

Is polyazulene cytocompatible? The synthesis method matters

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ABSTRACT

Polyazulene is an underrated conductive polymer characterized by its unique building blocks involving a fused-ring structure with an intrinsic dipole moment. It has been studied with a focus on the development of organic electronics, such as photovoltaic cells. However, the biological properties of polyazulene have not been reported to date. This study, the first of its kind, not only describes the properties of electrochemically and chemically synthesised polyazulenes and so-called true polyazulene, but also characterises their cytocompatibility. The physico-chemical properties of polyazulene powders and films were characterized by FTIR, Raman and UV-Vis spectroscopy, profilometry, scanning electron microscopy, voltammetry, van der Pauw conductivity measurement, and thermogravimetric analysis. Their cytocompatibility was explored using NIH/3T3 mouse embryonic fibroblasts, HaCaT human keratinocytes, and ES R1 mouse embryonic stem cells. Our results indicate that the tested polyazulenes exhibit an overall favourable short-term *in vitro* cytocompatibility with limitations varying according to the synthesis method and conditions. Electrochemically synthesized polyazulene formed continuous films with moderate conductivity under optimized deposition conditions, whereas chemically prepared films were inhomogeneous for reliable conductivity measurements. These differences in conductivity therefore primarily reflect variation in film morphology and deposition conditions rather than intrinsic bulk electronic properties of the respective polyazulene types.

1. Introduction

The successful application of polymers in medicine and biological research, especially tissue engineering, relies on an understanding of their interactions with cells. These material/cell interactions are strongly influenced by material properties with notable advantages provided by conductivity. Conductive scaffolds mimic the native conductivity of tissues and promote cell–cell communication, thus improving tissue integration and functionality in electrosensitive tissues such as cardiac or neural tissue. They also provide a platform for the controlled electrical stimulation, guiding cell behavior, enhancing growth and migration, accelerating nerve regeneration, and promoting cardiomyogenesis [1–4].

Conductive polymers have gained significant attention due to their unique combination of electron- and ion-based conductivity coupled

with low density and easy processability, features which metals lack. Conductive polymers are characterized by their conjugated π -bonds and doping ions, allowing them to switch between various redox states in response to environmental conditions. Given such versatility, most conductive polymers such as polypyrrole and polyaniline have been intensively studied and used in various fields of interest, including biomedicine. Contrary, polyazulene (PAz) has received much less attention and is largely overlooked up to this day.

The monomer used for PAz synthesis is azulene (Az), the bicyclo [5.3.0]decapentaene shown in Fig. 1. Az is an aromatic hydrocarbon, an isomer of naphthalene, containing 5- and 7-membered condensed rings. It has a characteristic dark blue color and specific physico-chemical properties, thanks to which Az and its derivatives are widely used in optoelectronic devices [5,6], solar cells [7,8], and sensors [9]. Regarding biological properties of Az and its derivatives, phototoxicity

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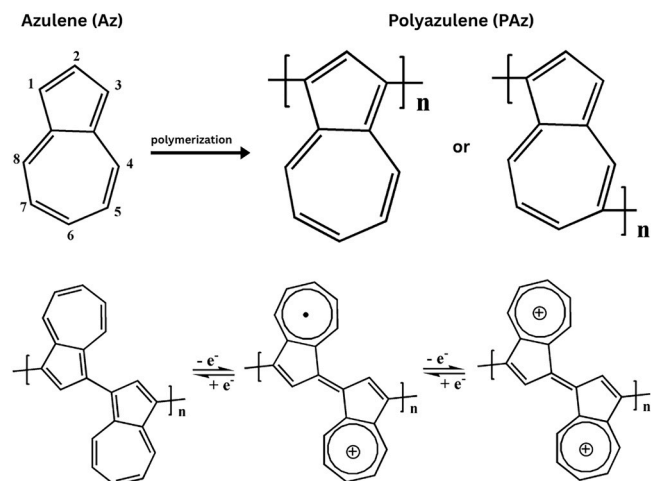


Fig. 1. Chemical structure of Az, PAz with 1,3 bonding, PAz with 1,5 bonding, and below, redox reactions of PAz.

[10,11], anti-inflammatory activity [12], antidiabetic activity [13], and also antineoplastic activity [14] have been studied [15,16].

Before starting any polymerization of Az, three facts must be considered as they may strongly influence PAz synthesis. First, the resonance delocalization of electrons in Az creates the electron-rich positions 1, 3, 5, and 7 that rather willingly react with electrophiles. The electron-poor positions 2, 4, 6, and 8, on the other hand, react more easily with nucleophiles [17,18]. Besides, the 7-membered ring carries a partial positive charge while the 5-membered ring carries a partial negative charge, which leads to a relatively large dipole moment of 1.08 D [19]. Last but not least, Az can be oxidized to an azulenylium carbocation, which is about 10 kcal mol⁻¹ more stable than Az and exhibits a strong electron-donating feature [17,20].

The most common approach to Az polymerization is electrochemical synthesis, usually carried out in tetrabutylammonium hexafluorophosphate [20–23] or tetrabutylammonium perchlorate electrolyte salts [24] dissolved in acetonitrile [20–22,24] or deoxygenated and distilled benzonitrile [23], resulting in PAz shown in Fig. 1. Additionally, PAz has also been prepared by a chemical polymerization of brominated or iodinated Az derivatives [21,25–28]. In an unique study, Grądzka et al. [29] reported on PAz synthesis by a chemical reaction of Az with an oxidizing agent, iron(III) chloride, in different solvents, namely water, ethanol, acetonitrile, dichloromethane, propylene carbonate, and dimethylformamide. The final PAz products were obtained in the form of a black powder or liquid [29].

Another unique study was carried out by Kihara et al. [30], who claimed that conventional PAz should be correctly referred to as polyazulenylene or dehydropolyazulene, and presented a cationic polymerization of Az resulting in the “true polyazulene” (truePAz) shown in Fig. 2. This synthesis was based on dissolving Az in heated trifluoroacetic acid, followed by precipitation in methanol with triethylamine. The obtained brown powder contained 71 mol. % of truePAz and 29 mol. % of truePAz–acetate complex [30].

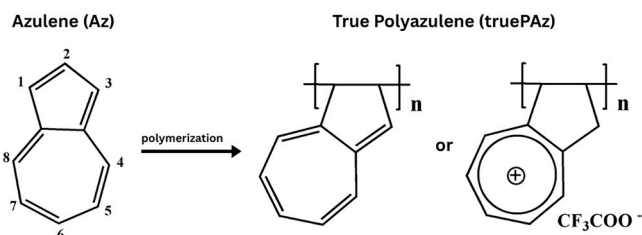


Fig. 2. Chemical structure of Az, truePAz, and truePAz–acetate complex.

Both PAz and truePAz have been reported as conductive. The conductivity of neat untreated PAz is, however, very low, from $< 10^{-11}$ to 10^{-1} S/cm. One strategy to increase the conductivity is by blocking 1,5 bonding in PAz. This may be achieved by substituting a bulky alkyl at the 5- or 6-position of the Az ring. This leads to strict 1,3 bonding in PAz, providing a planar configuration with a long conjugated chain. The conductivity of poly(5-isopropylazulene) can thus reach 7.3 S/cm [23]. The conductivity of PAz may also be enhanced after polymerization. For example, the exposure of untreated PAz to oxygen increases the conductivity up to 0.6 S/cm [26], which is attributed to protonation into various redox states [20]. In addition, PAz conductivity can be increased to 1.2 S/cm by exposure to iodine vapor [26]. The same approach can also be applied to truePAz, whose conductivity can thus be increased from approximately 10^{-8} S/cm to 10^{-3} S/cm [30]. Also, PAz can be made conductive by chemical or electrochemical doping. The introduction of charge carriers modifies the electron distribution, which also modifies the geometry of the conjugated backbone, as reflected in infrared and Raman spectra [31].

The method used to synthesise PAz and its particular conditions, as well as post-synthesis treatment, can all strongly affect PAz's chemical and physical properties, such as conductivity or its optical properties; however, they may also influence its biological properties. To the best of the authors' knowledge, no previous studies have reported on the biological testing of PAz. We hypothesized that, with respect to biological applications, hitherto overlooked PAz could be prepared and used not only as powders but also as conductive coatings that may exhibit cell-controlling properties. Our aim was to verify this hypothesis not only with respect to the form of the prepared PAz but also concerning to the method of its preparation.

In the present study, PAz powders and films were synthesized by electrochemical polymerization (PAz^{EP}) as reported in [32], and by chemical oxidative polymerization (PAz^{CHP}) as reported in [29], and the PAz precursor truePAz was prepared by cationic polymerization as reported in [30]. The physico-chemical properties of the PAz samples were then determined; however, the main novelty and strength of this study lie in its assessment of PAz's cytocompatibility. This study not only extends current knowledge on the material properties of PAz but, for the first time, brings completely new information on its effect on cellular behavior.

2. Materials and methods

2.1. Electrochemical synthesis of polyazulene

Powders: Electrochemically-synthesized PAz powders (PAz^{EP-P}) were prepared according to Österholm et al. (2008) [32]. An acetonitrile-based solution of Az (Sigma-Aldrich, USA) at a concentration of 5 mM with tetrabutylammonium hexafluorophosphate (Sigma-Aldrich, USA) at a concentration of 0.1 M was prepared. After the solution was purged with argon, electrochemical polymerization was carried out in a conventional three-electrode, one-compartment cell with a silver wire as the reference electrode (Ag RE) calibrated against ferrocene/ferrocenium (Fc/Fc⁺), a coiled platinum wire as a counter electrode (Pt CE), and a platinum wire as the working electrode (Pt WE) at temperature 24 ± 1 °C. It was only possible to produce PAz^{EP-P} in minimal quantities insufficient for a comprehensive characterization of its physico-chemical and biological properties.

Films: The preparation of electrochemically-synthesized PAz films (PAz^{EP-F}) followed the same synthesis used for PAz^{EP-P}, with the difference that indium-tin-oxide (ITO) glass (Biotain Hong Kong CO.) was used instead of Pt WE.

2.2. Chemical synthesis of polyazulene

Powders: Chemically-synthesized PAz powders (PAz^{CHP-P}) were prepared according to Grądzka et al. (2018) [29]. Ethanol (BC

Chemservis, Czech Republic) solutions of Az (Sigma-Aldrich, USA) at a concentration of 8.5 mM, and of the oxidizing agent anhydrous iron(III) chloride (Sigma-Aldrich, USA) at an equilibrium-exceeding concentration of 61.6 mM were prepared. The solutions were mixed and the polymerization reaction was proceeded for 24 h at 20 ± 1 °C while under slow shaking. Subsequently, the suspension was vacuum filtered while being thoroughly rinsed with 0.2 M hydrochloric acid (Penta Chemicals, Czech Republic) and methanol (Penta Chemicals, Czech Republic). The prepared PAz^{CHP-P} was then left to dry overnight in a vacuum evaporator at 20 °C.

Films: The preparation of PAz films (PAz^{CHP-F}) followed the same synthesis used for PAz^{CHP-P}. The only difference was the presence of ITO glass in the reaction mixture, which was not shaken during the reaction. After 24 h of this *in-situ* polymerization, PAz^{CHP-F} on the surface of ITO glass was gently rinsed and dried under the same conditions as PAz^{CHP-P}.

2.3. Synthesis of true polyazulene

Powders: Chemically synthesized truePAz powders (truePAz^P) were prepared according to Kihara et al. [30]. First, the monomer Az was dissolved in trifluoroacetic acid (Sigma-Aldrich, USA) at a concentration of 0.37 M and allowed to react under oxygen-free conditions at 60 °C and constantly stirred for 24 h. After this time, the reaction mixture was poured into methanol containing the same volume of triethylamine (Sigma-Aldrich, USA) as the volume of trifluoroacetic acid used beforehand. The precipitated suspension was then vacuum filtered while being thoroughly rinsed with methanol. The powders were dried overnight in a vacuum evaporator at 20 °C.

Films: Films of truePAz (truePAz^F) were prepared by dissolving the prepared truePAz^P in dichloromethane (Sigma-Aldrich, USA), followed by immediate casting on ITO glass. After solvent evaporation, truePAz^F on the surface of ITO glass was lightly rinsed and dried under the same conditions as truePAz^P.

All PAz powders and films (PAz^{EP}, PAz^{CHP}, truePAz) formed solids that were insoluble in water, phosphate-buffered saline and cell culture media, and only sparingly soluble or dispersible in selected organic solvents, in agreement with previous reports on chemically and electrochemically synthesized PAz [30,33–37].

2.4. Characterization of physico-chemical properties

Voltammetry: Differential pulse voltammetry (DPV) of Az oxidation was carried out using an Eco Chemie AutolabPGSTAT128n potentiostat (Metrohm AG) and Pt WE in a scan speed of 100 mV/s and over the potential range from 0 V to 2.2 V. Cyclic voltammetry (CV) of Az oxidation was processed using the same device and either Pt WE or ITO glass under various conditions: scan speed – 50, 100, 150, 200 mV/s; number of scans – 6, 14 or 26 steps (3, 7 or 13 cycles); potential range – from 0.01 V to 1.15, 1.55 or 1.95 V. All measurements were performed at 24 ± 1 °C.

UV-Vis spectroscopy: UV-Vis spectra of PAz^{EP-F} deposited on ITO glass were measured using a HITACHI U-2900 spectrophotometer with a special attachment for testing thin layers. The same instrument was also used for determining the UV-Vis spectra of the Az solution in acetonitrile, for which a 1 cm quartz cell was used.

