NOVEL AGAROSE/β-1,3-D-GLUCAN FOAM AS PROMISING BIOMATERIAL FOR SKIN REGENERATION APPLICATIONS

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Introduction

Skin wounds (especially chronic) often do not heal according to the expectations and provided treatment. In this case, it is necessary to use bioactive wound dressings. Currently, high hopes are connected with biomaterials based on β-glucans, which exhibit multifunctional properties, including moisturizing properties, antioxidant activity, anti-inflammatory and regenerative effects. Dressings made of β-glucans appear to be a suitable alternative in tissue engineering since they are very stable, flexible and resistant to proteases [1]. Recent reports indicated the possibility of using bacterial β-1,3-D-glucan (curdlan) in the production of biomaterials [2]. It is known that curdlan aqueous suspension create high-set thermal nonreversible gel at temperature > 80°C [3]. It can be also combined with other biocompatible polysaccharides (e.g. agarose, chitosan) to improve its biocompatibility, absorption ability, and ensure adequate gaseous exchanges. The main purpose of this work was to create a new agarose/curdlan matrix-based foam for regenerative medicine applications, including the treatment of exudative wounds.

Materials and Methods

Preparation of foam

Agarose/curdlan foam was prepared by mixing agarose and curdlan at the appropriate ratio to prepare suspension in deionized water. Obtained homogeneous mass was transferred to a mould, which was incubated in a water bath at 95°C for 20 min. Then, the biomaterial was cooled to 4-8°C, moved to -80°C for 1-2 hours, and lyophilized for 16 hours. The resulting foam material was subjected to further testing.

Cell culture test

The cell culture experiments were carried out using human normal skin fibroblasts (BJ) purchased from ATCC. To assess cytotoxicity of the produced foam, indirect test (MTT assay) using fluid extract of the agarose/curdlan biomaterial was conducted according to ISO 10993-5 (2009). Viability of cells growing next to the samples and on the biomaterial was investigated by fluorescent stained using Live/Dead Double Staining Kit following with manufacturer protocol. Stained cells were then evaluated qualitatively by observation under a confocal laser scanning microscope (Olympus Fluoview equipped with FV1000).

Results and Discussion

The conducted MTT test showed that the tested material is not-toxic to human skin fibroblasts. Compared to the control, the viability of cells was slightly reduced on the second day of the test to 94,31%. Visualization by confocal microscopy showed clusters of viable fibroblasts around the material and only single cells growing on the surface of the agarose/curdlan foam, indicating that developed biomaterial prevents adhesion of skin fibroblasts. It is desired feature when biomaterial act as a temporary dressing to cover the wound since it allows to remove the material after healing process without causing trauma to the wound bed.

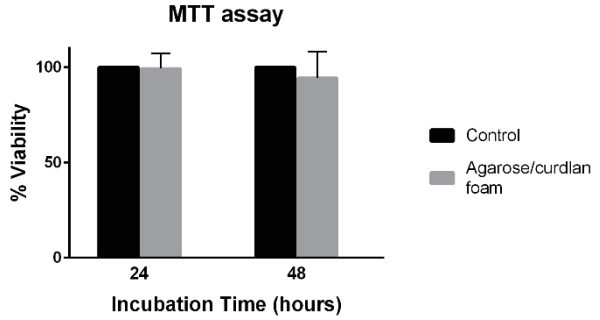

FIG. 1. MTT assay results for BJ cells cultured with fluid extract of the agarose/curdlan biomaterial.

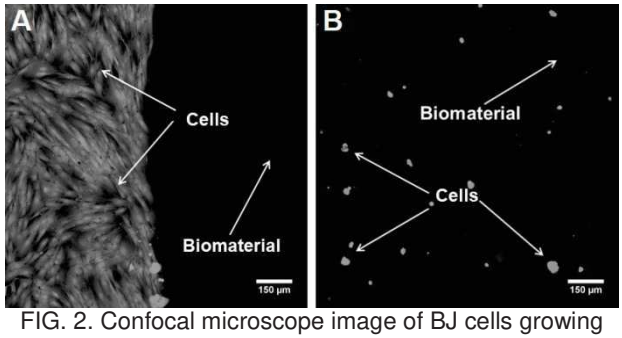

FIG. 2. Confocal microscope image of BJ cells growing next to the sample (A) and on the surface of the foam (B) after 2-day culture, magn. 100x, scale bar = 150 µm.

Conclusions

The obtained biomaterial is non-toxic and prevents the adhesion of skin fibroblasts to its surface. Due to specific surface properties of the material, it may be potentially used as a dressing, which when removed, does not affect the wound bed, preventing the scarring. Based on these studies, it can be assumed that the produced biomaterial have a promising potential to be used in skin regenerative medicine.

Acknowledgments

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