In vivo assessment of Nanobody® molecules using hydrogel-forming microarray patch technology

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CONTROLLED RELEASE SOCIETY
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THE FUTURE OF DELIVERY SCIENCE

Outline

1. Current biotherapeutics on the market
2. What are Nanobody® molecules?
3. Hydrogel-forming microarray patches (MAPs) (Brief overview)
4. Fabrication of Nanobody® based lyophilised wafers
5. *In vitro* permeation of Nanobody® molecules
6. *In vivo* assessment of Nanobody® molecules molecules using MAP technology
7. Conclusion

Introduction

- During the last 30 years, significant research and development funds and resources have focused on antibody-based therapies.
- Evidently, this is a financially lucrative field of development for drug manufacturers and its success has led to the development of improved treatment strategies.
- One such strategy involves the development of lower molecular weight (MW) protein-based macromolecules, such as Nanobody® molecules.

Table 1. Top 10 best selling drugs of 2022. *Sourced from: Drug Discovery & Development, 2023*

Rank	Product	Company	Pharmacological class	2022 worldwide sales ($millions)
1	Comirnaty	Pfizer/BioNTech	SARS-CoV-2 vaccine	55,918
2	Humira	AbbVie/Eisai	Anti-TNF mAb	21,237
3	Keytruda	Merck & Co.	Anti-PD1 mAb	20,937
4	Paxlovid	Pfizer	SARS-CoV-2 antiviral	18,933
5	Spikevax	Moderna	SARS-CoV-2 vaccine	18,435
6	Eliquis	Bristol Myers Squibb/Pfizer	Factor Xa inhibitor	18,269
7	Dupixent	Sanofi/Regeneron	Anti-IL-4/IL-13 mAb	17,417
8	Eylea	Regeneron	Anti-VEGF	12,721
9	Biktarvy	Gilead Sciences	HIV INSTI/NRTI/NtRTI	10,390
10	Revlimid	Bristol Myers Squibb/BeiGene	Immunomodulator	9,978

What are Nanobody® Molecules?

- Nanobody® molecules were developed following the discovery that camelidea species possess fully functional heavy-chain only antibodies (HCAbs). These unique, low molecular weight (∽15 kDa) variable domain protein structures have led to the generation of potent biological drugs.
- Ablynx N.V. currently have one Nanobody® therapeutic molecule on the market. Caplacizumab (Cablivi®), a bivalent domain which targets the ultra large von Willebrand factor, has been indicated for the treatment of thrombotic thrombocytopenic purpura (TTP), a rare blood disorder in which platelet aggregation leads to microvascular thrombosis.

