REFERENCES

Ahmet, Z., Warren, M., and Houang, E. T. (1995). Species identification of members of the Streptococcus milleri group isolated from the vagina by ID 32 Strep system and differential phenotypic characteristics. **J Clin Microbiol** 33, 1592-1595.

Al-Hendy, A., Toivanen, P., and Skurnik, M. (1991). Rapid method for isolation and staining of bacterial lipopolysaccharide. **Microbiol Immunol** 35, 331-333.

Aimes, R. T., and Quigley, J. P. (1995). Matrix metalloproteinase-2 is an interstitial collagenase. Inhibitor-free enzyme catalyzes the cleavage of collagen fibrils and soluble native type I collagen generating the specific 3/4- and 1/4-length fragments. **J Biol Chem** 270, 5872-5876.

Asensi, V., and Fierer, J. (1991). Synergistic effect of human lysozyme plus ampicillin or beta-lysin on the killing of Listeria monocytogenes. **J Infect Dis** 163, 574-578.

Attstrom, R., and Egelberg, J. (1970). Emigration of blood neutrophils and monocytes into the gingival crevices. **J Periodontal Res** 5, 48-55.

Azzam, H. S., and Thompson, E. W. (1992). Collagen-induced activation of the M(r) 72,000 type IV collagenase in normal and malignant human fibroblastoid cells. **Cancer Res 52**, 4540-4544.

Banbula, A., Bugno, M., Goldstein, J., Yen, J., Nelson, D., Travis, J., and Potempa, J. (2000). Emerging family of proline-specific peptidases of Porphyromonas gingivalis: purification and characterization of serine dipeptidyl peptidase, a structural and functional homologue of mammalian prolyl dipeptidyl peptidase IV. **Infect Immun** 68, 1176-1182.

Banbula, A., Mak, P., Smoluch, M., Travis, J., Potempa, J., Bugno, M., Silberring, J., Dubin, A., and Nelson, D. (2001). Arginine-specific cysteine proteinase from porphyromonas gingivalis as a convenient tool in protein chemistry prolyl tripeptidyl peptidase from Porphyromonas gingivalis. A novel enzyme with possible pathological implications for the development of periodontitis. **Biol Chem** 382, 1399-1404.

Bartold, P.M., and Narayanan, A.S. (1998). Biology of the periodontal connective tissue. Illinois. Quintessence.

Birkedal-Hansen, H. (1993a). Role of cytokines and inflammatory mediators in tissue destruction. **J Periodontal Res** 28, 500-510.

Birkedal-Hansen, H. (1993b). Role of matrix metalloproteinases in human periodontal diseases. **J Periodontol** 64, 474-484.

Birkedal-Hansen, H., Moore, W. G., Bodden, M. K., Windsor, L. J., Birkedal-Hansen, B., DeCarlo, A., and Engler, J. A. (1993). Matrix metalloproteinases: a review. **Crit Rev Oral Biol Med** 4, 197-250.

Bramanti, T. E., and Holt, S. C. (1991). Roles of porphyrins and host iron transport proteins in regulation of growth of Porphyromonas gingivalis W50. **J Bacteriol** 173, 7330-7339.

Brown, D. J., Bishop, P., Hamdi, H., and Kenney, M. C. (1996). Cleavage of structural components of mammalian vitreous by endogenous matrix metalloproteinase-2. **Curr Eye Res** 15, 439-445.

Bulkacz, J., Erbland, J. F., and MacGregor, J. (1981). Phospholipase A activity in supernatants from cultures of Bacteroides melaninogenicus. **Biochim Biophys Acta** 664, 148-155.

Carlsson, J., Herrmann, B. F., Hofling, J. F., and Sundqvist, G. K. (1984a). Degradation of the human proteinase inhibitors alpha-1-antitrypsin and alpha-2-macroglobulin by Bacteroides gingivalis. **Infect Immun** 43, 644-648.

Carlsson, J., Hofling, J. F., and Sundqvist, G. K. (1984b). Degradation of albumin, haemopexin, haptoglobin and transferrin, by black-pigmented Bacteroides species. **J Med Microbiol** 18, 39-46.

Han, Y. P., Tuan, T. L., Wu, H., Hughes, M., and Garner, W. L. (2001). TNF-alpha stimulates activation of pro-MMP2 in human skin through NF-(kappa)B mediated induction of MT1-MMP. **J Cell Sci** 114, 131-139.

Caton, J., Bouwsma, O., Polson, A., and Espeland, M. (1989). Effects of personal oral hygiene and subgingival scaling on bleeding interdental gingiva. **J Periodontol** 60, 84-90.

Chang, Y. C., Yang, S. F., Lai, C. C., Liu, J. Y., and Hsieh, Y. S. (2002). Regulation of matrix metalloproteinase production by cytokines, pharmacological agents and periodontal pathogens in human periodontal ligament fibroblast cultures. **J Periodontal Res** 37, 196-203.

Chen, H. A., Johnson, B. D., Sims, T. J., Darveau, R. P., Moncla, B. J., Whitney, C. W., Engel, D., and Page, R. C. (1991). Humoral immune responses to Porphyromonas gingivalis before and following therapy in rapidly progressive periodontitis patients. **J Periodontol** 62, 781-791.

Choi, B. K., Jung, J. H., Suh, H. Y., Yoo, Y. J., Cho, K. S., Chai, J. K., and Kim, C. K. (2001). Activation of matrix metalloproteinase-2 by a novel oral spirochetal species Treponema lecithinolyticum. **J Periodontol** 72, 1594-1600.

Choi, B. K., Lee, H. J., Kang, J. H., Jeong, G. J., Min, C. K., and Yoo, Y. J. (2003). Induction of osteoclastogenesis and matrix metalloproteinase expression by the lipooligosaccharide of Treponema denticola. **Infect Immun** 71, 226-233.

Condorelli, F., Scalia, G., Cali, G., Rossetti, B., Nicoletti, G., and Lo Bue, A. M. (1998). Isolation of Porphyromonas gingivalis and detection of immunoglobulin A specific to fimbrial antigen in gingival crevicular fluid. **J Clin Microbiol** 36, 2322-2325.

Costerton, J. W., Lewandowski, Z., DeBeer, D., Caldwell, D., Korber, D., and James, G. (1994). Biofilms, the customized microniche. **J Bacteriol** 176, 2137-2142.

Crawford, J. M., Wilton, J. M., and Richardson, P. (2000). Neutrophils die in the gingival crevice, periodontal pocket, and oral cavity by necrosis and not apoptosis. **J Periodontol** 71, 1121-1129.

Curtis, M. A., Slaney, J. M., Carman, R. J., and Johnson, N. W. (1991). Identification of the major surface protein antigens of Porphyromonas gingivalis using IgG antibody reactivity of periodontal case-control serum. **Oral Microbiol Immunol** 6, 321-326.

Curtis, M. A., Aduse-Opoku, J., and Rangarajan, M. (2001). Cysteine proteases of Porphyromonas gingivalis. **Crit Rev Oral Biol Med** 12, 192-216.

Dahan, M., Nawrocki, B., Elkaim, R., Soell, M., Bolcato-Bellemin, A. L., Birembaut, P., and Tenenbaum, H. (2001). Expression of matrix metalloproteinases in healthy and diseased human gingiva. **J Clin Periodontol** 28, 128-136.

Darveau, R. P., Tanner, A., and Page, R. C. (2000). The microbial challenge in periodontitis. **Periodontol** 14, 12-32.

