Electromechanically active polymer actuators based on biofriendly choline ionic liquids

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Abstract
Smart and soft electroactive polymer actuators have many beneficial properties, making them attractive for biomimetic and biomedical applications. However, the selection of components to fabricate biofriendly composites has been limited. Although biofriendly options for electrodes and membranes are available, the conventional ionic liquids (ILs) often used as the electrolytes in the actuators have been considered toxic in varying degrees. Here we present a smart electroactive composite with carefully designed and selected components that have shown low toxicity and a biofriendly nature. In the present study, polypyrrole-PVdF trilayer actuators using six different choline ILs were prepared and characterized. Choline ILs have shown promise in applications where low environmental and biological impact is critical. Despite this, the anions in ILs have a strong impact on toxicity. To evaluate how the anions effect the bioactivity of the ILs used to prepare the actuators, the ILs were tested on different microbial cultures (Escherichia coli, Staphylococcus aureus, Shewanella oneidensis MR-1) and HeLa cells. All of the selected choline ILs showed minimal toxic effects even at high concentrations. Electro-chemomechanical characterization of the actuators indicated that polypyrrole-PVdF actuators with choline ILs are viable candidates for soft robotic applications. From the tested ILs, choline acetate showed the highest strain difference and outperformed the reference system containing an imidazolium-based IL.

Supplementary material for this article is available online

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(Some figures may appear in colour only in the online journal)

1. Introduction

Technological development towards bioinspired soft systems requires replacement of traditional rigid actuators with more compliant and adaptive materials.

Electromechanically active polymers (EAPs) are a class of smart materials, which either respond to external electrical stimulus with change in shape or generate an electrical signal in response to mechanical deformation. A subgroup of EAPs are ionic electromechanically active polymers (IEAPs), in which the electrically driven motion of ions causes actuation. IEAP actuators can be prepared in different configurations showing either bending or linear movement. A typical bending actuator is a soft trilayer laminate consisting of two electrodes separated by an ion-permeable membrane containing an electrolyte responsible for the actuation. IEAPs have potential for biomimetic and medical applications due to the large strain values achieved already under very low voltage [1], relatively simple structure [1], silent operation...
IEAPs consisting of polypyrrole focused on applying low toxicity choline based ILs in trilayer biocompatible in any system. Therefore, the current study was composite materials cannot be expected to be biofriendly or without an electrolyte that has shown low toxicity, the smart EAPs and it is the best choice when biocompatibility is been widely studied in IEAPs different occasions. It is one of the main challenges for such applications is the preparation of biologically benign IEAPs, capable of working under ambient conditions [7]. More often than not, the materials used to fabricate EAPs are toxic at the very least. In the current study we demonstrate, that a careful selection of starting materials enables one to develop truly biofriendly IEAPs.

Figure 1 gives an overview of the materials used for the preparation of IEAPs and their fit with living matter. There are suitable materials available for biofriendly electrodes and membranes [8]. Without encapsulation [9], the highest obstacle until now has been the electrolyte. Ionic liquids (ILs) commonly used as the electrolytes in IEAPs are known to have toxicity issues [10]. For example a popular choice has been 1-alkyl-3-methylimidazolium tetrafluoroborates [11], which are related to serious environmental hazards and toxicity towards living matter [12]. Moreover, [BF₄]⁻ salts hydrolyze, releasing highly corrosive hydrogen fluoride [13]. Without an electrolyte that has shown low toxicity, the smart composite materials cannot be expected to be biofriendly or biocompatible in any system. Therefore, the current study was focused on applying low toxicity choline based ILs in trilayer IEAPs consisting of polypyrrole (PPy) electrodes and polyvinylidene fluoride (PVdF) membrane. Both materials have been widely studied in IEAPs [1]. PVdF is medically approved and the biocompatibility of PPy has been proven in different occasions.

Conductive polymers (CP), including PPy, are well established electrode materials for IEAPs due to several advantages: high conductivity (10²–10³ S cm⁻¹), low operation voltage (<1 V), stability in air, low cost, tailorable properties (e.g. biocompatibility and biodegradability [14]), and miniaturizability [15]. PPy has been widely used for EAPs and it is the best choice when biocompatibility is required. Furthermore, the dopant selection enables tuning of the mechanical, electrical, electrochemical, biological properties, as well as the stability of PPy films.

The IL interaction with PVdF membrane influences the ionic conductivity and the mechanical properties of the material in question, which in turn determine in large part the actuator displacement [16]. These PVdF-IL interactions can depend on the cation chain length but also from the conformation of the cation size [17]. Because of its non-cytotoxic nature towards C2C12 myoblast cell-line, PVdF has been proven to be useful in the biomedical field [18]. Interestingly, the incorporation of ILs into actuators with PVdF membranes can significantly increase their maximum bending capabilities [19].