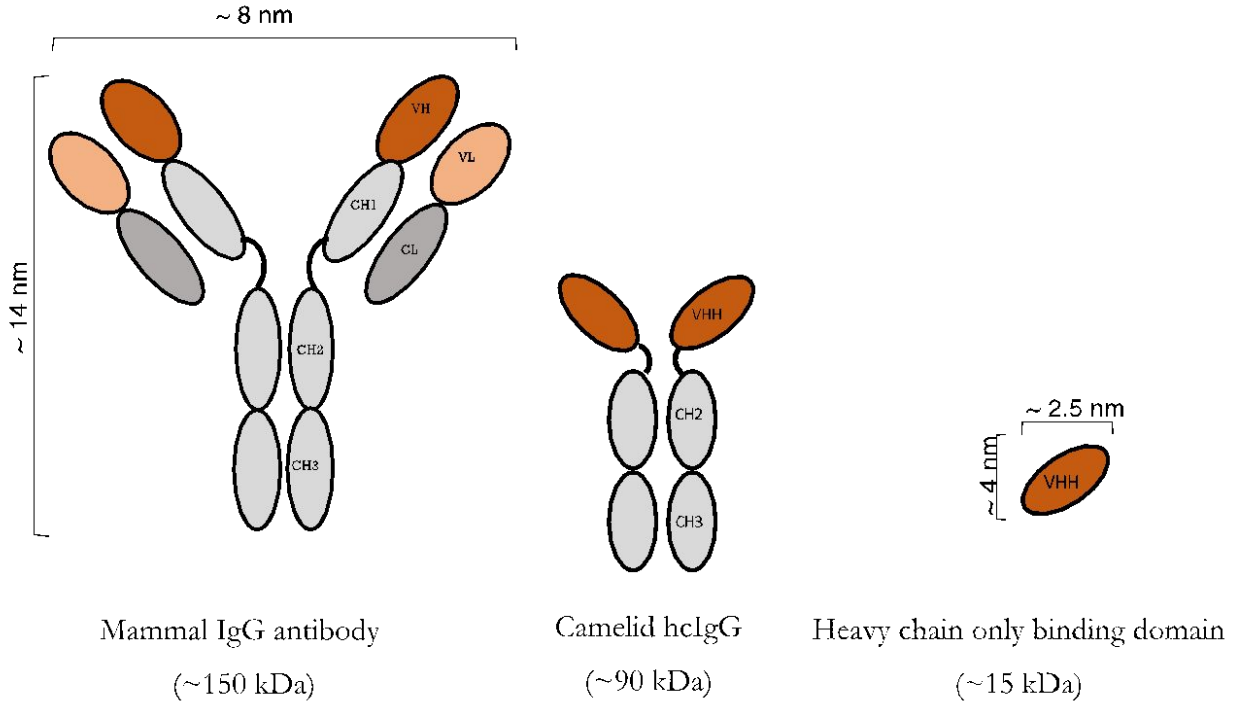

Figure 1. Schematic representation of a conventional IgG antibody, a heavy chain antibody and a heavy chain only single binding domain. Adapted from Safarzadeh Kozani *et al*. (2021).

Safarzadeh Kozani, P., and Rahbarizadeh, F., (2021). The Potential Applicability of Single-Domain Antibodies (VHH): From Checkpoint Blockade to Infectious Disease Therapy. *Trends in Medical Sciences*, **1**(2): 1–8.

Hydrogel-forming MAPs

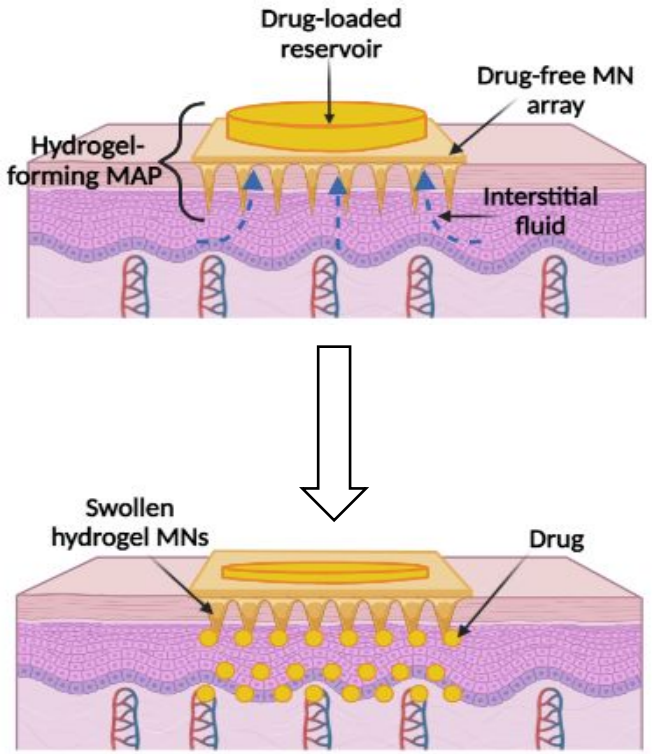

Figure 2. Schematic overview of a hydrogel-forming MAP facilitating the transdermal delivery of a compound retained within a drug reservoir.

- Microneedles contain no drug themselves
- Drug contained in a separate drug reservoir
- Microneedles are chemically crosslinked
- Rapid uptake of skin interstitial fluid
- Drug diffuses through swollen microneedles
- Rate of drug delivery determined by crosslink density and hydrogel type
- Reservoir properties can be altered to modulate drug delivery
- **Potential for higher doses and more prolonged delivery, for both hydrophilic and hydrophobic drugs**

