J Mater Sci: Mater Med (2009) 20:1571–1577
DOI 10.1007/s10856-009-3714-4

Macroporous hydrogels based on 2-hydroxyethyl methacrylate. Part 6: 3D hydrogels with positive and negative surface charges and polyelectrolyte complexes in spinal cord injury repair

A. Hejčl · P. Lesný · M. Přádný · J. Šedý · J. Zámečník · P. Jendelová · J. Michálek · E. Syková

Received: 31 July 2008 / Accepted: 9 February 2009 / Published online: 28 February 2009

Abstract Macroporous hydrogels are artificial biomaterials commonly used in tissue engineering, including central nervous system (CNS) repair. Their physical properties may be modified to improve their adhesion properties and promote tissue regeneration. We implanted four types of hydrogels based on 2-hydroxyethyl methacrylate (HEMA) with different surface charges inside a spinal cord hemisection cavity at the Th8 level in rats. The spinal cords were processed 1 and 6 months after implantation and histologically evaluated. Connective tissue deposition was most abundant in the hydrogels with positively-charged functional groups. Axonal regeneration was promoted in hydrogels carrying charged functional groups; hydrogels with positively charged functional groups showed increased axonal ingrowth into the central parts of the implant. Few astrocytes grew into the hydrogels. Our study shows that HEMA-based hydrogels carrying charged functional groups improve axonal ingrowth inside the implants compared to implants without any charge. Further, positively charged functional groups promote connective tissue infiltration and extended axonal regeneration inside a hydrogel bridge.

A. Hejčl (✉) · P. Lesný · J. Šedý · P. Jendelová · E. Syková
Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic
e-mail: ales.hejcl@mnul.cz

A. Hejčl · P. Lesný · M. Přádný · J. Šedý · P. Jendelová · J. Michálek · E. Syková
Department of Neuroscience and Centre for Cell Therapy and Tissue Repair, 2nd Medical Faculty, Faculty Hospital Motol, Charles University, Prague, Czech Republic

M. Přádný · J. Michálek
Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

J. Zámečník
Department of Pathology and Molecular Medicine, 2nd Medical Faculty, Faculty Hospital Motol, Charles University, Prague, Czech Republic

A. Hejčl
Department of Neurosurgery, Masaryk Hospital, Ústí nad Labem, Czech Republic

1 Introduction

Spinal cord injury (SCI) causes severe tissue damage resulting in a permanent neurological deficit. Spontaneous regeneration is severely limited. The glial scar, mesenchymal scar and posttraumatic pseudocysts form an obstacle for regeneration. Tissue engineering continues to assume greater importance in neuroscience, with the ultimate goal of restoring the morphology and function of damaged nervous tissue. Tissue engineering techniques help to create a permissive environment to promote the regeneration of neurons and axons, oligodendrocytes, and blood vessels.

Various biomaterials, including hydrogels, have been implanted inside a SCI in order to bridge the lesion [1, 2]. Hydrogels are synthetic 3-dimensional polymer scaffolds with pore sizes ranging from 10 to 100 μm. They have a high water content (70–90%). Diffusion parameters within implanted hydrogels attain values similar to those of developing neural tissue [3]. The physical and chemical properties of hydrogels can be modified to improve cell adhesion and tissue regeneration. Further, as synthetic materials, they can be produced in large quantities, and combined with allogenic or autogenic transplants. Previous

studies of ours and others have shown that hydrogels based on 2-hydroxyethyl methacrylate (HEMA) are promising biomaterials for CNS regeneration [1, 4–6]. Increasing knowledge about the pathophysiology of SCI, cell adhesion properties, molecular biology, and biomaterial science could lead to the development of implants that would successfully bridge a spinal cord lesion and lead to a complete recovery of locomotor, sensory, and autonomic functions.

It is well known that the physical and chemical properties of a surface can influence cellular adhesion [7]. Previous studies have shown that neurons preferentially adhere to and form neural networks on positively charged surfaces such as polylysine-coated glass slides [8–11]. In our previous study, we found that HEMA-based hydrogels with positively-charged functional groups promote the adhesion of mesenchymal stem cells [12], which have been shown to promote functional improvement and protect against tissue damage in experimental SCI and to increase the expression of growth and trophic factors in the ischemic rat brain [13, 14].

