory cell line, the OC-k3 cells [24], suggesting that 6-DMS at high concentration exerts a toxic effect on both cell lines, independently of their origin. The compound 8-DMS at dilution 1:50 did not affect the viability of differentiated PC12 cells, but at dilution 1:10 significantly reduced viability and this effect decreased with increasing exposure time. Previous results on OC-k3 cells showed the same effects [24], supporting the hypothesis that inner ear sensory and neuronal tissues may be able to counteract 8-DMS toxicity over time. A possible explanation for this toxicity reduction could be the reduction of PDMS oily film thickness, with increased O_2 flux and metabolic activity. Concerning 10-DMS, a dilution of 1:1000 did not affect the viability of differentiated PC12 cells, while a dilution 1:100 significantly reduced viability from an exposure time of 72 h onwards. In comparison, OC-k3 cells appeared more sensitive to 10-DMS at dilution 1:100, since in these cells the viability was reduced from 48 h onwards [24].

The observed effects of PDMS compounds on cell viability are

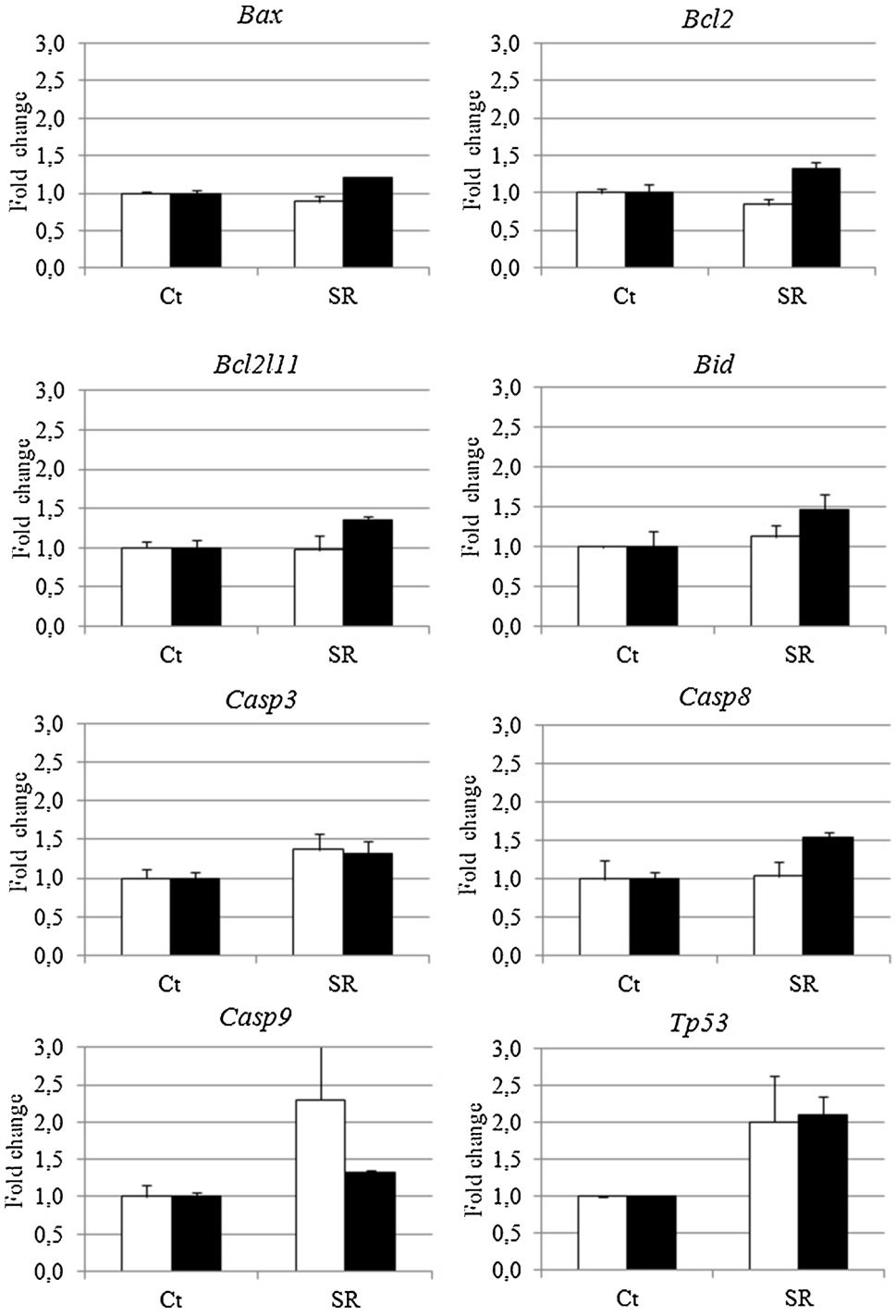

Fig. 4. Effects of the exposure to silicone rods on the expression of pro-apoptotic and anti-apoptotic markers (*Bax*, *Bcl2*, *Bcl2l11*, *Bid*, *Casp3*, *Casp8*, *Casp9* and *Tp53*) in differentiated PC12 cells. Cells were incubated for 24 h (white box) and 6 days (black box), in culture medium in presence of silicone rods (SR). Data were expressed as fold change of mRNA expression levels ± SEM *vs* control cells (Ct).

