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Polymer hydrogels usable for nervous tissue repair

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Abstract

The implantation of non-resorbable biocompatible polymer hydrogels into defects in the central nervous system can reduce glial scar formation, bridge the lesion and lead to tissue regeneration within the hydrogel. We implanted hydrogels based on crosslinked poly hydroxyethyl-methacrylate (pHEMA) and poly *N*-(2-hydroxypropyl)-methacrylamide (pHPMA) into the rat cortex and evaluated the cellular invasion into the hydrogels by means of immunohistochemical methods and tetramethylammonium diffusion measurements. Astrocytes and NF160-positive axons grew similarly into both types of hydrogels. We found no cell types other than astrocytes in the pHEMA hydrogels. In the pHPMA hydrogels, we found a massive ingrowth of connective tissue elements. These changes were accompanied by corresponding changes in the extracellular space volume fraction and tortuosity of the hydrogels.

Keywords: Astrocytes; Diffusion; Extracellular space; Image analysis; Macroporous hydrogel; Regeneration; Transplantation

1. Introduction

Severe injury of central nervous system (CNS) tissue results in the formation of a complex scar that prevents regenerating axons and glial cells from entering the lesion. Inducing regeneration in adult brain or spinal cord requires removing the scar tissue and developing a method to re-form the tissue structure. Biocompatible non-resorbable polymer hydrogels are suitable implantation materials used in medicine, and the implantation of these hydrogels can reduce scar tissue formation and bridge the lesion, creating an environment permissive for cellular ingrowth and the diffusion of various neuroactive substances including growth factors (Woerly et al., 2001, 1999b, 1998, 1996).

In the present study we have evaluated two different hydrogels (Woerly, 1999a; Woerly et al., 1999b, Přádný, unpublished results) based on crosslinked poly hydroxyethyl-methacrylate (pHEMA) and poly *N*-(2-hydroxypropyl)-methacrylamide (pHPMA) as implant materials designed to bridge defects in nervous tissue. We have implanted small blocks of hydrogel into the rat cortex and evaluated the cellular invasion into the implants by means of immunohistochemical methods and tetramethylammonium (TMA^+) diffusion measurements (Nicholson and Syková, 1998; Syková, 1997a,b; Syková et al., 2000).

2. Materials and methods

The pHEMA hydrogels were prepared by mixing 0.609 g of HEMA, 0.006 g of azobisisobutyronitril, 0.012 g of 1,2-ethyleneglycol dimethacrylate, 0.077 g of methacrylic acid, 4.76 g of poly(ethylene glycol), Mw =

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400 and 10 g of fractionated sodium chloride with a grain size of up to 0.03 mm. The mixture was compressed with a force of 10 Nm and heated at 80 °C for 8 h. The sodium chloride and the rest of the monomers were washed out by a physiological solution.

The pHPMA hydrogels were prepared under sterile conditions by a method developed earlier (Strohalm and Kopeček, 1978; Woerly, 1999a), by crosslinking radical polymerization of the monomer (HPMA) in a mixture of acetone–dimethyl sulfoxide. Due to the use of excess dimethyl sulfoxide, the resulting hydrogel had a macroporous homogeneous structure. The ratio between acetone and dimethyl sulfoxide (93:7 v/v) was chosen in order to create communicating pores. The concentration of HPMA in the polymerizing mixture was 30% by weight. The resulting hydrogels had fewer diffusion barriers and more space available for cellular ingrowth than did the pHPMA hydrogels used in previous studies (Woerly et al., 1999b).

Using the in vivo real-time ionthophoretic TMA^+ method (Nicholson and Syková, 1998; Syková, 1997a) the extracellular space (ECS) volume fraction, α ($\alpha =$ ECS volume/total tissue volume) and tortuosity $\lambda = (D/ADC)^{0.5}$ (where D is the free and ADC is the apparent diffusion coefficient), were measured in hydrogels prior to implantation and compared with the values in cortex. The α and λ values were also determined in both hydrogels post-implantation (Fig. 1).

