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# In vitro and in vivo evaluation of chemically modified degradable starch microspheres for topical haemostasis

Katarina Björses [a,*], Lars Faxälv [b], Carl Montan [a], Katarina Wildt-Persson [c], Peter Fyhr [c], Jan Holst [a], Tomas L. Lindahl [b]

[a] Vascular Center, Skane University Hospital Malmö, Lund University, Malmö, Sweden
[b] Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
[c] Magle Life Sciences, Kristianstad, Sweden

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ABSTRACT

Degradable starch microspheres (DSMs) are starch chains cross-linked with epichlorhydrin, forming glycerol–ether links. DSMs have been used for many years for temporary vascular occlusion and drug delivery in treatment of malignancies. They are also approved and used for topical haemostasis by absorbing excess fluid from the blood and concentrating endogenous coagulation factors, thereby facilitating haemostasis. This mechanism of action is not sufficient for larger bleedings in current chemical formulations of DSMs, and modification of DSMs to trigger activation of platelets or coagulation would be required for use in such applications. Chemical modifications of DSMs with *N*-octenyl succinic anhydride, chloroacetic acid, acetic anhydride, diethylaminoethyl chloride and ellagic acid were performed and evaluated in vitro with thrombin generation and platelet adhesion tests, and in vivo using an experimental renal bleeding model in rat. DSMs modified to activate platelets in vitro were superior in haemostatic capacity in vivo. Further studies with non-toxic substances are warranted to confirm these results and develop the DSM as a more effective topical haemostatic agent.



## 1. Introduction

Topical haemostatic agents are a heterogeneous group of products developed as a complement to traditional surgical techniques to achieve haemostasis during surgery. They are applied to the bleeding tissue and facilitate haemostasis by different modes of action. According to their working mechanism they can be classified as passive or active agents and combinations thereof [1]. Passive agents act by absorption of excess fluid from the blood and thus the concentration of endogenous coagulation factors increases at the site of bleeding. The material itself also provides a matrix for formation of a clot. Some of the passive agents also possess platelet-activating properties, which probably improve their haemostatic effect. Gelatine, collagen (porcine, bovine or equine) and oxidized cellulose are the most frequently used passive agents. After in vivo application gelatine and oxidized cellulose are degraded and absorbed by the body, the process requiring 4–8 weeks for oxidized cellulose and 12–16 weeks for collagen [2].

Active agents consist of exogenous coagulation factors such as thrombin and fibrinogen of either animal (bovine, equine) or human (plasma-derived or recombinant) origin. These haemostatic agents interact with the patient's coagulation system and accelerate the fibrin formation process, thereby facilitating the rapid formation of a strong haemostatic clot. Pure active agents do not provide a matrix that could protect the fresh clot from fragmentation during continuation of the surgical procedure. Combining active and passive agents generally improves their haemostatic capacity, however, the risk of negative side-effects may be increased by such manoeuvres. Side-effects may include antibody development after exposure to bovine thrombin, potentially causing iatrogenic coagulopathy [3]. Non-recombinant human-derived products also carry an increased risk of disease transmission.

Degradable starch microspheres (DSMs) have for many years been used for temporary vascular occlusion during co-administration of cytotoxic drugs in the treatment of malignancies. Since 2002 DSMs have also been approved for intra-operative applications and used clinically as a topical haemostatic agent.

The DSMs are formed by cross-linking starch chains with epichlorohydrin, forming glycerol–ether links (1–3 dioxypropanol). Starch is derived from plants as a branched glucose polymer (α4-glucose chains with α6 branches). The polymer consists of amylose (long chained with little branching) and amylopectin (highly branched and short chained). Amylopectin is very similar to glycogen, the animal equivalent to starch, only differing in a

* Corresponding author. Tel.: +46 733681538.
*E-mail address:* katarina.bjorses@med.lu.se (K. Björses).

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shorter branch length for the glycogen molecule [4]. These similarities make starch an ideal material as a suitable biomaterial for in vivo use. Furthermore, DSMs are rapidly degraded in vivo by plasma amylase into oligosaccharides, maltose and, eventually, glucose. The degradation rate depends on the endogenous amylase activity and degree of cross-linking of the spheres [5].

At present unmodified DSMs have a documented haemostatic effect in low flow bleeds [6,7]. However, in larger bleeds DSMs do not appear to have enough haemostatic capacity to stop the bleeding properly [8–11]. In previous experimental studies we have combined DSMs with recombinant factor VIIa and found evidence of an increased haemostatic effect [11], but still not sufficient to stem the larger bleeds [12]. Due to the insufficient effect of unmodified DSMs we conducted a small pilot study to evaluate whether DSMs actually trigger coagulation and/or platelets in vitro. No evidence of either enhanced coagulation or platelet activation by DSMs was found (data not shown).

DSMs are obtained as a biologically inert powder that contains no human or animal proteins, significantly reducing the risk of transmission of disease and development of antibodies. The powder is easy to handle and can be stored with a long shelf life. Therefore, DSMs have the potential to be a very attractive haemostatic agent, if they only performed better in larger bleeds. Would the haemostatic capacity of DSMs be improved if they were able to activate platelets and/or trigger coagulation? Is it possible to chemically modify the starch spheres to introduce such abilities?