FTIR spectroscopy: ATR FTIR spectra of powdered Az, PAz^{CHP-P} deposited on filtration paper, and PAz^{EP-F} deposited on ITO were obtained with a FTIR mikroskop Nicolet iN10 MX instrument using an MCT-A nitrogen-cooled detector in the region 4000–650 cm⁻¹ with a maximum spectral resolution of 0.4 cm⁻¹. The microATR technique with a spatial resolution of 25 µm was used. The ATR FTIR spectrum of powdered truePAz^P was analysed using a Nicolet 6700 spectrometer (Thermo-Nicolet, USA) equipped with a reflective GladiATR ATR extension (PIKE Technologies, USA) with a diamond crystal. Spectra were recorded in the 4000–400 cm⁻¹ region with a DLATGS (Deuterated L-alanine doped triglycine sulfate) detector at a resolution of 4 cm⁻¹,

using 64 scans and Happ-Genzel apodization. The spectra were corrected for carbon dioxide and humidity in the optical path.

Raman spectroscopy: Raman spectra of powdered Az, PAz^{CHP-P} deposited on filtration paper and PAz^{EP-F} deposited on ITO were collected using a Thermo Scientific DXR Raman microscope equipped with a 780 nm line. The spot size of the lasers was focused by a 50 × objective. The scattered light was analyzed by a spectrograph with a holographic grating with 400 lines per mm and a pinhole width of 50 µm. The acquisition time was 10 s with 10 repetitions.

Water contact angle: Static water contact angles were measured to assess the surface wettability of the PAz^{EP-F}, PAz^{CHP-F} and truePAz^F films. A droplet of deionized water (5 µL) was gently placed on the film surface at room temperature and imaged using a SEE system (Advex Instrument, Czech Republic). Contact angles were evaluated by fitting the droplet profile using the instrument software. For each film type, at least five independent droplets on different positions of three samples were analysed and average values ± standard deviation were reported.

Thermogravimetric analysis (TGA): Az and PAz powders underwent TGA on a Setaram LabSys Evo instrument equipped with a TG/DSC sensor in an air atmosphere (airflow 60 cm³min⁻¹), under a heating regime of 5 °C min⁻¹, and over a temperature range of 30–1000 °C.

Diffraction: Powder XRD patterns of TGA residues of PAz^{CHP-P} were recorded on a Rigaku MiniFlex 600 diffractometer equipped with a CoKα (λ = 1.7903 Å) X-ray tube (40 kV, 15 mA). Data processing was performed with Rigaku PDXL2 software.

Scanning electron microscopy (SEM): Surface images of ITO glass and PAz films were obtained by SEM using a Phenom XL G2 instrument (Thermo Fisher Scientific). Prior to imaging, the samples were sputter-coated with a gold and palladium layer in a Quorum SCSC7620 Mini sputter-coater (Quorum Technologies) in an argon atmosphere for 30 s to increase sample stability for analysis. Samples were analyzed at an acceleration voltage of 10 kV using a backscattered electron detector in Image mode.

Profilometry: The thickness of the PAz films was measured using a Dektak XT mechanical profilometer (Bruker) with a diamond tip with a radius of curvature of 2.5 µm and applying a pressure of 5 mg. The Rz and Rt parameters were calculated on the basis of ISO standard 4287. The average values of the films' thickness and mean standard deviations were determined from triplicates. For each sample, one representative profile was selected for graphical representation.

Conductivity: The conductivities of PAz films deposited on ITO glass substrates were determined by the four-point van der Pauw method. A Keithley 6517B electrometer, a Keithley 2410 source meter, and a Keithley 7002 switch (USA) were used at room temperature.

2.5. Characterization of biological properties

Cell lines and culture media: Three cell lines were used to fully characterise the cytocompatibility of PAz. First, the NIH/3T3 mouse embryonic fibroblast cell line (ECACC 93061524, England) was used. Dulbecco's modified Eagle's medium (DMEM) (BioSera, France) and sodium hydrogen carbonate (Penta, Czech Republic) were used as the basis for the fibroblast culture medium. Calf serum (BioSera, France) (at a concentration of 10% of the total volume of the medium) and the antibiotics penicillin/streptomycin (BioSera, France) at a concentration of 100 µg/mL (at a concentration of 1% of the total volume of the medium together with the serum) were then added to the DMEM.

Second, the HaCaT human keratinocyte cell line was used [38]. RPMI medium 1640 with L-glutamin (Gibco™, USA) and sodium hydrogen carbonate were used as the basis for the keratinocyte culture medium, which was enriched with fetal bovine serum (Gibco™, USA) (at a concentration of 10% of the total volume of the medium) and the antibiotics penicillin/streptomycin at a concentration of 100 µg/mL (at a concentration of 1% of the total volume of the medium together with the serum).

The third cell line used was the ES R1 mouse embryonic stem cell line

[39]. A DMEM-based culture medium (Gibco™, USA) was used to culture embryonic stem cells in an undifferentiated state. The following supplements were added to the DMEM: fetal bovine serum (at a concentration of 16.5% of the total volume of the medium), the antibiotics penicillin/streptomycin at a concentration of 100 µg/mL (at a concentration of 1% of the total volume of the medium together with the serum), 100 mM non-essential amino acids (Gibco™, USA), 0.05 mM beta-mercaptoethanol (Sigma-Aldrich, USA) and leukaemia inhibitory factor (LIF) (Chemicon, USA) at a concentration of 5 ng/mL.

All the used cell lines were cultured in a HERAcell 150i biological incubator (Thermo Scientific, USA) with a controlled atmosphere of 5% CO₂, a temperature of 37 °C, and constant relative humidity.

Extraction and MTT assay: Extracts of PAz powders (PAz^{CHP-P}, and truePAz^P) were prepared according to ISO standard 10993-12 with a defined mass-to-volume extraction ratio modified from 0.2 g/mL (required by the standard) to 3 mg/mL (diluted 66.7 times). Due to insufficient quantity of PAz^{EP-P} available for this experiment, the extraction was done from PAz^{EP-F} with a defined surface-to-volume ratio extraction ratio, which was adjusted in the same manner, specifically from 6 cm²/mL (required by the standard) to 0.09 cm²/mL (diluted 66.7 times) in order to obtain comparable data. The extraction was processed in the culture medium for 24 h at 37 °C under shaking. The parent extracts were diluted in the culture medium to obtain a series of dilutions: 75, 50, and 25%. All extracts were filtered before use (up to 24 h). Fibroblasts at a concentration of 1 × 10⁵ cells per 1 mL of the culture medium were seeded in 96-well plates for 24 h. Then, the medium was aspirated and replaced by the series of diluted extracts of Az, PAz^{CHP-P}, truePAz^P, and PAz^{EP-F}. Extract-free culture medium was used as references. Each experiment was conducted in at least five independent replicates. After 24 h, the extracts were removed and replaced with the culture medium and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (Duchefa Biochemie, Netherlands) solution in ultrapure water (5 mg/mL) in a ratio of 9:1. After at least 4 h, the content was replaced with dimethylsulfoxide (Duchefa Biochemie, Netherlands) and the absorbance values were measured on a NanoQuant infinite M200PRO spectrophotometer (Tecan, Switzerland) at 570 nm and with a reference wavelength of 690 nm. The results are expressed as a percentage reduction in cell viability relatively to the reference cells.

Cell adhesion and proliferation: Before use, PAz^{EP-F}, PAz^{CHP-F}, and truePAz^F were sterilized before use by rinsing in ethanol followed by 30 min of UV exposure. Fibroblasts, keratinocytes, or embryonic stem cells at a concentration of 1 × 10⁵ cells/mL in complete culture medium were seeded onto the surfaces of the PAz films and references (cell culture dishes). For embryonic stem cells, all tested substrates were coated with aqueous 0.1% gelatin from bovine skin (Sigma-Aldrich, USA). The samples with cell suspensions were then incubated for 4 d with medium being changed every 2 d. Cell morphology was determined using fluorescence microscopy. Cells grown on the films were fixed with aqueous 4% formaldehyde (Penta Chemicals, Czech Republic) and permeabilized with 0.5% Triton X-100 (Sigma-Aldrich, USA) in phosphate-buffered saline. Two fluorescent dyes were used for staining: 1) ActinRed 555 or ActinGreen 488 (Life Technologies, USA) (1 drop/mL), which bind to proteins in the cytoskeleton, 2) and Hoechst 33258 (Sigma-Aldrich, USA) (20 µL/mL), which penetrates the nuclei where it binds to DNA. The stained cells were observed using an Olympus IX 81 phase-contrast inverted fluorescence microscope (Olympus, Japan) with a Leica DFC480 camera (Leica Microsystems, Japan).

Cell image analysis: Fluorescence microscopy photographs of cells cultured on the PAz^{EP-F}, PAz^{CHP-F}, truePAz^F, and also on the reference, taken at 100 × magnification, were analyzed for further morphology qualification. Image analysis was performed using the open-access software CellProfiler 4.0.7 [40]. The analyzed morphological factors included area and perimeter. Area is expressed as the number of pixels in the region of interest (a cell or a nucleus). The perimeter represents the number of pixels around the boundary of the region of interest (a cell or a nucleus).

3. Results and discussion

3.1. Electrochemical polymerization of azulene leads to homogenous conducting films

The electrochemical polymerization of Az, resulting in PAz^{EP-P}, was conducted in a conventional three-electrode, one-compartment cell with Pt CE, Ag RE calibrated versus Fc/Fc⁺, and Pt WE. During Az oxidation on Pt WE using the DPV method (Fig. 3A), three distinct oxidation peaks were identified, these corresponding to Az's transformation into electroactive intermediates. The CV method shown in Fig. 3B illustrates on these processes by mapping the oxidation steps across varying potential ranges. The first potential range (up to 1.15 V) highlights the initial oxidation step, which likely forms azulenylium cations that initiates the polymerization process, resulting in the formation of PAz^{EP} on the Pt WE surface [24].

The deposition of PAz^{EP-F} on ITO glass was performed and studied using the CV method over a potential range from 0.01 V to 1.15 V. In Fig. 4A, the first cycle shows only one peak at 0.52 V of the corrected potential related to the Az oxidation. The second and third cycles reveal two other peaks: an anodic peak at 0.07 V and a cathodic peak at -0.04 V of the corrected potential. This suggests successful nucleation and polymer growth on the electrode surface [24]. Fig. 4B clearly shows a stable current density increase with more cycles applied, implying that a steady-state polymerization phase was reached. The blue line in Fig. 4B was obtained using a monomer-free solution (only acetonitrile with electrolyte without the monomer Az). The negligible change in current confirms that the previously-observed current changes were caused by polymer formation rather than electrolyte decomposition. In Supplementary material, Figure S1 shows more CV graphs with PAz^{EP-F} polymerization performed under different conditions.

The absorbance of PAz^{EP-F} on ITO glass and Az solution in acetonitrile with electrolyte was recorded by UV-Vis spectroscopy. The distinct shift in absorption peaks between Az (Fig. 5A) and PAz^{EP-F} (Fig. 5B) aligns with the formation of a conjugated π -electron system. These shifts indicate successful polymerization and the emergence of conductive properties [32,41]. In the Supplementary material, Figure S2 shows additional UV-Vis spectra of PAz^{EP-F} synthesized at different scan rates.

The four-point Van der Pauw method is commonly used to accurately measure the resistivity of thin films with arbitrary shapes, as it effectively eliminates the influence of contact resistance [42–44]. However, the conductivity calculation from these data strongly depends on the film thickness, which often fluctuates. The thickness of PAz^{EP-F} varied from 0.2 µm to 5.2 µm. The thickness of PAz^{EP-F} was measured by profilometry on the edge of the film formed at the reaction solution level – the thickness of the PAz^{EP-F} was calculated from the height difference between the bare ITO glass (above the solution level) and the ITO glass with deposited PAz^{EP-F} (below the solution level). See Supplementary material Figure S3 for profiles of PAz^{EP-F} on ITO glass prepared under various synthesis conditions. The conductivity measurements of PAz^{EP-F} varied within tens of S/cm, given the differences in synthesis conditions (Table S1). Importantly, these values reflect the behavior of continuous electrochemically grown films with non-uniform thickness and should not be interpreted as intrinsic bulk conductivities of an idealized PAz phase. In contrast, chemically synthesized PAz films (PAz^{CHP-F} and truePAz^F) were structurally discontinuous and therefore showed no detectable conductivity via the four-point van der Pauw method, even though earlier studies have reported low intrinsic conductivity in chemically synthesized PAz [29,30]. Together with the SEM data, this indicates that the apparent conductivity differences between PAz^{EP-F} and chemically formed PAz films are dominated by film continuity and microstructure, in line with observations in literature [37,45–47].

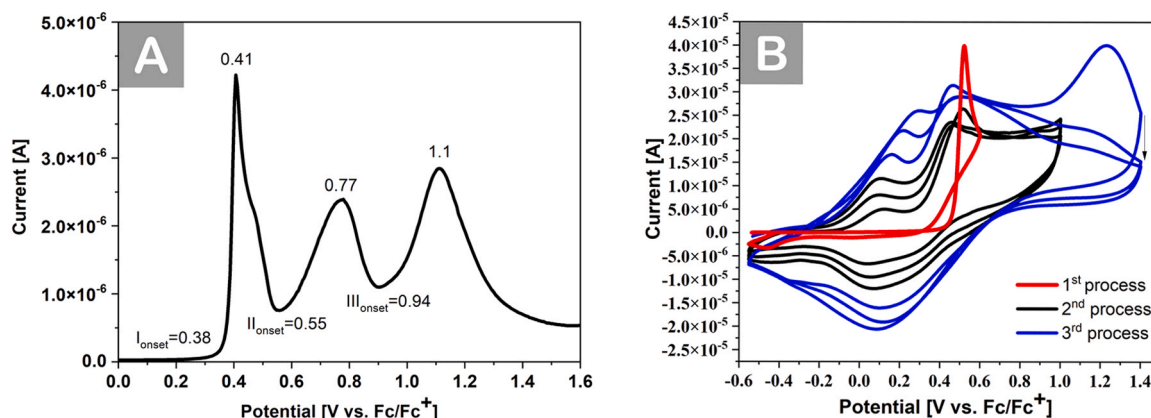


Fig. 3. (A) DPV of Az oxidation and PAz^{EP} polymerization on Pt WE to 2.2 V by 100 mV/s. (B) CV of Az oxidation PAz^{EP} polymerization on Pt WE to 1.15 V (1st process), to 1.55 V (2nd process), to 1.95 V (3rd process) each with 3 cycles.

