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| **Preparation of alginate scaffold loaded with biologics using freeze-drying method** |
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| **Background:**  There is considerable interest in developing local delivery methods for biologics that can maintain their stability and functional activity over extended periods. Novel biomaterial-based drug delivery vehicles have been designed to achieve (i) sustained release, (ii) modulate pharmacokinetics, and (iii) optimize the bioavailability of therapeutic agents. Alginate is a chemically inert, non-immunogenic, and hydrophilic biomaterial that can be crosslinked with non-toxic divalent cations, which offer an ideal platform for scaffold fabrication. The freeze-drying (lyophilization) process is a versatile method that can produce highly porous, multi-layered 3D scaffolds with complex pore morphologies, using various biomaterials. Lyophilized scaffolds made from alginate can serve as vehicles for the delivery of biologics, such as antibodies and derivatives. However, biologics tend to exhibit decreased stability and aggregation during the freeze-drying process, which presents challenges for formulation and bioprocess development. The aim of the present study was to prepare and optimize stable, freeze-dried 3D alginate biopolymer systems that can provide controlled delivery of IgG antibodies and antibody fragments. We hypothesized that alginate could minimize protein degradation and aggregation during the freeze-drying process and enable sustained release of therapeutics. |
| **Methods:** Tocilizumab, an anti-IL6R antibody, and Fab derived from tocilizumab were used as biologics in this study. Fab-Tocilizumab was obtained using papain digestion of full IgG. Different solutions were prepared from sodium alginate in distilled water, at concentrations of 4% (w/v) and mixed with tocilizumab (2.0 mg) and Fab-tocilizumab (2.0 mg), and then crosslinked with 5% (w/v) CaCl2. Control solution containing biologics without alginate was also prepared. Hyaluronic acid (HA: 30KDa, 3%) was used to enhance protein stability. The prepared solutions were then subjected to freeze-drying which was performed using primary drying at -20 0C for 12 hours at 100 μBar, followed by secondary drying at 20 0C for a further 2 hours. Release study of biologics was also performed using a peristaltic pump and investigated with HPLC. The stability of antibody released from lyophilised alginate scaffold was studied using SDS-PAGE and SEC analysis. |
| **Results:** Tocilizumab and the Fab-Tocilizumab were freeze-dried with alginate biomaterials. The stability study results revealed that alginate-based scaffolds minimize denaturation and aggregation of biologics during lyophilization. According to Nanodrop spectrometer data, a sustained release of drugs was observed, in which more than 90% of loaded drugs were slowly released up to two weeks. The presence of hyaluronic acid was associated with reduced mesh size and burst release of drugs. |
| **Conclusions:** Our results have demonstrated that freeze-dried alginate biopolymer systems can enable controlled and sustained release of IgG antibodies. Nevertheless, usage of the freeze-drying technique can result in heterogeneous pore structures in the scaffold with large variations in the average pore diameter at different locations within the structure. 3D bioprinting represents an attractive alternative method for scaffold fabrication as it offers a higher degree of reproducibility and precise control over scaffold pore size, geometry and interconnectivity, which will be used in the next phase of this project. |