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Alterations of Neutrophil F-Actin Kinetics by Cigarette Smoke

by

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THESIS

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DEDICATION

To my parents and my wife, Nita-

for your endless devotion and love.

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INTRODUCTION

Smoking as A Risk Factor for Systemic and Periodontal Diseases

Tobacco smoking is directly related to the incidence and prevalence of a variety of medical disorders including pulmonary, cardiovascular, gastrointestinal disease and cancer (Bartecci, *et al.* 1994). Tobacco smoking has also been identified as a potential risk factor for periodontal disease (Bergstrom & Preber 1994). Bergstrom, *et al.* (1989), using sites with periodontal pockets of >4 mm as "diseased sites", found that 56% of cases with periodontal disease and 34% of controls subjects were smokers. They also noticed that there was significantly higher frequency of periodontally involved teeth in smokers.

Haber and Kent (1992) reported on a questionnaire of smoking history in a periodontally diseased group, that the frequency of current smoking increased with increasing severity of periodontal disease. When patients with a history of diabetes were compared with non-diabetics, smoking was found to be associated with a higher prevalence of periodontitis within both the diabetic and non-diabetic groups (Haber, *et al.* 1993). The population attributable risk percent (PAR%) among the non-diabetics was 51% in the age group of 19-30 years and 32% in the age group of 31-40 years. Smoking in early onset periodontitis (EOP) patients was related to a higher proportion of deep pockets (>5mm), especially in the maxillary anterior teeth and premolar regions; and presented a significantly greater mean probing depth (PD) and attachment loss compared to non-smokers (Kamma, *et al.* 1999).

Cross-sectional studies have identified the following risk indicators for periodontal disease in elderly populations: colonization by *P. gingivalis, B. forsythus, A.*

actinomycetemcomitans (Grossi, *et al.* 1994) , or *P. intermedia* (Beck, *et al.* 1990), higher N-benzoyl-DL-arginine-2-naphthylamides (BANA) score (Beck, *et al.* 1990), poor general nutrition (Carlos and Wolfe 1989), age (Grossi, *et al.* 1994), memory loss, non-white race (Grossi, *et al.* 1994), lower socio-economic status (Beck, *et al.* 1990), male gender (Grbic, *et al.* 1991), poor oral hygiene (Burt, *et al.* 1990), smoking/smokeless tobacco use (Grossi, *et al.* 1994; Bergstrom and Eliasson 1987), diabetes mellitus (Grossi, *et al.* 1994) and osteoporosis (Jeffcoat and Chesnut 1993). Data from epidemiologic studies from Iowa (Levy, *et al.* 1987), North Carolina (Beck, *et al.* 1990) and Canada (Locker 1992; Locker and Leake 1991, 1993) all suggest that smoking is a risk indicator of periodontal disease.

Beck, *et al.* (1990,1995, 1997) found that the risk factors for progression of established disease (>3mm increase in attachment loss at sites with >3mm probing depths at baseline) was higher in people who were cigarette smokers, were BANA and subgingival bacteria (e.g., *P. gingivalis*) positive, and had economic problems. New lesions (>3mm increase in attachment loss at sites with <3mm probing depths at baseline) were more frequently found in smokeless tobacco users and patients with low income, with lower educational attainment, and with history of oral pain. They suggested that factors associated with the initiation of disease might be different from the ones involved in disease progression.

In order to identify smoking as a risk factor, longitudinal studies involving multi-variate analysis methods are necessary to offer high quality evidence (Papapanou 1996). An 18-month, short-term longitudinal study (Brown, *et al.* 1994) showed that in blacks: *P. gingivalis*, no flossing, worsening memory, and no dental visits for the past 3 years

were risk factors for progression of attachment loss. While in whites, risk factors for attachment loss were *P. gingivalis*, less medical care, depression, regular smoking, advanced disease at baseline. Ismail, *et al.* (1983, 1990) conducted a 28-yr follow-up of 526 subjects, using bivariate analysis and logistic regression analysis techniques, and showed that plaque (Odds Ratio:10.9), calculus (Odds Ratio:6.3), age (Odds Ratio:3.9-5.4), smoking (Odds Ratio:6.3), tooth mobility (Odds Ratio: 5.3) had significant higher risks for longitudinal mean loss of attachment increase of >2mm..

When the Odds Ratios were calculated, they showed smokers had higher risk of sites of deep pockets ranging from 2.5 (Bergstrom, *et al.* 1989) to 5.3 (Stoltenberg, *et al.* 1993) than non-smokers, depending on the pocket-depth threshold used. A recent meta-analysis of data from six cross-sectional and case-control studies by Papapanou (1996) demonstrated that smoking entailed an overall increased risk for severe disease with overall Odds Ratio of 2.82 for periodontal disease when comparing smokers with non-smokers. Etleret, *et al.* (1999) used multivariable Poisson regression models to identify etiologic factors for incident periodontal attachment loss from the 697 older blacks/whites in the Piedmont 65+ dental study over a 7-year period. Smoking was independently associated with 1.4 and 1.9 times higher rates of attachment loss for whites and blacks, respectively, and an estimated 28.5% attachment loss progression rate in older adults may be contributable to smoking at both the site-level and person-level.

Smoking and Host Systems

Smoking has been shown to have detrimental effects on immunity and health. Studies suggest that smoking is associated with higher rates of neoplastic disease (Shaw and Milton 1981) and infection (Burger, *et al.* 1993; Cohen, *et al.* 1993; Haynes, *et al.* 1966; Parnell, *et al.* 1966), increased chance of infection after exposure to HIV (Halsey, *et al.* 1992; Newell, *et al.* 1985) and acceleration of HIV disease progression (Nieman, *et al.* 1993; Royce and Winkelstein 1990).

Redistribution of leukocytes and lymphoid subsets, and changes in functional measures of all aspects of the immune system have also been observed in smokers. Miller, *et al.* (1982) found that the percent of total T-lymphocytes as well as the CD8 subset were increased, and the percent of CD4 cells were decreased in heavy smokers. In addition, exposure to tobacco smoke is associated with a decreased percentage and activity of natural killer (NK) cells in humans and animals (Ferson, *et al.* 1979; Sopori, *et al.* 1985; Tollerud, *et al.* 1989). Cigarette smoking affects immune and inflammatory responses by reducing antibody production (Finklfea, *et al.* 1971) and by inhibiting several peripheral blood neutrophil functions (MacFarlane, *et al.* 1992 ; Nowak and Ruta 1990; Ryder, *et al.* 1998), chemotactic and phagocytotic activities (Bridges, *et al.* 1977; Corberand, *et al.* 1979). Quinn (1996) showed that smoking could suppress the production of IgG2 in generalized EOP patients. Since IgG2 is mainly regulated by macrophages (Zhang 1996) and smoking has been shown to have an effect on macrophages (Soliman and Twigg 1992) and on T-lymphocyte subsets ratios (Mili, *et al.* 1991), smoking may compromise antibody production by modulating macrophage and T-helper cell functions.

However, serum IgE levels have been shown to be increased in adults (Burrows and Halonen 1981) and experimental animals (Zetterstrom, *et al.* 1985) exposed to tobacco smoke. Byron, *et al.* (1994) found that phytohaemagglutinin (PHA)-induced IL-4 production by peripheral blood mononuclear cells of smokers is significantly higher than that of non-smokers; and heavy smokers produce more IL-4 than light smokers. They suggested that an imbalance in cytokine production might be partly responsible for the increases in serum IgE.

Previous studies have shown that *in vitro* nicotine administration can produce changes in immunocytes such as decreased PHA-induced proliferation in human peripheral blood lymphocytes (PBL) (Neher 1974), reduced numbers of NK cells (Kusaka and Kondou 1992), the lysis of target cells by NK cells (Nair, *et al.* 1990), induced suppressor cell activity in human T- lymphocytes, a biphasic effect on the mitogenic responses of PBL (Menard and Rola-Pleszynski 1987), altered expression of certain surface proteins in T-cells (Zhang and Petro 1996) and decreased induction of antibody-forming cells and the proliferative response to anti-CD3, suggesting that nicotine treatment leads to T-cell anergy (Geng, *et al.* 1995, 1996).

The immunological effects of nicotine are believed to involve several possible mechanisms: 1) the glucocorticoid hypersecretion produced by nicotine exposure (Fuxe, *et al.* 89, Munck and Guyre 1991, Wilkins, et al. 1982; Winternitz and Quillen 1977; Caggiula, et al. 1992; McAllister-Sistilli, *et al.* 1998); 2) direct effect on lymphocytes through nicotinic cholinergic receptors (Maslinski 1989); 3) stimulation of a wide array of pituitary hormones and/or sympathetic nervous system activation, including the release of peripheral catecholamines (Fuxe, *et al.* 1989, Dantzer and Kelley 1989;

Madden and Livnat 1991); 4) as a hapten (Sudan and Sainte-Laudy 1990); 5) its effect on central nicotinic-cholinergic receptors (McAllister, *et al.* 1993)

Acute and chronic exposure to tobacco smoke also has effects on tissue other than peripheral blood. An expansion in the population of Langerhans cells in the lower respiratory tract of smokers (Casolaro, *et al.* 1988) and a decrease in the number of these cells in the cervix (Barton, *et al.* 1988) has been reported. Alveolar macrophages from smokers exhibit decreased antibody-dependent cell-mediated cytotoxicity (Lin, *et al.* 1989). Induced sputum from smokers was found to have a higher percent of macrophages and a lower proportion of neutrophils. The percent of macrophages expressing surface molecules associated with the antigen-presenting function (e.g., RFD1, HLA-DR, CD71 and CD54) was also found to be significantly lower in smokers than in non-smokers (Lensmar, *et al.* 1998).

Intraorally, (Bergstrom and Preber 1986, Bergstrom, *et al.* 1988) smoking was associated with less redness and fewer capillaries in the gingiva, supposedly causing a reduction in the gingival blood flow, a decreased number of circulating cells, and less oxygen reaching the gingiva, thus weakening its defense-reparative ability. Smoking has also been shown to reduce the functional activity of leukocytes and macrophages in saliva and crevicular fluid, as well as decreasing the chemotaxis and phagocytosis of blood and tissue polymorphonuclear neutrophils (PMNs) (Ryder, *et al.* 1998, Numabe, *et al.* 1998)

Other studies have shown that tobacco smoking was associated with a reduction in the short-term oxidation-reduction potentials (Eh) in dental plaque and oxygen levels, which may result in an increased proportion of anaerobic bacteria (Palmer 1988) and

decreased PMN mobility (Belenky, *et al.* 1993), thus contributing to increased susceptibility to bacterial infection.

Smoking and Bacteria

Although most cross-sectional studies have shown that smoking is significantly associated with higher frequency and prevalence of periodontal disease (Bergstrom 1989, Haber and Kent 1992, Haber, *et al.* 1993, Locker, *et al.* 1992, Jette, *et al.* 1993), no notable difference was found between smokers and non-smokers with respect to plaque (Bergstrom 1989) and subgingival microflora (Preber, *et al.* 1992, Stottenberg, *et al.* 1993). Preber, *et al.* (1992) reported no significant differences between 93 smokers and 62 non-smokers in the prevalence of periodontal pathogens, such as *A. actinomycetemcomitans, P. gingivalis*, or *P. intermedia.* Similar results were found by Stottenberg, *et al.* (1993).

Although previous studies have shown that there was little difference in prevalence of periodontal pathogens between smokers and non-smokers, attachment loss was associated with combination of smoking and subgingival microflora. A cross-sectional study of risk indicators of attachment loss in 1,426 subjects in Erie County, NY showed that increasing age, smoking, diabetes mellitus, and *P. gingivalis* and *B. forsythus* were risk indicators of attachment loss (Grossi et al 1994). Stottenberg, *et al.* (1993) also found that *A. actinomycetemcomitans, P. intermedia, E. corrodens* and smoking were significantly associated with deeper periodontal pockets. The authors speculated that there might be a higher susceptibility to certain bacterial infections in smokers. Zambon, *et al.* (1996) reported that the risk of subgingival infection with *B.*

forsythus and *P. gingivalis* was higher in current smokers than in non-smokers. The relative risk of infection with subgingival *B. forsythus* increased 1.18 times for each category of the amount of smoking (measured in pack years) increased from very light to heavy.

A recent study (Kamma, *et al.* 1999) showed that in a cultivable microbial profile of smokers and non-smokers in 60 early onset periodontitis (EOP) patients, smokers harbored a greater total number of bacteria. In addition, *S. aureus*, *P. micros, C. concisus, E. coli, B. forythus, C. gracilis, C. rectus, P. gingivalis, S. sputigena, C. albicans,* and *A. fumigatus* were found in significantly greater numbers and more frequently in smokers; while *S. intermedius, A. naeslundii, A. israellii* and *E. lentum* were detected more frequently and in higher proportion in non-smokers. They hypothesized that the presence of bacteria belonging to the exogenous, non-oral flora (e.g., *E. coli, C. albicans, A. fumigatus* and *S. aureus*) in smokers may be attributable to a modified local and systemic host resistance, and an altered ecology.

Smoking and Destructive Periodontal Disease

Previous studies have indicated that nicotine and its metabolite, cotinine, are associated with severity of periodontal disease (Haber and Kent 1992, Grossi, *et al.* 1994). A relationship has been demonstrated between smoking exposure and the prevalence and the severity of periodontal disease in various cross-sectional studies (Papapanou 1996, Grossi, *et al.* 1994).

A case-control study that compared the prevalence of smoking among patients in a periodontal practice and those in referring general dental offices (Haber and Kent 1992)

showed that current and former cigarette smoking was significantly associated with a higher prevalence of deep periodontal pockets and more severe periodontal destruction. Their data also showed a positive association between both amount smoked and duration of smoking and periodontal disease. When smoking was assessed seperately in diabetic and non-diabetic patients, the effects of smoking among insulin-dependent diabetes mellitus (IDDM) subjects were similar to those in the non-diabetic ones (Haber, *et al.* 1993). Gonzalez, *et al.* (1996) reported a quantitatively positive correlation between serum cotinine levels and the severity of periodontal attachment loss in smokers.

Smoking and Alveolar Bone Loss

Bolin, *et al.* (1986) found that over 10 years, smokers demonstrated a higher bone loss index compared to non-smokers. Stepwise multiple regression analysis showed that the dominant predictor for bone loss was the plaque index (PlI), followed by smoking. The difference between the bone loss indices for smokers and non-smokers significantly increased with increasing PlI and with daily tobacco consumption. Grossi, *et al.* (1995) conducted a cross-sectional study of risk indicators for alveolar bone loss in 1,361 subjects in Erie County, NY. They found a significant positive correlation between increase in smoking and increasing severity of bone loss. Approximately 56% of the non-smokers were in the healthy group (<2mm bone loss) and 7.5% in the severe bone loss group (≥ 4 mm bone loss), as compared to only 9.2% of the heavy smokers being in the healthy and 35.2% in the severe bone loss groups. On the other hand, fewer non-smokers (7.5%) were in the severe bone loss group compared to heavy smokers (35.2%). They also found that *P. gingivalis* and *B. forsythus* were associated with both periodontal

attachment loss as well as bone loss. Bergstrom, *et al.* (1991, 1995) suggested that the increasing bone support loss in smokers appeared to be independent of the presence of plaque. A recent longitudinal study in Jonkoping County, Sweden, showed that greater age, smoking, % periodontal pockets >4mm at baseline, female gender, and differences in income level, were significantly associated with severe periodontal disease progression. Current smokers and number of cigarettes/day were significantly associated with more pronounced periodontal bone loss with Odds Ratios of 5.66 and 2.89 respectively (Norderyd, *et al.* 1999).

The higher frequency and severity of bone loss in smokers might be associated with a reduction in bone mineral content due to a reduced systemic absorption of calcium and local factors (Grossi, *et al.* 1995). Rundgren and Mellstrom (1984) showed that smoking decreased the intestinal absorption of calcium, therefore affecting osteoblast function, leading to increasing bone loss. Lenz, *et al.* (1992) and Ramp, *et al.* (1991) reported that alkaline phosphatase, the enzyme associated with bone deposition and osteoblastic activity, is released from osteoblast-like cells and that this release was markedly inhibited by exposure of the cultures to smokeless tobacco extract (STE). Early onset periodontitis (EOP) smokers had significantly greater periodontal pockets, attachment loss and greater alveolar bone loss than EOP non-smokers (Kamma, *et al.* 1999). Monocytes from EOP patients have been shown to produced elevated amounts of PGE_2, TNF and IL-6, the major mediators of alveolar bone destruction (Shapira, *et al.* 1994). An *in vitro* study showed that secretion of PGE_2 and IL-1β from human peripheral monocytes might be potentiated up to 3.5-fold by the combination of bacterial lipopolysaccharide and nicotine (Payne, *et al.* 1996). Therefore, bone loss caused by

smoking may be due to both systemic and local effects from nicotine and other components of smoke.

Smoking and Tooth Loss

A 10-year longitudinal study of tooth loss in 273 subjects showed that oral hygiene, smoking habits, and age were strongly associated with tooth loss (Holm 1994). A cross-sectional and longitudinal study of 1,418 women in Sweden showed that smokers were more often edentulous than non-smokers and dentulous women smokers had significantly fewer remaining teeth than non-smokers. A negative correlation was found between the amount of cigarette consumption, as well as the number of years of smoking, and number of remaining teeth. When background variables were taken into account, the correlation remained. Authors concluded that smoking *per se* might play a causative role with respect to tooth loss (Ahlqwist, *et al.* 1986, 1989).

A recent study of tooth loss in 42 patients on maintenance care for 14 years demonstrated that a positive IL-1 genotype increased the risk of tooth loss by 2.7 times and heavy smoking by 2.9 times (McGuire and Nunn 1999). The combination effect of IL-1 genotype-positive and heavy smoking was multiplicative and increased the risk of tooth loss by 7.7 times.