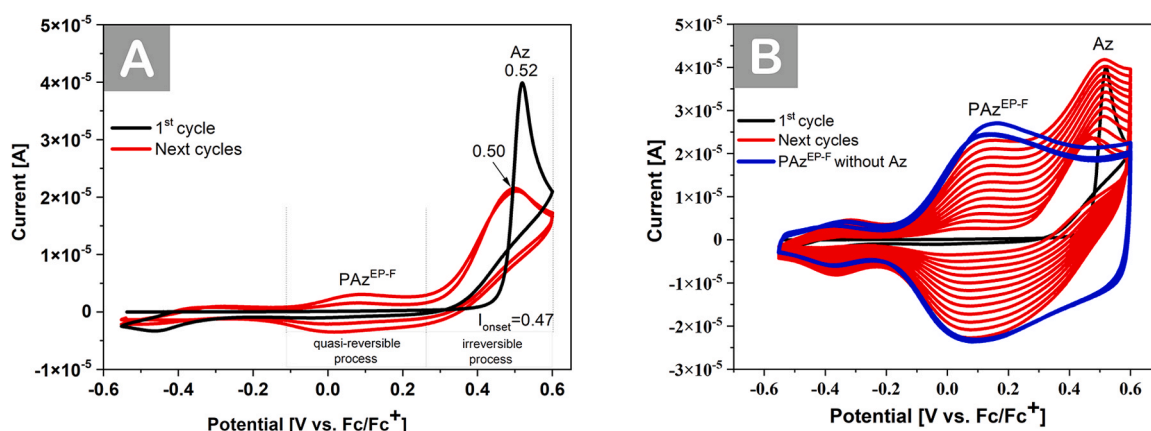


Fig. 4. CV of Az oxidation and PAz^{EP-F} polymerization on ITO glass to 1.15 V by 100 mV/s. (A) 3 cycles, (B) 13 cycles + 3 cycles without the presence of Az.

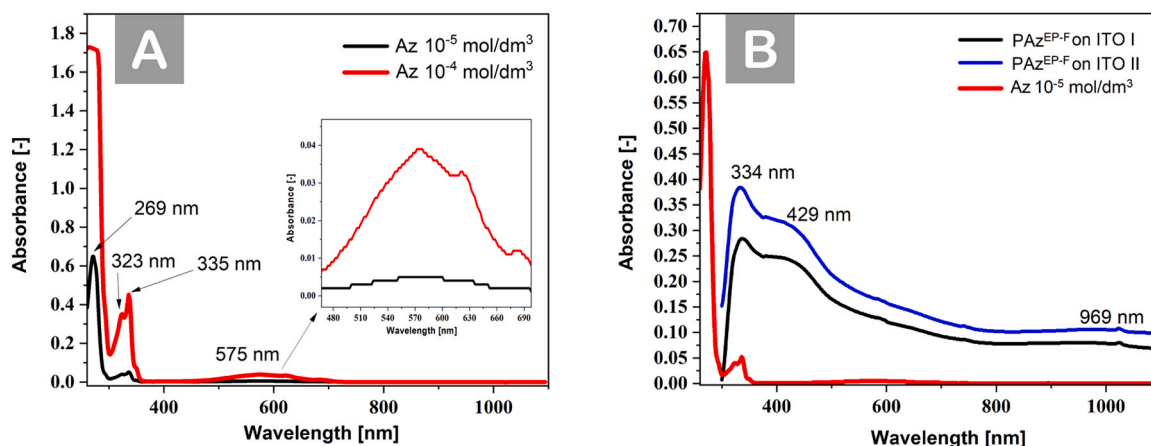


Fig. 5. UV-Vis spectroscopy of (A) Az in concentrations of 0.01 mM and 0.1 mM dissolved in acetonitrile with electrolyte, (B) PAz^{EP-F} on ITO glass (1.15 V, 100 mV/s, 13 cycles).

3.2. Different methods of azulene polymerization yield films with distinct properties

One of the factors that may strongly impact the conductivity of chemically-prepared thin films is structural inconsistency. The SEM analysis (Fig. 6) reveals a disrupted morphology in PAz^{CHP-F} and true-PAz^F, likely caused by drying-induced stresses (Figs. 6C and 6D, respectively). In contrast to these films, the electrochemical

polymerization of PAz^{EP-F} leads to a homogenous film with only slight fluctuations in its thickness (Fig. 6B).

Taking a closer look at PAz^{CHP-F} (Fig. 6C), its particle-based structure is obvious. Round PAz particles smaller than 1 μm aggregate to form a film that is seemingly homogeneous to the naked eye; however, the microscopic analysis shows that the film is structurally discontinuous. Although the individual particles are uniform in size, their aggregates vary considerably. This type of film is often used in polymer thick film

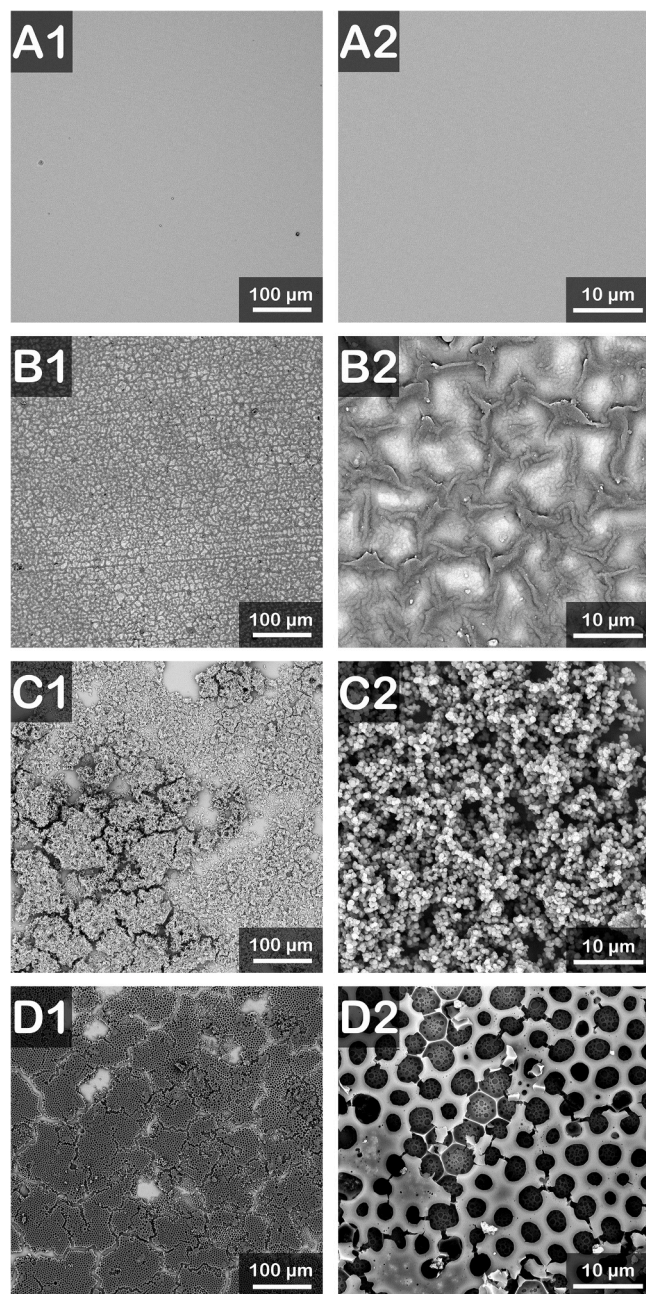


Fig. 6. SEM images of (A) clear ITO glass, (B) PAz^{EP-F} on ITO glass, (C) PAz^{CHP-F} on ITO glass, (D) truePAz^F on ITO glass; (1) Magnification 500 ×, (2) Magnification 6000 ×.

resistor technology [48–50].

Finally, let us consider truePAz^F (Fig. 6D), which self-assembled into a complex hierarchical structure. At the bottom, there are nano- to submicroscopic wells overlaid by a microscale breath-figure pattern of fairly uniform size (around 3 μm) with very thin walls forming hexagonal shapes. Above this is a thin smooth layer with round holes leading into the open spaces of the breath-figure structure. The breath-figure pattern self-assembles during the casting of a polymer solution (truePAz^P in dichloromethane, in this case), relying on the interplay of solvent evaporation, interfacial tension, and water condensation dynamics. When a volatile polymer solution is deposited on a solid surface under humid conditions, rapid solvent evaporation induces evaporative cooling, leading to the condensation of water droplets onto the polymer solution's surface. These droplets spontaneously arrange into a

hexagonally ordered array driven by interfacial energy minimization. Fundamental physical phenomena, such as evaporative cooling, interfacial tension differences, and Marangoni flows, contribute to the dynamic evolution on various scales – microscale ordering from the water droplet templating, and nanoscale modifications through the polymer's inherent self-assembly or resulting from the presence of inorganic particles [51–55]. Consequently, the use of hierarchical breath-figure structures represents an adaptable approach to fabricating porous polymer films with multi-scale order, promising applications in various fields, including tissue engineering.

Different methods of synthesising PAz thus result in variability not only in architecture but also in physico-chemical properties. To relate film morphology to surface wettability, we determined the static water contact angles of all three PAz film types. PAz^{EP-F} exhibited a mild hydrophobic character with a contact angle of approximately $70^\circ \pm 16^\circ$. Meanwhile, PAz^{CHP-F} and truePAz^F showed smaller contact angles of approximately $54^\circ \pm 7^\circ$ and $64^\circ \pm 4^\circ$, respectively, lying slightly below the Berg limit of 65° [56,57]. While the mild hydrophobicity of PAz^{EP-F} is consistent with the non-polar, hydrocarbon backbone of PAz, the lower contact angle of PAz^{CHP-F} indicates enhanced surface polarity. This difference arises from the polymerization pathway: chemical oxidative synthesis typically introduces a higher density of structural defects and residual polar functionalities, thereby increasing surface energy and water affinity in comparison to electrochemical synthesis. The behavior of truePAz^F suggests a combined influence of surface chemistry and morphology on the overall wetting properties. Although hierarchical breath-figure structures are present on truePAz^F and would generally be expected to increase apparent hydrophobicity, its intermediate contact angle suggests a competing contribution of surface chemistry [58–60]. These findings suggest that variations in synthesis route and resulting morphology influence interfacial properties, which may in turn play a role in modulating polymer–cell interactions in bio-related applications.

Furthermore, FTIR and Raman spectroscopy data of PAz^{EP-F}, PAz^{CHP-F}, and truePAz^P are shown in Fig. 7. Comparing the obtained spectra of each type of PAz to its monomer Az shows distinct differences supporting the idea of the creation of new bonding in the structure of the oligomer or polymer. A detailed description of the spectroscopy results can be found in the [Supplementary material](#) (SM 1.2).

Here, concisely, the infrared spectra of PAz^{EP-F} deposited on ITO glass (Fig. 7A1 and 7A2) showed the presence of tetrabutylammonium hexafluorophosphate from the electrolyte used in electropolymerization. Also, some newly-created infrared active vibration (IRAV) bands were observed, which indicates that PAz^{EP-F} is in a doped state because the introduction of polarons, bipolarons, or polaron pairs into the polymer disrupts the backbone symmetry. This notion is also supported by Raman spectra (Fig. 7A3) and by their comparison with the Raman spectrum observed by Meana-Esteban et al. [61].

The ATR FTIR spectrum of PAz^{CHP-F} (Fig. 7B1) shows several changes in comparison to Az, such as peaks associated with aromatic ring stretching vibrations, =C–H in-plane deformation vibrations, ring breathing vibrations, and =C–H ring out-of-plane deformation vibrations. These changes support the oxidation reaction of Az and the oligomeric structure of the product. Further, in the Raman spectrum of PAz^{CHP-F} (Fig. 7B2), a fluorescence background and a broadening of the bands of Az support the oxidation reactions. Broadened Az peaks in PAz^{CHP-F} and some new bands confirm aromatic vibrations and new modes of ring vibrations in the oligomeric structure.

