Preparation of alginate scaffold loaded with biologics using freeze-drying method

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INTRODUCTION

Alginate, a chemically inert, non-immunogenic, and hydrophilic biomaterial that can be crosslinked with non-toxic divalent cations, is an ideal material for scaffold fabrication. The freeze-drying process is a versatile method that can produce highly porous, multi-layered 3D scaffolds, using various biomaterials such as alginate.

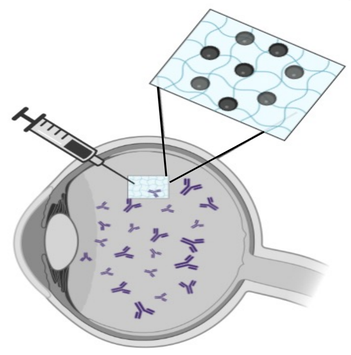

Figure 1. Schematic representation of intraocular of drugs delivered by degradable sustained delivery systems.

However, biologics tend to exhibit decreased stability and aggregation during freeze-drying, which presents challenges for formulation and bioprocess development.

Aims

To prepare and optimize stable, freeze-dried 3D alginate biopolymer systems that can provide controlled delivery of IgG antibodies and antibody fragments.

METHODOLOGY

Tocilizumab (TOC), anti-IL6R antibody, and Fab derived from tocilizumab (FAB-TOC) was mixed with sodium alginate and then crosslinked with $CaCl_2$ (Fig.2). Hyaluronic acid was used to enhance protein stability. The prepared solutions were then subjected to freeze-drying. Release study of biologics and stability of antibody were analysed using Nanodrop spectrometer, HPLC, SDS-PAGE and SEC analysis.

