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Soft macroporous hydrogel scaffolds based on copolymers of *N*-(2-hydroxypropyl)methacrylamide

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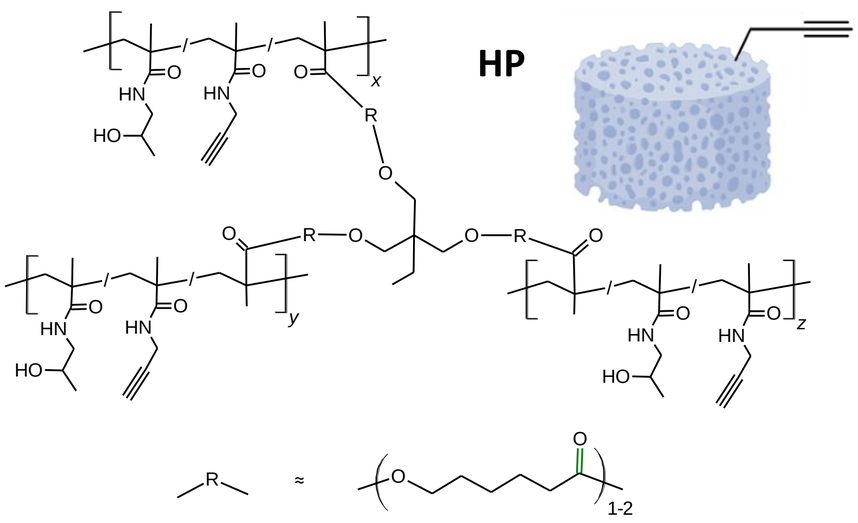
INTRODUCTION

Macroporous synthetic **hydrogels based on *N*-(2-hydroxypropyl) methacrylamide (HPMA) copolymers** form **3D scaffolds applicable in tissue engineering and regenerative medicine**, e.g. for the treatment of traumatic spinal cord injury.

Water-soluble copolymers of HPMA have been studied for several decades mainly as drug carriers for several decades. They **are biocompatible, non-immunogenic, and non-toxic**, but nevertheless, their polymer backbone is not biodegradable. HPMA has was also been utilized in the synthesis of hydrogels. The viscoelastic properties of the HPMA-based hydrogels are similar to those of the developing spinal cord with porous HPMA-based hydrogels allowing cell migration and new tissue growth composed of glial cells, blood vessels, axons, and dendrites. However, they **do not allow non-specific absorption of proteins (fouling)** from the medium or blood plasma and lead to uncontrolled cell attachment. Such properties of HPMA-based hydrogels enable to control cell of the fate and behavior of cells on the surface of the hydrogel matrix.

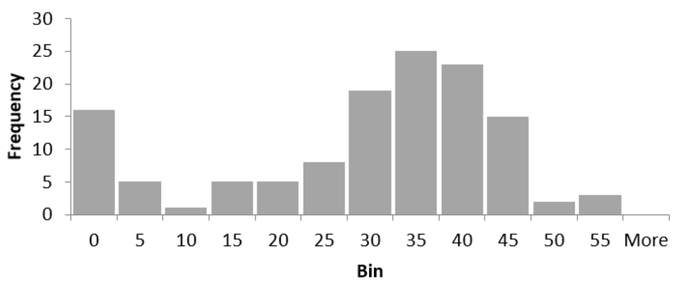
The surface of **HPMA-based hydrogels does not contain any domains promoting cell adhesion,** which can be overcome by employing selected signaling domains. In addition, **the surface can be functionalized with biomimetic motifs**, whereby cells specifically recognize the matrix surface and increase adhesion, engraftment, and/or differentiation. Modification by extracellular matrix (ECM) adhesion proteins such as laminin, fibronectin, or derived peptides (e.g., RGD oligopeptide) can also mainly alters the attractiveness of the material in the form of increased cell adhesion and cytoskeleton organization. One commonly used adhesive peptide is the **arginylglycylaspartic acid (RGD) peptide** derived from fibronectin is the most widely used biomimetic motif in tissue engineering.

