Evaluation of biocompatibility of the copolymer of 2-hydroxyethyl methacrylate with 2-(methylsulfanyl)ethyl methacrylate

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This study compares subcutaneous and intracerebral biocompatibilty of two hydrogels: copolymer of 2-hydroxyethyl methacrylate with 2-(methylsulfanyl)ethyl methacrylate and poly(2-hydroxyethyl methacrylate) as reference polymer. The experimental copolymer was more biologically inert than poly(2-hydroxyethyl methacrylate) in both the studied parameters, hence the former material is a suitable candidate for biomedical application.

1. Introduction

The history of medical exploitation of synthetic hydrogels is very long [1, 2]. Their chemical properties seem very convenient for the development of implantable devices, because the extracellular matrix of animal (including human) tissue can also be considered a hydrogel [3]. The biological behavior of synthetic materials including hydrogels is influenced by numerous properties of the implant, namely by its surface Gibbs energy, wettability and charge. These factors influence adsorption of biologically active proteins or glycoproteins such as fibrinogen, fibronectin and complement. These molecules are actively recognized by cell receptors which also influence colonization of these surfaces by cells, including cells of non-specific immune reaction [4, 5]. We demonstrated in our previous reports that chemical composition of hydrogels clearly influences adhesion of human monocytes *in vitro* as well as adhesion of macrophages and foreign-body giant multinucleate cell formation *in vivo* [6–9].

In the course of biological characterization of hydrophilic materials as candidates for biomedical application, biological properties of a copolymer of 2-hydroxyethyl methacrylate with 2-(methylsulfanyl)ethyl methacrylate [poly(HEMA-*co*-MSEM)] were studied by the subcutaneous implantation test. We chose the sulfur-containing comonomer because we were interested in the influence of heteroatom on properties of the studied implant. The intracerebral biocompatibility was evaluated after stereotactic implantation into the rat brain of specimens stained with Cresyl Violet and specimens stained for the detection of nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d). These results were compared with biological properties of poly(2-hydroxyethyl methacrylate) [poly(HEMA)].

2. Materials and methods

2.1. Preparation and characterization of monomers and polymers

2.1.1. Preparation of 2-(methylsulfanyl)ethyl methacrylate (MSEM)

MSEM was prepared [10] from methacryloyl chloride (Fluka) (1.05 mol) and 2-(methylsulfanyl)ethan-l-ol (Fluka) (1 mol) in ethyl acetate in the presence of trimethylamine (1.05 mol) at 0 °C. The pure product (97.9%, GC) was obtained by distillation (0.05 mm Hg, b.p. 32 °C), elemental analysis C 52.56%, H 7.47%, S 19.82%, theory C 52.45%, H 7.54%, S 20.00%. IR and NMR spectra are in Fig. 1.

2.1.2. Preparation of hydrogel strips

The crosslinking polymerization of 2-hydroxyethyl methacrylate (HEMA) and MSEM (10 wt % of MSEM) was carried out in a thermostatted block [11] of hard-aluminum flow forms with an area of 10 × 10 cm, fitted with reinforced polypropylene foils and firmly closed using screw clamps. The thickness of the unswollen original samples corresponded to the thickness of the silicone seals used. The polymerization proceeded for a period of 16 h at 60 °C. The initiator (2,2′-azobisisobutyronitrile) concentration in the polymerization mixture was 0.4 wt % in each case, the crosslinking agent

