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Syndecan-1 immunohistochemical expression in gingival tissues of chronic periodontitis patients correlated with various putative factors

S. Kotsovilis[1,2], S. Tseleni-Balafouta[3], A. Charonis[1], I. Fourmousis[2], D. Nikolidakis[4], J. A. Vrotsos[2]

[1]Department of Basic Research, Biomedical Research Foundation of the Academy of Athens, Athens, Hellas, [2]Department of Periodontology, School of Dentistry, University of Athens, Athens, Hellas, [3]Department of Pathology, Medical School, University of Athens, Athens, Hellas and [4]Department of Periodontology & Biomaterials, University Medical Center Nijmegen, Nijmegen, The Netherlands

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Background and Objective: Limited information is available on the expression and distribution of syndecan-1 within human gingival tissues/cells and on putative factors that might affect its expression. Therefore, the objective of the present study was to determine immunohistochemically the expression and distribution of syndecan-1 in the gingival tissues of patients with chronic periodontitis and to examine the correlation of syndecan-1 expression with various putative factors (environmental, patient/systemic and local factors).

Material and Methods: Gingival specimens were surgically excised from the area of the junctional/pocket epithelium (study group 1, including 30 chronic periodontitis patients) or the gingival oral epithelium (study group 2, comprising another 30 chronic periodontitis patients), adjacent to teeth with poor prognosis. Standard two-step immunohistochemistry and semi-quantitative evaluation of immunohistochemical staining were used to determine syndecan-1 expression. Statistical analyses on the impact of various putative factors were performed.

Results: In the junctional/pocket epithelium or the oral epithelium, syndecan-1 expression was weak to moderate in the suprabasal and basal epithelial cells and absent to weak in the internal basal lamina, external basal lamina and gingival connective tissue matrix. Syndecan-1 expression in the junctional/pocket epithelium was statistically significantly stronger than in the oral epithelium in inflammatory cells within the underlying gingival connective tissue (primarily plasma cells and lymphocytes) and in scattered fibroblast-like cells.

Conclusions: Syndecan-1 expression in the junctional/pocket epithelium or the oral epithelium can exhibit a significant positive correlation with the severity/degree of histologically evaluated local gingival inflammation, but in general is not significantly correlated with age, smoking, full-mouth and local clinical (probing pocket depth and clinical attachment level) and radiographical parameters (radiographical bone loss) of periodontal status.

Sotirios Kotsovilis, MSc Oral Biology, Periodontist, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Ephessiou, GR 11527, Athens, Hellas
Tel: +30 210 6042845
Fax: +30 210 7462124
e-mail: sot.kotsovilis@yahoo.gr

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Syndecans are a family of highly conserved proteoglycans, containing predominantly heparan sulfate (1,2) or, less frequently, chondroitin sulfate or dermatan sulfate glycosaminoglycans (1,3). So far, four members of this family have been identified and cloned in mammals (2): Syndecan-1, -2 (fibroglycan), -3 (N-syndecan) and -4 (ryudocan or amphiglycan). Syndecan-1 was the first member to be cloned (4) and until now remains the best characterized syndecan member. Most often, syndecans are located in the cell membrane, but in certain instances they might also be detected intracellularly (within the cell plasma or nucleus) or within the extracellular matrix (5–8).

Syndecans may interact with a plethora of extracellular components, such as fibronectin (1), interstitial type I and III collagen (3,9), thrombospondin (10) and tenascin (11), as well as growth factors, such as basic fibroblast growth factor (12). Syndecan-1, following its interaction with extracellular components, subsequently interacts with cytoskeletal elements, thus determining various cell functions (13). In particular, syndecan-1 may play a role in cell adhesion (2,13), inflammation (14), wound healing (7,14), cancer (15) etc.

In the oral cavity, syndecan-1 participates in epithelial–mesenchymal interactions during tooth development (16). Furthermore, syndecan-1 is expressed in the supporting periodontal tissues (periodontal ligament, alveolar bone and cementum) in Lewis rats (5,7) and in fibroblasts and osteoblasts of human periodontal ligament (17–19). Animal experiments have demonstrated the expression of syndecan-1 in all three types of gingival epithelia, i.e. the oral gingival epithelium, the oral sulcular epithelium and the junctional/pocket epithelium (5,7,8), and in the fibroblasts and extracellular matrix of the gingival connective tissue (7). Studies in humans have revealed that syndecan-1 is expressed in the oral gingival epithelium (15,20–24), the junctional/pocket epithelium (21) and the gingival connective tissue (19,21,22).

Limited information appears to be available on the expression and distribution of syndecan-1 within various tissues and cells of the human periodontium and, in particular, on putative factors that could potentially affect its expression.

Therefore, the objective of the present study was to determine immunohistochemically the expression and distribution of syndecan-1 in the gingival tissues of patients with chronic periodontitis, either in the area of the junctional/pocket epithelium (i.e. close to the tooth surface, where gingival/periodontal inflammation as a rule is more intense) or in the area of the oral gingival epithelium (i.e. not close to the tooth surface, where gingival/periodontal inflammation as a rule is less intense) and to examine the correlation of syndecan-1 expression with various putative factors, namely patient age, behavioural (e.g. smoking) and systemic factors (e.g. systemic diseases or drugs), the severity/degree of histological and clinical gingival/periodontal inflammation and full-mouth or local parameters of periodontal status, such as probing pocket depth, clinical attachment level and radiographical bone loss.

Material and methods

Patient selection: inclusion/exclusion criteria

Upon the commencement of the study, its protocol was reviewed by the Ethics and Research Committee, School of Dentistry, University of Athens, Hellas, and ethical approval was obtained for the experimental procedures applied in humans, in accordance with the provisions of the World Medical Association's Declaration of Helsinki of 1975, as revised in 2000. All patients included in the study signed an informed consent form.

The patient population of the study comprised 60 Caucasian volunteers (35 females and 25 males; age range 32–83 years; mean 56.83 ± 11.18 years), who presented for periodontal therapy in the Post-graduate Clinic, Department of Periodontology, School of Dentistry, University of Athens, Hellas and the private periodontal office of one of the authors (I.F.). Two groups, each with 30 patients, were included in the study. Patients were screened for inclusion in the study after treatment planning, on the basis of the following inclusion/exclusion criteria.

