Therapeutic Cells via Functional Modification: Influence of molecular properties of polymer grafts on in vivo circulation, clearance, immunogenicity and antigen protection

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Supporting information

1.1 Proton NMR analysis of HPG polymers:

Proton NMR of HPG polymers and HPG polymers functionalized with eight groups of carboxylic acid were provided in Figure 1S. Due to barboxylation two new peaks at 2.35 and 2.51 ppm was appeared. A proton NMR of succinimidyl succinate functionalized HPG was provided in a previous publication from our group.[1]

2.1. Influence of HPG graft properties on the surface protection of mouse RBCs

Currently, there is no information available on the surface characteristics of HPG grafted mouse RBCs. Such characteristics are important for understanding the clearance of HPG modified mouse RBCs, and can be used as a control to estimate the level of modification prior to the in vivo administration. The in vitro characterization can also provide reasons to why HPG 20 kDa and HPG 60 kDa modified RBCs gave comparable in vivo circulation, considering the significant differences in the hydrodynamic diameters of HPG 20 kDa (5.2 nm) and HPG 60 kDa (7.4 nm).

2.1.1 Charge protection by electrophoretic mobility

The electrophoretic mobility (EPM) of RBCs is governed by the surface charge density due to the presence of negatively charged sialic acids, and by the hydrodynamic flow in the region of double layer.[2,3] Results are shown in Figure 1SA. As expected, the increase in the molecular weight of HPG grafts and polymer graft concentration decreased the electrophoretic mobility of HPG grafted mouse RBCs compared to unmodified control cells. The decrease in the EPM with increase in polymer graft concentration due to the increase in the number of polymer molecules grafted to the surface.[4] a significant difference between mouse RBCs grafted with HPG 60 kDa and HPG 20 kDa was observed only at graft concentration of 0.75 mM and higher. The

difference, however, remained small. The small impact of the molecular weight in electrophoretic mobility reflected into small differences in the in vivo circulation (Figure 3A).

2.1.2 Changes in surface properties by aqueous two phase partitioning

Partition of RBCs in the Dextran110 kDa/PEG8 kDa aqueous two phase system supplemented with NaCl and sodium phosphate depends on the cell surface charge, modification level and on cells surface glycoprotein composition.[5] Since HPG is more compatible with PEG than dextran, the surface conjugation of HPG to RBCs will tend to enhance partition into the PEG-rich upper phase. As seen in Figure 1Sb, unmodified control mouse RBCs were located in the lower dextran phase. Mouse RBC modified with 0.5 and 1 mM of HPG 20 kDa and HPG 60 kDa moved slightly upward forming a clear region in the bottom of the dextran phase. Further modification with HPG 60 kDa and HPG 20 kDa (1.5 mM) replaced significant portion of cells in the upper PEG phase (Figure 2Sb). Since grafting of neutral polymers to the surface of RBCs does not alter the surface charge density, the movement of HPG grafted RBCs from the lower dextran phase to the upper PEG phase due to the interaction surface bound HPG with the PEG phase.[6,7] This results also confirm that the grafted HPG is accessible and the surface properties of RBCs were modified. The results of aqueous two phase partitioning experiment followed closely the results of the electrophoretic mobility of HPG grafted mouse RBCs.