In the high wavenumber region of ATR FTIR spectra of truePAz^P (Fig. 7C1 and 7C2), three bands indicating aromatic and aliphatic CH stretching vibrations are broader and some bands are missing in comparison to Az spectra due to new bonding in the oligomer structure. Below 1800 cm^{-1} , new bands appear, linked to COOH vibrations from carboxylate anions and CF₂ vibrations from trifluoroacetic acid. These spectral features suggest that the polymerization product is partially oxidized. The Raman spectrum of truePAz^P (Fig. 7C3) exhibits strong

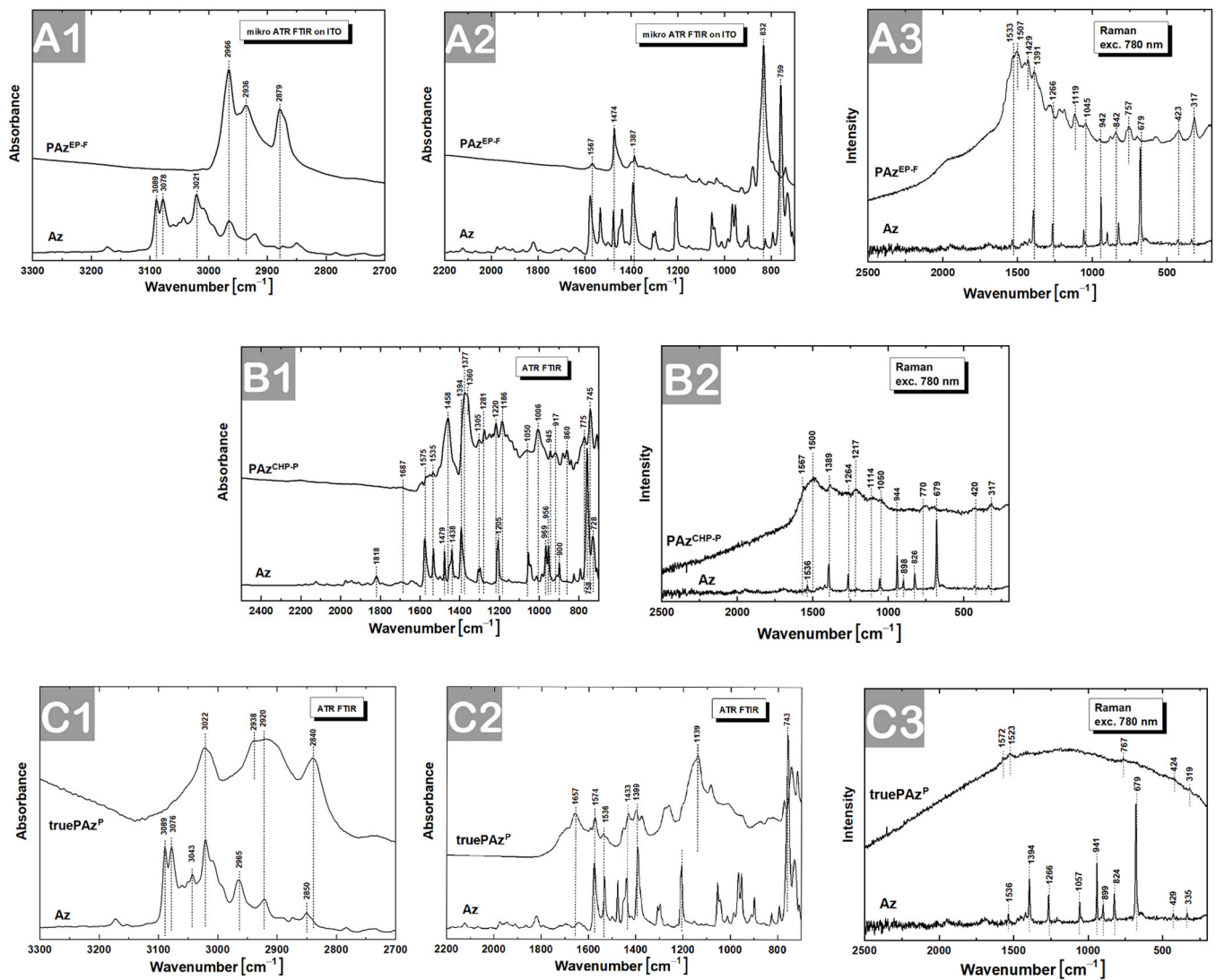


Fig. 7. (A1) FTIR spectra of $\text{PAz}^{\text{EP-F}}$ and Az in the wavenumber range $2700\text{--}3300\text{ cm}^{-1}$, (A2) FTIR spectra of $\text{PAz}^{\text{EP-F}}$ and Az in the wavenumber range $700\text{--}2200\text{ cm}^{-1}$, (A3) Raman spectra of $\text{PAz}^{\text{EP-F}}$ and Az in the wavenumber range $200\text{--}2500\text{ cm}^{-1}$; (B1) FTIR spectra of $\text{PAz}^{\text{CHP-P}}$ and Az in the wavenumber range $700\text{--}2500\text{ cm}^{-1}$, (B2) Raman spectra of $\text{PAz}^{\text{CHP-P}}$ and Az in the wavenumber range $200\text{--}2500\text{ cm}^{-1}$; (C1) FTIR spectra of $\text{truePAz}^{\text{P}}$ and Az in the wavenumber range $2700\text{--}3300\text{ cm}^{-1}$, (C2) FTIR spectra of $\text{truePAz}^{\text{P}}$ and Az in the wavenumber range $700\text{--}2200\text{ cm}^{-1}$, (C3) Raman spectra of $\text{truePAz}^{\text{P}}$ and Az in the wavenumber range $700\text{--}2500\text{ cm}^{-1}$.

fluorescence with only very weak bands observable with the excitation laser.

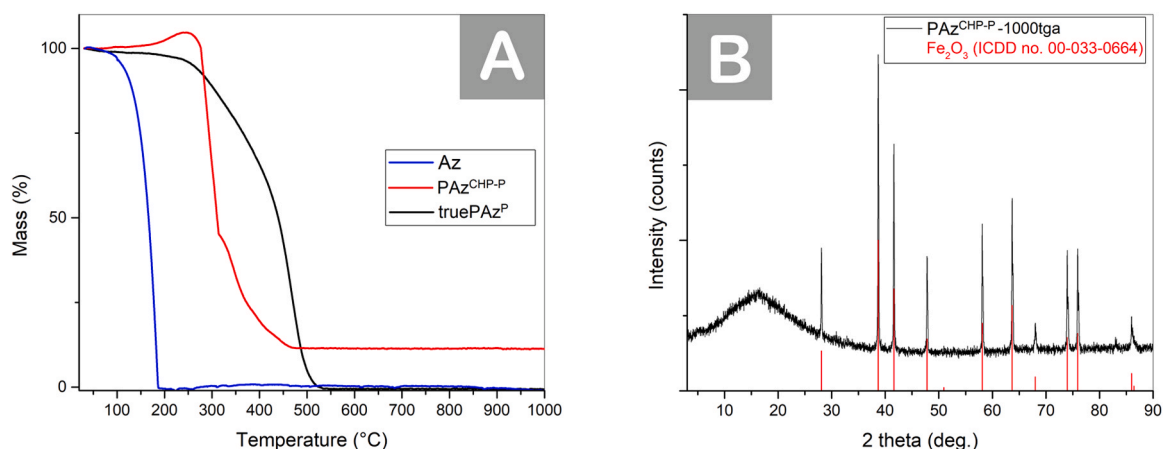


Fig. 8. (A) TGA of Az, $\text{PAz}^{\text{CHP-P}}$, and $\text{truePAz}^{\text{P}}$. (B) Diffractometry of TGA residues of $\text{PAz}^{\text{CHP-P}}$ compared to hematite.

PAz powders were also analyzed with TGA. The results (Fig. 8A) show distinct thermal decomposition behavior on the part of Az, PAz^{CHP-P}, and truePAz^P. The residue of PAz^{CHP-P} after TGA, identified as hematite via diffractometry (Fig. 8B), suggests that iron residues from the oxidizing agent remained within the polymer matrix. Residual catalysts may affect the thermal and electrical properties of conductive polymers. However, PAz^{CHP-P} had been thoroughly washed to remove residual iron(III) chloride, raising the question of the possibility of iron binding to the cyclopentadiene rings in the Az units of PAz to form ferrocene.

3.3. Residual impurities of electrochemical synthesis cause biphasic cytotoxicity

The MTT assay results provide insightful data regarding the cytotoxicity of extracts prepared from Az, PAz^{CHP-P}, truePAz^P, and PAz^{EP-F} in different concentrations according to ISO 10993-5, thereby probing the effects of leachable species.

As shown in Fig. 9A, Az extracts exhibited a slightly downward but statistically insignificant trend in relative cell viability, with a sharp drop down to a value of 0.51 for the undiluted Az extract. This suggests that high concentrations of Az are cytotoxic and thus significantly detrimental to cell survival. It is important to consider the cytotoxic effect of monomers, as polymers often contain residual monomers and other reactants and additives used during the polymerization. Similar dose-dependent cytotoxic effects have been observed in studies on

polyaniline and polypyrrole, where residual dopants and low-molecular-weight impurities were shown to play a critical role in the cytotoxic potential of these conductive polymers [62,63]. These findings thus underscores the necessity of optimizing electrochemical deposition methods to minimize the presence of toxic residuals while preserving the polymer's bioactive properties.

The Fig. 9B shows the results recorded on cells treated with for PAz^{EP-F} extracts, which exhibit a biphasic effect. Cell viability increased at 25% extract (specifically, the relative cell viability was 1.28) and dropped significantly to 0.44 at 100% extract. One plausible explanation is the extraction of residual chemicals from PAz^{EP-F}, such as tetrabutylammonium hexafluorophosphate, whose presence was confirmed by FTIR spectra. Similar biphasic responses have been described for low concentrations of inorganic species, where sub-toxic levels of residual chemicals stimulate cellular defense mechanisms and proliferation [64, 65]. However, our study did not directly measure stress markers, so this mechanism should be regarded as a hypothesis consistent with the literature. Previous work on conductive polymers reported predominantly monotonic, concentration-dependent increases in cytotoxicity [62,66,67].

Fig. 9C, representing the results of PAz^{CHP-P} extracts, shows that the relative cell viability decreased slightly to 0.90 at 25% extract concentration. However, as the concentration increased, cell viability returned to the reference level until the parent extract of PAz^{CHP-P} was used. The 100% concentrated extract reduced relative cell viability to observable but not statistically significant extent.

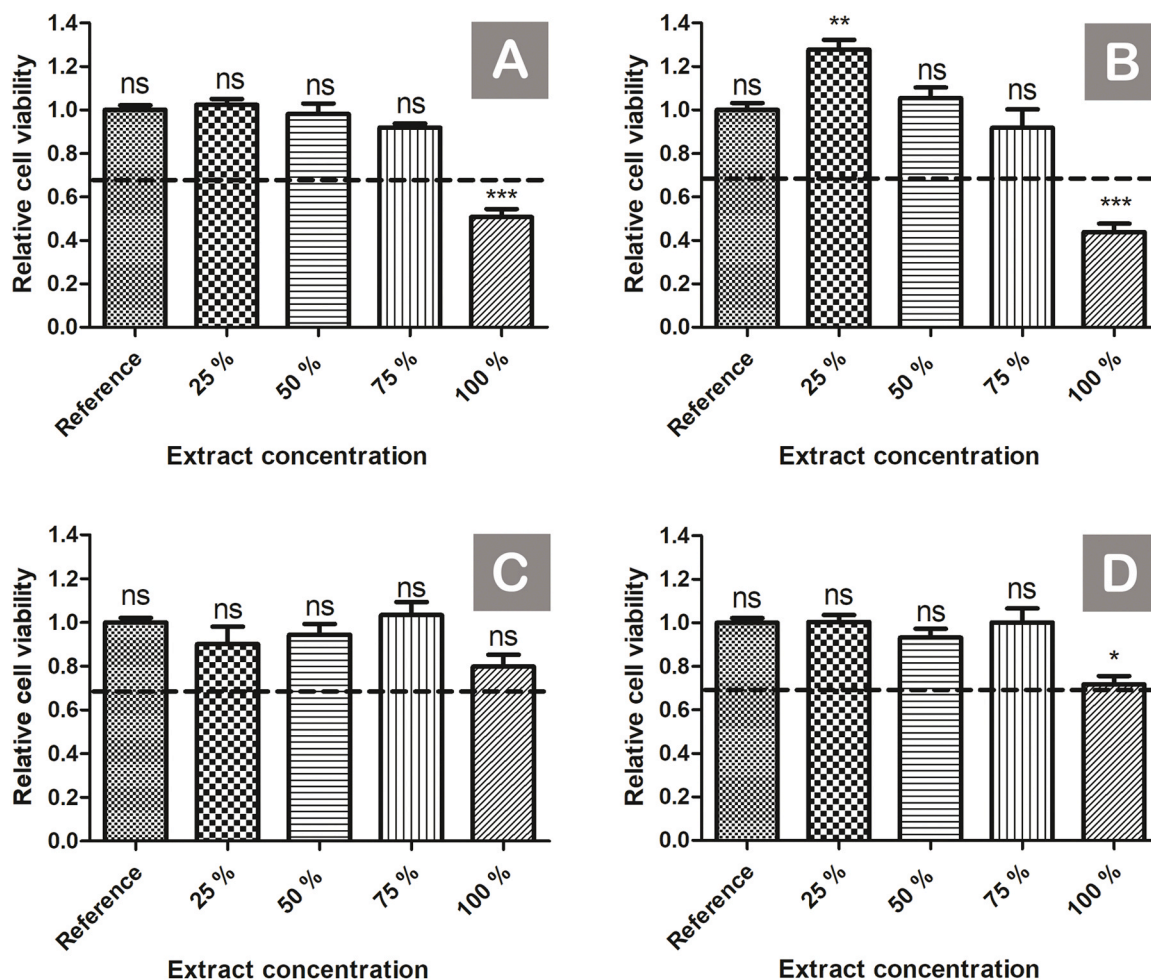


Fig. 9. MTT assay graphs of extracts prepared from (A) Az, (B) PAz^{EP-F} on ITO glass, (C) PAz^{CHP-P}, (D) truePAz^P. Reference cells were cultivated in culture medium containing no extract. Error bars show standard error of the mean. One-way ANOVA with post-hoc Tukey's test: ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

In the fourth graph (Fig. 9D), truePAz^P extracts at concentrations up to 75% maintain the relative cell viability close to the value 1. At 100% concentration, the cell viability drops to the cytotoxicity threshold value of 0.7. This suggests that non-diluted extract of truePAz^P may induce mild cytotoxic.

Since both PAz^{CHP-P} and truePAz^P were powders that underwent thorough purification, their overall better cytocompatibility compared to the biphasic response of PAz^{EP-F} suggests that residual synthesis by-products in PAz^{EP-F} significantly influence its cytotoxicity. Comparable findings have been reported in studies on polyaniline cryogels, where processing conditions drastically affected biocompatibility, and optimized fabrication steps reduced cytotoxicity [68,69].

Taken together, the extract-based MTT assay indicates that all three PAz types exhibit acceptable short-term cytotoxicity profiles under the tested conditions. Non-diluted Az and truePAz^P extracts reduced fibroblast viability towards or below the ISO 10993-5 threshold of 70%, whereas diluted extracts (25–75%) of PAz^{EP-F}, PAz^{CHP-P} and truePAz^P maintained viability at or above this threshold. The most pronounced effect was the biphasic response to PAz^{EP-F} extracts, while PAz^{CHP-P} and truePAz^P extracts showed only modest viability reductions, consistent with more extensive purification of these powders. These descriptive findings form the basis for our subsequent analysis of cell adhesion and morphology on the corresponding films.

