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Surface modification of copolymerized films from three-armed biodegradable macromers – An analytical platform for modified tissue engineering scaffolds

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ABSTRACT

The concept of macromers allows for a broad adjustment of biomaterial properties by macromer chemistry or copolymerization. Copolymerization strategies can also be used to introduce reactive sites for subsequent surface modification. Control over surface features enables adjustment of cellular reactions with regard to site and object of implantation. We designed macromer-derived polymer films which function as non-implantable analytical substrates for the investigation of surface properties of equally composed scaffolds for bone tissue engineering. To this end, a toolbox of nine different biodegradable, three-armed macromers was thermally cross-copolymerized with poly(ethylene glycol)-methacrylate (PEG-MA) to films. Subsequent activation of PEG-hydroxyl groups with succinic anhydride and *N*-hydroxysuccinimid allowed for covalent surface modification. We quantified the capacity to immobilize analytes of low (amino-functionalized fluorescent dye, Fcad, and RGD-peptides) and high (alkaline phosphatase, ALP) molecular weight. Fcad grafting level was controlled by macromer chemistry, content and molecular weight of PEG-MA, but also the solvent used for film synthesis. Fcad molar amount per surface area was twentyfive times higher on high-swelling compared to low-swelling films, but differences became smaller when large ALP (appr. 2:1) were employed. Similarly, small differences were observed on RGD peptide functionalized films that were investigated by cell adhesion studies.

Presentation of PEG-derivatives on surfaces was visualized by atomic force microscopy (AFM) which unraveled composition-dependent domain formation influencing fluorescent dye immobilization. Surface wetting characteristics were investigated via static water contact angle. We conclude that macromer ethoxylation and lactic acid content determined film swelling, PEG domain formation and eventually efficiency of surface decoration.

Statement of Significance

Surfaces of implantable biomaterials are the site of interaction with a host tissue. Accordingly, modifications in the composition of the surface will determine cellular response towards the material which is crucial for the success of innovations and control of tissue regeneration. We employed a macromer approach which is most flexible for the design of biomaterials with a broad spectrum of physicochemical characteristics. For ideal analytical accessibility of the material platform, we cross-copolymerized films on solid supports. Films allowed for the covalent immobilization of fluorescent labels, peptides and enzymes and thorough analytical characterization revealed that macromer hydrophilicity is the most relevant design parameter for surface analyte presentation in these materials. All analytical results were combined in a model describing PEG linker domain formation and ligand presentation.

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1. Introduction

Macromers are oligomeric, cross-polymerizable building blocks which typically allow for copolymerization with other components that contain compatible functional groups [1]. They give opportunity for the design of adjustable biomaterials [2] and are frequently employed in bone [3], cartilage [4] and skin [5] regeneration purposes. In a recent paper, we introduced a macromer platform that consisted of three-armed biodegradable macromers [6]. Variations in the degree of ethoxylation, lactic acid content and size of the macromers allowed for an adaptation of hydrophilicity and swelling of the materials [6]. Similar to other materials [7] they can be processed into macroporous tissue engineering scaffolds [6] by thermally initiated cross polymerization due to their terminal methacrylation. Copolymerization with other molecules, such as monomethacrylated poly(ethylene glycols) (PEG-MA) enables the adjustment of material compositions and properties [8] and widely adaptable surface reactivity. The incorporation of PEG-moieties is a common strategy to limit unspecific protein adsorption to lipophilic surfaces [9–11] which has been used to modify surface characteristics and biological interactions [12]. Effectiveness of these approaches depend on surface design, such as density [13] and molecular weight [14] of PEG-anchors as well as cell type related sensitivity towards these parameters [13,15,16].

The material surface is the site of immediate interaction with the biological environment and can therefore determine cell adhesion and function and in consequence tissue regeneration [17]. However, it is challenging to assess surface characteristics of porous three-dimensional scaffolds. In this study we therefore processed the macromer-based materials into films which were covalently grafted to methacrylated glass disks to provide stable, carrier-adherent film formulations independent of their composition. They are composed of the same building blocks and processed under similar conditions as our scaffolds [6]. Hence their characterization provides useful data to rationalize the material development and surface design of these scaffolds.

First we studied the influence of chemical surface modifications obtained by changes of polymerization conditions and material feed. General aspects of surface modification, such as solvent effects [18], impact of the interfacial conditions [19], PEG-amounts [20,21] and backfilling with shorter, endcapped PEG-derivatives [15] on the availability of PEGs for covalent surface modification were explored. PEG-MA was copolymerized in different ratios with the macromers to provide functional groups in adaptable surface density for efficient surface functionalization. Surface properties, such as availability of reactive sites were investigated by immobilization of a fluorescent probe using a high resolution fluorescence plate reader. Film topography and surface presence of PEG-moieties was evaluated by AFM as an effect of film polymerization conditions. Hydrophilicity and swelling behavior were evaluated by studies on the static water contact angle.

