MICROFLUIDIC BIOMIMETIC ARTERIOLAR NETWORKS TO STUDY DRUG ELUTION FROM EMBOLISATION BEADS

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ABSTRACT

Transarterial chemoembolisation (TACE) is a therapeutic procedure generally employed in the treatment of hypervascularised tumours. It is performed by arterial infusion of anticancer drugs followed by injection of embolic materials, thus combining the therapeutic effect of drugs with embolisation-induced tissue ischemia generated by vascular occlusion. Recently, drug-loaded microspherical beads have been introduced and employed as chemoembolic devices, allowing for improved process standardisation and spatially-controlled drug delivery. A range of different experimental methods have been employed for investigating the kinetics of drug elution from drug eluting beads (DEBs) and comparing the elution profiles of different embolic devices. However, no current available system faithfully reproduces the flow dynamics and the intricate architecture of microvascular networks. In the present study, the elution of doxorubicin hydrochloride from hydrogel DEBs was investigated by means of a biomimetic microfluidic device. With the developed system, new insights into the mechanisms of drug elution at clinically-relevant fluidic conditions were obtained, including the effect of the spatial location of the embolic site on the spatio-temporal evolution of the drug elution process.

KEYWORDS

Chemoembolisation; Biomimetics; Microchannel network; Hydrogel bead; Bifurcation; Drug elution

INTRODUCTION

Despite its widespread clinical usage, traditional systemic delivery of chemotherapeutic drugs suffers from a range of drawbacks, including low drug concentration achieved in the target tumour tissue, non-specific drug toxicity and the development of drug resistance. This results in patients undergoing chemotherapy treatment potentially suffering from diverse systemic side effects. In order to minimise the occurrence of these side effects, locoregional therapies have been proposed, which allow for increased concentration of drug delivered to the tumour and reduced systemic exposure compared to conventional chemotherapy. Among these techniques, transarterial chemoembolisation (TACE) has been used for over 30 years and is performed by local arterial infusion of the chemotherapeutic agent and subsequent injection of embolic materials, thus synergistically combining the potential cytotoxic effect of drugs to bland embolisation, and minimising the systemic toxic effects [1]. Whilst the advantages of TACE with respect to systemic chemotherapy have been widely demonstrated, there still persists a lack of method standardisation in terms of properties and type of embolic device and administration techniques to be employed. In order to address these issues, DEBs in the form of calibrated deformable microspheres have been recently introduced [2]. DEBs generally consist of a biocompatible, non-degradable polymer capable of sequestering specific molecules (i.e. anticancer drugs) and release them for prolonged time. DEBs allow for spatially-controlled and simultaneous delivery of the anticancer drug and the embolic device in a single step, by means of microcatheters, providing improved controllability and reduced drug clearance compared to conventional TACE. A range of different experimental techniques have been developed for quantifying the kinetics of drug elution from DEBs. However, currently available techniques do not faithfully reproduce the *in-vivo* flow dynamic environment, which potentially limits the optimisation of chemoembolic devices. In the present study, the elution of doxorubicin hydrochloride from hydrogel embolic beads (DC Bead®, Biocompatibles UK Ltd) was investigated within a biomimetic microchannel network which reproduced typical features of tumour microvascular systems [3]. The microfluidic-based device was previously observed to allow for a faithful reproduction of embolisation mechanisms, including the discrimination between single-bead and multi-bead vascular embolisation [3].

EXPERIMENTAL

The biomimetic microfluidic device consisted of a microchannel network reproducing typical features of tumour arteriolar systems, including bifurcation architecture, vessel curvature and the significant reduction of vessel diameter along the network [3]. The device was fabricated by bonding two specular micromilled poly(methyl methacrylate) (PMMA) layers in order to obtain circular channel cross-section (inset, Fig. 1). Channels inner diameter varied from 1000μm (proximal) to 200μm (distal). A model of commercially-available DEB (DC Bead®, 500-700μm diameter range, Biocompatibles UK Ltd) was employed for drug elution studies.

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DC Bead® consists of a polyvinyl alcohol (PVA) polymer hydrogel which has been modified by addition of a sulfonic acid-containing component. This is capable of actively sequestering oppositely charged molecules (i.e. doxorubicin hydrochloride) by ion-exchange mechanism, and subsequently release them when exposed to Na^+-rich fluidic environments (i.e. plasma). Physiological buffered saline (PBS) solution was employed as working fluid, reproducing the physical properties and the ionic strength of plasma.