3.4. Polyazulene prepared in different ways induces different cellular behaviour

In contrast to the extract-based MTT assay, which assesses cytotoxicity of leachables, the following experiments address cytocompatibility in terms of direct cell adhesion, proliferation and morphology on the respective PAz films.

NIH/3T3 mouse embryonic fibroblasts were seeded onto PAz^{EP-F}, PAz^{CHP-F}, and truePAz^F. Cells successfully adhered and proliferated on all films, as shown in Fig. 10; however, differences in their morphology were noticeable. As a reference substrate (Fig. 10A), plastic cell culture dishes were used. Reference cells thus displayed their standard morphology typical of 2D *in vitro* cultivation, characterized by flattened cell bodies, evenly distributed nuclei, and well-organized cytoskeletons

with visible actin filaments.

Fibroblasts on PAz^{EP-F} (Fig. 10B) exhibited similar morphology to the reference, only with more distinct differences in size. The conductivity of PAz^{EP-F}, as confirmed by van der Pauw measurements, may play a role in these morphological changes, since substrate conductivity has already been associated with stimulating downstream signaling pathways that regulate cytoskeletal organization and cell spreading [70]. In addition to electroactive properties, surface microstructure and chemical heterogeneity significantly contribute to cell behavior by altering protein adsorption. These changes affect integrin-mediated adhesion, cytoskeletal organization, and cell spreading [71]. The resulting local microenvironments on the polymer surface lead to diverse cell morphologies and sizes.

In comparison to the reference, cells cultured on PAz^{CHP-F} (Fig. 10C) had an increased number and, more importantly, an increased length of processes, especially filopodia, extending over 200 µm. Furthermore, there was an observable variation in the size of the cytoskeleton on PAz^{CHP-F}, although the nuclei were of normal size. These morphological changes may be attributed to the specific topography of PAz^{CHP-F} since micro- or nanopatterning can affect cell spreading and cytoskeletal architecture. For example, studies on nanostructured surfaces showed that specific topographical features can serve as “guiding posts” for elongating filopodia and lamellipodia formation [72]. Similarly, micro-patterned substrates have been used to mimic the heterogeneity of the extracellular environment, resulting in modulating actin network assembly, which contributed to the variability in cytoskeletal size [73].

Cells grown on truePAz^F (Fig. 10D) exhibited compact and rounded morphologies with prominent actin filaments, lamellipodia replacing filopodia, and nuclei that appeared enlarged compared to the reference. In the literature, nuclear enlargement has been associated with oxidative and/or mechanical stress, while the shift from filopodia-dominant to lamellipodia-dominant protrusions has been connected to the altered Rho GTPase signaling [74–76]. Also, a similar modulation of focal adhesion distribution and cytoskeletal dynamics have been reported on other hierarchical breath-figure substrates [77]. Although we did not directly quantify reactive oxygen species, mechano-transduction pathways or GTPase activity in this study, our data suggest that the partial oxidization of truePAz^F (shown in the FTIR spectrum) combined with its

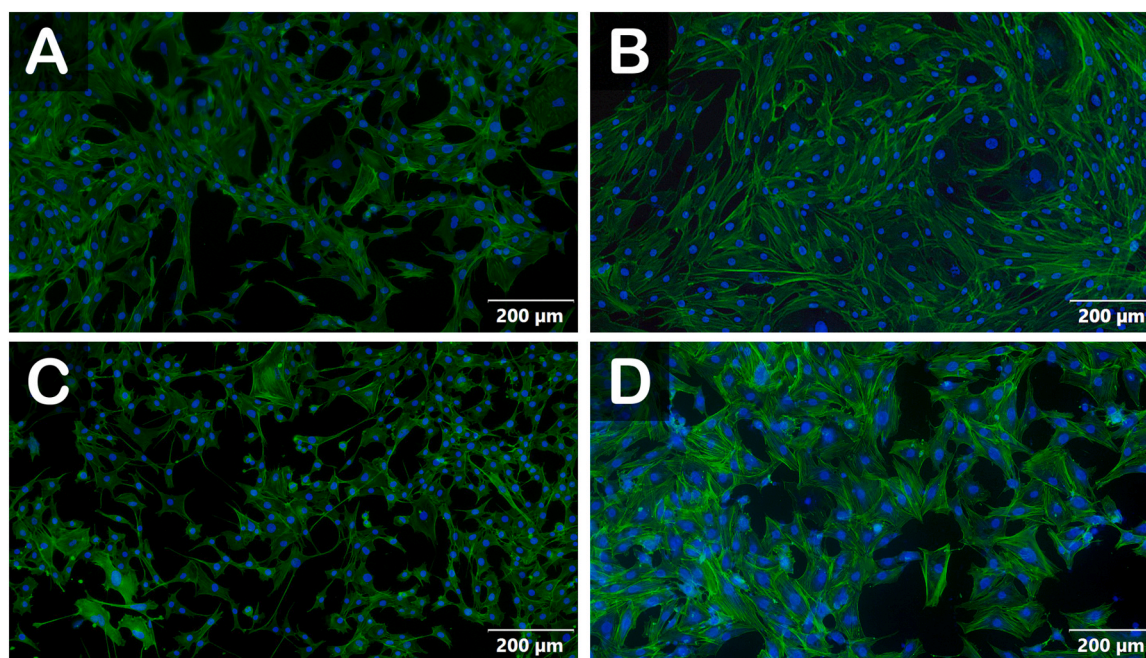


Fig. 10. Fluorescence microscopy images of NIH/3T3 mouse embryonic fibroblasts cultivated on: (A) Reference; (B) PAz^{EP-F}; (C) PAz^{CHP-F}; (D) truePAz^F. Reference cells were cultivated in cell culture dishes. Green color: ActinGreen – actin, Blue color: Hoechst – DNA. Magnification: 100 × .

hierarchical microstructure (shown in the SEM images) may contribute to the observed substrate-dependent differences in cell and nuclear shape.

The cell-image analysis of NIH/3T3 mouse fibroblasts was carried out using CellProfiler software [40]. Results in Fig. 11 show that fibroblasts cultured on $\text{PAz}^{\text{EP-F}}$ were significantly larger (took up more area) compared to other cells, while their perimeter was only a slightly larger, pointing to the compactness of the cells. In contrast, an average fibroblast cultured on $\text{PAz}^{\text{CHP-F}}$ occupied a very small area with a perimeter comparable to cells on other substrates. This is because of the high number and length of processes. Finally, it should be highlighted that the cells on $\text{truePAz}^{\text{F}}$ exhibited undeniably enlarged nuclei in (indicated by a large nuclei area), especially in comparison to the size of cells, which was similar to the area and perimeter of reference cells. The outcomes of this analysis align with the visual evaluation of the cell images above while offering precise, quantitative insights to facilitate a deeper understanding.

HaCaT human keratinocytes were seeded onto $\text{PAz}^{\text{EP-F}}$, $\text{PAz}^{\text{CHP-F}}$, and $\text{truePAz}^{\text{F}}$. Cells successfully adhered and proliferated on all films as shown in Fig. 12, including the reference substrate (Fig. 12A) – plastic cell culture dishes. Reference cells showed a typical cobblestone-like morphology with evenly-distributed actin filaments and normal nuclear sizes typical of 2D *in vitro* cultivation.

On the surface of $\text{PAz}^{\text{EP-F}}$ (Fig. 12B), keratinocytes adhered well, with moderate spreading. Although some studies have examined the effects of substrate topography and chemical composition on keratinocyte behavior [78,79], the direct evidence for enhanced proliferative and secretory responses in HaCaT cells is most convincingly demonstrated by studies involving conductive materials [80]. Conductive substrates enhance HaCaT keratinocyte attachment, proliferation, and cytokine secretion, which stem from the activation of intracellular signaling pathways like ERK1/2, supporting the role of electrically-active materials in wound healing and skin regeneration [80,81].

The surface of $\text{PAz}^{\text{CHP-F}}$ (Fig. 12C), on the other hand, repressed HaCaT cell adhesion, with fewer cells observed. The cells grew preferably in tight contact with the substrate (made of PAz particles) and even within its larger aggregates (highlighted with arrows in the figure). The cells possessed an elongated morphology, suggesting effective adhesion and maturation. This observation aligns with findings from a study on similar microporous substrates, which reported enhanced cell–substrate contacts and focal adhesion formation due to micro/nano-porosity that promotes local interactions and cell ingrowth into the pores [82]. However, the reduced proliferation could be attributed to a surface chemistry that supports tight adhesion but may limit the adsorption of attachment proteins, reducing cellular proliferation [83]. Similar behavior has been observed in studies in which porous substrates favored cell anchorage and spreading over cell proliferation [78,84].

Finally, keratinocytes seeded on $\text{truePAz}^{\text{F}}$ (Fig. 12D) exhibited

compact, rounded morphologies with limited actin organization and enlarged nuclei, similar to fibroblasts on the same substrate. These similarities indicate that the unique hierarchical topography and chemistry of $\text{truePAz}^{\text{F}}$ significantly affect cell shape and nuclear size. In other systems, comparable phenotypes have been linked to combined mechanical and oxidative cues [75]. In the present work, we therefore interpret these changes as substrate-induced alterations in cell behaviour, while acknowledging that the underlying molecular pathways (e.g. mechano-transduction or oxidative signalling) remain to be elucidated in future studies.

What first catches the eye in the HaCaT image analysis (Fig. 13) is the differences in cell sizes. As reported, an enhanced cell area arises with micro- or nanoscale surface features, promoting focal adhesion and cytoskeletal reorganization, and increasing cytoskeletal tension and integrin clustering in HaCaT cells [85,86]. This description is in line with the fact that the largest cells and nuclei were observed on $\text{PAz}^{\text{CHP-F}}$. However, $\text{truePAz}^{\text{F}}$, which is also full of micro- or nano-scale features, led to quite the opposite result – cells smaller than the reference cells. These findings highlight the complex interplay between substrate topography, surface chemistry, and cell behaviour, where morphological changes often occur at the expense of proliferation [87].

ES R1 mouse embryonic stem cells in the presence of LIF were also seeded onto $\text{PAz}^{\text{EP-F}}$, $\text{PAz}^{\text{CHP-F}}$, and $\text{truePAz}^{\text{F}}$, which had previously been coated with gelatine. Cells successfully adhered and proliferated on all films as shown in Fig. 14. Reference cells cultivated on gelatinized cell culture dishes (Fig. 14A) showed their standard behaviour – the formation of rounded clusters with clearly defined boundaries, as is typical of 2D *in vitro* cultivation.

Stem cells on $\text{PAz}^{\text{EP-F}}$ (Fig. 14B) formed loosely organized clusters with actin filaments visible within the clusters, and the nuclei of normal size. The cells maintained typical morphology, except for an occasional large binucleated cell. In adherent cells, proper cytokinesis is tightly regulated by integrin-mediated adhesion and cytoskeletal dynamics; perturbations in these interactions may compromise the final abscission step of cell division, leading to the formation of binucleated cells [88]. These data may imply that while the conductive polymer substrate adequately supports overall stem cell adhesion and survival, minor alterations in the biophysical cues might interfere with the integrin signaling critical for cytokinesis, resulting in a single, enlarged cell harbouring two nuclei. However, this hypothesis requires further experimental validation.

Stem cells seeded on $\text{PAz}^{\text{CHP-F}}$ (Fig. 14C) form more dissociated clusters with significant spreading and prominent actin filaments. The increased surface roughness and topographical heterogeneity of $\text{PAz}^{\text{CHP-F}}$ enhance cell–substrate interactions, promoting a more pronounced actin cytoskeletal organization and stress fiber formation [89], and also reduce cell–cell junctional contacts, leading to the formation of more dissociated clusters [90]. The observed actin remodeling and enhanced cell spreading suggest that the substrate induces a shift toward a

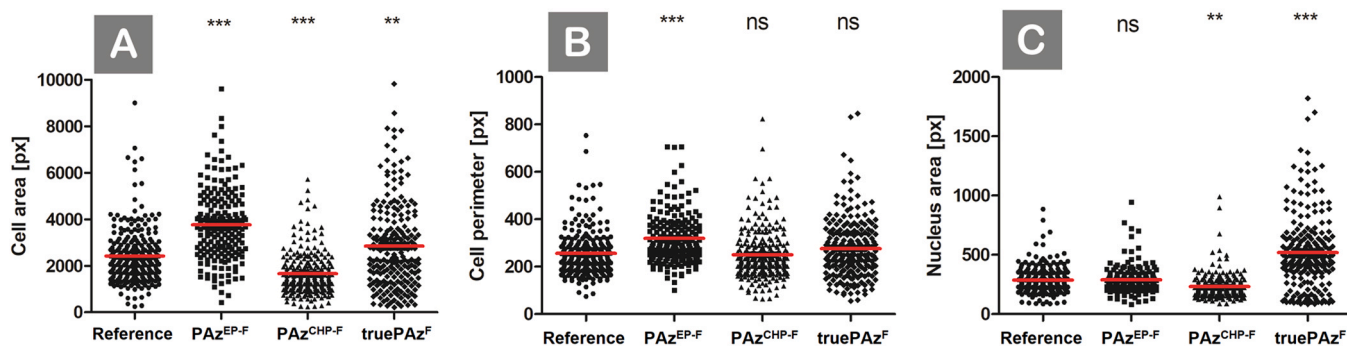


Fig. 11. Results of image analyses performed by CellProfiler software comparing (A) cell area, (B) cell perimeter, and (C) nucleus area of NIH/3T3 mouse embryonic fibroblasts cultivated on reference, $\text{PAz}^{\text{EP-F}}$, $\text{PAz}^{\text{CHP-F}}$, and $\text{truePAz}^{\text{F}}$. Reference cells were cultivated in cell culture dishes. One-way ANOVA with post-hoc Tukey's test: ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