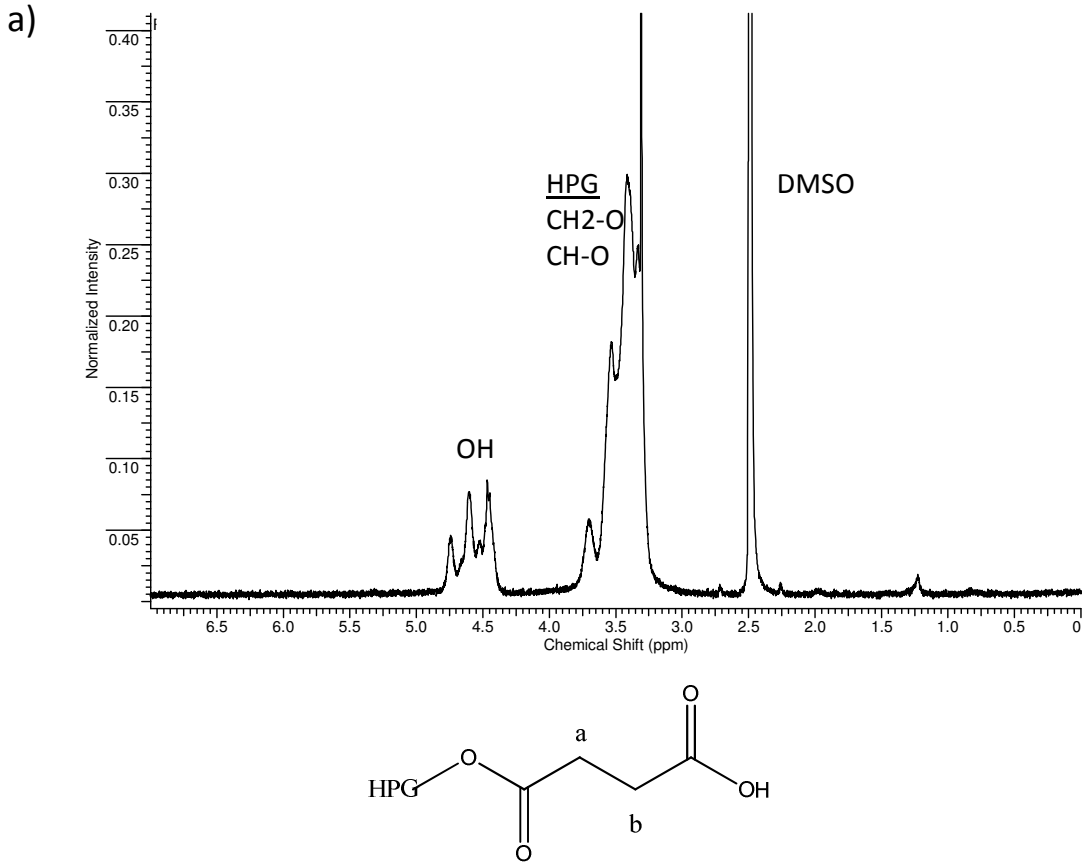

Figure 1S: a) Proton NMR (d6-DMSO) of hyperbranched polyglycerol (M_n =60 kDa), and b) Proton NMR (D_2O) hyperbranched polyglycerol (M_n = 60 kDa), functionalized with eight groups of carboxylic acid.