Table 3
Ratio of Bax/Bcl2 mRNA transcript levels.

Compound	6-DMS		8-DMS		10-DMS		GR	SR
Dilutions	1:10	1:5	1:50	1:10	1:1000	1:100		
After 24 h	0.99	0. 98	0.99	0.96	0.97	0.96	1.00	1.06
After 6 days	1.00	1.00	0.99	0.99	0.99	0.97	1.00	0.91

Bax/Bcl2 was expressed as mean value fold *vs* control cells. GR, glass rods, SR, silicone rods.

variable, probably due to the differences in viscosity. The 6-DMS at dilution 1:5 produced an oily film that covered the entire well, on the contrary of 10-DMS at dilution 1:100. The 8-DMS at dilution 1:10 showed an intermediate behaviour. According to the results of cell viability assay, at 24 h the oily film causes significant stress in cells treated with 6-DMS and a minor stress in cells treated with 8-DMS and 10-DMS. Along with treatment time, the oily film progressively dissolved on the surface of culture medium. The 6-DMS showed similar toxic effects at all exposure times; the 10-DMS was increasingly toxic along time, while the 8-DMS was decreasingly toxic.

Silicone rods affected viability of differentiated PC12 cells only at 24 h similarly to glass rods, suggesting that the reduction of viability is not associated to the material but rather to a mechanical stress exerted by the contact with rods. Concerning silicone rods, these results agree with previously published ones on OC-k3 cells, although the reduction of viability occurred later, from 72 h of exposure to silicone rods onwards [24]. Moreover, our results agree with previous data obtained on differentiated PC12 cells exposed to similar solid PDMS rods: no toxic effect was detected and these cells were able to adhere, growth, differentiate and extend their neurites over these materials [27].

Morphological alterations were detected at the lowest dilutions of liquid PDMS. The compound 6-DMS apparently affected cell viability more than neurite morphology, while the contrary occurred for 8-DMS. The effects of 10-DMS were apparently the same on both cell viability and neurite morphology. Overall, these results indicate that the liquid PDMS compounds may exert their effects on differentiated PC12 cell line through different cellular pathways.

To further investigate the biocompatibility of liquid PDMS compounds we evaluated the mRNA expression levels of apoptotic markers. Gene expression alteration is known as a key event in adverse conditions such as drug-induced toxicity [28–30] and biomaterial-induced alterations [31]. No significant effects were detected on mRNA expression profiles of the apoptotic markers *Bax*, *Bcl2*, *Bcl2l11*, *Bid*, *Casp3*, *Casp8*, *Casp9* and *Tp53* when differentiated PC12 cells were exposed to the three PDMS compounds and silicone and glass rods at any dilution and time interval tested. Based on these results, the reduction in cell viability and the morphological alterations observed in differentiated PC12 cells treated with PDMS or silicone rod could not be ascribed to alterations of mRNA levels of the key components of the apoptotic machinery as confirmed by the Bax/Bcl2 ratio, an indicator of cell death [24,29,32]. Apoptosis can be initiated by an extrinsic or intrinsic signaling pathway: the first one is characterized by the activation of Caspase-8 that in turn directly activates the executioner Caspase-3 [33,34]. The intrinsic signaling pathway is characterized by repression of the anti-apoptotic Bcl-2 protein and activation of the pro-apoptotic BAX protein, resulting in the activation of Caspase-9 and later of Caspase-3 [33,34]. The Bcl-2-like protein 11 is a pro-apoptotic molecule that represents a regulating switch between the extrinsic and intrinsic pathway: once activated by Caspase-8, it may activate BAX and/or repress Bcl-2 [33,34]. Another key marker in the apoptotic process is cellular tumor antigen p53 that promotes apoptosis in response to hypoxia, DNA damage or improper oncogene expression, regulating the main components of the extrinsic and intrinsic signalling pathway [35].

