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Biocompatibility of polyaniline

Petr Humpolicek[a,b,*], Vera Kasparkova[a,c], Petr Saha[a], Jaroslav Stejskal[d]

[a] Centre of Polymer Systems, Polymer Centre, Tomas Bata University in Zlin, T.G. Masaryk Sq. 5555, 760 05 Zlin, Czech Republic
[b] Polymer Centre, Faculty of Technology, Tomas Bata University at Zlin, T.G.M. Sq. 275, 762 72 Zlin, Czech Republic
[c] The Department of Fat, Surfactant and Cosmetics Technology, Faculty of Technology, Tomas Bata University at Zlin, T.G.M. Sq. 275, 762 72 Zlin, Czech Republic
[d] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovsky Sq. 2, 162 06 Prague 6, Czech Republic

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ABSTRACT

Both the non-conducting polyaniline, emeraldine base, and its conducting form, polyaniline hydrochloride, were tested for their biocompatibility in terms of skin irritation, sensitization and cytotoxicity performed on human immortalized non-tumorigenic keratinocyte and human hepatocellular carcinoma cell lines. The testing was carried out on extracts of polyaniline powders in agreement with requirements of international standards applicable for testing of medical devices. The results can be hence generally employed in all types of materials and devices containing polyaniline in various concentrations. The study confirmed that polyaniline has not induced any sensitization and skin irritation either. In contrast, both polyaniline forms showed considerable cytotoxicity, which was higher for polyaniline hydrochloride compared to polyaniline base and was observed on both cell lines. Differences between cytotoxicity found on human immortalized non-tumorigenic keratinocyte cell line and human hepatocellular carcinoma cell line were attributed to variability in specific metabolic capabilities of the respective cell lines. Significant reduction of cytotoxicity was achieved through deprotonation and reprotonation procedure, used as an additional purification step after polymer synthesis. Accordingly, the cytotoxicity is thus caused rather by the reaction by-products and residues than by polyaniline itself.

1. Introduction

In the last decade, conducting polymers in general, and polyaniline (PANI) in particular, have become extensively studied materials [1]. The original interest in the conductivity has gradually shifted to the studies of electrochemical behavior and of their ability to respond by conductivity changes to external stimuli. Applications in corrosion protection [2], catalysis of organic reactions [3], in fuel cells [4,5], supercapacitors [6], or analytical electrodes [7] have become more frequent. The fact that conducting polymers produce rich choice of nanostructures [1,8–12], such as nanotubes [9,13,14] or nanofibers [4,10,15], make them of interest in nanotechnologies. In practical application, the mixed electron and proton conductivity of PANI [16] seems to be another factor of excitement. Polyaniline has recently been investigated for the uses involving biomedical applications, connected with its conductivity [17], such as cardiac [18] or neural [19] tissue engineering, and in neural probes [20]. The use of conducting polymers in controlled drug delivery has also been reviewed [21] and their role as free radical scavengers in biomedical applications was assessed [22].

The term biocompatibility refers generally to ability of material to coexist with living organisms and tissues without harming them. As almost any foreign material may trigger the defence of organism in terms of immune response, biological testing of materials coming in the contact with body is extremely important. Biocompatibility of medical devices can be assessed both through a process of evaluating individual materials used in the manufacture of medical devices as well as through the process of final product evaluation. As medical devices form significantly heterogeneous category of products and accessories, the materials testing seems to be advantageous.

Though PANI is a conducting polymer of a wide application potential in biotechnology and medicine, data on its biocompatibility are scarce. The reason is possibly based on the fact that PANI has often been regarded with caution because the monomer (aniline) and reaction intermediates (aniline dimers and oligomers) are aromatic amines that can be physiologically active or even harmful. The carcinogenic effect of aniline dimer, benzidine, is the most recognized potential threat. However, PANI alone, being absolutely insoluble in aqueous media, can hardly be cytotoxic, but the above mentioned low-molecular-weight reaction by-products may cause problems.

The published studies dealing with PANI biocompatibility can be divided into two main groups. The first group is focused on *in vivo* testing of implantability and post-implant changes of tissues surrounding the implant [23–25]. The second, prevailing group of test methods, is dealing with assessment of *in vitro* proliferation and/or differentiation of cells on PANI surfaces [26–28]. These

* Corresponding author. Tel.: +420 57 603 8035; fax: +420 57 6031444.
E-mail address: humpolicek@uni.utb.cz (P. Humpolicek).

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studies refer mainly to testing of (1) PANI films cast on various carrier surfaces [27,28]; (2) PANI complexes [29], (3) PANI composites [18,30,31] or (4) electrospun blends [19]. The publications cover not only PANI samples in various oxidation or protonation states, but also different types of tests (*in vivo*, *in vitro*, different cell lines, etc.). The studies dealing with such combined materials and modified PANI surfaces can provide only limited information on the behavior of PANI as such. As a consequence, rather inconsistent results were encountered and unambiguous conclusions can be hardly drawn regarding PANI cytotoxicity. Discrepancies in results from the published *in vivo* studies can be demonstrated by the following examples. While in the study of Kamalesh et al. [24], PANI films in emeraldine oxidation state assessed through a subcutaneous implantation into male Sprague-Dawley rats for a period ranging from 19 to 90 weeks did not cause any inflammatory responses, study of Wang et al. [23] for PANI emeraldine films implanted into identical animal model beneath the dorsal skin, for a period ranging from 19 to 50 weeks, showed a sign of inflammation response. The histological examinations of the above tissues, 24 weeks after implantation, revealed that the film was encapsulated by fibrous tissue. Finally, in the study conducted by Mattioli-Belmonte et al. [25], PANI implanted subcutaneously in rats induced fibrous encapsulation and inflammatory response of tissue.