Hydrogels prepared by free radical polymerization



Porogen NaCl (30-50 µm) - 78 wt.%; 1-dodecanol – 8 wt.%

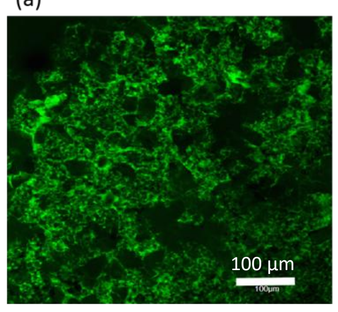
Histogram of pore size (HP)



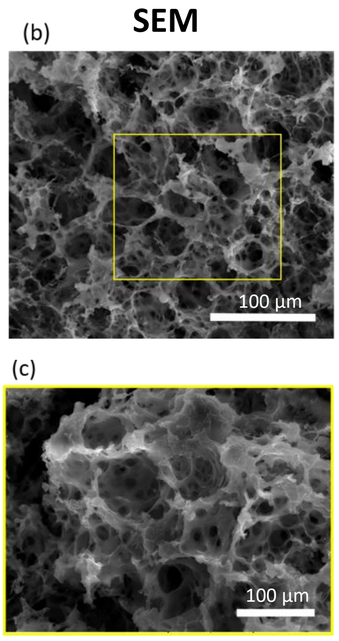
Average pore size - 25±7 µm
Pore volume fraction Φp = 0.88