Experiments were performed on 6-week-old Wistar rats. Following sodium pentobarbital anesthesia (50 mg/kg), a rat was placed in a stereotactic apparatus, a bone flap 2 mm in diameter was removed and the dura incised. A block of cortical tissue ($1.5 \times 1.5 \times 1.5$ mm^3) was removed, and the hydrogel implanted into the cavity under sterile conditions. The bone flap was returned and the wound closed. The rats were given antibiotics (ampiciline 200–400 mg/kg) prior to surgery and analgesics (karprofen 4 mg/kg) at the end of the surgical procedure.

Either 4 or 8 weeks after surgery, the rats were deeply anesthetized with sodium pentobarbital and perfused through the heart with PBS, followed by a fixative containing 4% paraformaldehyde. The brains with implanted hydrogels were then processed for haematoxylin–eosin and cresyl violet staining and for fluorescence immunohistochemistry using antibodies to GFAP (astrocytes), NF160 (axons), lectin (microglia) and chondroitin sulfate proteoglycans. The tissue sections were scanned using an AxioScope fluorescent microscope to allow further processing by the Image analysis toolbox in Matlab 5.3 (The MathWorks, Inc.). The extent of the ingrowth of astrocytes into the hydrogel was determined as the total area occupied by GFAP-positive astrocytic processes in individual histological sections.

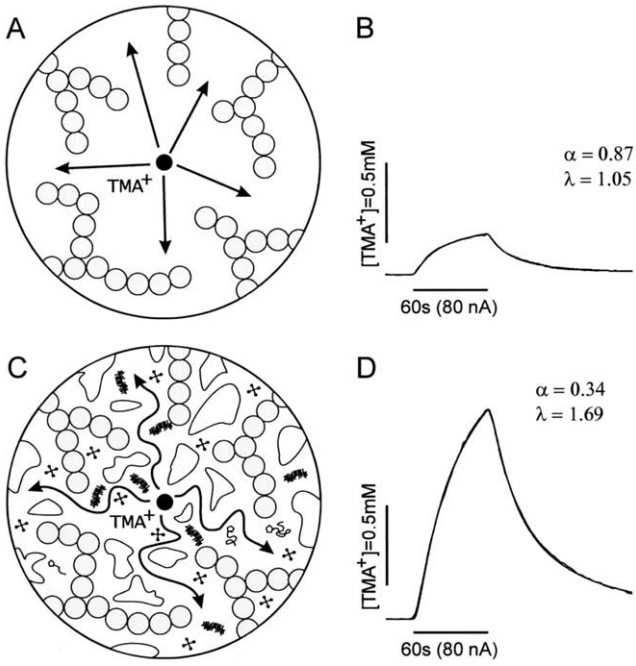

Fig. 1. Concept of extracellular space volume fraction (α) and tortuosity (λ). The concentration of a diffusing substance, such as TMA^+, released from a microelectrode and confined to the ECS is modified by the volume fraction and tortuosity. In the schematic drawings of the hydrogel prior to implantation (A) and postimplantation (C), the volume fraction is represented by the space between the building components of the hydrogel (A) or by the space between the building components of the hydrogel, ingrowing cells and elements of the extracellular matrix (C). The TMA^+ molecule paths, representing the tortuosity, are longer in the hydrogel postimplantation, showing increased hindrance for the TMA^+ molecules, than in the hydrogel before implantation. The TMA^+ ions are iontophoresed into the hydrogel by an iontophoretic micropipette, and their concentration is measured at a known distance by a TMA^+-selective microelectrode. The acquired diffusion curves (B, D) are fitted using a non-linear curve-fitting algorithm to determine the values of volume fraction (α), tortuosity (λ) and non-specific uptake (k′) (Nicholson and Phillips, 1981). The concentration scale is linear and the theoretical diffusion curve is superimposed on each data curve.

3. Results

Diffusion analysis revealed that in polymer hydrogels prior to implantation, the volume fraction of the gel space available for diffusion was 0.46 ± 0.01 in the pHEMA and 0.87 ± 0.01 in the pHPMA hydrogels. The tortuosity prior to implantation was 1.00 ± 0.01 in the pHEMA and 1.05 ± 0.01 in the pHPMA hydrogels. These values show that the diffusion of a rather small molecule such as TMA^+ is only slightly more hindered in polymer hydrogels than in diluted agar, where volume fraction and tortuosity both equal 1.00 (Fig. 1).