In the present study, we evaluated the effect of positively and negatively charged functional groups in HEMA-based hydrogels on cellular adhesion and neuronal and astrocytic regeneration in experimental spinal cord injury repair. We employed four types of hydrogels based on HEMA. The main difference in the properties of the hydrogels was the presence of differently charged polar groups on their surfaces. Type 1 copolymers had negative surface charges (HEMA−), caused by the presence of carboxylic groups; type 2 copolymers had positive charges (HEMA+), caused by the presence of quaternary ammonium groups. The type three terpolymers (HEMA±) and the type 4 polyelectrolyte complexes (PEC) had both positive and negative charges in their polymer chains. The difference between types 3 and 4 was that the counter-ions of the type 3 hydrogels were low-molecular weight (Na^+, Cl^-), while the type 4 hydrogels had counter-ions bound on a macromolecular chain of linear poly[2-(methacryloyloxy)ethyl]trimethylammonium [poly(MOETA$^+$)]. Therefore, the type 4 hydrogels' charges were strongly shielded by the polymer counter-ions, resulting in properties similar to those of uncharged hydrogels, as reported previously [15, 16].

2 Materials and methods

2.1 Hydrogel synthesis

Four series of macroporous hydrogels based on crosslinked copolymers of HEMA were prepared as described previously [12]. The hydrogels were prepared under GMP conditions. In brief, the crosslinked copolymer of HEMA with methacrylic acid in the sodium salt form (MA^-) was prepared according to [17] (HEMA−). The crosslinked copolymer of HEMA with MOETA$^+$ (HEMA+), the terpolymer of HEMA with MA^- and MOETA$^+$ (HEMA±) and the polyelectrolyte complex of HEMA-MA^- crosslinked copolymer with linear poly(MOETA$^+$) (PEC) were prepared similarly in a pelleting apparatus in the presence of fractionated sodium chloride particles [16, 17]. The average pore size in all the prepared hydrogels was 40 μm. After polymerization they were thoroughly washed with water and saline (2 weeks) in order to remove unreacted monomers and solvent, autoclaved (120°C, 20 min) and cut into blocks approximately 1 mm^3 (under sterile conditions).

2.2 Hydrogel implantation

Twenty-nine male rats (Wistar, Velaz, Czech Republic) with a weight of 250–300 g underwent a hemisection at the level of the 8th thoracic vertebra (Th8). The animals were intraperitoneally injected with pentobarbital for anesthesia (solution of 1 g/100 ml, 6 ml/1 kg of animal weight); one dose of ATB (ampicilin 0.3 ml s.c.), atropine (0.2 ml s.c., 1:5) and mesocain to enhance local anesthesia (0.3 ml s.c. + i.m.) was administered preoperatively. A linear skin incision was performed above the spinous processes of Th6–10; the paravertebral muscles were detached from the laminae Th6–10, and a Th8 laminectomy was performed. The dura was incised, and less than 1 mm^3 of spinal cord tissue was dissected to form a hemisection cavity. Four types of hydrogels [HEMA + ($n = 6$), HEMA− ($n = 7$), HEMA± ($n = 5$), PEC ($n = 7$)] were properly trimmed to adjust to the size and shape of the cavity. The hydrogel was implanted in such a way as to ensure that it would firmly adhere to the edges of the hemisection cavity without causing any undue pressure onto the surrounding spinal cord tissue. In four animals no hydrogel was implanted inside the cavity and they served as controls. The dura was sutured with Ethilon 8/0 thread (Ethicon, Johnson & Johnson). The muscles and skin were sutured, and the animals were housed two in a cage with food and water ad libitum. This study was performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC) regarding the use of animals in research and was approved by the Central Commission for Animal Protection of the Academy of Sciences of the Czech Republic in Prague.

2.3 Tissue processing and histology

At the 28th postoperative day, animals ($n = 25$) were deeply anesthetized with an intraperitoneal injection of

overdose pentobarbital and perfused with physiological saline followed by 4% paraformaldahyde in 0.1 M phosphate buffer. The spinal cord was left in bone overnight, then removed and postfixed in the same fixative for at least 1 week. In addition, four other animals [HEMA+ ($n = 2$), PEC ($n = 2$)] were allowed to survive until postoperative day 180 and then processed using the same method.