Hydrogel-forming MAPs

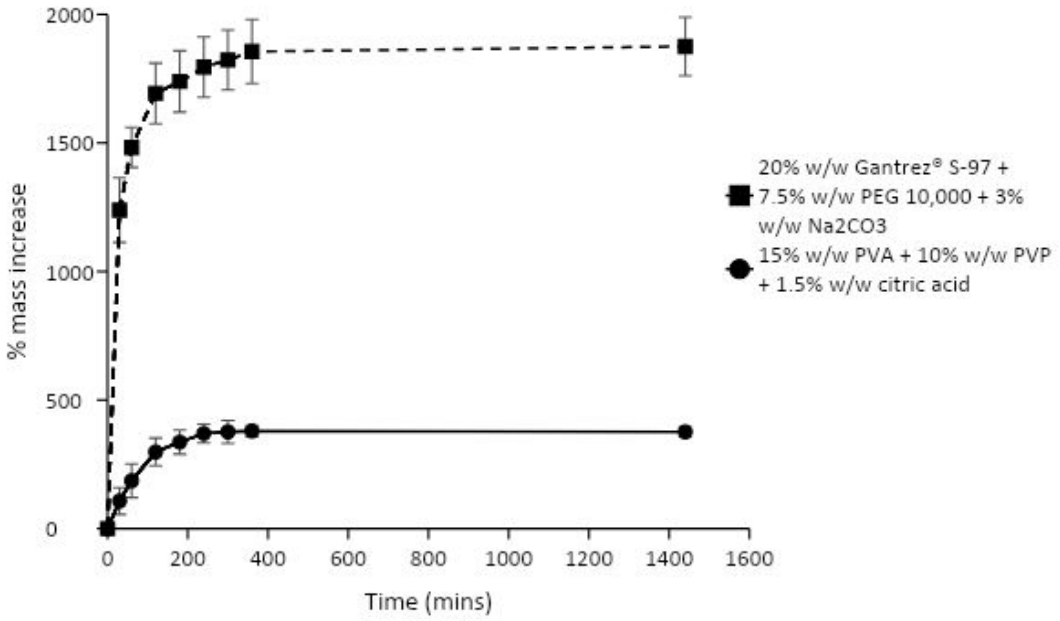

Figure 3. Comparison of percentage swelling over 24 hrs with 20% w/w Gantrez® S-97, 7.5% w/w PEG 10,000 + 3% w/w Na_2CO_3 ('super swellable') and 15% w/w PVA + 10% w/w PVP + 1.5% w/w citric acid hydrogel formulations in PBS (pH 7.4). Means ± SD., *n* = 3.

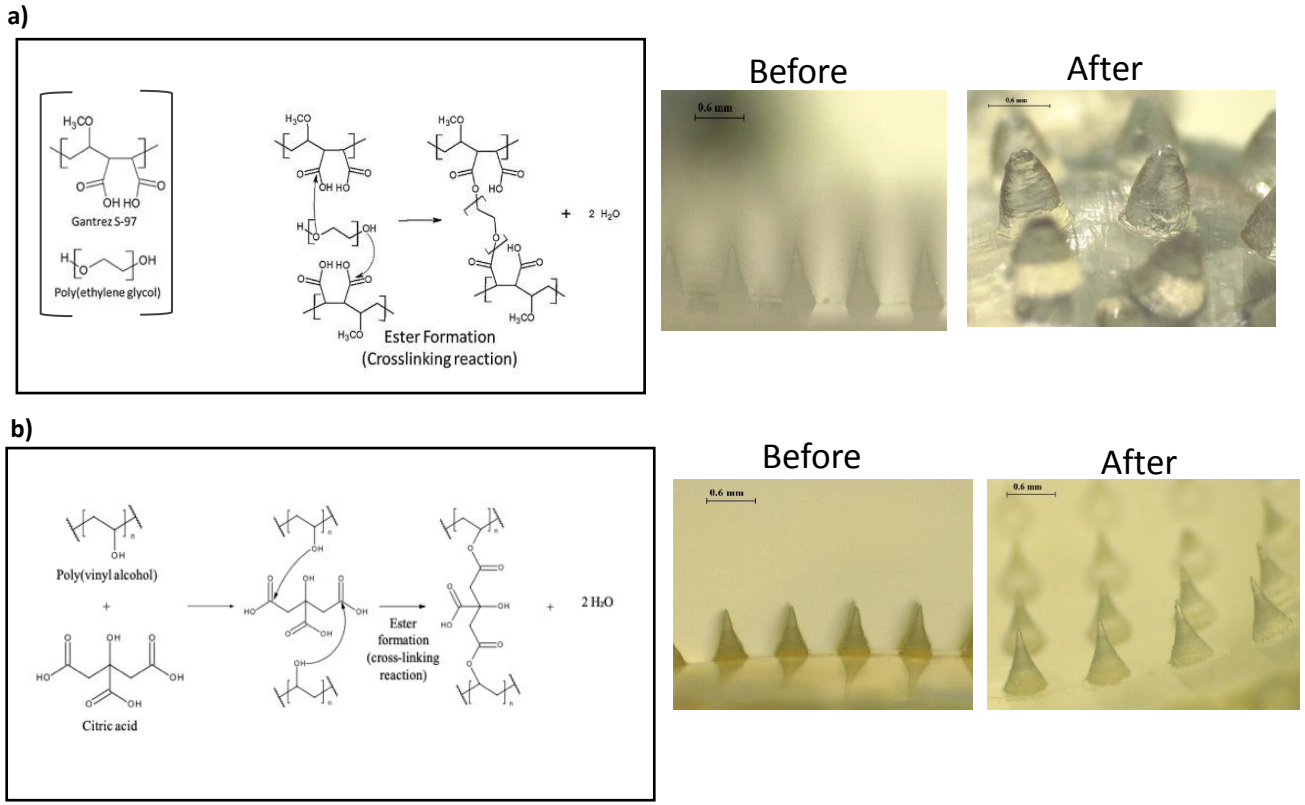

Figure 4. Crosslinking reaction equations and light microscope images taken before and after *In vitro* insertion into dermatomed neonatal porcine skin for both 20% w/w Gantrez® S-97, 7.5% w/w PEG 10,000 + 3% w/w Na_2CO_3 ('super swellable') and 15% w/w PVA + 10% w/w PVP + 1.5% w/w citric acid MAPs.