DeCarlo, A. A., Jr., Windsor, L. J., Bodden, M. K., Harber, G. J., Birkedal-Hansen, B., and Birkedal-Hansen, H. (1997). Activation and novel processing of matrix metalloproteinases by a thiol-proteinase from the oral anaerobe Porphyromonas gingivalis. **J Dent Res** 76, 1260-1270.

Ding, Y., Uitto, V. J., Firth, J., Salo, T., Haapasalo, M., Konttinen, Y. T., and Sorsa, T. (1995). Modulation of host matrix metalloproteinases by bacterial virulence factors relevant in human periodontal diseases. **Oral Dis** 1, 279-286.

Dzink, J. L., Socransky, S. S., and Haffajee, A. D. (1988). The predominant cultivable microbiota of active and inactive lesions of destructive periodontal diseases. **J Clin Periodontol** 15, 316-323.

Evans, R. T., Klausen, B., Sojar, H. T., Bedi, G. S., Sfintescu, C., Ramamurthy, N. S., Golub, L. M., and Genco, R. J. (1992). Immunization with Porphyromonas (Bacteroides) gingivalis fimbriae protects against periodontal destruction. **Infect Immun** 60, 2926-2935.

Firth, J. D., Putnins, E. E., Larjava, H., and Uitto, V. J. (1997). Bacterial phospholipase C upregulates matrix metalloproteinase expression by cultured epithelial cells. **Infect Immun** 65, 4931-4936.

Frank, R. M., and Voegel, J. C. (1978). Bacterial bone resorption in advanced cases of human periodontitis. **J Periodontal Res** 13, 251-261.

Fravalo, P., Menard, C., and Bonnaure-Mallet, M. (1996). Effect of Porphyromonas gingivalis on epithelial cell MMP-9 type IV collagenase production. **Infect Immun** 64, 4940-4945.

Freshney, R.I. (1994). Culture of animal cells: A manual of basic techniques. Third edition. New York. Wiley-Liss.

Fujimura, S., Shibata, Y., Hirai, K., and Nakamura, T. (1995). Some binding properties of the envelope of Porphyromonas gingivalis to hemoglobin. **FEMS Immunol Med Microbiol** 10, 109-114.

Garcia, A., Garcia, T., and Perez, J. L. (1997). Proline-aminopeptidase test for rapid screening of Clostridium difficile. **J Clin Microbiol** 35, 3007.

Gemmell, E., and Seymour, G. J. (1994). Modulation of immune responses to periodontal bacteria. **Curr Opin Periodontol**, 28-38.

Genco, R. J., Evans, R. T., and Ellison, S. A. (1969). Dental research in microbiology with emphasis on periodontal disease. **J Am Dent Assoc** 78, 1016-1036.

Gessani, S., Testa, U., Varano, B., Di Marzio, P., Borghi, P., Conti, L., Barberi, T., Tritarelli, E., Martucci, R., Seripa, D., and et al. (1993a). Enhanced production of LPS-induced cytokines during differentiation of human monocytes to macrophages. Role of LPS receptors. **J Immunol** 151, 3758-3766.

Gessani, S., Testa, U., Varano, B., Di Marzio, P., Borghi, P., Conti, L., Barberi, T., Tritarelli, E., Martucci, R., Seripa, D., et al. (1993b). Enhanced production of LPS-induced cytokines during differentiation of human monocytes to macrophages. Role of LPS receptors selective alteration of the turnover of interferon beta mRNA in peritoneal macrophages from LPS-hyporesponsive mice and its role in the defective expression of spontaneous interferon. **J Immunol** 151, 3758-3766.

Gilles, C., Polette, M., Seiki, M., Birembaut, P., and Thompson, E. W. (1997). Implication of collagen type I-induced membrane-type 1-matrix metalloproteinase expression and matrix metalloproteinase-2 activation in the metastatic progression of breast carcinoma. **Lab Invest** 76, 651-660.

Golub, L. M., Lee, H. M., Greenwald, R. A., Ryan, M. E., Sorsa, T., Salo, T., and Giannobile, W. V. (1997). A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. **Inflamm Res** 46, 310-319

Gomez, D. E., Alonso, D. F., Yoshiji, H., and Thorgeirsson, U. P. (1997). Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. **Eur J Cell Biol** 74, 111-122.

Graves, D. T., Nooh, N., Gillen, T., Davey, M., Patel, S., Cottrell, D., and Amar, S. (2001). IL-1 plays a critical role in oral, but not dermal, wound healing. **J Immunol** 167, 5316-5320.

Grenier, D., and McBride, B. C. (1989). Surface location of a Bacteroides gingivalis glycylprolyl protease. **Infect Immun** 57, 3265-3269.

Grenier, D., and Mayrand D. (2001). Inactivation of tissue inhibitor of metalloproteinase-1 (TIMP-1) by Porphyromonas gingivalis. **FEMS Micro Biol Letters** 203: 161-164.

Gutsmann, T., Muller, M., Carroll, S. F., MacKenzie, R. C., Wiese, A., and Seydel, U. (2001). Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. **Infect Immun** 69, 6942-6950.

Haffajee, A. D., and Socransky, S. S. (2000). Microbial etiological agents of destructive periodontal diseases. **Periodontol** 5, 78-111.

Han, Y. P., Tuan, T. L., Wu, H., Hughes, M., and Garner, W. L. (2001). TNF-alpha stimulates activation of pro-MMP2 in human skin through NF-(kappa)B mediated induction of MT1-MMP. **J Cell Sci** 114, 131-139.

Hancock, R. E. (1984). Alterations in outer membrane permeability. **Annu Rev Microbiol** 38, 237-264.

Hamada, S., Fujiwara, T., Morishima, S., Takahashi, I., Nakagawa, I., Kimura, S., and Ogawa, T. (1994). Molecular and immunological characterization of the fimbriae of Porphyromonas gingivalis. **Microbiol Immunol** 38, 921-930.

Hayashi, J., Masaka, T., Saito, I., and Ishikawa, I. (1996). Soluble CD14 mediates lipopolysaccharide-induced intercellular adhesion molecule 1 expression in cultured human gingival fibroblasts. **Infect Immun** 64, 4946-4951.

Hayashi, A., Shinohara, M., and Ohura, K. (1999). Effect of insulin on naturally occurring gingivitis rats with diabetes. **J Osaka Dent Univ** 33, 1-7.

Holt, S. C., and Bramanti, T. E. (1991). Factors in virulence expression and their role in periodontal disease pathogenesis. **Crit Rev Oral Biol Med** 2, 177-281.

Imamura, T., Pike, R. N., Potempa, J., and Travis, J. (1994). Pathogenesis of periodontitis: a major arginine-specific cysteine proteinase from Porphyromonas gingivalis induces vascular permeability enhancement through activation of the kallikrein/kinin pathway. **J Clin Invest** 94, 361-367.

Imamura, T., Potempa, J., Pike, R. N., Moore, J. N., Barton, M. H., and Travis, J. (1995). Effect of free and vesicle-bound cysteine proteinases of Porphyromonas gingivalis on plasma clot formation: implications for bleeding tendency at periodontitis sites. **Infect Immun** 63, 4877-4882.

Imatani, T., Kato, T., and Okuda, K. (2001). Production of inflammatory cytokines by human gingival fibroblasts stimulated by cell-surface preparations of Porphyromonas gingivalis. **Oral Microbiol Immunol** 16, 65-72.

