Soft Robotic Drug Delivery System Controls Delivery of VEGF to Stimulate Neovascularisation at an Islet Transplantation Site

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Introduction

Over 60% of islets transplanted in macroencapsulation devices are lost immediately post transplantation due to hypoxia from inadequate early vascularisation. Prevascularisation of an implant site by spatiotemporally delivering vascular endothelial growth factor (VEGF) is a potential solution. VEGF has a half-life of only 30-50 mins at body temperature if delivered systemically meaning multiple, large doses would be required which risk the formation of unstable, leaky blood vessels and adverse off-target effects.

Our aim is to stabilise VEGF by electrostatically interacting with a carboxymethylcellulose-sodium alginate (CMC-SA) based hydrogel and loading into a soft robotic drug delivery (SRDD) device (Figure 1).

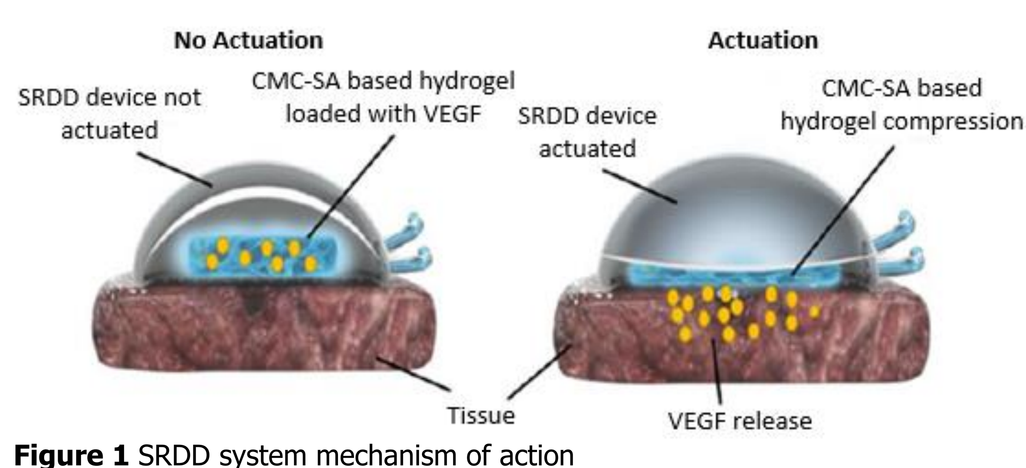

Figure 1 SRDD system mechanism of action

Actuation of the SRDD device will release VEGF in a controlled manner with the aim of stimulating neovascularisation at an implant site for future islet transplantation.

Methodology

Upon development of a novel negatively charged CMC-SA based hydrogel and SRDD device *in vitro* release studies were performed using Fluorescein isothiocyanate–Diethylaminoethyl–Dextran (FITC-DEAE-Dextran), of the same charge and molecular weight as VEGF to optimise an actuation regime for controlled release of VEGF (Figure 2):