Fabrication of Nanobody® molecule based lyophilised reservoirs

Lyophilised wafers containing IRR-VHH (F1-F8) and HLE-IRR-VHH (H1) were prepared using different iterations of gelatin, mannitol and NaCl. Both IRR-VHH (F8) and HLE-IRR-VHH (H1) formulations produced homogenous, intact wafers with dissolutions times < 5 mins and ~ 100% API recovery.

Table 2. F1-F8 lyophilised reservoirs produced using gelatin, mannitol, NaCl and IRR-Nb. *H1 represents a lyophilised reservoir loaded with HLE-IRR-Nb

ID	Gelatin (% w/w)	Mannitol (% w/w)	NaCl (% w/w)	IRR-VHH (mg)
F1	1.12	-	5.61	8.5
F2	1.12	5.61	5.61	8.5
F3	1.12	11.21	5.61	8.5
F4	2.80	11.21	5.61	8.5
F5	2.80	16.82	5.61	8.5
F6	5.61	16.82	5.61	8.5
F7	2.8	20	5.61	15
F8	5.61	20	5.61	15
H1	5.61	20	5.61	15*

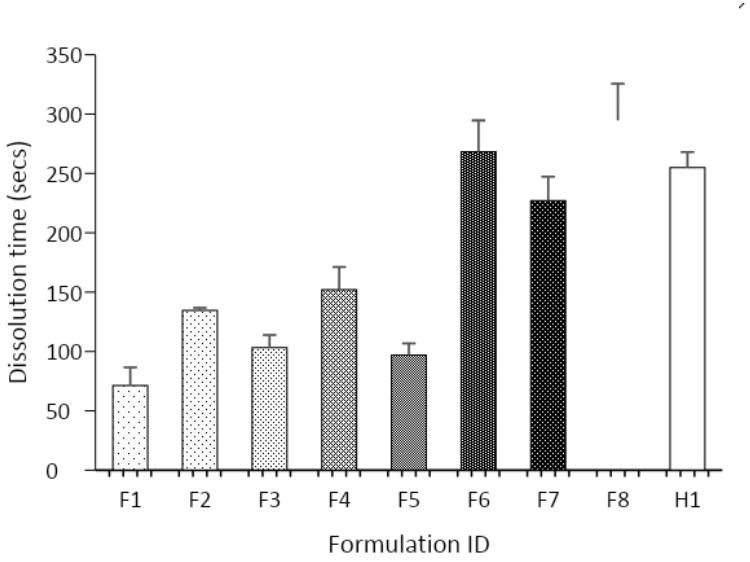

Figure 5. Dissolution times for F1-F8 + H1 lyophilised wafers in PBS (pH 7.4) at 37^{0}C and stirred at 200 rpm. Means + SD., *n* = 3

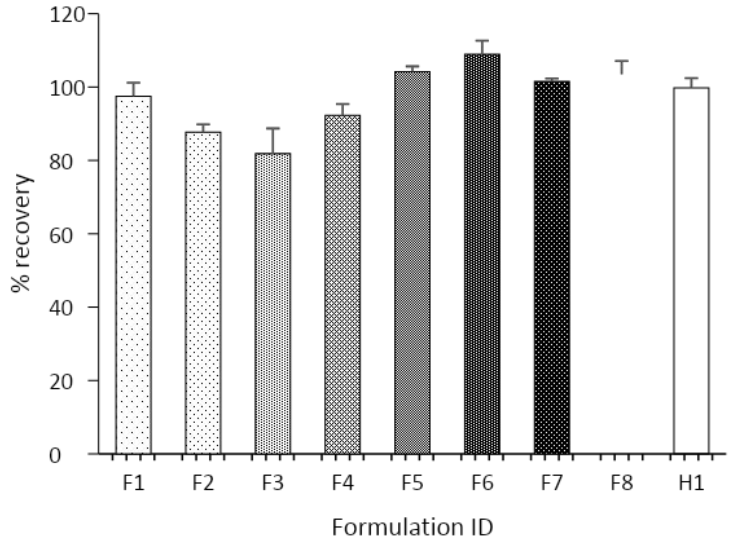

Figure 6. % recovery for F1-F8 + H1 lyophilised wafers in PBS (pH 7.4) at 37^{0}C and stirred at 200 rpm. Means + SD., *n* = 3