Smoking and Response to Periodontal Therapy

Previous studies have shown that tobacco smokers have a less favorable response to periodontal treatment, including scaling and root planing (Ah, *et al.* 1994, Bergstrom and Preber 1985, 1986, 1990, 1994; Grossi, *et al.* 1996, 1997; Newman, *et al.* 1994;

Preber, *et al.* 1995). Axtelius, *et al.* (1999) used multilevel or hierarchical modeling which involved three distinct levels (e.g. site, tooth, and individual) to analyze 22 periodontitis patients with different responses to periodontal therapy. "Number of cigarettes consumed/day" was significantly related to a lesser periodontal pocket reduction. Their previous study also showed that "age when smoking began" was related to non-response to periodontal treatments (Axtelius, *et al.* 1997).

Adjunctive antimicrobials with smoking and non-smoking subjects have been evaluated (Kinane and Radvar 1997; Palmer, *et al.* 1999). Kinane and Radvar (1997) investigated the treatment effect of adjunctive minocycline gel, metronidazole gel and tetracycline fibers in sites which failed to respond to scaling/root planing and found that pocket reductions were significantly less in smokers, regardless of the antimicrobial regimen. Similarly, when Palmer, *et al.* (1999) compared the clinical and microbial parameter changes in untreated moderate to advanced periodontitis sites (PD>5mm) to scaling and root planing, with or without subgingival 25% metronidazole gel or systemic metronidazole in smokers and non-smokers, they found that the reduction in PD at 6 months was significantly less in smokers than non-smokers. A reduction in the proportion of spirochetes was significantly less in smokers. Multiple linear regression analysis of PD at 6 months demonstrated that smoking was a significant explanatory factor for poor treatment outcome.

Poor clinical response to non-surgical therapy in smokers might be due to less gingival shrinkage in smokers (Kinane and Radvar 1997), less inflammation at the base of pocket (Biddle, *et al.* 1998) and failure to eliminate the periodontal pathogens (Grossi, *et al.* 1996, Palmer, *et al.* 1999).

Smoking and Regeneration

There is some evidence showing that smoking has a detrimental effect on periodontal healing following regenerative therapy in either intrabony or furcation defects (Cortellini, *et al.* 1996, Rosenberg, *et al.* 1994, Tonetti, *et al.* 1995). It has been shown that guided tissue regeneration (GTR) therapy in intrabony defects with either non-resorbable or resorbable barriers resulted in significant and predictable clinical improvements in both the short-term and long-term studies (Cortellini, *et al.* 1994,1996; Tonetti, *et al.* 1995,1996,1998). However, poor patient compliance with supportive periodontal therapy, poor oral hygiene, patients with multiple attachment loss sites , and smoking were found to have detrimental effects on clinical outcomes of regenerative treatments (Cortellini, *et al.* 1996, Tonetti, *et al.* 1995).

A retrospective study of the effect of smoking on the healing response following GTR in 71 deep intrabony defects was conducted by Tonetti, *et al.* (1995). At one-year follow-up, smokers demonstrated statistically significant less clinical attachment gains (2.1 mm vs 5.2 mm) and less percent of clinical attachment level (CAL) gains (51% vs 81%) than non-smokers. Multivariate analysis indicated that smoking status in itself was a significant factor in determining CAL gain. The authors speculated that smoking might play a causative role in the reduced healing response. Another 5-year long-term study (Cortellini, *et al.* 1996) also demonstrated that patients with good oral hygiene maintenance could maintain stable CAL in both GTR and scaling root planing sites. However, 10 patients in which both sites lost CAL also showed poor oral hygiene, attachment loss in multiple sites, poor compliance with recall schedule, and were smokers. The authors suspected that the toxic constituents in smoke might have

deleterious effects on wound healing by inhibiting tissue perfusion, cell-proliferation, and metabolism.

Smoking and Refractory Periodontitis

MacFarlane, *et al.* (1992) compared the chemotaxis and phagocytic function of peripheral PMNs from patients with refractory periodontitis and matched healthy subjects. PMNs from refractory patients demonstrated significantly impaired phagocytic function and 90% of the refractory patients (28/31) were found to be smokers. A 5-year follow-up of refractory periodontitis patients treated with combination therapy of SRP, systemic metronidazole and maintenance therapy demonstrated that smokers responded less favorably to such combined therapy than non-smokers (Soder, *et al.* 1999).

Smoking and Implantology

A 6-year retrospective study of 2,194 Branemark implants in 540 patients showed an overall failure rate of 5.92%. Smokers had a significantly higher failure rate (11.3%) than non-smokers (4.8%). By site, smokers had higher failure rates in all regions except the posterior mandible, highest in posterior maxilla and the least in the anterior mandible (Bain and Moy 1993). When smokers were on cessation programs, there was a statistically significant lower failure rate in smokers who were on cessation programs than in smokers who continued to smoke (Bain 1996). However, most failing implants in the previous studies were relatively short ones that had been placed in type 4 bone. Albrektsson, *et al.* (1986) showed that overall implant failure rates were highest in the posterior maxillae and lowest in anterior areas. Similar results were found in a

retrospective analysis of 208 Branemark implants which showed that, although there was no effect of smoking on failure of implants placed in mandibles, implant failures in maxillae were significantly higher in smokers than in non-smokers (9% vs. 1%). De Brayn and Callaert (1994) reported that 31% of smokers had implant failures in spite of excellent bone quality, the use of long implants, or good initial implant stability.

Lindquist, *et al.* (1996,1997) studied radiographic marginal bone loss around Branemark implants in mandibles for 10-15 years. The cumulative success rates of the implants were 98.9%, and overall mean marginal bone loss was 0.9 mm and 1.2 mm after 10 and 15 years, respectively. Poor oral hygiene, anterior placement of implants, and smoking were associated with bone loss around implants. Wilson and Nunn (1999) tested clinical factors on survival of implants in 71 patients. Their data showed no association between implant loss and either age or IL-1 genotype. However, smoking was shown to be a strong risk factor for implant failure with a relative risk of 2.5 compared to non-smokers.

Smoking/ Nicotine and Periodontal Ligament (PDL) Cells

The unfavorable response to periodontal treatment and implant placement in smokers may be attributable partly to the detrimental effects of tobacco and tobacco metabolites on PDL cells and impairment of the host defense system. Several studies *in vivo* have shown that the cell most likely to provide a major contribution to periodontal regeneration is the PDL cell (Boyko, *et al.* 1981, Myer 1986, Nyman, *et al.* 1982). It was suggested that any factor able to inhibit the functions of such cells, would also impair tissue repair and regeneration.

Previous *in vitro* studies had demonstrated that nicotine could be detected on the root surfaces of periodontally diseased teeth (Cuff, *et al.* 1989) and nicotine could be stored in human gingival fibroblasts and inhibit alkaline phosphatase production and cause alteration in their morphology (Hanes, *et al.* 1991). Exposure of human gingival fibroblasts to both nicotine and cotinine (the primary metabolite of nicotine), has been shown to impair cellular attachment and growth of human periodontal ligament fibroblasts, to decrease synthesis of extracellular matrix from those cells, and to cause morphological alteration and cell death (Chamson, *et al.* 1980; Cuff, *et al.* 1989; Giannopoulou, *et al.* 1999; Hanes, *et al.* 1991; James, *et al.* 1999, Raulin, *et al.* 1988,1989; Tipton and Dabbous 1995).

Smoking and Polymorphonuclear Neutrophils (PMNs)

PMNs constitute the first-line defense system against invading microbes. An average-sized person produces over 100 million mature neutrophils every day (Cartwright, *et al.* 1964). Before PMNs reach target sites, they need to move out of the bone marrow, into the blood circulation and then migrate out of the vasculature in search of microorganisms or necrotic tissue. They follow chemotactic gradients to sites of infection or injury, where they nonspecifically kill invading bacteria by releasing oxygen radicals ("respiratory burst") and/or digestive enzymes. Shape change and motility of PMNs are essential for host defense. In order to migrate though narrow endothelial spaces, PMNs have to deform themselves.

The migration of neutrophils to the site of inflammation is a complicated process that involves several steps: 1) induction , 2) protrusion and adhesion, 3) contraction and

detachment, and finally 4) propagation. Different chemoattractants, growth factors and other stimuli are responsible for induction of cell movement. (Stossel 1994) Chemoattractants such as bioactive lipids and polypeptide cytokines are mainly produced by macrophages. Examples of other stimulant factors are blood-clotting factors, C5a, kinins, leukotreine B4, interleukin-8 and N-formyl oligopeptides, mitogens (PDGF, EGF, ILGF-1, TGF-β1), extracellular matrix fragments and nonspecific chemical and physical perturbations (e.g., glass, cold and electrical field).

After initial "rolling" along the endothelium migration, PMNs develop a special organelle-excluding region surrounding the cell periphery known as the cortex, and may be in the form of flat sheets (lamellae or lamellipodia) or more bulbous form (pseudopodia or lobopodia). During locomotion of cells these protrusions are localized at the front end of the moving cell.

Adhesion provides a tight binding to the endothelium and traction force to permit the cell body to pull forward. Adhesion molecules such as integrins and selectins are responsible for cell adhesion and detachment. Crowley, *et al.* (1980) found an inherited abnormality of PMN adhesion was determined by a deficiency in expression of CD11b/CD18.

Tobacco smoking exerts various effects on the PMNs and monocytes. Previous studies have demonstrated that acute exposure to smoke might delay the passage of PMNs within the pulmonary circulation (MacNee, *et al.* 1989, Selby, *et al.* 1991), affect cell deformity (MacNee, *et al.* 1989), cell diameter (Lannan, *et al.* 1992) and chemotaxis (Bridges, *et al.* 1977). Oral PMNs harvested from saliva of smokers show impaired phagocytosis (Numabe, *et al.* 1998). Morphological changes related to smoking have

been demonstrated in both oral and peripheral PMNs (Eichel and Shahrik 1969, Kenny, *et al.* 1975, 1977, Lannan, *et al.* 1992). MacFarlane, *et al.* (1992) found an impaired phagocytosis of peripheral blood PMNs and suggested that there is an association between a PMN defect and refractory periodontitis and that tobacco use may contribute to this association.

In vitro and *in vivo* experiments have demonstrated that cigarette smoke might affect PMN migration (Bridges, *et al.* 1977; Kraal, *et al.* 1977). An *in vitro* study demonstrated that individual components of smoke might have different inhibitory effects on chemotaxis of human PMNs. The unsaturated aldehydes acrolein and crotonaldehyde were the most potent inhibitors, whereas nicotine, cyanide, acetaldehyde, and furfural were the next most potent ones. Different concentrations of nicotine might affect PMN chemotactic responses (Bridges, *et al.* 1977). Nowak, *et al.* (1990) found that low concentrations of nicotine enhanced PMN migration to chemoattractants while higher concentrations inhibited chemotactic response and spontaneous migration *in vitro.* When human PMNs were exposed to nicotine, PMNs demonstrated an impaired ability to kill *A. naeslundii, A. actinomycetemcomitans*, and *F. nucleatum* due to possible affected oxygen-dependent killing mechanisms (Pabst, *et al.* 1995). It was suggested that nicotine may block oxygen uptake and synthesis of superoxide and peroxide and directly absorb any superoxide produced.

F-actin and Cell Mobility

During cell movement, a cell is polarized with formation of a lamellipodia or a pseudopodia localized at the front edge facing the direction of movement. A protrusive

ruffling activity at the leading edge and following retrograde flow coupled to adhesion of filopodia (hair-like projections) near the leading edge is thought to be a driving force for cell movement (Waterman-Storer and Salmon 1999).

A "Sol-Gel" transformation and gel contraction model has been used to describe such activities during cell movements (Stossel 1994). It is believed that the cell movement is driven by changes of the cell substance between liquid-like ("sol") and solid-like ("gel") states. When a cell is stimulated, the cellular components in sols will undergo nucleation, elongation, assembly, gelation and contraction. After removal of stimuli, the gel will be depolarized and reverse into the sol form. Repeated cycles of sol-gel transformation result in cell movement.

Actin is the perfect candidate for the sol-gel model. It represents from 5% to 20% of cell protein and filamentous actin ("F-actin") is the major cytoskeleton component. Since actin filaments are concentrated in the cell periphery and inhibited by cytochalasins, which also block cellular locomotion, they are believed to play an important role in cell migration. When neutrophils are stimulated by chemotactic factors or by substratum contact, they change their shape by lamelloid extension and retraction cycles and these oscillations are paralleled by corresponding oscillations in F-actin content (Ehrengruber, *et al.* 1996). When cells are activated by stimuli, actin filaments assemble from monomeric subunits into relatively short linear filaments. These filaments are cross-linked by actin-binding proteins (ABPs) and other cross-linking proteins (e.g., fimbrin, villin, fascin, α-actinin) into a three-dimensional network. Cell locomotion therefore involves actin assembly, gelation and contraction.

Using fluorescent phalloidin to bind actin filaments, it has been found that actin polymerization is followed by depolymerization. Actin polymerization can be modulated by calcium transients, pH changes, protein phosphorylation reactions, phospholipid turnover, lipoxygenase activity, GTP-binding proteins and others. Actin polymerization involves a nucleation reaction and is then followed by elongation with "barbed" and "pointed" end polarity. Two general types of capping proteins have been identified: the gelsolin protein superfamily and a highly conserved capping protein named *capZ*. Elongation of actin is determined by uncapping of actin filament barbed ends.

Depolymerization of F-actin involves severing actin filaments by proteins of the gelsolin family and other actin-disassembling proteins, such as cofilin, actin-depolymerizaing factor (ADF), depactin and actophorin. Calcium and low pH (< 6.5) have been shown to activate gelsolin proteins to cause severing and capping of actin fibers, leading to disintegration of the gel network. The gel network reforms when gesolin proteins are uncapped from the ends of actin filaments in response to phosphoinositides, low Ca^{2+} concentration, and normal pH (Hartwig, *et al.* 1983; Lamb, *et al.* 1993; Kwiatkowski 1999).

During normal phagocytosis, receptor-ligand binding between PMN and microbes activates the actin, myosin, and actin-binding proteins and leads to polymerization. This polymerization causes puckering of the plasma membrane at the site of contact and formation of pseudopodia. The pseudopodia surround the particle and produce a phagocytic vacuole.

The effects of tobacco use have been linked mostly with impairment of PMN functions including alterations in their phagocytic ability, chemotactic migration and

bactericidal capacity (Sasagawa, *et al.* 1985). Both animal and human studies have shown that smoking results in accumulation of PMNs in lungs and release of elastase and other proteolytic enzymes that destroy alveolar tissue. It is believed that smoking can attract PMNs to the lungs either directly or indirectly via interaction with alveolar macrophages. Other studies suggest that cigarette smoking can activate an alternative pathway of complement C5 (C5a) which induces PMN chemotaxis, autoaggreation, increased adherence, and oxygen free-radical generation. Totti, *et al.* (1984) demonstrated that nicotine in smoke enhanced neutrophil responsiveness to C5a.

Very little is known regarding the relationship between smoking and actin organization in PMNs. An *in vitro* study showed that exposure of PMNs to smoke causes changes in cell size and shape ("blebbing") (Lannan 1992), which is associated with disruption of active binding sites for actin-binding proteins, decrease in F-actin and a concomitant increase in G-actin. Ryder (1994) found that acute nicotine exposure and/or chemotactic peptides could stimulate F-actin formation and elevate intracellular calcium levels in a dose-dependent pattern. The cellular changes may contribute to decreased deformability and delayed washout of PMNs from lungs.

The purpose of the present study was to test the hypothesis that acute exposure of human peripheral PMNs to smoke may have qualitative and quantitative effects on F-actin content and polymerization-depolymerization kinetics. Chronic effects of components of cigarette smoke on F-actin content and polymerization-depolymerization kinetics were also assessed in PMNs from peripheral blood in chronic cigarette smokers.

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MATERIAL AND METHODS

Isolation of Human Peripheral PMNs

The population for this study compromised 14 healthy adult volunteers. Seven smokers (3 women and 4 men) aged 28-49 years (mean 46.8 and SD 10.3) and 7 age-matched nonsmokers (4 women and 3 men) aged 34-57 years (mean 48.7 and SD 12.2). The medical and smoking histories of each subject were obtained by interview. Inclusion criteria for smokers was regular daily cigarette smoking with a current consumption at least one pack per day and a smoking duration at least 10 years (pack years; mean 29.2 and SD 8.9). Non-smokers were subjects who had never smoked. Clinical periodontal status and intra-oral radiographs were not assessed. All subjects read and signed a consent form for participating in the study. The study protocol was approved by the University of California at San Francisco Committee for Human Research.

Cell Preparation

Approximately 20 ml of peripheral blood was obtained from subjects in heparinized Vacutainer® tubes (Becton Dickinson, Rutherford, NJ, USA) through venipuncture. Whole blood was centrifuged at 1,600 rpm for 10 minutes and the leukocyte-rich layer ("buffy coat") and plasma were collected for further processing. PMNs were separated from monocytes, platelets and erythrocytes by a double-gradient centrifugation with Histopaque 1119 and Histopaque 1077 (Procedure No. 1119, Sigma Diagnostics, St. Louis, MO, USA) at 1,700 rpm for 30 minutes at room temperature. The

purified PMNs were collected, washed with phosphate-buffered saline (PBS), centrifuged at 1800 rpm for 10 minutes, and resuspended in PBS.