5. Conclusion

Our results show that liquid PDMS compounds and silicone rods did not affect the gene expression of any apoptotic marker tested. The reduction in cell viability and the morphological alterations observed in this study on differentiated PC12 cells cannot therefore be ascribed to alterations of mRNA levels of the key components of the apoptotic machinery. A more likely explanation of the effects on cell viability and neurite alterations detected in our study is the ability of these compounds to form films over the surface of the cell culture medium: these films may interfere with air exchange, thus impairing oxygen uptake and cell metabolism. This hypothesis is supported by the fact that silicone rods never had significant effects on cell viability, morphology and apoptotic processes in our tests, confirming its biocompatibility in contact with neurons and its usefulness in cochlear implants.

Our results show that liquid PDMS compounds could be toxic at high concentrations. If PDMS are released during silicone ageing, in order to exert toxic effects on neuronal cells they should be released at high concentrations, which are unlikely in the case of cochlear implants. Overall, in this study we did not find sufficient proofs to discourage use of silicone in cochlear implants.

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References

[1] World Health Organization, Deafness and Hearing Loss, (2017) (Fact sheet, Updated February 2017), http://www.who.int/mediacentre/factsheets/fs300/en/.
[2] A. Ciorba, L. Astolfi, C. Jolly, A. Martini, Cochlear implants and inner ear based therapy, Eur. J. Nanomed. 2 (2009) 25–28.
[3] J.P. Roche, M.R. Hansen, On the horizon: cochlear implant technology, Otolaryngol. Clin. North Am. 48 (2015) 1097–1116.
[4] S. Ghiselli, S. Nedic, S. Montino, L. Astolfi, R. Bovo, Cochlear implantation in postlingually deafened adults and elderly patients: analysis of audiometric and speech perception outcomes during the first year of use, Acta Otorhinolaryngol. Ital. 36 (2016) 513–519.
[5] P. Giordano, S. Hatzopoulos, N. Giarbini, S. Prosser, J. Petruccelli, E. Simoni, C. Faccioli, L. Astolfi, A. Martini, A soft-surgery approach to minimize hearing damage caused by the insertion of a cochlear implant electrode: a guinea pig animal model, Otol. Neurotol. 35 (2014) 1440–1445.
[6] L. Astolfi, E. Simoni, N. Giarbini, P. Giordano, M. Pannella, S. Hatzopoulos, A. Martini, Cochlear implant and inflammation reaction: safety study of a new steroid-eluting electrode, Hear. Res. 336 (2016) 44–52.
[7] V. Scheper, R. Hessler, M. Hütten, M. Wilk, C. Jolly, T. Lenarz, G. Paasche, Local inner ear application of dexamethasone in cochlear implant models is safe for auditory neurons and increases the neuroprotective effect of chronic electrical stimulation, PLoS One 12 (2017) e0183820.
[8] T. Stöver, T. Lenarz, Biomaterials in cochlear implants, GMS Curr. Top. Otorhinolaryngol. Head Neck Surg. 8 (2009) Doc10.
[9] C. Hassler, T. Boretius, T. Stieglitz, Polymers for neural implants, J. Polym. Sci. Part B: Polym. Phys. 49 (2011) 18–33.
[10] J.W. Swanson, J.E. Lebeau, The effect of implantation on the physical properties of silicone rubber, J. Biomed. Mater. Res. 8 (1974) 357–367.
[11] B. Dolezel, L. Adamírová, Z. Náprstek, P. Vondrácek, *In vivo* degradation of polymers. I. Change of mechanical properties in polyethylene pacemaker lead insulations during long-term implantation in the human body, Biomaterials 10 (1989) 96–100.
[12] L.J. Leslie, M.J. Jenkins, D.E. Shepherd, S.N. Kukureka, The effect of the environment on the mechanical properties of medical grade silicones, J. Biomed. Mater. Res. B Appl. Biomater. 86 (2008) 460–465.
[13] S. Puri, J.L. Dornhoffer, P.E. North, Contact dermatitis to silicone after cochlear implantation, Laryngoscope 115 (2005) 1760–1762.
[14] L.D. Kunda, K.R. Stidham, M.M. Inserra, P.S. Roland, D. Franklin, J.B.Jr. Roberson, Silicone allergy: a new cause for cochlear implant extrusion and its management, Otol. Neurotol. 27 (2006) 1078–1082.
[15] L. Migirov, E. Dagan, J. Kronenberg, Surgical and medical complications in different cochlear implant devices, Acta Otolaryngol. 129 (2009) 741–744.
[16] K. Felix, S. Janz, J. Pitha, J.A. Williams, E.B. Mushinski, G.W. Bornkamm, M. Potter, Cytotoxicity and membrane damage *in vitro* by inclusion complexes between γ-cyclodextrin and siloxanes, in: M. Potter, N.R. Rose (Eds.), Immunology of Silicones, Springer eBooks, 1996, pp. 93–99.
[17] Z. Huang, H. Lin, X. Peng, Z. Wang, R. Wang, Z. Li, P. Fang, Aging behaviors of silicone rubber composite materials under outdoor environment, J. Wuhan Univ. Technol.-Mat. Sci. Edit. 31 (2016) 1289–1293.
[18] K. Mojsiewicz-Pieńkowska, M. Jamrógiewicz, K. Szymkowska, D. Krenczkowska, Direct human contact with siloxanes (silicones) - safety or risk part 1. Characteristics of siloxanes (silicones), Front. Pharmacol. 7 (2016) 132.
[19] N. Jongkamonwiwat, M.N. Rivolta, The development of a stem cell therapy for deafness, in: G. Steinhoff (Ed.), Regenerative Medicine. From Protocol to Patient, Springer eBooks, 2013, pp. 793–821.
[20] E. Simoni, G. Orsini, M. Chicca, S. Bettini, V. Franceschini, A. Martini, L. Astolfi, Regenerative medicine in hearing recovery, Cytotherapy 19 (2017) 909–915.
[21] Z. Yue, X. Liu, P.J. Molino, G.G. Wallace, Bio-functionalisation of polydimethylsiloxane with hyaluronic acid and hyaluronic acid-collagen conjugate for neural interfacing, Biomaterials 32 (2011) 4714–4724.
[22] K.B. Anderson, S.T. Halpin, A.S. Johnson, R.S. Martin, D.M. Spence, Integration of multiple components in polystyrenebased microfluidic devices part II: cellular analysis, Analyst 138 (2013) 137–143.
[23] J.M. Łopacińska, J. Emnéus, M. Dufva, Poly(dimethylsiloxane) (PDMS) affects gene expression in PC12 cells differentiating into neuronal-like cells, PLoS One 8 (2013) e53107.
[24] L. Astolfi, E. Simoni, A. Martini, OC-k3 cells, an *in vitro* model for cochlear implant