Inclusion criteria

- Caucasian patients aged ≥ 18 years
- Diagnosis of chronic periodontitis, according to the definition settled in the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions (25)
- Presence of one or more teeth with a poor prognosis for any reason (presence of periodontitis, caries, endodontic or prosthetic reasons etc.) or scheduled for extraction, according to the predetermined treatment plan

Exclusion criteria

- History of previous periodontal therapy within 3 mo before the screening examination (gingival tissues selected for sampling should not be in a healing process/phase)
- Presence of a periodontal abscess or a combined periodontal–endodontic lesion in the gingival sampling area
- General contra-indications for periodontal surgery (e.g. psychiatric problems, pregnant or breast-feeding females) or presence of life-threatening conditions (e.g. malignant tumours or radiotherapy in the cervico-facial area, either current or within the previous 6 mo)

Screening examination

A screening examination was carried out during the initial patient visit. A clinical examination of periodontal tissues comprised measurements of the following clinical parameters.

- Full-mouth plaque score (26)

Full-mouth plaque score was recorded as the percentage of total surfaces (six aspects per tooth) that revealed the presence of plaque, when running a periodontal probe tip on the tooth surface along the gingival margin.

- Full-mouth bleeding on probing score (27)

Full-mouth bleeding on probing score was recorded as the percentage of total surfaces (six aspects per tooth) that revealed the presence of bleeding

within 30 s after probing at the base (the most apical border) of each periodontal pocket (28).

- Full-mouth probing pocket depth and local probing pocket depth

Probing pocket depth was measured as the distance between the free gingival margin and the base of each periodontal pocket. The category (shallow, moderate or deep) of full-mouth probing pocket depth, as well as local probing pocket depth at the sampling site of the tooth scheduled for extraction were both determined. By definition, shallow pockets exhibited probing pocket depth of 3 mm up to and including 4 mm, moderate pockets presented probing pocket depth > 4 mm up to and including 6 mm, and deep pockets had probing pocket depth > 6 mm.

- Full-mouth clinical attachment level and local clinical attachment level

Clinical attachment level was measured as the distance between the cemento-enamel junction and the base of each periodontal pocket. If the cemento-enamel junction was not visible/usable as a reference point, a restoration margin or another alternative stable landmark served as the reference point for measurement. The category (slight, moderate or advanced) of full-mouth clinical attachment level, as well as local clinical attachment level at the sampling site of the tooth scheduled for extraction were both determined. By definition, slight clinical attachment level was ≤ 4 mm, moderate clinical attachment level was > 4 mm up to and including 6 mm, and advanced clinical attachment level was > 6 mm.

All clinical measurements were carried out on six aspects (sites) per tooth: mesio-facial (-buccal), mid-facial (-buccal), disto-facial (-buccal), mesio-lingual (-palatal), mid-lingual (-palatal) and disto-lingual (-palatal), by the same single calibrated and masked investigator (S.K.), using the same type of periodontal probe (UNC-15, Hu-Friedy, Chicago, IL, USA) with a mild probing force, corresponding to approximately 25 N.

Finally, a radiographical examination of all teeth present was accomplished by means of an orthopantomograph and/or peri-apical radiographs taken using the paralleling cone technique, whenever deemed necessary. The radiographical examination of periodontal tissues provided the percentage of mean full-mouth radiographical bone loss, as well as the percentage of local radiographical bone loss at the sampling site of the tooth scheduled for extraction.

Calibration (intra-examiner reproducibility/agreement)

Calibration for the evaluation of the intra-examiner reproducibility of periodontal probing had already been performed prior to the screening examination. Five patients, each having at least 10 teeth (single- and multi-rooted) with probing pocket depth ≥ 6 mm on at least one aspect, were used to calibrate the examiner. The examiner carried out a measurement of probing pocket depth and clinical attachment level in duplicate and 48 h apart. The examiner was considered to be reproducible at an acceptable level, because at least 90% of the recordings at baseline and after 48 h were reproduced within a difference of 1 mm (28).

Collection of gingival specimens

The collection of gingival specimens measuring 2–3 mm in each of the three dimensions was carried out concomitantly with tooth extraction. According to the location of the sampling area, two study groups were formed. Study group 1 comprised 30 chronic periodontitis patients (19 females and 11 males; age range 35–80 years; mean 57.83 ± 10.74 years), and study group 2 included another 30 chronic periodontitis patients (16 females and 14 males; age range 32–83 years; mean 55.83 ± 12.07 years), thus providing a total of 60 patients (35 females and 25 males; age range 32–83 years; mean 56.83 ± 11.18 years) included in the present study.

In study group 1 the gingival specimens dissected were in close contact with the surface of the tooth extracted and comprised the entire zone of gingiva above the level of the alveolar bone crest, therefore including the oral gingival epithelium, the junctional/pocket epithelium and the supracrestal part of the gingival connective tissue. In contrast, in study group 2 the gingival specimens dissected were not in contact with the surface of the tooth extracted and therefore comprised exclusively the oral gingival epithelium and the underlying gingival connective tissue. Thus, gingival specimens in study group 2 differed from the specimens in study group 1 in that the junctional/pocket epithelium was not included. Since the specimens in study group 1 extended within the area of the periodontal pocket, whereas the specimens in study group 2 were located at a distance from the periodontal pocket, the specimens in study group 1 were anticipated, as a rule, to exhibit more intense clinical signs of gingival/periodontal inflammation compared with those in study group 2.

Sample processing

The gingival samples were fixed in 10% buffered formalin for a maximum of 24 h and subsequently embedded in paraffin. Microtome serial sections (5 μm thick) were cut and mounted on glass poly-D-lysine-coated slides. One section was stained with haematoxylin and eosin, in order to observe the histological features and to score the grade of gingival/periodontal inflammation, whereas the others underwent a standard two-step immunohistochemical procedure to detect the expression of syndecan-1.

Immunohistochemistry

On the first day of the immunohistochemical procedure, deparaffinization and rehydration was followed by a pretreatment stage (antigen/epitope retrieval), carried out by placing the slides into a 10 mM citrate buffer (pH = 6) and heating for 10 min in a microwave oven. The slides were incubated in 1.5% hydrogen peroxide solution for 30 min in the dark, with the aim of blocking endogenous peroxidase activity within tissue sections. Subsequently, the test slides were incubated with the primary antibody, a mouse anti-human CD138 monoclonal antibody against syndecan-1 core protein (Code CBL588; CHEMICON® Europe, CHEMICON®

International, Inc., Serologicals® Corporation, Hampshire, UK), diluted 1:50 using phosphate-buffered saline (pH = 7), overnight within a humidified chamber at 4°C.