Four as well as 8 weeks after implantation, both types of hydrogel implants firmly adhered to the host brain tissue and were surrounded by a dense glial scar consisting mostly of GFAP-positive astrocytes and a large amount of chondroitin-sulfate proteoglycan. The

average area of hydrogel measured in one tissue section was 1.66 ± 0.23 mm^2. In both hydrogels, GFAP-positive astrocytic processes were invading the hydrogel implants, primarily from the region closest to the corpus callosum (Fig. 2). The total area occupied by astrocytic processes in the pHEMA hydrogels was 6 ± 3 μm^2 in the 4-week group and 35 ± 6 μm^2 in the 8-week group. In the pHPMA hydrogels the area occupied by astrocytic processes increased from 9 ± 2 μm^2 in the 4-week group to 42 ± 7 μm^2 in the 8-week group. We conclude that the ingrowth of astrocytes into the implants was not significantly different between the two types of hydrogel.

Histological examination revealed a massive invasion of various connective tissue elements (fibroblasts, collagen, blood vessels) into the pHPMA hydrogels 4 as well as 8 weeks after implantation. The meninges constituted one of the most probable sources of the connective tissue together with the blood vessels growing into the implant from the immediate vicinity. No microglia or oligodendroglia were observed inside the hydrogels, but occasionally, a small number of NF160-positive axons were observed crossing the glial scar and entering the implants. These processes grew erratically, however, and did not form any synapses within the hydrogel.

In the pHEMA hydrogels, no cell types other than GFAP-positive astrocytes and occasional NF160-positive axons were found 4 or 8 weeks after implantation. The astrocytic processes and cell bodies grew into the hydrogel from the surrounding tissue. The middle part of the implanted hydrogel did not contain any con-