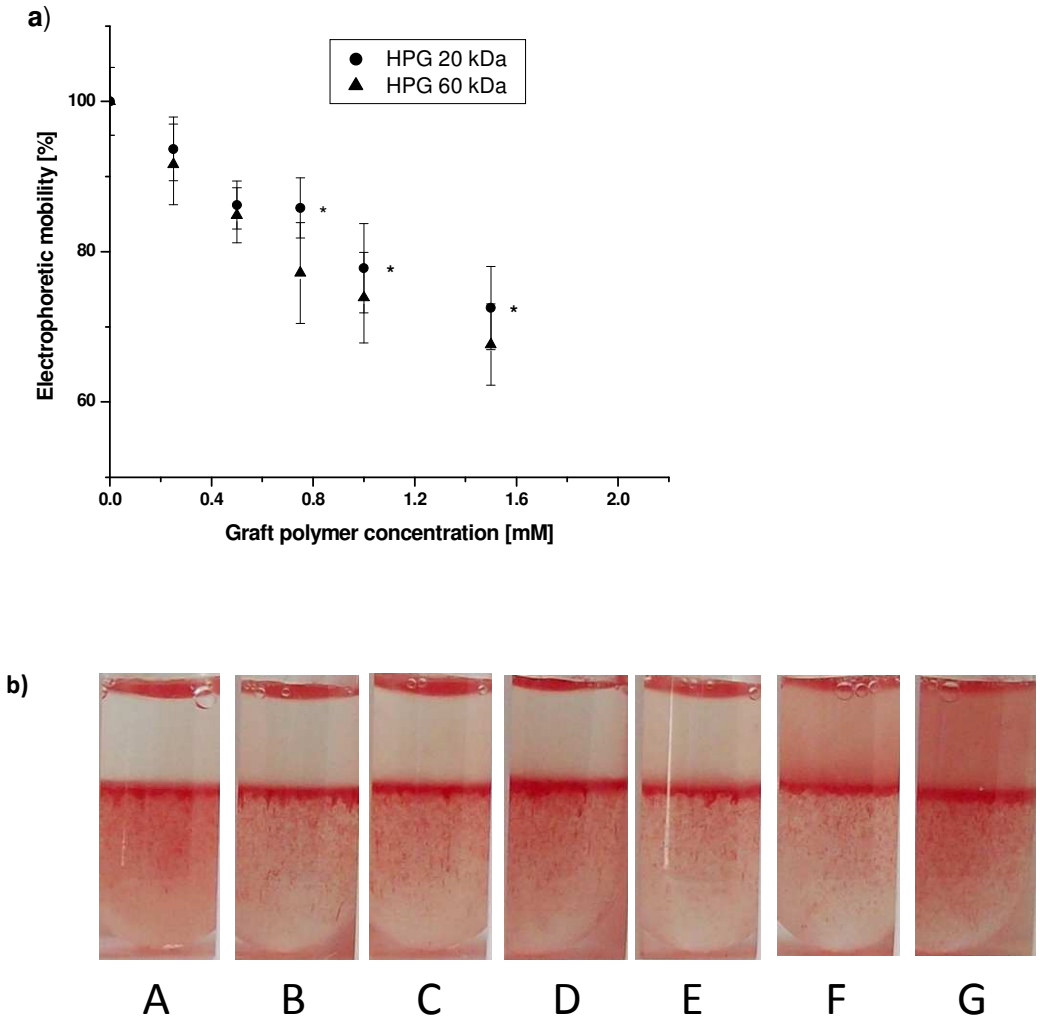

Figure 2S: In vitro characterization of mouse RBCs grafted with HPGs of different molecular weights and densities. A) Impact of HPG molecular weight and graft density on the electrophoretic mobility of mouse RBCs. Results represent the average of at least 6 independent measurements, and error bars represent the standard deviation around the mean, HPG 60 kDa grafted RBCs with electrophoretic mobility values that are significantly different from HPG 20 kDa grafted RBCs are indicated by * ($p < 0.05$). B) Impact of HPG molecular weight and graft density on the localization of cells in the PEG/dextran aqueous two phase partitioning system. A: control, B, D and F: RBCs grafted consequently with 0.75, 1, and 1.5 mM of HPG 20 kDa, C, E and G: mouse RBCs grafted consequently with 0.75, 1, and 1.5 mM of HPG 60 kDa.

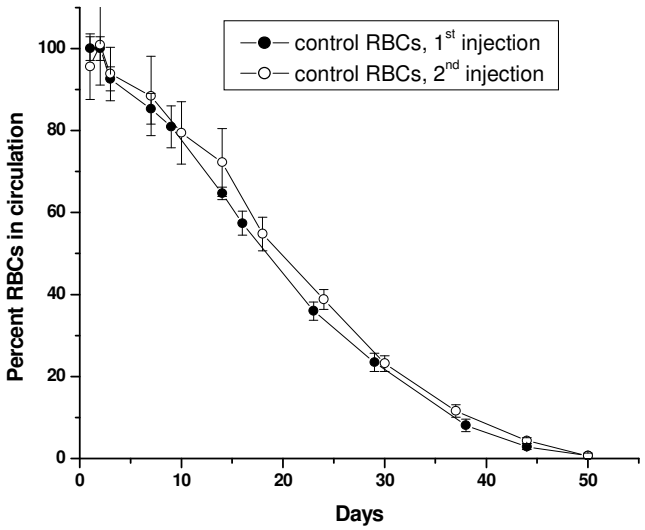

Figure 3S: The impact of repeated administration of control RBCs on the circulation in vivo. Results represent the average of 4 independent measurements, and error bars represent the standard deviation around the mean. Control RBCs were functionalized with PKH 26 lipid marker.