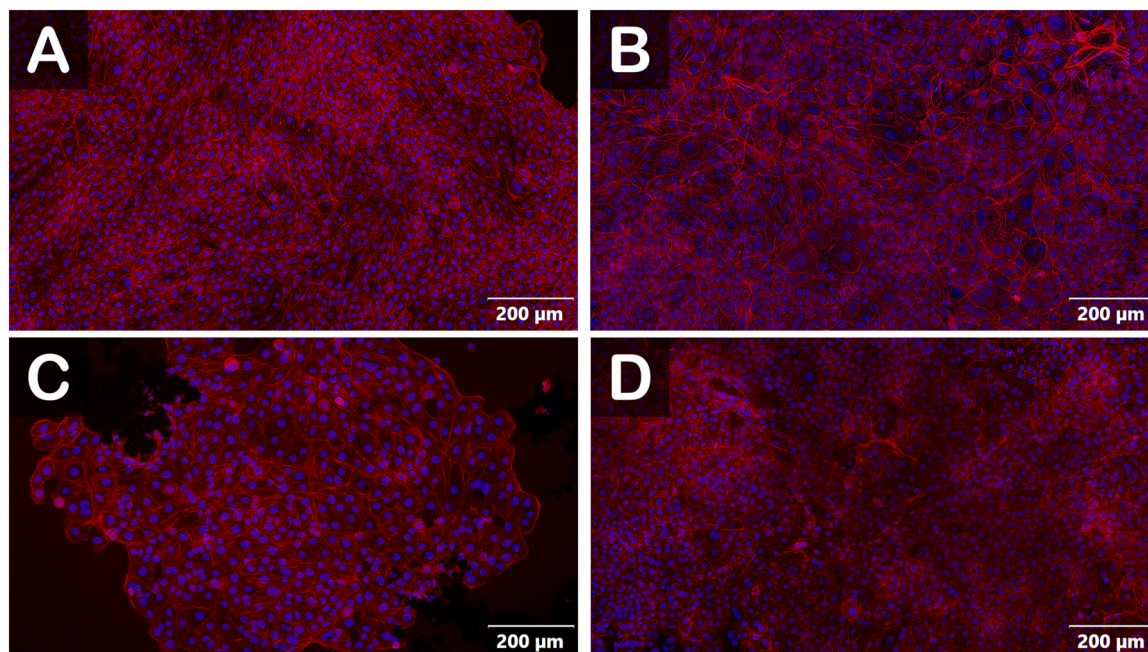


Fig. 12. Fluorescence microscopy images of HaCaT human keratinocytes cultivated on: (A) Reference; (B) PAz^{EP-F}; (C) PAz^{CHP-F}; (D) truePAz^F. Reference cells were cultivated in cell culture dishes. Red color: ActinRed – actin, Blue color: Hoechst – DNA. Magnification: 100 × .

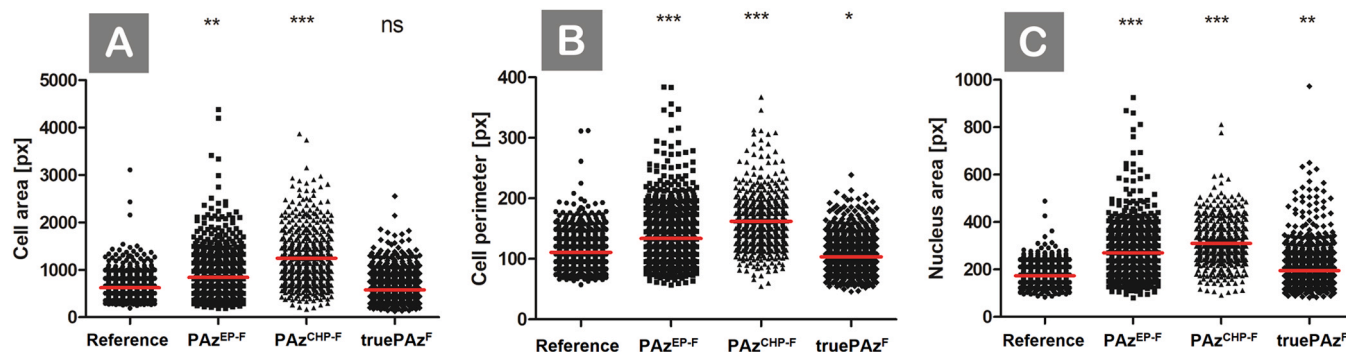


Fig. 13. Results of image analyses performed by CellProfiler software comparing (A) cell area, (B) cell perimeter, and (C) nucleus area of HaCaT human keratinocytes cultivated on reference, PAz^{EP-F}, PAz^{CHP-F}, and truePAz^F. Reference cells were cultivated in cell culture dishes. One-way ANOVA with post-hoc Tukey's test: ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

mesenchymal-like phenotype [91].

The specific topography of truePAz^F (Fig. 14D), on the other hand, led stem cells to form compact clusters with limited spreading, dense actin filaments, and enlarged nuclei. Similar combinations of enhanced actomyosin contractility, pronounced focal adhesion formation and nuclear remodelling have been discussed in reviews of stem cell mechanobiology as key features of substrates that deliver strong mechanical cues [92]. Our observations are therefore consistent with a scenario in which the hierarchical architecture of truePAz^F provides pronounced mechanical stimuli; however, we did not directly probe the associated signalling pathways or oxidative status, and these mechanisms remain to be confirmed.

In Fig. 15, the image analysis of ES R1 mouse embryonic stem cells in LIF revealed some differences. Trends in cell area and perimeter follow each other, suggesting the cells were rounded with no significant changes in cytoskeleton shapes. However, it is noticeable that cells on PAz^{CHP-F} gained significantly smaller area than cells on any other substrate. This substrate-induced stimulation promotes extensive focal adhesion and stress fiber assembly, which can increase intracellular tension and contractility. Such mechanical stimuli are known to

influence cell morphology by restricting the overall cell footprint, resulting in more compact cells relative to reference conditions, where smoother and more homogeneous surfaces allow for greater spreading and larger cell areas [93,94].

Across all three cell lines and all three film types, cells were able to adhere and proliferate, but with clearly substrate-specific morphologies. On PAz^{EP-F}, fibroblasts, keratinocytes and embryonic stem cells generally displayed larger projected cell areas and morphologies similar to the reference, consistent with good adhesion on a continuous, moderately conductive film. On PAz^{CHP-F}, the particle-based microstructure favoured elongated cells with long processes and, in some cases, reduced proliferation, particularly for keratinocytes and stem cells, which preferentially grew along particle aggregates. On truePAz^F, the hierarchical breath-figure architecture induced compact, rounded cell shapes with enlarged nuclei across all three cell types. The most robust conclusion from our biological data is that PAz films prepared by different synthetic routes are short-term cytocompatible *in vitro*, but elicit distinct, substrate-specific adhesion and morphology patterns rather than a uniform cell response.

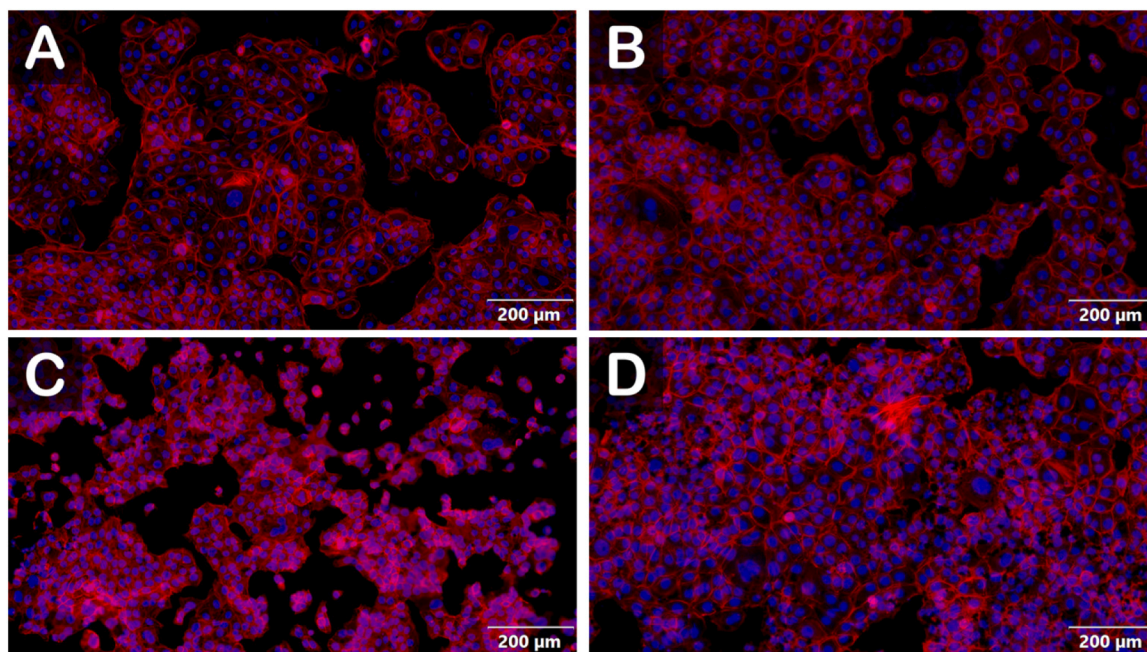


Fig. 14. Fluorescence microscopy images of ES R1 mouse embryonic stem cells in the presence of LIF cultivated on: (A) Reference; (B) PAz^{EP-F}; (C) PAz^{CHP-F}; (D) truePAz^F. Reference cells were cultivated in cell culture dishes. All surfaces were gelatinized in advance. Red color: ActinRed – actin, Blue color: Hoechst – DNA. Magnification: 100 × .

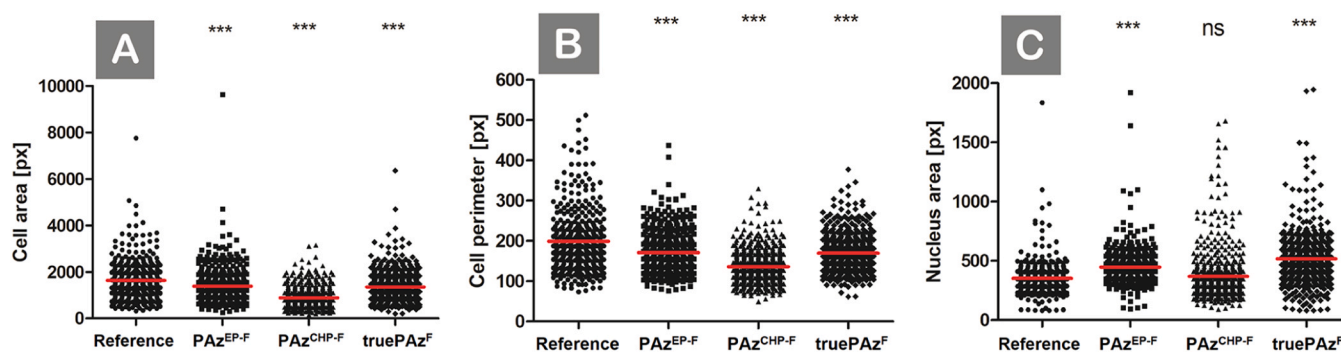


Fig. 15. Results of image analyses performed by CellProfiler software comparing (A) cell area, (B) cell perimeter, and (C) nucleus area of ES R1 mouse embryonic stem cells in the presence of LIF cultivated on reference, PAz^{EP-F}, PAz^{CHP-F}, and truePAz^F. Reference cells were cultivated in cell culture dishes. One-way ANOVA with post-hoc Tukey's test: ns = $p > 0.05$, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

4. Conclusions

This study represents, to the best of our knowledge, the first systematic *in vitro* cytotoxicity and cytocompatibility investigation of PAz, providing novel insights into its biological properties and interactions with cells. As hypothesized, we successfully prepared three different types of PAz films, each synthesized by a different method, resulting in materials with unique structures and properties.

First, electrochemical polymerization of Az led to a continuous PAz film with moderate conductivity in the tens of S/cm range under optimal deposition conditions. Because the film thickness and continuity vary with the electrochemical parameters, these conductivity values should be regarded as effective film properties rather than intrinsic bulk parameters of PAz. The cytotoxicity analysis suggested that the combination of surface microstructure, electroactivity, and possible residual impurities from synthesis may have influenced cell morphology and proliferation.

Second, chemically-*in situ*-synthesized PAz was fabricated in a specific structure consisting of uniform aggregated submicron particles. This topography resulted in increased focal adhesion and intracellular

tension that manifested in morphological changes that varied depending on the type of studied cell line.

Third, so-called true PAz was prepared by chemical synthesis with followed by dissolution and casting, resulting in films. Self-assembly of a hierarchical breath-figure pattern occurred, which significantly impacted cell morphology, inducing nuclei enlargement.

Each type of PAz exhibited a completely different structure with specific physico-chemical properties, resulting in varied cell behaviour on each of the surfaces. While our results indicate that all three PAz film types and their extracts are compatible with the tested cell lines over short culture periods, a comprehensive evaluation of long-term and *in vivo* biocompatibility, immune responses, and degradation behaviour will require additional dedicated studies. However, in the absence of prior studies available for comparison, this research thus provides valuable understanding and insights into the cytocompatibility of PAz, and establishes a foundation for further exploring PAz-based materials for biomedical applications.

CRediT authorship contribution statement

Věra Kašpárková: Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Leona Mahelová:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Petr Humpolíček:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **David Škoda:** Software, Investigation. **Karolína Kocourková:** Software, Investigation, Formal analysis. **Miroslava Trchová:** Methodology, Investigation, Formal analysis. **Sonia Kotowicz:** Supervision, Software, Methodology, Investigation, Formal analysis. **Zdenka Víchová:** Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Jan Vícha:** Validation, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.synthmet.2026.118139](https://doi.org/10.1016/j.synthmet.2026.118139).

Data availability

Data will be made available on request.