Ingman, T., Sorsa, T., Michaelis, J., and Konttinen, Y. T. (1994). Immunohistochemical study of neutrophil- and fibroblast-type collagenases and stromelysin-1 in adult periodontitis. **Scand J Dent Res** 102, 342-349.

Isaac, L., Florido, M. P., Fecchio, D., and Singer, L. M. (1999). Murine alpha-2-macroglobulin increase during inflammatory responses and tumor growth. **Inflamm Res** 48, 446-452.

Ishida, H., Shinohara, H., Amabe, Y., Tojo, H., Nagata, T., and Wakano, Y. (1993). Effects of interleukin 1 beta, tumor necrosis factor alpha and transforming growth factor beta on group II phospholipase A2 activity in rat gingival fibroblasts. **J Periodontal Res** 28, 517-520.

Jeffcoat, M. K., and Reddy, M. S. (1991). A comparison of probing and radiographic methods for detection of periodontal disease progression. **Curr Opin Dent** 1, 45-51.

Jones, W. A., and O'Leary, T. J. (1978). The effectiveness of in vivo root planing in removing bacterial endotoxin from the roots of periodontally involved teeth. **J Periodontol** 49, 337-342.

Kiili, M., Cox, S. W., Chen, H. W., Wahlgren, J., Maisi, P., Eley, B. M., Salo, T., and Sorsa, T. (2002). Collagenase-2 (MMP-8) and collagenase-3 (MMP-13) in adult periodontitis: molecular forms and levels in gingival crevicular fluid and immunolocalisation in gingival tissue. **J Clin Periodontol** 29, 224-232.

Kilian, M. (1981). Degradation of immunoglobulins A2, A2, and G by suspected principal periodontal pathogens. **Infect Immun** 34, 757-765.

Kinder, S. A., and Holt, S. C. (1989). Characterization of coaggregation between Bacteroides gingivalis T22 and Fusobacterium nucleatum T18. **Infect Immun** 57, 3425-3433.

Kolenbrander, P. E., Andersen, R. N., and Moore, L. V. (1990). Intrageneric coaggregation among strains of human oral bacteria: potential role in primary colonization of the tooth surface. **Appl Environ Microbiol** 56, 3890-3894.

Komatsu, T., Kubota, E., and Sakai, N. (2001). Enhancement of matrix metalloproteinase (MMP)-2 activity in gingival tissue and cultured fibroblasts from Down's syndrome patients. **Oral Dis** 7, 47-55.

Kornman, K. S., Page, R. C., and Tonetti, M. S. (2000). The host response to the microbial challenge in periodontitis: assembling the players. **Periodontol** 14, 33-53.

Korostoff, J. M., Wang, J. F., Sarment, D. P., Stewart, J. C., Feldman, R. S., and Billings, P. C. (2000). Analysis of in situ protease activity in chronic adult periodontitis patients: expression of activated MMP-2 and a 40 kDa serine protease. **J Periodontol** 71, 353-360.

Kubota, T., Nomura, T., Takahashi, T., and Hara, K. (1996). Expression of mRNA for matrix metalloproteinases and tissue inhibitors of metalloproteinases in periodontitis-affected human gingival tissue. **Arch Oral Biol** 41, 253-262.

Kunimatsu, K., Mine, N., Kato, I., Hase, T., Aoki, Y., and Yamamoto, K. (1993). Possible functions of human neutrophil serine proteinases, medullasin and cathepsin G, in periodontal tissue breakdown. **J Periodontal Res** 28, 547-549.

Kurita-Ochiai, T., Fukushima, K., and Ochiai, K. (1997). Butyric acid-induced apoptosis of murine thymocytes, splenic T cells, and human Jurkat T cells. **Infect Immun** 65, 35-41.

Laemmli, U.K. (1970). Clevage of structural proteins during the assembly of the head of bacteriophage T4. **Nature** 227: 680-685.

Lah, T. T., and Kos, J. (1998). Cysteine proteinases in cancer progression and their clinical relevance for prognosis. **Biol Chem** 379, 125-130.

Lala, A., Amano, A., Sojar, H. T., Radel, S. J., and De Nardin, E. (1994). Porphyromonas gingivalis trypsin-like protease: a possible natural ligand for the neutrophil formyl peptide receptor. **Biochem Biophys Res Commun** 199, 1489-1496.

Lamont, R. J., Chan, A., Belton, C. M., Izutsu, K. T., Vasel, D., and Weinberg, A. (1995). Porphyromonas gingivalis invasion of gingival epithelial cells. **Infect Immun** 63, 3878-3885.

Lamont, R. J., and Jenkinson, H. F. (1998). Life below the gum line: pathogenic mechanisms of Porphyromonas gingivalis. **Microbiol Mol Biol Rev** 62, 1244-1263.

Lee, W., Aitken, S., Sodek, J., and McCulloch, C. A. (1995). Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: role of active enzyme in human periodontitis. **J Periodontal Res** 30, 23-33.

Lepine, G., and Progulske-Fox, A. (1996). Duplication and differential expression of hemagglutinin genes in Porphyromonas gingivalis. **Oral Microbiol Immunol** 11, 65-78.

Lertchirakarn, V., Birner, R., and Messer, H. H. (1998). Effects of interleukin-1 beta on human pulpal fibroblast proliferation and collagen synthesis. **J Endod** 24, 409-413.

Liljenberg, B., Lindhe, J., Berglundh, T., Dahlen, G., and Jonsson, R. (1994). Some microbiological, histopathological and immunohistochemical characteristics of progressive periodontal disease. **J Clin Periodontol** 21, 720-727.

Listgarten, M. A. (1986). Pathogenesis of periodontitis. **J Clin Periodontol** 13, 418-430.

Listgarten, M. A. (1987). Nature of periodontal diseases: pathogenic mechanisms. **J Periodontal Res** 22, 172-178.

Loos, B. G., and Dyer, D. W. (1992). Restriction fragment length polymorphism analysis of the fimbrillin locus, fimA, of Porphyromonas gingivalis. **J Dent Res** 71, 1173-1181.

Loos, B. G., Dyer, D. W., Whittam, T. S., and Selander, R. K. (1993). Genetic structure of populations of Porphyromonas gingivalis associated with periodontitis and other oral infections. **Infect Immun** 61, 204-212.

Loos, B. G., Mayrand, D., Genco, R. J., and Dickinson, D. P. (1990). Genetic heterogeneity of Porphyromonas (Bacteroides) gingivalis by genomic DNA fingerprinting. **J Dent Res** 69, 1488-1493.

Lorenz, E., Patel, D. D., Hartung, T., and Schwartz, D. A. (2002). Toll-like receptor 4 (TLR4)-deficient murine macrophage cell line as an in vitro assay system to show TLR4-independent signaling of Bacteroides fragilis lipopolysaccharide. **Infect Immun** 70, 4892-4896.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. **J Biol Chem** 193: 265-275.

Lukashev, M. E., and Werb, Z. (1998). ECM signalling: orchestrating cell behaviour and misbehaviour. Trends Cell Biol 8, 437-441.

Lynch, M. C., and Kuramitsu, H. K. (1999). Role of superoxide dismutase activity in the physiology of Porphyromonas gingivalis. **Infect Immun** 67, 3367-3375.

Makela, M., Salo, T., Uitto, V. J., and Larjava, H. (1994). Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. **J Dent Res** 73, 1397-1406.