The toxicity and bioactivity of ILs have been intensely studied since the previous decade. The general consensus is that the toxicity of ILs depends on both the specific anions and cations [10]. According to the current data, the best candidates to replace the bio-harmful ILs are choline (N,N,N-trimethylammonium) based salts. Choline has many biological functions in the human body [20], for example it is vital for building up cell membranes and neurotransmitters [21]. Choline chloride is probably the most tested and applied choline salt, it also has low toxicity to human cell lines [22]. Other choline ILs have also been found to be harmless enough for medical applications, like choline phosphate [23] and choline dihydrogen phosphate [24]. There is a diverse body of data available concerning the harmlessness of choline salts towards various phyla of living organisms. Choline ILs have low toxicity towards different microbial cultures like Escherichia coli, Staphylococcus aureus, Salmonella enteritidis, and Listeria monocytogenes [25]. Cytotoxicity of different choline ILs has also been found to be low in case of different human tissue culture cells including cervix carcinoma cells (HeLa). It is also important to note when considering the biodegradability of materials, that choline salts can be decomposed by microorganisms [26]. Thus, designing choline-based ILs for use as electrolytes in IEAP actuators is a promising route to biologically benign electroactive composites. Although there are few examples about the usage of choline ILs in various electrochemical systems [27], to the best of our knowledge, choline ILs have not been tested in IEAP actuators so far.

Our first focus was on synthesizing choline ILs, which have an advantage over solid electrolytes. Room temperature ILs can be used outside of a solvent. This feature combined with the low-toxicity of ILs consisting of choline cations and organic anions can form the basis for harmless bio-actuators working in open air.

In this work, trilayer PPy-PVdF actuators containing choline ILs as the electrolytes were prepared and investigated, to evaluate the potential of biofriendly actuators (figure 2). Six different choline ILs were synthesized, characterized, and tested against an imidazolium-based IL. Evaluation of the electrochemomechanical performance of the actuators revealed significant differences in actuator properties in case of different ILs. In addition, harmlessness of the specific choline ILs used in this study was investigated on three
2. Materials and methods

2.1. Choline ILs

Choline ILs were synthesized from choline bicarbonate (Sigma-Aldrich, 80% in H2O) and the respective carboxylic acids (Sigma-Aldrich, used as received) using an established method [28]. Choline bicarbonate was mixed in 1:1 molar ratio with the carboxylic acids dissolved in water under ambient conditions for 12 h. The obtained product was purified by extraction with ethyl acetate. Water was removed by evaporation at 60 °C under vacuum. An overview of the synthesized ILs and their properties is given in table 1. 1-Ethyl-3-methylimidazolium trifluoromethanesulfonate ([EMIM][OTf]) (purity >99.5%, Solvionic) (water content 1.1%), 1-Ethyl-3-methylimidazolium bis (fluorosulfonfyl)imide ([EMIM][FSI]) (purity >99.5%, Solvionic) and 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide ([EMIM][TFSI]) (purity >98%, Fluka) did not undergo extra purification.

The water content of the choline ILs was determined by Karl Fischer coulometric titration (Mettler Toledo DL32). 1H NMR spectra were recorded with a Bruker Avance II 400 MHz spectrometer using D2O as the solvent. The IR spectra were recorded with a Bruker Tensor 27 FTIR spectrometer in the scanning range 400–4000 cm⁻¹, with a resolution of 4 cm⁻¹. For each spectrum, 64 scans were averaged.

The ionic conductivities of choline ILs were calculated from the high-frequency real resistance obtained by electrochemical impedance spectroscopy performed using a Parstat 2273 potentiostat-galvanostat. The measurements were carried out using a Micrux microfluidic chip with interdigitated gold electrodes. A small drop of each IL was placed on the electrodes and the impedance of the IL was determined. The impedance was determined by scanning in forward (from low to high frequency) and in reverse (from high to low frequency) directions. Each measurement was repeated six times. The influence of water uptake on the ionic conductivity of choline ILs was evaluated by repeated experiments. The impedance was measured for all synthesized choline ILs directly after synthesis, after 10 min, 1 h, and 3 weeks under ambient conditions (35.5 RH%, 25 °C, normal atmospheric pressure).

2.2. Bacterial strains and HeLa cells

Uropathogenic strain of E. coli (CFT073) and S. oneidensis MR-1 (ATCC700550) were used as model organisms for Gram-negative bacteria. S. aureus DSM2569 (ATCC2013) was used as a model organism for Gram-positive bacteria.

