Phosphate aggregation of PBS buffer affects heparin-protein interactions in 3D hydrogels

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Abstract

Three-dimensional (3D) hydrogels have attracted significant attention as a new and more efficient strategy for the cell cultures related to the manufacture of cellular products to combat cancer through cellular (immuno)therapies. A new class of hydrogel has recently been reported as shown in Figure 1. Heparin has been chosen based on its capacity to interact with positively charged proteins such as the cytokine CCL21 (in blue in Figure 1) which can affect T cell migration and proliferation [1-2].

The design of the new hydrogel has been studied "in silico" through molecular dynamics simulations, focusing on the electrostatic interactions between heparin and the newly added functionalization cytokine (CCL21). To our surprise, we found that the widely used phosphate buffered saline (PBS) can reduce the stability of interactions of CCL21 and heparin through phosphate aggregation, see Figure 2. Instead of the Protocol 1 in which the PBS buffer was used through out the wet-lab experiments, here we propose a new protocol (Protocol 2 of Figure 2): firstly establishing the interactions of CCL21 with heparin in the physiological environment (150 mM NaCl solution), then refilling PBS buffer along with the T cell re-infusion in wet-lab experiments. In this way, the stability of the complex of CCL21 and heparin is increased and the binding domain of CCL21 to which CCR7 receptor of T cells is exposed for the later-on landing of T cells in the 3D hydrogels. Preliminary experimental results are in good agreement with the predictions from our simulations.

References

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[2] Del Río, Eduardo Pérez, et al. Biomaterials 259 (2020): 120313.

Figures

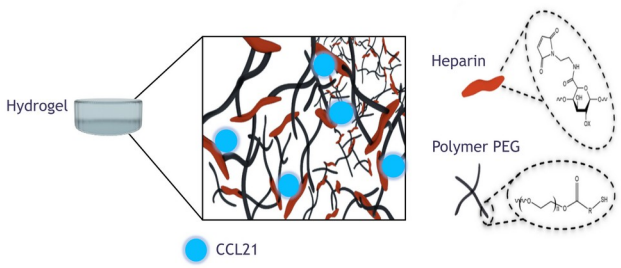

Figure 1. The poly(ethylene)glycol (PEG) is used to provide the structural and mechanical properties, where negatively charged heparin is used as an anchor for positively charged cytokin CCL21. CCL21 is known for binding a cell surface chemokin receptor known as CCR7 of T cells [1]. In this way CCL21 can affect T cell expansion in a CCL21-loaded PEG-heparin hydrogel.

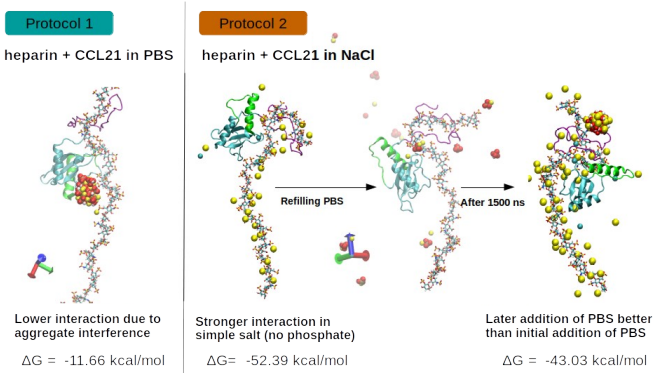

Figure 2. Phosphate aggregates interfere the interactions of CCL21 with heparin if initially considering PBS in the hydrogel. Protocol 1 is used in the previous wet-lab experiments, here we propose a new protocol that will increase the interactions of CCL21 with heparin, herein more T cells will be reproduced in 3D hydrogels.

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