A 3 cm long segment of the spinal cord containing the lesion site was dissected out, and a series of 40 μm thick longitudinal sections was collected. Hematoxylin-eosin (HE) and cresyl violet (CV) stainings were performed using standard protocols, and the slides were specifically evaluated for an adverse foreign-body type granulomatous reaction, an inflammatory response and the presence of connective and nervous tissue elements inside the hydrogel. Selected sections were processed for immunohistochemistry with the following antibodies: Cy3-conjugated mouse anti-Glial Fibrillary Acidic Protein (GFAP, 1:200, Sigma-Aldrich), which specifically labels astrocytes and mouse anti-neurofilament (NF) 160 (1:200, Sigma-Aldrich), which specifically labels neuronal processes (axons/dendrites). All these sections were incubated overnight at 4°C with one of the primary antibodies pre-diluted in phosphate-buffered saline (PBS 4°C, pH 8) containing 0.1% bovine serum albumin (BSA, Sigma) and 0.2%-Triton X100. After incubation, the sections were washed at 3 × 10 min and the sections labeled with anti-NF 160 were incubated in the presence of an anti-mouse antibody Alexa Fluor 488 goat anti-mouse IgG (1:200, Invitrogen). Control sections in which the primary antibodies were omitted were routinely prepared to check for non-specific staining.

3 Results and discussion

3.1 Hydrogel infiltration and biocompatibility

All four types of hydrogels formed a bridge across the lesion as they adhered well to the spinal cord tissue on the cranial, caudal, and medial aspects of the hemisection pseudocyst. CV and HE staining showed a small amount of tissue and a small number of cell nuclei in the negatively charged hydrogels (HEMA−) (Fig. 1a). In many parts of the hydrogel, the pores were empty with a few cells growing only along the walls of the pores (Fig. 1b). In the HEMA± implants, some parts of the hydrogel were infiltrated diffusely with connective tissue elements while others showed only minimal infiltration of the pores with a few cells residing along the walls (Fig. 1e, 1f). In the positively charged hydrogels (HEMA+) the ingrowing tissue filled all the pores within the hydrogel on the HE-stained sections (Fig. 1i); the number of cell nuclei on the CV-stained sections was much higher compared to the negatively charged hydrogel. The connective tissue completely and diffusely infiltrated the pores of the hydrogel (Fig. 1j). The PEC implants, having properties similar to uncharged hydrogels, were infiltrated with only minimal cells both 1 and 6 months after implantation (Fig. 1m, n). The control group (hemisection only) showed a large tissue defect, a pseudocystic cavity, without any cellular infiltration (Fig. 1q, r). The data are summarized in Table 1.

At the periphery of the HEMA+ and PEC implants, a significant foreign body-type giant-cell granulomatous reaction was noted when evaluated 1 month after implantation (Fig. 2a). The two types of hydrogel with a foreign-body reaction (HEMA+ and PEC) were also evaluated 6 months after hydrogel implantation. The foreign-body granulomatous reaction was significantly reduced with only a minimal or no giant cell reaction, indicating that this type of inflammatory response to the implant subsided over time (Fig. 2b). Both hydrogels adhered to the pseudocysts and formed a bridge across the cavity. While the HEMA+ hydrogels were completely filled with connective tissue infiltrating the pores of the hydrogel, the PEC hydrogels remained minimally infiltrated, so no progress of tissue ingrowth was observed since the 1 month evaluation. Thus, the foreign body reaction did not have any negative effect on the quality and long-term stability of the hydrogels.

Cellular adhesion to implants is important as it mediates many aspects of biocompatibility. Surface charge is considered to be one of the factors that influence cellular adhesion [18]. There have been several studies comparing cellular adhesion on negatively or positively charged surfaces with varying results. Fibronectin adsorption was found to be greater on positively charged films compared with negatively charged ones [19]. While Richert et al. found increased cell adhesion on polycation-terminated films [20, 21], Kidambi et al. found the strongest hepatocyte attachment and spreading on negatively charged, sulfonated polymer-terminated multilayers [22]. Woerly et al. detected increased connective tissue deposition in hydrogels with negatively charged functional groups [23], while other studies have reported no terminal layer effect [24, 25]. As we can conclude from these studies, there is no universal finding concerning the relationship between the charge of the polymer and cellular adhesion.