Nanobody® molecule stability in lyophilised reservoirs

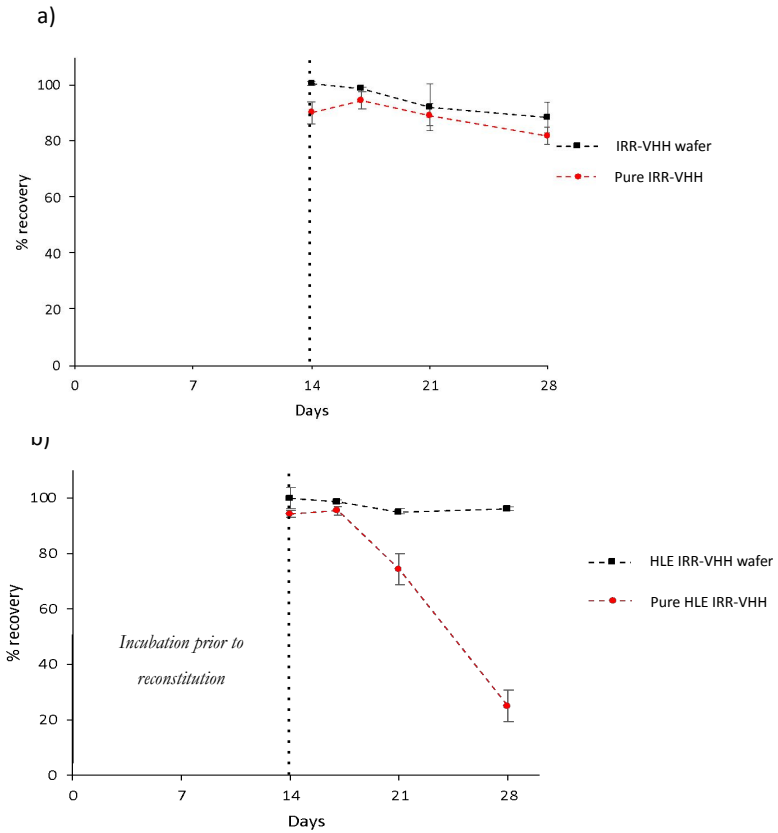

Figure 7. Line graph showing the percentage recovery of a) IRR-VHH F8 lyophilised wafers + pure IRR-VHH in PBS (pH 7.4) and b) HLE-IRR-VHH loaded lyophilised wafers and pure HLE-IRR-VHH in PBS (pH 7.4) during incubation at 37^{0}C over 28 days. Means ± S.D., *n* = 3.

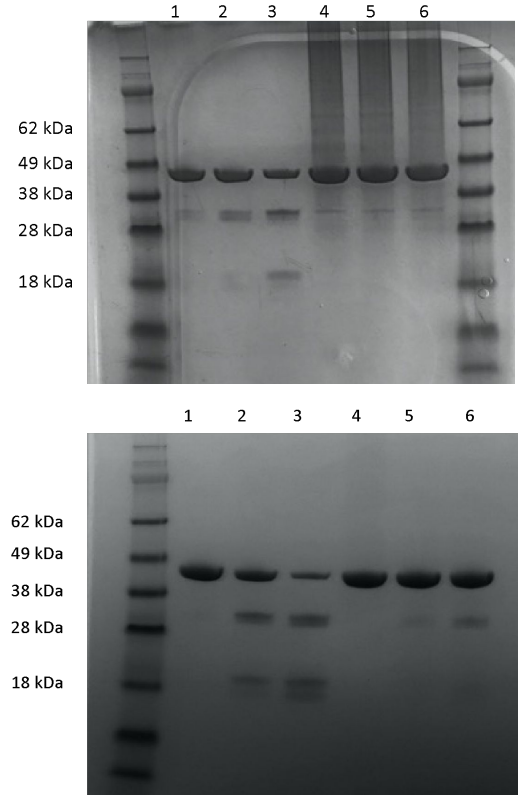

Figure 8. SDS-PAGE analysis of a) pure IRR-VHH and IRR-VHH loaded lyophilised wafers and b) pure HLE-IRR-VHH and HLE-IRR-VHH loaded lyophilised wafers following incubation at 37^{0}C for 28 days. Lane 1, pure VHH day 14; Lane 2, pure VHH day 21; Lane 3, pure VHH day 28; Lane 4, VHH wafer day 14; Lane 5, VHH wafer day 21; Lane 6, VHH wafer day 28.