To assess the cytotoxicity of chosen ILs, a widely exploited cell line—human cervical cancer epithelial cells—HeLa was used.

2.3. Minimum inhibitory concentration (MIC) assessments and HeLa CC50 assessments

Assessments of MIC towards microbial cultures were done according to a procedure adapted from elsewhere [29, 30]. Cell viability was assessed using a 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich).

For both experiments (done in triplicate), the details can be found in the Supplementary Information section.

2.4. pH of choline IL solutions

The pH of the IL solutions was studied at three different concentrations: 1 M, 125 mM, and 15.65 mM, using three different solvents: pure water (B. Braun), Mueller Hinton Broth (MHB, cation adjusted, BD) and 0.4 M 3-(N-morpholino)propanesulfonic acid (MOPS, Sigma-Aldrich) and 0.02171 M tricin (Sigma-Aldrich) buffer pH 7.2 (MHB + MOPS), and fresh Dulbecco’s Modified Eagle’s Medium (high glucose, Sigma-Aldrich) (DMEM).

The pH of these solutions was measured with a pH/conductivity meter (CPC-411, Elmetron).
2.5. Fabrication of PPy-PVdF actuators

PPy-PVdF actuators were fabricated by depositing PPy electrodes electrochemically on PVdF and immersing the obtained trilayer polymer laminates into ILs used as the electrolyte.

2.6. PVdF membranes

Commercial PVdF membranes (Millipore Immobilon-P, Sigma-Aldrich) had a thickness of 110 μm, pore size of 0.45 μm and a porosity of 70%.

The ionic conductivities of the membranes in the ILs were determined from the impedance spectroscopy measurements as described in section 2.1. The commercial PVdF membranes were immersed in the various ILs under nitrogen atmosphere for 48 h prior to the experiments. Thereafter, the soaked membrane was placed between two round gold contacts (d = 8 mm) that were attached to a thickness gauge and the sample thickness was also registered during each experiment. Any excess IL was wiped off with a filter paper prior to each measurement. The dry conductivity corresponds to the soaked sample being taken from the nitrogen atmosphere and impedance measured immediately. After the first measurement, the samples were allowed to absorb humidity from the ambient air until equilibrium was reached after 10 min, and then the impedance was measured again.

2.7. Electrochemical synthesis of PPy electrodes

PPy electrodes were electrochemically deposited on the membranes. PVdF membranes were initially sputter coated with a thin (20 nm) gold layer using a Leica EM ACE600 to make the membrane surface conductive.

The following electrochemical synthesis was done according to the procedure described earlier by our group [31]. The membranes went through the same galvanostatic electrochemical synthesis, where dodecylbenzenesulfonate-doped PPy was deposited on both sides (30 mm × 30 mm) of the membrane. The synthesis was carried out in a solution containing 0.2 M pyrrole (Py) (Sigma-Aldrich, distilled at reduced pressure before use) and 0.2 M sodium dodecylbenzenesulfonate (NaDBS) (Sigma-Aldrich, technical grade) in a mixture of MilliQ water and mono ethylene glycol (MEG) (Fluka, 99% purity) in a volume ratio 1:1. The synthesis was controlled with PARSTAT 2273 potentiostat/galvanostat and carried out in a one-compartment electrochemical cell at lowered temperature (−23 °C ± 2 °C). The sputter-coated membrane was the anode and symmetrical stainless steel (AISI 316 L) mesh sheets were the cathodes. PPy was synthesized applying current density of 0.1 mA cm⁻² for 20 000 s. The prepared composite was washed with ethanol and MilliQ water, and then dried at 1 mbar at room temperature for 24 h.

Each resulting sheet of material was cut into 3 × 14 mm strips, which were immersed in various ILs for at least 72 h before electromechanical characterization.

Table 1. The synthesized ILs and their properties.

| Ionic liquid          | Anion structure | Yield, % | Water content, % |
|-----------------------|-----------------|----------|------------------|
| Choline acetate ([Ch][Ac]) |                  | 97       | 5.4              |
| Choline isobutyrate ([Ch][Ib]) |              | 96       | 3.7              |
| Choline isovalerate ([Ch][Iv]) |                    | 93       | 3.6              |
| Choline 2-methylbutyrate ([Ch][2 mb]) |                   | 81       | 3.7              |
| Choline malonate ([Ch][Mal]) |                    | 95       | 0.1              |
| Choline glutarate ([Ch][Glu]) |                   | 89       | 0.5              |
| Choline citrate ([Ch][Cit]) |                    | 86       | 0.7              |
Scanning electron microscopy (SEM) images and energy-dispersive x-ray (EDX) sulfur mapping of the cross-sections (samples broken in liquid nitrogen) of the synthesized materials were obtained using a Hitachi TM3000 microscope (acceleration voltage of 15 kV, back-scattered electron detector) with an Oxford Instruments SwiftED 3000 EDX analyzer.