On the second day, the slides were incubated for 30 min with the secondary antibody, a one-step polymer horseradish peroxidase antibody, suitable for use with both rabbit and mouse primary antibodies (Code K5007; ChemMate™ Dako EnVision™ Detection Kit, DakoCytomation Denmark A/S, Glostrup, Denmark). For the visualization of the reaction product, the chromogen diaminobenzidine was used. Counterstaining was done with haematoxylin. Human tonsil was used as the positive control tissue. In negative control slides, instead of the primary antibody, phosphate-buffered saline solution (pH = 7) was used.

Evaluation of immunohistochemistry

Syndecan-1 expression was evaluated semi-quantitatively (5,7), according to the intensity and extent of immunohistochemical staining in various components of gingival tissues, by two independent investigators (S.T.-B. and S.K.) and in duplicate, using a light microscope (Eclipse 50i POL Polarizing Microscope; Nikon Corporation, Tokyo, Japan). Both examiners were masked with regard to patient (donor) characteristics (e.g. patient code, age, medical history), as well as the clinical (e.g. full-mouth plaque score, full-mouth bleeding on probing score, full-mouth probing pocket depth) and radiographical characteristics (e.g. full-mouth radiographical bone loss) of the periodontal tissues of each patient. Intra-examiner agreement (κ score) was first determined, separately for each examiner, using previously described methods (29–32). After the calculation of intra-examiner agreement for each examiner, each investigator subsequently formed only one (the final) list of his/her individual scorings. The two final lists of the two examiners were compared, and interexaminer agreement (κ score) was determined, using previously described methods (29–32). Finally, any disagreements between the two examiners were resolved by discussion and consequently a final consensus (i.e. in complete agreement) list of study scorings was formulated, intended to be used in the statistical analysis at a later stage of the study.

For histological sections of study group 1, the components of gingival tissues examined were the suprabasal and basal epithelial cells, the internal basal lamina, the external basal lamina and the inflammatory cells and matrix of the gingival connective tissue. For histological sections of study group 2, the components of gingival tissues examined were the same, except for the internal basal lamina, which is never present in the area of the oral gingival epithelium.

For the evaluation of syndecan-1 expression, a four-grade scale (−, +, ++, +++) was used (5,7) as follows:

- No expression (−); negative-to-weak specific immunohistochemical staining in < 5% of cells
- Weak expression (+); weak specific immunohistochemical staining in 5–30% of cells or strong specific staining in < 5% of cells
- Moderate expression (++); weak specific immunohistochemical staining in 30–100% of cells or strong specific staining in 5–75% of cells
- Strong expression (+++); strong specific immunohistochemical staining in 75–100% of cells

Statistical analysis

The statistical analysis was performed with commercially available software (Instat® 2000, version 3.05, GraphPad Software Inc., San Diego, CA, USA). Mean values with standard deviations were calculated for all variables in each group, based on the subject as the statistical unit. Furthermore, ordinal data were expressed as median, minimum and maximum value. Intergroup statistical comparisons were performed using the Mann–Whitney *U*-test. Intragroup statistical comparisons were applied by using the Friedman test (non-parametric repeated-measures ANOVA) with Dunn's *post hoc* test. Intragroup statistical correlations were analysed by using Spearman's non-parametric correlation test. Differences were considered to be statistically significant when the p value was ≤ 0.05.

Results

Histological evaluation of gingival/periodontal inflammation

Gingival tissues in the area of the junctional/pocket epithelium (study group 1) contained a more pronounced inflammatory cell infiltrate than those in the area of the oral gingival epithelium (study group 2). The majority of inflammatory cells were lymphocytes, plasma cells and polymorphonuclear cells.

In cases of severe gingival/periodontal inflammation, at the boundary between the junctional/pocket epithelium and its underlying gingival connective tissue, the presence of epithelial ridges projecting into the gingival connective tissue, so-called rete pegs, was a characteristic histological sign of gingival/periodontal inflammation in the junctional/pocket epithelium (Fig. 1A), in contrast to non-inflamed gingiva, where rete pegs are normally lacking in the junctional epithelium.

Evaluation of immunohistochemistry (Table 1 and Figs 1–3)

The results of the evaluation of immunohistochemistry by two independent investigators (S.T.-B. and S.K.), as well as intra-examiner and interexaminer agreement (κ score; 29–32) are summarized in Table 1. As deduced from Table 1, for the vast majority of scorings, κ score was higher than 0.80 and therefore indicated an 'almost perfect' strength/level of intra-examiner or interexaminer agreement beyond chance, based on proposed standards (30) for the interpetation of the magnitude of κ score as a measure of the strength/level of agreement beyond chance.

As shown in Table 1, in study group 1 the expression of syndecan-1, as represented by the four-grade intensity of immunohistochemical staining (−, 0; +, 1; ++, 2; and +++, 3), was weak to moderate (mean values ranging from 1 to 2) in the suprabasal and basal cells of the junctional/pocket epithelium

