THE EFFECT OF NANO-HA-BASED BIOMATERIAL ON MACROPHAGE POLARIZATION AND OSTEOGENIC DIFFERENTIATION IN CO-CULTURE SYSTEM *IN VITRO*

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Introduction

Biomaterials for tissue engineering applications should not induce inflammatory reactions after implantation, avoiding implant loosening. Macrophages play a critical role in the regulation of biomaterial-induced inflammatory response. Phenotype 1 macrophages (M1) were described as the pro-inflammatory type, whereas phenotype 2 macrophages (M2) were considered as the anti-inflammatory type [1]. The aim of this study was to evaluate the effect of the novel nanoHA-based bone scaffold (Polish Patent no. 235822) on the macrophage polarization and osteogenic differentiation.

Materials and Methods

The tested biomaterial was composed of chitosan, agarose, and nanohydroxyapatite (nanoHA) and it was prepared in accordance with the method described previously [2]. Macrophages were obtained by the differentiation of human acute monocytic leukaemia cells (THP-1, ATCC) in response to phorbol 12-myristate 13-acetate (PMA) stimulation. THP-1-derived macrophages (M0 phenotype – nonpolarized macrophages) were polarized to M1 and M2 phenotypes by exposure to LPS/INF-γ and IL-4/IL-13, respectively, as shown in FIG. 1. Macrophage characterization was conducted by assessment of levels of proinflammatory (IL-1β, IL-6) and anti-inflammatory (IL-4, Il-10, IL-13, TGF-β1) cytokines using commercially available human-specific ELISAs and by fluorescent staining of nuclei with DAPI and F-actin filaments with AlexaFluor635phalloidin.

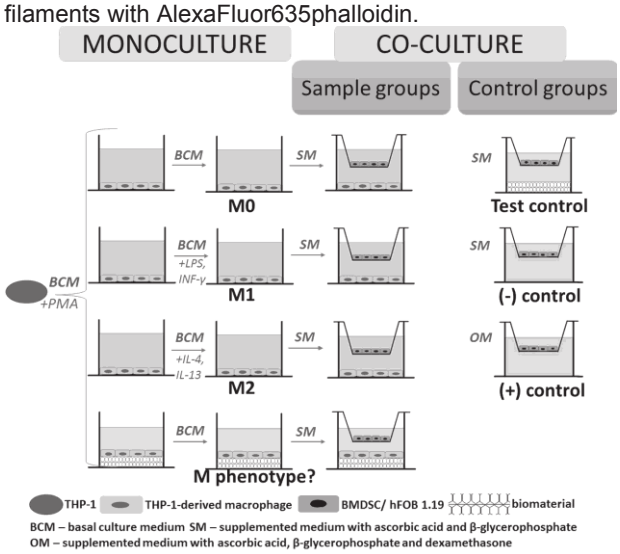

FIG. 1. Scheme showing the main idea of the co-culture experiment [3].

The co-culture experiments were conducted by co-culturing the THP-1-derived macrophages (M0, M1, and M2 macrophages seeded into PS wells and onto the surface of the biomaterial) with human bone marrow-derived stem cells (BMDSC, ATCC) to confirm the paracrine effect of macrophages on osteogenic differentiation, as shown in FIG. 1. On the 6th day of culture, collagen type I (Col I) and bone alkaline phosphatase (bALP) levels were assessed using commercially available human-specific ELISAs. On the 21st day, osteocalcin (OC) and bALP levels in the cell lysates were determined.

Results and Discussion

Comparative analysis of the secretion profile of cytokines and growth factors in monoculture of M0 (nonpolarized macrophages), M1, M2 macrophages and cells cultured on the surface of the developed bone scaffold revealed that macrophages grown on the tested biomaterial released a high level of anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGF-β1), which is typical of the M2 phenotype. Moreover, an assessment of cell morphology confirmed M2 polarization of the macrophages on the surface of the biomaterial (FIG. 2). In turn, evaluation of the level of typical osteogenic markers showed that BMDSC co-cultured with macrophages-seeded biomaterial produced a significantly higher amount of Col I, bALP, and OC than monoculture of BMDSCs grown in the presence of biomaterial (test control). Thus, it was demonstrated that M2 macrophages had a positive effect on the osteogenic differentiation of BMDSCs.

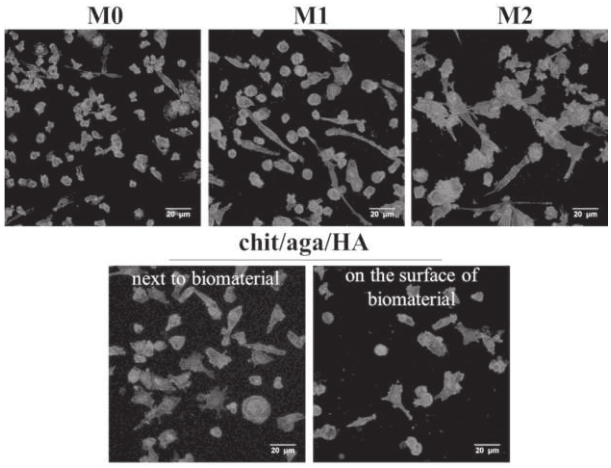

FIG. 2. Macrophages morphology after 3-day culture in the polystyrene wells (M0, M1, and M2 macrophages), on the developed scaffold, and next to the biomaterial [3].

Conclusions

In this study we demonstrated that the novel developed bone scaffold induced M2 polarization. The co-culture of macrophages-seeded nanoHA-based biomaterial with mesenchymal stem cells enhanced their osteogenic ability, approving the immunomodulatory effect of the macrophages on the osteogenic differentiation process. Moreover, we proved that developed biomaterial carries a low risk of inflammatory reactions and thus is a very promising bone scaffold for regenerative medicine applications.

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References

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