Smoke Exposure

Aliquots of neutrophils in PBS were placed in a flat 24-well plate (Cell Wells TM, Corning R, Corning, NY, USA) and enclosed in a smoking box designed for a previous study (Ryder, *et al.* 1998). Cigarette smoke was generated from the lit unfiltered end of a commercially available cigarette (Marlboro® class A filtered cigarettes, Philip Morris Inc., Richmond, VA, USA). The source of cigarette smoke was separated from the plate containing neutrophils by 3 layers of a 0.5 mm plastic mesh screen to prevent large particulate matter from entering the PMN suspension. In addition, two layers of the same screen were placed over the 24-well plate. Smoke was introduced into the chamber through intermittent injection of air through the cigarette "puffs" at the rate of 5 puffs per minute. The smoke was allowed to circulate over the neutrophils by introducing a gentle stream of air at one end of the chamber and pumping the air out by vacuum through the other end the chamber at a negative pressure of approximately 5 pounds per square inch (psi). During the time of smoke exposure, the chamber was placed on a rotary shaker platform operating at approximately 60 rotations per minute. Aliquots of PMNs in PBS were then removed from the smoking chamber at time intervals of 1, 2, 3, 4, and 5 minutes. Aliquots of PMNs from 5 minutes of smoke exposure and non-exposed PMN controls were subjected to F-actin stimulation using formyl-methionyl-leucyl-phenylalanine (fMLP).

F-Actin Staining with NBD-Phallacidin

For each experiment, aliquots of 500 μl of $5X10^5$ PMNs in PBS were used. An aliquot was removed before smoke and/or fMLP stimulation to determine the basal F-actin levels. Cells obtained from smoke exposure at different time intervals were immediately mixed and permeabilized in 2.0% para-formaldehyde and 0.2% Triton X-100 in PBS for 1 hour. For fMLP stimulation experiments, 10^{-7} M fMLP was added and aliquots of PMNs from control and 5-minute exposure groups were then removed at 30, 60, 120, 240, and 360 seconds after stimulation and fixed and permeablized for F-actin staining. Following fixation and permeabilization, the PMNs were washed once with PBS and stained for F-actin with 0.3 μg/mL N-(7-ritrobenz-2-oxa-1,3-diazol-4-y-(NBD)-phallacidin (Molecular Probes, Junction City, OR, USA) for 20 minutes in the dark. Stained PMNs were washed with PBS, centrifuged, and resuspended in 400 μl PBS.

Flow Cytometry Analysis

F-actin levels of neutrophils were analyzed on a FASCAN flow cytometer (Becton-Dickinson, Rutherford, NJ, USA) equipped with a 448-nm argon ion laser coupled with a 533/30 BP filter for fluorescein. For each sample, 10,000 events were collected. Debris and other cells were gated out of the quantitative analysis of PMNs on the basis of forward and orthogonal scatter. For the F-actin staining assay, the mean channel NBD phallacidin fluorescent values were collected in log scale and converted to linear scale.

Statistical Analysis

The results were expressed as mean (± s.d.) for each sample of neutrophils at each time interval. F-actin levels obtained at different time intervals after smoke exposure and/or fMLP stimulation were compared to baseline levels. Between-group comparisons for F-actin levels were conducted between control (no smoke) and experimental (5-minute smoke exposure) groups at different time intervals after fMLP stimulation. Comparisons between smokers and non-smokers for control and experimental were also performed. Significant differences ($P<0.05$) for relative F-actin stain were determined using one-way analysis of variance (ANOVA).

Results

Table I shows the means of relative F-actin stains of PMNs in smokers and non-smokers after 1-5 minute smoke exposure. Neither group showed a statistically significant decrease in mean fluorescence intensity compared to baseline. Tables II and III show the means of relative F-actin stains of PMNs in smokers and non-smokers after fMLP stimulation with and/or without preincubation with smoke for 5 minutes. Both control and 5-minute smoking exposure groups show that fMLP stimulation induces a rapid initial rise in F-actin stains then is followed by a decline to a slight elevation over baseline at 6 minutes after fMLP stimulation. Compared to control group, acute smoking exposure results in lower mean fluorescence intensity at baseline and at various time intervals after fMLP stimulation.

Figure 1 represents the sample flow cytometry histogram of F-actin stains of PMNs in a non-smoker at baseline and after exposure to smoke from 1-5 minutes. A gradual decrease in mean F-actin content is seen from baseline with increasing duration of smoke exposure. Figure 2 represents the sample flow cytometry histogram of F-actin stains of PMNs in a non-smoker at baseline and 30-360 seconds following fMLP stimulation. A rapid increase in F-actin stains is seen immediately after exposure to fMLP (at 30 seconds after exposure), this is followed by a gradual decrease with mean levels returning to baseline value at 6 minutes after fMLP stimulation. PMNs from all 14 subjects in this study showed similar patterns as in Figure 1 and 2. However, the magnitude of changes from baseline varied widely in different subjects. Figure 3 shows that 1-5 minute acute smoking exposure resulted in a gradual decrease in F-actin content

in both smoker and non-smoker groups. Smokers had slightly higher F-actin values than their matching controls at baseline and at all time intervals. However, there were no statistically significant differences between smokers and non-smokers at any intervals. Compared to baseline, neither group showed a statistically significant decrease in content of F-actin.

For smokers, in the 1-5 minute smoke exposure, there was a continuous decrease in F-actin stain with a 30% decrease at 5 minutes nonsignificant (NS) compared to values at baseline (Figure 3). Control PMNs incubated with fMLP demonstrated a 138% and 146% rise in F-actin stain at 30 seconds and 60 seconds ($p<0.05$:*t*-test), respectively, which gradually declined to 34% elevation over baseline at 5 minutes ($p>0.05$). By contrast, PMNs pre-incubated for 5 minutes with smoke, followed by stimulation with fMLP demonstrated a 303% burst of F-actin rise over baseline ($p<0.05$) that was followed by a rapid decline at 60 seconds and 120 seconds with levels decreasing to 34% over baseline at 5 minutes ($p>0.05$, Figure 5).

For matching non-smoker group, in the 1-5 minute smoke exposure, there was a continuous decrease of F-actin stain with an approximately 38% decrease at 5 minutes (NS) compared to values at baseline (Figure 3). Control PMNs incubated with fMLP demonstrated a 192% and 144% rise in F-actin stain at 30 seconds and 60 seconds ($p<0.05$:t-test), maintained at 139% elevation at 2 minutes, then followed by a decline to 15% elevation over baseline at 5 minutes. PMNs preincubated with 5-minute smoke showed an abrupt 269% rise in F-actin stain ($p<0.05$), followed by a rapid decline at 1 minute to 119% over baseline, to 23% over baseline at 2 minutes a gradual decrease over the next 4 minutes (Figure 4).

Inter-group comparison showed (Figures 3,6,7) no significant differences between smoker and non-smoker groups for 1-5 minute exposure, in both control and test groups. Fig 6 shows that without smoke exposure, both smokers and non-smokers had a similar baseline values and after fMLP stimulation, both groups demonstrated similar kinetic pattern of F-actin polymerization-depolymerization. Although non-smokers had higher F-actin values at 30 seconds and at 2 minutes than smokers, there were no statistically significant differences found between these 2 groups. Acute smoke exposure for 5 minutes caused slightly lower baseline F-actin values, and consistently lower F-actin values were found after fMLP challenge in non-smoker groups than in smoker groups . However, the differences between them were not found to be statistically significant (Figure 7).

Relative F-actin values obtained from both smokers and non-smoker controls were highly variable. Figures 8 to 13 show the distribution of F-actin values of smoker and non-smoker individuals at baseline, 1-5-minute smoke exposure, and after fMLP stimulation. As seen in Fig 8, most non-smokers had scattered baseline values, one individual had a significantly higher relative F-actin of 261 mean fluorescence intensity. After 5-minute smoke exposure, distribution of individual F-actin values became more clustered and ranged from 22 to 134 mean fluorescence intensity.

Without preincubation with smoke, PMNs from non-smokers had a dramatic rise in F-actin content after fMLP stimulation. F-actin values of 43% (3/7) of the subjects fell below 400 mean fluorescence intensity and 57% were above 600 mean fluorescence intensity at 30 seconds. Individuals with lower F-actin values continued to have values the lower range and individuals with higher F-actin values became more scattered as time

increased (Figure 9). A 5-minute preincubation with smoke caused more similar baseline values, ranging from 41 to 144 mean fluorescence intensity. However, after fMLP challenge, divergent values were evidenced at 30- and 60-second intervals, followed by more clustering of F-actin values after 2 minutes (Figure 10).

Compared to non-smokers, smokers had a more scattered distribution of F-actin values at baseline and at different time intervals of smoke exposure, after fMLP stimulation (Figures 11-13). 1-5 minute acute smoke exposure did not significantly affect the pattern of distribution of individual F-actin values. Two individuals in the control group demonstrated a dramatic rise in F-actin stains after fMLP stimulation and maintained at a higher level for 6 minutes compared to values of PMNs in other smokers (Figure 12). Individual F-actin values were more clustered in the 5-minute smoke exposure group. However, one individual continued to show a significantly higher F-actin value at 30 and 60 seconds after fMLP challenge (Figure 13).

Discussion

The F-actin kinetics of smoker's PMNs in response to 1-5 minute acute cigarette smoke exposure and to fMLP stimulation prior to and after 5-minute smoke incubation were compared to those from non-smoker PMNs by flow cytometry. None of these differences were statistically significant. However, the present data suggest a tendency for altered actin kinetics in association with acute exposure to smoke in both smokers and non-smokers. The absence of statistically significant differences between smokers and non-smokers may imply that chronic smoke exposure is unable to alter the F-actin kinetics in response to fMLP challenge.

The absence of statistically significant differences between smokers and non-smokers may also be due to the small sample size (N=7 in each group) and highly variable F-actin response from one subject to another. Similar results were also observed in a recent study which demonstrated that PMNs isolated from peripheral blood of 14 patients with localized juvenile periodontitis (LJP) and 12 matching control subjects had similar actin polymerization-depolymerization patterns (Champagne, *et al.* 1998). Their data showed that no statistically significant differences were detectable between the control and LJP groups, and found no correlation between the kinetics of actin polymerization-depolymerization and the abnormal chemotactic response observed in LJP PMNs. Like this study, they had also found highly variable individual F-actin response to fMLP stimulation. Packman and Lichtman (1990) described that about 80% of the cells showed an increase in F-actin content, with 20% showing unchanged levels. Champagne, *et al.* (1998) also showed a variable proportion of non-responding PMNs to fMLP stimulation.

The highly variable inter-subject F-actin kinetics of PMN response to fMLP may be due to other systemic factors and due to local modulation of PMNs: these factors include age, presence of cytokines and other mediators, bacterial challenge, genetic racial variation and some medications/medical conditions (Battig 1981; Omann, *et al.* 1987; Grunberg, *et al.* 1991; Ashkenazi, *et al.* 1992; Beck, *et al.* 1994; Chancy, *et al.* 1995; Perkins 1996; De Clerk, *et al.* 1997; Hassell, *et al.* 1997; Matsuba, *et al.* 1998; McAllister-Sistilli, *et al.* 1998; Quinn, *et al.* 1996, 1998; Ratasirayakorn 1999).

Gender differences in nicotine metabolism and/or sensitivity have been reported for both humans and laboratory animals (McAllister-Sistilli, *et al.* 1998). For example, men metabolize nicotine more quickly than women (Grunberg, *et al.* 1991) Nicotine alone and combination of nicotine and alcohol induced a significant thermogenic effect in men but not in women (Perkins 1996). Animal models have shown that nicotine sensitivity may be linked to genetic factors reflected in the distinct pattern of response associated with certain rat and mouse strains (Battig 1981).

Genetic and /or racial differences may be partially contributing to differential response to smoke. Beck, *et al.* (1994) found that Caucasian smokers had a higher likelihood of developing periodontal disease than African-American smokers with the Odds Ratios of 6.7 and 2.8, respectively. Race also appeared to be an important factor on the influence of smoking on serum and subgingival IgG levels (Quinn, *et al.* 1996, 1998). Their data demonstrated that white smokers had significantly lower levels of serum IgG2 than their matched non-smokers. However, young black EOP patients in general were not affected by smoking. The authors further identified that white adult periodontitis and healthy control smokers had greater mean attachment loss/site and less serum IgG2 levels

than their non-smoking counterparts. In black adult periodontitis patients, smoking did not reduce the relation between the periodontal destruction and serum IgG2 levels.

Age. Some studies indicated that PMNs undergo substantial functional changes as they age in the circulation. Using L-selectin expression as a marker for aging, Tanji-Matsuba, *et al.* (1998) found that, although there was no difference in the F-actin content at baseline between aged and young PMNs, aged PMNs had impaired ability to assemble F-actin, to stiffen and to change shape upon fMLP stimulation compared to young PMNs

Nitric oxide (NO). Prostaglandin E_2 (PGE_2) and nitric oxide are important proinflammatory mediators. Tissue obtained from patients with moderate periodontitis has been shown to have an increased PGE_2 –synthesis and changes in NO concentrations as compared to gingival tissue from healthy controls (Matejka, *et al.* 1998). When human PMNs were pretreated with NO, a decreased fMLP-induced F-actin assembly accompanied by an inhibition of adherence in cells was noted (Chancy, *et al.* 1995).

Medication. F-actin content and kinetics of F-actin polymerization could be affected by certain medications. De Clerk, *et al.* (1997) compared the basal F-actin content and kinetics of actin polymerization of PMNs in rheumatoid arthritis (RA) patients on non-steroidal anti-inflammatory drugs (NSAID) with those in RA patients not on NSAIDs and age-matched controls. A statistically significant lower basal F-actin content was found in the RA patients on NSAID. However, upon stimulation by fMLP, the F-actin polymerization curve of RA patients on NSAID was higher than that of RA patients not on NSAID and controls. They concluded that NSAIDs might affect PMN function by altering F-actin polymerization-depolymerization cycles.

Priming with Cytokines. Studies have shown that PMN antimicrobial activity can be unregulated ("primed") by TNF-α and several other locally produced inflammatory mediators (Steinbeck and Roth 1989, Hallett and Lloyds 1995, Lloyds, *et al.* 1995). On the other hand, other mediators may antagonize the priming effect. Ratasirayakorn (1999) recently found that levels of polyamines such as putrescine, may locally inhibit PMN priming by TNF-α. In addition, putrescine can enhance superoxide release by unprimed fMLP-activated PMNs by 38%. Different inflammatory mediators could modulate PMN functions by either priming /or deactivating PMNs. Leukotriene B_4 has been shown to be able to induce short-lived F-actin polymerization (Omann, *et al.* 1987).

Bacterial Challenge. Lipopolysaccharides activate actin polymerization of monocytes and make them stiff enough to be retained in the lung (Janmey and Chaponnier 1995). Studies have also reported that microorganisms *in vitro* might alter cellular morphology and f-actin rearrangement in gingival fibroblasts and epithelial cells. (Hassell, *et al.* 1995; Beahni, *et al.* 1992; Finlay, *et al.* 1991; Phillips, *et al.* 1990). Bacterial extracts have also shown an effect on F-actin polymerization (Ashkenazi, *et al.* 1992; Hassell, *et al.* 1997). Preincubation of healthy human PMNs with an extract of *A. actinomycetemcomitans* for 15 minutes induced increased F-actin polymerization at baseline and following fMLP or PMA stimulation compared to controls without a bacterial extract (Ashkenazi, *et al.* 1992). Exposure of gingival fibroblasts from paired twins to cell-free extracts from *F. nucleatum* and *P. gingivalis* for 8-48 h resulted in dramatic alterations in cell morphology and F-actin distribution compared to control cultures. The authors suggested that perturbation of cytoskeletal proteins may adversely

affect cell function, tissue homeostasis, wound healing or host defense (Hassell, *et al.* 1997).

The true mechanism of actin organization has never been completely understood. However, based on previous studies, Aspenstrom (1999) proposed a model of signaling pathways which involve the complex receptor-effector cascades. Upon stimulating different receptors with respective external stimuli, such as bradykinin, PDGF, EGF, insulin, LPA, leads to the activation of Rho GTPases. The activated Rho GTPases, including 14 distinct members in mammalian cells, may further pass signals through their respective effector proteins and trigger various cellular functioning, such as actin organization, apoptosis, Jun amino-terminal kinase (JNK) activation, cell cycle progression, NFkB activation, cell transformation, NADPH oxidase regulation and phosphoinositide metabolism.

Gelsolin, Ca^{2+}, D3 and D4 phosphoinositides, pH and possibly lysophosphatic acid also regulate actin organization. Gelsolin is an enzyme and actin-binding protein which has been known for its involvement in regulation of actin cytoskeleton assembly and/or dissembly. Gelsolin severs assembled actin filaments and caps the free ends of the severed filament, which is regulated by intracellular Ca+ level and pH. Phosphoinositides of the D3 and D4 types, on the other hand, release gelsolin from the free ends of filaments and allow actin filaments to assemble (Kwiatkowski 1999). An interesting finding from a study by Ryder (1994) demonstrated that nicotine exposure may elevate intracellular Ca^{2+} level and F-actin formation in human neutrophils.

Other actin-binding proteins involved in stabilizing actin or actin-membrane interactions in cell transformation or malignancy have been studied, which include

thymosin β-4, tropomysin, α-catinin, merlin, vinculin, gelsolin, L-plastin, and lymphocyte-specific protein (LSP1). (Janmey and Chaponnier 1995). Some oncogene products such as *c-Abl* have been shown to be able to bind to F- and G-actin and possibly regulate actin polymerization and depolymerization (Van Etten, *et al.* 1994; McWhirter and Wang 1993).

Whether nicotine affects F-actin polymerization directly via its receptor-effector signaling pathway or indirectly affects Ca^{2+} level, pH, or other secondary messengers and consequently regulates F-actin organization needs to be further studied. Migration of PMNs into the gingival sulcus is regulated by complement fragments, arachidonic acid metabolites, formyl peptides and other bacterial products, and also the presence and gradient of ICAM-1 and IL-8 across junctional epithelium (Tonetti 1997). Therefore, the effects of smoking on such local mechanisms also need further investigation.