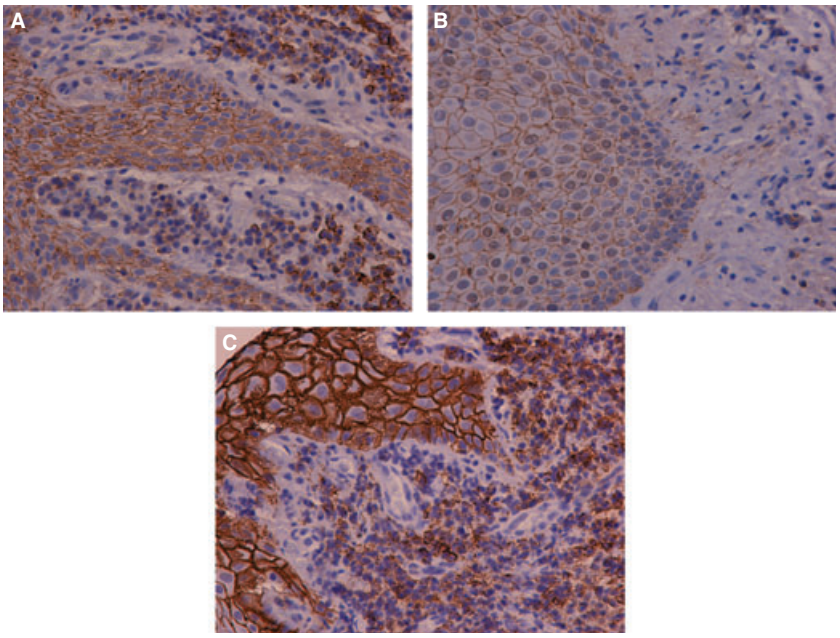

Fig. 1. (A) Specific immunohistochemical staining for syndecan-1 in the region of the junctional epithelium towards the gingival connective tissue, on a histological section derived from study group 1 (magnification ×400). At the boundary between the junctional epithelium and its underlying gingival connective tissue, the presence of rete pegs projecting into the gingival connective tissue is a characteristic histological sign of inflammation in the junctional epithelium. (B) Specific immunohistochemical staining for syndecan-1 in the region of the junctional epithelium towards the gingival connective tissue, on a histological section of the same gingival specimen (magnification ×400). In contrast to (A), the absence of rete pegs is concordant with the presence of a limited degree of gingival inflammation. Compared with (A), syndecan-1 expression appears to be less intense. (C) Specific immunohistochemical staining for syndecan-1 in the region of the junctional epithelium towards the tooth surface, on a histological section of the same gingival specimen (magnification ×400). Syndecan-1 expression appears to be more intense in the region of the junctional epithelium towards the tooth surface compared with that towards the gingival connective tissue (A,B).

(Figs 1,3A), as well as in inflammatory cells, primarily plasma cells (Fig. 3C) and lymphocytes, but not in polymorphonuclear cells (Fig. 3D) and, furthermore, in scattered fibroblast-like cells (Fig. 2) within the underlying gingival connective tissue. In contrast, syndecan-1 expression was absent to weak (mean values ranging from 0 to 1) in the internal basal lamina, the external basal lamina and the matrix of the gingival connective tissue (Table 1 and Figs 1,2,3A).

As demonstrated in Table 1, in study group 2 the expression of syndecan-1 was weak to moderate (mean values ranging from 1 to 2) in the suprabasal and basal cells of the oral gingival epithelium. In contrast, syndecan-1 expression was absent to weak (mean values ranging from 0 to 1) in the internal basal lamina, the external basal lamina and the inflammatory cells and matrix of the gingival connective tissue. Compared with the corresponding values in study group 1, the expression of syndecan-1 in study group 2 was less intense in the basal cells of the oral gingival epithelium, as well as in the inflammatory cells located within the underlying gingival connective tissue (Table 1).

At a cellular level, positive syndecan-1 immunostaining was observed predominantly in the cell membrane, as expected, and to a lesser degree within the cytoplasm, but not in the nucleus of cells (Figs 1A,B,C,3C).

Results of statistical analysis (Tables 1–3)

A statistically significant difference ($p < 0.05$) in syndecan-1 expression between the two study groups was demonstrated exclusively in the inflammatory cells within the gingival connective tissue, but not ($p > 0.05$) in other components of gingival tissues (Table 1). As demonstrated in Table 1, a statistically significant ($p < 0.05$) difference in the severity/degree of histological gingival/periodontal inflammation (−, +, ++ and +++) was revealed between the two study groups, constituting a major intergroup difference.

All feasible intragroup statistical comparisons among components of gingival tissues for syndecan-1 expression are summarized in Table 2. Differences between the above-mentioned components of gingival tissues with weak-to-moderate expression, on the one hand, and components of gingival tissues with absent-to-weak expression, on the other hand, reached statistical significance at varying levels ($p < 0.05$, $p < 0.01$ or $p < 0.001$; Table 2).

A statistically significant positive correlation was demonstrated between the expression of syndecan-1 and the following factors (Table 3):

- Patient age, in study group 2, exclusively in the matrix of the gingival connective tissue ($p \leq 0.01$)
- Severity/degree of histological gingival/periodontal inflammation (−, +, ++ and +++), in study group 1 and in the internal basal lamina ($p < 0.05$) and the inflammatory cells located within the gingival connective tissue ($p \leq 0.001$), as well as in study group 2 and in the suprabasal ($p < 0.001$) and basal cells ($p < 0.05$) of the oral gingival epithelium and in the inflammatory cells within the underlying gingival connective tissue ($p < 0.01$)
- Full-mouth probing pocket depth or full-mouth clinical attachment level, in study group 1, exclusively in the internal basal lamina ($p < 0.05$)

A statistically significant negative correlation was found between the expression of syndecan-1 and local probing pocket depth or local clinical attachment level ($r = -0.39$ and $r = -0.44$, respectively) in study group 1, exclusively in the matrix of the gingival connective tissue ($p < 0.05$).

Table 1. Intergroup statistical comparisons, using Mann–Whitney *U*-test, for the severity/degree of histological gingival inflammation and for syndecan-1 expression in each component of gingival tissues (A–F)

Study group	HGI (0–3)	S1E (0–3) A	S1E (0–3) B	S1E (0–3) C	S1E (0–3) D	S1E (0–3) E	S1E (0–3) F
SG1 (n = 30)	1.9 ± 0.9 & 2 (0,3)	1.8 ± 0.5 & 2 (1,3)	1.2 ± 0.9 & 1 (0,3)	0.5 ± 1.0 & 0 (0,3)	0.0 ± 0.1 & 0 (0,1)	1.4 ± 1.0 & 2 (0,3)	0.2 ± 0.4 & 0 (0,1)
SG2 (n = 30)	1.3 ± 0.9 & 1 (0,3)	1.9 ± 0.4 & 2 (1,3)	1.0 ± 0.6 & 1 (0,2)	C does not exist in SG2	0 ± 0 & 0 (0,0)	0.8 ± 0.8 & 1 (0,2)	0.2 ± 0.4 & 0 (0,1)
p value	0.012[a]	0.5025 (n.s.)	0.4042 (n.s.)	No statistical test (C does not exist in SG2)	No statistical test (data set = 0 in SG2)	0.0334[a]	0.9939 (n.s.)
INTRA-AGR (κ score) for SG1	Examiner 1[b]: κ = 0.952 Examiner 2[c]: κ = 0.952	Examiner 1[b]: κ = 0.923 Examiner 2[c]: κ = 0.862	Examiner 1[b]: κ = 0.951 Examiner 2[c]: κ = 0.899	Examiner 1[b]: κ = 0.860 Examiner 2[c]: κ = 0.726	Examiner 1[b]: κ = 1.000 Examiner 2[c]: κ = 1.000	Examiner 1[b]: κ = 0.954 Examiner 2[c]: κ = 0.909	Examiner 1[b]: κ = 0.902 Examiner 2[c]: κ = 0.902
INTRA-AGR (κ score) for SG2	Examiner 1[b]: κ = 0.904 Examiner 2[c]: κ = 0.906	Examiner 1[b]: κ = 0.841 Examiner 2[c]: κ = 0.718	Examiner 1[b]: κ = 0.863 Examiner 2[c]: κ = 0.788	C does not exist in SG2	Examiner 1[b]: κ = 1.000 Examiner 2[c]: κ = 1.000	Examiner 1[b]: κ = 0.948 Examiner 2[c]: κ = 0.895	Examiner 1[b]: κ = 1.000 Examiner 2[c]: κ = 1.000
INTER-AGR (κ score) for SG1	κ = 0.904	κ = 0.788	κ = 0.899	κ = 0.725	κ = 1.000	κ = 0.863	κ = 0.902
INTER-AGR (κ score) for SG2	κ = 0.905	κ = 0.841	κ = 0.925	C does not exist in SG2	κ = 1.000	κ = 0.948	κ = 1.000