2.8. Electro-chemo-mechanical characterization of PPy-PVdF actuators

2 mm of the actuators was mounted sideways between gold contacts and the rest (12 mm) was free to move. The displacement of the actuator was measured at 5 mm from the fixed end using a laser displacement meter. The actuators were driven using the following signals: a square wave potential steps signal between ±1 V at 0.001 Hz, with neutral voltage period before each polarization step; and triangular voltage signals between ±1 V with scan rates of 5 mV s⁻¹ and 50 mV s⁻¹. Maximum strain differences were calculated relative to the position of a full bending cycle according to the formula [32]:

$$\varepsilon = \frac{2 \cdot D \cdot W}{L^2 + D^2},$$

where ε is the strain difference, D is half of the peak to peak displacement from graph, W is the thickness of the actuator (assuming constant thickness) that was measured with a micrometer, and L is the distance from the fixed end of the actuator to the projection of the laser beam to the middle position of the actuator.

Cyclic voltammetry (CV) response of the actuators was measured in a two-electrode system using a PARSTAT 2273 potentiostat. Five cycles in the range of ±1 V at a scan rate of 5 and 50 mV s⁻¹ were recorded.

All measurements described here were carried out under ambient conditions (35.5 RH%, 25 °C, normal atmospheric pressure).

2.9. Assessment of harmlessness of PPy-PVdF actuators

The harmlessness of the PPy-PVdF actuators was assessed with the disk diffusion method [33, 34] using E. coli described in 2.2 as the test organism. The density of the E. coli suspension was adjusted to 0.5 McFarland standard ($A_{600} = 0.063$; between $1 \times 10^8$ and $2 \times 10^8$ CFU ml⁻¹) using Ultrospec 7000 (Biochrom LTD, UK) at 600 nm wavelength.

E. coli suspension was smeared on the surface of the LB agar. Actuators previously immersed in ILs were cut into 4 × 4 mm squares and placed on the surface of the inoculated agar with sterilized forceps. All six choline ILs liquid at room temperature were tested against [EMIM][OTf], which was used in the reference actuators. Two additional imidazolium ILs popular in soft actuators were used as negative controls (1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide ([EMIM][TFSI]), 1-Ethyl-3-methylimidazolium bis (fluorosulfonyl)imide ([EMIM][FSI])). After incubation at 37°C for 22 h, the plates were photographed on a black non-reflecting background and their inhibition halo diameters assessed. This experiment was done in a triplicate.

3. Results and discussion

The goal of the present study was to analyze IEAP actuators prepared from biocompatible and biofriendly starting materials and to evaluate how their performance is influenced by the different choline ILs as the electrolytes. First, the choline ILs were tested separately form the PPy-PVdF actuators to assess their biological influence on bacteria and human cells. Choline ILs were also characterized for ionic conductivity individually and in PVdF membrane. This was followed by the thorough characterization of the electrochemomechanical properties of the PPy-PVdF actuators with choline ILs. Finally, assessment of the influence of PPy-PVdF actuators with choline ILs on bacterial growth was carried out.

3.1. Yields and ionic conductivity of choline ILs

The yields, the residual water content in choline ILs after drying, and the melting points are presented in Table 1. At room temperature, all the synthesized choline ILs, except choline citrate, were liquids with varying viscosities. The ILs were obtained with high yields, >90% for most of the compounds. Although choline ILs are very hygroscopic, only for [Ch][Ac] the water content after drying exceeded 5%. The structures and the purities of the obtained choline ILs were confirmed with $^1$H NMR and IR (supplementary information is available online at stacks.iop.org/SMS/29/055021/mmedia).

Although the ionic conductivities are not the only factors concerning the performance of choline ILs in actuators for producing large strain difference, they still are an important part of the overall description of the system [16]. In Table 2, the ionic conductivities of all the synthesized ILs and the membranes saturated with the ILs are presented. The latter is more determinant for obtaining a high-performance actuator, being a function of ion mobility and the interactions of ILs with the membrane material [19].

