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Alginate–lanthanide microspheres for MRI-guided embolotherapy

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ABSTRACT

In cancer therapy, a promising treatment option to accomplish a high tumor-to-normal-tissue ratio is endovascular intervention with microsized particles, such as embolotherapy. In this study, alginate microspheres (ams) were prepared with the JetCutter technique, which is based on cutting a sodium alginate solution jet stream into small droplets of uniform size which are then cross-linked with different lanthanides or iron-III, resulting in microspheres of a predefined size which can be visualized by magnetic resonance imaging (MRI). The microspheres were investigated for their size and morphology (light microscopy and scanning electron microscopy analysis), cation content and MRI properties. The lanthanide-ams formulations, with a uniform size of 250 μm and a cation content between 0.72–0.94%, showed promising results for MR imaging. This was further demonstrated for Ho^{3+}-cross-linked alginate microspheres (Ho^{3+}-ams), the most potent microsphere formulation with respect to MR visualization, allowing single sphere detection and detailed microsphere distribution examination. Intravascular infusion of Ho^{3+}-ams by catherization of ex vivo rabbit and porcine liver tissue and assessment of the procedure with MRI clearly showed accumulation and subsequently embolization of the targeted vessels, allowing accurate monitoring of the microsphere biodistribution throughout the tissue. Therefore, the different alginate–lanthanide microsphere formulations developed in this study show great potential for utilization as image-guided embolotherapy agents.

1. Introduction

In cancer chemotherapy, treatment techniques aimied at local drug delivery are gaining increasing interest. Ideally, successful tumor targeting will lead to a high tumor-to-normal-tissue ratio, enhancing treatment efficacy while toxicity and side effects are reduced [1,2]. A promising treatment option for efficient tumor targeting is embolotherapy, in which microsized particles are administered selectively into the tumor-feeding artery by catheterization. During this procedure, a catheter is advanced intra-arterially to the desired location. Using radiopaque contrast medium, the procedure can be performed under fluoroscopy guidance. When the tumor-feeding artery is reached with the catheter, the microsized particles are infused. As a result, the blood flow is reduced or completely obstructed, which causes a lack of nutrients and oxygen and eventually leads to necrosis of the affected tissue. Different embolization agents, such as polyvinyl alcohol embolization particles and tris-acryl gelatin embolization microspheres, have been developed and are currently utilized for various indications, such as treatment of uterine fibroids, arteriovenous malformations and hypervascularized tumors [3,4]. Furthermore, embolization microspheres can also be exploited as a carrier of therapeutic agents, e.g. drug-eluting beads, which have been used recently in transarterial chemo-embolization [5–7]. These microspheres enabled visualization with fluoroscopy and X-ray computed tomography (CT) imaging, which would allow "real-time" image guidance of the transcatheter embolization procedure [8,9]. However, these ionizing radiation-based imaging techniques lack soft tissue contrast, which is important for tumor identification. The possibility to non-invasively assess the biodistribution of microspheres in conjunction with the local anatomy is of major importance, because it allows for superior treatment monitoring as well as validation of the targeting efficiency. Recently, our group demonstrated proof-of-concept of an embolization particle based on alginate containing a magnetic resonance imaging (MRI) contrast agent (Ho^{3+}) [10]. MRI is a promising modality for image-guided embolotherapy and biodistribution assessment of microspheres within the patient due to its relatively high spatial and temporal resolution and superior soft tissue contrast properties [11] as compared to CT, which allows visualization of lower quantities of the contrast agent and improves localization with respect to soft tissue target organs. After intra-arterial infusion of microspheres, clustering and accumulation of the microspheres within the tissue can occur, which may

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lead to saturation of the MR signal, especially when using high-field MRI and microspheres with a high magnetic susceptibility. Therefore, alginate microspheres with a low susceptibility may be more suitable for the quantification of higher microsphere concentrations. Alginate, the main component of these microspheres, is a biocompatible hydrophilic polysaccharide consisting of alternating (1,4)-linked β-D-mannuronic (M) acid and α-L-guluronic acid (G) units and can interact with different cations, resulting in the formation of a hydrogel [12]. Alginate hydrogels have been widely investigated in the biomedical research field and have subsequently been successfully employed as matrix devices for the loading and release of drugs [13–17] and growth factors [18–21], as well as for the entrapment of cells [22–25]. The development of different micro-sized spherical particles which have the potential to be used, for instance, as drug carriers and additionally can be visualized with MRI will offer a great improvement in minimally invasive transcatheter embolization therapy.

In this comparative study, different lanthanides were investigated for their ability to form alginate-based MRI-detectable microspheres, exploiting the paramagnetic behavior of the lanthanides [26–28]. All microsphere formulations were characterized with light microscopy, scanning electron microscopy (SEM) and complexometric cation content analysis. Extensive qualitative and quantitative MRI assessment of the microsphere formulations was of particular interest. Furthermore, ex vivo embolization studies with rabbit and porcine liver were performed and the possibility of "single sphere detection" was explored, which would allow detailed microsphere biodistribution examination.