In this study, three types of hydrogels contained charged polar groups in their structure, while the PEC hydrogel had characteristics similar to uncharged hydrogels. All three types of hydrogels were infiltrated with connective tissue elements while the PEC hydrogel showed only minimal cellular infiltration. There is evidence, in accordance with our study, that plain hydrogels do not elicit good tissue adhesion or cellular infiltration without the addition of charged polar groups [23, 26] Even further, few unmodified hydrogels were found within the implantation site and

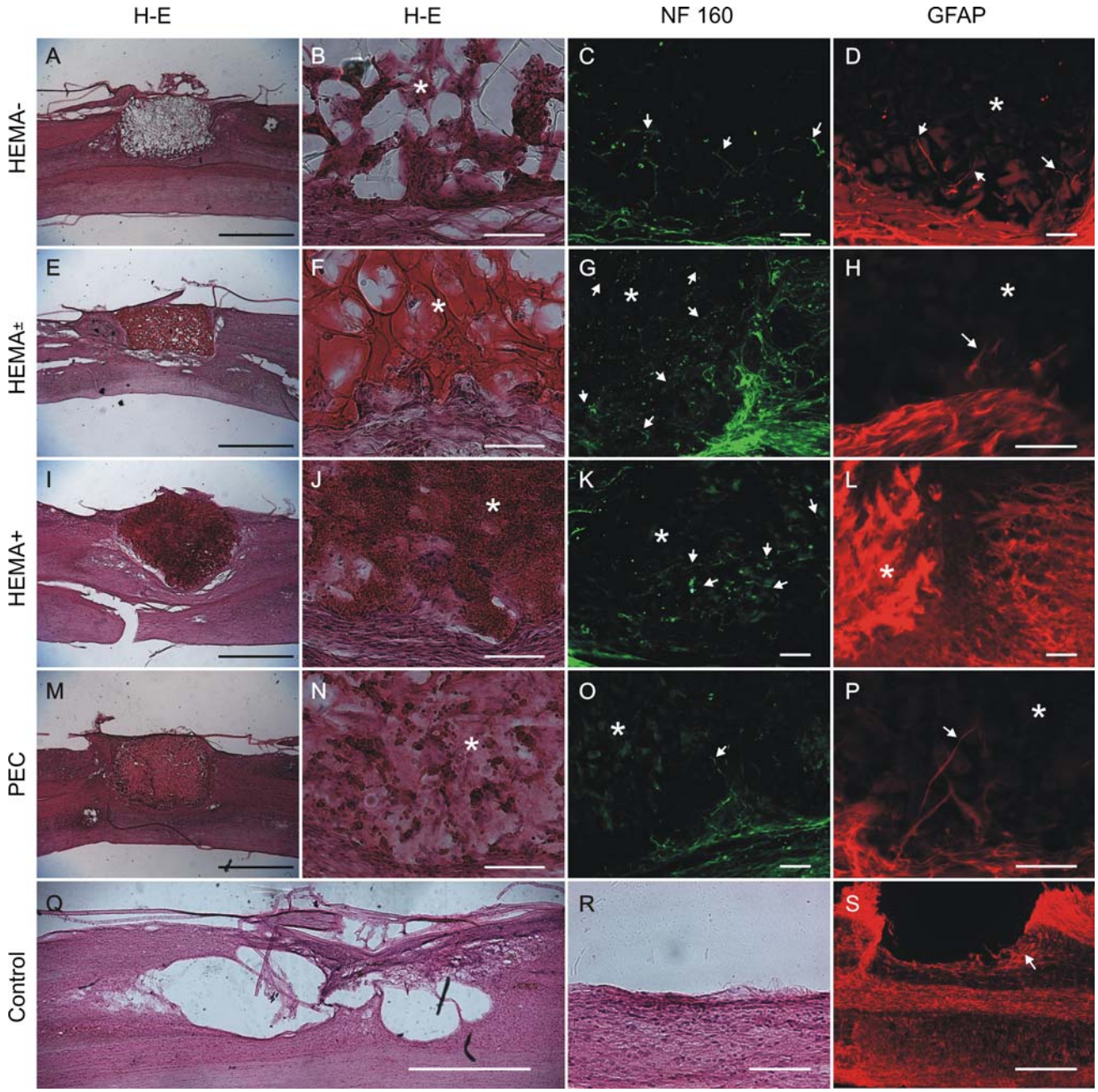

Fig. 1 Tissue infiltration in the four HEMA-based hydrogels with different surface charges. In the negatively charged hydrogels, there was only a small amount of connective tissue in the hydrogels visible on hematoxylin-eosin stained sections (**a**, scale bar = 1 mm, **b**, scale bar = 50 μm). The amount of connective tissue elements increased in hydrogels with both positive and negative charges (**e**, scale bar = 1 mm, **f**, scale bar = 50 μm) and was highest in the positively-charged hydrogels (**i**, scale bar = 1 mm, **j**, scale bar = 50 μm). In the PEC implants, minimal connective tissue elements infiltrated the implant (**m**, scale bar = 1 mm, **n**, scale bar = 50 μm). The pseudocystic cavity dominated the lesion after hemisection without hydrogel implantation (**q**, scale bar = 1 mm), with a sharp border between the pseudocystic cavity and the residual tissue (**r**, scale bar = 100 μm) Neurofilaments (arrows) were found in hydrogels with functional groups (**c**, **g**, **k**, scale bar = 100 μm) in contrast to PEC implants (**o**, scale bar = 100 μm). We found axons infiltrating the peripheral parts of the implants in all hydrogels with functional groups (**c**), while hydrogels with positive charges (**g**) had many axons also infiltrating the central parts of the implants. Few astrocytic processes were found especially in the HEMA- (**d**, scale bar = 100 μm) and PEC implants (**p**, scale bar = 100 μm) as compared with minimal astrocytic processes in the HEMA± (**h**, scale bar = 100 μm) and HEMA + (**l**, scale bar = 100 μm). The astrocytes formed a glial scar (white arrow) around the hemisection cavity (**s**, scale bar = 400 μm). Hydrogels are marked with an asterisk