Cell adhesion and movement are essential functions of PMNs in host defense and require both intact cell surface receptors (e.g., L-selectin, CD11/CD18) and a functioning cytoskeleton. Acute smoke exposure has been shown to impair PMN adhesion and migration *in vitro* (Drost, *et al.* 1992; Selby, *et al.* 1992). Since PMA- or fMLP-stimulated PMN adherence was significantly inhibited by disruption of the microfilaments of cytoskeleton by cytochalasin B or by blockade of CD18, the authors suspected that the cell adherence involves cytoskeletal/membrane function (Sebly, *et al.* 1992). Because changes in actin kinetics affect cell deformability, regulation of actin polymerization/ depolymerization may affect PMN rigidity and functions.

Results from studies of the effects of the acute smoke exposure on PMN cell surface receptors have been conflicting. While Selby, *et al.* (1992) did not find a

significant change in expression of CD18 following *in vitro* smoke exposure, Ryder, *et al.* (1998) reported that acute smoke exposure resulted in a 15-20% increase in CD18 expression and similar expression of L-selectin in both smokers and non-smokers. However, both studies did not demonstrate statistically significant differences between smokers and non-smokers in expression of cell surface receptors. Results from the present study demonstrated that acute exposure of PMNs to smoke caused a significant suppression of F-actin polymerization-depolymerization kinetics in both smokers and non-smokers. However, comparison of F-actin kinetics of control PMNs from smokers and non-smokers after fMLP stimulation showed no statistically significant differences in F-actin kinetics between smokers and non-smokers. Lack of statistically significant differences in F-actin kinetics after fMLP stimulation between PMNs of smokers and non-smokers was also found in 5-minute acute exposure of PMNs to smoke. Data from the present and previous studies may suggest that acute and chronic smoke exposure might affect PMN functions differently.

Lannan, *et al.* (1992) concluded that a cell surface change ("zeinosis") in PMNs by acute smoke exposure was caused by oxidative injury and alteration in the configuration of actin. Acute smoke may impair initial PMN functions, such as adherence and migration by "fixing" the actin pool in one of its two states and prevent normal sol-gel transformation (Ryder 1994). Acute smoke exposure may also affect the oxidative burst of unstimulated and stimulated PMNs. Ryder, *et al.* (1998) showed that *in vitro* acute smoke exposure resulted in a time-related suppression of PMA-stimulated PMN superoxide and H_2O_2 production and a time-related elevation in these oxidative products in unstimulated smoke-exposed PMNs. Previous studies have shown that when F-actin in

fMLP-stimulated PMN's was fixed in the polymerized form by cytosolic phalloidin, oxidase activation was inhibited (al-Mohanna and Hallett 1990). Therefore, alteration of PMN oxidative burst is associated with suppressed F-actin polymerization-depolymerization kinetics. Excessive products of the oxidative burst may directly damage tissue cells, stimulate local production and release of chemotactic factors, upgrade the expression of certain cell surface receptors, activate proteolytic enzymes, and inhibit enzyme inhibitors (Ryder, *et al.* 1998). Chronic smoke exposure may result in recruitment of PMNs in the tissue, active release of elastase and altered physical activities. PMNs isolated from smokers and patients with chronic obstructive pulmonary disease (COPD) contained significantly higher concentrations of plasma proteinase elastase and enhanced proteolysis of fibronectin (Hind, *et al.* 1991; Burnett, *et al.* 1987). Therefore, the tissue damage in chronic smokers may be associated with the larger number and the increased proteolytic activity of PMNs in the tissue.

Host resistance to pathogenic bacteria may be impaired by smoking-related alteration of PMN activities, such as chemotaxis, adherence, migration, phagocytosis, oxidative burst, degranulation and activating of other components of the secondary messenger system. This study found a decrease in formation of F-actin and inhibition of F-actin polymerization-depolymerization kinetics in human PMNs after acute smoke exposure. This may be associated with promotion and progression of destructive periodontal disease. These results might provide further insight into the role of tobacco smoking in periodontal disease.

Conclusion

In vitro acute exposure of human peripheral PMNs (from smokers and non-smokers) to cigarette smoke resulted in a gradual decrease in F-actin content. With 5-minute preincubation with cigarette smoke, fMLP-induced F-actin kinetics of PMNs in smokers and non-smokers was significantly suppressed compared to control PMNs. No statistically significant differences in F-actin kinetics after exposure to cigarette smoke *in vitro* were found in PMNs from smokers and non-smokers.

BIBLIOGRAPHY

Ah, MK; Johnson, GK; Kaldahl, WB; Patil, KD; Kalkwarf, KL. The effect of smoking on the response to periodontal therapy. *Journal of Clinical Periodontology* 1994; 21:91-7.

Ahlqwist, M; Bengtsson, C; Hollender, L; Lapidus, L; Osterberg, T. Smoking habits and tooth loss in Swedish women. *Community Dentistry and Oral Epidemiology* 1989; 17:144-7

Ahlqwist, M; Bengtsson, C; Greondahl, HG; Halling, A. Tooth mortality in women in a 12-year follow-up of a population sample in Gothenburg, Sweden. *Community Dentistry and Oral Epidemiology* 1986; 14:358-61

Albrektsson, T; Jansson, T; Lekholm, U. Osseointegrated dental implants. *Dental Clinics of North America* 1986; 30:151-74.

al-Mohanna, FA; Hallett, MB. Actin polymerization in neutrophils is triggered without a requirement for a rise in cytoplasmic Ca2+. *Biochemical Journal* 1990; 266:669-74.

Ashkenazi, M; White, RR; Dennison, DK. Neutrophil modulation by Actinobacillus actinomycetemcomitans. I. Chemotaxis, surface receptor expression and F-actin polymerization. *Journal of Periodontal Research* 1992; 27:264-73.

Aspenstrom, P. Effectors for the Rho GTPases. *Current Opinion in Cell Biology*, 1999; 11:95-102.

Axtelius, B; Soderfeldt, B; Edwardsson, S; Attstrom, R. Therapy-resistant periodontitis (II). Compliance and general and dental health experiences. *Journal of Clinical Periodontology* 1997b; 24: 646-653.

Axtelius, B; Seoderfeldt, B; Attstreom, R. A multilevel analysis of factors affecting pocket probing depth in patients responding differently to periodontal treatment. *Journal of Clinical Periodontology* 1999; 26:67-76

Baehni, PC; Song, M; McCulloch, CA; Ellen, RP. Treponema denticola induces actin rearrangement and detachment of human gingival fibroblasts. *Infection and Immunity* 1992; 60:3360-8.

Bain, CA; Moy, PK. The association between the failure of dental implants and cigarette smoking. *International Journal of Oral and Maxillofacial Implants* 1993; 8:609-15.

Bain, CA. Smoking and implant failure--benefits of a smoking cessation protocol. *International Journal of Oral and Maxillofacial Implants* 1996; 11:756-9.

Bartecchi, CE; MacKenzie,TD; Schrier, RW. The human costs of tobacco use. I. *New England Journal of Medicine* 1994; 331:907-912.

Barton, SE; Maddox, PH; Jenkins, D; Edwards, R; Cuzick, J; Singer, A. Effect of cigarette smoking on cervical epithelial immunity: a mechanism for neoplastic change? *Lancet* 1988; 2:652-4.

Beattig, K. The smoking habit and psychopharmacological effects of nicotine. *Activitas Nervosa Superior* 1980; 22:274-88.

Beck, JD; Koch, GG; Rozier, RG; Tudor, GE. Prevalence and risk indicators for periodontal attachment loss in a population of older community-dwelling blacks and whites. *Journal of Periodontology* 1990; 61:521-8.

Beck, JD; Koch, GG; Offenbacher, S. Attachment loss trends over 3 years in community-dwelling older adults. *Journal of Periodontology* 1994; 65:737-43.

Beck, JD; Koch, GG; Offenbacher, S. Incidence of attachment loss over 3 years in older adults--new and progressing lesions. *Community Dentistry and Oral Epidemiology* 1995; 23 :291-6

Beck, JD; Sharp, T; Koch, GG; Offenbacher, S. A study of attachment loss patterns in survivor teeth at 18 months, 36 months and 5 years in community-dwelling older adults. *Journal of Periodontal Research* 1997; 32:497-505

Beck, JD; Cusmano, L; Green-Helms, W; Koch, GG; Offenbacher, S. A 5-year study of attachment loss in community-dwelling older adults: incidence density. *Journal of Periodontal Research* 1997; 32:506-15

Beck, JD; Sharp, T; Koch, GG; Offenbacher, S. A 5-year study of attachment loss and tooth loss in community-dwelling older adults. *Journal of Periodontal Research* 1997; 32:516-23.

Belenky, SN; Robbins, RA; Rennard, SI; Gossman, GL; Nelson, KJ; Rubinstein, I. Inhibitors of nitric oxide synthase attenuate human neutrophil chemotaxis *in vitro*. *Journal of Laboratory and Clinical Medicine* 1993; 122:388-94.

Bergstrom, J. Short-term investigation on the influence of cigarette smoking upon plaque accumulation. *Scandinavian Journal of Dental Research* 1981; 89:235-8.

Bergstrom, J; Preber, H. The influence of cigarette smoking on the development of experimental gingivitis. *Journal of Periodontal Research* 1986; 21:668-76.

Bergstrom, J; Eliasson, S. Noxious effect of cigarette smoking on periodontal health. *Journal of Periodontal Research* 1987; 22:513-7.

Bergstrom, J; Eliasson, S. Cigarette smoking and alveolar bone height in subjects with a high standard of oral hygiene. *Journal of Clinical Periodontology* 1987; 14 :466-9.

Bergstrom, J; Persson, L; Preber, H. Influence of cigarette smoking on vascular reaction during experimental gingivitis. *Scandinavian Journal of Dental Research* 1988; 96:34-9

Bergstrom, J. Cigarette smoking as risk factor in chronic periodontal disease. *Community Dentistry and Oral Epidemiology* 1989; 17:245-7

Bergstrom, J. Oral hygiene compliance and gingivitis expression in cigarette smokers. *Scandinavian Journal of Dental Research* 1990; 98:497-503

Bergstrom, J; Eliasson, S; Preber, H. Cigarette smoking and periodontal bone Loss. *Journal of Periodontology* 1991; 62:242-6

Bergstrom, J; Preber, H. Tobacco use as a risk factor. *Journal of Periodontology* 1994; 65:545-50.

Bolin, A; Lavstedt, S; Frithiof, L; Henrikson, CO. Proximal alveolar bone loss in a longitudinal radiographic investigation. IV. Smoking and some other factors influencing the progress in individuals with at least 20 remaining teeth. *Acta Odontologica Scandinavica* 1986; 44:263-9.

Bolin, A; Eklund, G; Frithiof, L; Lavstedt, S. The effect of changed smoking habits on marginal alveolar bone loss. A longitudinal study. *Swedish Dental Journal* 1993; 17:211-6.

Bostreom, L; Linder, LE; Bergstrom, J. Influence of smoking on the outcome of periodontal surgery. A 5-year follow-up. *Journal of Clinical Periodontology* 1998; 25:194-201

Boyko, GA; Melcher, AH; Brunette, DM. Formation of new periodontal ligament by periodontal ligament cells implanted *in vivo* after culture *in vitro*. A preliminary study of transplanted roots in the dog. *Journal of Periodontal Research* 1981; 16:73-88.

Bridges, RB; Kraal, JH; Huang, LJT; Chancellor, MB. Effects of cigarette smoke components on in vitro chemotaxis of human polymorphonuclear leukocytes. *Infection and Immunity* 1977; 16:240-248.

Brown, LF; Beck, JD; Rozier, RG. Incidence of attachment loss in community-dwelling older adults. *Journal of Periodontology* 1994; 65:316-23

Burrows, B; Halonen, M; Barbee, RA; Lebowitz, MD. The relationship of serum immunoglobulin E to cigarette smoking. *American Review of Respiratory Disease* 1981; 124:523-5.

Burnett, D; Chamba, A; Hill, SL; Stockley, RA. Neutrophils from subjects with chronic obstructive lung disease show enhanced chemotaxis and extracellular proteolysis. *Lancet* 1987; 2:1043-6.

Burt, BA; Ismail, AI; Morrison, EC; Beltran, ED. Risk factors for tooth loss over a 28-year period. *Journal of Dental Research* 1990; 69:1126-30.

Byron, KA; Varigos, GA; Wootton, AM. IL-4 production is increased in cigarette smokers. *Clinical and Experimental Immunology* 1994; 95:333-6.

Caggiula, A; McAllister, C; Epstein, L; Antelman, S; Knopf, S; Saylor, S; Perkins, K. Nicotine suppresses the proliferative response of peripheral blood lymphocytes in rats. *Drug Development Research* 1992; 26:473-479.

Carlos, JP; Wolfe, MD. Methodological and nutritional issues in assessing the oral health of aged subjects. *American Journal of Clinical Nutrition* 1989; 50:1210-8

Cartwright, GE; Athens, JW; Wintrobe, MM. The kinetics of granulopoiesis in normal man. *Blood* 1964;24:780.

Casolaro, MA; Bernaudin, JF; Saltini, C; Ferrans, VJ; Crystal, RG. Accumulation of Langerhans' cells on the epithelial surface of the lower res iratory tract in normal subjects in association with cigarette smoking. *American Review of Respiratory Disease* 1988; 137:406-11.

Champagne, CM; Vaikuntam, J; Warbington, ML; Rose, L; Daniel, MA; Van Dyke, TE. Cytoskeletal actin reorganization in neutrophils from patients with localized juvenile periodontitis. *Journal of Periodontology* 1998; 69:209-18.

Chamson, A; Frey, J; Hivert, M. Effects of tobacco smoke extracts on collagen biosynthesis by fibroblast cell cultures. *Journal of Toxicology and Environmental Health* 1982; 9:921-32.

Chen, WY; Lu, CC. Effect of maternal labor and mode of delivery on neutrophil actin response to N-formylmethionyl-leucyl-phenylalanine in healthy neonates. *Kao-Hsiung i Hsueh Ko Hsueh Tsa Chih* [*Kaohsiung Journal of Medical Sciences*] 1996; 12:83-7.

Cohen, S; Tyrrell, DA; Russell, MA; Jarvis, MJ; Smith, AP. Smoking, alcohol consumption, and susceptibility to the common cold. *American Journal of Public Health* 1993; 83:1277-83.

Corberand, J; Nguyen, F; Do, AH; Dutau, G; Laharrague, P; Fontanilles, AM; Gleizes, B. Effect of tobacco smoking on the functions of polymorphonuclear leukocytes. *Infection and Immunity* 1979; 23:577-81.

Cortellini, P; Pini-Prato, G; Tonetti, M. Periodontal regeneration of human infrabony defects V . Effect of oral hygiene on long-term stability. *Journal of Clinical Periodontology* 1994; 21:606-10.

Cortellini, P; Paolo, G; Prato, P; Tonetti, MS. Long-term stability of clinical attachment following guided tissue regeneration and conventional therapy. *Journal of Clinical Periodontology* 1996; 23:106-11.

Cortellini, P; Pini Prato, G; Tonetti, MS. Periodontal regeneration of human intrabony defects with bioresorbable membranes. A controlled clinical trial. *Journal of Periodontology* 1996; 67:217-23.

Crowley, CA; Curnutte, JT; Rosin, RE; Andrae-Schwartz, J; Gallin, JI; Klempner, M; Snyderman, R; Southwick, FS; Stossel, TP; Babior, BM. An inherited abnormality of neutrophil adhesion. Its genetic transmission and its association with a missing protein. *New England Journal of Medicine* 1980; 302:1163-8.

Cuff, MJ; McQuade, MJ; Scheidt, MJ; Sutherland, DE; Van Dyke, TE. The presence of nicotine on root surfaces of periodontally diseased teeth in smokers. *Journal of Periodontology* 1989; 60:564-569.

Dantzer, R; Kelley, KW. Stress and immunity: an integrated view of relationships between the brain and the immune system. *Life Sciences* 1989; 44:1995-2008.

De Bruyn, H; Collaert, B. The effect of smoking on early implant failure. *Clinical Oral Implants Research* 1994; 5:260-4

De Clerck, LS; Mertens, AV; De Gendt, CM; Bridts, CH; Stevens, WJ. Actin polymerisation in neutrophils of rheumatoid arthritis patients in relation to treatment with non-steroidal anti-inflammatory drugs. *Clinica Chimica Acta* 1997; 261:19-25.

Drost, EM; Selby, C, Lannan, S; Lowe, GDO; MacNee, W. Changes in neutrophil deformability following in vitro smoke exposure: mechanism and protection. *Am J Respir Cell Mol Biol* 1992; 6:287-295.

Ehrengruber, MU; Deranleau, DA; Coates, TD. Shape oscillations of human neutrophil leukocytes: characterization and relationship to cell motility. *Journal of Experimental Biology* 1996; 199:741-7

Eichel, B; Shahrik, HA. Tobacco smoke toxicity: loss of human oral leukocyte function and fluid-cell metabolism. *Science* 1969; 166:1424-8.

Eklund, SA; Burt, BA. Risk factors for total tooth loss in the United States; longitudinal analysis of national data. *Journal of Public Health Dentistry* 1994; 54:5-14.

Engelberg, M; Elder, JP; Hammond, N; Boskin, W; Molgaard, CA. Procuring incentives for community health promotion programs. *Journal of Community Health* 1987; 12:56-65.

Ferson, M; Edwards, A; Lind, A; Milton, GW; Hersey, P. Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. *International Journal of Cancer* 1979; 23:603-9.

Fuxe, K; Andersson, K; Eneroth, P; Hearfstrand, A; Agnati, LF. Neuroendocrine actions of nicotine and of exposure to cigarette smoke: medical implications. *Psychoneuroendocrinology* 1989; 14:19-41.

Geng, Y; Savage, SM; Johnson, LJ; Seagrave, J; Sopori, ML. Effects of nicotine on the immune response. I. Chronic exposure to nicotine impairs antigen receptor-mediated signal transduction in lymphocytes. *Toxicology and Applied Pharmacology* 1995; 135:268-78.

