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# Photocrosslinkable diazoresin/pectin films – Synthesis and application as cell culture supports

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ABSTRACT

Multilayer polymeric films composed of diphenylamine-4-diazoresin (DR), a synthetic polycation, and pectin (Pec), a polyanionic natural polysaccharide, were obtained using a layer-by-layer method (LbL). The films was photocrosslinked by the irradiation with 350 nm light which resulted in their stabilization against different solvents. The nanostructure of the films was studied using AFM microscopy. Irradiation of the films resulted also in the change of the zeta potential of both DR and Pec outermost layer to more negative values and in the increase of the film hydrophobicity as found from the contact angle measurements. The DR/Pec films have been shown to support cell growth and osteogenic potential of cell culture supports in tissue engineering, as found from the experiments with human bone marrow stromal cells (hMSCs).



## 1. Introduction

There is a growing interest in the development of the functional materials which could be useful for various nanotechnological and biomedical applications. The search for new materials is accompanied by the studies directed towards the development of new methods of their preparation. One of the methods which is getting considerable attention is so called layer-by-layer (LbL) technique. This technique is useful for the construction of multilayer structures through electrostatic [1], hydrogen bonding [2,3], coordination [4,5], and charge–transfer [6,7] interactions between the molecules (macromolecules) forming subsequent layers. The layers are alternatingly deposited on a charged substrate (support). The attractiveness of LbL method is related to the fact that it allows the control over the film fabrication on the nanometer scale in a relatively easy and environmentally friendly (using aqueous solutions) way [1,8]. However, formation of very stable and linearly growing films is achieved mainly for strong polyelectrolytes with relatively flexible backbones [9]. Those conditions are usually fulfilled by synthetic polymers. Natural polymers, like polysaccharides, have been found rather difficult to form such stable LbL films in a regular manner. Recently, we have demonstrated that chitosan-based multilayered structures can be obtained from modified chitosan derivatives [10]. Here, we present a modified approach to the formation of stable polysaccharide-containing multilayered films. The films were formed using polycationic diphenylamine-4-diazoresin (DR), a synthetic polymer, and pectin (Pec), a natural anionic polysaccharide (see Scheme 1).

DR is synthesized in a polycondensation reaction of formaldehyde and diphenylamine-4-diazonium salt [11]. It has found practical applications, e.g. as a photosensitive material in lithographic printing [11]. DR absorbs light

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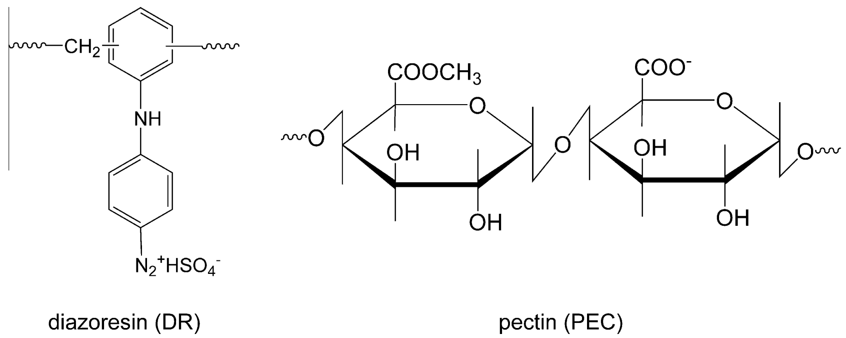


