Poster Number 02M0930

Storage stability studies of anti-VEGF FpF antibody mimetics

Hanieh Khalili[1,3], Steve Brocchini[3], Peng Tee Khaw [2], Sergey Filippov[4]

[1]UEL School of Health, Sport and Bioscience, London, United Kingdom, [2]National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom; [3]UCL School of Pharmacy, London, United Kingdom, [4]Institute of Macromolecular Chemistry, Prague, Czech Republic.

2016 AAPS ANNUAL MEETING AND EXPOSITION
NOVEMBER 13 –17, 2016
COLORADO CONVENTION CENTER, DENVER

PURPOSE

We have developed a bivalent IgG antibody mimetic called Fab-PEG-Fab (FpF) (Fig 1) [1]. The Fc region of the IgG is replaced in the FpF with a flexible, hydrophilic poly(ethylene glycol) (PEG) scaffold. The conjugation is thought to enhance the stability and the PEG scaffold to reduce the propensity for aggregation. The potential increased stability of FpFs compared to IgG antibodies is being determined in an effort to develop more stable and useful FpF dosage forms (e.g. depots) than may be possible with IgGs.

The aim of this study was to evaluate the stability of FpFs in storage and in a freeze-dried form in buffer only.

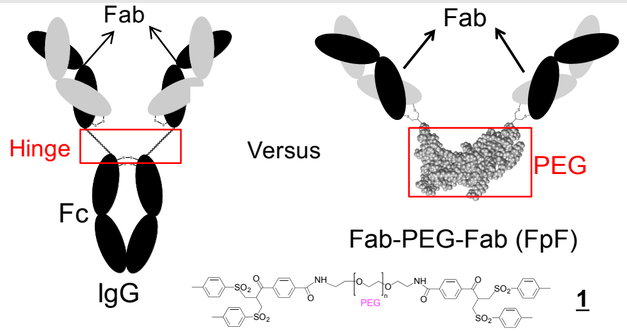


Figure 1. Structure of IgG and IgG mimetic (FpF).

METHOD

- FpFs were prepared as described [1] using the anti-vascular endothelial growth factor (anti-VEGF) ranibizumab (Fab_{rani}), Fab_{beva} and PEG-di(*bis*-sulfone) **1**.
- Fab_{beva} obtained from enzymatic digestion of anti-VEGF bevacizumab.
- Storage stability studies were conducted in glass vials at 4°C with the Fab_{beva}, and FpF_{beva} at concentration of 5 μM for a period of 60 days.
- Bevacizumab and Fab_{rani} (40 μM) were dialysed against PBS pH 7.4 before being freeze-dried to remove their formulation excipients. FpF_{rani} was prepared at concentration of 40 μM in PBS pH 7.4 buffer with no excipients.
- Freeze-drying was conducted with primary drying at -20 °C for 24 hours at 100μBar vacuum pressure and followed by secondary drying at 20 °C for 2 hours. Lyophilised bevacizumab, Fab_{rani} and FpF_{rani} were then reconstituted into water (0.3 mL) to study the aggregation.
- Aggregation and light/heavy chain dissociation were studied using a dynamic light scattering (DLS) DynoPro plate reader II, gel electrophoresis SDS-PAGE analysis and size-exclusion chromatography (SEC).