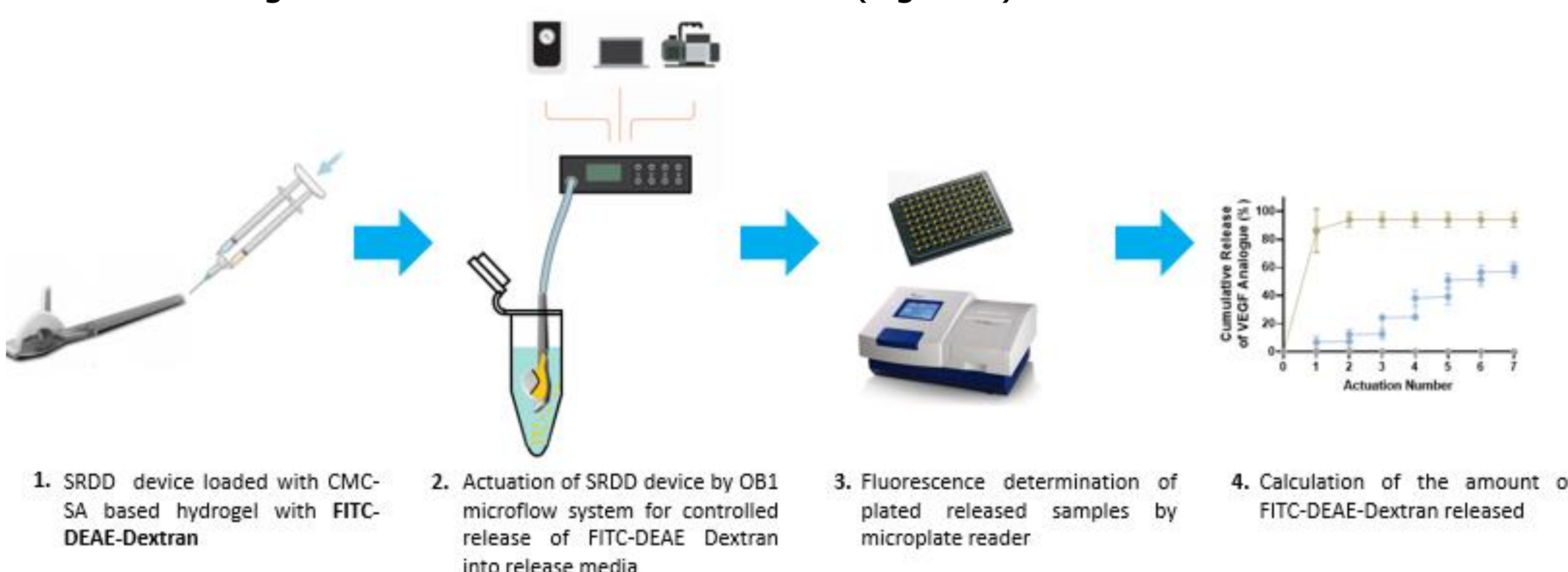

Figure 2 Schematic of *in vitro* release studies to optimise an actuation regime to achieve sufficient release of VEGF

Phase I preclinical animal study (Authorisation number 719/2020-PR) was carried out whereby SRDD devices were loaded with CMC-SA based hydrogel without or with 13.5 µg/ml VEGF and subcutaneously implanted in female Sprague-Dawley rats (Figure 3). Implanted SRDD devices were actuated once daily for 7 days using the optimised actuation regime. Explanted samples were stained with CD31 and αSMA for histological analysis of angiogenesis to determine if VEGF could stimulate neovascularisation at an implant site in healthy Sprague Dawley rats:

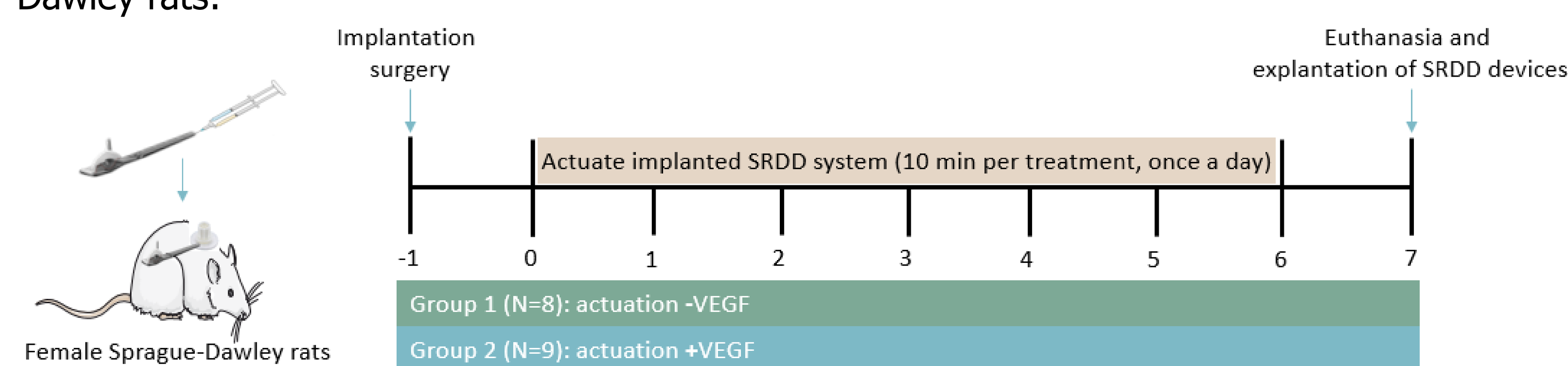

Figure 3 Overview of phase I preclinical study

Conclusions

We developed a new delivery vehicle, CMC-SA based hydrogel, to stabilise VEGF and established a SRDD device for controlled delivery of VEGF at a target site. Actuation of our SRDD system was modifiable and facilitated controlled release of VEGF to stimulate neovascularisation at implant sites. Future preclinical studies will have an extended follow-up period to establish whether these neovessels persist and mature to prevascularise an implant site for future islet transplantation.