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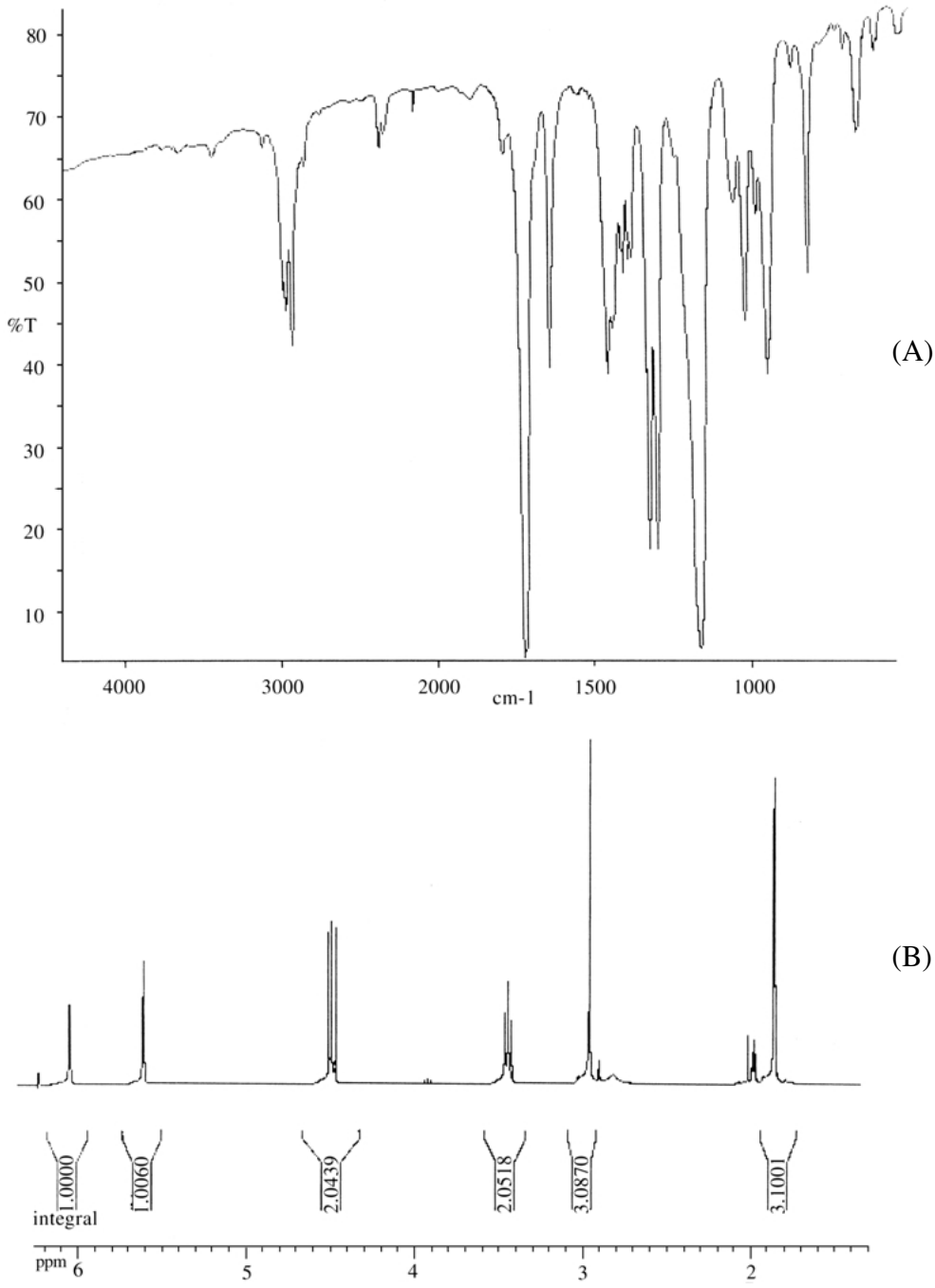

Figure 1 IR (A) and NMR (B) spectra of MSEM.

(ethylene dimethacrylate) concentration was 0.2 wt % relative to the monomers in each case. The 1 mm thick foil obtained was strong and transparent in the unswollen state. Before biological experiments, the foil was washed with water (at least 10 times, 5 h each) and finally with physiological saline. The equilibrium water content in swollen hydrogel was 25.5 wt %, Young's modulus 0.67 MPa. The corresponding values for pure HEMA hydrogel without MSEM are 39.4 wt % and 0.53 MPa.

2.2. Subcutaneous implantation into the rat

Strips 4 × 8 mm of experimental polymers ($n = 12$) and of poly(HEMA) ($n = 6$) were subcutaneously implanted into the interscapular region of Wistar laboratory rats of both sexes weighing 350 g as described [12]. The animals were sacrificed 14 days later, when the foreign-body reaction in the subcutaneous region of the rat is maximal [12]. The strips were fixed with 4% paraformaldehyde, washed with water and observed as a whole mount specimen [6]. The capsule of connective tissue surrounding all the implants was routinely embedded in paraffin, sectioned and stained with hematoxylin eosin.

2.3. Intracerebral implantation into the rat

A total of 15 adult Wistar rats of 300 g were used. In six rats, unilateral and in two rats, bilateral stereotactic implantation of polymers were made in the striatum, two rats were sham-operated (craniotomy without a lesion) and five rats were used for control of NADPH-d staining only. All surgical procedures were performed employing stereotactic apparatus under Phenobarbital anesthesia

(0.2 mg/100 g of zbody weight). A small hole was drilled in the skull and a 1 mm wide cannula was brought into the striatum using Fifkova and Maršala [13], or Paxinos [14] and Watson [13] coordinates. Small pieces of polymer ($1.0 \times 0.5 \times 0.5$ mm) inserted in the tip of cannula were pushed out into the nervous tissue of striatum. Afterwards, the cannula was removed, the skin wound closed and the rats were allowed to survive for 14 days. After this postoperative survival time, the rats were sacrificed under deep ether anesthesia and perfused transcardially with 4% paraformaldehyde in phosphate buffer (pH 7.4). The brains were quickly removed, cut into 1.0 cm slices and stored overnight in the same fixative. The following day the slices were placed into a 30% sucrose solution in phosphate buffer for cryoprotection. After three to four days, the slices were cut into 40 µm frontal sections on a freezing microtome. The sections were used for Cresyl Violet staining and for NADPH-d histochemistry.

