ELISA detection of glycosaminoglycan(GAG)-linked proteoglycans(PG) in gingival crevicular fluid(GCF). Watari Nishino, Toshiaki Shibutani, Yoshinobu Murahashi, Masafumi Shiraki and Yukio Iwayama. Department of Periodontology, School of Dentistry, Asahi University.
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The purpose of this study was to develop ELISA assay to Chondroitin sulfate isomers(CH-4S, CH-6S and DS) proteoglycan in GCF associated with experimentally induced periodontitis in the dog.

(Materials and Methods) Monoclonal antibodies, 3B3 and 9A2, were developed by Caterson et al. (1985). Combinations of these antibodies and specific enzymatic digestion have made possible the identification of CH-4S, CH-6S and DS.

Experimental periodontitis was induced by silk ligature placement below the gingival margin of the dog molar. GCF was collected in microcapillary tubes at 0, 7, 21 and 60 days after ligature in three mongrel dogs. Samples were diluted 5x10 in 0.15M PBS pH 7.2.

The method of indirect ELISA assay was as follows. 50µl of diluted samples were added and coated in a 96 well microtiter plate. Each well was treated with Chondroitinase ABC, ACII and B (SEIKAGAKU KOGYO) (0.1-0.5 U/ml). After enzymatic digestion, 1% BSA was added in order to inhibit non specific reaction. The first antibody, 3B3 and 9A2 (1:8000), were added. The second antibody, affinity purified peroxydase conjugated goat anti mouse IgG and IgM(CAPPEL) (1/1000), was added. The enzyme substrate (o-phenilenediamine and H₂O₂) was added and this reaction was stopped with 4N H₂SO₄. The absorbance at 492 nm was read with an ELISA plate reader (TOSO). Standard curve and detection levels of GAG-PG were examined in a preliminary study using bovine nasal cartilage proteoglycan monomer (SEIKAGAKU KOGYO).

(Result) Detection levels in these ELISAs were from 15ng to 1000ng for CH-4S (Fig.1) The quantity of CH-4S, CH-6S and DS was fluctuated with advancing inflammation. In particular, CH-4S was increased in the acute phase and then decreased gradually (Fig.2).
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(Conclusion) The present study has confirmed that the ELISA assay made it possible to detect GAGs in GCF associated with experimentally induced periodontitis in the dog.

![Graph 1](image1.png)

**Fig. 1.** ELISA assay bovin nasal cartilage proteoglycan monomer when using monoclonal antibody 9-A-2.

![Graph 2](image2.png)

**Fig. 2.** ELISA assay for glycosaminoglycan proteoglycan in GCF.