IN VITRO EVALUATION OF CELLULAR RESPONSE TO NOVEL AGAROSE/CHITOSAN/HA SCAFFOLD

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[ENGINEERING OF BIOMATERIALS 153 (2019) 18]

Introduction

Mechanical properties, roughness, wettability, functional groups, ion release and charge of surface scaffold are considered as relevant factors, having impact on cell behaviour (including attachment, proliferation, differentiation, and formation of an extracellular matrix – ECM). It is well known, that evaluation of cells' response to biomaterials under *in vitro* environment allows for better understanding of potential host-implant response under *in vivo* conditions [1,2]. The aim of this study was to evaluate cytotoxicity, adhesion, and proliferation of osteoblast cells and osteogenic differentiation of mesenchymal stem cells on the surface of developed novel scaffold for bone tissue engineering applications.

Materials and Methods

Preparation of scaffolds

The composition and production method of chitosan/agarose/nanohydroxyapatite (marked as chit/aga/HA) is protected by Polish Patent application no. P.426788. Briefly, chit/aga/HA was fabricated by mixing of chitosan (2 wt.%) and agarose (5 wt.%) suspension in acetic acid with hydroxyapatite nanopowder (70 wt.%) and sodium bicarbonate. Obtained paste was subjected to heating, then the sample was cooled and frozen. Frozen sample was lyophilised, followed by its neutralization in sodium hydroxide and finally left to dry in air. Microstructure of chit/aga/HA was visualized by stereoscopic microscope (Olympus SZ61TR) (FIG. 1).

Cell culture experiments

The cytotoxicity, adhesion and proliferation tests were carried out using normal human foetal osteoblast cell line (hFOB 1.19; ATCC). The cytotoxicity of the scaffold was evaluated by indirect test (MTT assay) using fluid extracts prepared in accordance with ISO 10993-5 (2009). Cell viability on the surface of the scaffold was assessed by fluorescent staining using Live/Dead Staining Kit. Cell adhesion and proliferation was determined by fluorescent staining of nuclei with DAPI and F-actin filaments with AlexaFluor635phalloidin. The number of cells on the surface of the scaffold was estimated via counting of nuclei using ImageJ software. Stained cells were analysed under confocal laser scanning microscope (CLSM, Olympus Fluoview equipped with FV1000). The osteogenic differentiation was conducted using human bone marrow-derived mesenchymal stem cells (BMDSCs; ATCC). BMDSCs were seeded directly on the scaffold and cultured in osteogenic medium for 21 days. On the 3rd, 7th, and 21st day, collagen type I (Col I), bone alkaline phosphatase (bALP) and osteocalcin (OC) level in the cell lysates were determined using ELISAs.

Results and Discussion

MTT assay showed that osteoblast viability was near 100% compared to the control cells. Also, confocal microscope visualization confirmed that chit/aga/HA biomaterial is non-toxic. Additionally, fluorescent staining of cells on the surface of the scaffold showed that new biomaterial is favourable to cell adhesion since cells were well spread and had lengthened shape (FIG. 2). Cell proliferation experiment revealed good growth and proliferation of hFOB 1.19 cells on the scaffold surface as during 9-day cultivation, amount of cell number increased from 2 x 10^4 to 10.77 x 10^4 cells per cm^2 of the sample. Osteogenic differentiation assessment revealed that BMDSCs cultured on the scaffold synthesised osteogenic markers what confirmed osteoinductive ability of developed biomaterial (TABLE 1).

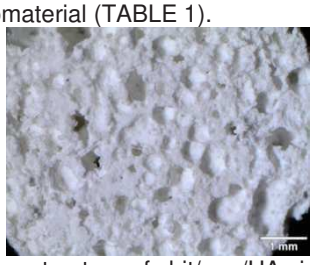

FIG. 1. Microstructure of chit/aga/HA visualized by stereoscopic microscope (scale bar = 1 mm).

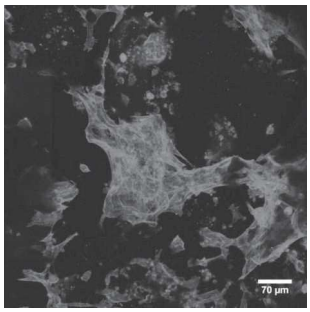

FIG. 2. Fluorescent staining of hFOB 1.19 cells growing on the surface of the chit/aga/HA after 9-day culture (magn. 200x, scale bar = 70 µm).

TABLE 1. Production of osteogenic differentiation markers by BMDSCs cultured on polystyrene (PS) and on the chit/aga/HA scaffold.

Time [day]	*Sample*	*Col I [ng/ml]*	*bALP [ng/ml]*	*OC [ng/ml]*
3	PS	10.62	0.09	0.98
	chit/aga/HA	4.88*	0.17*	2.36*
7	PS	6.11	1.15	4.34
	chit/aga/HA	8.26*	0.43*	7.27*
21	PS	7.40	4.46	15.36
	chit/aga/HA	8.84	0.19*	11.99*

*statistically significant results compared to PS, $P < 0.05$, unpaired t-test

Conclusions

Obtained results showed that fabricated scaffold is characterized by high biocompatibility. The surface of chit/aga/HA supports osteoblast adhesion, spreading and proliferation. Furthermore, novel scaffold has osteopromotive properties as it slightly enhances BMDSCs differentiation into osteoblastic lineage. This indicates that fabricated scaffold is a promising candidate for bone tissue engineering application.

Acknowledgments

The study was supported by the National Science Centre (NCN) in Poland within OPUS 16 grant no. UMO-2018/31/B/ST8/00945 and by using the equipment purchased within agreement no. POPW.01.03.00-06-010/09-00 Operational Program Development of Eastern Poland 2007–2013, Priority Axis I, Modern Economy, Operations 1.3. Innovations Promotion.

References

[1] N. Sachot, E. Engel, O. Castano, Curr. Org. Chem. 18 (2014) 2299–2314.
[2] A. Przekora, Mater. Sci. Eng. C. 97 (2019) 1036-1051.