Acknowledgements

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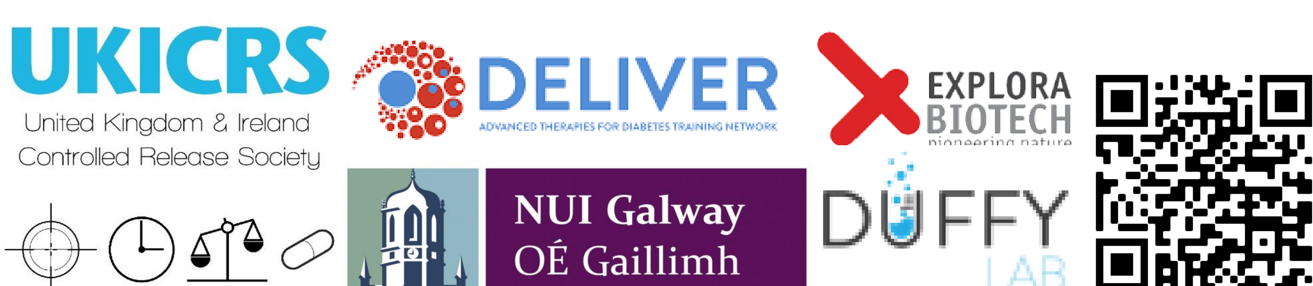

Results

The negatively charged CMC-SA based hydrogel could electrostatically interact with VEGF and gelate within 60 secs of polymer and crosslinker dispersion mixing (Figure 4a).

The porous membrane of the SRDD device (Figure 4b) developed for subcutaneous implantation in rats had 5 evenly distributed 0.3048 mm diameter laser cut pores incorporated to achieve uniform porosity (Figure 4c).

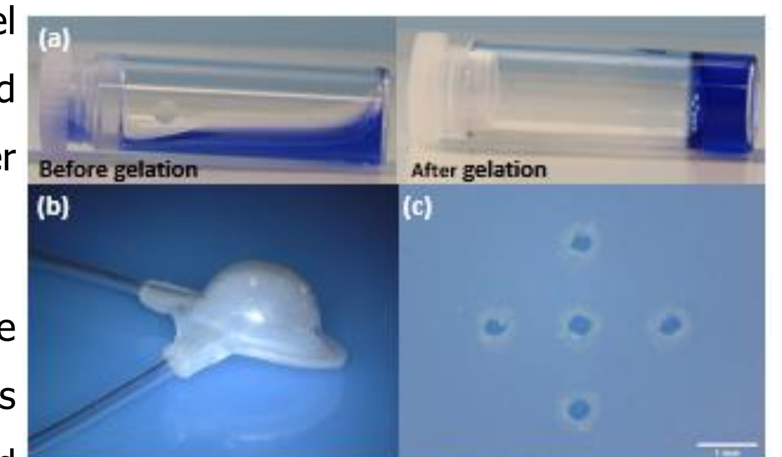

Figure 4 (a) Gelation of CMC-SA based hydrogel, (b) SRDD device, (c) laser cut 0.3048 mm diameter pores in porous membrane of SRDD device

Pressure of 10 psi was chosen for incorporation into the actuation regime as it could disrupt electrostatic interactions and release 2.68% of the loaded FITC-DEAE Dextran (Figure 5a). A ramp time of 5 secs increased FITC-DEAE Dextran release to 17.17% (Figure 5b). Increasing cycle number to 10 cycles further increased FITC-DEAE Dextran release to 53.61% (Figure 5c). The optimised actuation regime consists of 10 cycles of 10 psi, 10 sec on and 90 sec off (Figure 5d) and when combined with SRDD devices of uniform porosity resulted in the controlled release of 60% of the total loaded FITC-DEAE-Dextran – this controlled release was only achieved with the combination of SRDD device, CMC-SA based hydrogel and actuation (Figure 5e).