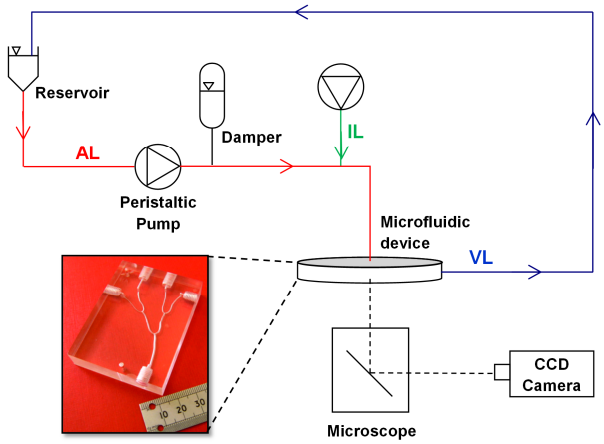

Figure 1: Experimental set-up (AL: arterial line, IL: injection line, VL: venous line).

Fig. 1 illustrates the experimental set-up. The working fluid was injected into the arterial line (AL) using a peristaltic pump followed by a damper. Steady inlet flow rate was varied within a range of physiological Reynolds numbers (Re) between 71.28 and 287.27. A single bead was injected into AL through the injection line (IL). The fluid flow was recirculated through the venous line (VL). Embolisation was performed at different locations within the network, including proximal embolisation in channel C2 (e.g. bead partially penetrated within the embolised channel, Fig. 2*e1*) and distal embolisation in channel C3 (e.g. bead completely penetrated within the embolised channel, Fig. 2*e2*).

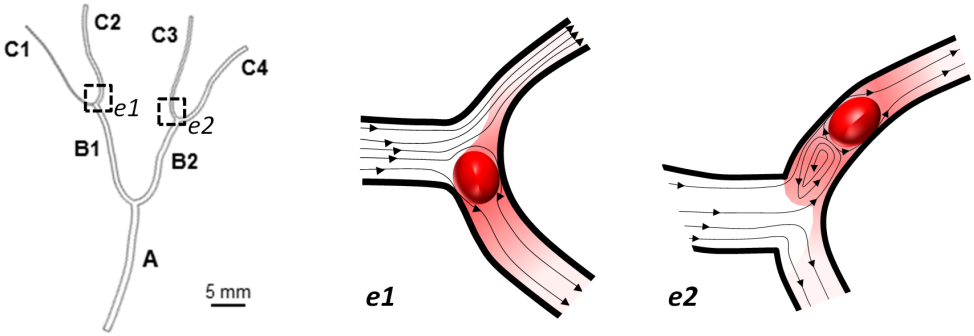

Figure 2: Schematic depiction of proximal embolisation (e1) and distal embolisation (e2) within the network.

Quantification of the spatio-temporal evolution of drug elution was achieved by two means: 1. *On-chip* fluorescent determination was achieved by positioning the microfluidic device on the stage of an inverted microscope (IX 71, Olympus) and recording subsequent fluorescent images of the bead. An in house MATLAB code was designed for quantification of drug elution kinetics and the time evolution of bead location within the embolised channel. 2. *Off-chip* on-line spetrofluorimetric recordings were obtained by connecting the venous line to a flow cell. Furthermore, flow visualisation experiments using 1μm diameter polystyrene fluorescent particles were performed in order to investigate the contribution of the flow dynamic environment on drug elution mechanisms.

RESULTS AND DISCUSSION

With the developed biomimetic microfluidic device, relevant information regarding the mechanisms of drug elution from hydrogel embolic beads was obtained. Notably, the fractional amount of eluted drug (ϕ) from single-bead was quantified at different Re (Fig. 3a). Results showed that ϕ increased with time, which was represented in a reduction of fluorescence intensity on the bead surface (contours of Fig. 3b-d). When proximal embolisation was achieved (i.e. bead partially exposed to fluid flowing from the feeding channel, Fig. 2*e1*) a significant amount of drug was eluted within three hours from embolisation. Additionally, ϕ was observed to increase by increasing Re from 71.28 to 213.84, likely due to increased advection phenomena (Fig. 3a). However, increasing Re above 213.84 resulted in a counter-intuitive reduction in the amount of eluted drug after three hours (Fig. 3a). This observation was likely due to the correlation between drug content and bead mechanical properties (i.e. compressibility). With this respect, drug elution resulted in reduced drug content and,

in turn, increased bead compressibility likely due to water uptake. To demonstrate this hypothesis, bead surface was divided in two regions, namely an *advection-dominated* surface (A) exposed to fluid flow from the feeding channel, and a *diffusion-dominated* surface towards the embolised channel (D) (Fig. 3e-f). The time evolution of the ratio α = A/D was quantified (Fig. 3g) and displayed a marked reduction with increasing Re; which resulted from bead penetrating more distally as a consequence of drug elution. This corroborates with the observed Re-dependence of drug elution kinetics profiles. Conversely, distal embolisation (Fig. 2*e2*) provided a more sustained and durable release (data not shown), and bead position remained substantially unvaried during the elution process.