The aim of this study was to chemically modify DSMs and thoroughly examine the possible effect these modifications have on the haemostatic capacity. The examination involved initial in vitro testing to study modification-dependent activation of coagulation and triggering of platelet binding. To further evaluate the haemostatic efficiency of those DSMs that did indeed demonstrate platelet and/or coagulation activation in vitro we used an experimental bleeding model in the rat in which modified DSMs were compared with unmodified DSMs. The chemical modification was aimed at generating DSMs with different basic surface properties, such as a negative/positive charge and hydrophilic/hydrophobic moieties. These basic surface properties have previously proved to greatly influence protein adsorption mechanisms in the initial response during blood–material contact [13]. Protein adsorption is essential in the blood-borne haemostatic response to foreign materials. Known blood–material interactions are, for example, platelet adherence to positively charged surfaces [14] and the ability of negatively charged surfaces to induce coagulation initiated via autoactivation of coagulation factor XII [13].

## 2. Materials and methods

### 2.1. Materials

Hydrolyzed starch was obtained from Lyckeby Starch AB (Kristianstad, Sweden). All other chemicals and reagents were of analytical grade and obtained from Merck unless otherwise noted.

### 2.2. Preparation of degradable starch microspheres

The DSMs used in the experiments were obtained from Magle AB (Kristianstad, Sweden). DSMs were prepared by emulsion cross-linking of hydrolysed starch with epichlorohydrin in toluene. The DSMs were subsequently washed repeatedly with ethanol followed by distilled water and finally successively dehydrated with increasing concentrations of ethanol and dried overnight at 60 °C.

### 2.3. Modification of DSMs

The same batch of DSMs was used for all modifications except No. 9, which had a higher level of cross-linking.

#### 2.3.1. Surface modifications

See Fig. 1.

#### 2.3.2. Octenylsuccinate (negative and hydrophobic)

Eighty grams of DSMs were suspended in purified water, *N*-octenylsuccinic anhydride (Pentagon, Widnes, UK) was added to 0.08 g $g^{-1}$ dry DSMs and the reaction was allowed to continue for 3 h. The pH was maintained above 7.4 by addition of 0.75 M NaOH. The resulting material was washed eight times with 2000 ml of purified water, thereafter dehydrated with increasing concentrations of ethanol and finally dried overnight at 60 °C [15].

#### 2.3.3. Carboximethylation (negative)

Fifty grams of DSMs were suspended in purified water, chloroacetic acid was added to 0.1 g $g^{-1}$ dry DSMs and the reaction

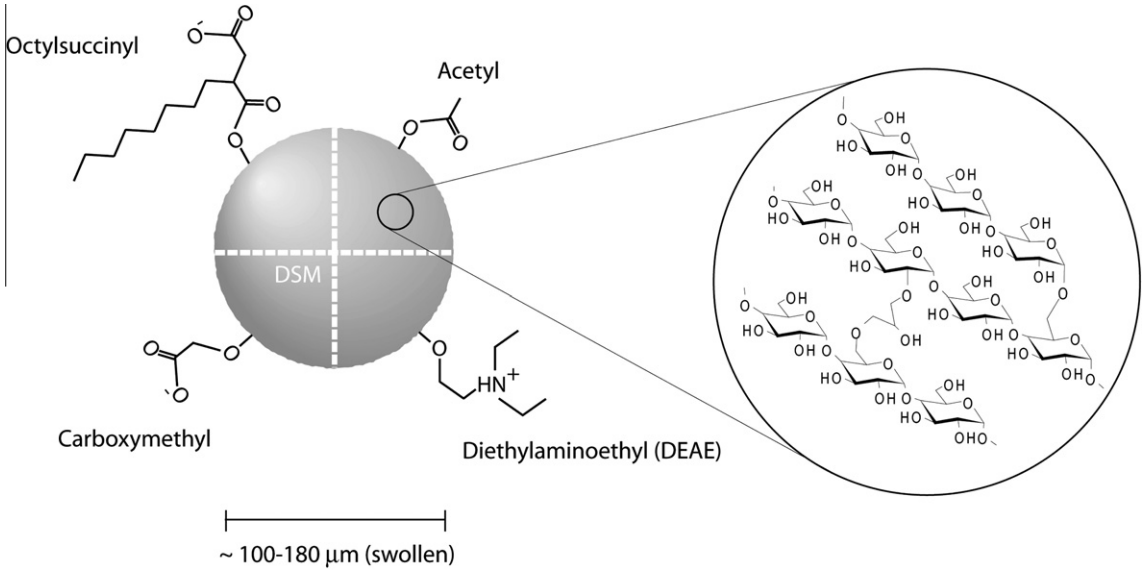


**Fig. 1.** Schematic drawing of a degradable microsphere (DSM) and the chemical modifications performed in this study.

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