Biomaterials 33 (2012) 8943–8954

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

The calcium-dependent regulation of spheroid formation and cardiomyogenic differentiation for MSCs on chitosan membranes

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ARTICLE INFO

Article history:
Received 13 August 2012
Accepted 30 August 2012
Available online 15 September 2012

Keywords:
Adipose derived adult stem cells (ADAS)
Spheroids
Chitosan
Intracellular calcium
Cardiomyogenesis

ABSTRACT

Mesenchymal stem cells (MSCs) were recently found to form three-dimensional (3D) multicellular spheroids on chitosan membranes. The exact mechanism of spheroid formation, however, remains unclear. In this study, the regulation of spheroid formation for adipose derived adult stem cells (ADAS) grown on chitosan membranes was examined. By varying the membrane thickness, calcium concentration in culture medium, and acetylation extent of chitosan, the physico-chemical characteristics of chitosan that modulated spheroid formation was elucidated. The capacity of cardiomyogenic differentiation was further evaluated. Results suggested that the calcium binding capacity of chitosan may affect the cell–substrate and cell–cell interactions and critically influence the dynamics of spheroid formation. The intracellular calcium level was elevated for ADAS spheroids on chitosan. Chitosan-bound calcium was observed to enter the cells. The expression of N-cadherin was upregulated for ADAS spheroids on chitosan, evidenced by quantitative RT-PCR and Western blot. After the induction by 5-aza, the expression levels of cardiac marker genes (Gata4, Nkx2.5, Tnnt2, and Myh6) were remarkably enhanced (about four-fold) for ADAS on chitosan vs. tissue culture polystyrene or polyvinyl alcohol. Immunofluorescence staining confirmed the expression of cardiac-associated tight junction protein ZO-1 for ADAS grown on chitosan membranes. The gene expression of Wnt11 was significantly upregulated for ADAS spheroids on chitosan at 3 days and 12 days. We suggested that Wnt11 may be involved in the spheroid formation and cardiomyogenic differentiation of MSCs on chitosan membranes. Spheroids formed on the acetylated chitosan or polyvinyl alcohol membranes failed to show such behavior. The properties of MSC spheroids were therefore determined by the culture substrate.

1. Introduction

The cellular microenvironment that stem cells reside in can determine the cell behavior. The differentiation of stem cells is highly regulated by their niche, which includes both the intrinsic and extrinsic signals [1]. Stem cells are commonly cultured as two-dimensional (2D) monolayer by traditional tissue culture techniques. The 2D culture method yet has difficulty in controlling the cell fate, which results in cells of low differentiation capacity [2]. Therefore, the development of three-dimensional (3D) cell culture techniques is an important recent trend in stem cell research.

Chitosan is the deacetylated derivative of chitin, composed of β(1–4) linked D-glucosamine residues with different contents of N-acetyl-glucosamine group. Because of its biocompatibility and biodegradability, chitosan has been extensively studied as a scaffolding material for tissue engineering. Researches have indicated that the amount of amino groups in the structure of chitosan can influence its cell-contacting performance and is considered as one of the major factors that affect the cellular response [3–7].

Mesenchymal stem cells (MSCs) have the capacities of self-renewal and multi-lineage differentiation. They are considered as a potential cell source for regenerative medicine. Adipose derived adult stem cells (ADAS) are an abundant source of MSCs which are easily accessible from subcutaneous adipose tissue via liposuction [8] and can differentiate into cells of multiple lineages including osteoblasts, chondrocytes, adipocytes, myocytes, neurons, and endothelial cells [9–12]. ADAS and bone marrow-derived MSCs were recently found to form 3D spheroids on chitosan membranes [13–15]. These cells attached and spread on chitosan membranes before they retracted their pseudopodia to form multicellular spheroids [13]. This process of spheroid formation was quite different from that occurred in suspension [16,17], on non-adherent

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http://dx.doi.org/10.1016/j.biomaterials.2012.08.069