Malek, R., Fisher, J. G., Caleca, A., Stinson, M., van Oss, C. J., Lee, J. Y., Cho, M. I., Genco, R. J., Evans, R. T., and Dyer, D. W. (1994). Inactivation of the Porphyromonas gingivalis fimA gene blocks periodontal damage in gnotobiotic rats. **J Bacteriol** 176, 1052-1059.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganism. **J Mol Biol** 3: 208-218.

Marsh, P. D., and Bradshaw, D. J. (1995). Dental plaque as a biofilm. **J Ind Microbiol** 15, 169-175.

Mayrand, D., and Holt, S. C. (1988). Biology of asaccharolytic black-pigmented Bacteroides species. **Microbiol Rev** 52, 134-152.

Mayrand, D., and Grenier, D. (1989). Biological activities of outer membrane vesicles. **Can J Microbiol** 35, 607-613.

Mayrand, D., and Holt, S. C. (1988). Biology of asaccharolytic black-pigmented Bacteroides species. **Microbiol Rev** 52, 134-152.

McCawley, L. J., and Matrisian, L. M. (2000). Matrix metalloproteinases: multifunctional contributors to tumor progression. **Mol Med Today** 6, 149-156.

McKee, A. S., McDermid, A. S., Baskerville, A., Dowsett, A. B., Ellwood, D. C., and Marsh, P. D. (1986). Effect of hemin on the physiology and virulence of Bacteroides gingivalis W50. **Infect Immun** 52, 349-355.

Menard, C., and Mouton, C. (1995). Clonal diversity of the taxon Porphyromonas gingivalis assessed by random amplified polymorphic DNA fingerprinting. **Infect Immun** 63, 2522-2531.

Merill, C.R., Goldmann, D., Sedmann, S.A., and Ebert, M.H. (1981). Utrasensitive stain for proteins in polyacrylamide gels shows reginal variations in serebrospinal fluid protein. **Science** 211,17-22.

Mitchell, T. I., Jeffrey, J. J., Palmiter, R. D., and Brinckerhoff, C. E. (1993). The acute phase reactant serum amyloid A (SAA3) is a novel substrate for degradation by the metalloproteinases collagenase and stromelysin. **Biochim Biophys Acta** 1156, 245-254.

Mouton, C., Ni Eidhin, D., Deslauriers, M., and Lamy, L. (1991). The hemagglutinating adhesin HA-Ag2 of Bacteroides gingivalis is distinct from fimbrilin. **Oral Microbiol Immunol** 6, 6-11.

Nagase, H. (1998). Cell surface activation of progelatinase A (proMMP-2) and cell migration. **Cell Res** 8, 179-186.

Nilsson, T., Carlsson, J., and Sundqvist, G. (1985). Inactivation of key factors of the plasma proteinase cascade systems by Bacteroides gingivalis. **Infect Immun** 50, 467-471.

Offenbacher, S. (1996). Periodontal diseases: pathogenesis. **Ann Periodontol** 1, 821-878.

Offenbacher, S., and Salvi, G. E. (1999). Induction of prostaglandin release from macrophages by bacterial endotoxin. **Clin Infect Dis** 28, 505-513.

Okada, H., and Murakami, S. (1998). Cytokine expression in periodontal health and disease. **Crit Rev Oral Biol Med** 9, 248-266.

Overall, C. M., and Sodek, J. (1987). Initial characterization of a neutral metalloproteinase, active on native 3/4-collagen fragments, synthesized by ROS 17/2.8 osteoblastic cells, periodontal fibroblasts, and identified in gingival crevicular fluid. **J Dent Res** 66, 1271-1282.

Overall, C. M., Wiebkin, O. W., and Thonard, J. C. (1987). Demonstration of tissue collagenase activity in vivo and its relationship to inflammation severity in human gingiva. **J Periodontal Res** 22, 81-88.

Page, R. C. (1986). Gingivitis. **J Clin Periodontol** 13, 345-359.

Page, R. C. (1995). Critical issues in periodontal research. **J Dent Res** 74, 1118-1128.

Page, R. C., and Beck, J. D. (1997). Risk assessment for periodontal diseases. **Int Dent J** 47, 61-87.

Page, R. C., and Kornman, K. S. (2000). The pathogenesis of human periodontitis: an introduction. **Periodontol** 14, 9-11.

Page, R. C., Narayanan, A. S., and Schroeder, H. E. (1980). Connective tissue composition and collagen synthesis in diseased and normal gingiva of adult dogs with spontaneous periodontitis. **Arch Oral Biol** 25, 727-736.

Paster, B. J., Boches, S. K., Galvin, J. L., Ericson, R. E., Lau, C. N., Levanos, V. A., Sahasrabudhe, A., and Dewhirst, F. E. (2001). Bacterial diversity in human subgingival plaque. **J Bacteriol** 183, 3770-3783.

Payne, W. A., Page, R. C., Ogilvie, A. L., and Hall, W. B. (1975). Histopathologic features of the initial and early stages of experimental gingivitis in man. **J Periodontal Res** 10, 51-64.

Potempa, J., Pavloff, N., and Travis, J. (1995). Porphyromonas gingivalis: a proteinase/gene accounting audit. **Trends Microbiol** 3, 430-434.

Progulske-Fox, A., Rao, V., Han, N., Lepine, G., Witlock, J., and Lantz, M. (1993). Molecular characterization of hemagglutinin genes of periodontopathic bacteria. **J Periodontal Res** 28, 473-474.

Pulyaeva, H., Bueno, J., Polette, M., Birembaut, P., Sato, H., Seiki, M., and Thompson, E. W. (1997). MT1-MMP correlates with MMP-2 activation potential seen after epithelial to mesenchymal transition in human breast carcinoma cells. **Clin Exp Metastasis** 15, 111-120.

Ramamurthy, N. S., Rifkin, B. R., Greenwald, R. A., Xu, J. W., Liu, Y., Turner, G., Golub, L. M., and Vernillo, A. T. (2002). Inhibition of matrix metalloproteinase-mediated periodontal bone loss in rats: a comparison of 6 chemically modified tetracyclines. **J Periodontol** 73, 726-734.

Reddy, M. S., and Jeffcoat, M. K. (1993). Periodontal disease progression. Curr Opin Periodontol, 52-59.

Reynolds, J. J., and Meikle, M. C. (1997). The functional balance of metalloproteinases and inhibitors in tissue degradation: relevance to oral pathologies. **J R Coll Surg Edinb** 42, 154-160.

Reynolds, J. J., and Meikle, M. C. (2000). Mechanisms of connective tissue matrix destruction in periodontitis the functional balance of metalloproteinases and inhibitors in tissue degradation: relevance to oral pathologies. **Periodontol** 14, 144-157.

Riviere, G. R., Smith, K. S., Carranza, N., Jr., Tzagaroulaki, E., Kay, S. L., and Dock, M. (1995). Subgingival distribution of Treponema denticola, Treponema socranskii, and pathogen-related oral spirochetes: prevalence and relationship to periodontal status of sampled sites. **J Periodontol** 66, 829-837.

Ruangpanit, N., Chan, D., Holmbeck, K., Birkedal-Hansen, H., Polarek, J., Yang, C., Bateman, J. F., and Thompson, E. W. (2001). Gelatinase A (MMP-2) activation by skin fibroblasts: dependence on MT1-MMP expression and fibrillar collagen form. **Matrix Biol** 20, 193-203.

