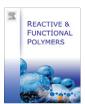
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# Cell adhesion on surface patterns generated by the photocrosslinking of hyperbranched polyesters with a trisdiazonium salt

Miguel Lomba<sup>a</sup>, Luis Oriol<sup>a,\*</sup>, Carlos Sánchez-Somolinos<sup>b,\*</sup>, Valeria Grazú<sup>c</sup>, María Moros<sup>c</sup>, José Luis Serrano<sup>c</sup>, Jesús Martínez De la Fuente<sup>c</sup>

<sup>a</sup> Instituto de Ciencia de Materiales de Aragón (ICMA), CSIC – Universidad de Zaragoza, Departamento de Química Orgánica, Facultad de Ciencias, C/Pedro Cerbuna 12, 50009 Zaragoza, Spain

<sup>b</sup> Instituto de Ciencia de Materiales de Aragón (ICMA), CSIC – Universidad de Zaragoza, Departamento de Física de la Materia Condensada, Facultad de Ciencias, C./Pedro Cerbuna 12, 50009 Zaragoza, Spain

<sup>c</sup> Instituto Universitario de Nanociencia de Aragón, Campus Río Ebro (Edificio I+D), 50018 Zaragoza, Spain

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### 1. Introduction

#### ABSTRACT

Aliphatic hyperbranched polyesters of different generation, having different nucleophilic groups (such as hydroxyl or sulfonate) in the periphery, have been photocrosslinked by using a trifunctional low molecular weight diazonium salt. The photocrosslinking process, that does not require photoinitiator, allows to prepare polymeric materials that have shown not to cause cytotoxicity when used as substrate for cell growth. Furthermore, cell adhesion studies carried out in polymeric micropatterns generated in glass by direct laser writing, show that cells preferentially proliferate on the structure demonstrating cell affinity for the material.

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Photopolymerization of multifunctional monomers represents a widely used strategy for the fabrication of biomaterials. Lightinduced polymerization allows the generation of materials with tunable properties [1] whose microstructure can also be engineered by the application of light-structuring techniques [2,3]. Additionally, photopolymerization may be carried out at physiological conditions and allow to encapsulate cells into the generated material [4,5], which represents an advantage in tissue engineering applications.

As macromolecular precursors for the fabrication of biomaterials by photocrosslinking, either natural or synthetic functionalized polymers have been widely investigated. Natural polymers can intrinsically show bioactivity [6,7], while synthetic polymers allow material properties modulation by an appropriated design of the chemical structure of the polymer [8,9].

Either with natural or synthetic polymers, the preparation of biomaterials by photocrosslinking has been mainly carried out by a radical chain photopolymerization of polymers functionalized

with acrylate groups. However, when biodegradable materials are sought, this reaction generates acrylic chains that cannot be degraded and they could be difficult to eliminate from the body [10]. Alternative photoinduced reactions, having a step-growth mechanism, are being explored to avoid this drawback. With this aim, macromonomers functionalized with alkene or alkyne groups have been photocrosslinked using a multifunctional thiolated molecule by thiol-ene and thiol-yne photochemistry [11-13].

Other photoreactions can also be applied for the photocrosslinking of polymers using a step growth mechanism. In this way, diazonium salt photoinduced decomposition has been demonstrated as a feasible strategy for the crosslinking of polymers having nucleophilic groups such as sulfonate [14-16], carboxylate [17,18] and hydroxyl groups [19,20]. To date, this reaction has been mainly applied for the fabrication of negative presensitized plates [15,20] and electrooptical applications [17,18,21,22] by using a polymeric diazonium salt, although some biological applications such as enzyme encapsulation [23] and DNA microarrays generation [24,25] have also been described. Recently, we have demonstrated the photocrosslinking of HA (naturally having nucleophilic hydroxyl and carboxylate groups) without further chemical modification by using a low molecular weight trisdiazonium salt for the preparation of biomaterials. The use of this low molecular

<sup>\*</sup> Corresponding authors. Tel.: +34 976762276; fax: +34 976239302 (L. Oriol), tel.: +34 976762742; fax: +34 976761229 (C. Sánchez-Somolinos).