**Scheme 1.** Structures of diazoresin and pectin.

with the wavelength of about 380 nm due to $\pi$–$\pi^*$ transition in the diazonium group [12]. Since one of the atoms in the diazonium group has a lone electron pair (the other one carries a positive charge), DR is able to form LbL films based on hydrogen bonds with strong hydrogen donors such as the phenolic hydroxy group [13–15]. However, DR may be used for the fabrication of much more stable multilayer films than those based on hydrogen bonds. The diazonium group of DR is a good leaving group and it is cleaved forming phenyl cation under UV irradiation with a 380 nm light source. Thus, upon irradiation it may be substituted by nucleophilic groups present in polyanions, such as carboxylic, phosphate or sulfonate groups. Since this is accompanied with the formation of a covalent bond, the polyanionic layer(s) in contact with the DR layer may be photochemically crosslinked and consequently strengthened [16,17]. This photoreaction taking place between the layers plays a key role in achieving a stable film structure, which is otherwise sensitive to etching with DMF or electrolyte aqueous solutions [18]. This method was previously used to obtain stable films from DR and synthetic anionic polymers e.g. poly(sodium styrene sulfonate) (PSSS) [16,19], poly(acrylic acid) [20,21], poly(aniline-*co*-*o*-anthranilic acid), and carboxylic polyaniline [22]. Films were fabricated from DR and low-molecular weight compounds, both organic, e.g. 5,10,15,20-tetrakis(4-hydroxyphenyl) porphyrin [13], brilliant yellow [23], and inorganic, e.g. CdS [24] and $Fe_3O_4$ [25]. DR was used to prepare LbL films on flat surfaces, microspheres [26], nanospheres [25,27] as well as more complicated systems, e.g. a sandwich-type structures composed of latex particles containing carboxylic groups deposited on quartz [18] or single-wall nanotubes (SWNT) grafted with PSSS [19].

Pectin, the anionic polymer used in the present studies, is a polysaccharide functionalized with carboxylic groups (Scheme 1). It is an ubiquitous component of the cell walls of land plants and green algae [28]. Its main role is to provide mechanical strength for the cell walls. It was also found to play an important role in various processes in cells, e.g. binding of water, morphological development and fruit ripening [29]. Moreover, it displays potent physiological activity in humans. For example it shows anticancer properties [30–33] and has been applied to prepare films for the attachment, proliferation, and differentiation of mesenchymal stem cells (MSCs) [34] or differentiation of osteoblasts on pectin-coated titanium [35], scaffolds for bone tissue engineering [36], films and scaffolds for controlled release of pentoxifylline [37], and wound healing dressings [38]. An interesting example of pectin application is within the microarray technology [39]. Therefore, it is of practical importance to design new methods of the fabrication of stable pectin films, especially because the studies on the application of pectin for LbL film formation are still scarce.

The potential applications in biotechnology and medicine of the polyelectrolyte multilayer (PEM) films fabricated by the LbL deposition method are more and more intensively studied. The example applications include the engineering of liver [40], bone and cartilage [41] and vascular [42] tissues. The applicability of the PEMs in these areas is largely due to the ease of their preparation while their properties (physicochemical, mechanical, topography, stability, etc.) can be fine-tuned using a variety of methods (pH change during film deposition [43,44], covalent crosslinking [45]). By controlling the properties of the PEM films both the initial cell adhesion [46,47] and differentiation [48,49] can be influenced. Moreover, it is possible to obtain detachable PEMs which facilitates manipulation of the cell cultures [50]. Among polymers the polysaccharides are of particular interest as the main film components due to their biocompatibility, availability and ease of functionalization [51].

This paper describes the studies on the alternatingly deposited films of DR and Pec stabilized by the photochemical covalent bond formation between DR and Pec films. The formation of diazoresin–pectin (DR/Pec) polymeric films, whose zeta potential can be changed from positive to negative (or to more negative) simply by photoirradiation was described with particular regard to their use as cell culture supports.

To the best of our knowledge up till now pectin is the first polysaccharide immobilized using this method, DNA being the only biopolymer immobilized using DR [52].

## 2. Materials and methods

### 2.1. Reagents

Pectin (Pec) (Sigma Aldrich), sodium chloride (POCH Gliwice), degree of esterification 70.2%, 4-diazodiphenylamine sulfate (Sigma Aldrich), paraformaldehyde (POCH