The ionic conductivities of the membranes saturated with the ILs followed the same general trend as the ionic conductivities of the pure ILs (Table 2), with [Ch][Ac] having the highest ionic conductivity in both cases, and [Ch][Glu] the lowest. Hence, the ionic conductivity of the PVdF membranes immersed in ILs was primarily determined by the ionic conductivity of the ILs inside, indicating that there are no specific interactions between the membrane and ILs, and therefore, the ions in the IL are free to move in an applied electric field. The ionic conductivities increased in time due to the absorbed water as the ILs and the saturated PVdF membranes were kept under ambient conditions.

3.2. Toxicological testing of choline ILs

Apart from the evidence that choline ILs can be relatively safe for most tested systems, there has been no analysis on the interplay of the effects of choline and the anions in the ILs.
Table 2. Ionic conductivities ($\kappa$) of the ILs and the membranes saturated with the ILs. For the ILs, the conductivity was measured after synthesis; after 10 min under ambient conditions; after 1 h under ambient conditions; after 3 weeks under a fume hood. For membranes, the conductivity was measured directly after immersing in inert atmosphere and after 10 min under ambient conditions.

| Ionic liquid | Ionic conductivity of ILs $\kappa$/mS cm$^{-1}$ | Ionic conductivity of ILs in PVdF membranes $\kappa$/mS cm$^{-1}$ |
|--------------|-----------------------------------------------|--------------------------------------------------|
|              | AS$^{a}$ 10 min 1 h 3 weeks PVdF PVdF, 10 min |                                    |
| [Ch][Ac]    | 1.67 1.82 3.88 4.28 0.44 0.55               |                                    |
| [Ch][Ib]    | 0.64 1.70 2.28 2.40 0.01 0.05               |                                    |
| [Ch][Iv]    | 0.28 0.99 1.80 1.87 0.02 0.09               |                                    |
| [Ch][2 mb]  | 0.55 1.44 1.72 1.89 0.04 0.09               |                                    |
| [Ch][Mal]   | 0.12 0.11 0.60 2.65 0.03 0.12               |                                    |
| [Ch][Glu]   | 0.02 0.02 0.20 0.67 0.00 0.02               |                                    |

$^{a}$AS—as synthesized.

Table 3. The MIC values after 22 h anoxic, and 48 h of anoxic and anaerobic exposure of the tested choline ILs in respect to Escherichia coli, Staphylococcus aureus, and Shewanella oneidensis MR-1. CC$\text{_{50}}$ values of the tested choline ILs in respect to HeLa cells. Values expressed in mM.

| IL      | Escherichia coli Anoxic 22 h MIC | Staphylococcus aureus Anoxic 22 h MIC | Shewanella oneidensis MR-1 Anoxic 22 h MIC | Anaerobic 48 h MIC | HeLa CC$\text{_{50}}$ |
|---------|---------------------------------|--------------------------------------|-----------------------------------------------|-------------------|--------------------|
| [Ch][Ac] | 600                             | >1000                                | 250                                          | 350               | 350                | 57                 |
| [Ch][Ib] | 500                             | >1000                                | 200                                          | 300               | 300                | 64                 |
| [Ch][Iv] | 400                             | 600                                  | 300                                          | 350               | 350                | 25                 |
| [Ch][2 mb]| 450                             | 850                                  | 150                                          | 300               | 300                | 50                 |
| [Ch][Mal]| 140                             | 125                                  | 45                                           | 45                | 45                 | 23                 |
| [Ch][Glu]| 125                             | 90                                   | 45                                           | 45                | 45                 | 28                 |
| [Ch][Cit]| 125                             | 62                                   | 22.5                                         | 45                | 45                 | 10                 |

There is also no ground to compare the sensitivity of different organisms towards particular choline ILs. Hence, additional systematic studies on the biofriendliness of choline ILs are needed where the toxicity of the same IL structures towards different organisms is compared. Moreover, the majority of studies have been focused on bacteria and marine life [35]. Mammalian cells in comparison have gained significantly less attention although being more relevant concerning potential applications of ILs in medicine and biotechnology. Without knowing the full extent of the effects on various phyla of living organisms, the applications of ILs will remain more limited than expected based on their extraordinary physico-chemical properties. As the ILs tested in this study contain the same cation, differences in the toxicity are caused by the anions. The anions can be differentiated according to the length and branching of the alkyl chain, and the number of carboxyl groups. All these characteristics are expected to affect the physico-chemical properties of the ILs, and thus, toxicity.