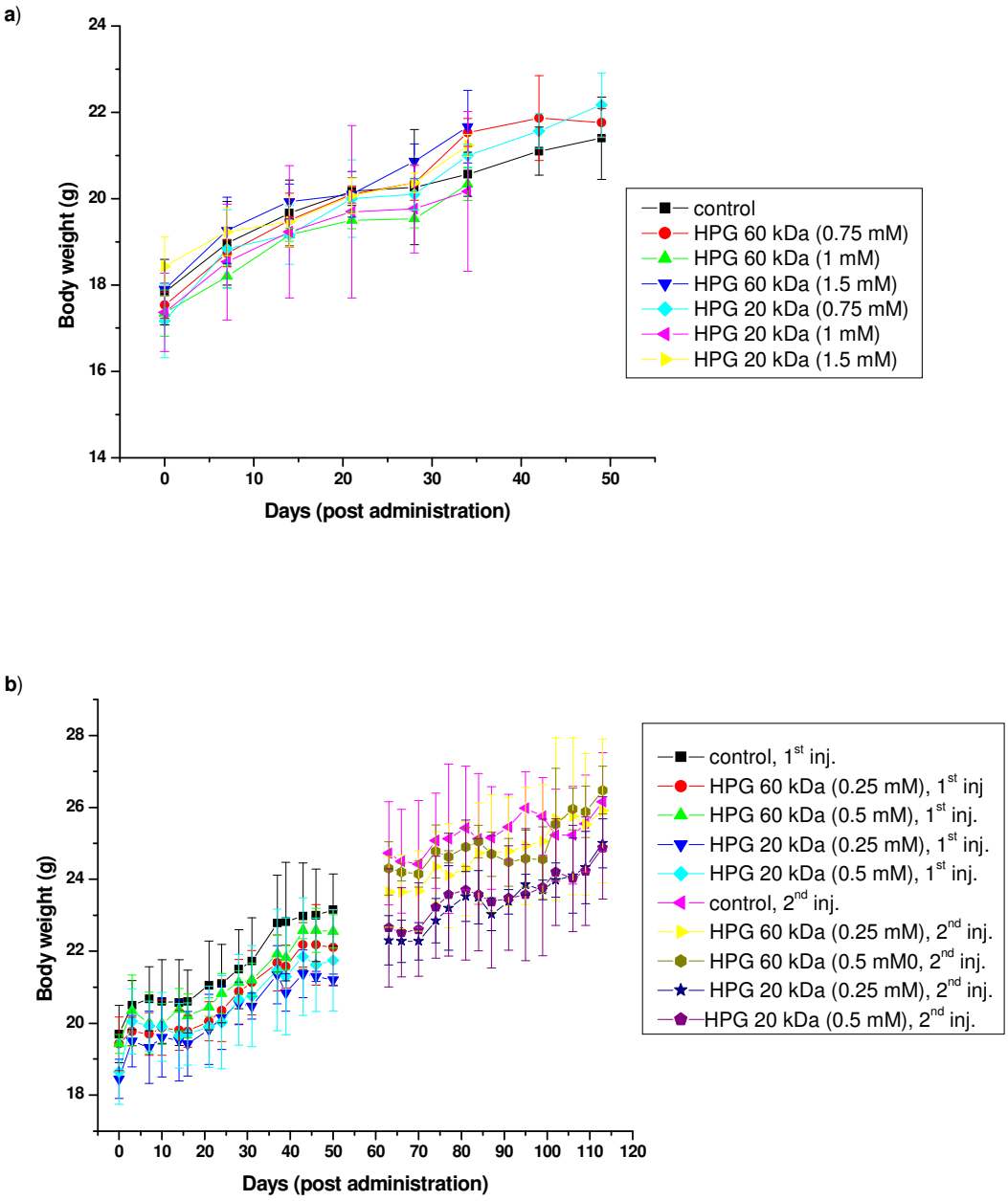

Figure 4S: A) Impact of graft HPG molecular weight (20 kDa and 60 kDa) and density (0.75, 1 and 1.5 mm) on the post administration body weight gain of animals transfused with HPG grafted RBCs at day 0. B) Impact of graft HPG molecular weight (20 kDa and 60 kDa) and density (0.25 and 0.5 mM) and repeated administration on the post administration body weight gain of animals. Mice were transfused with approximately 10 % of whole blood mass, based on the weight of the animals and the volume of RBC suspension infused.