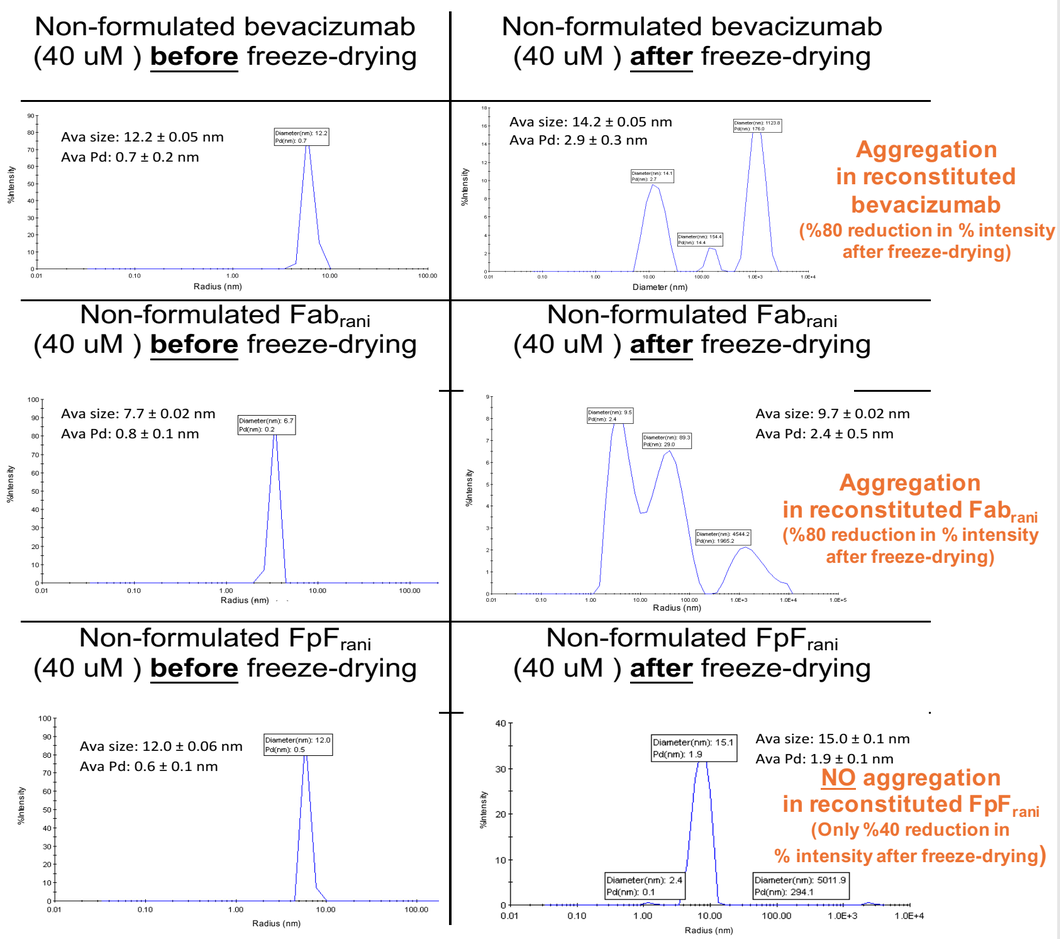
Acknowledgments/Reference

Medical Research Council, National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology. HK acknowledges funding from University of East London (UEL) School of Health, Sport and Bioscience.

[1] Khalili, H. *et all (2013)*, Fab-PEG-Fab as a potential antibody mimetic. *Bioconjugate Chem.*, **24**(11), 1870–1882.

RESULTS

(A) DynoPro; De-formulated bevacizumab, Fab_{rani} and FpF_{rani} before/after freeze-drying



(B) SEC for Fab_{rani} and FpF_{rani} at 40 μM after freeze-drying.

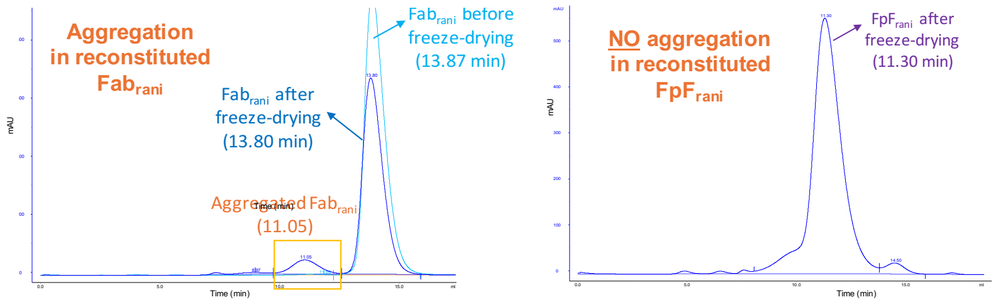


Figure 2. Stability of lyophilised anti-VEGF FpF_{rani}, de-formulated bevacizumab and Fab_{rani}. Lyophilised powders were reconstituted in water prior to analysis by DLS **(A)** and SEC **(B)** analysis.

RESULTS

Storage stability of Fab_{beva} and FpF_{beva} after 60 days at 4°C

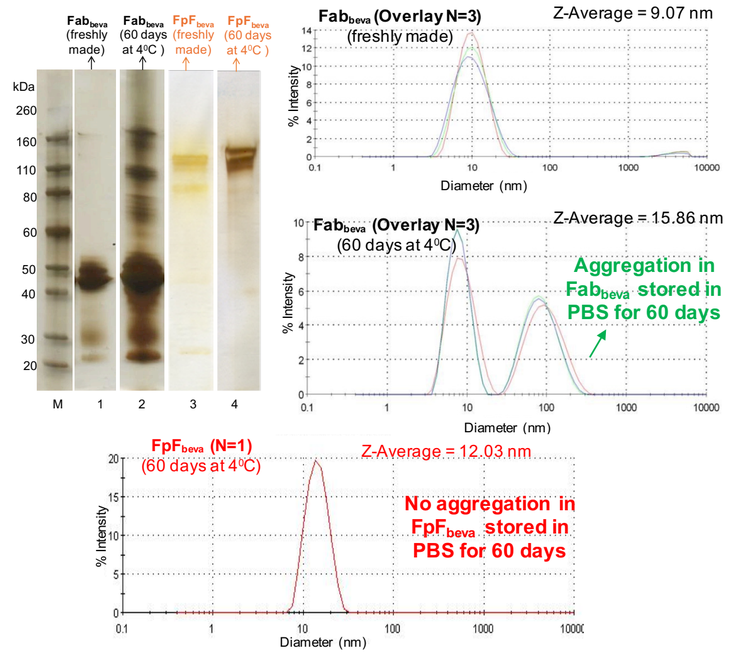


Figure 3. Storage Stability of anti-VEGF FpF_{beva} and Fab_{beva} in liquid form in PBS over period of 60 days at 4°C.

The non-formulated FpF_{rani} did not show any aggregation or light / heavy chain dissociation after being freeze-dried DynoPro and SEC) (Fig. 2 (A) and (B)). In contrast, the de-formulated bevacizumab and Fab_{rani} displayed evidence of aggregation after freeze-drying (Fig. 2A-B) Fab_{beva} that was freshly prepared from the proteolytic digestion of bevacizumab appeared as a band at 50 kDa (SDS-PAGE) with no trace of aggregation and a single DLS peak (Fig. 3, lane 1). Fab_{beva}, however, aggregated when it was stored in PBS buffer for 60 days at 4°C as shown by the higher molecular weight bands in Fig. 2 (A) lane 2. FpF_{beva} did not aggregate or display any light /heavy chain dissociation when it was stored in the same buffer and storage conditions as Fab_{beva} (5 μM; Fig. 3, lanes 3-4, single DLS peak).

CONCLUSION

These preliminary results suggest that FpFs are more stable in liquid form and when freeze-dried without the addition of excipients (e.g. trehalose). The apparent increased stability of FpFs provides an opportunity to formulate these antibody mimetics in high concentration for use in tissue implantable depot forms.