2.4. NADPH-diaphorase histochemistry and Cresyl Violet staining

For the demonstration of NADPH-d activity, a modified Scherer-Singler [16, 17] method was used. In our series, we can distinguish two main types of NADPH-d positive neurons and three types of NADPH-d fibers in the neuropil:

i. Golgi-like neurons – blue black with distinct dendritic arborization of the processes, with slightly pale appearance in the nucleus.

ii. Nissl-like neurons – light blue-violet neurons with easily distinguishable nuclei, but without stained processes.

iii. In neuropil: smooth, spindle or beaded fibers. Studying NADPH-d positivity, we traced the changes of this reaction in the nitric oxide synthase (NOS)-positive neurons, characteristic of normal striatum.

Every other section was stained with Cresyl Violet. The location and the extent of the implanted synthetic material were checked. The structure and presence of neurons and glial reaction in the neighborhood of the implants and in the neighborhood of the cannula tracks were studied in the Cresyl Violet-stained sections.

3. Results and discussion

The surface of poly(HEMA-*co*-MSEM) implants was colonized with rare macrophages with a very limited number of foreign-body giant multinucleate cells (approx. 5 per implant). The implants prepared from poly(HEMA) were covered extensively with inflammatory cells, mainly with macrophages. However, the lymphocytes were also observed. The foreign-body giant multinucleate cells were the very frequent cell element on the surface of poly(HEMA) implants (Fig. 2).

The connective tissue capsule surrounding both the implanted polymers was very thin, formed by collagen tissue without polymer-dependent differences (not demonstrated).

In close environment of poly(HEMA-*co*-MSEM) and poly(HEMA) implants used in our experiments, ''normal'' neurons stainable with Cresyl Violet were *always* present (Fig. 3). At the contact point of the polymer implant and nervous tissue, the glial response was not detectable. A weak glial reaction was found in the environment of the cannula track. In some few cases, the glial response slightly passed from cannula track also towards the peak of implant area (Fig. 4).

The NADPH-d positive neurons with the reaction product present in processes surrounded the surface of implanted poly(HEMA-*co*-MSEM) in contrast to implants prepared from poly(HEMA). The NADPH-d positive neurons were not present in the close vicinity of poly(HEMA) implants and no detectable reaction product was observed in processes of these cells (Fig. 3).

The subcutaneous and intracerebral biocompatibility of the tested copolymer poly(HEMA-*co*-MSEM) was

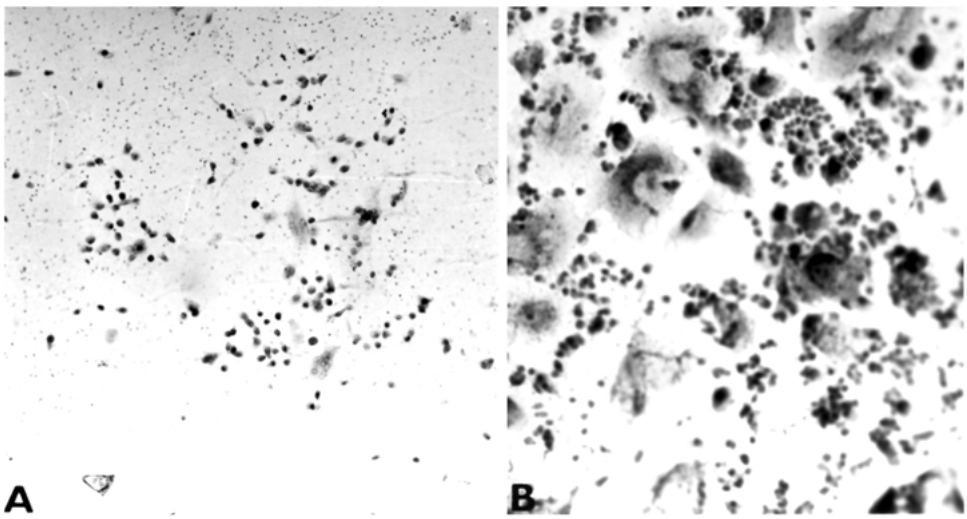

Figure 2 Comparison of the extent of foreign-body reaction on the surface of poly(HEMA-*co*-MSEM) (A) and poly(HEMA) (B). Foreign-body giant multinucleate cells are denoted with arrows. Magnification × 200.