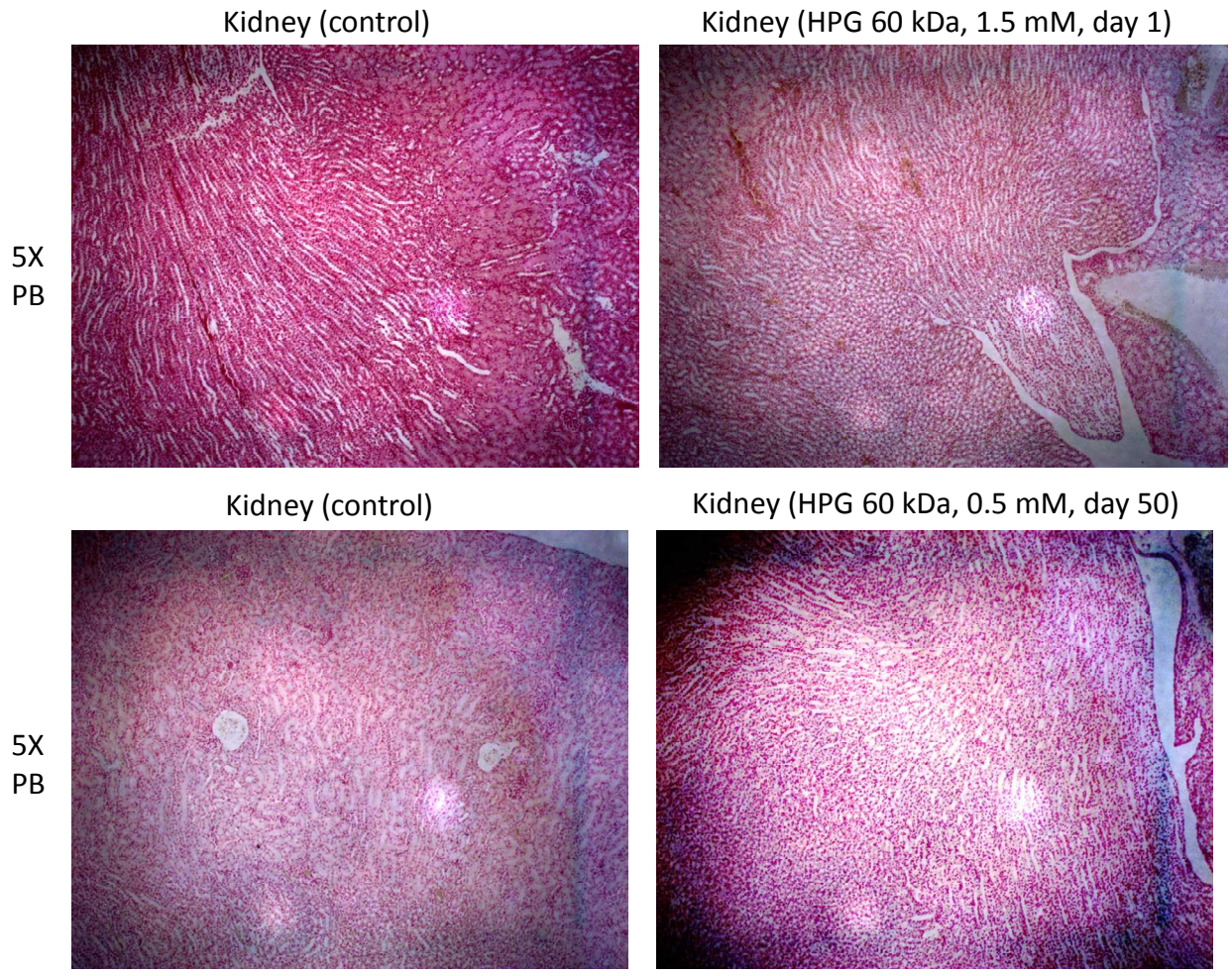

Figure 5S: Histological sections of the kidneys of animals administered with RBCs grafted with 1.5 mM HPG 60 kDa, and sacrificed at day 1 and RBCs grafted with 0.5 mM HPG 60 kDa, and sacrificed at day 50. Tissues were stained Perussian blue to detect iron deposits (blue stains). No iron deposits were detected in the kidneys of animals that received HPG grafted RBCs.