References

- [1] M. Gajendiran, et al., Conductive biomaterials for tissue engineering applications, *J. Ind. Eng. Chem.* 51 (Jul. 2017) 12–26, <https://doi.org/10.1016/j.jiec.2017.02.031>.
- [2] G. Shi, M. Rouabhi, Z. Wang, L.H. Dao, Z. Zhang, A novel electrically conductive and biodegradable composite made of polypyrrole nanoparticles and polylactide, *Biomaterials* 25 (13) (Jun. 2004) 2477–2488, <https://doi.org/10.1016/j.biomaterials.2003.09.032>.
- [3] J. Huang, et al., Electrical stimulation to conductive scaffold promotes axonal regeneration and remyelination in a rat model of large nerve defect, *PLoS One* 7 (6) (Jun. 2012) e39526, <https://doi.org/10.1371/journal.pone.0039526>.
- [4] L. Mohammadi Amirabad, et al., Enhanced cardiac differentiation of human cardiovascular disease patient-specific induced pluripotent stem cells by applying unidirectional electrical pulses using aligned electroactive nanofibrous scaffolds, *ACS Appl. Mater. Interfaces* 9 (8) (Mar. 2017) 6849–6864, <https://doi.org/10.1021/acsami.6b15271>.
- [5] J.-X. Dong, H.-L. Zhang, Azulene-based organic functional molecules for optoelectronics, *Chin. Chem. Lett.* 27 (8) (Aug. 2016) 1097–1104, <https://doi.org/10.1016/j.ccllet.2016.05.005>.
- [6] H. Xin, et al., Incorporation of 2,6-connected azulene units into the backbone of conjugated polymers: towards high-performance organic optoelectronic materials, *Angew. Chem. Int. Ed.* 57 (5) (2018) 1322–1326, <https://doi.org/10.1002/anie.201711802>.
- [7] P. Cowper, A. Pockett, G. Kociok-Köhn, P.J. Cameron, S.E. Lewis, Azulene – thiophene – cyanoacrylic acid dyes with donor- π -acceptor structures. Synthesis, characterisation and evaluation in dye-sensitized solar cells, *Tetrahedron* 74 (22) (May 2018) 2775–2786, <https://doi.org/10.1016/j.tet.2018.04.043>.
- [8] H. Nishimura, et al., Hole-Transporting Materials with A Two-dimensionally Expanded π -system around an azulene core for efficient perovskite solar cells, *J. Am. Chem. Soc.* 137 (50) (Dec. 2015) 15656–15659, <https://doi.org/10.1021/jacs.5b11008>.
- [9] G.-O. Buica, et al., Azulene-ethylenediaminetetraacetic acid: A versatile molecule for colorimetric and electrochemical sensors for metal ions, *Electrochim. Acta* 263 (Feb. 2018) 382–390, <https://doi.org/10.1016/j.electacta.2018.01.059>.
- [10] H.-M. Chiang, et al., Photoirradiation of azulene and guaiazulene-Formation of reactive oxygen species and induction of lipid peroxidation, *J. Photochem. Photobiol. A Chem.* 211 (2–3) (Apr. 2010) 123–128, <https://doi.org/10.1016/j.jphotochem.2010.02.007>.
- [11] T. Damrongrungruang, et al., Effects of photodynamic therapy with azulene on peripheral blood mononuclear cell viability and singlet oxygen formation, *Photo. Photodyn. Ther.* 24 (Dec. 2018) 318–323, <https://doi.org/10.1016/j.pdpdt.2018.10.015>.
- [12] D. Ma, J. He, D. He, Chamazulene reverses osteoarthritic inflammation through regulation of matrix metalloproteinases (MMPs) and NF- κ B pathway in in-vitro and in-vivo models, *Biosci. Biotechnol. Biochem.* 84 (2) (Feb. 2020) 402–410, <https://doi.org/10.1080/09168451.2019.1682511>.
- [13] K. Ikegai, et al., Synthesis and biological evaluation of C-glucosides with azulene rings as selective SGLT2 inhibitors for the treatment of type 2 diabetes mellitus: Discovery of YM543, *Bioorg. Med. Chem.* 21 (13) (Jul. 2013) 3934–3948, <https://doi.org/10.1016/j.bmc.2013.03.067>.
- [14] A. Russo, M. Bruno, R. Avola, V. Cardile, D. Rigano, Chamazulene-Rich Artemisia arborescens Essential Oils Affect the Cell Growth of Human Melanoma Cells, *Plants* 9 (8) (Aug. 2020), <https://doi.org/10.3390/plants9081000>.
- [15] P. Bakun, B. Czarczynska-Goslinska, T. Goslinski, S. Lijewski, vitro and in vivo biological activities of azulene derivatives with potential applications in medicine, *Med Chem. Res* 30 (4) (Apr. 2021) 834–846, <https://doi.org/10.1007/s00044-021-02701-0>.
- [16] L.C. Murfin, S.E. Lewis, Azulene—A Bright Core for Sensing and Imaging, *Molecules* 26 (2) (Jan. 2021), <https://doi.org/10.3390/molecules26020353>.
- [17] H.N. Zeng, Z.M. Png, J. Xu, Azulene in Polymers and Their Properties, *Chem. Asian J.* 15 (13) (2020) 1904–1915, <https://doi.org/10.1002/asia.202000444>.
- [18] T. Tsuchiya, T. Hamano, M. Inoue, T. Nakamura, A. Wakamiya, Y. Mazaki, Intense absorption of azulene realized by molecular orbital inversion, *Chem. Commun.* 59 (71) (2023) 10604–10607, <https://doi.org/10.1039/D3CC02311G>.
- [19] T. Shoji, S. Ito, Chapter One - The Preparation and Properties of Heteroarylazulenes and Hetero-Fused Azulenes, in: E.F.V. Scriven, C.A. Ramsden (Eds.), *Advances in Heterocyclic Chemistry*, 126, Academic Press, 2018, pp. 1–54, <https://doi.org/10.1016/bs.aihch.2018.02.002>.
- [20] R.-M. Latonen, B. Meana Esteban, C. Kvarnström, A. Ivaska, Electrochemical polymerization and characterization of a poly(azulene)-TiO₂ nanoparticle composite film, *J. Appl. Electrochem* 39 (5) (May 2009) 653–661, <https://doi.org/10.1007/s10800-008-9705-1>.
- [21] G. Nöll, C. Lambert, M. Lynch, J. Daub, Electronic Structure and Properties of Poly- and Oligoazulenes, *J. Phys. Chem. C* 112 (6) (Feb. 2008) 2156–2164, <https://doi.org/10.1021/jp074376b>.
- [22] A. Österholm, B.M. Esteban, C. Kvarnström, A. Ivaska, Spectroelectrochemical study of the redox reactions of polyazulene on aluminum substrates, *J. Electroanal. Chem.* 613 (2) (Feb. 2008) 160–170, <https://doi.org/10.1016/j.jelechem.2007.10.022>.
- [23] K. Iwasaki, K. Matsumoto, S. Hino, M. Yasunami, Electrochemical polymerization of alkyl-substituted azulenes, *Synth. Met.* 55 (2) (Mar. 1993) 1062–1066, [https://doi.org/10.1016/0379-6779\(93\)90200-G](https://doi.org/10.1016/0379-6779(93)90200-G).
- [24] Y.-B. Shim, S.-M. Park, Electrochemistry of Conductive Polymers: XXII. Electrochemical and Spectroelectrochemical Studies of Polyazulene Growth and Its Characterization, *J. Electrochem. Soc.* 144 (9) (Sep. 1997) 3027, <https://doi.org/10.1149/1.1837954>.
- [25] K.G. Neoh, E.T. Kang, T.C. Tan, Chemical synthesis and characterization of electroactive and partially soluble polyazulene, *Polym. Bull.* 19 (4) (Apr. 1988), <https://doi.org/10.1007/BF00263932>.
- [26] F. Wang, Y.-H. Lai, N.M. Kocherginsky, Yu. Yu Koteski, “The First Fully Characterized 1,3-Polyazulene: High Electrical Conductivity Resulting from Cation Radicals and Polycations Generated upon Protonation, *Org. Lett.* 5 (7) (Apr. 2003) 995–998, <https://doi.org/10.1021/ol0274615>.
- [27] R. Kaewchingduang, N. Paradee, A. Sirivat, S. Niamlang, Effects of conductive polyazulene and plasticizer embedded in deproteinized natural rubber transdermal patch on electrically controlled naproxen release-permeation, *Int. J. Pharm.* 561 (Apr. 2019) 296–304, <https://doi.org/10.1016/j.ijpharm.2019.02.046>.
- [28] S. Ghasimi, K. Landfester, K.A.I. Zhang, Water Compatible Conjugated Microporous Polyazulene Networks as Visible-Light Photocatalysts in Aqueous Medium, *ChemCatChem* 8 (4) (2016) 694–698, <https://doi.org/10.1002/cctc.201501102>.
- [29] E. Gradzka, P. Makowska, K. Winkler, Chemically formed conducting polyazulene: From micro- to nanostructures, *Synth. Met.* 246 (Dec. 2018) 115–121, <https://doi.org/10.1016/j.synthmet.2018.10.002>.
- [30] N. Kihara, H. Nakayama, T. Fukutomi, True Polyazulene: Soluble Precursor of So-Called ‘Polyazulene’, *Macromolecules* 30 (20) (Oct. 1997) 6385–6387, <https://doi.org/10.1021/ma970510m>.
- [31] A. Österholm, B. Meana-Esteban, C. Kvarnström, A. Ivaska, situ Resonance Raman Spectroscopy of Polyazulene on Aluminum, *J. Phys. Chem. B* 112 (20) (May 2008) 6331–6337, <https://doi.org/10.1021/jp0762828>.
- [32] A. Österholm, A. Petr, C. Kvarnström, A. Ivaska, L. Dunsch, The Nature of the Charge Carriers in Polyazulene as Studied by in Situ Electron Spin Resonance—UV—Visible—Near-Infrared Spectroscopy, *J. Phys. Chem. B* 112 (45) (Nov. 2008) 14149–14157, <https://doi.org/10.1021/jp805813s>.
- [33] S. Ghasimi, K. Landfester, K.A.I. Zhang, Water Compatible Conjugated Microporous Polyazulene Networks as Visible-Light Photocatalysts in Aqueous