Geng, Y; Savage, SM; Razanai-Boroujerdi, S; Sopori, ML. Effects of nicotine on the immune response. II. Chronic nicotine treatment induces T cell anergy. *Journal of Immunology* 1996; 156:2384-90.

Giannopoulou, C; Geinoz, A; Cimasoni, G. Effects of nicotine on periodontal ligament fibroblasts in vitro. *Journal of Clinical Periodontology* 1999; 26:49-55.

Gonzaalez, YM; De Nardin, A; Grossi, SG; Machtei, EE; Genco, RJ; De Nardin, E. Serum cotinine levels, smoking, and periodontal attachment loss. *Journal of Dental Research* 1996; 75:796-802.

Grbic, JT; Lamster, IB; Celenti, RS; Fine, JB. Risk indicators for future clinical attachment loss in adult periodontitis. Patient variables. *Journal of Periodontology* 1991; 62:322-9.

Grossi, SG; Zambon, J; Machtei, EE; Schifferle, R; Andreana, S; Genco, RJ; Cummins, D; Harrap, G. Effects of smoking and smoking cessation on healing after mechanical periodontal therapy. *Journal of the American Dental Association* 1997; 128:599-607

Grossi, SG; Skrepcinski, FB; DeCaro, T; Zambon, JJ; Cummins, D; Genco, RJ. Response to periodontal therapy in diabetics and smokers. *Journal of Periodontology* 1996;67 10:1094-102.

Grossi, SG; Genco, RJ; Machtei, EE; Ho, AW; Koch, G; Dunford, R; Zambon, JJ; Hausmann, E. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *Journal of Periodontology* 1995; 66:23-9

Grossi, SG; Zambon, JJ; Ho, AW; Koch, G; Dunford, RG. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *Journal of Periodontology* 1994; 65:260-7

Grunberg, NE; Winders, SE; Wewers, ME. Gender differences in tobacco use. *Health Psychology* 1991; 10:143-53.

Gunsolley, JC; Quinn, SM; Tew, J; Gooss, CM; Brooks, CN; Schenkein, HA. The effect of smoking on individuals with minimal periodontal destruction. *Journal of Periodontology* 1998; 69:165-70

Haber, J; Wattles, J; Crowley, M; Mandell, R; Joshipura, K; Kent, RL. Evidence for cigarette smoking as a major risk factor for periodontitis. *Journal of Periodontology* 1993; 64:16-23.

Haber, J; Kent, RL. Cigarette smoking in a periodontal practice. *Journal of Periodontology* 1992; 63:100-6

Hallett, MB; Lloyds, D. Neutrophil priming: the cellular signals that say 'amber' but not 'green'. *Immunology Today* 1995; 16:264-8.

Halsey, NA; Coberly, JS; Holt, E; Coreil, J; Kissinger, P; Moulton, LH; Brutus, JR; Boulos, R. Sexual behavior, smoking, and HIV-1 infection in Haitian Women. *Journal of the American Medical Association* 1992; 267:2062-6.

Hanes, PJ; Schuster, GS; Lubas, S. Binding, uptake, and release of nicotine by human gingival fibroblasts. *Journal of Periodontology* 1991; 62:147-152.

Hartwig, JH; Stossel, TP. Structure of macrophage actin-binding protein molecules in solution and interacting with actin filaments. *Journal of Molecular Biology* 1981; 145: 563-81.

Hartwig, JH; Yin, HL; Stossel, TP. How phagocytic leukocytes move. *Journal of Clinical Chemistry and Clinical Biochemistry* 1983; 21:535-44.

Hassell, TM; Harris, EL. Genetic influences in caries and periodontal diseases. *Critical Reviews in Oral Biology and Medicine* 1995; 6:319-42.

Hassell, TM; Baehni, P; Harris, EL; Walker, C; Gabbiani, G; Geinoz, A. Evidence for genetic control of changes in f-actin polymerization caused by pathogenic microorganisms: *in vitro* assessment using gingival fibroblasts from human twins. *Journal of Periodontal Research* 1997; 32:90-8.

Haynes, WF Jr; Krstulovic, VJ; Bell, AL Jr. Smoking habit and incidence of respiratory tract infections in a group of adolescent males. *American Review of Respiratory Disease* 1966; 93:730-5.

Hind, CR; Joyce, H; Tennent, GA; Pepys, MB; Pride, NB. Plasma leucocyte elastase concentrations in smokers. *Journal of Clinical Pathology* 1991; 44(3):232-5.

Holm, G. Smoking as an additional risk for tooth loss. *Journal of Periodontology* 1994; 65: 996-1001.

Ismail, AI; Burt, BA; Eklund, SA. Epidemiologic patterns of smoking and periodontal disease in the United States. *Journal of the American Dental Association* 1983; 106:617-21.

Ismail, AI; Morrison, EC; Burt, BA; Caffesse, RG; Kavanagh, MT. Natural history of periodontal disease in adults: findings from the Tecumseh Periodontal Disease Study, 1959-87. *Journal of Dental Research* 1990; 69 2 :430-5.

James, JA; Sayers, NM; Drucker, DB; Hull, PS. Effects of tobacco products on the attachment and growth of periodontal ligament fibroblasts. *Journal of Periodontology* 1999; 70: 518-525.

Janmey, PA; Chaponnier, C. Medical aspects of the actin cytoskeleton. *Current Opinion in Cell Biology* 1995; 7:111-7.

Jeffcoat, MK; Chesnut, CH 3d. Systemic osteoporosis and oral bone loss: evidence shows increased risk factors. *Journal of the American Dental Association* 1993; 124:49-56.

Jette, AM; Feldman, HA; Tennstedt, SL. Tobacco use: a modifiable risk factor for dental disease among the elderly. *American Journal of Public Health* 1993; 83:1271-6.

Kamma, JJ; Nakou, M; Baehni, PC. Clinical and microbiological characteristics of smokers with early onset periodontitis. *Journal of Periodontal Research* 1999; 34:25-33

Kenney, EB; Kraal, JH; Saxe, SR; Jones, J. The effect of cigarette smoke on human oral polymorphonuclear leukocytes. *Journal of Periodontal Research* 1977; 12:227-34

Kenney, EB; Saxe, SR; Bowles, RD. The effect of cigarette smoking on anaerobiosis in the oral cavity. *Journal of Periodontology* 1975; 46:82-5.

Kepley, CL; Wilson, BS; Oliver, JM. Identification of the Fc epsilonRI-activated tyrosine kinases Lyn, Syk, and Zap-70 in human basophils. Journal of Allergy and *Clinical Immunology* 1998;102: 304-15.

Kinane, DF; Radvar, M. The effect of smoking on mechanical and antimicrobial periodontal therapy. *Journal of Periodontology* 1997; 68:467-72.

Kraal, JH; Chancellor, MB; Bridges, RB; Bemis, KG; Hawke, JE. Variations in the gingival polymorphonuclear leukocyte migration rate in dogs induced by chemotactic autologous serum and migration inhibitor from tobacco smoke. *Journal of Periodontal Research* 1977; 12:242-249.

Kusaka, Y; Kondou, H; Morimoto, K. Healthy lifestyles are associated with higher natural killer cell activity. *Preventive Medicine* 1992; 21:602-15.

Kwiatkowski, DJ. Functions of gelsolin: motility, signaling, apoptosis, cancer. *Current Opinion in Cell Biology* 1999; 11:103-108.

Lamb, JA; Allen, PG; Tuan, BY; Janmey, PA. Modulation of gelsolin function. Activation at low pH overrides Ca2+ requirement. *Journal of Biological Chemistry* 1993; 268:8999-9004.

Lannan, S; McLean, A; Drost, E; Gilloly, M; Donaldson, K; Lamb, D; MacNee, W. Cjhanges in neutrophil morphology and morphometry following exposure to cigarette smoke. *International Journal of Experimental Pathology* 1992; 73:183-191.

Lensmar, C; Elmberger, G; Sandgren, P; Skeold, CM; Eklund, A. Leukocyte counts and macrophage phenotypes in induced sputum and bronchoalveolar lavage fluid from normal subjects. *European Respiratory Journal* 1998; 12:595-600.

Levy, SM; Heckert, DA; Beck, JD; Kohout, FJ. Multivariate correlates of periodontally healthy teeth in an elderly population. *Gerodontics* 1987; 3:85-8.

Lin, CC; Huang, WC; Lin, CY. Chemiluminescence and antibody-dependent, cell-mediated cytotoxicity between human alveolar macrophages and peripheral blood monocytes in smokers, nonsmokers, and lung cancer patients. *Chest* 1989; 95:553-7.

Lindquist, LW; Carlsson, GE; Jemt, T. Association between marginal bone loss around osseointegrated mandibular implants and smoking habits: a 10-year follow-up study. *Journal of Dental Research* 1997; 76:1667-74

Lindquist, LW; Carlsson, GE; Jemt, T. A prospective 15-year follow-up study of mandibular fixed prostheses supported by osseointegrated implants. Clinical results and marginal bone loss. *Clinical Oral Implants Research* 1996; 7:329-36.

Lloyds, D; Brindle, NP; Hallett, MB. Priming of human neutrophils by tumour necrosis factor-alpha and substance P is associated with tyrosine phosphorylation. *Immunology* 1995; 84:220-6.

Locker, D,Leake, J, Hamilton, M, Hicks, T, Lee, J, Main, P. The oral healh status of older adults in four Ontario communties. *Journal of Candian Dental Society* 1991; 57727-732.

Locker, D. Smoking and oral health in older adults. Canadian Journal of Public Health. *Revue Canadienne de Sante Publique* 1992; 83:429-32

Locker, D; Leake, JL. Risk indicators and risk markers for periodontal disease experience in older adults living independently in Ontario, Canada. *Journal of Dental Research* 1993; 72:9-17.

Loesche, WJ; Bretz, WA; Lopatin, D; Stoll, J; Rau, CF; Hillenburg, KL; Killoy, WJ; Drisko, CL; Williams, R; Weber, HP; et al. Multi-center clinical evaluation of a chairside method for detecting certain periodontopathic bacteria in periodontal disease. *Journal of Periodontology* 1990; 61:189-96.

Maaslianski, W. Cholinergic receptors of lymphocytes. *Brain, Behavior, and Immunity* 1989; 3:1-14.

MacFarlane, GD; Herzberg, MC; Wolff, LF; Hardie, NA. Refractory periodontitis associated with abnormal polymorphonuclear leukocyte phagocytosis and cigarette smoking. *Journal of Periodontology* 1992; 63:908-13

MacNee, W; Wiggs, B; Belzberg, AS; Hogg, JC. The effect of cigarette smoking on neutrophil kinetics in human lungs. *New England Journal of Medicine* 1989; 321:924-8.

Madden, KS; Felten, SY; Felten, DL; Hardy, CA; Livnat, S. Sympathetic nervous system modulation of the immune system. II. Induction of lymphocyte proliferation and migration in vivo by chemical sympathectomy. *Journal of Neuroimmunology* 1994; 49:67-75.

Madden, KS; Moynihan, JA; Brenner, GJ; Felten, SY; Felten, DL; Livnat, S. Sympathetic nervous system modulation of the immune system. III. Alterations in T and B cell proliferation and differentiation in vitro following chemical sympathectomy. *Journal of Neuroimmunology* 1994; 49:77-87.

Maenard, L; Rola-Pleszczynski, M. Nicotine induces T-suppressor cells: modulation by the nicotinic antagonist D-tubocurarine and myasthenic serum. *Clinical Immunology and Immunopathology* 1987; 44:107-13.

Matejka, M; Partyka, L; Ulm, C; Solar, P; Sinzinger, H. Nitric oxide synthesis is increased in periodotnal disease. *Journal of Periodontal Research* 1998; 33:517-518.

Matsuba, T; Keicho, N; Higashimoto, Y; Granleese, S; Hogg, JC; Hayashi, S; Bondy, GP. Identification of glucocorticoid- and adenovirus E1A-regulated genes in lung epithelial cells by differential display. *American Journal of Respiratory Cell and Molecular Biology* 1998; 18:243-54.

McAllister, CG; Caggiula, AR; Knopf, S; Epstein, LH; Miller, AL; Antelman, SM; Perkins, KA. Immunological effects of acute and chronic nicotineadministration in rats. *Journal of Neuroimmunology* 1994; 50:43-9.

McAllister-Sistilli, CG; Caggiula, AR; Knopf, CS; Rose, CA; Miller, AL; Donny, EC. The effects of nicotine on the immune system. *Psychoneuroendocrinology* 1998; 23:175-187.

McGuire, MK; Nunn, ME. Prognosis versus actual outcome. IV. The effectiveness of clinical parameters and IL-1 genotype in accurately predicting prognoses and tooth survival. *Journal of Periodontology* 1999; 70:49-56.

McWhirter, JR; Wang, JY. An actin-binding function contributes to transformation by the Bcr-Abl oncoprotein of Philadelphia chromosome-positive human leukemias. *Embo Journal* 1993; 12:1533-46.

Mili, F; Flanders, WD; Boring, JR; Annest, JL; Destefano, F. The associations of race, cigarette smoking, and smoking cessation to measures of the immunesystem in middle-aged men. *Clinical Immunology and Immunopathology* 1991; 59:187-200.

Miller, LG; Goldstein, G; Murphy, M; Ginns, LC. Reversible alterations in immunoregulatory T cells in smoking. Analysis by monoclonal antibodies and flow cytometry. *Chest* 1982; 82:526-9.

Myer, JR. The regenerative potential of the periodontal ligament. *Journal of Prosthetic Dentistry* 1986; 55: 260-5.

Nair, MP; Kronfol, ZA; Schwartz, SA. Effects of alcohol and nicotine on cytotoxic functions of human lymphocytes. *Clinical Immunology and Immunopathology* 1990; 54:395-409.

Numabe, Y; Ogawa, T; Kamoi, H; Kiyonobu, K; Sato, S; Kamoi, K; Deguchi, S. Phagocytic function of salivary PMN after smoking or secondary smoking. *Annals of Periodontology* 1998; 3:102-7.

Neher, GH. Nicotine-induced depression of lymphocyte growth. *Toxicology and Applied Pharmacology* 1974; 27:253-8.

Newman, MG; Kornman, KS; Holtzman, S. Association of clinical risk factors with treatment outcomes. *Journal of Periodontology* 1994; 65:489-97

Newell, GR; Mansell, PW; Wilson, MB; Lynch, HK; Spitz, MR; Hersh, EM. Risk factor analysis among men referred for possible acquired immune deficiency syndrome. *Preventive Medicine* 1985; 14:81-91.

Nieman, RB; Fleming, J; Coker, RJ; Harris, JR; Mitchell, DM. The effect of cigarette smoking on the development of AIDS in HIV-1-seropositive individuals. *Aids* 1993; 7:705-10.

Norderyd, O; Hugoson, A; Grusovin, G. Risk of severe periodontal disease in a Swedish adult population. A longitudinal study. *Journal of Clinical Periodontology* 1999; 26:608-615.

Nowak, D; Ruta, U. Nicotine inhibits alpha-1-proteinase inhibitor inactivation by oxidants derived from human polymorphonuclear leukocytes. *Experimental Pathology* 1990; 38:249-55.

Nowak, D; Ruta, U; Piaseecka, G. Nicotine increases human polymorphonuclear leukocytes chemotactic response--a possible additional mechanism of lung injury in cigarette smokers. *Experimental Pathology* 1990; 39:37-43.

Nyman, S; Gottlow, J; Karring, T; Lindhe, J. The regenerative potential of the periodontal ligament. An experimental study in the monkey. Journal of Clinical Periodontology 1982; 9:257-65.

Omann, GM; Swann, WN; Oades, ZG; Parkos, CA; Jesaitis, AJ; Sklar, LA. N-formylpeptide-receptor dynamics, cytoskeletal activation, and intracellular calcium response in human neutrophil cytoplasts. *Journal of Immunology* 1987; 139:3447-55.

Pabst, MJ; Pabst, KM; Collier, JA; Coleman, TC; Lemonns-Prince, ML; Godat; Waring, MB; Babu, JP. Inhibition of neutrophil and monocyte defective functions by nicotine. *Journal of Periodontology* 1995; 66:1047-1055.

Packman, CH; Lichtman, MA. Activation of neutrophils: measurement of actin conformational changes by flow cytometry. *Blood Cells* 1990; 16:193-205

Palmer, RM. Tobacco smoking and oral health. *British Dental Journal* 1988; 164:258-60.

Palmer, RM; Matthews, JP; Wilson, RF. Non-surgical periodontal treatment with and without adjunctive metronidazole in smokers and non-smokers. *Journal of Clinical Periodontology* 1999; 26:158-63.

Papapanou, PN. Periodontal diseases: epidemiology. *Annals of Periodontology* 1996; 1:1-36

Parnell, JL; Anderson, DO; Kinnis, C. Cigarette smoking and respiratory infections in a class of student nurses. *New England Journal of Medicine* 1966; 274:979-84.

Paulin, L; McPherson, L; McQuade, M; Hanson, B. The effect of nicotine the attachment of human fibroblasts to glass and human root surfaces of periodontally diseased diseased teeth in smokers. *Journal of Periodontology* 1989; 60:564-569.

Payne, JB; Johnson, GK; Reinhardt, RA; Dyer, JK; Maze, CA; Dunning, DG. Nicotine effects on PGE2 and IL-1b release by LPS-treated human monocytes. *Journal of Periodontal Research* 1996; 31:99-104.

Perkins, KA; Sexton, JE; Dimarco, A. Acute thermogenic effects of nicotine and alcohol in healthy male and female smokers. *Physiology and Behavior* 1996; 60:305-9.

Phillips, JR; Nadim, HS; Layman, DL. Alterations in cell morphology and cytoskeletal proteins in gingival fibroblasts exposed to a Bacteroides gingivalis extract. *Journal of Periodontal Research* 1990; 25:339-46.