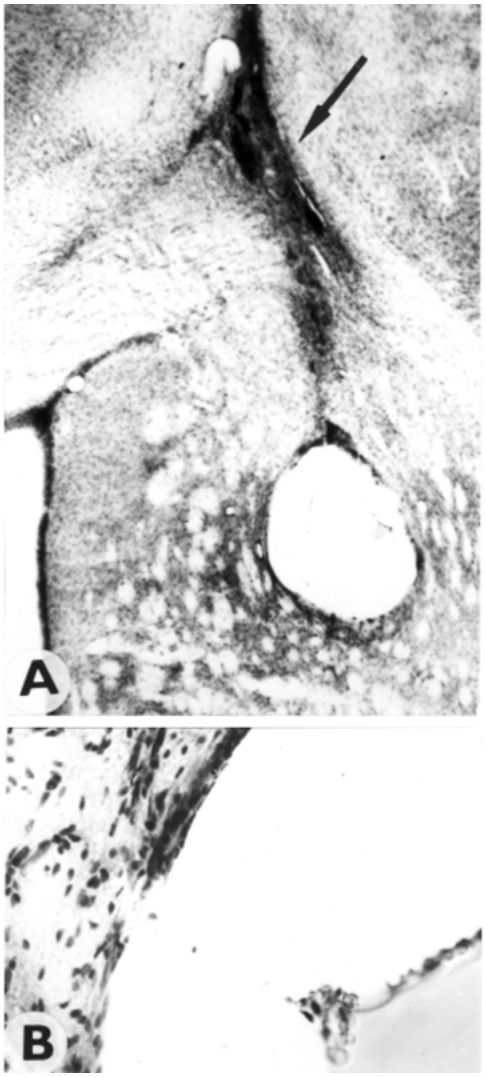

Figure 3 Comparison of the extent of foreign-body reaction on the surface of poly(HEMA-*co*-MSEM) (A) and poly(HEMA) (B). Foreign-body giant multinucleate cells are denoted with arrows. Magnification × 200.

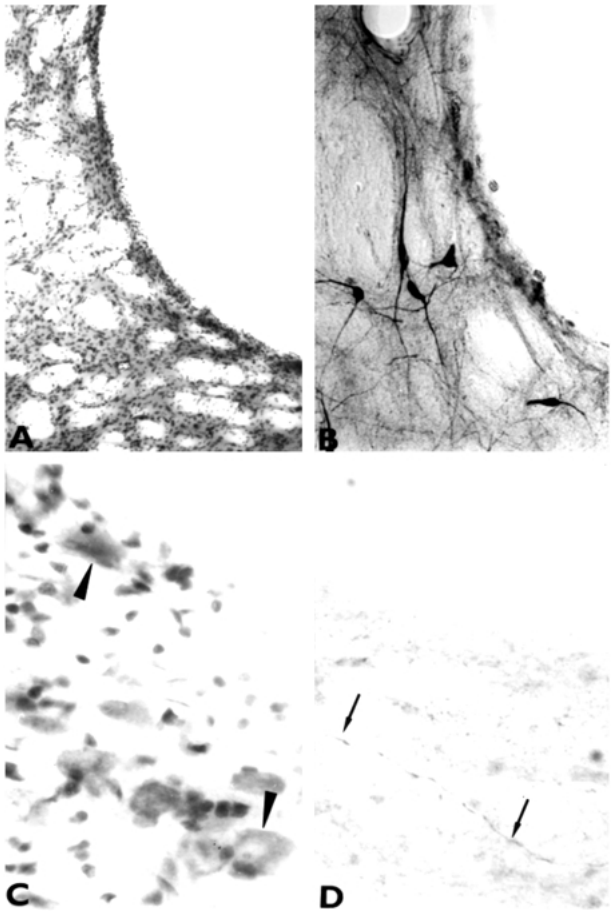

Figure 4 Comparison of the extent of foreign-body reaction on the surface of poly(HEMA-*co*-MSEM) (A) and poly(HEMA). Foreign-body giant multinucleate cells are denoted with arrows. Magnification × 200.

significantly better than the biological tolerance of poly(HEMA) only and it was compatible with the results obtained from experiments with implantation of copolymer of HEMA with sodium methacrylate which was characterized as biologically inert in our previous studies [6–9]. The similar biological behavior of polymeric crosslinked materials with different chemical structure suggests the existence of some general polymer surface property improving the biological non-reactivity of implanted polymers.

Acknowledgments

This study was supported by the Grant Agency of the Czech Republic (project No. 304/97/1072). We would like to thank Mrs K. Patková, Mrs R. Kobianová and Mrs Eva Vancová for their technical assistance in experiments and for their assistance in preparation of this manuscript.

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Received 15 March 2000
and accepted 11 May 2001