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Release characteristics of cellulose sulphate capsules and production of cytokines from encapsulated cells

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ABSTRACT

The size and speed of release of proteins of different sizes from standard cellulose sulphate capsules (Cell-in-a-Box®) was investigated. Proteins with molecular weights of up to around 70kD can be released. The conformation, charge and concentration of the protein being released play a role in the release kinetics. Small proteins such as cytokines can be easily released. The ability to produce cytokines at a sustained and predefined level from encapsulated cells genetically engineered to overexpress such cytokines and implanted into patients may aid immunotherapies of cancer as well as infectious and other diseases. It will also allow allogeneic rather than autologous cells to be used. We show that cells encapsulated in polymers of cellulose sulphate are able to release cytokines such as interleukin-2 (IL-2) in a stimulated fashion e.g. using phorbol 12-myristate 13-acetate (PMA) plus ionomycin. Given the excellent documented safety record of cellulose sulphate in patients, these data suggest that clinical usage of the technology may be warranted for cancer treatment and other diseases.

1. Introduction

The implantation of encapsulated cells overexpressing specific biomolecules offers a means to treat a variety of diseases (for reviews see (Acarregui et al., 2013; Gurruchaga et al., 2015; Gonzalez-Pujana et al., 2017), including cancer (Salmons and Gunzburg, 2010; Salmons et al., 2010). Encapsulated cells have been evaluated in clinical trials for the treatment of diabetes (Orlando et al., 2014; Salmons et al., 2014) as well as for the treatment of pancreatic (Löhr et al., 2014) and mammary cancer (Michalowska et al., 2014).

A number of different materials can be used for encapsulation of cells, such as alginate, agarose, polysulphone (for a review of materials see (Gasperini et al., 2014). We have focussed on using cellulose sulphate as an encapsulation material (Dautzenberg et al., 1999) since it has a number of advantages over other encapsulation materials (Dangerfield et al., 2013; Salmons et al., 2014).

Cellulose sulphate encapsulation of mammalian cells has been shown to provide a means to protect cells from rejection by the immune system, to localize the cells at the site of implantation, to provide a long term microenvironment for survival of the cells, and to allow release of biomolecules from the capsules, as well as entry of food and nutrients for the cells (Dangerfield et al., 2013). Moreover, cellulose sulphate encapsulated human cells have been shown to be safe and efficacious in 27 human patients in clinical trials (Löhr et al., 2014).

To date however, there has been no systematic characterization of the size of the molecules released from standard cellulose sulphate capsules (Cell-in-a-Box®). This would be important since the size of the molecules that can be released will dictate which molecules can be successfully used therapeutically without changing the chemistry of the capsules.

The ability to produce, for instance, cytokines at physiological concentrations in patients could be of benefit in at least two major areas: (i) boosting or improving immune responses as a result of vaccination (important for combatting infectious agents as well as of potential use in combination with therapeutic vaccines for the treatment of tumours) and (ii) to directly combat tumours. Immuno-stimulatory cytokines have been administered to boost the immunogenic potential of other agents such as immune checkpoint-blocking antibodies, anticancer vaccines, oncolytic viruses and immunogenic chemotherapeutics (Vacchelli et al., 2014).

A number of cytokines have been used for cancer treatment including interferon alpha (IFN-α), interferon beta (IFN-β), gamma-interferon (γ-IFN) and interleukin-1 (IL-1) as well as IL-2, and IL-12. These cytokines demonstrate their efficacy by inducing apoptosis and other anticancer functions in the tumour microenvironment and some of these mechanisms of action have been recently reviewed (Kumar and Chandra, 2014).

The first reproducibly effective human cancer immunotherapies

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have been obtained using the pro-inflammatory cytokine IL-2 as a means to expand tumour infiltrating, anti-tumour T cells. These anti-tumour T cells lead to durable, complete, and apparently curative regressions in patients with metastatic melanoma and renal cancer (Rosenberg, 2014).

IL-2 is currently manufactured using recombinant DNA technology and is marketed as a protein therapeutic called aldesleukin. It has been approved by the Food and Drug Administration (FDA) and in several European countries for the treatment of cancers (such as malignant melanoma and renal cell cancer) in large intermittent doses and has been extensively used in continuous doses (Bhatia et al., 2009). Different dosages of IL-2 are recommended for the treatment of patients, depending on the country, and the efficiency and side effects of different dosages is often a point of disagreement. In the U.S., patients are typically given high dosages for five consecutive days, three times a day, for fifteen minutes, with a 10 day recovery period between treatment dosages. IL-2 is delivered intravenously (i.v.) for this type of dosing and hospitalization and even intensive care is required throughout due to the side effects. Alternatively, a lower regimen can be given as an injection of IL-2 under the skin at home or in the doctor's office. It is also possible to receive this dosage by i.v. delivery in the hospital over 1–3 days, similar to and often including the delivery of chemotherapy (MedlinePlus, 2013). IL-2 has a narrow therapeutic window, and the level of dosing usually determines the severity of toxicity and the side effects, leading to the search for other means of delivery (Shaker and Younes, 2009).

Tumour necrosis factor-α (TNF-α) is also a potential anticancer cytokine however, systemic use in humans has been limited due to significant toxicities as well as a lack of efficacy. More recently, the use of TNF-α as a facilitator, rather than as a direct actor, is being explored and this has been reviewed by Roberts and colleagues (Roberts et al., 2011).