Preber, H; Bergstreom, J. Occurrence of gingival bleeding in smoker and non-smoker patients. *Acta Odontologica Scandinavica* 1985; 43:315-20

Preber, H; Bergstreom, J. Effect of non-surgical treatment on gingival bleeding in smokers and non-smokers. *Acta Odontologica Scandinavica* 1986; 44:85-9

Preber, H; Bergstreom, J. The effect of non-surgical treatment on periodontal pockets in smokers and non-smokers. *Journal of Clinical Periodontology* 1986; 13:319-23

Preber, H; Bergstreom, J. Cigarette smoking in patients referred for periodontal treatment. *Scandinavian Journal of Dental Research* 1986; 94:102-8

Preber, H; Bergstreom, J; Linder, LE. Occurrence of periopathogens in smoker and non-smoker patients. *Journal of Clinical Periodontology* 1992;19 :667-71

Preber, H; Linder, L; Bergstreom, J. Periodontal healing and periopathogenic microflora in smokers and non-smokers. *Journal of Clinical Periodontology* 1995; 22:946-52.

Quinn, SM; Zhang, JB; Gunsolley, JC; Schenkein, HA; Tew, JG. The influence of smoking and race on adult periodontitis and serum IgG2 levels. *Journal of Periodontology* 1998; 69: 171-7

Quinn, SM; Zhang, JB; Gunsolley, JC; Schenkein, JG; Schenkein, HA; Tew, JG. Influence of smoking and race on immunoglobulin G subclass concentrations in early-onset periodontitis patients. *Infection and Immunity* 1996; 6:2500-5.

Ratasirayakorn, W; Leone, P; Leblebicioglu, B; Walters, JD. Polyamines found in the inflamed peiodontium inhibit priming and apoptosis in human polymorphonuclear leukocytes. *Journal of Periodontology* 1999; 70:179-184.

Rosenberg, ES; Cutler, SA. The effect of cigarette smoking on the long-term success of guided tissue regeneration: a preliminary study. *Annals of the Royal Australasian College of Dental Surgeons* 1994; 12:89-93.

Royce, RA; Winkelstein, W Jr. HIV infection, cigarette smoking and CD4+ T-lymphocyte counts: preliminary results from the San Francisco Men's Health Study. *Aids* 1990; 4:327-33

Rundgren A, Mellstrom, D. The effect of tobacco smoke on the bone mineral content of the aging skeleton. *Mech Aging Dev* 1984; 28:273-277.

Ryder, MI. Nicotine effects on neutrophil F-actin formation and calcium release: implications for tobacco use and pulmonary diseases. *Experimental Lung Research* 1994; 20:283-96.

Ryder, MI; Fujitaki, R; Johnson, G; Hyun, W. Alterations of neutrophil oxidative burst by in vitro smoke exposure: implications for oral and systemic diseases. *Annals of Periodontology* 1998; 3:76-87.

Ryder, MI; Fujitaki, R; Lebus, S; Mahboub, M; Faia, B; Muhaimin, D; Hamada, M; Hyun, W. Alterations of neutrophil L-selection and CD18 expression by tobacco smoke: implications for periodontal diseases. *Journal of Periodontal Research* 1998; 33:359-68.

Selby, C; Drost, E; Wraith, PK; MacNee, W. In vivo neutrophil sequestration within the lungs of humans is determined by in vitro 'filterability'. *Journal of Appl Physiology* 1991; 71:1996-2003.

Selby, C; Drost, E; Brown,D; Howie, S; MacNee, W. Inhibition of neutrophil adherence and movement by acute cigarette smoke exposure. *Experimental Lung Research* 1992; 18:813-827.

Seoder, B; Nedlich, U; Jin, LJ. Longitudinal effect of non-surgical treatment and systemic metronidazole for 1 week in smokers and non-smokers with refractory periodontitis: a 5-year study. *Journal of Periodontology* 1999; 70:761-71.

Shapira, L; Soskolne, WA; Sela, MN; Offenbacher, S; Barak, V. The secretion of PGE_2, IL-1β, IL-6, and TNFα by adherent mononuclear cells from early onset periodontitis patients. *Journal of Periodontology* 1994; 65:139-46.

Shaw, HM; Milton, GW. Smoking and the development of metastases from malignant melanoma. *International Journal of Cancer* 1981; 28:153-6.

Soliman, DM; Twigg, HL 3d. Cigarette smoking decreases bioactive interleukin-6 secretion by alveolar macrophages. *American Journal of Physiology* 1992; 263:L471-8.

Sopori, ML; Gairola, CC; DeLucia, AJ; Bryant, LR; Cherian, S. Immune responsiveness of monkeys exposed chronically to cigarette smoke. *Clinical Immunology and Immunopathology* 1985; 36:338-44.

Steinbeck, MJ; Roth, JA. Neutrophil activation by recombinant cytokines. *Reviews of Infectious Diseases* 1989; 11:549-68.

Stoltenberg, JL; Osborn, JB; Pihlstrom, BL; Herzberg, MC; Aeppli, DM; Wolff, LF; Fischer, GE. Association between cigarette smoking, bacterial pathogens, and periodontal status. *Journal of Periodontology* 1993; 64:1225-30

Stossel, TP. The mechanery of blood cell movements. *Blood* 1994; 84:367-379

Sudan, BJ; Sainte-Laudy, J. *Nicotine and immunology.* In, *Drugs of Abuse and Immune Function*, ed. R.B. Watson, CRC Press, Boca Raton 1990; PP. 113-123.

Tipton, DA; Dabbous MKh. Effects of nicotine on proliferation and extracellular matrix production of human gingival fibroblasts in vitro. *Journal of Periodontology* 1995; 66:1056-1064.

Tollerud, DJ; Clark, JW; Brown, LM; Neuland, CY; Mann, DL; Pankiw-Trost, LK; Blattner, WA; Hoover, RN. Association of cigarette smoking with decreased numbers of circulating natural killer cells. *American Review of Respiratory Disease* 1989; 139:194-8.

Tonetti, MS; Cortellini, P; Carnevale, G; Cattabriga, M; de Sanctis, M; Pini Prato, GP. A controlled multicenter study of adjunctive use of tetracycline periodontal fibers in mandibular class II furcations with persistent bleeding. *Journal of Clinical Periodontology* 1998; 25:728-36

Tonetti, MS; Cortellini, P; Suvan, JE; Adriaens, P; Baldi, C; Dubravec, D; Fonzar, A; Fourmousis, I; Magnani, C; Muller-Campanile, V; Patroni, S; Sanz, M; Vangsted, T; Zabalegui, I; Pini Prato, G; Lang, NP. Generalizability of the added benefits of guided tissue regeneration in the treatment of deep intrabony defects. Evaluation in a multi-center randomized controlled clinical trial. *Journal of Periodontology* 1998; 69:1183-92.

Tonetti, MS; Prato, GP; Cortellini, P. Factors affecting the healing response of intrabony defects following guided tissue regeneration and access flap surgery. *Journal of Clinical Periodontology* 1996; 23:548-56

Tonetti, MS; Pini-Prato, G; Cortellini, P. Effect of cigarette smoking on periodontal healing following GTR in infrabony defects. A preliminary retrospective study. *Journal of Clinical Periodontology* 1995; 22:229-34.

Tonetti, MS. Molecular factors associated with compartmentalization of gingival immune responses and transepithelial neutrophil migration. *Journal of Periodontal Research* 1997; 32: 104-9

Totti, N; Mccusker, KT; Campbell,E; Griffin, CL; Senior, RM. Nicotine is chemotactic for neutrophils and enhanceds neutrophil responsiveness to chemotatic peptides. *Science* 1984; 223:169-171.

Van Etten, RA; Jackson, PK; Baltimore, D; Sanders, MC; Matsudaira, PT; Janmey, PA. The COOH terminus of the *c-Abl* tyrosine kinase contains distinct F- and G-actin binding domains with bundling activity. *Journal of Cell Biology* 1994; 124:325-40.

Waterman, CM; Salmon, ED. Positive feedback interactions between microtubule and actin dynamics during cell motility. *Current Opinion in Cell Biology* 1999; 11:61-7.

Wilkins, JN; Carlson, HE; Van Vunakis, H; Hill, MA; Gritz, E; Jarvik, ME. Nicotine from cigarette smoking increases circulating levels of cortisol, growth hormone, and prolactin in male chronic smokers. *Psychopharmacology* 1982; 78:305-8.

Wilson, TG; Nunn, M. The relationship between the interleukin-1 periodontal genotype and implant loss. Initial data. *Journal of Periodontology* 1999; 70:724-729.

Winternitz, WW; Quillen, D. Acute hormonal response to cigarette smoking. *Journal of Clinical Pharmacology* 1977; 17:389-97.

Zambon, JJ; Grossi, SG; Machtei, EE; Ho, AW; Dunford, R; Genco, RJ. Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. *Journal of Periodontology* 1996; 67:1050-4

Zetterstreom, O; Nordvall, SL; Bjeorkstaen, B; Ahlstedt, S; Stelander, M. Increased IgE antibody responses in rats exposed to tobacco smoke. *Journal of Allergy and Clinical Immunology* 1985; 75:594-8.

Zhang, S; Petro, TM. The effect of nicotine on murine CD4 T cell responses. *International Journal of Immunopharmacology* 1996; 18:467-78.

Zhang, JB; Quinn, SM; Rausch, M; Gunsolley, JC; Schenkein, HA; Tew, JG. Hyper-immunoglobulin G2 production by B cells from patients with localized juvenile periodontitis and its regulation by monocytes. *Infection and Immunity* 1996; 64:2004-9.

Subject I.D.	Smoking Status	Smoke Exposure Duration (minutes)					
		0	1	2	3	4	5
1	Smoker	200.00	167.27	171.06	157.89	139.47	124.95
2	Smoker	88.76	101.27	68.94	75.88	66.75	53.52
3	Smoker	156.00	108.91	106.43	108.59	N/A	84.64
4	Smoker	90.32	89.38	79.30	62.24	61.24	55.97
5	Smoker	159.91	167.29	163.06	159.40	160.45	138.95
6	Smoker	208.59	216.61	208.85	188.66	181.56	189.40
7	Smoker	329.75	291.68	262.71	279.49	247.55	224.18
	Mean	**176.19**	**163.2**	**151.48**	**147.45**	**142.84**	**124.52**
	SD	**82.55**	**72.6**	**70.98**	**74.47**	**71.04**	**65.42**
8	Nonsmoker	77.29	90.14	76.82	70.77	61.95	58.30
9	Nonsmoker	65.20	53.81	47.90	46.04	38.86	34.35
10	Nonsmoker	81.55	54.79	46.87	37.80	39.57	22.27
11	Nonsmoker	163.98	N/A	119.57	89.07	101.78	61.91
12	Nonsmoker	261.80	264.06	268.89	149.95	134.99	133.87
13	Nonsmoker	134.43	136.12	98.64	95.72	99.97	88.23
14	Nonsmoker	70.36	215.28	172.23	136.00	185.30	132.15
	Mean	**122.09**	**120.7**	**118.7**	**89.34**	**94.63**	**75.87**
	SD	**71.87**	**78.18**	**79.32**	**42.36**	**53.45**	**44.32**

TABLE I. Mean fluorescence intensity of F-actin stains in PMNs of smokers and non-smokers after 1-5 minute smoke exposure. Data relates to graph in Figure 3.**N/A** denotes undetectable F-actin levels after centrifugation procedure. Neither group showed statistically significant decrease in mean fluorescence intensity compared to baseline. Smokers had higher mean fluorescence intensity than nonsmokers at baseline and at all time intervals. However, no statistically significant difference in mean fluorescence intensity was found between-group comparisons.

Subject I.D.	Study Group	fMLP Stimulation Duration (Seconds)					
		0	30	60	120	240	360
8	Control	89.44	325.32	283.02	195.99	112.46	89.60
9	Control	62.88	174.26	156.52	136.65	95.99	97.15
10	Control	80.59	170.89	169.93	144.57	105.80	67.22
11	Control	168.52	704.81	682.23	605.63	362.34	N/A*
12	Control	368.82	736.95	701.49	752.79	437.67	398.43
13	Control	191.51	653.64	513.11	262.50	199.10	220.92
14	Control	235.18	734.55	N/A*	759.82	539.92	311.49
	Mean	**170.99**	**500.06***	**417.72***	**408.28#**	**264.75**	**197.47**
	SD	**108.16**	**265.09**	**24.94**	**286.02**	**180.92**	**136.09**
8	5-min smoke	62.14	249.54	123.43	70.33	88.09	93.64
9	5-min smoke	49.22	168.82	123.28	47.75	49.74	54.65
10	5-min smoke	41.14	180.30	83.26	27.81	92.12	42.62
11	5-min smoke	73.01	672.22	513.29	153.34	148.41	109.12
12	5-min smoke	144.39	377.90	212.80	172.19	189.66	197.80
13	5-min smoke	115.25	119.89	21.52	140.20	130.99	42.30
14	5-min smoke	74.89	296.92	148.60	83.90	89.15	82.75
	Mean	**80.01**	**295.08***	**175.17**	**99.36**	**112.59**	**88.98**
	SD	**37.06**	**187.52**	**160.14**	**55.90**	**46.69**	**54.49**

TABLE II. Mean fluorescence intensity of F-actin stain in PMNs of non-smokers after 0-6 minute fMLP stimulation. Data relates to graph in Figure 4. **N/A** denotes undetectable F-actin levels after centrifugation procedure. * denotes statistically significant difference in mean fluorescence intensity within group comparison to baseline ($p<0.05$). # denotes statistically significant difference in mean fluorescence intensity denotes statistically significant difference in mean fluorescence intensity in between-group comparisons at baseline and various time intervals($p<0.05$).

Subject I.D.	Study Group	fMLP Stimulation Duration (Seconds)					
		0	30	60	120	240	360
1	Control	164.43	642.71	646.24	518.54	342.12	378.93
2	Control	93.95	228.00	N/A	220.63	111.02	110.66
3	Control	114.21	368.69	314.84	194.45	166.61	136.44
4	Control	119.17	359.59	307.07	199.95	100.36	74.96
5	Control	173.92	273.13	283.69	268.92	229.59	217.97
6	Control	220.75	347.63	350.29	291.50	251.78	267.85
7	Control	327.15	666.58	656.79	616.02	482.75	443.09
	Mean	**173.37**	**412.33***	**426.49***	**330.00*#**	**240.60**	**232.85**
	SD	**80.33**	**173.18**	**175.64**	**168.23**	**136.09**	**139.13**
1	5-min Smoke	124.58	498.79	197.42	106.22	106.93	82.45
2	5-min Smoke	55.23	216.81	66.16	44.28	80.09	78.13
3	5-min Smoke	82.22	398.30	189.22	94.20	103.79	105.36
4	5-min Smoke	57.41	219.64	74.81	75.66	80.91	80.54
5	5-min Smoke	101.17	417.66	352.42	175.53	126.25	180.12
6	5-min Smoke	131.45	494.64	405.59	275.50	202.75	218.34
7	5-min Smoke	178.05	699.18	539.29	238.30	251.88	247.97
	Mean	**104.30**	**420.72***	**260.70***	**144.24**	**136.09**	**141.84**
	SD	**44.14**	**169.13**	**177.26**	**87.27**	**65.86**	**72.18**

TABLE III. Mean fluorescence intensity of F-actin stain in PMNs of smokers after 0-6 minute fMLP stimulation. Data relates to graph in Figure 5. **N/A** denotes undetectable F-actin levels after centrifugation procedure. * denotes statistically significant difference in mean fluorescence intensity within group comparison to baseline ($p<0.05$). # denotes statistically significant difference in mean fluorescence intensity in between-group comparisons at baseline and various time intervals ($p<0.05$).

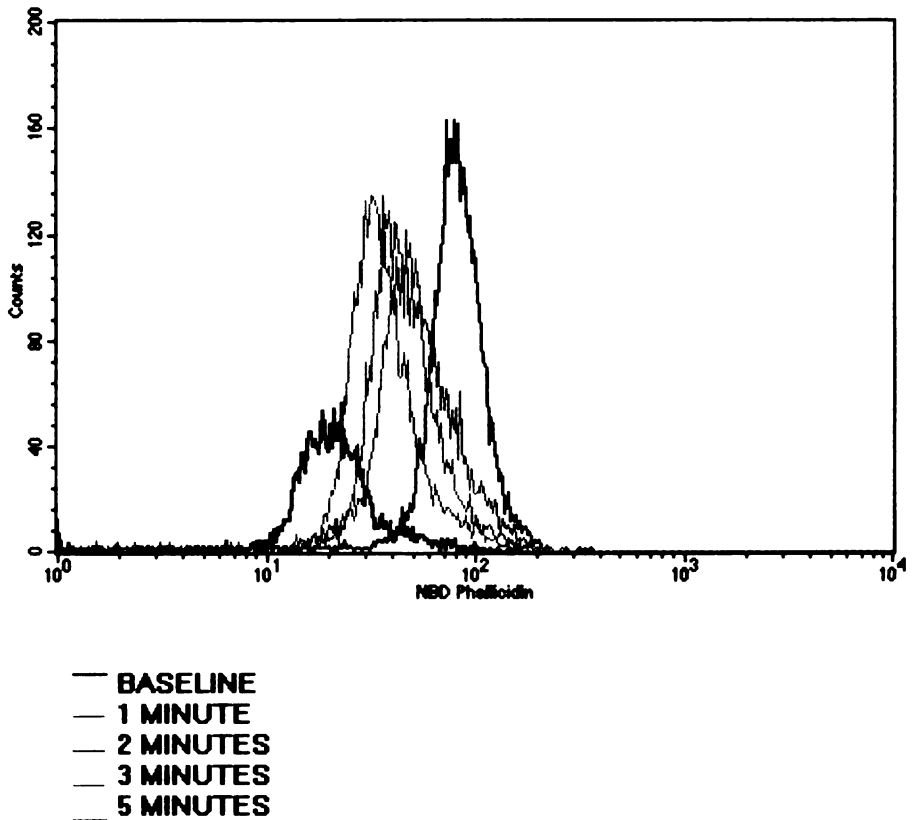


Figure 1. Sample flow cytometry histogram of F-actin stains of PMNs of a non-smoker at baseline and after exposure to smoke from 1-5 minutes. A gradual decrease in mean F-actin content is seen from baseline with increasing duration of smoke. This figure shows results for PMNs of a non-smoker. PMNs from all 14 smokers and non-smokers in this study showed the same pattern of changes in F-actin content on exposure to cigarette smoke. However, the magnitude of chnages varied significantly in different subjects.