Values are expressed as means ± SD and median (minimum, maximum value), respectively.

Abbreviations: A, suprabasal cells of junctional/pocket epithelium (in SG1) or gingival oral epithelium (in SG2); B, basal cells of junctional/pocket epithelium (in SG1) or gingival oral epithelium (in SG2); C, internal basal lamina (towards tooth surface); D, external basal lamina (towards gingival connective tissue); E, inflammatory cells within gingival connective tissue; F, gingival connective tissue matrix; n.s., no statistically significant difference ($p > 0.05$) between SG1 and SG2; HGI, severity/degree of histological gingival inflammation (–, 0; +, 1; + +, 2; + + +, 3); INTER-AGR, inter-examiner agreement; INTRA-AGR, intra-examiner agreement; S1E, syndecan-1 expression, as represented by the four-grade intensity of immunohistochemical staining (–, 0; +, 1; + +, 2; + + +, 3); n, number of patients included in SG1 or SG2; SG1, study group 1; and SG2, study group 2.

[a] Statistically significant difference ($p \leq 0.05$) between SG1 and SG2.

[b] Examiner 1 was the author S.T.-B.

[c] Examiner 2 was the author S.K.

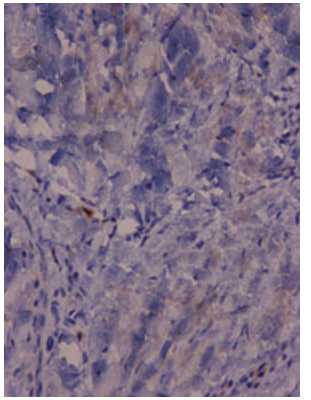

Fig. 2. Specific immunohistochemical staining for syndecan-1 in fibroblast-like cells scattered within the gingival connective tissue under the junctional epithelium, on a slide derived from study group 1 (magnification ×400). The staining within the matrix of the gingival connective tissue appears to be diffuse, indicating a weak expression of syndecan-1.

In both study groups, no statistically significant correlation ($p > 0.05$) was revealed between the expression of syndecan-1 and patient smoking status in any component of the gingival tissues (Table 3). As initially anticipated, the amount of data finally acquired in relation to any specific patient systemic disease or drug administered was not sufficient for a reliable statistical evaluation of a potential correlation between syndecan-1 expression and a certain systemic disease or drug.

Discussion

The present study demonstrates the expression of syndecan-1 in human gingival epithelial cells, both in the area of the junctional/pocket epithelium (study group 1) and in the oral gingival epithelium (study group 2) in chronic periodontitis patients.

Previous studies have reported syndecan-1 expression for the non-inflamed junctional epithelium in the rat (7) and the mouse (8) and the inflamed junctional/pocket epithelium in chronic periodontitis patients (21) and for the non-inflamed oral gingival epithelium in the rat (5), the mouse (8) and periodontally healthy subjects (15,20,23,24), as well as in the clinically healthy oral gingival epithelium (i.e. without clinical signs of gingival/periodontal inflammation but with minimal gingival/periodontal inflammation at

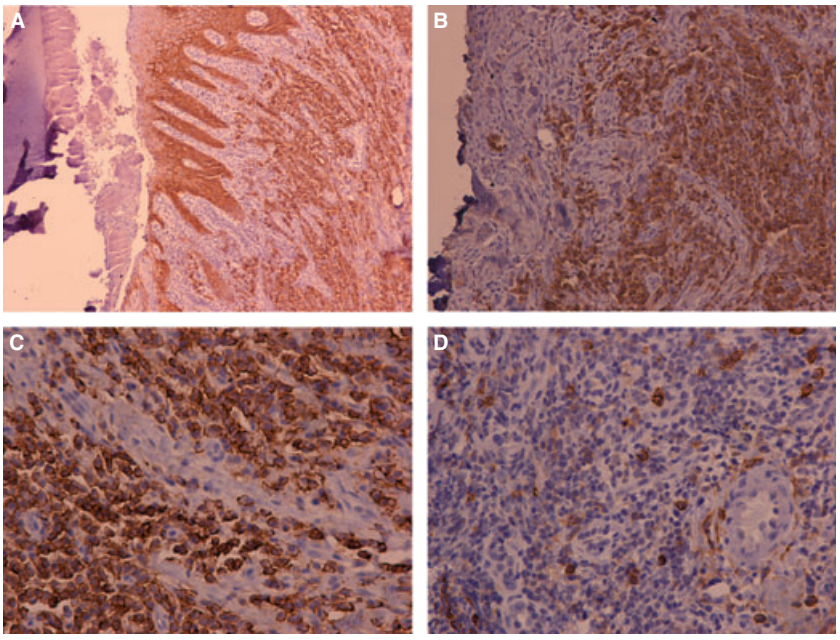

Fig. 3. (A) Specific immunohistochemical staining for syndecan-1 in the region of the junctional epithelium towards the tooth surface (left), on a slide derived from study group 1 (magnification ×100). Characteristic intense rete pegs are visible, indicating a high degree of gingival inflammation, and the expression of syndecan-1 also appears to be intense. (B) Same slide, higher magnification (magnification ×200). (C) Intense specific immunohistochemical staining for syndecan-1 in plasma cells of the inflammatory infiltrate within the gingival connective tissue, on a slide of the same gingival specimen (magnification ×400). (D) Absence of specific immunohistochemical staining for syndecan-1 in polymorphonuclear cells and vascular endothelium, on a slide of the same gingival specimen (magnification ×400).