Table 1 The relative amount of cellular infiltration inside the four types of HEMA-based hydrogels with different surface charges

	Axons		Astrocytes	Connective tissue
	Peripheral part of hydrogel	Central part of hydrogel		
HEMA+	2	2	0	3
HEMA±	2	2	0	2
HEMA−	2	1	1	1
PEC	1	0	1	0

0—no or minimal cells in the implant, 1—few cells or cellular processes in the implant, 2—many cells or cellular processes in the implant, 3—diffusely infiltrated implant with cells, pores completely filled

those that were found were not attached to host tissue and showed no cellular infiltration within their structure and the gels were encapsulated by host astrocytes [27]. Therefore, hydrogel modification with functional polar groups is beneficial for the proper integration of the biomaterial after implantation.

3.2 Axons

We evaluated the ingrowth of axons into the 3D porous structure of the hydrogels. In the three hydrogels with surface charges, axons infiltrated the pores of the hydrogel implants (HEMA−, HEMA+, HEMA±) (Fig. 1c, g, k). Many axons grew in the peripheral parts of implants with quite a few axonal processes reaching the central parts. The most axons were found in the central parts of the hydrogels carrying functional groups with a positive charge (HEMA+ and HEMA±). The hydrogels based on PEC, in contrast, were infiltrated with only few axonal processes (Fig. 1o). Most axons were found in the peripheral parts of the PEC implants with scarce processes reaching the deeper parts of the implant; no axons were found in the center of the implants (Table 1).

Additionally, we evaluated the relationship between the pores of the hydrogels and the ingrowth of axonal processes, specifically the relationship between the neural cell processes and the surface of the hydrogel in all types of implants. In the HEMA− hydrogels, the axons grew in close contact with the surface of the hydrogel (Fig. 2c). In