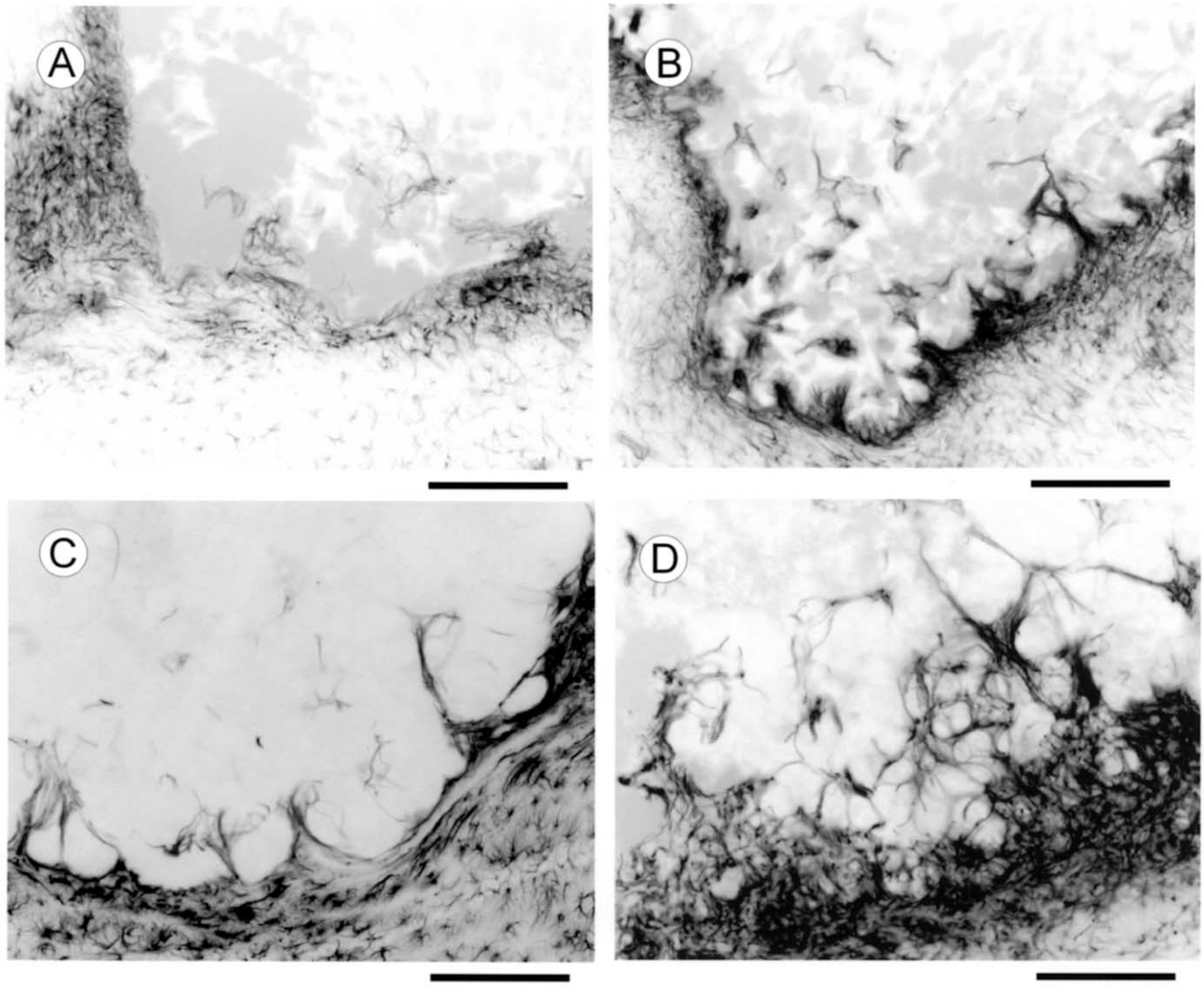

Fig. 2. Hydrogels 4 and 8 weeks after implantation into rat cortex. Astrocytes stained for GFAP at the border of a hydrogel implant. Four weeks after implantation some astrocytic processes are seen growing into the hydrogel implants pHEMA (A) and pHPMA (C). Eight weeks after implantation the number of astrocytic processes has grown larger, forming a net-like structure in both the hydrogels—pHEMA (B) and pHPMA (D). There is no significant difference in the extent of the processes between the pHEMA and pHPMA hydrogels. Scale bar = 200 μm.

nective tissue, cells or chondroitin sulphate proteoglycans.

The diffusion parameters were measured in the center of the pHEMA and pHPMA hydrogel implants. The diffusion parameters of the pHEMA hydrogels did not change after implantation. This is in good agreement with the absence of tissue elements in the central part of the pHEMA hydrogels. The diffusion measurements in the center of the pHPMA hydrogels in the 8-week group showed that the ECS volume fraction decreased to 0.34 ± 0.01. This value, when compared to the volume fraction in pHPMA hydrogels before implantation, reflects the new tissue formation in the hydrogels. The value is, however, still larger than the volume fraction in adult rat cortex, which is 0.21 ± 0.01 (Lehmenkühler et al., 1993; Nicholson and Syková, 1998; Syková, 1997a; Syková et al., 2000), but it is similar to the volume fraction during early postnatal development (Lehmenkühler et al., 1993; Syková, 1997a). The tortuosity increased from 1.05 ± 0.01 to 1.69 ± 0.03, showing the development of new diffusion barriers that hinder the diffusion of TMA^+ in the hydrogel. This hindrance is even larger than in adult rat cortex, where tortuosity is 1.59 ± 0.02 (Lehmenkühler et al., 1993; Nicholson and Syková, 1998; Syková et al., 2000).

4. Discussion

Non-resorbable polymer hydrogels are a group of materials that have considerable potential for tissue replacement and possibly axonal regeneration in the CNS. They allow tissue formation within that portion of the total hydrogel volume expressed as the volume fraction before implantation (about 87% in pHPMA and 46% in pHEMA) and provide ingrowing cells with mechanical stability and a large surface area (Woerly et al., 1996).

The changes in the diffusion properties of both hydrogels following implantation correspond to the new tissue formation within the hydrogels. The diffusion parameter values observed in the pHPMA hydrogels are closer to the normal diffusion parameter values in rat cortex (Nicholson and Syková, 1998) than the values obtained previously in a different pHPMA hydrogel (Woerly et al., 1999b). The difference between the values in the previous study (Woerly et al., 1999b) and those found in the present study can be explained by the different structures of the hydrogels that were used.