3.2.1. Growth inhibition of bacteria by choline IL. To investigate the toxicological effects of the ILs, to be used in harmless actuators, microorganisms are excellent candidates, because of their environmental and industrial impact, and the robustness of the toxicity assays. It has been shown previously that ILs interact differently with Gram-negative and Gram-positive bacteria, and therefore, it is important to test both [36]. Besides, the toxicity of ILs towards Shewanella oneidensis MR-1, a bacterium with reducing capacity, was evaluated. To the best of our knowledge, choline ILs have not been tested before on the facultatively anaerobic Shewanella strains that are highly diverse in their metabolic activities [37]. Hence Shewanella oneidensis MR-1 was chosen to test if it can possibly chemically modify choline ILs and, as a result, change their toxic effects.

It can be clearly seen from the average MIC values in table 3 that, despite having the same choline cation, the ILs tested in this research showed various toxicological effects against Escherichia coli, Shewanella oneidensis MR-1, and Staphylococcus aureus. The highest MIC values (table 3) for all the test organisms were found for [Ch][Ac], which has been shown previously to have high MIC values for other bacterial strains [25]. In general, the MIC values from the current study are very high compared to the results reported for other types of substances (figure 3). Thus, the current study confirms the low toxicity of choline ILs toward bacteria, which is in good agreement with the existing data related to choline ILs with other anions, e.g. based on other carboxylates [38] and amino acids [25].

Many of the ILs with monocarboxylic anions showed higher MIC values with the decrease in alkyl side chain lengths. Similar trends in literature for IL anions [38, 41] and cations [36, 42] have been related to the interactions with biological membranes [43]. This is a very general structure-toxicity relationship in agreement with results outside the IL field [44] and the interaction affinity between ILs and cells.
[45]. Comparison between [Ch][IV] and [Ch][2 mb] indicated that branching of the alkyl chains also plays a role as both anions have the same number of carbons in a chain, but a methyl group is positioned differently. However, the differences arising from alkyl chain length and branching are minor compared to the toxic effect of additional monocarboxylic groups. MICs for [Ch][Mal], [Ch][Glu], and [Ch][Cit] were significantly lower than the values for the monocarboxylates. It can partially be related to the decreased pH of the growth media (supplementary onformation).

Noticeably, the data in table 3 indicates higher harmful effect of choline monocarboxylates to Gram-negative E. coli, than Gram-positive S. aureus. Although this seems contradictory to the general trend in the literature, there are also studies, which have suggested a different kind of process occurring when it comes to choline IL interactions with Gram-negative E. coli and S. enteritidis versus Gram-positive S. aureus and L. monocytogenes [25]. In this study, the Gram-negative bacteria were more sensitive to the effects of choline amino acid ILs than the Gram-positive ones. The same kind of effect has been confirmed by another study [46].

Although the S. oneidensis MR-1 showed more sensitivity towards all the tested choline ILs after 22 h in anoxic environment compared to E. coli and S. aureus, the measured MICs were still high enough to consider the tested choline ILs as harmless substances. There was no difference in the anoxic and anaerobic 48 h MICs (table 3) despite its genus having the capability to couple organic matter oxidation to the reduction of a wide range of electron acceptors [37]. This shows that S. oneidensis MR-1 has no effect on the harmfulness of the tested choline ILs.

It was hypothesized that the change of pH in growth media due to the addition of choline ILs was causing adverse effects on bacteria (and human cells). pH measurements were carried out in pure distilled water, DMEM and MHB + MOPS at different IL concentrations (supplementary information). The pH of choline ILs with monocarboxylic anions remained around neutral. These ILs also showed higher MIC and CC values (table 3). The lowered pH was very probably the main cause of the observed growth inhibition of ILs that have di- and tricarboxylic anions.

3.2.2. The response of HeLa cells to choline ILs. For practical applications involving interaction with living organisms, the ILs used need to have outstanding biocompatibility. Cytotoxicity is among the key values to assess the harmlessness of ILs. In this study, the toxicity of the selected choline ILs was determined on HeLa cells, which are widely used to test the cytotoxicity of various compounds. The CC50 values of the studied choline ILs are summarized in table 3.

Similarly to the results with bacteria, monocarboxylic choline ILs presented the lowest cytotoxicity. According to the data in table 3, the most benign ILs were [Ch][Ac], [Ch][Br], and [Ch][2 mb]. Interestingly, the structurally similar [Ch][Iv] gave a CC50 value comparable to choline ILs with dicarboxylic and tricarboxylic anions. The difference between the monocarboxylic choline IL CC values may be caused by the varying carbon chains on choline IL anions. However, it is surprising that the different branching in [Ch][Iv] and [Ch][2 mb] leads to such a remarkable difference in toxicity. Similarly to the results with bacteria, it is suspected that the toxic effect of [Ch][Mal], [Ch] [Glu], and [Ch][Cit] was mainly caused by pH change in the growth media, which in case of di- and tricarboxylic salts occurs already at relatively low concentrations comparable with the measured CCs. Although most of the cell-lines are capable of sustaining themselves in mildly acidic environments, diversion of pH from the optimal range can still affect the metabolic cycle and cellular growth. Change of even one pH unit has been shown to severely affect the growth of HeLa cells [47]. In case of the monocarboxylates, much higher concentrations are needed to decrease the pH, indicating that there are other additional mechanisms behind the cytotoxicity.

