Versatile Polymer Microspheres for Injection Therapy: Aspects of X-Ray Visibility and Biofunctionalization

Ketie Saralidze, Menno L.W. Knetsch, Leo H. Koole
Department of Biomedical Engineering/Biomaterials Science, Faculty Health and Life Sciences,
Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands

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 2-propenal (acrolein), and the iodine-containing monomer 2-[4-iodobenzoyloxy] ethyl methacrylate (4-IEMA). These microspheres feature *intrinsic radiopacity (X-ray visibility)*, due to incorporation of covalently bound iodine. Surface-aldehyde groups introduced by the addition of acrolein can be 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. 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 propenal (Figure 1). A series of microspheres with varying 2-propenal content were synthesized.

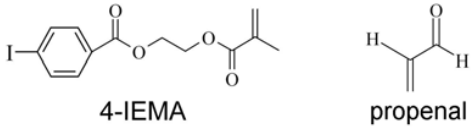

Figure 1. Chemical structure 4-IEMA (2-[4-iodobenzoyloxy] ethyl methacrylate) and propenal.

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 were investigated. Control or collagen-coated microspheres were incubated on a layer cells for 48 hours. The cell-layers with attached microspheres were put on a 45 degree angle and the retention of the microspheres on the layer was tested by rinsing with sterile PBS at 20 ml/min.

Results

Smooth microspheres with the desired compositions were synthesized. Radiopacity was verified by X-ray fluoroscopy, with the microspheres demonstrating higher radiopacity than isolated porcine bone tissue. Dinitrophenylhydrazine coupling was used to demonstrate and quantify the available aldehyde groups on the surface. XPS demonstrated the presence of collagen I on the surface of the spheres. Furthermore, FITC-labeled collagen I was coupled to the microspheres to confirm successful covalent coupling to the surface of the microspheres (Figure 2). There was an optimum for collagen coupling at 10-20 % propenal.

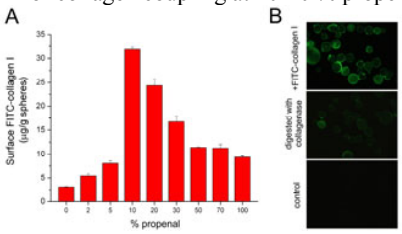

Figure 2. A) Coupling of FITC-collagen to microspheres with varying acrolein contents. B) Fluorescent micrographs of microspheres with FITC-collagen I (top) that can be partly removed by incubation with collagenase (middle).

Mouse fibroblasts attached and proliferated at normal speed only on the collagen-modified spheres. The surface coupled collagen firmly attached the radiopaque microspheres to a monolayer of mouse fibroblasts grown on a glass coverslip (Figure 3).

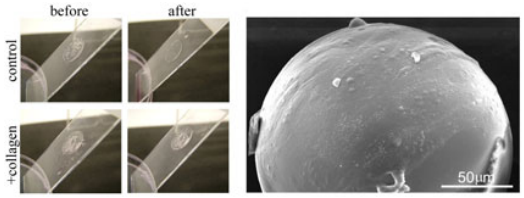

Figure 3. (A) Cell attachment to control or collagen-coupled microspheres. Only collagen-spheres remain attached to a cell layer after rinsing. (B) SEM micrograph of collagen-microspheres incubated on a monolayer of fibroblasts.

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 2-propenal enables the attachment of bioactive compounds that may result in improved anchoring of the microspheres in the surrounding soft tissue, which may prevent particle migration away from the site of injection.

References

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2. Sivakumar M. Int J Appl Polym Sci 2002;83:3045-3054.
3. Slomkowski S. Prog. Polym Sci 1998;23:815-878.

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