2. Materials and methods

2.1. Materials

All chemicals and polymers were from commercial sources and were used as obtained. Sodium alginate (Protanal LF 240 D, Ph. Eur.) was a generous gift from FMC Biopolymer Ltd. (Girvan, Ayrshire, UK). Calcium(II) chloride dihydrate (⩾99.0%), ferric(III) chloride hexahydrate (⩾98%), gadolinium(III) chloride hexahydrate (99%), dysprosium(III) chloride hexahydrate (99.9%), Eriochrome® Black T (ACS reagent), 1,10-phenanthroline (⩾99%), ammonium iron(II) sulphate hexahydrate (Mohr's salt, ACS reagent; 99%) and sodium chloride (ACS reagent, ⩾99.8%) were obtained from Sigma Aldrich (Steinheim, Germany). Holmium(III) chloride hexahydrate (99.9%), ytterbium(III) chloride hexahydrate (99.9%), thulium(III) chloride hexahydrate (99.9%), europium(III) chloride hexahydrate (99.9%) and terbium(III) chloride hexahydrate (99.9%) were purchased from Metall rare earth Ltd. (Shenzhen, China). Agarose MP was obtained from Roche Applied Science (Mannheim, Germany). Nitric acid (65%), potassium nitrate (99.9%), ethylene dinitrilotetraacetic acid (EDTA; >99.0%), xylenol-orange (Ph. Eur. 99.9%), hydroxylamine hydrochloride (⩾96%) and manganese(II) chloride tetrahydrate (ACS reagent) were obtained from Merck (Darmstadt, Germany). Sodium hydroxide (99.9%) was purchased from Riedel-de Haën (Seelze, Germany), Magnesium (II) chloride hexahydrate was obtained from Boom Lab (Meppel, the Netherlands).

2.2. Alginate microsphere preparation

An important advantage of alginate gels is their ease of preparation, allowing the formation of gels with different shapes and sizes [29]. The JetCutter technique allows for the preparation of monodisperse alginate microspheres (ams) (Fig. 1), as previously described by Prusse et al. [30], resulting in the preparation of spherical particles with a predetermined size which can be utilized as embolization agents [10,31,32]. The JetCutter uses a mechanical cutting step in combination with a jet of fluid, which is pressed through a nozzle with a small diameter to create identical cylindrical segments that will become spherical due to the surface tension of the fluid [10]. In order to produce ams with the JetCutter, sodium alginate was dissolved in demineralized water under magnetic stirring at a concentration of 2% (w/v). Subsequently, the alginate solution was processed with the JetCutter using a nozzle diameter of 120 μm and a rotor speed set to 5000 rpm. The JetCutter was equipped with a cutting tool consisting of 120 wires with a diameter of 100 μm. An alginate flow rate of 0.12 ml s^{-1} was used to prepare microspheres with a target size of around 250 μm. The droplets were collected into various solutions containing 25 mM concentrations of the chloride salts of alkaline earth metals ($CaCl_2$), transition metals ($FeCl_3$) or lanthanides ($HoCl_3$, $GdCl_3$, $DyCl_3$, $YbCl_3$, $TmCl_3$, $EuCl_3$ and $TbCl_3$). Ca^{2+}- and Fe^{3+}-ams were used as control formulations, as Ca^{2+} is often used for alginate hydrogel formation [29] and Fe^{3+} is frequently used in non-lanthanide-based MRI contrast agents [33,34]. All droplets were allowed to cross-link for 2 h under gentle magnetic stirring. After three washing steps with demineralized water to remove excess cations, the microspheres were collected and stored in demineralized water at room temperature. In order to determine the influence of the cross-linking time and cross-linking solution concentration on cation content, the ams were also allowed to cross-link for 2 up to 24 h or to cross-link in a 100 mM cross-linking solution.

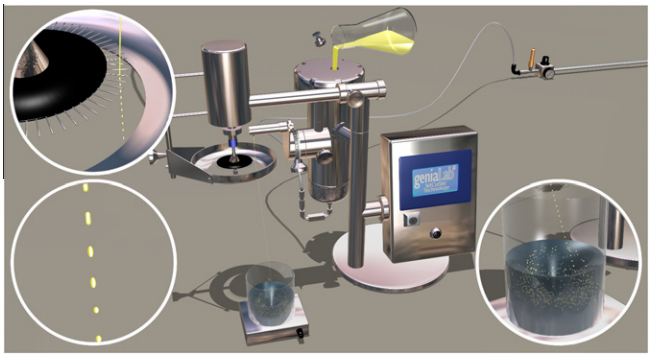

Fig. 1. The JetCutter system used in this study to prepare monodisperse ams with different cross-linking agents. The cutting disk cuts the alginate jet stream into small cylinders (top left). The cylinders become spherical when falling down (bottom left). When the droplets come into contact with the cross-linking solution, microspheres are formed (bottom right).

2.3. Characterization of the alginate microspheres

2.3.1. Light microscopy

Morphological examination of the formed ams was performed using light microscopy (magnification 10 × 10). To calculate the mean size, the diameters of 25 randomly selected microspheres were determined. Size distribution was calculated as the coefficient of variation (C.V.), which is defined as the ratio between the standard deviation and the mean. A C.V. of less than 5% is the commonly accepted definition of monodispersity [35].

2.3.2. Scanning electron microscopy

For SEM analysis of the surface morphology of the different ams, they were subjected to critical point drying, which allows the examination of the microspheres under vacuum. Therefore, freshly prepared microspheres were centrifuged at 3000 rpm for 2 min and subsequently subjected to dehydration steps for 5 min each in an increasing series of ethanol (30%, 50%, 70%, 80%, 90%, 96% and 100%). Next, ethanol was replaced by acetone in three

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