It is clear, that the selected choline ILs demonstrate low toxicity toward HeLa cells. This is in line with previous studies carried out with other choline ILs [48].

3.3. Characterization of PPy-PVdF actuators

3.3.1. Structure and morphology of PPy-PVdF actuators. The structure and morphology of PPy-PVdF actuators was assessed from SEM images. The overall thickness of the actuators was around 130 µm and did not change significantly after immersing in the ILs, although during immersion, the mass of PPy-PVdF actuators doubled. The surface of the PPy-PVdF actuators was compact but still ion permeable.

The PPy layer was additionally investigated with EDX sulfur mapping, visualizing the distribution of [DBS]− anions in the materials. Higher concentration of [DBS]− anions in the outer layers of the actuators indicates the presence of PPy (figure 4).

3.3.2. Electro-chemo-mechanical properties PPy-PVdF actuators. PPy was synthesized via the oxidation of the monomer and it was, therefore, deposited on the electrode in its charged conducting form. For electrical neutrality, anions
(dopants) must be introduced into the polymer layer during synthesis. There are two main mechanisms for the contraction and expansion of PPy films under electrical stimulation. First, if a small and mobile dopant (e.g. Cl) has been used, it is expelled from the polymer matrix during reduction and reintroduced from the surrounding medium during subsequent oxidation. This has been a common approach for doping PPy in biological systems, since chloride anions are biologically compatible and typically readily available in the medium for reintroduction [49].

Another approach, the one also applied in the present study, involves a larger dopant (e.g. dodecylbenzenesulfonate ([DBS]−)) that remains mostly trapped in the polymer matrix. The main benefit of a larger dopant is that it cannot leach out or be exchanged with other ions from the surrounding environment. During reduction of the PPy layer, the charge balance is maintained by the introduction of cations from the medium (e.g. choline from the IL), which are expelled during the subsequent oxidation of the electrode.

For many systems, a combination of both mechanisms occurs [50], which is usually undesirable, as it lowers the net displacement as well as makes controlling such actuators difficult. In any case, even for mainly cation-active actuators, the anion of the electrolyte still strongly influences the overall performance either by counteracting the cation activity or supplementing it. This phenomenon is also seen in the current study. [DBS]− was chosen for the synthesis as the immobile anion for promoting cation transport.

The displacement of the PPy-PVdF actuators was studied using different driving signals as described in section 2.8. First, the preliminary measurements were carried out by driving all the actuators with square wave potential. Based on these results, the actuators with three ILs were chosen for further in-depth characterization: actuators with [Ch][Ac] and [Ch][Ib] were chosen to represent systems with monocarboxylic choline ILs; the one with [Ch][Mal] was chosen to represent an actuator with dicarboxylic choline ILs. In addition, [EMIM][OTf] was used as a conventional IL reference to compare with the biofriendly choline ILs. The selected actuators were then driven with triangular voltage signal while recording the current response.

**Figure 4.** Cross-sectional SEM image (A) and EDX sulfur map (distribution of [DBS]− anions) (B) of PPy-PVdF.

**Table 4.** Strain difference (%) calculated from the displacement according to (1). sq—actuated with square wave voltage between ±1 V at 0.001 Hz; CV 5—actuated with triangle wave CV signal between ±1 V at 5 mV s−1; CV 50—actuated with triangle wave CV signal between ±1 V at 50 mV s−1.

| Ionic liquid | sq  | CV 5 | CV 50 |
|--------------|-----|------|-------|
| [Ch][Ac]     | 0.67 ± 0.09 | 0.65 ± 0.05 | 0.30 ± 0.03 |
| [Ch][Ib]     | 0.19 ± 0.08  | 0.49 ± 0.07  | 0.15 ± 0.05  |
| [Ch][Iv]     | 0.42 ± 0.10  | NTa          | NT            |
| [Ch][2-mbs]  | 0.37 ± 0.03  | NT           | NT            |
| [Ch][Mal]    | 0.11 ± 0.02  | 0.06 ± 0.01  | 0.02 ± 0.00   |
| [Ch][Glu]    | 0.05 ± 0.01  | NT           | NT            |
| [EMIM][OTf]  | 0.16 ± 0.02  | 0.05 ± 0.01  | 0.04 ± 0.01   |

a NT—not tested.