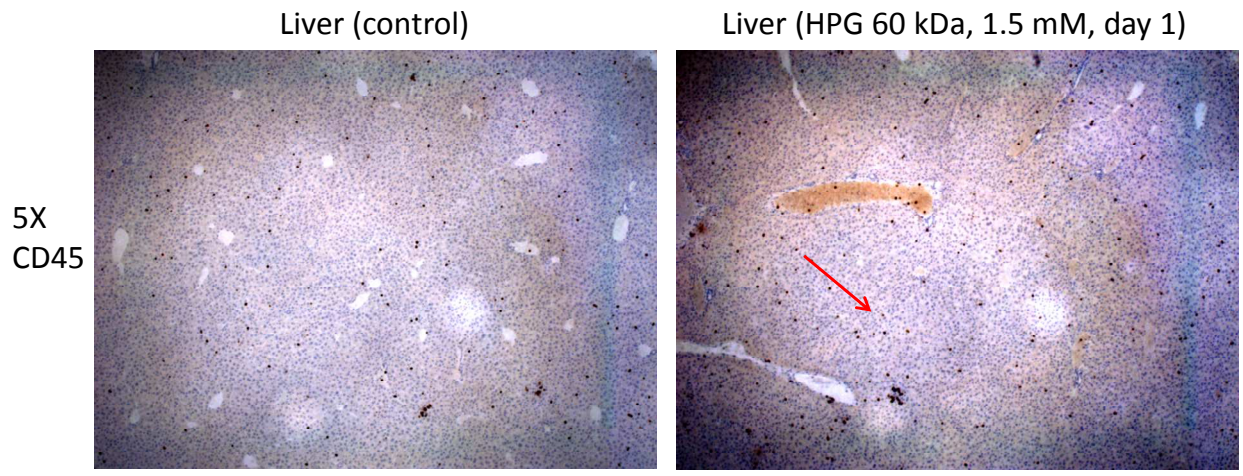

Figure 6S: Liver tissue stained with CD45 in animals that were administered with RBCs grafted with 1.5 mM HPG 60 kDa, and sacrificed at day 1. The arrow indicates toCD45 expressing cell (brown stains). The number of cells that expressed CD45 was ~ 30 % higher in the liver of animals administered with HPG compared to the control, quantified using the Northern Eclipse 6.0 image analysis software.