Rudek, W., and Haque, R. U. (1976). Extracellular enzymes of the genus Bacteroides. **J Clin Microbiol** 4, 458-460.

Sambrook, J., E.F. Fritsch, and T. Maniatis. (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sato, H., Kinoshita, T., Takino, T., Nakayama, K., and Seiki, M. (1996). Activation of a recombinant membrane type 1-matrix metalloproteinase (MT1-MMP) by furin and its interaction with tissue inhibitor of metalloproteinases (TIMP)-2. **FEBS Lett** 393, 101-104.

Schenkein, H. A., and Berry, C. R. (1988). Production of chemotactic factors for neutrophils following the interaction of Bacteroides gingivalis with purified C5. **J Periodontal Res** 23, 308-312.

Schroder, N. W., Opitz, B., Lamping, N., Michelsen, K. S., Zahringer, U., Gobel, U. B., and Schumann, R. R. (2000). Involvement of lipopolysaccharide binding protein, CD14, and Toll-like receptors in the initiation of innate immune responses by Treponema glycolipids. **J Immunol** 165, 2683-2693.

Schwartz, Z., Kieswetter, K., Dean, D. D., and Boyan, B. D. (1997). Underlying mechanisms at the bone-surface interface during regeneration. **J Periodontal Res** 32, 166-171.

Sfakianakis, A., Barr, C. E., Kreutzer, D. L., Laine, M. L., Farre, M. A., Gonzalez, G., van Dijk, L. J., Ham, A. J., Winkel, E. G., Crusius, J. B., et al. (2001). Actinobacillus actinomycetemcomitans-induced expression of IL-1alpha and IL-1beta in human gingival epithelial cells: role in IL-8 expression **J Med Microbiol** 51, 1080-1089.

Shah, H. N., and Gharbia, S. E. (1989). Lysis of erythrocytes by the secreted cysteine proteinase of Porphyromonas gingivalis W83. **FEMS Microbiol Lett** 52, 213-217.

Shah, H. N., and Gharbia, S. E. (1993). Studies on the physiology and ecology of black-pigmented gram-negative anaerobes which may be important in disease development

Shapiro, S. D. (1994). Elastolytic metalloproteinases produced by human mononuclear phagocytes. Potential roles in destructive lung disease. **Am J Respir Crit Care Med** 150, S160-164.

Simonson, L. G., Goodman, C. H., Bial, J. J., and Morton, H. E. (1988). Quantitative relationship of Treponema denticola to severity of periodontal disease. **Infect Immun** 56, 726-728.

Singer, R. E., and Buckner, B. A. (1981). Butyrate and propionate: important components of toxic dental plaque extracts. **Infect Immun** 32, 458-463.

Skopek, R. J., and Liljemark, W. F. (1994). The influence of saliva on interbacterial adherence. **Oral Microbiol Immunol** 9, 19-24.

Slots, J. (1977). Microflora in the healthy gingival sulcus in man. Scand **J Dent Res** 85, 247-254.

Slots, J. (1981). Enzymatic characterization of some oral and nonoral gram-negative bacteria with the API ZYM system. **J Clin Microbiol** 14, 288-294.

Slots, J., and Genco, R. J. (1984). Black-pigmented Bacteroides species, Capnocytophaga species, and Actinobacillus actinomycetemcomitans in human periodontal disease: virulence factors in colonization, survival, and tissue destruction. **J Dent Res** 63, 412-421.

Slots, J., and Reynolds, H. S. (1982). Long-wave UV light fluorescence for identification of black-pigmented Bacteroides spp. **J Clin Microbiol** 16, 1148-1151.

Smalley, J. W., and Birss, A. J. (1991). Extracellular vesicle-associated and soluble trypsin-like enzyme fractions of Porphyromonas gingivalis W50. **Oral Microbiol Immunol** 6, 202-208.

Smalley, J. W., Birss, A. J., McKee, A. S., and Marsh, P. D. (1998). Hemin regulation of hemoglobin binding by Porphyromonas gingivalis. **Curr Microbiol** 36, 102-106.

Smolian, H., Aurer, A., Sittinger, M., Zacher, J., Bernimoulin, J. P., Burmester, G. R., and Kolkenbrock, H. (2001). Secretion of gelatinases and activation of gelatinase A (MMP-2) by human rheumatoid synovial fibroblasts. **Biol Chem** 382, 1491-1499.

Socransky, S. S., Haffajee, A. D., Goodson, J. M., and Lindhe, J. (1984). New concepts of destructive periodontal disease. **J Clin Periodontol** 11, 21-32.

Socransky, S. S., and Haffajee, A. D. (1992). The bacterial etiology of destructive periodontal disease: current concepts. **J Periodontol** 63, 322-331.

Socransky, S. S., Haffajee, A. D., Cugini, M. A., Smith, C., and Kent, R. L., Jr. (1998). Microbial complexes in subgingival plaque. **J Clin Periodontol** 25, 134-144.

Sorsa, T., Ingman, T., Suomalainen, K., Haapasalo, M., Konttinen, Y. T., Lindy, O., Saari, H., and Uitto, V. J. (1992). Identification of proteases from periodontopathogenic bacteria as activators of latent human neutrophil and fibroblast-type interstitial collagenases. **Infect Immun** 60, 4491-4495.

Sorsa, T., Uitto, V. J., Suomalainen, K., Vauhkonen, M., and Lindy, S. (1988). Comparison of interstitial collagenases from human gingiva, sulcular fluid and polymorphonuclear leukocytes. **J Periodontal Res** 23, 386-393.

Sosroseno, W., and Herminajeng, E. (1995). The immunopathology of chronic inflammatory periodontal disease. **FEMS Immunol Med Microbiol** 10, 171-180.

Srinivas, P. R., Kramer, B. S., and Srivastava, S. (2001). Trends in biomarker research for cancer detection. **Lancet Oncol** 2, 698-704.

Stein, S. H., Borke, J. L., and Cummings, L. A. (1999). Effects of chronic adult periodontitis and endotoxin (LPS) on gingival fibroblast plasma membrane Ca++-pump. **Connect Tissue Res** 40, 59-66.

Sternlicht, M. D., and Werb, Z. (2001). How matrix metalloproteinases regulate cell behavior. **Annu Rev Cell Dev Biol** 17, 463-516.

Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Grant, G. A., and Goldberg, G. I. (1995). Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. **J Biol Chem** 270, 5331-5338.

Suchett-Kaye, G., Morrier, J. J., and Barsotti, O. (1998). Interactions between non-immune host cells and the immune system during periodontal disease: role of the gingival keratinocyte. **Crit Rev Oral Biol Med** 9, 292-305.

Sundqvist, G., Bengtson, A., and Carlsson, J. (1988a). Generation and degradation of the complement fragment C5a in human serum by Bacteroides gingivalis. **Oral Microbiol Immunol** 3, 103-107.

Sundqvist, G., Bengtson, A., Carlsson, J., Hofling, J. F., and Sundqvist, G. K. (1988b). Generation and degradation of the complement fragment C5a in human serum by Bacteroides gingivalis

Takahashi, N., and Sato, T. (2002). Dipeptide utilization by the periodontal pathogens Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens and Fusobacterium nucleatum. **Oral Microbiol Immunol** 17, 50-54.