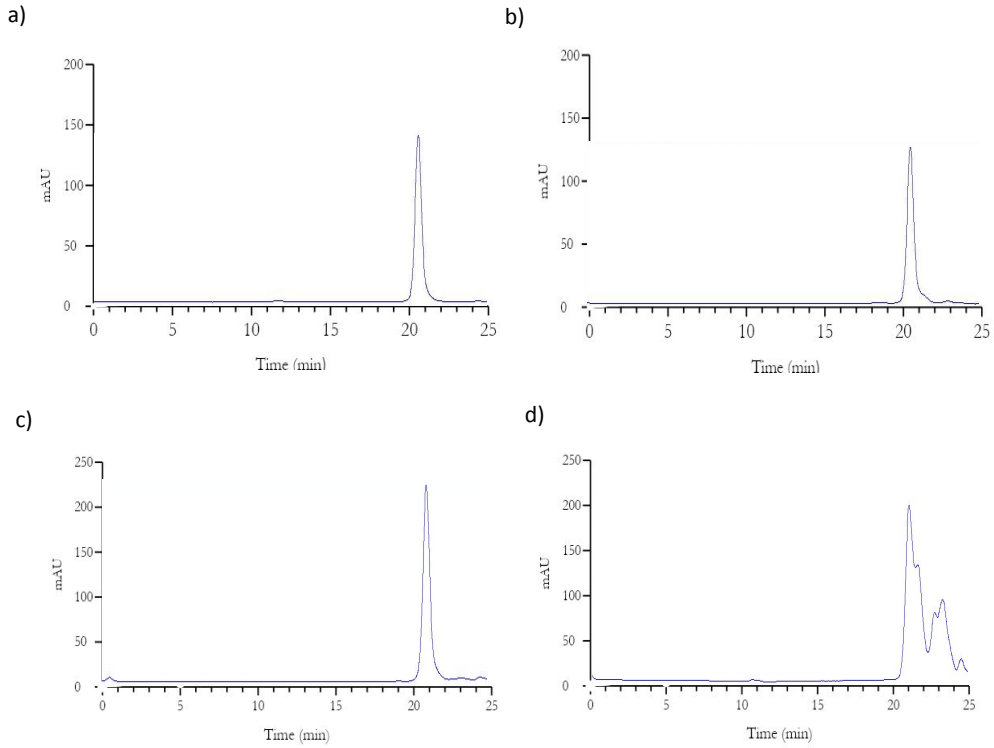

Figure 9. SEC traces of a) IRR-VHH loaded F8 lyophilised wafer, b) pure IRR-VHH, c) HLE-IRR-VHH H1 lyophilised wafer and d) pure HLE-IRR-VHH following incubation at 37^{0}C for 28 days.