Secondly, we immobilized functional biomolecules to the film surface in order to generate specific cell interactions. Changes in hydrophilicity [22], surface topography [23], charge [24], and pattern of molecular structures [25] are known to strongly influence cellular responses towards biomaterials. We grafted calf intestinal alkaline phosphatase (ALP) to the films. The enzyme is an active compound with relevance for calcified tissues and consequently for bone regeneration [26]. ALP was covalently immobilized by carbodiimide chemistry and, in a second approach, via the biotin-streptavidin concept. While the latter opens a platform for immobilization of other biotinylated effector molecules to the surfaces, a comparison of both methods was done to determine changes in activity of ALP by direct covalent immobilization.

RGD peptides are a frequently used means to improve cell adhesion to non-adherent surfaces [27]. Knowledge about design parameters for such peptides [28] and conformation-related specificity is available [29]. Thus, adhesion peptides can be used for proof of concept studies for biofunctionalization. Cell adhesion to these polymer films was studied with osteoblast-like SaOS-2 cells and human adipose tissue derived stromal cells (hASC). Improved cell adhesion via grafted RGD peptide is considered as a proof for functional surface modification of the upmost film layer.

2. Materials and methods

2.1. Fabrication of polymer films and generation of activated esters

Macromer synthesis has been described elsewhere [6]. Chemical structure of three-armed macromers is displayed in Fig. 1. In brief, macromers were composed of trimethylolpropane as core molecule. This core was provided at different ethoxylation degrees (a,b,c) and thus molecular weights (described as T134, T170, T450) from Sigma-Aldrich. Core hydroxyl groups were esterified with lactic acid side chains of variable block length (m) – LAx. X describes the number of lactic acids per arm of the macromer. Lactic acid chains were capped with methacrylate groups allowing for thermally induced radical polymerization reactions.

Film polymerization took place in a thin layer between common glass object slides and circular glass disks of 3 cm^2 area as shown in Fig. 2. The circular glass disks functioned as covalent carrier of the polymer films and were silanized with 3-(trimethoxysilyl)propyl methacrylate following an established procedure [30]. The object slides were silanized with 2-(carbomethoxy) ethylmethyldimethoxysilane if not described otherwise. Exceptional untreated glass cover slips or a hexadecyl trimethoxypropylsilan decoration were employed in order to study the effect of silanization. Films consisted of macromers and, when specified, PEG-MA of different molecular weight ("reactive anchor", 500 g/mol, 1000 g/mol) and PEG-methyl ether methacrylate (mPEG-MA) ("unreactive filler", 475 g/mol). The polymerization took place for 60 min at 50 °C and was initiated using 2% (per mol methacrylate of the macromers) of each benzoyl peroxide and 2-(4-dimethylami nophenyl)-ethanol. Each film was polymerized from 5 µl of reaction batch which was homogenized in mixtures of different solvents including ethanol (EtOH), tetrahydrofuran(THF), dioxan, dimethyl formamide (DMF), a mixture of acetone and methylene chloride (A + M, 5 + 3 volume parts), toluene, and hexafluoroisopropanol (HFIP). As some of these are non-solvents for benzoyl peroxide, the initiator was always dissolved in a low amount of THF representing 5% of the reaction batch volume.

Hydroxyl groups of the reactive anchors were derivatized to activated esters for conjugation of analytes bearing primary amines on the basis of previously described method [30]. Carboxylated surfaces were generated by incubating the films in a 0.1 M solution of succinic anhydride in THF over night at room temperature. After subsequent washing in THF the activated esters were formed by incubation of these films in 0.1 M *N,N′*-dicyclohexylcarbodiimide (DCC) and 0.05 M *N*-hydroxysuccinimide (NHS) for 5.5 h in THF. Films were washed with methylene chloride. We refer to carboxylated films as blank and to the activated esters as verum.

2.2. Film hydrophilicity by static water contact angle

Surface hydrophilicity of polymer films was studied via static water contact angle measurements using a Krüss G10 goniometer. The contact angles of 8 µl droplets of Milli-Q water on dry films were determined 1 and 10 min after deposition.

In order to determine the behavior of fully equilibrated films, samples were stored under high humidity before analysis. A chamber was filled with a stirred and oversaturated solution of potassium nitrate which created an atmosphere of nearly 90%

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