Prospects of Therapeutic Cells via the Surface Grafting of Hyperbranched Poly(glycerol)s to Red Blood Cells

+[1]Chapanian, R; [1,3]Constantinescu, I; [1,2,3]Scott, MD; [1,2,4]Brooks, DE; [1,2,4*] Kizhakkedathu, JN
[1]Centre for Blood Research, [2]Department of Pathology and Laboratory Medicine, [3]Canadian Blood Services, [4]Department of Chemistry, University of British Columbia, Canada

Introduction:
Therapeutic cells have applications in stem cell transplantation, cancer, vascular diseases and transfusion[1,2]. Cell surface grafting of functional polymers has emerged as a practical technique to prepare therapeutic cells[1]. Hyperbranched poly(glycerol)s (HPG)s are attractive candidates in cell surface modification due to their biocompatibility, high hydration, the presence of derivatizable hydroxyl groups on the periphery, and the compact structure[3,4]. The objectives of the current study are to investigate the immunogenicity of HPG grafted red blood cells (RBC)s, to study the impact of polymer graft concentration and molecular weight on the *in vivo* circulation, and to elucidate factors that govern HPG-grafted RBC clearance.

Materials and Methods:
Reagents and solvents used for the polymer synthesis and modification include glycidol, potassium methylate, succinic anhydride, 4- dimethylaminopyridine, N,N′-diisopropylcarbodiimide and N-hydroxysuccinimide, pyridine, dimethyl formamide, methanol, acetone, purchased from Sigma Aldrich (ON, Canada), and trimethylolpropane obtained from Fluka (ON, Canada). PKH-26 Fluorescent cell lipid membrane marker was purchased from Sigma Aldrich. FITC labeled rat Anti-mouse CD47 was purchased from BD Pharmingen (ON, Canada), and for the histology studies, a rat anti-mouse CD45 antibody was purchased from BD Pharmingen (ON, Canada). HPG was synthesized using the anionic ring opening multi-branching polymerization of glycidol[3,4]. HPGs with different molecular weights were synthesized, and grafted to cell surface proteins, after converting an optimum number of hydroxyls in HPGs to carboxyls, and activating them with N-hydroxy succinimide. To investigate the *in vivo* circulation, RBCs were functionalized with PKH 26 lipid membrane marker prior to HPG grafting. HPG-grafted RBCs, equivalent to 10 % of the whole blood mass, were transfused via the tail vein injection. The clearance of HPG grafted RBCs was followed by flow cytometry. The immunogenicity of HPG grafted RBCs was investigated by repeated injection of HPG grafted RBCs in mice and evaluation of the circulation half-life. The mechanism of clearance was investigated by histology and the protection of CD47 self-proteins expression.

Results:
At low polymer graft concentrations (0.25 and 0.5 mM), the circulation half-lives of HPG 20 kDa and HPG 60 kDa grafted RBCs were comparable to the control. At graft concentration of 0.75 mM and above, a significant reduction in the circulation time of HPG grafted RBCs was observed even though they were stable *in vitro* (Fig 1).The polymer graft density had a greater impact on the circulation of HPG grafted RBCs than the molecular weight. HPG grafted RBCs were not immunogenic as evidenced from similar long time (50 days) circulation profiles upon a repeated administration in mice. Histological examination of the spleen, liver and kidneys

indicated no difference compared to the control, when animals were injected with RBCs grafted with low concentration of HPG 60 kDa (0.5 mM) and sacrificed on day 50. Distinct differences compared to the control were observed, when animals were injected with RBCs grafted with high concentration of HPG 60 kDa (1.5 mM). The amount of iron deposition was comparable in the white pulp region, but significantly higher in the red pulp region.

Discussion:

Both reductions in the CD47 self-protein accessibility and alteration in membrane mechanical properties are potential reasons in the faster removal of HPG grafted RBCs at high graft concentrations. Our results indicate that HPG grafted RBCs were not removed via antibody opsonization. Grafted RBCs were non-immunogenic and showed long circulation half-life similar to control cells. No alterations in the organs of mice at the cellular level were observed upon administration of HPG modified RBCs. In this study, a cell compatible modification chemistry based on HPG was developed. It was concluded that antigen camouflaged cells have potential application in blood transfusion and therapeutic drug delivery.

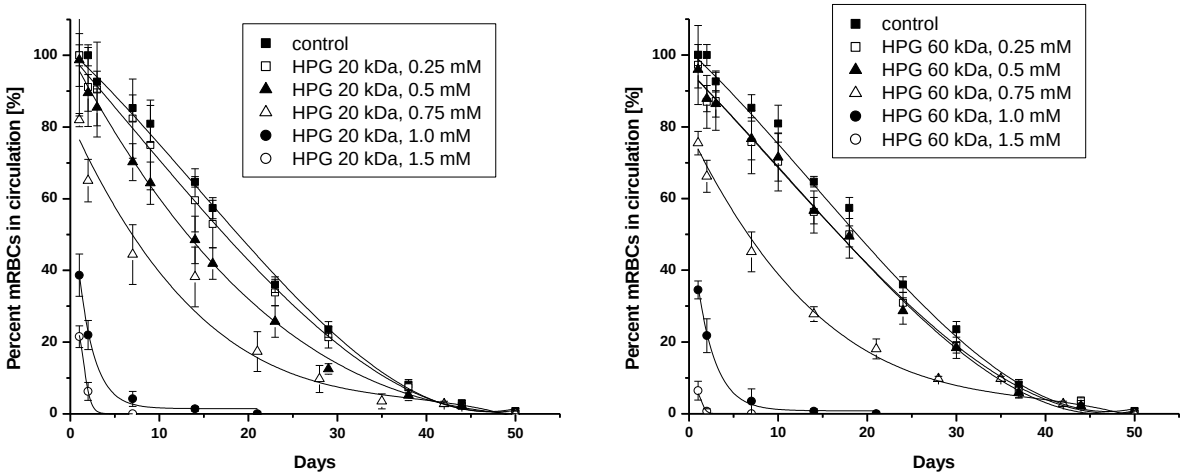

Figure 1: Circulation of HPG grafted RBCs in mice at different graft concentrations. A) HPG 20 kDa , B) HPG 60 kDa. Results represent the average of 4 independent measurement, error bars represent the standard deviation around the mean. The Lines represent fitted mathematical models to experimental data, used to calculate the circulation half-lives.

References:

1. Stephan MT. *et al.*, Nano Today (2011),6:309-25
2. Muzykantov VR. Expert Opin Drug Deliv (2010),7:403-27
3. Chapanian R. *et al.*, Biomaterials (2012),33:7871-83
4. Rossi NA. *et al.*, Biomaterials (2010),31:4167-78

Acknowledgment:

We would like to acknowledge the Canadian Blood Services, Health Canada, the Canadian Institutes of Health Science, Michael Smith Foundation for Health Research, and LMB Macromolecule Hub at the UBC Centre for Blood research.