Nanobody® molecule *in vitro* permeation

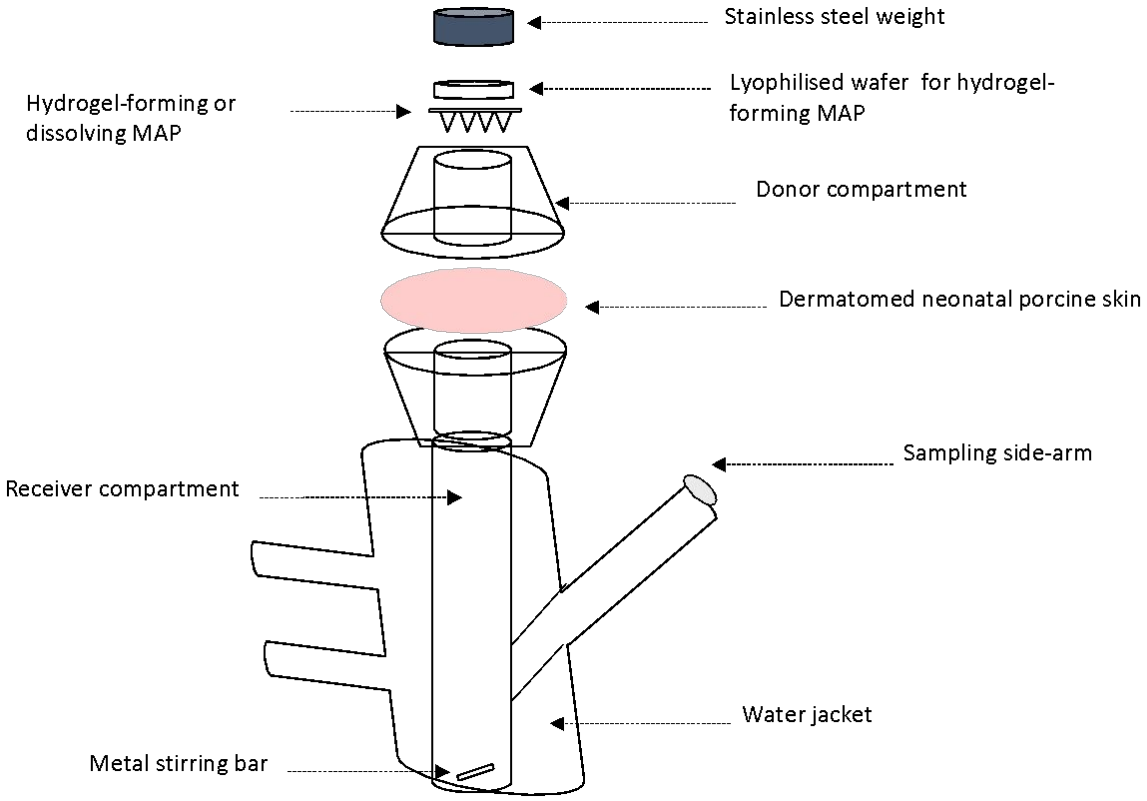

Figure 10 . Schematic representation of Franz cell diffusion apparatus

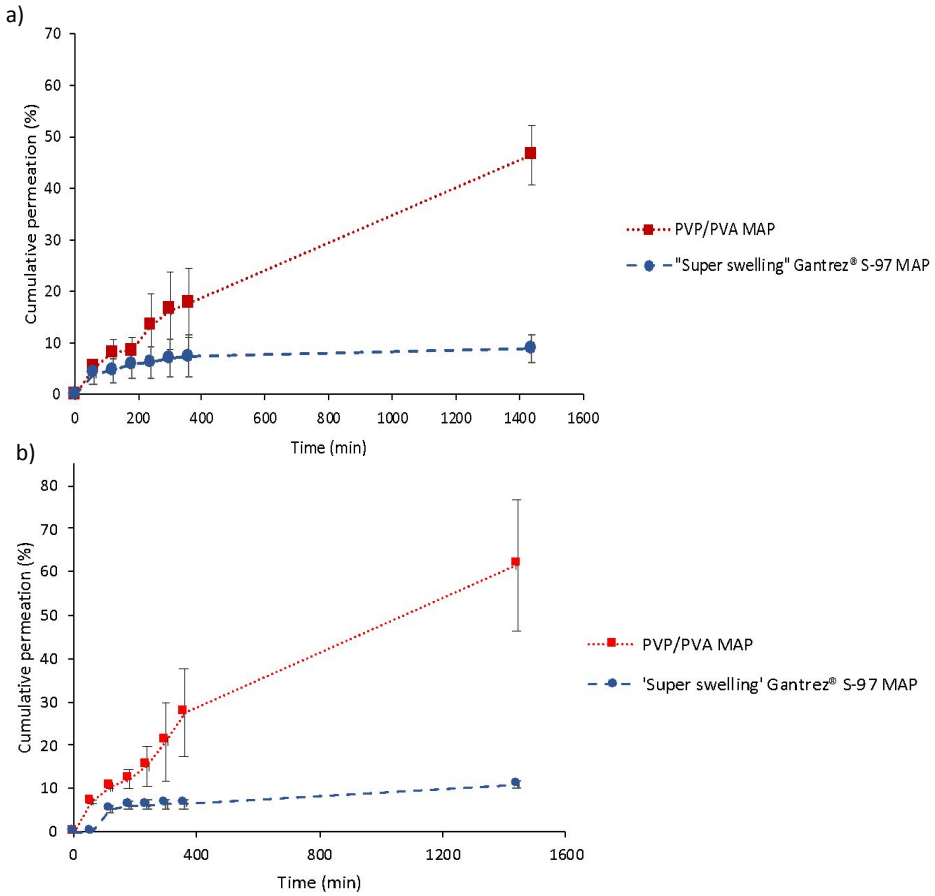

Figure 11. Line graph showing cumulative a) IRR-VHH and b) HLE-IRR-VHH permeation (%) across dermatomed neonatal porcine skin over 24 h using PVP/PVA and 'super swellable' Gantrez® S-97 hydrogel-forming MAPs. Means ± SD, *n* = 3.