Swelling – 94%

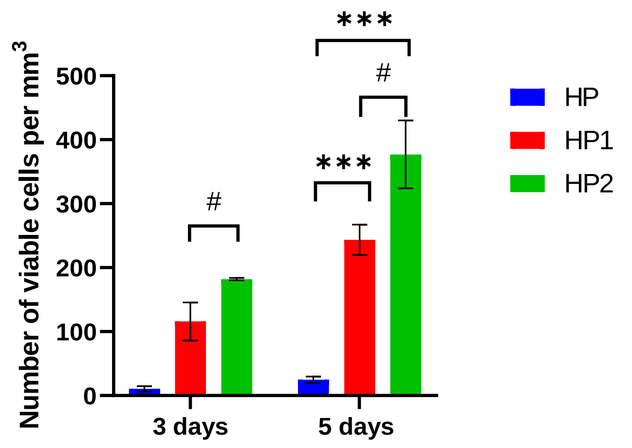
LCSM



SEM

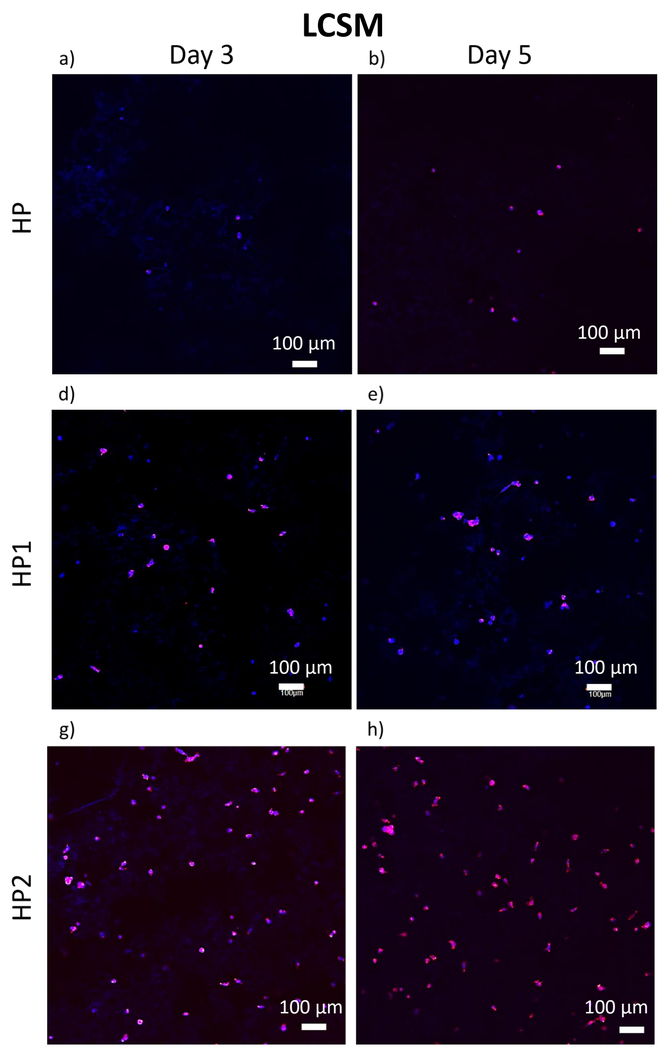


Cultivation of hydrogels with mesenchymal stem cells

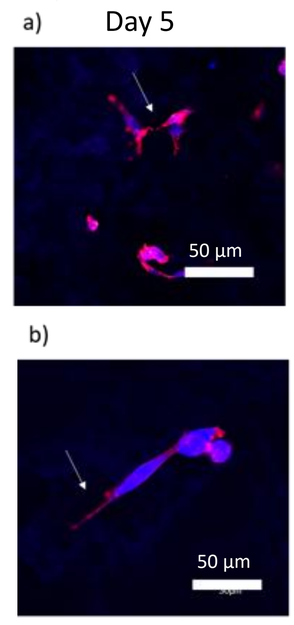


One-way ANOVA followed by Tukey's test was used for statistical analysis (# $P < 0.05$). Dunnett's method was used for individually modified hydrogels versus HP (*** $P < 0.001$).