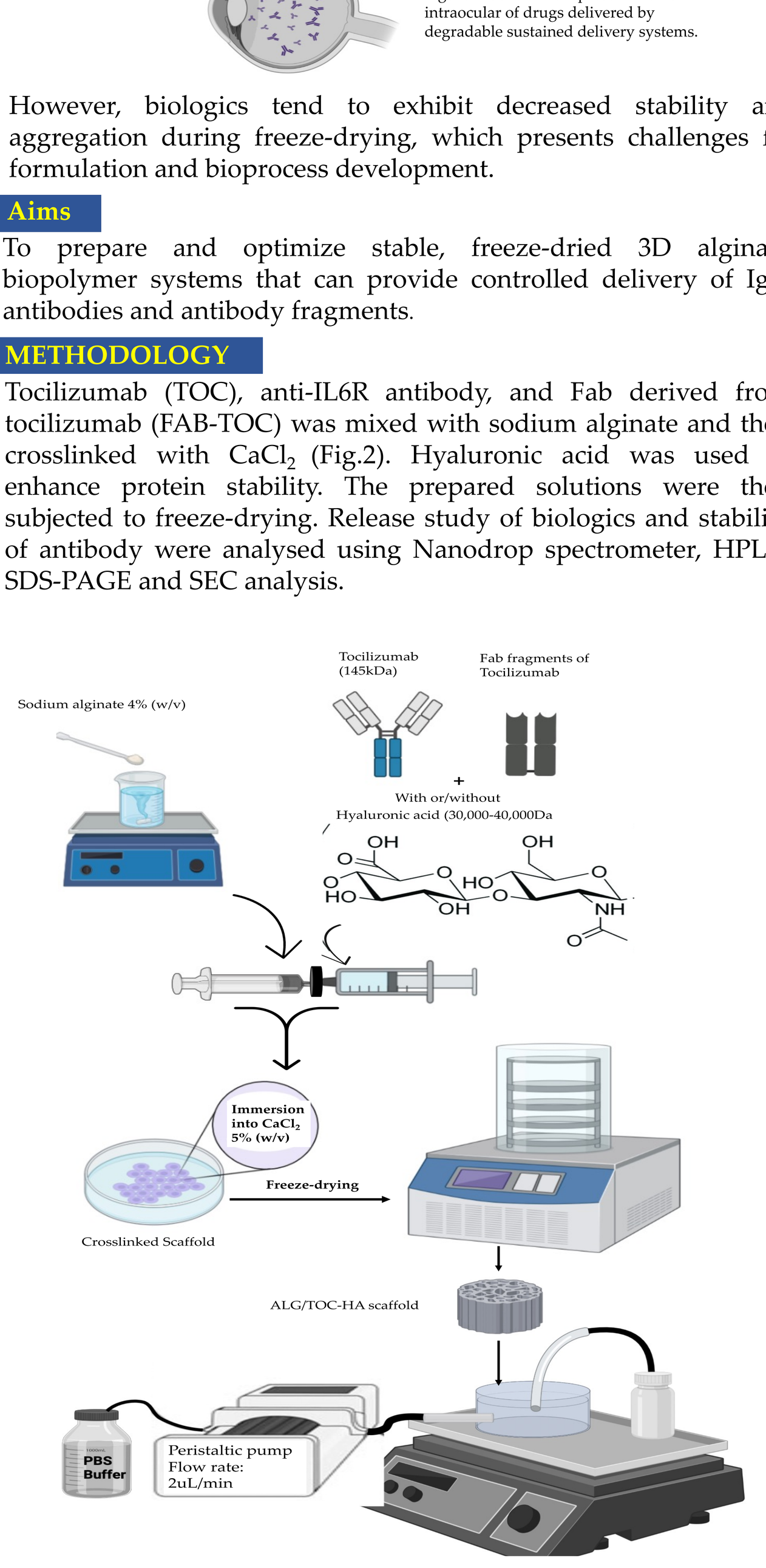

Figure 2. A schematic diagram of preparation of Alginate-Tocilizumab with or without HA using freeze-drying technique.

RESULTS

TOC and the FAB-TOC were freeze dried with and without alginate biomaterials. Based on HPLC analysis ,when tocilizumab was formulated alone, more than 21% of total concentration was degraded during freeze-drying methods. The Freeze-dried ALG-TOC or FAB-TOC scaffolds turned into a soft, hydrogel fibril after approximately 10 minutes by the exchange of Ca^{2+} ions in present of PBS buffer (Fig.3)

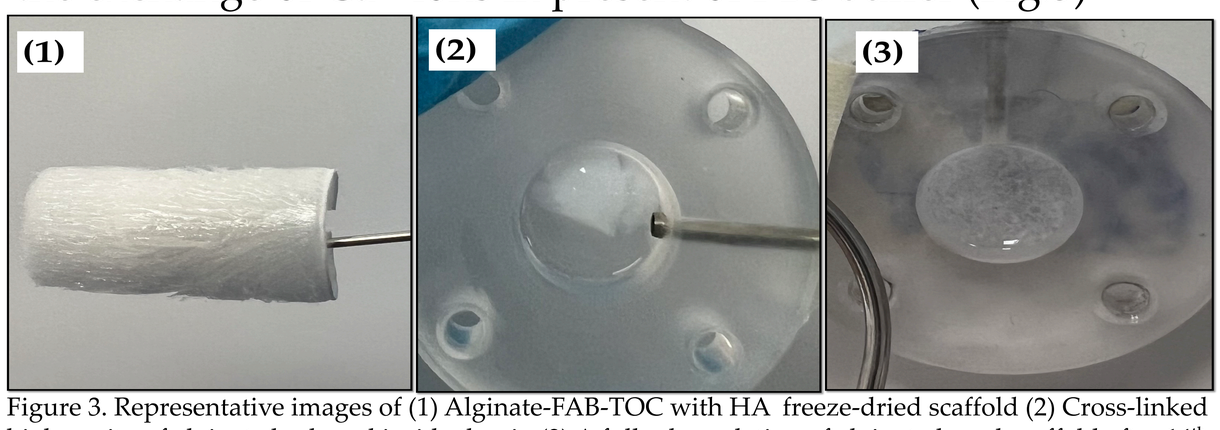

Figure 3. Representative images of (1) Alginate-FAB-TOC with HA freeze-dried scaffold (2) Cross-linked high purity of alginate hydrogel inside the rig (3) A fully degradation of alginate-based scaffold after 14th days.

The stability study results revealed that alginate-based scaffolds reduced denaturation and aggregation of biologics during lyophilization(Fig.4).