In vivo delivery of IRR-VHH and HLE-IRR-VHH using hydrogel-forming MAPs

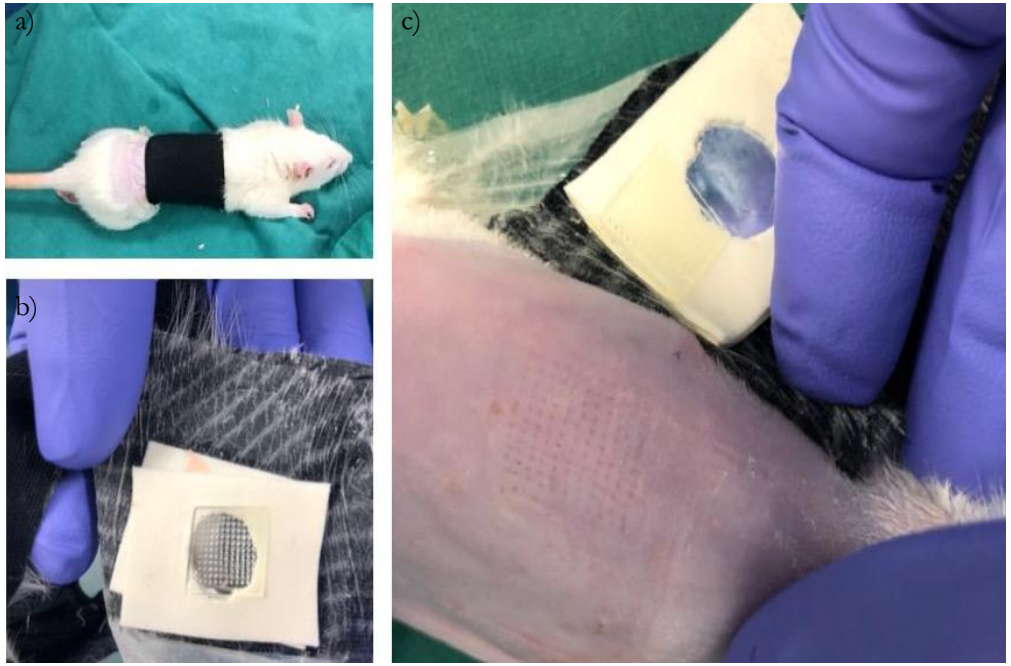

Figure 12. Digital images following *in vivo* delivery of IRR-VHH and HLE-IRR-VHH using hydrogel-forming MAPs depicting; a) a rat after application of hydrogel forming MAP and lyophilised wafer, secured in place with an adhesive foam border, Tegaderm™ film and Kinesiology™ tape, b) adhesive foam border with no visible residue on the MAP or release lining film and c) visible microchannels at the application site immediately after MAP removal.

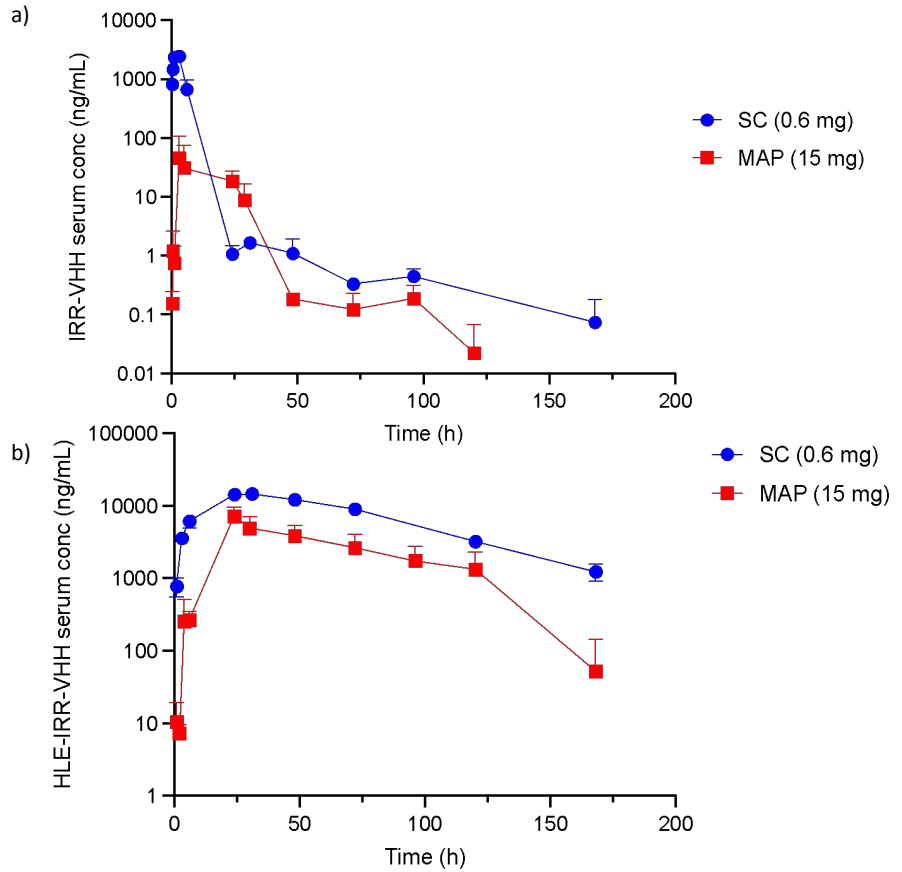

Figure 13. *In vivo* serum profiles of a) IRR-VHH in female Sprague Dawley rats administered using PVP/PVA hydrogel-forming MAPs or subcutaneous (SC) injection and b) HLE-IRR-VHH administered using PVP/PVA hydrogel-forming MAPs or SC injection. (Means + S.D., *n*=2 at 15 min, 30 min, 1 h, 3 h, 5 h, 31 h, 48 h, 72 h, 96 h, 144 h & 168 h; *n*=4 at 24 h).

Conclusion

Advances in antibody-based engineering has led to the production of low MW biotherapeutics. As these novel proteins are cheaper to manufacture and are not restricted to systemically accessible targets, they are now beginning to be considered as potential alternatives to mAbs in autoimmune and cancer-based therapies.

For the first time, a 'next generation' biologic has been successfully delivered *in vitro* using MAP technology. Using hydrogel-forming MAPs, Nanobody® molecules were successfully delivered across dermatomed porcine skin *in vitro.*

Whilst these Nanobody® molecules have no therapeutic effect, the levels achieved in this *in vivo* study suggest that hydrogel-forming MAPs can deliver therapeutically relevant doses if the patch was left in place for >24 h. This presents an opportunity to circumvent some of the problems associated with traditional hypodermic needle and syringe administration, enabling the delivery of biologics to be taken out of the in-patient setting and into the hands of patients in their own homes.

Acknowledgments

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Department for the Economy

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