Table 2. Intragroup statistical comparisons, using Friedman test (non-parametric repeated-measures ANOVA) with Dunn's *post hoc* test, among components of gingival tissues for syndecan-1 expression in each group separately

CGTs compared	SG1 ($n = 30$)	SG2 ($n = 30$)
A vs. B	$p > 0.05$ (n.s.)	$p < 0.01$ (**)
A vs. C	$p < 0.001$ (***)	No statistical test (C does not exist in SG2)
A vs. D	$p < 0.001$ (***)	$p < 0.001$ (***)
A vs. E	$p > 0.05$ (n.s.)	$p < 0.001$ (***)
A vs. F	$p < 0.001$ (***)	$p < 0.001$ (***)
B vs. C	$p < 0.05$ (*)	No statistical test (C does not exist in SG2)
B vs. D	$p < 0.001$ (***)	$p < 0.001$ (***)
B vs. E	$p > 0.05$ (n.s.)	$p > 0.05$ (n.s.)
B vs. F	$p < 0.01$ (**)	$p < 0.01$ (**)
C vs. D	$p > 0.05$ (n.s.)	No statistical test (C does not exist in SG2)
C vs. E	$p < 0.05$ (*)	No statistical test (C does not exist in SG2)
C vs. F	$p > 0.05$ (n.s.)	No statistical test (C does not exist in SG2)
D vs. E	$p < 0.001$ (***)	$p < 0.05$ (*)
D vs. F	$p > 0.05$ (n.s.)	$p > 0.05$ (NS)
E vs. F	$p < 0.01$ (**)	$p > 0.05$ (NS)

Abbreviations: CGTs, components of gingival tissues; otherwise, as in Table 1.
* Statistically significant difference ($p \leq 0.05$).
** Statistically significant difference ($p \leq 0.01$).
*** Statistically significant difference ($p \leq 0.001$).

the histological level) in periodontally clinically healthy subjects (22) and in chronic periodontitis patients (21) and, finally, the inflamed oral gingival epithelium in gingivitis and chronic periodontitis patients (22).

The observation that in both study group 1 (Figs 1, 3A) and study group 2, the expression of syndecan-1 was close to moderate in the suprabasal cells, but close to weak in the basal cells, i.e. was higher in the suprabasal cells, could be attributed to the fact that syndecan-1 is induced during keratinocyte differentiation. A previous immunohistochemical study on human keratinocytes of the oral gingival epithelium (21) also reported that differentiating suprabasal keratinocytes demonstrated intense immunoreactivity for syndecan-1, whereas basal cells immunostained rather weakly. Similar results have been reported by other studies, as well (5,33).

In the present study, syndecan-1 was weakly expressed in the internal basal lamina (in study group 1) but demonstrated no expression at all in the external basal lamina, either in the vicinity of the junctional/pocket epithelium (study group 1) or of the oral gingival epithelium (study group 2). In rat gingival tissues, no immunohistochemical staining at all was observed for syndecan-1 in either the internal or the external basal lamina (5). During tooth development in the mouse, no immunostaining was detected in the inner layer of non-inflamed newly forming junctional epithelium, i.e. against the internal basal lamina, derived from reduced ameloblasts in all ages (stages of tooth eruption) examined (8).

Furthermore, in the present study in both study groups, weak-to-moderate syndecan-1 expression was observed in inflammatory cells, primarily plasma cells (Fig. 3C) and lymphocytes, but not in polymorphonuclear cells (Fig. 3D), in contrast to a previous immunohistochemical study (7) in rat gingival tissues during a postsurgical wound-healing process, in which weakly positive immunostaining for syndecan-1 was observed on B-lymphocytes, but also on polymorphonuclear cells. In agreement with the findings of this study, a previous study (21) demonstrated that syndecan-1 was strongly expressed in extravasating and infiltrating leukocytes. The expression of syndecan-1 in B-lymphocytes/plasma cells (21,22), but not in

Table 3. Intragroup statistical correlations for both study groups, using Spearman's non-parametric correlation test, between syndecan-1 expression in each component of gingival tissues (A–F) and various putative factors