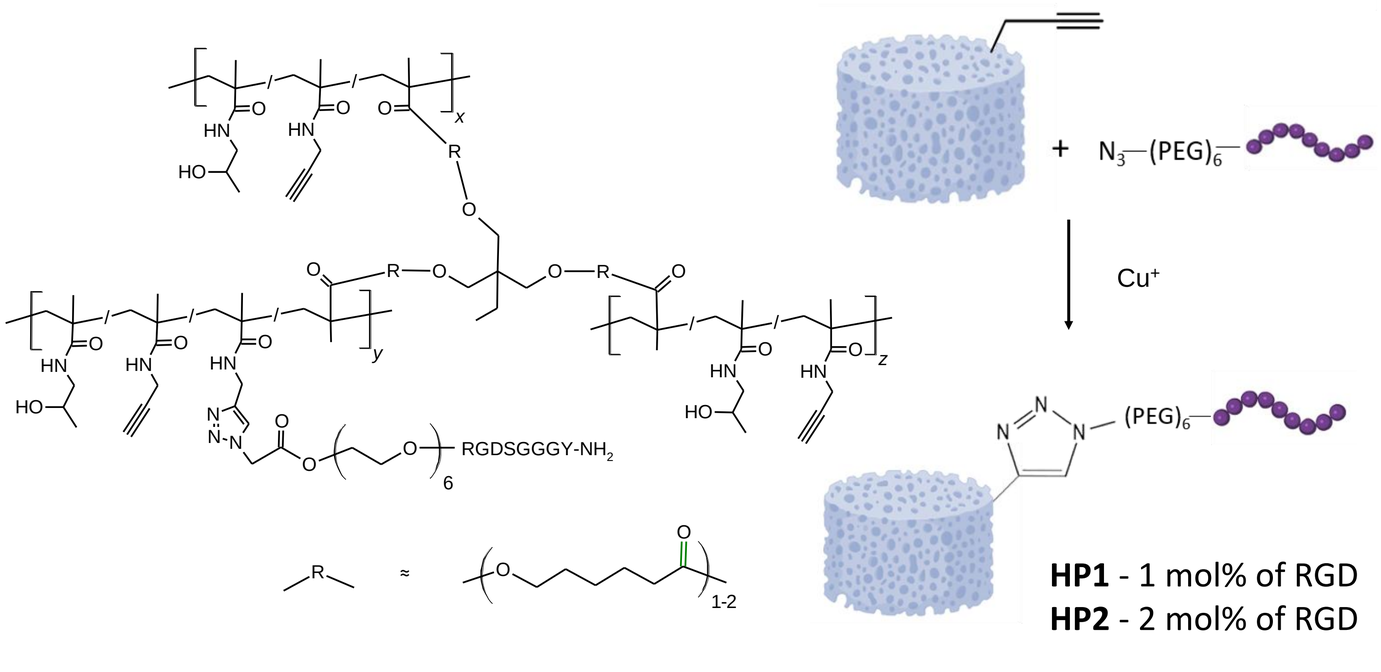
LCSM



The growth of rat mesenchymal stem cells (rMSCs) on the hydrogels obtained by LSCM. Cells were fixed, and cell nuclei were stained with DAPI (blue) and actin filaments with Rhodamine phalloidin (red).

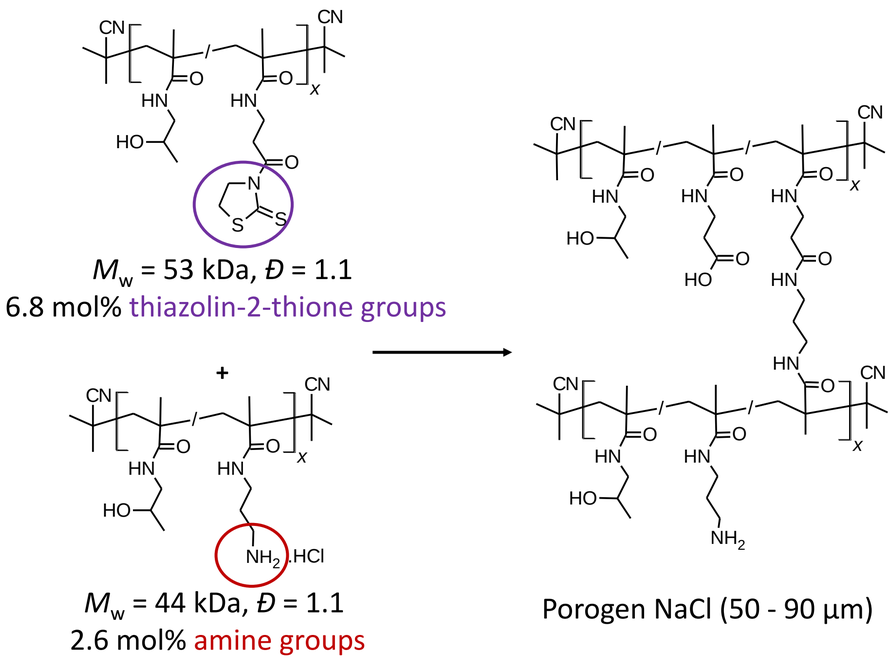


Modification of hydrogels by RGD oligopeptides using click-chemistry

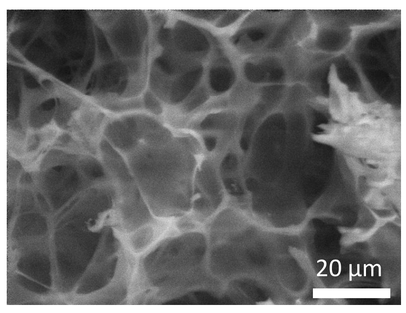


HP1 - 1 mol% of RGD
HP2 - 2 mol% of RGD

Preparation of hydrogels by cross-linking of polymer precursors



SEM



Compressive Modulus (Stiffness) – ca 3 kPa

Conclusion

- Porous soft HPMA-based hydrogels were prepared and characterized.
- Hydrogels were succesfully modified by the oligopeptide containing RGD motif.
- Hydrogel matrices are biocompatible and allow the growth of rMSC when modified RGD oligopeptide.
- The hydrogels can be prepared also from defined polymer precursors.
- Developed hydrogels are suitable candidates for further testing *in vivo* and offer potential use as scaffolds for neuronal tissue repair.

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