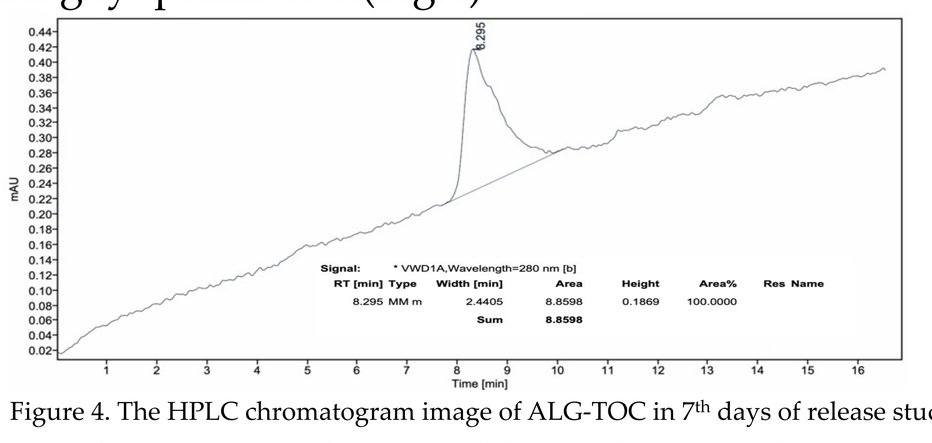

Figure 4. The HPLC chromatogram image of ALG-TOC in 7th days of release study

As is shown in Figure (5) and 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 14th day. The presence of hyaluronic acid associated with reduced the mesh size and burst release of drugs.

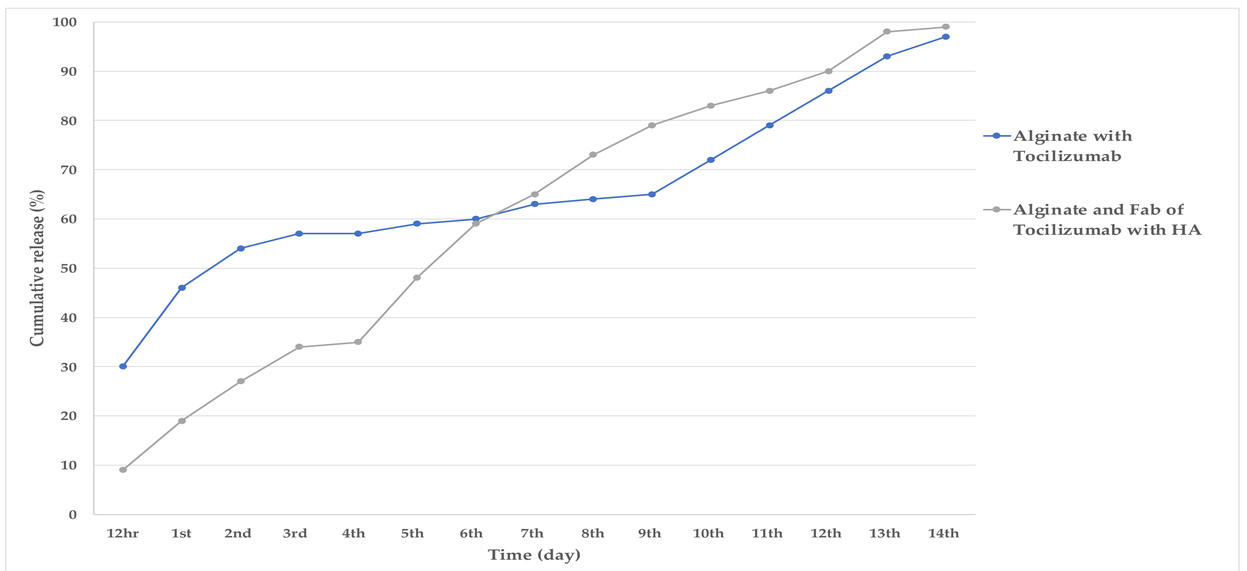

Figure 5. Cumulative release of TOC and FAB-TOC from alginate –based scaffold with or without HA. All data is presented as its mean (n=2).

Conclusion

Our results have demonstrated freeze-dried alginate biopolymer systems can enable controlled and sustained release of TOC and FAB-TOC. Freeze-drying technique tend to exhibit heterogeneous pore structures in scaffold with large variations in the average pore diameter at different locations within the structure.

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