Culture of osteoblast on polyelectrolyte complexes (PECs) composed of polysaccharides

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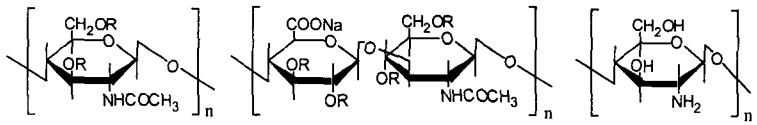
Abstract

Osteoblasts (MC3T3-E1) were cultured on polysaccharide type polyelectrolyte complex (PEC). The growth of the MC3T3-E1 on the PEC with carbxyl group (c-type) was slightly suppressed and exhibited aggregation morphology. On the other hand, cell growth on the PEC with sulfate group (s-type) was enhanced and the cell exhibited spreading form. Differentiation markers of osteoblast (ALPase activity, calcification, expression of osteocalsin) were enhanced and localized around cell aggregates on c-type PECs. These results suggest that PEC has the ability to control osteoblast proliferation and differentiation.



CMCHN(Carboxymethyl Chitin) R=CH_2COO^- or H, D.S.=1.0
SCHN(Sulfated Chitin) R=SO_3^- or H, D.S.=1.1
HA R=H
SHA(Sulfated Hyaluronan) R=SO_3^- or H, D.S.=2.1

Fig.1 Structures of polysaccarides

Introduction

It is well known that cells *in vivo* are surrounded by many types of extracellular matrix (ECM) ; adhesive proteins and proteoglycans are major components of ECM which control cellular adhesion, morphological changes, proliferation, and differentiation. Glycosaminoglycans (GAG) are an integral part of the proteoglycans and linear polymers of repeated disaccharides, and control the cell functions through growth factors interacted with[1]. The component polysaccharides (chitin derivatives as polyanions and chitosan as a polycation) which are components of a polyelectrolyle complex (PEC) have a structure and properties similar to GAG [2,3]. It has been previously reported that such PECs control the cell adhesion, morphologies, proliferation, and differentiation of human periodontal ligament fibroblasts [4,5].

In this study, we will discuss the functional control of osteoblast (MC3T3-E1) by PECs, which consist of chitosan and chemically substituted chitin and hyaluronan with sulfate or carboxyl groups.

Experimental

We employed carboxymethylated chitin and hyaluronan (abbreviated as c-type polyanions) and sulfated chitin and sulfated hyaluronan (as s-type polyanions) and chitosan as a polycation. Tissue culture dish (TCD) was coated with PECs, which were formed by mixing equimolar aqueous solutions of these polyanions and polycations(Fig1). MC3T3-E1 was cultured on these dishes in the presence of L-ascorbic acid and β-grycerolophosphate and evaluated in the viewpoints of growth, morphology and differentiation.

Results

More than 90% of the MC3T3-E1 was adhered onto all PEC coated dishes after 1day incubation. LDH release ratio, which was an indicator of cell membrane damage, of MC3T3-E1 cultured on PECs showed no significant difference among the PECs coating dishes and control dish (TCD). These results suggest that the PECs have a low cytotoxicity and high adhesion performance to MC3T3-E1.

Fig.2 shows the growth curves of MC3T3-E1 on various PECs. The growth of the MC3T3-E1 on c-type PEC was slightly suppressed and nodule like structure consisted of multiple cell layers, in

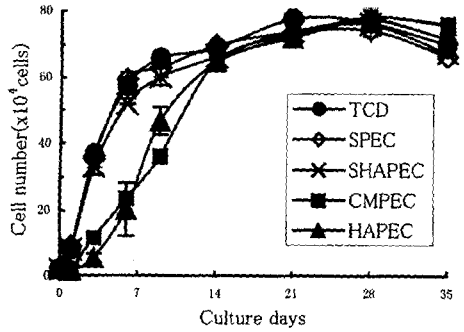


Fig.2 Growth curves of MC3T3-E1 cultured on various PECs

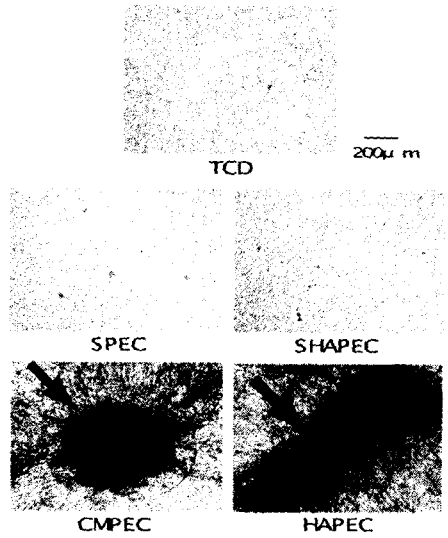


Fig.3 Von Kossa staining of MC3T3-E1 cultured on various PECs for 21days

which calcification occurred, was formed. On the other hand, cell growth on s-type PEC was enhanced and the cell exhibited spreading form.

Alkaline phosphatase (ALPase) activity was determined as an indicator of osteoblastic differentiation of MC3T3-E1 cells cultured on PECs. ALPase activity on the c-type PECs were significantly higher than on the s-type PECs and TCD. Calcification is also an important indicator of osteoblastic differentiation. Fig3. shows the localization of calcium by Von Kossa staining method. Calcium depositions were observed especially at the part of cell aggregate on the c-type PECs. It was suggested that calcification was locally induced by c-type PECs.

The expression of osteocalsin, a differentiation marker protein of osteoblast, was determined by detection of its mRNA using a RT-PCR method. The mRNA of osteocalsin was enhanced on the PEC containing hyaluronan as a polyanion.

Conclusions

As the result, MC3T3-E1 was proliferated and formed spreading morphology on the s-type PECs similar to control dish. On the contrary, MC3T3-E1 cultured on c-type PECs was low in cell growth and formed cell aggregates. These aggregates expressed high ALPase activity. That is, aggregate formation of MC3T3-E1 might be a primary step to the calcification and mainly due to the existence of carboxyl group.

References

1. W. H. Burgess, and T. Maciag, *Annu. Rev. Biochem.* **58**, 575 (1989)
2. S. Nishimura, and S.Tokura, *Int. J. Biol. Macromol.*, **9**, 225 (1987)
3. J. Numata, I. Saiki, K. Matsumoto, S.Tokura, and I.Azuma, *Jpn. J. Cancer Res.* , **81**, 506 (1990)
4. T. Hamano, A. Teramoto, E. Iizuka, and K. Abe, *J. Macromol. Sci. -Chem.*, **A35(3)**, 439 (1998)
5. T. Hamano, D. Chiba, A. Teramoto, Y. kondo and K. Abe, *J. of Biomate. Sci. Polym. Ed.*, **9**, 985(1998)