Takeda, M., Imada, K., Sato, T., and Ito, A. (2000). Activation of human progelatinase A/promatrix metalloproteinase 2 by Escherichia coli-derived serine proteinase. **Biochem Biophys Res Commun** 268, 128-132.

Tanner, A. (1992). Microbial etiology of periodontal diseases. Where are we? Where are we going? **Curr Opin Dent** 2, 12-24.

Tatakis, D. N. (1993). Interleukin-1 and bone metabolism: a review. **J Periodontol** 64, 416-431.

Teanpaisan, R., Douglas, C. W., Eley, A. R., and Walsh, T. F. (1996). Clonality of Porphyromonas gingivalis, Prevotella intermedia and Prevotella nigrescens isolated from periodontally diseased and healthy sites. **J Periodontal Res** 31, 423-432.

Tervahartiala, T., Pirila, E., Ceponis, A., Maisi, P., Salo, T., Tuter, G., Kallio, P., Tornwall, J., Srinivas, R., Konttinen, Y. T., and Sorsa, T. (2000). The in vivo expression of the collagenolytic matrix metalloproteinases (MMP-2, -8, -13, and -14) and matrilysin (MMP-7) in adult and localized juvenile periodontitis. **J Dent Res** 79, 1969-1977.

Theilade, E. (1984a). Sampling, cultivation and identification of microorganisms from dental plaque. **Dtsch Zahnarztl Z** 39, 611-614.

Theilade, E., Wright, W. H., Jensen, S. B., and Loe, H. (1966). Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. **J Periodontal Res** 1, 1-13.

Theilade, J. (1984b). Development and structure of dental plaque. **Dtsch Zahnarztl Z** 39, 606-610.

Thompson, E. W., Yu, M., Bueno, J., Jin, L., Maiti, S. N., Palao-Marco, F. L., Pulyaeva, H., Tamborlane, J. W., Tirgari, R., Wapnir, I., and et al. (1994). Collagen induced MMP-2 activation in human breast cancer. **Breast Cancer Res Treat** 31, 357-370.

Tomasek, J. J., Halliday, N. L., Updike, D. L., Ahern-Moore, J. S., Vu, T. K., Liu, R. W., and Howard, E. W. (1997). Gelatinase A activation is regulated by the organization of the polymerized actin cytoskeleton. **J Biol Chem** 272, 7482-7487.

Tonzetich, J., and McBride, B. C. (1981). Characterization of volatile sulphur production by pathogenic and non-pathogenic strains of oral Bacteroides. **Arch Oral Biol** 26, 963-969.

Toth, M., Bernardo, M. M., Gervasi, D. C., Soloway, P. D., Wang, Z., Bigg, H. F., Overall, C. M., DeClerck, Y. A., Tschesche, H., Cher, M. L., et al. (2000). Tissue inhibitor of metalloproteinase (TIMP)-2 acts synergistically with synthetic matrix metalloproteinase (MMP) inhibitors but not with TIMP-4 to enhance the (Membrane type 1)-MMP-dependent activation of pro-MMP-2. **J Biol Chem** 275, 41415-41423.

Tournier, J. M., Polette, M., Hinnrasky, J., Beck, J., Werb, Z., and Basbaum, C. (1994). Expression of gelatinase A, a mediator of extracellular matrix remodeling, by tracheal gland serous cells in culture and in vivo. **J Biol Chem** 269, 25454-25464.

Uitto, V. J., Larjava, H., Heino, J., and Sorsa, T. (1989). A protease of Bacteroides gingivalis degrades cell surface and matrix glycoproteins of cultured gingival fibroblasts and induces secretion of collagenase and plasminogen activator. **Infect Immun** 57, 213-218.

Uitto, V. J., Overall, C. M., and McCulloch, C. (2000). Proteolytic host cell enzymes in gingival crevice fluid. **Periodontol** 31, 77-104.

van Dalen, P. J., van Deutekom-Mulder, E. C., de Graaff, J., and van Steenbergen, T. J. (1998). Pathogenicity of Peptostreptococcus micros morphotypes and Prevotella species in pure and mixed culture. **J Med Microbiol** 47, 135-140.

van Winkelhoff, A. J., Clement, M., and de Graaff, J. (1988). Rapid characterization of oral and nonoral pigmented Bacteroides species with the ATB Anaerobes ID system. **J Clin Microbiol** 26, 1063-1065.

Wang, P. L., Azuma, Y., Shinohara, M., and Ohura, K. (2000). Toll-like receptor 4-mediated signal pathway induced by Porphyromonas gingivalis lipopolysaccharide in human gingival fibroblasts. **Biochem Biophys Res Commun** 273, 1161-1167.

Wang, P. L., Oido-Mori, M., Fujii, T., Kowashi, Y., Kikuchi, M., Suetsugu, Y., Tanaka, J., Azuma, Y., Shinohara, M., and Ohura, K. (2001). Heterogeneous expression of Toll-like receptor 4 and downregulation of Toll-like receptor 4 expression on human gingival fibroblasts by Porphyromonas gingivalis lipopolysaccharide. **Biochem Biophys Res Commun** 288, 863-867.

Weinberg, A., Belton, C. M., Park, Y., and Lamont, R. J. (1997). Role of fimbriae in Porphyromonas gingivalis invasion of gingival epithelial cells. **Infect Immun** 65, 313-316.

Westphal O, and Jann K. (1965). Bacterial lipopolysaccharides: extraction with phenol-water and further applications of the procedure. **Methods in Carbohydrate Chemistry** 5,83–91.

Whittaker, C. J., Klier, C. M., and Kolenbrander, P. E. (1996). Mechanisms of adhesion by oral bacteria. **Annu Rev Microbiol** 50, 513-552.

Williams, R. C. (1990). Periodontal disease. **N Engl J Med** 322, 373-382.

Yamada, M., Nakae, H., Yumoto, H., Shinohara, C., Ebisu, S., Matsuo, T., Greenwell, H., Bissada, N. F., Schaumann, R., Sommer, K., et al. (2002). N-acetyl-D-galactosamine specific lectin of Eikenella corrodens induces intercellular adhesion molecule-1 (ICAM-1) production by human oral epithelial cells **J Med Microbiol** 51, 1080-1089.

Yamazaki, K., Nakajima, T., Kubota, Y., Gemmell, E., Seymour, G. J., and Hara, K. (1997). Cytokine messenger RNA expression in chronic inflammatory periodontal disease. **Oral Microbiol Immunol** 12, 281-287.

Yoshimura, A., Hara, Y., Kaneko, T., and Kato, I. (1997). Secretion of IL-1 beta, TNF-alpha, IL-8 and IL-1ra by human polymorphonuclear leukocytes in response to lipopolysaccharides from periodontopathic bacteria. **J Periodontal Res** 32, 279-286.

Yun, P. L., DeCarlo, A. A., Collyer, C., and Hunter, N. (2002). Modulation of an interleukin-12 and gamma interferon synergistic feedback regulatory cycle of T-cell and monocyte cocultures by Porphyromonas gingivalis lipopolysaccharide in the absence or presence of cysteine proteinases. **Infect Immun** 70, 5695-5705.

Yoneda, M., Maeda, K., and Aono, M. (1990). Suppression of bactericidal activity of human polymorphonuclear leukocytes by Bacteroides gingivalis. **Infect Immun** 58, 406-411.

Zambon, J. J. (1996). Periodontal diseases: microbial factors. **Ann Periodontol** 1, 879-925.