Conducting polymers, such as PANI, have demonstrated potential for electrical stimulation of electro-sensitive tissues, which has shown benefit in many regenerative medicine strategies including neural and cardiac tissue engineering [32]. The cell-line sensitive to electrical stimulation, namely H9c2 cardiac myoblast, was used by Bidez et al. [26], to investigate *in vitro* cell adhesion and proliferation on PANI substrate. The study resulted in the conclusion that PANI allows cell attachment and proliferation. Irrespective of the fact that initial adhesion of H9c2 cells was somewhat reduced, the overall rate of cell proliferation was similar to a tissue-culture-treated polystyrene control and, after six days, the cells formed uniform, homogenous monolayer.

Different cell-line, PC-12 pheochromocytoma cells, not responding to electrical stimuli, was used in biocompatibility study of Wang et al. [27]. In the study, PANI films prepared either by direct polymerization deposition or by casting on a surface of polytetrafluoroethylene substrate were studied for cell proliferation and adhesion. Though in both cases proliferation and adhesion were acceptable, directly polymerized films showed much better ability for cells to adhere on the surface compared to the cast films. PC-12 cells were also used for investigation of biocompatibility of PANI films prepared by surface polymerization by Liu et al. [28]. The cells were cultured onto PANI-coated silicon wafer and bare wafer for one and two days. After one day of culture it was obvious that coated surface enables good cell adhesion and proliferation compared to silicon surface without coating. Cells persisted to proliferate on the PANI surface also after two days of culture and fluorescence microscopy images demonstrated that number of cells was increasing. Enhanced proliferation of PC-12 cells on PANI compared to reference surface was related to the outstanding biocompatibility of PANI.

Contrary to previously published studies, current study is focused on testing of powder PANI solely. This enables better generalization of the obtained results and their application in cases, where PANI is used in combination with other polymers or materials. Cytotoxicity, as well as irritation and sensitization of PANI, have been tested in the present study in agreement with the procedures given by EN ISO 10993-5 and EN ISO 10993-10, respectively. Among these tests, cytotoxicity is crucial, as it can provide a good approximation of basic cellular response to tested substances. The combination of the above methods is primarily intended for materials and medical devices coming in contact with intact skin, mucosal membrane, and breached or compromised skin surface. As practical examples non-invasive electrodes, biometric sensors or urinary catheters for short-term use can be named. In addition these methods can also provide valuable information on PANI applications in more advanced products. Differences between various PANI forms and their biological responses are also provided and information on the extent, to which tested samples exhibit a destructive action on chosen cell cultures, allergenic potential or sensitizing capacity, are discussed. This study, hence, addresses queries related to biocompatibility of PANI and the prospects of potential use of this polymer in life sciences.

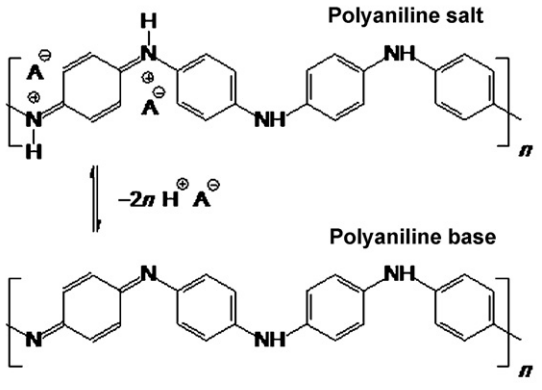
2. Experimental

2.1. Sample preparation

Aniline hydrochloride (Fluka, Switzerland) and ammonium peroxydisulfate (Lach-Ner, Czech Republic) were used as delivered. All PANI samples were synthesized using procedure recommended by IUPAC Technical Report [33]. Protonated, conducting PANI hydrochloride (PANI-H) was prepared by oxidation of aniline hydrochloride with ammonium peroxydisulfate in aqueous medium. Aniline hydrochloride (p.a.; 2.59 g, 20 mmol) was dissolved in water in a volumetric flask to 50 ml of solution. Ammonium peroxydisulfate (p.a.; 5.71 g, 25 mmol) was dissolved in water also to 50 ml of solution. Both solutions were mixed in a beaker, briefly stirred, and aniline was left at rest to polymerize. Next day, the green PANI precipitate was collected on a filter, washed with three 100-ml portions of 0.2 M hydrochloric acid, and similarly with acetone. Polyaniline hydrochloride powder was dried in air and then in desiccator over silica gel.

Polyaniline hydrochloride was converted to PANI base (PANI-B) (Scheme 1) by suspension of powder in large excess of 1 M ammonium hydroxide for 24 h, and the alkaline reaction of the residual medium was checked. Thus obtained blue PANI base was collected on a filter, washed with acetone, and dried as above. The physicochemical characterization of corresponding materials can be found in [33,34].

In order to test influence of possible impurities present in the samples on biocompatibility, the above prepared PANI base was further treated to get samples with improved impurity profile. As the transition between PANI base and PANI hydrochloride is reversible (Scheme 1), the PANI base was reprotonated by immersion in excess 1 M hydrochloric acid. The acidic reaction of the residual medium was checked and reprotonated PANI hydrochloride (R-PANI-H) was collected, washed with acetone, and dried as above. A part of this sample was deprotonated again with 1 M ammonium hydroxide and obtained PANI base (DR-PANI-B) was



Scheme 1. Polyaniline salt converts to polyaniline base in alkaline media. HA is an arbitrary acid.