Effect on Osteoblast Responses of the Self-Assembly and Cross-linking of Collagen Immobilized on Titanium

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Purpose of Study: Collagen, as a major organic constituent of human hard tissues, has been considered as an important biomaterial [1,2]. As such, collagen has been studied much as a coating moiety on Ti hard tissue implants. However, very little work has been made on the control of the characteristics of the collagen. In fact, some controversies are encountered as to the biological effects of collagen on a series of cellular responses, including cell attachment, proliferation and protein synthesis. This was mainly associated with the structural and chemical properties / variances of the collagen. Therefore, this study aims to address the effects of fibrillar assembly and cross-linking of collagen immobilized onto Ti on the chemical stability and the subsequent osteoblastic responses.

Methods: Commercially pure Ti (grade II) was used as a substrate for the collagen immobilization. Acid soluble type I collagen was dissolved in acetic acid at 2.5 % w/v. Fibrillar assembly of the collagen was conducted in a diluted PBS medium (pH ~4.2) with incubation at 37 ^{o}C for different periods. The degree of the fibrillar assembly with the incubation time was quantified. The assembled collagen solutions were homogenized and immobilized on Ti discs. Parts of the collagen were cross-linked with EDC/NHS solution [3]. The concentration of immobilized collagen was quantified by Sirius Red dye assay. The morphology and assembly level of the collagen were characterized with SEM and TEM. The chemical stability of the collagen was measured in collagenase medium. Human osteoblastic cellular responses were assessed on the differently controlled collagen. Cell attachment and proliferation level was measured by MTT assay [3]. Cell morphology was observed with SEM. Statistical analysis was carried out by Student's t-test for n=6.

Results / Discussion: The fibrillar assembly of the collagen sol was controlled with varying the incubation time at 37 ^{o}C. As monitored by a turbidity change, the assembly level was observed to be saturated within several hours. The collagen sols assembled at different levels were immobilized on Ti and subsequently cross-linked. The partial assembly of the collagen featured fibrils with various diameters and many non-fibrillar aggregates on TEM image (Fig. 1, left). On the other hand, the fully assembled collagen represented complete formation of fibrils with diameter of ~100-200 nm (Fig. 1, right). Moreover, high resolution image showed periodic patterns within the fibrils, characterizing native collagen fibers. The level of fibrillar assembly of the collagen significantly influenced the chemical stability and cellular responses. When assembled well into fibrillar, the collagen immobilized onto Ti preserved chemical stability, as well as improved cell attachment and proliferation significantly. In particular, without the fibrillar assembly, the collagen on Ti adversely affected the cell responses (less than the un-immobilized Ti). When the unassembled collagen was cross-linked well, the chemical stability was preserved similarly; however the cellular responses were recovered to or even higher than the level of un-immobilized Ti. On the other hand, on the well-assembled collagen, the cross-linking did not affect significantly in terms of cellular responses, although the chemical stability was more improved. Taken together, it is deduced that the chemical stability of collagen would not directly correlate with the cellular responses. However, the cross-linking step was highly effective in the case of unassembled-collagen. In any case, the fibrillar assembly of collagen is required to obtain sufficient chemical stability and to stimulate favorable cellular responses.

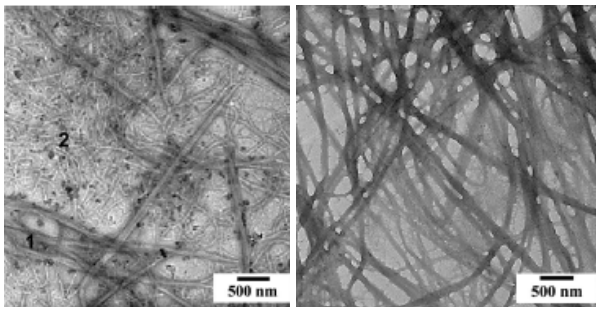

Fig. 1

Conclusions: We observed the fibrillar assembly and cross-linking of the collagen immobilized onto Ti significantly influenced the chemical stability and osteoblastic cellular responses. In particular, the native fibrillar assembly of collagen is strongly suggested and should be carefully controlled in its use as biomedical material, such as an immobilization moiety on Ti implants.

Acknowledgement: Grant from Korea Research Foundation (R08-2004-000-10192-0)

References:

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