Zhao, L., Ohtaki, Y., Yamaguchi, K., Matsushita, M., Fujita, T., Yokochi, T., Takada, H., and Endo, Y. (2002). LPS-induced platelet response and rapid shock in mice: contribution of O-antigen region of LPS and involvement of the lectin pathway of the complement system. **Blood** 100, 3233-3239

Appendix

Appendix A. Gram-staining

The visualization of bacteria by means of staining procedures is an important tool for identification and classification of isolates. Of all of the various staining methods for bacteria, by far the most important is Gram stain. Bacteria are grouped on the basis of their Gram reaction (positive or negative), a definitive characteristic in the organism identification process. The examination of bacterial cells isolated in pure culture from clinical specimens. The difference of characteristic morphology and staining properties can be used for preliminary identification.

Staining procedure

1. Prepare a thin, evenly distributed on the microscope slide to be Gram stained.
    a. Growth from bacterial colonies can be picked with an inoculating loop or needle and emulsified in a drop of water placed on the slide
    b. Bacteria growing in broth culture can be applied directly to a microscope slide with a swab or inoculating loop.
2. Allow the smear to completely air dry without heat, and then heat -fix by quickly passing the slide through the (low) flame of a Bunsen burner 2 or 3 times. (the slide should be just hot when touched to the wrist for correct smear fixation; if exposed to excess heat, bacteria may not stain properly)
3. Cover the smear with crystal violet solution and allow the stain to act for approximately 1 minute.
4. Remove the excess stain by briefly rinsing the slide with softly flowing tap water.

5. Shake off the excess water, flood the smear with iodine solution, and allow to stand for 1 minute or longer.
6. Rinse off the iodine solution with tap water and carefully apply decolorized solution just until no more color is being washed from the smear. Quickly rinse off any remaining decolorizer with tap water. Do not over decolorize.
7. Shake off any excess water and apply safranin counterstain solution for approximately 1 minute.
8. Wash the slide in tap water, blot dry without rubbing and examine microscopically.

Microscopic examination

Using light microscope to examine the color and morphology of bacteria. The organisms that retain the primary stain-mordant complex will appear microscopically blue to purple are termed "gram-positive" whereas the organisms that are decolorized and therefore take up the counter stain microscopically will appear pink to red are termed "gram-negative."

Reagents

1. Crystal violet solution:

   0.4% crystal violet in an aqueous alcohol solution
2. Iodine solution:

   13% PVP-iodine complex in 1.9% aqueous potassium iodide

3. Decolorized solution:

   Ethyl alcohol: Acetone (3:1)

4. Safranin solution:

   0.25% safranin in 20% ethyl alcohol

Appendix B. Chemical reagent for LPS isolation

1. TAE buffer:

   40 mM Tris-acetate, pH 8.5 – 2 mM EDTA

2. Alkaline solution:

   3 g SDS, 0.6 g Trizma base and 6.4 ml 2 N NaOH in 100 ml H_2O

3. Phenol- chloroform :

   1:1 (vol/vol)

4. Proteinase K

5. 3 M sodium acetate (pH 5.2)

6. Ethanol

7. H_2O

8. 50mM Tris-HCl,pH 8.0-100 mM sodium acetate

Sample buffer (10 ml)

0.01 M Tris-HCl (Mw 157.64)............................15.7 mg

1% SDS, w/v; ....0.1 g

0.029% EDTA , w/v;..2.9 mg

0.005% Bromophenol blue, w/v;.........................0.5 mg

20% glycerol v/v;..2 ml

5% 2-mercaptoethanol, v/v;...............................0.5 ml

Adjust pH to 8.0

Appendix C. Rapid ID 32 A : Reading table

couple	Test	Reaction	Negative -result	Positive-result
1.0	URE	UREase	Yellow	Red
1.1	ADH	Arginine Dihydrolase	colourless	Yellow
1.2	αGAL	alpha Galactosidase	colourless	Yellow
1.3	βGAL	beta Galactosidase	colourless	Yellow
1.4	βGP	beta Galactosidase 6 phosphate	colourless	Yellow
1.5	αGLU	alpha Glucosidase	colourless	Yellow
1.6	βGLU	beta Glucosidase	colourless	Yellow
1.7	αARA	alpha Arabinose	colourless	Yellow
1.8	βGUR	beta Glucuronidase	colourless	Yellow
1.9	βNAG	beta N-Acetyl-Glucosaminidase	colourless	Yellow
1.A	MNE	Manose fermentation	Red	Yellow-orange
1.B	RAF	Rafinose fermentation	Red	Yellow-orange
1.C	GDC	Glutamic ac.decarboxilase	Yellow-green	Blue
1.D	αFUC	alpha Fucosidase	colourless	yellow
1.E		empty cupule		
1.F		empty cupule		
0.0	NIT*	Reduction of Nitrates	colourless	Red
0.1	IND*	Indole production	colourless	Pink
0.2	PAL*	Phosphatase alkaline	colourless or pale orange	Orange
0.3	ArgA	Arginine Arylamidase	colourless or pale orange	Orange
0.4	ProA	Proline Arylamidase	colourless or pale orange	Orange

0.5	LGA	Leucyl Glycine Arylamidase	colourless or pale orange	Orange
0.6	PheA	Phenyl alanine Arylamidase	colourless or pale orange	Orange
0.7	LeuA	Leucine Arylamidase	colourless or pale orange	Orange
0.8	PyrA	Pyroglutamic ac. Arylamidase	colourless or pale orange	Orange
0.9	TyrA	Tyrosine Arylamidase	colourless or pale orange	Orange
0.A	AlaA	Alanine Arylamidase	colourless or pale orange	Orange
0.B	GlyA	Glycine Arylamidase	colourless or pale orange	Orange
0.C	HisA	Histidine Arylamidase	colourless or pale orange	Orange
0.D	GGA	Glutamyl Glutamic ac. Arylamidase	colourless or pale orange	Orange
0.E	SerA	Serine Arylamidase	colourless or pale orange	Orange
0.F		empty cupule		

Add

NIT* : (NIT1+NIT2) reagents / 5 -10 min

IND* : JAMES reagent / 5 -10 min

PAL* : FB reagent / 5 -10 min

Appendix D. E-TOXATE Test

Endotoxin detection procedure

1. Mix endotoxin standard stock solution (4000 EU/ml) using the vortex mixer. All endotoxin dilutions should be prepared in sterile, capped polystyrene tubes.
2. Preparedilutions of endotoxin standard stock using endotoxin-free water as indicated below:

Tube No.	Endotoxin	Endotoxin-free water (ml)	Final concentration (EU/ml)
1	0.2 ml endotoxin standard stock solution	1.8	400
2	0.2 ml from Tube No1.	1.8	40
3	0.2 ml from Tube No2.	1.8	4
4	0.3 ml from Tube No3.	2.1	0.5
5	1.0 ml from Tube No4.	1.0	0.25
6	1.0 ml from Tube No5.	1.0	0.125
7	1.0 ml from Tube No6.	1.0	0.06
8	1.0 ml from Tube No7.	1.0	0.03
9	1.0 ml from Tube No8.	1.0	0.015

Vortex dilutions for 30-6- seconds prior to further dilution assay. Any endotoxin solution standard for more than 30 minutes should be vortexed prior to use.

Endotoxin standard dilution containing 400 or more EU/ml, are generally stable for at least one week stored in refrigerator if kept free from contamination. All other dilution should be prepared fresh daily.