Putative factors	A	B	C	D	E	F
Patient age						
SG1 (n = 30)	r = −0.29	r = −0.10	r = −0.24	r = −0.09	r = 0.02	r = −0.12
(r (95% CI)	(−0.60 to 0.09)	(−0.46 to 0.28)	(−0.56 to 0.14)	(−0.44 to 0.29)	(−0.36 to 0.38)	(−0.47 to 0.26)
& p value)	p = 0.122 (n.s.)	p = 0.585 (n.s.)	p = 0.196 (n.s.)	p = 0.652 (n.s.)	p = 0.932 (n.s.)	p = 0.517 (n.s.)
SG2 (n = 30)	r = −0.19	r = 0	No statistical test	No statistical test	r = −0.20	r = 0.46
(r (95% CI)	(−0.53 to 0.19)	(−0.37 to 0.37)	(C does not exist in SG2)	(data set = 0 in SG2)	(−0.53 to 0.18)	(0.10 to 0.72)
& p value)	p = 0.304 (n.s.)	p = 0.978 (n.s.)			p = 0.282 (n.s.)	p = 0.010 (**)
Patient smoking status						
SG1 (n = 30)	r = 0.16	r = −0.12	r = −0.11	r = 0.33	r = 0	r = 0.22
(r (95% CI)	(−0.22 to 0.50)	(−0.47 to 0.27)	(−0.46 to 0.27)	(−0.04 to 0.63)	(−0.37 to 0.37)	(−0.16 to 0.55)
& p value)	p = 0.387 (n.s.)	p = 0.544 (n.s.)	p = 0.564 (n.s.)	p = 0.072 (n.s.)	p = 0.984 (n.s.)	p = 0.240 (n.s.)
SG2 (n = 30)	r = −0.24	r = −0.13	No statistical test	No statistical test	r = −0.02	r = −0.23
(r (95% CI)	(−0.56 to 0.14)	(−0.47 to 0.26)	(C does not exist in SG2)	(data set = 0 in SG2)	(−0.39 to 0.35)	(−0.55 to 0.15)
& p value)	p = 0.193 (n.s.)	p = 0.508 (n.s.)			p = 0.924 (n.s.)	p = 0.223 (n.s.)
Severity/degree of histological gingival/periodontal inflammation (−, +, ++, +++)						
SG1 (n = 30)	r = 0.21	r = 0.23	r = 0.38	r = −0.22	r = 0.69	r = 0.04
(r (95% CI)	(−0.17 to 0.54)	(−0.16 to 0.55)	(0.01 to 0.66)	(−0.54 to 0.17)	(0.44 to 0.85)	(−0.34 to 0.40)
& p value)	p = 0.261 (n.s.)	p = 0.230 (n.s.)	p = 0.037 (*)	p = 0.251 (n.s.)	p < 0.001 (***)	p = 0.839 (n.s.)
SG2 (n = 30)	r = 0.56	r = 0.40	No statistical test	No statistical test	r = 0.50	r = 0.25
(r (95% CI)	(0.24 to 0.77)	(0.03 to 0.67)	(C does not exist in SG2)	(data set = 0 in SG2)	(0.16 to 0.73)	(−0.13 to 0.57)
& p value)	p = 0.001 (***)	p = 0.029 (*)			p = 0.005 (**)	p = 0.179 (n.s.)
Severity/degree of clinical gingival/periodontal inflammation (full-mouth bleeding score)						
SG1 (n = 30)	r = −0.10	r = −0.30	r = 0.12	r = 0.03	r = 0.06	r = 0.12
(r (95% CI)	(−0.45 to 0.28)	(−0.61 to 0.07)	(−0.26 to 0.47)	(−0.34 to 0.40)	(−0.31 to 0.42)	(−0.26 to 0.47)
& p value)	p = 0.609 (n.s.)	p = 0.103 (n.s.)	p = 0.533 (n.s.)	p = 0.865 (n.s.)	p = 0.737 (n.s.)	p = 0.529 (n.s.)
SG2 (n = 30)	r = 0.03	r = 0.13	No statistical test	No statistical test	r = −0.07	r = −0.03
(r (95% CI)	(−0.35 to 0.40)	(−0.25 to 0.48)	(C does not exist in SG2)	(data set = 0 in SG2)	(−0.43 to 0.31)	(−0.40 to 0.34)
& p value)	p = 0.879 (n.s.)	p = 0.498 (n.s.)			p = 0.725 (n.s.)	p = 0.865 (n.s.)
Full-mouth probing pocket depth						
SG1 (n = 30)	r = 0.25	r = 0.08	r = 0.39	r = −0.01	r = 0.28	r = −0.02
(r (95% CI)	(−0.13 to 0.57)	(−0.29 to 0.44)	(0.07 to 0.67)	(−0.40 to 0.36)	(−0.11 to 0.59)	(−0.39 to 0.35)
& p value)	p = 0.176 (n.s.)	p = 0.656 (n.s.)	p = 0.032 (*)	p = 0.952 (n.s.)	p = 0.139 (n.s.)	p = 0.899 (n.s.)
SG2 (N = 30)	r = 0.14	r = 0.05	No statistical test	No statistical test	r = −0.16	r = −0.08
(r (95% CI)	(−0.25 to 0.48)	(−0.33 to 0.41)	(C does not exist in SG2)	(data set = 0 in SG2)	(−0.50 to 0.22)	(−0.44 to 0.30)
& p value)	p = 0.476 (n.s.)	p = 0.802 (n.s.)			p = 0.396 (n.s.)	p = 0.679 (n.s.)

Table 3. (Continued)

Full-mouth clinical attachment level						
SG1 (n = 30)	r = 0.25	r = 0.08	r = 0.39	r = −0.01	r = 0.28	r = −0.02
(r (95% CI)	(−0.13 to 0.57)	(−0.29 to 0.44)	(0.07 to 0.67)	(−0.40 to 0.36)	(−0.11 to 0.59)	(−0.39 to 0.35)
& p value)	p = 0.176 (n.s.)	p = 0.656 (n.s.)	p = 0.032 (*)	p = 0.952 (n.s.)	p = 0.139 (n.s.)	p = 0.899 (n.s.)
SG2 (n = 30)	r = 0.15	r = 0.04	No statistical test	No statistical test	r = −0.15	r = −0.01
(r (95% CI)	(−0.24 to 0.49)	(−0.33 to 0.41)	(C does not exist in SG2)	(data set = 0 in SG2)	(−0.50 to 0.23)	(−0.45 to 0.28)
& p value)	p = 0.439 (n.s.)	p = 0.820 (n.s.)			p = 0.420 (n.s.)	p = 0.899 (n.s.)
Full-mouth radiographical bone loss						
SG1 (N = 30)	r = 0.22	r = 0.00	r = −0.02	r = −0.12	r = 0.17	r = −0.04
(r (95% CI)	(−0.16 to 0.55)	(−0.37 to 0.37)	(−0.39 to 0.36)	(−0.47 to 0.26)	(−0.21 to 0.51)	(−0.40 to 0.34)
& p value)	p = 0.242 (n.s.)	p = 0.991 (n.s.)	p = 0.927 (n.s.)	p = 0.532 (n.s.)	p = 0.359 (n.s.)	p = 0.848 (n.s.)
SG2 (N = 30)	r = 0.22	r = −0.07	No statistical test	No statistical test	r = −0.13	r = −0.07
(r (95% CI)	(−0.16 to 0.55)	(−0.42 to 0.31)	(C does not exist in SG2)	(data set = 0 in SG2)	(−0.48 to 0.25)	(−0.43 to 0.31)
& p value)	p = 0.242 (n.s.)	p = 0.733 (n.s.)			p = 0.492 (n.s.)	p = 0.718 (n.s.)
Local probing pocket depth						
SG1 (n = 30)	r = 0.27	r = −0.11	r = 0.20	r = −0.23	r = 0.20	r = −0.39
(r (95% CI)	(−0.11 to 0.59)	(−0.46 to 0.27)	(−0.19 to 0.53)	(−0.55 to 0.15)	(−0.19 to 0.53)	(−0.66 to 0.02)
& p value)	p = 0.142 (n.s.)	p = 0.568 (n.s.)	p = 0.296 (n.s.)	p = 0.220 (n.s.)	p = 0.315 (n.s.)	p = 0.035 (*)
Local clinical attachment level						
SG1 (n = 30)	r = 0.15	r = −0.17	r = 0.12	r = −0.24	r = 0.11	r = −0.44
(r (95% CI)	(−0.23 to 0.49)	(−0.51 to 0.21)	(−0.26 to 0.47)	(−0.56 to 0.14)	(−0.27 to 0.46)	(−0.69 to −0.08)
& p value)	p = 0.428 (n.s.)	p = 0.357 (n.s.)	p = 0.525 (n.s.)	p = 0.205 (n.s.)	p = 0.578 (n.s.)	p = 0.016 (*)
Local radiographical bone loss						
SG1 (n = 30)	r = 0.28	r = −0.05	r = 0.13	r = −0.13	r = 0.11	r = −0.35
(r (95% CI)	(−0.10 to 0.59)	(−0.41 to 0.33)	(−0.26 to 0.48)	(−0.48 to 0.25)	(−0.27 to 0.46)	(−0.64 to 0.03)
& p value)	p = 0.128 (n.s.)	p = 0.801 (n.s.)	p = 0.503 (n.s.)	p = 0.496 (n.s.)	p = 0.550 (n.s.)	p = 0.060 (n.s.)