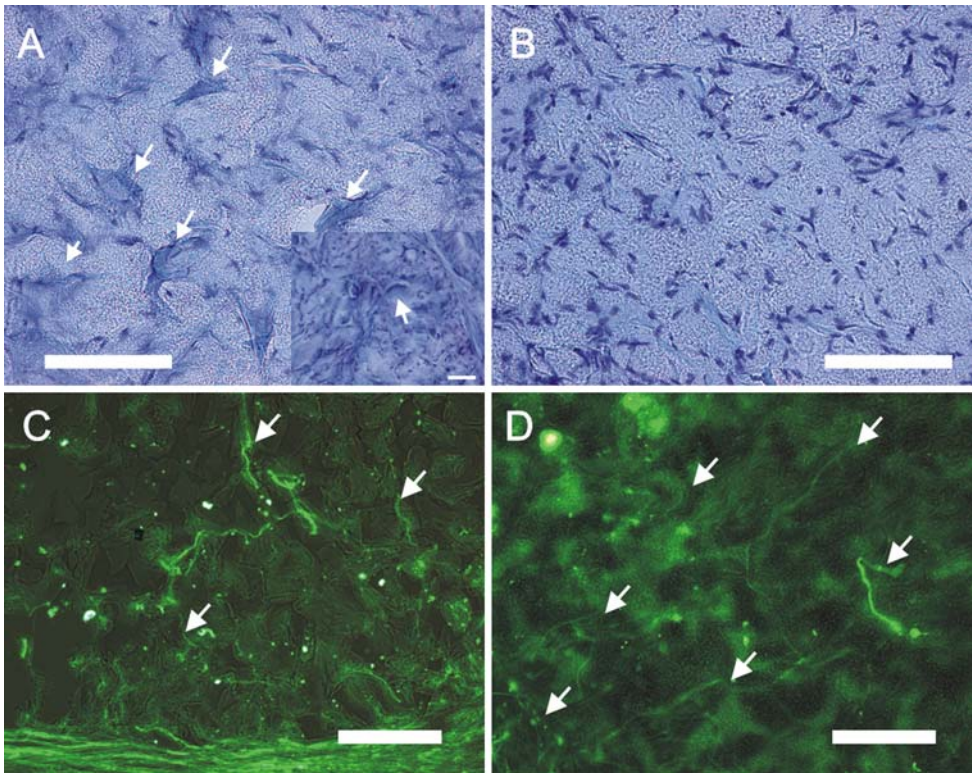

Fig. 2 Foreign body reaction in a HEMA + based hydrogel (**a**, **b**) and the relationship of axons and the pores of the implants (**c**, **d**). Foreign body reaction. In some parts the HEMA+ hydrogels were positive for a foreign body reaction, as documented by giant multinucleated cells (arrows) 1 month after hydrogel implantation. In the inset, a detail of the multi-nucleated cell is apparent (**a**, scale bar = 100 μm, inset scale bar = 50 μm). The hydrogel HEMA+ 6 months after implantation without the foreign body reaction. (**b**, scale bar = 100 μm). The relationship of axons and the pores of the implants. In the peripheral part of a negatively charged implant (HEMA−) axons grew along the walls of the pores (**c**, scale bar = 50 μm). Axons in the central part of a positively charged implant (HEMA+) spanned the pores of the hydrogel (**d**, scale bar = 50 μm)

contrast, in the HEMA+ hydrogels, where the cell count was higher, the axons grew longer and spanned the pores of the hydrogel (Fig. 2d).

Our results demonstrate that axonal regeneration was promoted by hydrogels with positively charged functional groups compared to hydrogels with negative ones. Positively charged functional groups (HEMA+ and HEMA±) induced extended axonal ingrowth into the central parts of the implant. Further, all three types of hydrogels with charged functional groups (HEMA+, HEMA±, HEMA−) promoted axonal regeneration compared to hydrogels without a charge. In accordance with our study, previous studies showed that neurons preferentially adhere to and form neural networks on positively charged surfaces such as polylysine-coated glass slides [8–11]. Cellular attachment and axonal outgrowth were most pronounced on poly(ethylenimine)-coated (PEI) surfaces. Furthermore, an estimation of the adhesion and proliferation rates of rat neuronal cultures indicated that PEI had a greater effect than other positively charged polymers [28]. A possible reason for this is that the cell membrane is negatively charged, and the deposition of round neurons in suspension will be accelerated if the surface is covered with positively charged amino groups. Once the cell has attached itself onto the surface, it will not change position or migrate readily. To some extent, the PEI-coated glass coverslips are equivalent to patterned laminin grids. If the position of neurons and the path of axons need to be controlled effectively, the use of charged groups on the surface needs to be considered. The improved nerve cell affinity might be due to both the increased surface charge and the hydrophilicity of composite materials [29].

Axonal regeneration is also influenced by surface chemical modifications. Woerly et al. studied a poly[N-(2-hydroxypropyl methacrylamide)] PHPMA hydrogel modified with an attached oligopeptide sequence (RGD). The PHPMA-RGD implant showed stronger adhesion to the host tissue and promoted the ingrowth and spread of astrocytes and neurofilaments inside the hydrogel [30]. Dorsal root ganglia (DRG) cultured on an agarose hydrogel with a covalently bound chitosan (polycationic polysaccharide) showed a significant increase in the length of regenerating axons [31].

3.3 Astrocytes

Generally, the ingrowth of astrocytes inside the hydrogel implants was very limited. The greatest number of astrocytic processes was found in the HEMA− and PEC hydrogels (negative and no charge). The processes were found only in the peripheral parts of the implants (Fig. 1d, p). No astrocytic processes grew deeper into the implants or into the central part. There were fewer astrocytic processes in the peripheral part of hydrogels based on HEMA± terpolymer (Fig. 1h) and almost no astrocytes in the HEMA+ hydrogels (Fig. 1l). The ingrowth of astrocytes inside the implant was very limited in the positively charged hydrogels (HEMA+ and HEMA±), in contrast to axonal ingrowth. We found no astrocytic processes in the central part of either type of hydrogel (Table 1). This is in accordance with the results of a study in which positively and negatively charged hydrogels were implanted in an experimental brain injury. In that study, hydrogels with negatively charged functional groups were shown to promote astrocytic ingrowth [23].