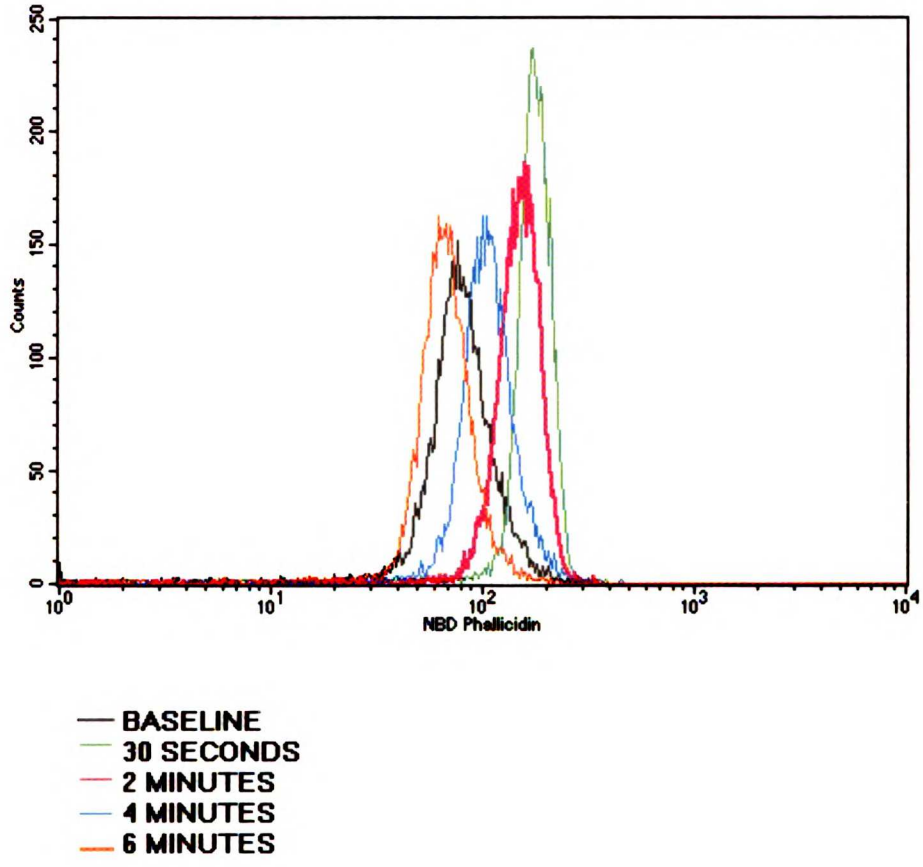


Figure 2. Sample flow cytometry histogram of F-actin stains of PMNs of a non-smoker at baseline and at 30-360 seconds following fMLP stimulation. A rapid increase in F-actin stains is seen immediately after exposure to fMLP, this is followed by a gradual decrease with mean levels returning to baseline value at 6 minutes after exposure. This figure shows results for PMNs of a non-smoker. PMNs from all 14 smokers and non-smokers in this study showed the same pattern of changes in F-actin content on exposure to cigarette smoke. However, the magnitude of increase varied significantly in different subjects.

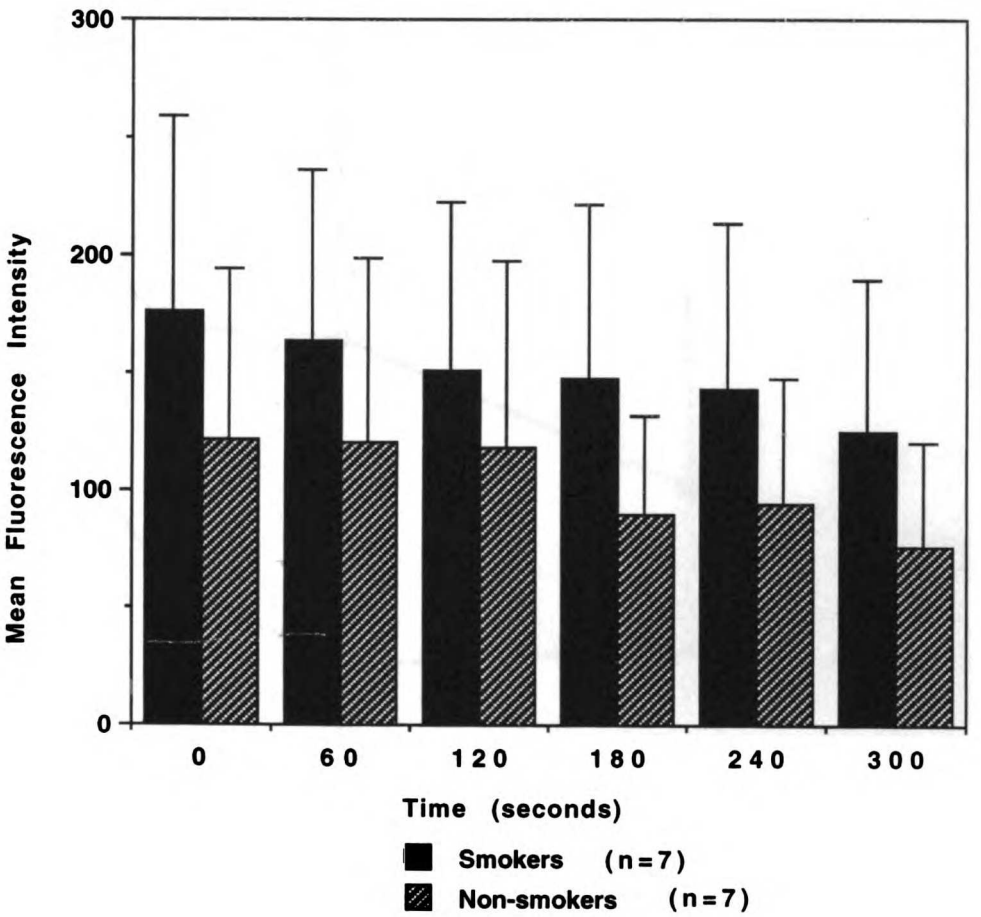
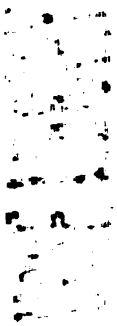


Figure 3. F-actin kinetics of PMNs in smokers and non-smokers at baseline and after 1-5 minute smoke exposure (± SD).

1-5 minute acute smoke exposure resulted in a gradual decrease in F-actin content in both smoker and non-smoker groups. Smokers had slightly higher F-actin values than their matching controls at baseline and at all time intervals. However, there were no statistically significant differences between smokers and non-smokers at any time intervals. Compared to baseline, neither group showed a statistically significant decrease in content of F-actin.



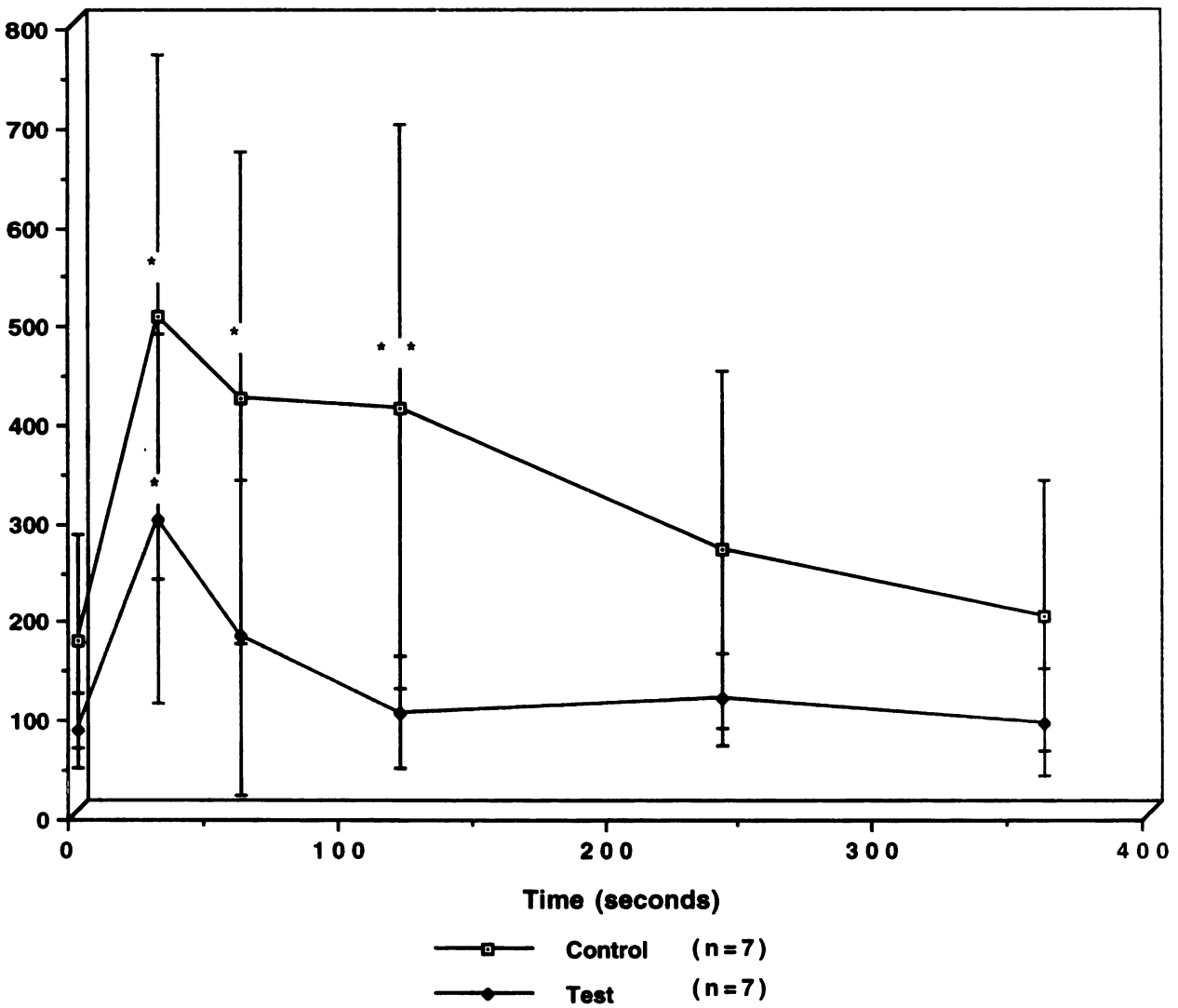


Figure 4. F-actin kinetics of control and 5-minute smoke-exposed PMNs in non-smokers at baseline and at 30-360 seconds following fMLP stimulation (± SD).
Control PMNs showed a 192% and 144% rise in F-actin stain at 30 and 60 seconds ($p<0.05$), maintained at 139% elevation at 2 minutes, then followed by a decline to 15% elevation over baseline at 5 minutes after fMLP stimulation. PMNs preincubated with 5-minute smoke showed an abrupt 269% rise in F-actin stain ($p<0.05$), followed by a rapid decline at 1 minute to 119% over baseline, to 23% over baseline at 2 minutes and a gradual decrease over the next 4 minutes.

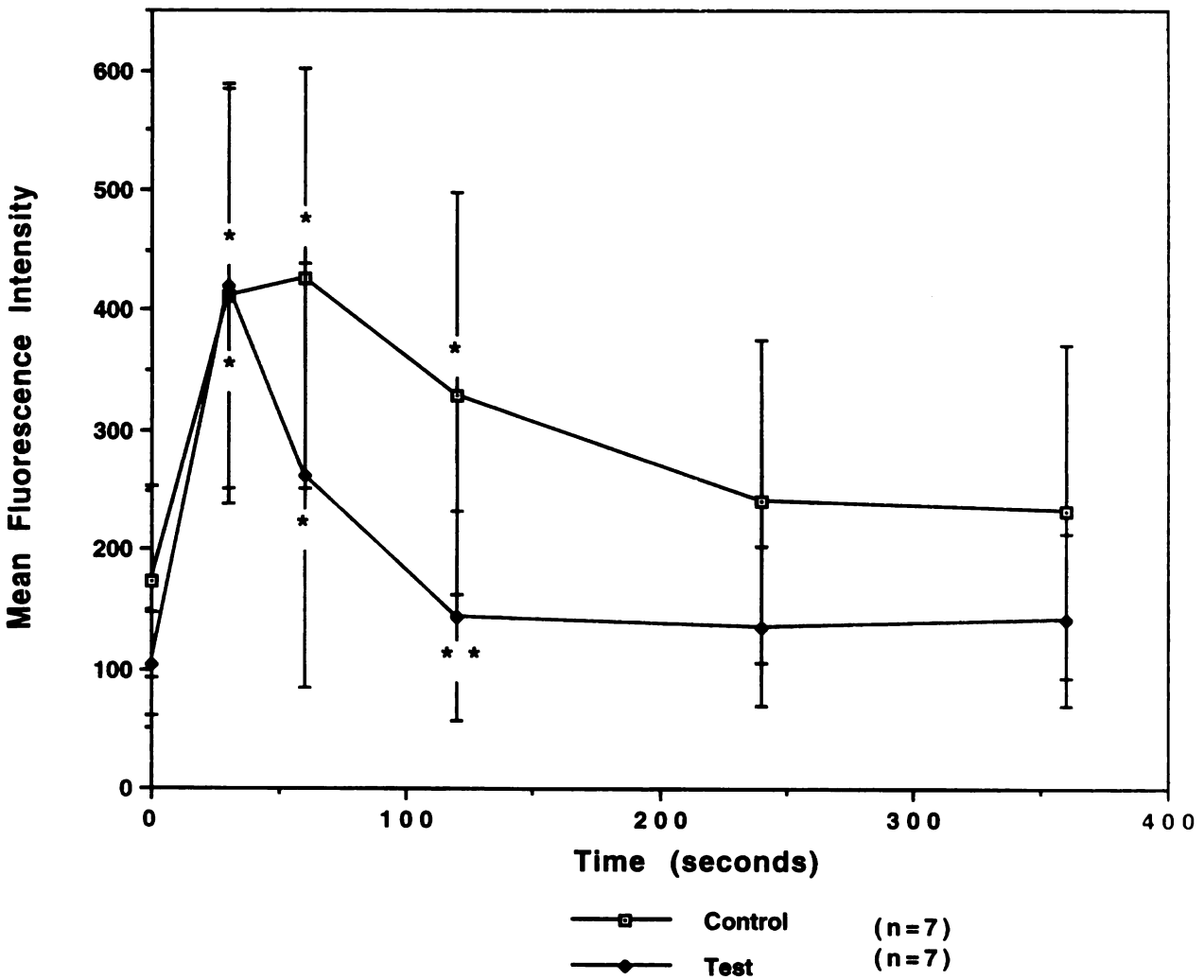


Figure 5. F-actin kinetics of control and 5-minute smoke-exposed PMNs in smokers at baseline and at 30-360 seconds following fMLP stimulation.

Control PMNs incubated with fMLP showed a 138% and 146% rise in F-actin stain at 30 and 60 seconds ($p<0.05$), respectively, which gradually decline to 34% elevation over baseline at 5 minutes after fMLP stimulation. PMNs preincubated with 5-minute smoke showed an abrupt 303% rise in F-actin stain ($p<0.05$) that was followed by a rapid decline at 1 and 2 minutes with levels decreasing to 34% over baseline at 5 minutes after fMLP stimulation.

