Radiopaque Microspheres containing Acrolein for Protein Attachment to improve Cell Adhesion and Blood Coagulation

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Introduction

Polymeric microspheres find use in minimally invasive treatments. These spheres are often used as fillers and bulking agents to treat acne scars and stress urinary incontinence respectively. Also in interventional radiology polymeric microspheres find widespread use as embolic agents, e.g. for precise occlusion of a tumor- or uterine fibroid-feeding artery.

A frequently encountered problem of currently used microspheres is problematical detection and migration away from the injection site. Here we present the synthesis of non-toxic, biocompatible, stable polymeric microspheres that contain acrolein (2-propenal), and the iodine-containing monomer 2-[4-iodobenzoyloxy] ethyl methacrylate (4-IEMA). These microspheres feature *intrinsic radiopacity*, due to incorporation of covalently bound iodine. The surface-aldehyde groups, which are introduced by the addition of acrolein, were used to covalently couple protein to the surface. Microspheres decorated with collagen I were shown to support the adhesion and growth of cells on their surface, which leads to anchoring of the spheres in the surrounding tissue. Also the immobilized collagen I induced blood-coagulation, enhancing the embolic effect of these spheres. The here presented microspheres combine *intrinsic radiopacity* with the possibility of surface bio-functionalization, and present a new alternative for use as filler, bulking agent and embolic particles.

Methods

Microspheres were produced by suspension polymerization[1,2]. The microspheres are based on a ter-polymer consisting of the monomers methyl-methacrylate (MMA), iodine containing methacrylate 4-IEMA and acrolein (Figure 1).

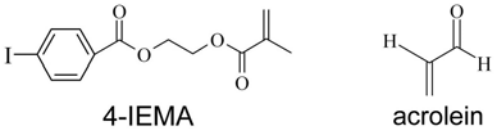

Figure 1. Chemical structure of iodine containing monomer, 2-[4-iodobenzoyloxy] ethyl methacrylate (4-IEMA), and acrolein (2-propenal).

Coupling of collagen-I or FITC-labeled collagen-I, to the aldehyde groups was performed at pH 9.0 in carbonate buffer, followed by reduction with $NaCNBH_3$ of the formed Schiff-base, resulting in a stable amide bond.[3] Presence of collagen-I was confirmed by fluorescence microscopy and XPS. Furthermore the adhesion and growth of mouse fibroblasts on the spheres and the induction of blood-coagulation by the collagen modified microspheres were tested.

Results

Smooth microspheres with defined composition were synthesized and analyzed by scanning electron microscopy (SEM), XPS, X-ray fluoroscopy and dinitrophenylhydrazine coupling to confirm radiopacity and the presence of available aldehyde groups on the surface. FITC-labeled collagen I was coupled to the microspheres to demonstrate successful covalent coupling to the surface of the microspheres (Figure 2).

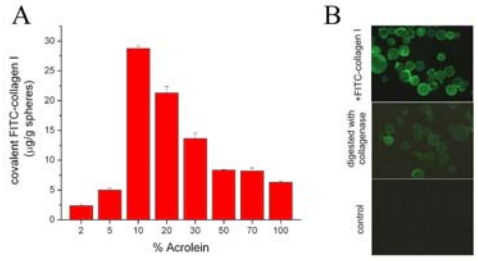

Figure 2. A) Coupling of FITC-collagen to microspheres with varying acrolein contents. B) Fluorescent micrographs of microspheres with FITC-collagen I (top) that can be partly removed by incubation with collagenase (middle).

Mouse fibroblasts attached and proliferated at normal speed only on the collagen-modified spheres (Figure 3A). The surface coupled collagen firmly attached the radiopaque microspheres to a monolayer of mouse fibroblasts. Onset of blood coagulation was strongly accelerated by the collagen-microspheres. These spheres incorporated readily in a fibrin clot when incubated in human platelet-rich-plasma (Figure 3B).

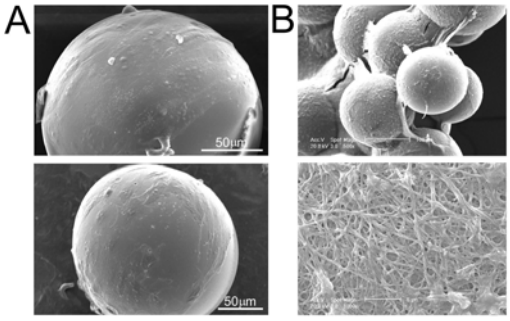

Figure 3. SEM micrographs of collagen-microspheres: A) incubated 48 hours on a monolayer of mouse fibroblasts B) incubated for 8 minutes with human platelet-rich-plasma.

Discusssion

The polymeric microspheres presented here combine several features that make their use safer and more precise. The 4-IEMA monomer provides X-ray visibility, guiding the surgeon during the intervention and preventing misplacement. The acrolein enables the attachment of bioactive compounds that may result in improved anchoring of the microspheres in the surrounding soft tissue or the embolized blood vessel.

References

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3. Slomkowski S. Prog. Polym Sci 1998;23:815-878.