4 Conclusions

Macroporous hydrogels based on HEMA are able to bridge a spinal cord lesion when implanted inside a hemisection cavity. Charged functional groups, especially the positive ones, promote connective tissue infiltration inside the implants. Hydrogels with positively or negatively-charged functional groups promote axonal regeneration inside the implant, while minimal axons infiltrate hydrogels without any charge. Hydrogels with positively charged functional groups show increased axonal ingrowth into the central parts of the implant. Astrocytes infiltrate only those hydrogel implants with a negative or no charge, and most are found in the peripheral zones only. By modifying the properties of scaffolds used in SCI repair, we can improve tissue regeneration. Modifying the physical properties of biomaterials can influence and direct the growth of different types of cellular elements. As we have also shown in our previous study, HEMA-based hydrogels with different surface charges play a significant role in interaction with mesenchymal stem cells [12]. In conclusion, according to our studies the surface charge of scaffolds should be considered an important factor in tissue engineering.

Acknowledgments We thank James Dutt for reading of the manuscript. This work was supported by the following grants: AVOZ50390703 from the Academy of Sciences of the Czech Republic, 1M0538 from the Ministry of Education of the Czech Republic, GAAV IAA500390902 from the Grant agency of the Czech Academy of Science, 1A8697-5 from the Ministry of Health of the Czech Republic, and the EC FP6 project RESCUE (LSHB-CT-2005-518233).

References

1. Hejčl A, Lesný P, Přádný M, Michálek J, Jendelová P, Syková E. Biocompatible hydrogels in spinal cord injury repair. Physiol Res. 2008;57(Suppl 3):S121–32.
2. Schmidt CE, Leach JB. Neural tissue engineering: strategies for repair and regeneration. Annu Rev Biomed Eng. 2003;5:293–347.
3. Woerly S, Petrov P, Syková E, Roitbak T, Šimonová Z, Harvey AR. Neural tissue formativ within porous hydrogels implanted in