Both pHEMA and pHPMA hydrogels were gradually colonized by astrocytes, whose processes grew into both hydrogels with similar speed. However, the two hydrogels differed markedly in the reaction of the surrounding connective tissue. The pHPMA hydrogel was invaded by various connective tissue elements shortly after implantation. Although a small amount of connective tissue is necessary, various connective tissue elements can negatively influence the regeneration of nervous tissue after an injury. In contrast, some chemical or physical properties of the pHEMA apparently hindered connective tissue ingrowth into the hydrogel, while still allowing astrocytes to enter the implant. This cell-specific feature of the pHEMA hydrogel could be useful in the future design of hydrogel implants.

Among many other issues, the further development of hydrogel implants capable of bridging nervous tissue defects should concentrate on influencing the cell populations growing into the implant. This might be accomplished by altering the chemical properties of the hydrogel surface by binding growth-supporting (or conversely, growth-inhibiting) factors onto it. Alternatively, the growth dynamics of the cells in the implanted hydrogel could be modified by the continuous infusion of growth factors.

5. Further perspectives

Engineering neural tissue based on hydrogel technology has future applications such as hybrid cell–polymer constructs (using fetal or stem cells) that would combine the features of biocompatible polymer matrices, e.g. stability, a porous structure and hydrophilicity, with the properties of physiologically active cells, such as the expression of biospecific surface receptors and the synthesis of bioactive molecules. Hydrogel implantation may serve as an alternative to conventional grafting technologies, benefiting from advances in surface chemistry and the cell–cell or cell–matrix interactions that occur during development or regeneration. Further experiments are underway to compare the suitability of hydrogels with various chemical structures and different physical characteristics (porosity, tortuosity) for preventing the formation of diffusion barriers evoked by injury.

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References

Lehmenkühler, A., Syková, E., Svoboda, J., Zilles, K., Nicholson, C. 1993. Extracellular space parameters in the rat neocortex and subcortical white matter during postnatal development determined by diffusion analysis. Neuroscience 55, 339–351.

Nicholson, C., Phillips, J.M. 1981. Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. J. Physiol. 321, 225–257.

Nicholson, C., Syková, E. 1998. Extracellular space structure revealed by diffusion analysis. Trends Neurosci. 21, 207–215.

Strohalm, J., Kopeček, J. 1978. Poly[*N*-(2-hydroxypropyl)methacrylamide]. Angew. Makromol. Chem. 70, 109.

Syková, E. 1997a. The extracellular space in the CNS: its regulation, volume and geometry in normal and pathological neuronal function. The Neuroscientist 3, 28–41.

Syková, E. 1997b. Extracellular space volume and geometry of the rat brain after ischemia and central injury. Adv. Neurol. 73, 121–135.

Syková, E., Mazel, T., Vargová, L., Vořišek, I., Prokopová-Kubínová, Š. 2000. Extracellular space diffusion and pathological states. Prog. Brain Res. 125, 155–178.

Woerly, S. (1999a) Implantable polymer hydrogels for therapeutic use, US Patent 5,863,551 (1999a).

Woerly, S., Doan, V.D., Sosa, N., de Vellis, J., Espinosa, A. 2001. Reconstruction of the transected cat spinal cord following Neuro-Gel implantation: axonal tracing, immunohistochemical and ultrastructural studies. Int. J. Dev. Neurosci. 19, 63–83.

Woerly, S., Petrov, P., Syková, E., Roitbak, T., Šimonová, Z., Harvey, A.R. 1999b. Neural tissue formation within porous hydrogels implanted in brain and spinal cord lesions: ultrastructural, immunohistochemical, and diffusion studies. Tissue Eng. 5, 467–488.

Woerly, S., Pinet, E., De Robertis, L., et al. 1998. Heterogeneous PHPMA hydrogels for tissue repair and axonal regeneration in the injured spinal cord. J. Biomater. Sci. Polym. Ed. 9, 681–711.

Woerly, S., Plant, G.W., Harvay, A.R. 1996. Neural tissue engineering: from polymer to biohybrid organs. Biomaterials 17, 301–310.