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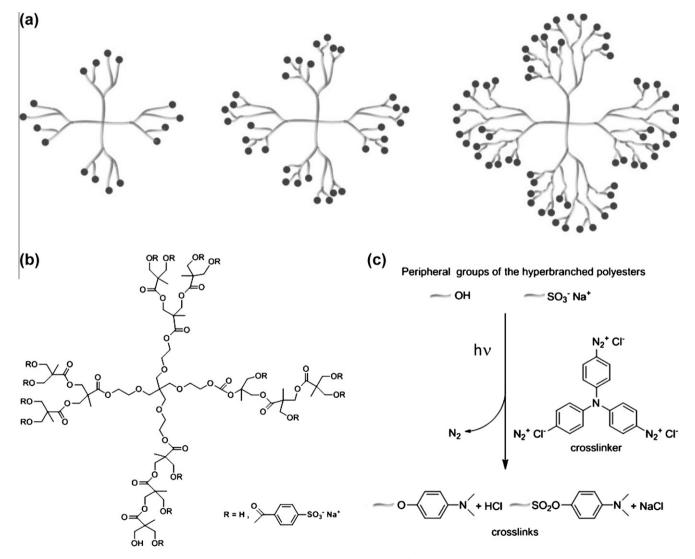
weight crosslinker (instead of tipically used polymeric diazonium salts in combination with a degradable polymer) shows advantages since degradation of this can lead to small molecules easier to excrete by the body. Additionally, this photoinduced reaction takes places in water and without need of photoinitiator, which represents an advantage for biomedical applications since the photoinitiator (or its generated byproducts) could produce an inflammatory response [26].

In this paper, the photoinduced decomposition of diazonium salt is applied to the photocrosslinking of different aliphatic polyesters (Scheme 1), that are known to be biocompatible and biodegradable materials widely used in biomedical applications [27,28]. The selected polyesters have a hyperbranched polymeric architecture and, consequently, they are highly functionalized with ending groups which facilitates the formation of a polymeric network. Thus, the peripheral hydroxyl groups allow the direct photocrosslinking of the polymer by photoinduced reaction with the trifunctional low molecular weight diazonium salt previously studied [29,30]. Furthermore, the hyperbranched polymers can also be modified with other nucleophilic groups such as sulfonate that can photoreact with the diazonium crosslinker to obtain materials with tunable properties. With this aim, the photocrosslinking of aliphatic hyperbranched polyesters of different generations, bearing hydroxyl groups or functionalized with sulfonate groups in the periphery, using a trisdiazonium salt as a crosslinker has been studied to obtain biomaterials. The biocompatibility of the resulting materials was studied with two different cell lines, HeLa tumoral cells and COS-7 fibroblasts as a healthy cell line. Two dimensional surface patterns were also generated by direct laser writing (DLW) to study cell interaction with the polymeric material.

#### 2. Experimental

## 2.1. Materials

The hyperbranched aliphatic polyesters derived from 2,2di(hydroxymethyl)propionic acid with 16, 32 and 64 hydroxyl groups (coded as HB16OH, HB32OH and HB64OH respectively), having an estimated molecular weight of 1750, 3600 and 7300, respectively, according to the supplier) were purchased from Aldrich. Potassium 4-hydroxycarbonilbenzenesulfonate (Aldrich)



**Scheme 1.** (a) Schematic representation of the hyperbranched polyesters of second, third and fourth generation (bearing 16, 32 and 64 peripheral reactive groups respectively): polyesters having ending hydroxyl groups were coded as HB160H, HB320H and HB640H respectively; sulfonated polyesters were coded as HB16S, HB32S and HB64S respectively. (b) Average chemical structure of hyperbranched polyesters HB160H and HB16S of second generation (as an example). (c) Photoinduced crosslinking process of the trisdiazonium salt crosslinker and peripheral reactive groups.

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