POLY(L-DOPA)-MODIFICATION OF CURDLAN HYDROGEL FOR SAFE AND EFFECTIVE DRUG BINDING AND RELEASE

Anna Michalicha[1]*, Agata Roguska[2], Agata Przekora[3], Barbara Budzyńska[4], Anna Belcarz[1]

[1] Chair and Department of Biochemistry and Biotechnology, Medical University of Lublin, Chodzki 1, 20-093 Lublin, Poland
[2] Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland
[3] Independent Unit of Tissue Engineering and Regenerative Medicine, Chair of Biomedical Sciences, Medical University of Lublin, Chodzki 1, 20-093 Lublin, Poland
[4] Independent Laboratory of Behavioral Studies, Medical University of Lublin, Chodzki 4a, 20-093 Lublin, Poland
*e-mail: anna.michalicha@gmail.com

[Engineering of Biomaterials 163 (2021) 46]

Introduction
Observation of clams adhesion to many types of surfaces contributed to significant progress in the surface modification area. It has been shown that the main agent responsible for this extremely strong adhesion are dopamine and proteins lysine-rich. PDA (polydopamine) coating has the ability to deposit on many types of substrates and has ability to increase their functionality [1]. Covering the surface of biopolymers with a polycatecholamine layer significantly improves their functionality [2,3]. However, L-DOPA being a dopamine precursor in the biochemical synthesis pathway (with free carboxyl group in its structure) also forms highly adhesive coatings but it is very rarely used for surface functionalization [4]. Therefore, we decided to make a wide-ranging characterization of matrices obtained using L-DOPA as a curdlan modifier.

Materials and Methods
Functionalization of curdlan hydrogel was performed by L-DOPA polymerization from 2 or 4 mg/ml solution for 24 h at 25°C. L-DOPA monomer was added to curdlan matrix before its gelation (sample 2-LD-BG and 4-LD-BG) or after (2-LD-AG). The matrices were characterized using the FTIR and XPS methods and the soaking capacity was evaluated in defined time points. The biological safety of the modified hydrogels was verified after contact with blood, fibroblasts cell lines and in zebrafish model, in relation to their possible application as wound dressings. Secondly, modified hydrogels were coupled with gentamicin and the amount of bound drug was estimated spectrophotometrically, based on gentamicin derivatization by phthaldialdehyde. Drug release from each variant of modified hydrogels was evaluated by incubation in PBS pH 7.4 at 37°C, with daily exchange of buffer and replacement it by the new portion. Antibacterial activity of functionalized curdlan matrices was evaluated by indirect method, in extracts collected in similar way as in drug release test (PBS was replaced by Mueller-Hinton Broth). The extracts were inoculated by three reference bacterial strains and allowed at 37°C for 24 h. Then the bacterial growth was estimated in daily collected extracts as optical density at 660 nm in Synergy H4 plate reader. The samples of hydrogel after incubated with MH Broth medium were used to indirect bacterial adhesion test. Experiments were performed in triplicate.

Results and Discussion
Blackish colour of modified hydrogels was the indicator of the successful deposition of the functional poly(L-DOPA) coating. XPS also confirmed the presence of poly(L-DOPA) coating on the hydrogels. The increased soaking capacity of poly(L-DOPA)-modified curdlan hydrogels manufactured by AG method seemed to be related to the specific method of curdlan modification (where L-DOPA monomer was introduced into curdlan matrix after curdlan gelling process). Poly(L-DOPA)-modified curdlan hydrogels were stable in blood and plasma as well as in the wide range pH buffers (excluding alkaline media). Poly-levodopa coatings on curdlan were nontoxic in zebrafish model and did not negatively change the crucial blood parameters. Modified hydrogels were nontoxic for primary fibroblasts cultures and inhibited their adhesion for some modification variants. All modified hydrogels showed ability to bind gentamicin and exhibited high antibacterial properties.

Conclusions
We assumed that coating formed by L-DOPA may impart new beneficial features of the curdlan hydrogels and that kind of modification is safe and promising for future curdlan medical applications as wound dressings. Use of L-DOPA monomer for curdlan modification process allows for a more efficient drug binding in comparison with dopamine for that purpose (Michalicha et al., 2021).

Acknowledgments
This study was financed from DS6/2021 grant (Medical University of Lublin, Poland).

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