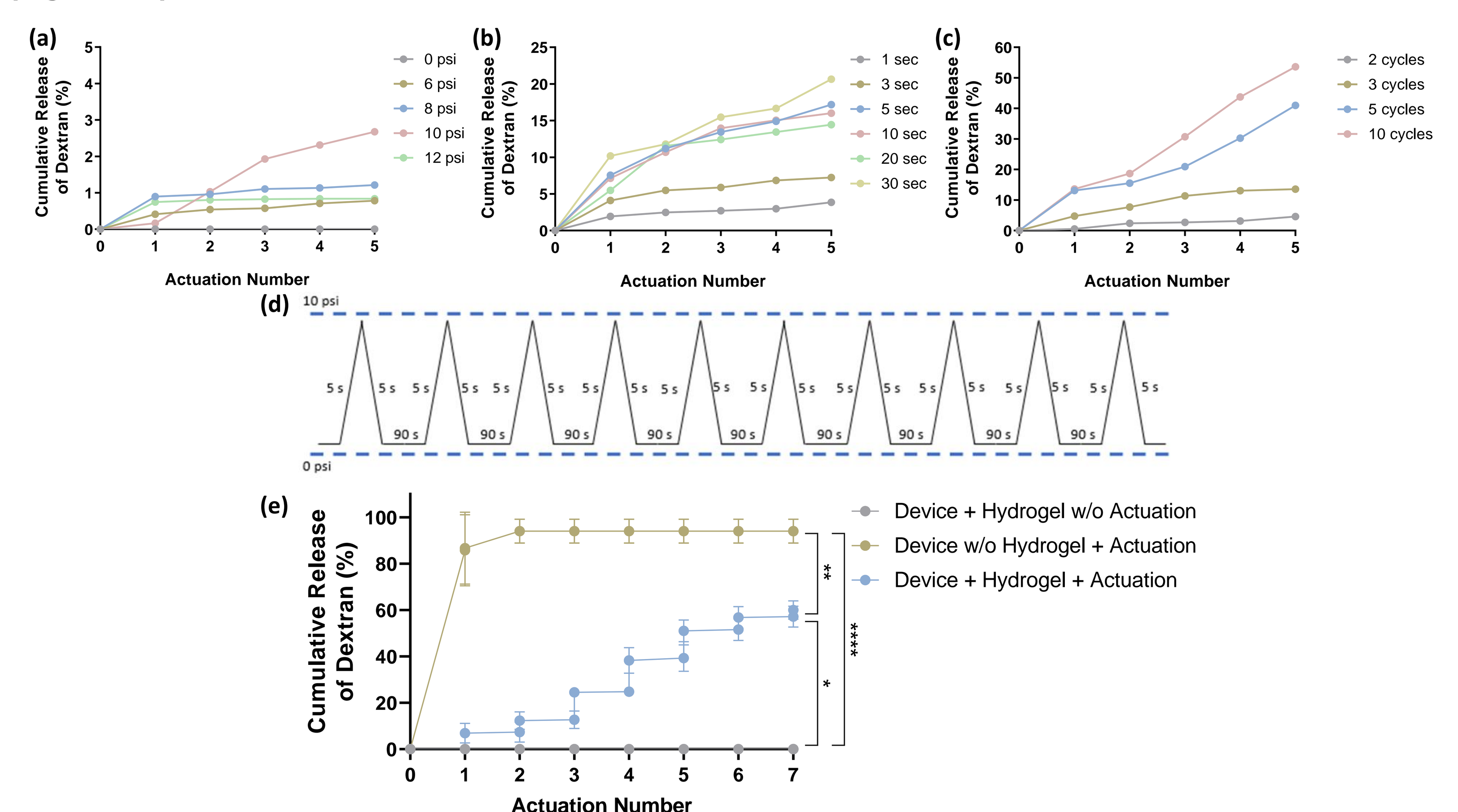

Figure 5 Optimisation of actuation regime parameters demonstrates that FITC-DEAE Dextran release from SRDD system is tuneable. (a) Pressure optimisation, (b) ramp optimisation, (c) cycle optimisation, (d) optimised actuation regime, (e) rationale for the combination of SRDD device, CMC-SA based hydrogel and actuation. N=3 per group. Kruskal-Wallis, *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001.

The controlled release of VEGF over 7 days in healthy rats significantly increased CD31+ neovessels abundance (p=0.0310, Figure 6b) and length density (p=0.0310, Figure 6c) and significantly reduced radial diffusion distances (p=0.0460, Figure 6d) compared to the no VEGF controls. VEGF also significantly increased the diameter of CD31+ blood vessels (p=0.0130, Figure 6e). However, the percentage of αSMA+ blood vessels was not significantly increased (p=0.3030, Figure 6f).