- Medium, *ChemCatChem* 8 (4) (2016) 694–698, <https://doi.org/10.1002/cctc.201501102>.
- [34] V.S. Shetti, Chemical syntheses and salient features of azulene-containing homo- and copolymers, *Beilstein J. Org. Chem.* 17 (1) (Aug. 2021) 2164–2185, <https://doi.org/10.3762/bjoc.17.139>.
- [35] G. Nöll, C. Lambert, M. Lynch, M. Porsch, J. Daub, Electronic Structure and Properties of Poly- and Oligoazulenes, *J. Phys. Chem. C* 112 (6) (Feb. 2008) 2156–2164, <https://doi.org/10.1021/jp074376b>.
- [36] R. Yewale, P. Damlin, C. Kvarnström, Effect of Oxidants on Properties of Electroactive Ultrathin Polyazulene Films Synthesized by Vapor Phase Polymerization at Atmospheric Pressure, *Langmuir* 38 (49) (Dec. 2022) 15165–15177, <https://doi.org/10.1021/acs.langmuir.2c02215>.
- [37] S. Wang, X. Meng, H. Luo, L. Yao, X. Song, Z. Liang, Post-synthetic modification of conjugated microporous polymer with imidazolium for highly efficient anionic dyes removal from water, *Sep. Purif. Technol.* (Feb. 2022), <https://doi.org/10.1016/j.seppur.2021.120245>.
- [38] P. Boukamp, R.T. Petrussevska, D. Breitkreutz, J. Hornung, A. Markham, N. E. Fusenig, Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line, *J. Cell Biol.* 106 (3) (Mar. 1988) 761–771, <https://doi.org/10.1083/jcb.106.3.761>.
- [39] A. Nagy, J. Rossant, R. Nagy, W. Abramow-Newerly, J.C. Roder, Derivation of completely cell culture-derived mice from early-passage embryonic stem cells, *PNAS* 90 (18) (Sep. 1993) 8424–8428, <https://doi.org/10.1073/pnas.90.18.8424>.
- [40] A.E. Carpenter, et al., CellProfiler: image analysis software for identifying and quantifying cell phenotypes, *Genome Biol.* 7 (10) (2006) R100, <https://doi.org/10.1186/gb-2006-7-10-r100>.
- [41] R.-M. Latonen, B. Meana Esteban, C. Kvarnström, A. Ivaska, Electrochemical polymerization and characterization of a poly(azulene)-TiO₂ nanoparticle composite film, *J. Appl. Electrochem* 39 (5) (May 2009) 653–661, <https://doi.org/10.1007/s10800-008-9705-1>.
- [42] N.K. Guimard, N. Gomez, C.E. Schmidt, Conducting polymers in biomedical engineering, *Prog. Polym. Sci.* 32 (8) (Aug. 2007) 876–921, <https://doi.org/10.1016/j.progpolymsci.2007.05.012>.
- [43] T. Patois, B. Lakard, N. Martin, P. Fievet, Effect of various parameters on the conductivity of free standing electrosynthesized polypyrrole films, *Synth. Met.* 160 (19) (Oct. 2010) 2180–2185, <https://doi.org/10.1016/j.synthmet.2010.08.005>.
- [44] V. Kaspárková, et al., Cell-compatible conducting polyaniline films prepared in colloidal dispersion mode, *Colloids Surf. B Biointerfaces* 157 (Sep. 2017) 309–316, <https://doi.org/10.1016/j.colsurfb.2017.05.066>.
- [45] G. Cai, et al., Molecular level assembly for high-performance flexible electrochromic energy-storage devices, *ACS Energy Lett.* 5 (4) (2020) 1159–1166, <https://doi.org/10.1021/acseenergylett.0c00245>.
- [46] D. Gogoi, et al., Development of advanced antimicrobial and sterilized plasma polypropylene grafted mussel (antheraea assama) silk as suture biomaterial, *Biopolymers* 101 (4) (2014) 355–365, <https://doi.org/10.1002/bip.22369>.
- [47] K.D. O'Neil, B. Shaw, O.A. Semenikhin, On the origin of mesoscopic inhomogeneity of conducting polymers, *J. Phys. Chem. B* 111 (31) (Aug. 2007) 9253–9269, <https://doi.org/10.1021/jp071564t>.
- [48] B. Poornaiyah, G.S. Rao, A.V.P. Kumar, Y.S. Rao, Low Frequency Noise and TCR Mechanisms in Polymer Thick Film Resistors, *J. Microelectron. & Elect. Pkg* 7 (2) (Apr. 2010) 105–110, <https://doi.org/10.4071/1551-4897-7.2.105>.
- [49] Y. Ye, K.Y. Choi, Preparation of Micron-Sized Spherulitic Bisphenol A Polycarbonate Particles in Thin Films, *Macromol. Mater. Eng.* 294 (12) (2009) 847–854, <https://doi.org/10.1002/mame.200900165>.
- [50] Y.S. Rao, Studies on temperature coefficient of resistance (TCR) of polymer thick film resistors, *Microelectron. Int.* 25 (3) (2008) 33–36, <https://doi.org/10.1108/13565360810889601>.
- [51] A. Böker, et al., Hierarchical nanoparticle assemblies formed by decorating breath figures, *Nat. Mater.* 3 (5) (May 2004) 302–306, <https://doi.org/10.1038/nmat1110>.
- [52] L.A. Connal, G.G. Qiao, Preparation of Porous Poly(dimethylsiloxane)-Based Honeycomb Materials with Hierarchical Surface Features and Their Use as Soft-Lithography Templates, *Adv. Mater.* 18 (22) (2006) 3024–3028, <https://doi.org/10.1002/adma.200600982>.
- [53] E. Bormashenko, Breath-Figure Self-Assembly, a Versatile Method of Manufacturing Membranes and Porous Structures: Physical, Chemical and Technological Aspects, *Membranes* 7 (3) (Sep. 2017), <https://doi.org/10.3390/membranes7030045>.
- [54] N. Benoot, et al., Hierarchically organized honeycomb films through block copolymer directed self-assembly in 'breath figure' templating and soft microwave-triggered annealing, *Soft Matter* 14 (23) (Jun. 2018) 4874–4880, <https://doi.org/10.1039/C8SM00137E>.
- [55] H. Ma, L. Kong, X. Guo, J. Hao, Dynamic insights into formation of honeycomb structures induced by breath figures, *RSC Adv.* 1 (7) (Nov. 2011) 1187–1189, <https://doi.org/10.1039/C1RA00367D>.
- [56] J. Berg, L. Eriksson, P. Claesson, K. Borve, 3-Component Langmuir-Blodgett-Films with a Controllable Degree of Polarity, *Langmuir* 10 (4) (Apr. 1994) 1225–1234, <https://doi.org/10.1021/la00016a041>.
- [57] E.A. Vogler, Structure and reactivity of water at biomaterial surfaces, *Adv. Colloid Interface Sci.* 74 (1) (Feb. 1998) 69–117, [https://doi.org/10.1016/S0001-8686\(97\)00040-7](https://doi.org/10.1016/S0001-8686(97)00040-7).
- [58] L. He, et al., Highly robust supramolecular polymer networks crosslinked by a tiny amount of metallacycles, *Nat. Commun.* 15 (Apr. 2024) 3050, <https://doi.org/10.1038/s41467-024-47333-1>.
- [59] R.-X. Yang, T.-T. Wang, W.-Q. Deng, Extraordinary Capability for Water Treatment Achieved by a Perfluorinated Conjugated Microporous Polymer, *Sci. Rep.* 5 (May 2015) 10155, <https://doi.org/10.1038/srep10155>.
- [60] S. Ghasimi, K. Landfester, K.A.I. Zhang, Water Compatible Conjugated Microporous Polyazulene Networks as Visible-Light Photocatalysts in Aqueous Medium, *ChemCatChem* 8 (4) (2016) 694–698, <https://doi.org/10.1002/cctc.201501102>.
- [61] B. Meana-Esteban, C. Lete, C. Kvarnström, A. Ivaska, Raman and in Situ FTIR-ATR Characterization of Polyazulene Films and Its Derivate, *J. Phys. Chem. B* 110 (46) (Nov. 2006) 23343–23350, <https://doi.org/10.1021/jp0631811>.
- [62] V. Kaspárková, P. Humpolíček, J. Stejskal, J. Kopecká, Z. Kuceková, R. Moučka, Conductivity, impurity profile, and cytotoxicity of solvent-extracted polyaniline, *Polym. Adv. Technol.* 27 (2) (2016) 156–161, <https://doi.org/10.1002/pat.3611>.
- [63] V. Kaspárková, et al., Exploring the Critical Factors Limiting Polyaniline Biocompatibility, *Polymers* 11 (2) (Feb. 2019), <https://doi.org/10.3390/polym11020362>.
- [64] C.M. Schmidt, C.N. Cheng, A. Marino, R. Konsoula, F. A Barile, Hormesis effect of trace metals on cultured normal and immortal human mammary cells, *Toxicol. Ind. Health* 20 (1–5) (Feb. 2004) 57–68, <https://doi.org/10.1191/0748233704th192oa>.
- [65] E.J. Calabrese, L. A. Baldwin, Inorganics and Hormesis, *Crit. Rev. Toxicol.* 33 (3–4) (Jan. 2003) 215–304, <https://doi.org/10.1080/10713611040>.
- [66] K. Skopalová, et al., Modulation of Differentiation of Embryonic Stem Cells by Polypyrrole: The Impact on Neurogenesis, *Int. J. Mol. Sci.* 22 (2) (Jan. 2021), <https://doi.org/10.3390/ijms22020501>.
- [67] S. Yin, J. Zhou, J. Wang, B. Xia, G. Chen, Preparation and performance of electrically conductive decellularized nerve matrix hydrogel conduits, *J. Biomater. Appl.* 38 (4) (Oct. 2023) 471–483, <https://doi.org/10.1177/08853282231200963>.
- [68] P. Humpolíček, et al., Polyaniline cryogels: Biocompatibility of novel conducting macroporous material, *Sci. Rep.* 8 (Jan. 2018) 135, <https://doi.org/10.1038/s41598-017-18290-1>.
- [69] K. Siczek, The Toxicity of Secondary Lithium-Sulfur Batteries Components, *Batteries* 6 (3) (Sep. 2020), <https://doi.org/10.3390/batteries6030045>.
- [70] A. Inoue, H. Yuk, B. Lu, X. Zhao, Strong adhesion of wet conducting polymers on diverse substrates, *Sci. Adv.* (Mar. 2020), <https://doi.org/10.1126/sciadv.aay5394>.
- [71] A. Furuhashi, Y. Ayukawa, I. Atsuta, Y.D. Rakhmatia, K. Koyano, Soft Tissue Interface with Various Kinds of Implant Abutment Materials, *J. Clin. Med.* 10 (11) (Jan. 2021), <https://doi.org/10.3390/jcm10112386>.
- [72] D.-H. Kim, P. Kim, K.Y. Suh, S.K. Choi, S.H. Lee, B. Kim, Modulation of Adhesion and Growth of Cardiac Myocytes by Surface Nanotopography, 2005 IEEE Eng. Med. Biol. 27th Annu. Conf. (Jan. 2005) 4091–4094, <https://doi.org/10.1109/IEMBS.2005.1615362>.
- [73] F. Xing, et al., Regulation of actin cytoskeleton via photolithographic micropatterning, *J. Innov. Opt. Health Sci.* 16 (02) (Mar. 2023) 2244005, <https://doi.org/10.1142/S1793545822440059>.
- [74] D. Septiadi, F. Crippa, T.L. Moore, B. Rothen-Rutishauser, A. Petri-Fink, Nanoparticle-Cell Interaction: A Cell Mechanics Perspective, *Adv. Mater.* 30 (19) (2018) 1704463, <https://doi.org/10.1002/adma.201704463>.
- [75] E. Griesser, V. Vemula, A. Mónico, D. Pérez-Sala, and M. Fedorova, "Dynamic posttranslational modifications of cytoskeletal proteins unveil hot spots under nitroxidative stress," *Jan. 26, 2021, bioRxiv*. doi: 10.1101/2021.01.24.427971.
- [76] P. Aspenström, The Role of Fast-Cycling Atypical RHO GTPases in Cancer, *Cancers* 14 (8) (Jan. 2022), <https://doi.org/10.3390/cancers14081961>.
- [77] K. Yang, et al., Multiscale, Hierarchically Patterned Topography for Directing Human Neural Stem Cells into Functional Neurons, *ACS Nano* 8 (8) (Aug. 2014) 7809–7822, <https://doi.org/10.1021/nm501182f>.
- [78] D. Dutta, J. Markhoff, N. Suter, K. Rezwan, D. Brüggemann, Effect of Collagen Nanofibers and Silanization on the Interaction of HaCat Keratinocytes and 3T3 Fibroblasts with Alumina Nanopores, *ACS Appl. Bio Mater.* 4 (2) (Feb. 2021) 1852–1862, <https://doi.org/10.1021/acsbm.0c01538>.
- [79] A. Eickenscheidt, et al., Effect of Poly(Oxanorbonene)- and Poly(Methacrylate)-Based Polyzwitterionic Surface Coatings on Cell Adhesion and Gene Expression of Human Keratinocytes, *Macromol. Biosci.* 22 (11) (2022) 2200225, <https://doi.org/10.1002/mabi.202200225>.
- [80] S. Cui, M. Rouabhi, A. Semlali, Z. Zhang, Effects of electrical stimulation on human skin keratinocyte growth and the secretion of cytokines and growth factors, *Biomed. Mater.* 16 (6) (Oct. 2021) 065021, <https://doi.org/10.1088/1748-605X/ac2bba>.
- [81] E.M. Stewart, et al., Cell attachment and proliferation on high conductivity PEDOT-glycol composites produced by vapour phase polymerization, *Biomater. Sci.* 1 (4) (Mar. 2013) 368–378, <https://doi.org/10.1039/C2BM00143H>.
- [82] D. Narayan, S.S. Venkatraman, Effect of pore size and interpore distance on endothelial cell growth on polymers, *J. Biomed. Mater. Res. Part A* 87A (3) (2008) 710–718, <https://doi.org/10.1002/jbm.a.31749>.
- [83] A. Sh. Asran, Khashayar Razghandi, N. Aggarwal, G.H. Michler, T. Groth, Nanofibers from Blends of Polyvinyl Alcohol and Polyhydroxy Butyrate As Potential Scaffold Material for Tissue Engineering of Skin, *Biomacromolecules* 11 (12) (Dec. 2010) 3413–3421, <https://doi.org/10.1021/bm100912v>.
- [84] A. Joshi, T. Nuntapramote, D. Brüggemann, Self-Assembled Fibrinogen Scaffolds Support Cocultivation of Human Dermal Fibroblasts and HaCat Keratinocytes, *ACS Omega* 8 (9) (Mar. 2023) 8650–8663, <https://doi.org/10.1021/acsomega.2c07896>.
- [85] L. Francis, et al., The Influence of Tetracycline Loading on the Surface Morphology and Biocompatibility of Films Made from P(3HB) Microspheres, *Adv. Eng. Mater.* 12 (7) (2010) B260–B268, <https://doi.org/10.1002/adem.201080020>.

- [86] J.R. McMillan, et al., Small-Diameter Porous Poly (ϵ -Caprolactone) Films Enhance Adhesion and Growth of Human Cultured Epidermal Keratinocyte and Dermal Fibroblast Cells, *Tissue Eng.* 13 (4) (Apr. 2007) 789–798, <https://doi.org/10.1089/ten.2006.0321>.
- [87] S. Chandrasekaran, U.-B.T. Giang, M.R. King, L.A. DeLouise, Microenvironment induced spheroid to sheeting transition of immortalized human keratinocytes (HaCaT) cultured in microbubbles formed in polydimethylsiloxane, *Biomaterials* 32 (29) (Oct. 2011) 7159–7168, <https://doi.org/10.1016/j.biomaterials.2011.06.013>.
- [88] T. Pellinen, et al., Integrin trafficking regulated by Rab21 Is necessary for cytokinesis, *Dev. Cell* 15 (3) (Sep. 2008) 371–385, <https://doi.org/10.1016/j.devcel.2008.08.001>.
- [89] M. Tanaka, A. Takayama, E. Ito, H. Sunami, S. Yamamoto, M. Shimomura, Effect of pore size of self-organized honeycomb-patterned polymer films on spreading, focal adhesion, proliferation, and function of endothelial cells, *J. Nanosci. Nanotechnol.* 7 (3) (Mar. 2007) 763–772, <https://doi.org/10.1166/jnn.2007.514>.
- [90] C.N. Berger, K. S. Sturm, Self renewal of embryonic stem cells in the absence of feeder cells and exogenous leukaemia inhibitory factor, *Growth Factors* 14 (2–3) (Jan. 1997) 145–159, <https://doi.org/10.3109/08977199709021517>.
- [91] S. Sugita, M. Hozaki, T.S. Matusi, Y. Ujihara, S. Deguchi, M. Nakamura, Retardation can quantify tension in single stress fibers? *Biophys. J.* 118 (3) (Feb. 2020) 601a–602a, <https://doi.org/10.1016/j.bpj.2019.11.3251>.
- [92] N. Shah, Y. Morsi, R. Manasseh, From mechanical stimulation to biological pathways in the regulation of stem cell fate, *Cell Biochem. Funct.* 32 (4) (2014) 309–325, <https://doi.org/10.1002/cbf.3027>.
- [93] E.K.F. Yim, E.M. Darling, K. Kulangara, F. Guilak, K.W. Leong, Nanotopography-induced changes in focal adhesions, cytoskeletal organization, and mechanical properties of human mesenchymal stem cells, *Biomaterials* 31 (6) (Feb. 2010) 1299–1306, <https://doi.org/10.1016/j.biomaterials.2009.10.037>.
- [94] L.C.Y. Lee, et al., Nanotopography controls cell cycle changes involved with skeletal stem cell self-renewal and multipotency, *Biomaterials* 116 (Feb. 2017) 10–20, <https://doi.org/10.1016/j.biomaterials.2016.11.032>.