CELLULOSE BEADS PREPARED IN DEEP EUTECTIC SOLVENT FUNCTION AS CARRIER AND CYTOPROTECTIVE ENCAPSULATION MATRIX FOR CAR-T CELLS IN T-CELL THERAPY AGAINST GLIOBLASTOMA CELLS

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BACKGROUND

Malignant brain tumors, including glioblastoma, represent some of the most difficult to treat of solid tumors. Chimeric antigen receptor (CAR) T cells are a promising immunotherapeutic modality, which utilizes the tumor targeting specificity of any antibody or receptor ligand to redirect the cytolytic potency of T cells. CAR-T cells have been recently used successfully to treat various hematologic malignancies. The use of CAR-T in solid tumours have been so far limited, but many development programs are currently underway[1].

The unique properties of the central nervous system limit T cell entry and risks of immune-based toxicities in highly sensitive environments. This is especially the case for CAR-T cells due to their lability during transfer, manipulation, and storage. Convection Enhanced Delivery (CED) is a technique used to infuse sensitive therapeutic agents directly into the intracranial area continuously under pressure with an improved turnover. Although CED successfully delivers small therapeutic agents, this technique has failed to effectively deliver cells largely due to cell sedimentation or damage during delivery.

CAR-T cell encapsulation has recently been suggested as a simple but versatile method for effectively preserving and protecting living cells from degradation in vitro. The encapsulation matrix requires to be non-toxic and readily biodegradable. Cellulose is one of the most abundant green materials on earth and readily modifiable for pharmaceutical applications. Deep eutectic solvents (DES) are mixtures of solid compounds, typically hydrogen bond donors and acceptors, which form liquids due to a large depression of the melting point as mixtures. DESs enable preparation of cellulose hydrogels, which can be utilized to encapsulate and protect sensitive biological components *in vivo*.

The aim of our study was to develop a cellulose bead delivery matrix for encapsulating CAR-T cells and improving their stability during storage and in vivo applications, such as CED. We also studied the influence of an improved CAR-T turnover on tumour volume and treatment response.

FIGURE 1. Preparation of cellulose beads from a cellulose-DES hydrogel

METHODS

The cellulose-DES hydrogel was prepared by mixing microcrystalline cellulose and ethyl glycidyl ether at high consistency using a twin-helicon mechanical stirrer. Aqueous NaOH was used as a catalyst. The washed mixture was transferred to a previously prepared 1:3 DES mixture of boric acid and choline chloride to form a 5 % w/v mixture. Cellulose beads were formed as reported previously by coagulating the hydrogel in acidic ethanol[2].

The human glioma cell line U87MG, as well as the Epidermal Growth Factor Receptor variant III (EGFRvIII) expressing subline, U87MG-ΔEGFR were purchased from Sigma-Aldrich. CAR-T cells were purchased from Creative Biolabs and used as received. The process for encapsulating CAR T cells in cellulose beads is currently being patented. For the simulation of CED mimicking a clinical setup, a KDS single-syringe pump (series 100, Sigma-Aldrich) with 20 ml syringes (Hamilton) and 20 cm long silicon tubes were used. Infusion rate was adjusted to 500 µl/hr. The number of cells and their viability was counted.

5.0×10^6 U87MG and U87MG-EGFR glioma cells were labelled with 100 µCi of Cr51 at 37°C for 90 minutes to define tumour cell cytotoxicity. The cells were washed twice in culture medium, co-incubated with cellulose bead carriers containing specific CAR-T cells at a ratio of 10:1 (CAR-T cells: tumor cells), and incubated at 37°C for a minimum of 4 hours.

All animal experiments were carried out in accordance with the National Recommendations in the Guide for the Use of Laboratory Animals of The Institutional Animal Ethics Committee (IIMS). Mouse Toxicity studies were done in non-tumor bearing 7–8 week old C57BL/6 mice. Xenograft models were prepared as reported by Iwami et al[3]. The tumor-bearing mice were randomly divided into three groups (n = 5) when the tumor volume reached 350 mm^3. One group received tail vein injections of 1×10^7 CAR-T cells, one group a similar volume of cells encapsulated in cellulose beads, and the third group a similar amount of cellulose bead encapsulated cells delivered with the aid of CED (mini-osmotic pumps Alzet®)[4].

RESULTS

Cellulose reacts readily under dry conditions with ethyl glycidyl ether, forming a derivative with improved solubility. The derivative can be processed into a hydrogel with the aid of a DES mixture, and the hydrogel further coagulates into cellulose beads without the need to solubilize cellulose during the process. The prepared cellulose beads were studied for maintaining the stability and viability of CAR-T cells when utilizing CED. CAR-T cells submitted to CED were counted and the efficiency of delivery was determined. It was shown that the delivery efficacy was higher compared to saline and CAR-T cells remained viable after the CED delivery (Figure 2) showing improved stability. In addition, the encapsulated CAR-T cells maintained cytotoxicity towards human glioma cells after the simulated CED delivery (Figure 3). There were no observed additional toxicity signs associated with the cellulose beads compared to saline after an injection of the components directly into caudate nucleus in mice (Figure 4). The encapsulated CAR-T cells induced similar tumour shrinkage as native CAR-T cells, with a statistically non-inferior result. CED delivery improved the speed of response statistically significantly, resulting in the highest overall decrease in tumour volume (Figure 5).