Immuno-protective encapsulation of cells is an ideal way to achieve delivery of cytokines at physiological concentrations in patients. Moreover, it obviates the need for individualized *ex vivo* processing of autologous cells, thus allowing well characterised, defined and pre-tested cytokine secreting human allogeneic cells to be implanted (Schwenter et al., 2011). The feasibility of this approach has been demonstrated by encapsulating granulocyte-macrophage colony-stimulating factor (GM-CSF) producing K562 cells in alginate (Schwenter et al., 2011). Side effects of using purified cytokines and targeting to the site of action also favour the use of encapsulated cells for the production of cytokines.

Interleukin-6 (IL-6) is a cytokine with pleiotropic effects that plays a central role in normal and abnormal hepatic function and response. Moran and colleagues (Moran et al., 2006) reported the encapsulation of genetically modified CHO cells in alginate followed by intraperitoneal (i.p.) implantation in a rat model of hepatocellular carcinoma (HCC). They observed a significant increase in the circulating and intra-hepatic levels of human IL-6 up to 4 days post-implantation but this was not accompanied by a significantly altered rate of overall tumour progression. Stat3 activity was however significantly increased in both normal liver and HCC tissue resected from animals implanted with the IL-6 producing CHO cells. These data demonstrate the short-term advantage of using cell encapsulation technology to generate high levels of active circulating and intra-hepatic cytokines as well as raising the possibility of modifying specific signal transduction cascades that have been identified as important during tumour progression (Moran et al., 2006).

Chang and colleagues (Cirone et al., 2004) investigated a combined immunotherapy and angiostatic therapy in the B16-F0/neu melanoma mouse model using intraperitoneally implanted, alginate poly-L-lysine-alginate (APA) microencapsulated mouse myoblasts (C2C12), genetically modified to deliver angiostatin and an interleukin 2 fusion protein (sFvIL-2). The data that they obtained was promising in that the combination treatment improved survival of the mice. In addition, the treatment delayed tumour growth, and this was associated with increased histological indices indicative of apoptosis and necrosis. Moreover, the combination of immuno- and anti-angiogenic therapies delivered by immune-isolated cells was superior to individual treatments and gave rise to unexpected protection against the adverse side effects of the single treatments alone (Cirone et al., 2004).

Recently, Werner and colleagues (Werner et al., 2015) encapsulated a human leukemia T lymphocyte Jurkat cell line in cellulose sulphate capsules. The encapsulated cells divided more actively and showed less propensity to undergo programmed cell death (apoptosis).

It has been reported that stable transfection of a subclone of HEK293 cells with various cytokines and chemokine expression vectors, including GM-CSF, IFNγ and hIL-15 and alginate encapsulation resulted in the stable production of relevant cytokines and chemokines from the encapsulated cells for at least 2 weeks *in vitro* (Hamilton et al., 2006; Huang, 2005). Moreover, in anti-tumour therapy experiments in C57BL/6 mice with either B16 melanoma, EL4 or EG7 tumours, GM-CSF produced from alginate encapsulated cells was particularly potent in stimulating anti-tumour, cellular mediated, immune responses (Huang, 2005).

Currently the evaluation of encapsulated cells to boost anti-tumour immune responses is being tested in an ongoing clinical trial. In this immunotherapy study, patients with advanced stage solid tumours (ovary, pancreas, head and neck, colon, prostate) are being given encapsulated cells producing GM-CSF subcutaneously as a strong immune booster for the co-administered vaccine consisting of $4x10^6$ of the patients' own irradiated tumour cells. In this study, two "theracyte" macrocapsules made of polysulphone ester and each producing 20 ng GM-CSF/24hr are implanted in each patient. Data from 15 treated patients has recently been reported. As well as showing safety (no Serious Unexpected Suspected Adverse Reactions (SUSAR) nor Systemic suspected Adverse Drug Reactions (SADR) reported), 2 of the 15 patients treated to date showed partial responses and another 6 patients showed stable disease (Mach et al., 2016). The anecdotal results from the first successful immunotherapy treatment of patients with a chordoma were also recently published (Migliorini et al., 2017).

Here we show for the first time that cells encapsulated in cellulose sulphate are able to produce interleukin-2.

2. Methods

2.1. Fluorescent dextran study

Fluorescein isothiocyanate (FITC) coupled dextran of different molecular weights (4, 10, 20, 40 and 70 kDa) was encapsulated in cellulose sulphate essentially as outlined previously (Hauser et al., 2006; Ortner et al., 2012). The resulting capsules were placed in the wells of cell culture plates (5, 10 or 30 capsules per well) in 200 µl phosphate buffered saline (PBS) and analysed by fluorescence microscopy and photography at various time points.

In a separate experiment, 10 or 30 capsules per well were incubated in 200 µl of PBS and 24 h or 48 h after incubation (37 °C), 100 µl of supernatant of each well was transferred into another well and the fluorescence measured by spectrophotometry. The fluorescence is proportional to the amount of 10, 20 and 40 kDa Dextran-FITC that has left the capsules.

2.2. Protein release from capsules

A mix of proteins from SDS6H2 SIGMA, Molecular Weight Marker (M.W. 30,000–200,000) was encapsulated and 3000 capsules incubated in 2 ml PBS for periods between 2 h and 7 days at 37 °C. At fixed time points, the supernatant was removed from the capsules, lyophilized and taken up in non-denaturing loading buffer before being loaded onto a polyacrylamide gel and separated by electrophoresis. Proteins were visualized by silver staining. Separately, β-Galactosidase (116kD) and