As seen from the results, there were significant differences in the actuator performance, despite [Ch]+ being the mobile species responsible for actuation in all of them. Strain differences calculated from the displacements with different driving signals are summarized in table 4. Change in the displacement amplitude of the actuators in time was negligible. The best candidates among the tested choline ILs in terms of displacement were [Ch][Ac] and [Ch][Ib].

CV was used to obtain more insight about the electrochemical processes in the actuators, which are directly related to the performance. In figure 5, selected cyclic voltammograms (CVs; second cycle) for PPy-PVdF actuators are presented. There was no noticeable CV surface area change from cycle to cycle. While other ILs made use of the whole potential range, the optimal range for actuators with [Ch][Ac] was found to be just −0.4 to 0.4 V, as the charging/discharging decreased considerably beyond that. The voltage range may be tuned to taste by altering the porosity of PPy by modifying the synthesis conditions [51].

While [Ch][Ac] did show the highest ionic conductivity both separately and in PVdF, which is reflected in the exchanged currents and actuation performance, the outstanding results of [Ch][Ib] with the CV 5 signal, in comparison to [Ch][Mal]
cannot be traced back to differences in ionic conductivity. It is likely that either charge distribution, interactions in ion clusters or some other specific effects had to be behind these remarkably different results.

3.3.3. Antimicrobial properties of PPy-PVdF actuators. Despite the individual components of PPy-PVdF actuators being established as harmless compounds, the whole composite has to be tested for biological effects as well. For that purpose, disk diffusion tests were conducted. Bacterial disk diffusion is a quick and elegant way of testing the biological impact of a material. *E. coli* was chosen as the test organism because of its biological and medical relevance and short generation time. Because [*Ch*][*Cit*] is a solid and thus cannot be tested in PPy-PVdF actuators under normal conditions, [*Ch*][*Cit*] was excluded from this experiment.

No inhibition rings were observed for PPy-PVdF actuators with choline ILs of monocarboxylate anions (figure 6). For choline ILs with dicarboxylic anions, there was minimal development of rings after 22 h, indicating their more harmful nature towards *E. coli*. Two imidazolium ILs, namely [*EMIM*][*TFSI*] and [*EMIM*][*FSI*], showed the largest signs of inhibition. One possibility is, that this indicates more efficient diffusion of the chosen imidazolium ILs into the solid agar medium, compared to choline ILs. The other possibility is simply higher toxicity of the ILs. Dicarboxylic choline ILs can also increase the acidity of the solid agar medium, and thus, damage the bacterial cell membrane. The disk diffusion experiment corroborated the MIC and EC50 values, where monocarboxylic choline ILs also showed the lowest inhibition of bacterial growth. These results show that PPy-PVdF actuators applying choline ILs with monocarboxylic anions as the electrolytes, are viable candidates for harmless soft polymer actuators.
4. Conclusion

The aim of this study was to develop biofriendly IEAP actuators made of biofriendly components with most favorable properties in terms of actuation performance. This was achieved through careful selection of these materials. Six choline ILs were tested as the electrolytes in PPy-PVDF actuators. Comparison with a reference system containing an imidazolium IL indicated that such combination of materials resulted in high-performance actuators, which in case of choline acetate and choline isobutyrate even exceeded the performance of the reference.

The toxicity of seven choline carboxylate ILs was tested against three bacteria (E. coli, S. aureus, S. oneidensis MR-1) and HeLa cells. Although all the tested ILs were found to have low toxicity, there were remarkable differences between ILs depending on the number of carboxylic groups in the anions. Dian and tricarboxylic choline ILs decreased the pH of the growth media, which correlated with the increase of their harmful effect towards bacteria and mammalian cells. Although the monocarboxylates showed very low toxicity, there were still differences between the various structures related to the length and branching of the alkyl chain. S. oneidensis MR-1 was evaluated for the first time. According to the results, the reduction-capable bacteria did not cause changes in the chemical structure of the ILs, as no significant increase in their toxicity was detected. Although the MICs in case of S. oneidensis MR-1 were lower compared to those of E. coli and S. aureus, all the selected choline ILs can be considered benign towards the tested bacteria and human cells.

In addition to the harmlessness of individual choline IL the actuators containing choline ILs showed either no or minimal inhibition of the growth of E. coli, hence the selection of biosafe starting materials proved to be efficient in the fabrication of harmless soft polymer actuators.

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Conflicts of interest

Declarations of interest

None.

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