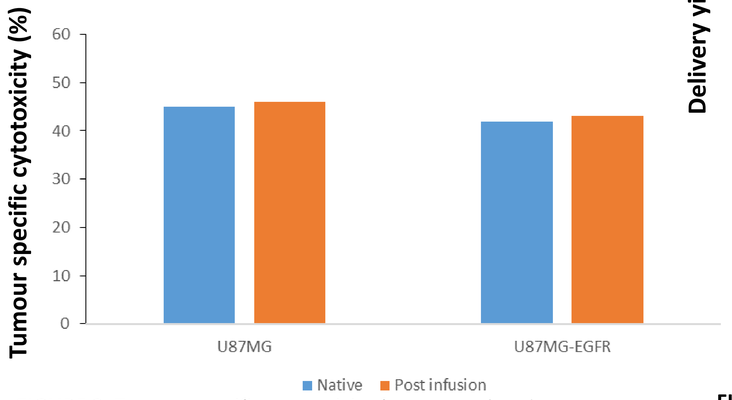

FIGURE 3. Tumour specific cytotoxicity for encapsulated CAR-T cells before and after CED infusion

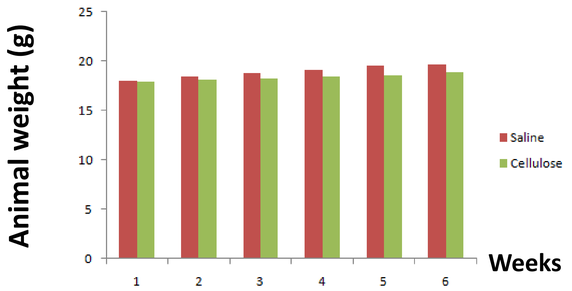

FIGURE 4. Toxicity of cellulose beads as evaluated by influence on female mice weight (n=5 in each group)

CONCLUSIONS

We developed a cellulose-based hydrogel matrix with the aid of deep eutectic solvents and further processed the hydrogel into beads suitable for encapsulating CAR-T cells. The biodegradable cellulose beads were capable of retaining CAR-T cells in suspension and enhanced cell viability in clinically relevant settings, while maintaining biological activity for therapeutic efficacy towards glioblastoma cell lines. The cellulose beads enable the use of CED for targeted delivery of CAR-T cells directly into the intracranial space, notably increasing speed of response and leading to a decrease in tumour volume. In summary, the cellulose beads provide a non-toxic green matrix for improving the efficacy and viability of CAR-T cells for treating glioblastoma in preclinical animal models.

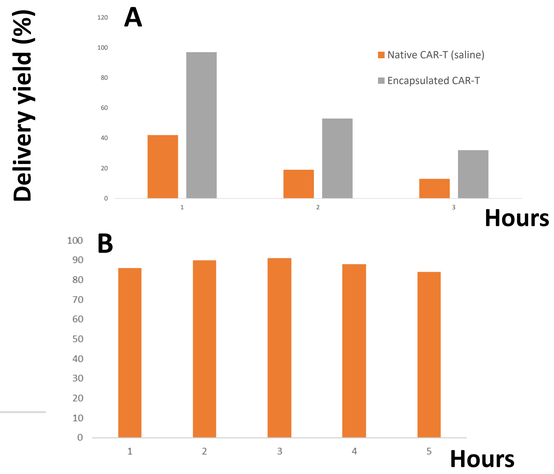

FIGURE 2. A) CAR-T cell CED delivery yield (%)
B) Cell viability (%) after CED for the encapsulated CAR-T cells

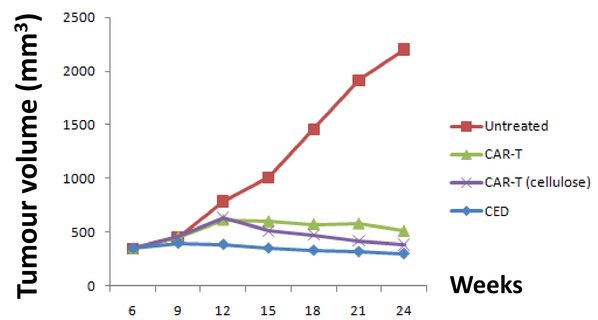

FIGURE 5. Comparison of CAR-T treatments in tumour (U87MG-EGFR) volume

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