biocompatibility, Hearing Balance Commun. 13 (2015) 166–174.
[25] C. Batich, D. DePalma, J. Marotta, G. Latorre, Silicone degradation reactions, in: M. Potter, N.R. Rose (Eds.), Immunology of Silicones. Curr. Top. Microbiol. Immunol. vol 210, Springer, Berlin, Heidelberg, 1996.
[26] K.P. Das, T.M. Freudenrich, W.R. Mundy, Assessment of PC12 cell differentiation and neurite growth: a comparison of morphological and neurochemical measures, Neurotoxicol. Teratol. 26 (2004) 397–406.
[27] R.N. Palchesko, L. Zhang, Y. Sun, A.W. Feinberg, Development of polydimethylsiloxane substrates with tunable elastic modulus to study cell mechanobiology in muscle and nerve, PLoS One 7 (2012) e51499.
[28] C. Garnis, T.P. Buys, W.L. Lam, Genetic alteration and gene expression modulation during cancer progression, Mol. Cancer 3 (2004) 9.
[29] S.F. Tadros, M. D'Souza, X. Zhu, R.D. Frisina, Apoptosis-related genes change their expression with age and hearing loss in the mouse cochlea, Apoptosis 13 (2008) 1303–1321.
[30] Y. Cui, R.S. Paules, Use of transcriptomics in understanding mechanisms of drug-induced toxicity, Pharmacogenomics 11 (2010) 573–585.
[31] M. Liu, S. Gu, Y. Zhou, Cell morphology modified by biomaterial induces miR-21 expression and apoptosis, J. Biosci. Med. 4 (2016) 42–48.
[32] K.N. Hoang, C.T. Dinh, E. Bas, S. Chen, A.A. Eshraghi, T.R. Van De Water, Dexamethasone treatment of naïve organ of Corti explants alters the expression pattern of apoptosis-related genes, Brain Res. 1301 (2009) 1–8.
[33] R.J. Youle, A. Strasser, The BCL-2 protein family: opposing activities that mediate cell death, Nat. Rev. Mol. Cell Biol. 9 (2008) 47–59.
[34] D. Westphal, G. Dewson, P.E. Czabotar, R.M. Kluck, Molecular biology of Bax and Bak activation and action, Biochim. Biophys. Acta 1813 (2011) 521–531.
[35] J.S. Fridman, S.W. Lowe, Control of apoptosis by p53, Oncogene 22 (2003) 9030–9040.