Using E-TOXATE multiple vial

All assay using multiple vials are performed in 10x75 mm glass culture tubes (not siliconized). The mouths of tubes may be covered with small squares of foil or parafilm during incubation. Unless incubation environment is extremely contaminated, covering the mouths of tubes may be unnecessary.

1. Label 9 tubes as I chart bellow. One set of tubes A and B are need for each sample to be tested. Tubes D, E, F, G, H and I are used to determine the sensitivity of the E-TOXATE working solution and also serve as positive controls. Tube B may be omitted if sample has been previously shown to be free of lysate inhibitor.
2. Make additions of sample, water and endotoxin standard dilution directly to the bottom of tubes(volumes as indicated below).
3. Add E-TOXATE working solution to each tube by inserting pipette to just above the contents and allowing lysate to flow down the side of tube, thereby avoiding contact and possible contamination. The addition of lysate to tube containing least (expected) endotoxin first, i.e., tube C followed by tube A, then lowest through highest positive standard(s) and finally tube B, will reduce possible contamination.
4. Mix tube contents gently. Cover mouths of tubes with foil or parafilm and incubate for 1 hour undisturbed at 37 ^{o}C.
5. Reading the results after 1 hour incubation.

   (+) = Hard gel

   (-) = Absence of hard gel

(Tube) Sample	Endotoxin-free water (ml)	Endotoxin standard dilution	E-TOXATE Working solution
(A) Samples			
0.1 ml	-	-	0.1 ml
(B) Inhibitor in sample			
0.1 ml	-	0.01 ml of 4 EU/ml	0.1 ml
(C) Negative control			
0.1 ml	-	-	0.1 ml
(D) Standard	-	0.01 ml of 0.5 EU/ml	0.1 ml
(E) Standard	-	0.01 ml of 0.25 EU/ml	0.1 ml
(F) Standard	-	0.01 ml of 0.125 EU/ml	0.1 ml
(G) Standard	-	0.01 ml of 0.06 EU/ml	0.1 ml
(H) Standard	-	0.01 ml of 0.03 EU/ml	0.1 ml
(I) Standard	-	0.01 ml of 0.015 EU/ml	0.1 ml

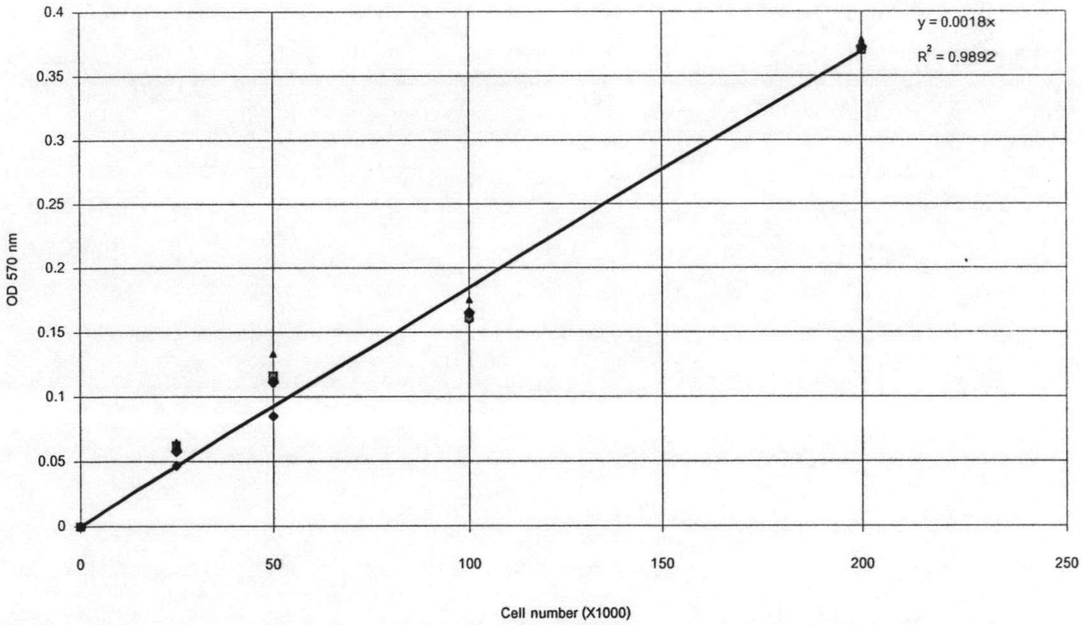
6 Interpretation:

Endotoxin (EU/ml) =(1/the inverse of the highest dilution of sample) x Final concentration of endotoxin standard dilution which has a positive result (Appearance of hardening gel)

Appendix E. Standard curve: MTT assay (OD 570 nm)

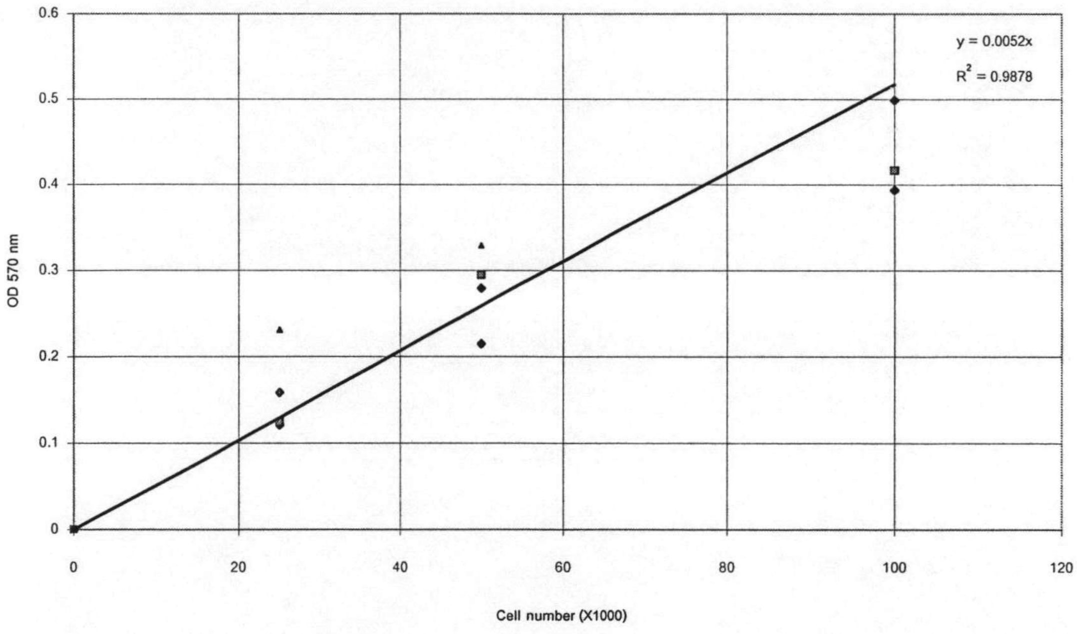
PDL cells

Cell No. (X1000)	OD1	OD2	OD3	mean
0	0	0	0	0
25	0.047	0.062	0.065	0.058
50	0.085	0.116	0.133	0.111
100	0.16	0.161	0.175	0.165
200	0.37	0.37	0.378	0.373



Gingival fibroblasts (GF)

Cell No. (X1000)	OD1	OD2	OD3	mean
0	0	0	0	0
25	0.121	0.124	0.231	0.159
50	0.215	0.295	0.329	0.280
100	0.394	0.417	0.687	0.499



VITA

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