brain and spinal cord lesions: ultrastructural, immunohistochemical, and diffusion studies. Tissue Eng. 1999;5:467–88.
4. Bakshi A, Fisher O, Dagci T, Himes BT, Fischer I, Lowman A. Mechanically engineered hydrogel scaffolds for axonal growth and angiogenesis after transplantation in spinal cord injury. J Neurosurg Spine. 2004;1:322–9.
5. Dalton PD, Flynn L, Shoichet MS. Manufacture of poly(2-hydroxyethyl methacrylate-co-emthylmethacrylate) hydrogel tubes for use as nerve guidance channels. Biomaterials. 2002;23:3843–51.
6. Lesný P, De Croos J, Přádný M, Vacík J, Michálek J, Woerly S, et al. Polymer hydrogels usable for nervous tissue repair. J Chem Neuroanat. 2002;23:243–7.
7. Han D-W, Lee MS, Park BJ, Kim JK, Park JC. Enhanced neurite outgrowth of rat neural cortical cells on surface-modified film of poly(lactic-co-glycolic acid). Biotechnol Lett. 2005;27:53–8.
8. Bledi Y, Domb AJ, Linial M. Culturing neuronal cells on surfaces coated by a novel polyethyleneimine-based polymer. Brain Res Brain Res Protoc. 2000;5:282–9.
9. Lakard S, Herlem G, Propper A, Kastjer A, Michel G, Valles-Villarreal N, et al. Adhesion and proliferation of cells on new polymers modified biomaterials. Bioelectrochemistry. 2004;62:19–27.
10. Oliva AA Jr, James CD, Kingman CE, Craighead HG, Banker GA. Patterning axonal guidance molecules using a novel strategy for microcontact printing. Neurochem Res. 2003;28:1639–48.
11. Branch DW, Wheeler BC, Brewer GJ, Leckband DE. Long-term maintenance of patterns of hippocampal pyramidal cells on substrates of polyethylene glycol and microstamped polylysine. IEEE Trans Biomed Eng. 2000;47:290–300.
12. Lesný P, Přádný M, Jendelová P, Michálek J, Vacík J, Syková E. Macroporous hydrogels based on 2-hydroxyethyl methacrylate. Part: 4 growth of rat bone marrow stromal cells in three-dimensional hydrogels with positive and negative surface charges and in polyelectrolyte complexes. J Mater Sci Mater Med. 2006;17:829–33.
13. Urdzíková L, Jendelová P, Glogarová K, Burian M, Hájek M, Syková E. Transplantation of bone marrow stem cells as well as mobilization by granulocyte-colony stimulating factor promotes recovery after spinal cord injury in rats. J Neurotrauma. 2006;23:1379–91.
14. Chen X, Li Y, Wang L, Katakowski M, Zhang L, Chen J, et al. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. Neuropathology. 2002;22:275–9.
15. Přádný M, Lesný P, Smetana K Jr, Vacík J, Šlouf M, Michálek J, et al. Macroporous hydrogels based on 2-hydroxyethyl methacrylate. Part II. Copolymers with positive and negative charges, polyelectrolyte complexes. J Mater Sci Mater Med. 2005;16:767–73.
16. Michálek J, Přádný M, Artyukhov A, Šlouf M, Smetana K Jr. Macroporous hydrogels based on 2-hydroxyethyl methacrylate. Part III. Hydrogels as carriers for immobilization of proteins. J Mater Sci Mater Med. 2005;16:783–6.
17. Přádný M, Lesný P, Fiala J, Vacík J, Šlouf M, Michálek J, et al. Macroporous hydrogels based on 2-hydroxyethyl methacrylate. Part 1. Copolymers of 2-hydroxyethyl methacrylate with methacrylic acid. Collect Czech Chem Commun. 2003;68:812–22.
18. Hallab NJ, Bundy KJ, O'Connor K, Clark R, Moses RL. Cell adhesion to biomaterials: correlations between surface charge, surface roughness, adsorbed protein, and cell morphology. J Long Term Eff Med Implants. 1995;5:209–31.
19. Wittmer CR, Phelps JA, Saltzman WM, Van Tassel PR. Fibronectin terminated multilayers films: protein adsorption and cell attachment studies. Biomaterials. 2007;28:851–60.
20. Richert L, Boulmedais F, Lavalle P, Mutterer J, Ferreux E, Decher G, et al. Improvement of stability and cell adhesion properties of polyelectrolyte multilayers films by chemical cross-linking. Biomacromolecules. 2004;5:284–94.
21. Richert L, Engler AJ, Discher DE, Picart C. Elasticity of native and cross-linked polyelectrolyte multilayers films. Biomacromolecules. 2004;5:1908–16.
22. Kidambi S, Lee I, Chan C. Controlling primary hepatocyte adhesion and spreading on protein-free polyelectrolyte multilayer films. J Am Chem Soc. 2004;126:16286–7.
23. Woerly S, Lavallée C, Marchand R. Intracerebral implantation of ionic synthetic hydrogels: effect of polar substrata on astrocytosis and axons. J Neural Transplant Plast. 1992;3:21–34.
24. Mendelsohn JD, Yang SY, Hiller J, Hochbaum AI, Rubner MF. Rational design of cytophilic and cytophobic polyelectrolyte multilayer thin films. Biomacromolecules. 2003;4:96–106.
25. Hwang JJ, Jelacic S, Samuel NT, Maier RV, Campbell CT, Castner DG, et al. Monocyte activation on polyelectrolyte multilayers. J Biomater Sci Polym Ed. 2005;16:237–51.
26. Smetana K Jr, Vacík J, Součková D, Krčová Z, Šulc J. The influence of hydrogel functional groups on cell behavior. J Biomed Mater Res. 1990;24:463–70.
27. Plant GW, Woerly S, Harvey AR. Hydrogels containing peptide or aminosugar sequences implanted into the rat brain: influence on cellular migration and axonal growth. Exp Neurol. 1997;143:287–99.
28. Lakard S, Herlem G, Valles-Villareal N, Michel G, Propper A, Gharbi T, et al. Culture of neural cells on polymers coated surfaces for biosensor applications. Biosens Bioelectron. 2005;20:1946–54.
29. Mingyu C, Kai G, Jiamou L, Yandao G, Nanming Z, Xiufang Z. Surface modification and characterization of chitosan film blended with poly-L-lysine. J Biomater Appl. 2004;19:59–75.
30. Woerly S, Laroche G, Marchand R, Pato J, Šubr V, Ulbrich K. Intracerebral implantation of hydrogel-coupled adhesion peptides: tissue reaction. J Neural Transplant Plast. 1995;5:245–55.
31. Dillon GP, Yu X, Sridharan A, Ranieri JP, Bellamkonda RV. The influence of physical structure and charge on neurite extension in a 3D hydrogel scaffold. J Biomater Sci Polym Ed. 1998;9:1049–69.