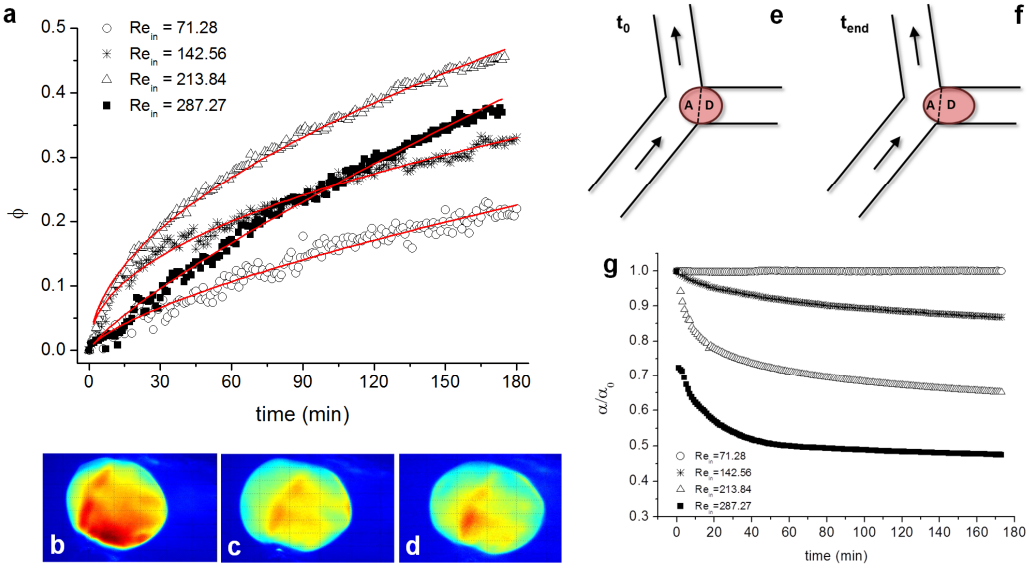

Figure 3. (a) Time evolution of fractional eluted drug (ϕ) at different Re. (b-d) Fluorescence intensity contours after 180 min from embolisation, at Re = 71.28 (b), 142.56 (c) and 213.84 (d). (e-f) Schematic depiction of the time evolution of bead surface area as a consequence of drug elution. (g) Time evolution of α=A/D at different Re. Values are normalized with respect to $\alpha(t_0) = \alpha_0$.

Interestingly, the dynamics of the drug elution process from the bead was observed to strongly depend on the spatial location of the occlusion site. Whilst for proximal embolisation the elution process was observed to be uniform within the whole bead, distal embolisation was instead characterised by a markedly inhomogeneous elution process (Fig. 4a-c). Fig. 4b clearly shows a decrease of fluorescence intensity level on the bead portion facing the feeding channel, while it decreased to a lower extent in the other areas of the bead. Flow visualisation experiments (Fig. 4d) revealed the formation of laminar vortices in close proximity to distal beads, which may cause enhanced elution of drug from the bead surface exposed to the vortex (i.e. vortex-enhanced elution). This may have relevant implications on the spatial distribution of drug within microvascular networks.

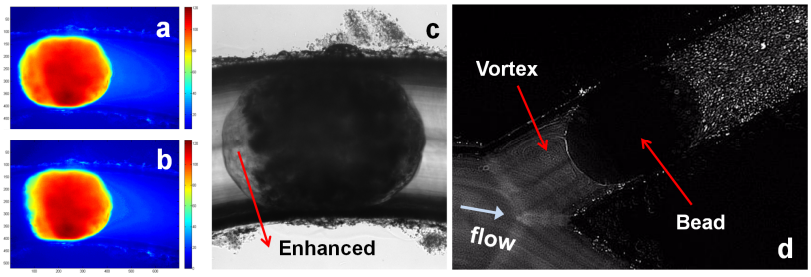

Figure 4. (a-b) Fluorescence intensity contours at $t=t_0$ (a) and t=18 hours (b). (c) Bright field image of single distal bead at t=18 hours. (d) Microscope image of fluorescent particles during flow visualisation experiments.

CONCLUSION

The developed microfluidic device, coupled with non-invasive fluorescence-based analysis techniques, allowed for obtaining unique insights into the dynamics of drug elution from embolic beads under biomimetic fluidic conditions and, particularly, for investigating the influence of bead location on the drug elution process.

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