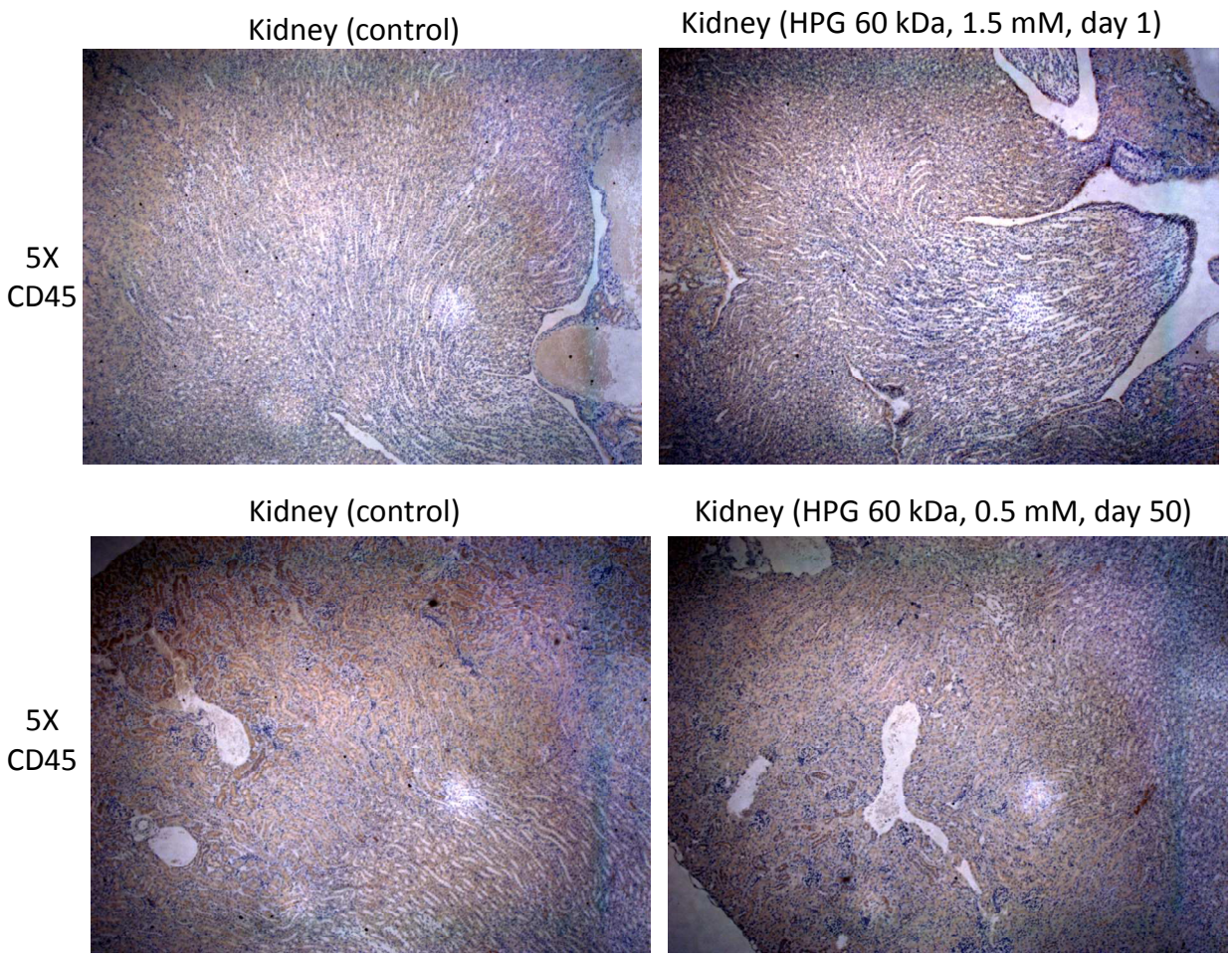

Figure 7S: Histological sections of the kidneys of animals administered with RBCs grafted with 1.5 mM HPG 60 kDa, and sacrificed at day 1 and RBCs grafted with 0.5 mM HPG 60 kDa, and sacrificed at day 50. Tissues were stained for CD45, no differences compared to the control were observed in the kidney samples that received HPG grafted RBCs.

REFERENCES

(1) Rossi, N. A. A.; Constantinescu, I.; Kainthan, R. K.; Brooks, D. E.; Scott, M. D.; Kizhakkedathu, J. N. *Biomaterials* **2010,** 31 (14), 4167-4178.
(2) Levine, S.; Levine, M.; Sharp, K. A.; Brooks, D. E. *Biophys. J.* **1983**, 42 (2), 127-135.
(3) Neu, B.; Armstrong, J. K.; Fisher, T. C.; Baumler, H.; Meiselman, H. J. *Biorheology* **2001**, 38(5-6), 389-403.
(4) Chapanian, R.; Constantinescu, I.; Brooks, D. E.; Scott, M. D.; Kizhakkedathu, J. N. *Biomaterials* **2012**, 33 (10), 3047-3057.
(5) Walter, H.; Krob, E. J.; Brooks, D. E. *Biochemistry* **1976**, 15 (14), 2959-2964.
(6) Bradley, A. J.; Murad, K. L.; Regan, K. L.; Scott, M. D. *Biochim. Biophys. Acta* **2002**, 1561 (2), 147-158.
(7) Brooks, D. E., Greig, R. G., Janzen, J. in Erythrocyte mechanics and blood flow; Cokelet, G. R., Meiselman, H. J., Brooks D. E., Eds.; Alan R Liss Inc.: New York, USA, 1980; p 119-140.