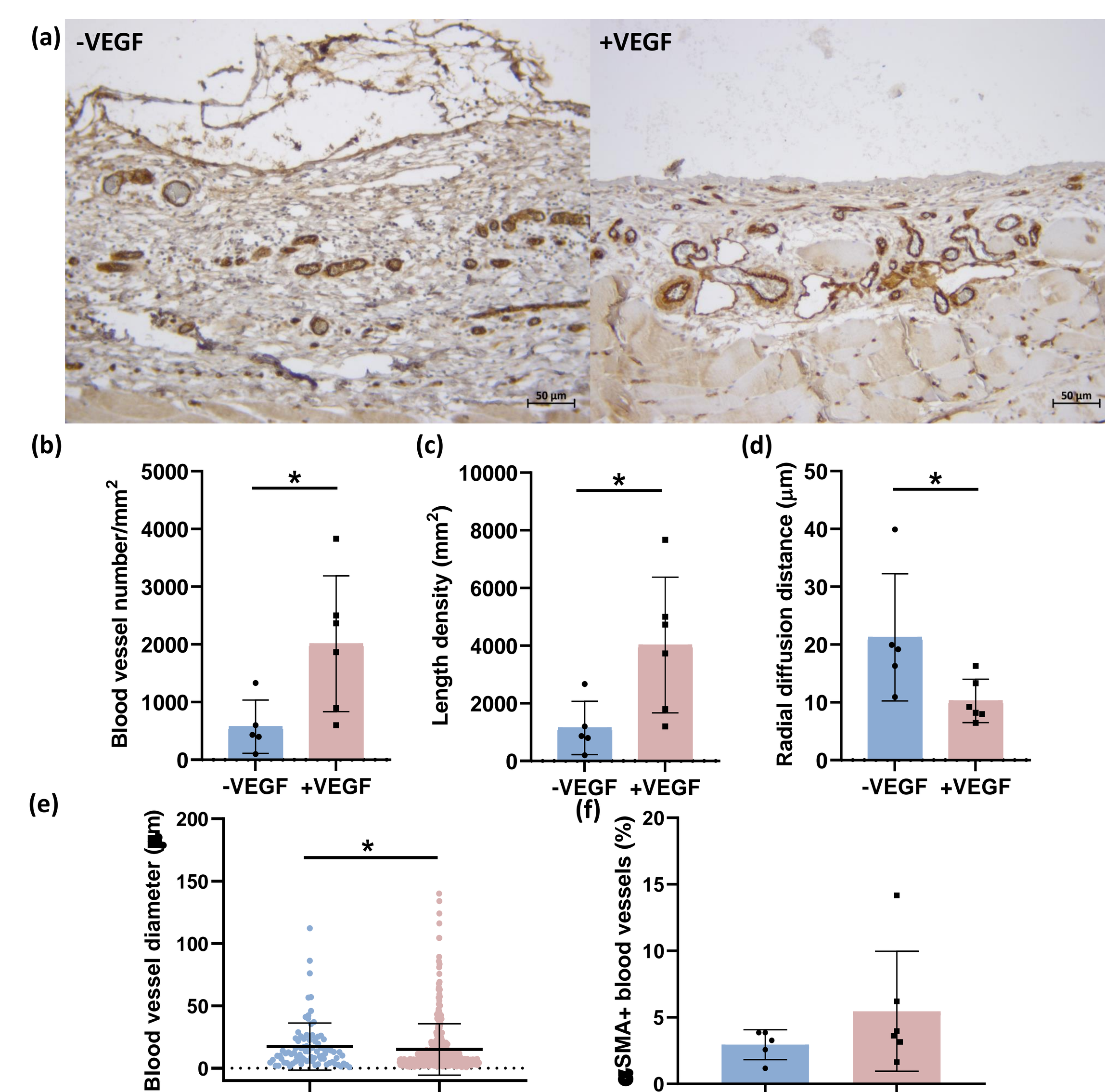

Figure 6 (a) Representative images of CD31 staining of vasculature surrounding –VEGF (left) and +VEGF (right) SRDD devices, 50 µm scalebar. (b) CD31+ blood vessel number per mm². (c) CD31+ length density. (d) CD31+ radial diffusion distances. (e) CD31+ blood vessel diameters, N>200 blood vessels/group. (f) Percentage of αSMA+ vessels. No VEGF, N=5; VEGF, N=6; data represented as means ± SD, * p < 0.05