Abbreviations: CI, confidence intervals; r, correlation coefficient; otherwise, as in Table 1.

* Statistically significant difference ($p \leq 0.05$).

** Statistically significant difference ($p \leq 0.01$).

*** Statistically significant difference ($p \leq 0.001$).

T-lymphocytes (22), within the gingival tissues of gingivitis (22) and chronic periodontitis patients (21,22) has been previously reported. Syndecan-1 expression has been proposed as a marker for plasma cells in chronic endometritis (34).

In the present study, weak-to-moderate (in study group 1, area of the junctional/pocket epithelium; Fig. 2) or weak syndecan-1 expression (in study group 2, area of the oral gingival epithelium) was observed in fibroblast-like cells scattered within the gingival connective tissue. Syndecan-1 was, in general, moderately expressed in active rat gingival connective tissue fibroblast-like cells in the area beneath newly forming long junctional epithelium during postsurgical wound healing (7), in mature non-inflamed rat fibroblasts of the gingival connective tissue (5) or in inflamed human gingival fibroblasts (17–19). Especially in the area underlying the junctional epithelium and close to or attached to the tooth surface, syndecan-1 immunostaining was intense and extensive, suggesting a potential role in cell–cell and cell–matrix interactions in this area (5). However, a study in chronic periodontitis patients (21) failed to immunolocalize syndecan-1 in gingival connective tissue fibroblasts beneath clinically healthy oral gingival epithelium (i.e. without clinical signs of gingival/periodontal inflammation but with minimal gingival/periodontal inflammation at the histological level).

Syndecan-1 expression within the matrix of the gingival connective tissue in chronic periodontitis patients was weak in the present study in both study group 1 (Fig. 2) and study group 2, as reported previously in the rat for the matrix of the gingival connective tissue beneath newly forming junctional epithelium during postsurgical wound healing (7). Moderate syndecan-1 expression within mature non-inflamed rat gingival connective tissue matrix has been previously observed (5).

In general, in the vast majority of the components of gingival tissues examined, it appeared that patient demographic (age) and behavioural parameters (smoking) had no statistically significant correlation with syndecan-1 expression. Similarly, a previous immunohistochemical study (21) reported no clear differences in the results in relation to patient age or sex.

The present study demonstrated that the severity/degree of histological gingival/periodontal inflammation is a factor that can significantly affect syndecan-1 expression, at least in certain components of gingival tissues. On the contrary, generally it appeared that full-mouth clinical (full-mouth probing pocket depth and full-mouth clinical attachment level) and radiographical periodontal parameters (full-mouth radiographical bone loss) were not statistically significantly correlated with the expression of syndecan-1. These findings might be interpreted by the fact that gingival specimens were collected from the areas of teeth with poor prognosis, which inevitably could not be representative of the periodontal status of the entire dentition, or that chronic periodontitis is a site-specific inflammatory disease (35).

The results of this study demonstrated a statistically significant difference in syndecan-1 expression between the two study groups exclusively in the inflammatory cells within the gingival connective tissue (Table 1). Taking into account that the severity/degree of histological gingival/periodontal inflammation constituted a major, statistically significant difference between the two study groups, this finding could be explained by the fact that the severity/degree of histological gingival/periodontal inflammation was positively correlated with syndecan-1 expression in the inflammatory cells within the gingival connective tissue in both study groups.

The finding that in study group 1 a statistically significant negative correlation between the expression of syndecan-1 and local probing pocket depth was found in the matrix of the gingival connective tissue might appear intriguing, because deeper periodontal pockets are usually associated with an increased severity/degree of gingival/periodontal inflammation (36), which in turn might show a significant positive correlation with syndecan-1 expression, as documented above. A plausible explanation of this inverse correlation between syndecan-1 expression and local probing pocket depth could be that specific conditions within the ecological environment of deep pockets, such as increased concentrations of inflammatory molecules, might negatively affect syndecan-1 expression.

Even though the two study groups included in the present design follow a previously published design (21), nonetheless the inclusion of a third study group, comprising periodontally healthy individuals, could have aided in the clarification of the effect of periodontal/gingival inflammation on syndecan-1 expression to a greater extent.

Taking into account that syndecan-1 is a cell adhesion molecule (2,13) and is expressed in the junctional/pocket epithelium, as demonstrated in the present study, the induction of syndecan-1 overexpression could promote the epithelial attachment to the tooth surface, thereby potentially having periodontal therapeutic applications. Another study (8) has also suggested the potential of using syndecans in therapeutic applications, such as tissue engineering. The conduction of related *in vitro* studies would be of particular interest in the future.

Conclusions

Within the limits of the present immunohistochemical study, the following conclusions may be drawn with regard to chronic periodontitis patients.

- In the area either of the junctional/pocket epithelium or the gingival oral epithelium, syndecan-1 expression is weak to moderate in the suprabasal and basal epithelial cells and absent to weak in the internal basal lamina, the external basal lamina and the matrix of the gingival connective tissue.
- Compared with syndecan-1 expression in the area of the gingival oral epithelium, the corresponding syndecan-1 expression in the area of the junctional epithelium is significantly stronger in the inflammatory cells located within the underlying gingival connective tissue (primarily plasma cells and lymphocytes) and in scattered fibroblast-like cells.

- Syndecan-1 expression in the area of either the junctional or the gingival oral epithelium can show a significant positive correlation with the severity/degree of local gingival inflammation at the histological level, but in general, in the vast majority of components of gingival tissues, is not significantly correlated with age, smoking, full-mouth and local clinical (probing pocket depth and clinical attachment level) and radiographical parameters (radiographical bone loss) of periodontal status.

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