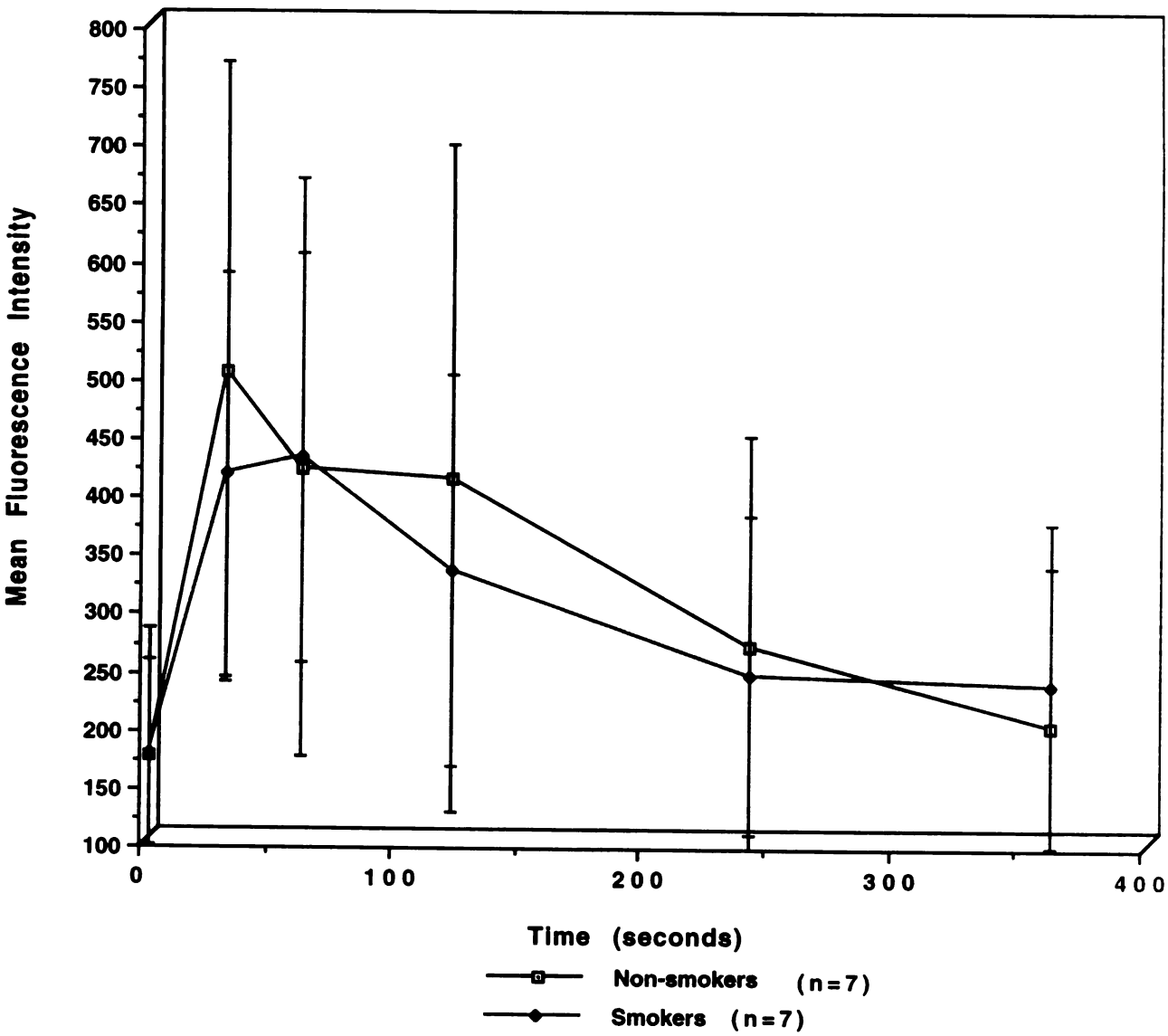
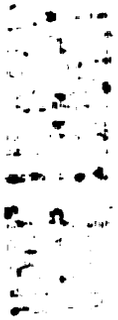


Figure 6. Comparison of F-actin kinetics of control PMNs between smokers and non-smokers at baseline and at 30-360 seconds following fMLP stimulation (± SD).
Without smoke exposure, both smokers and non-smokers had similar baseline values and after fMLP stimulation, both groups showed similar kinetic pattern of F-actin polymerization-depolymerization. There were no statistically significant differences found between these 2 groups.



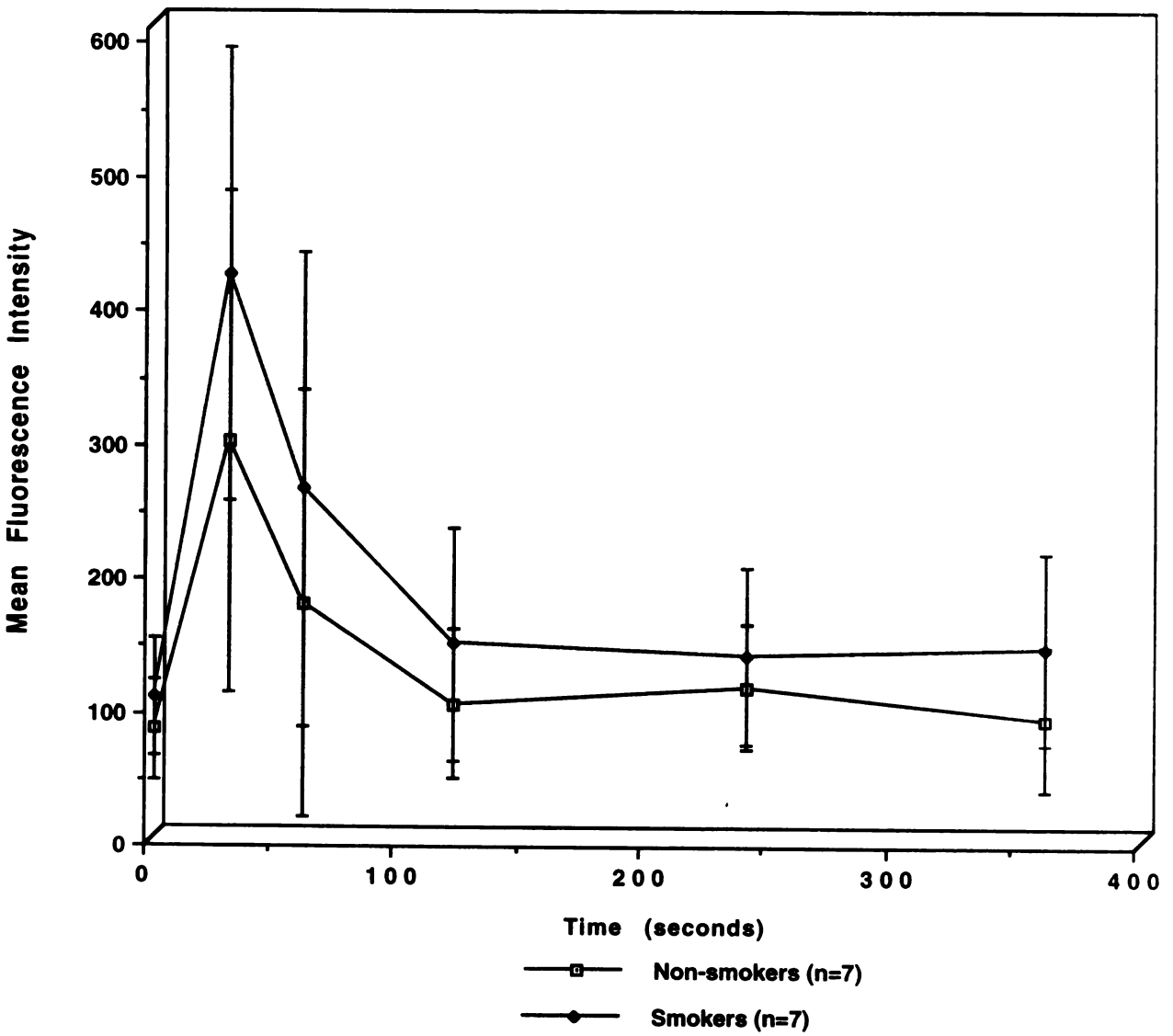
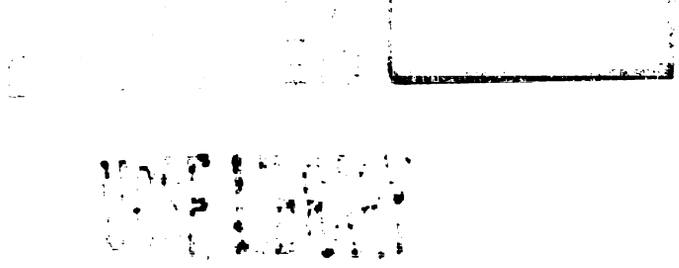


Figure 7. Comparison of F-actin kinetics of 5-minute smoke-exposed PMNs between smokers and non-smokers at baseline and at 30-360 seconds following fMLP stimulation (± SD).

Without 5- minute smoke exposure, both smokers and non-smokers had slightly lower baseline F-actin values, compared to their control groups. Consistently lower F-actin values were found after fMLP stimulation in non-smokers than in smokers. However, the differences between them were not found to be statistically significant.



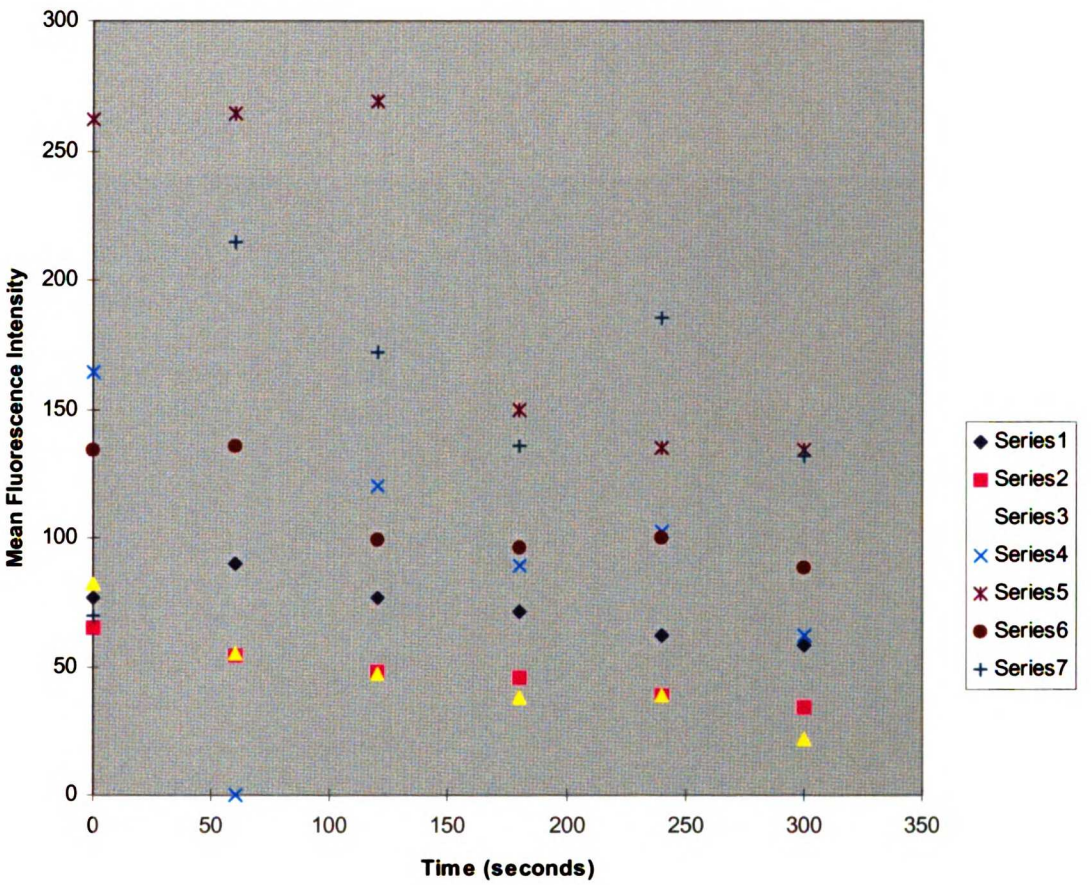
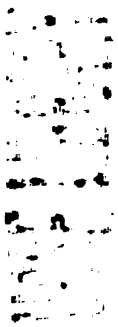


Figure 8. Distribution of F-actin stain of PMNs in non-smoking individuals at baseline and at 1-5 minutes following smoke exposure.

Non-smokers had scattered baseline values. One individual had a significantly higher F-actin of 261 mean fluorescence intensity. After 5-minute smoke exposure, distribution of individual F-actin values became more clustered and ranged from 22-134 mean fluorescence intensity.



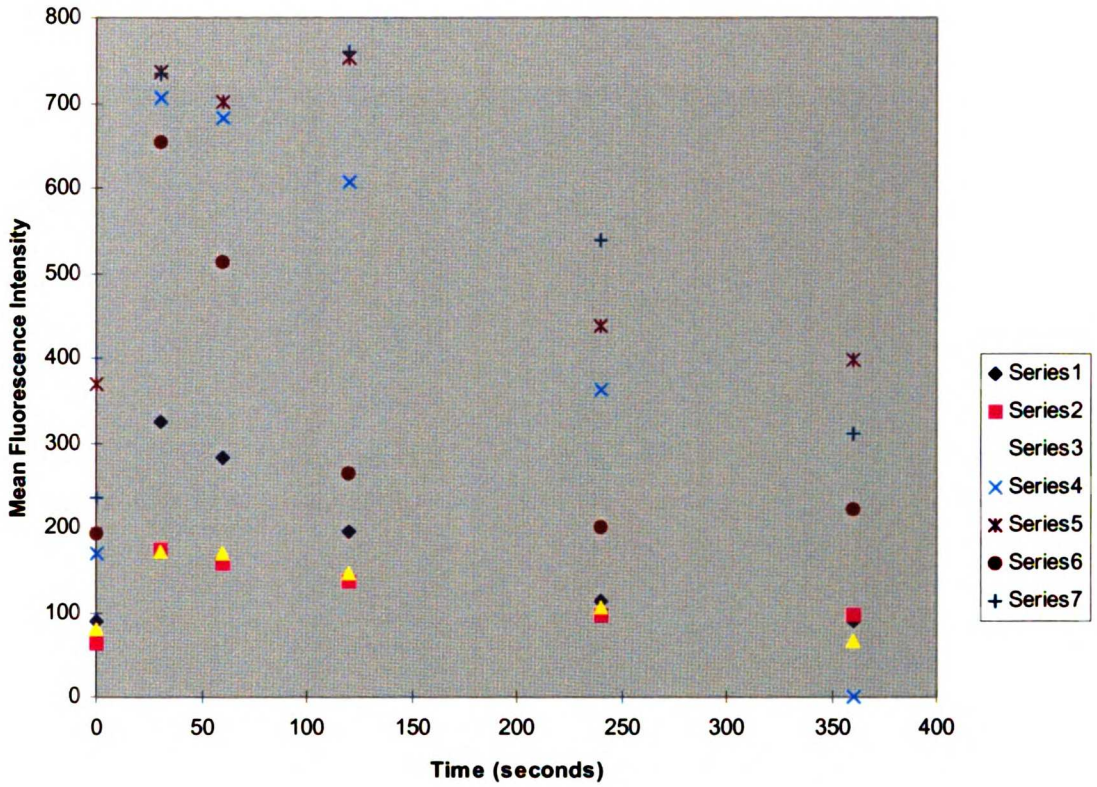
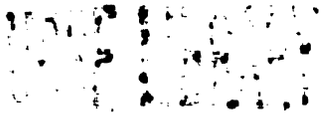


Figure 9. Distribution of F-actin stain of PMNs in non-smoking individuals from control group at baseline and at 30-360 seconds following fMLP stimulation.

A dramatic rise in F-actin stain after fMLP stimulation was found. 43% of the subjects' F-actin values fell below 400 mean fluorescence intensity and 57% were above 600 mean fluorescence intensity. Individuals with lower F-actin values continued to have values in the lower range and individuals with higher values became more scattered as time increased.



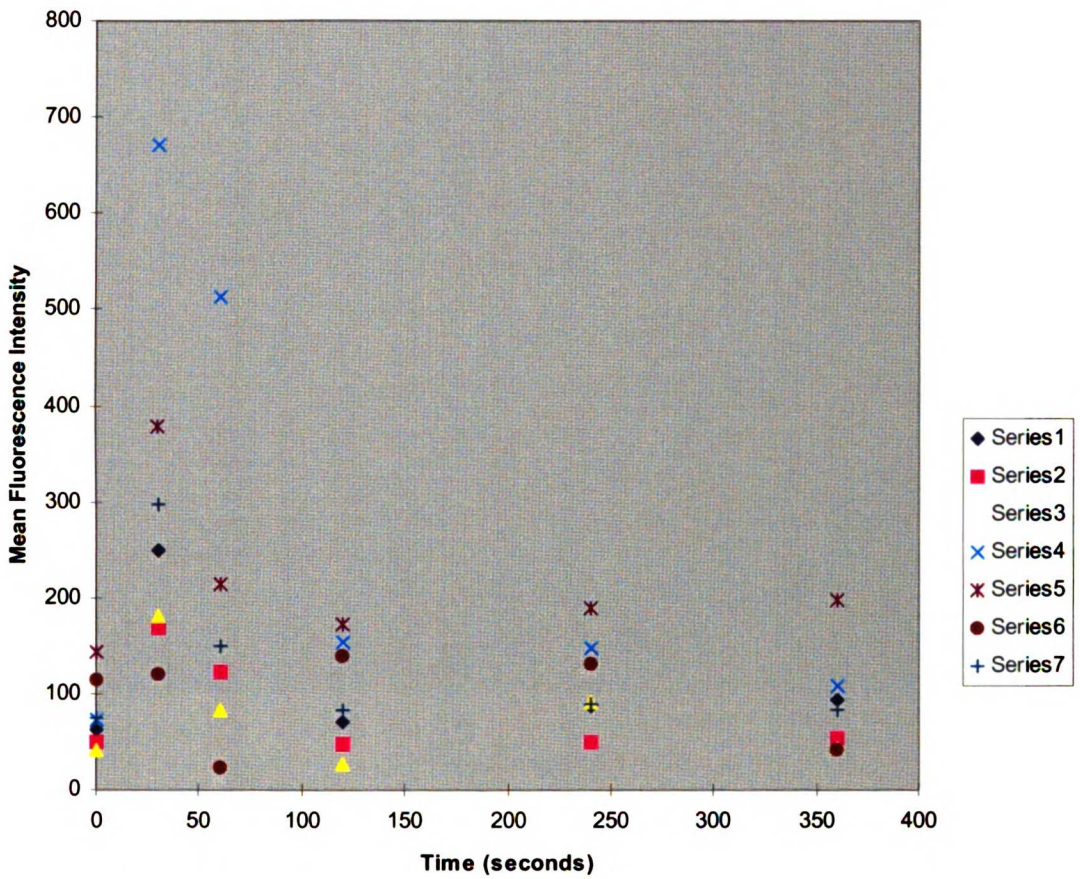
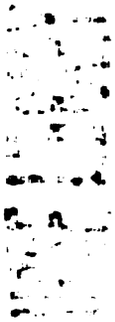


Figure 10. Distribution of F-actin stain of PMNs in non-smoking individuals from test group at baseline and at 30-360 seconds following fMLP stimulation.

PMNs preincubated with cigarette smoke for 5 minutes had similar baseline values, ranging from 41 to 144 mean fluorescence intensity. However, following fMLP stimulation, divergent values were found at 30- and 60-second intervals, followed by more clustering of F-actin values after 2 minutes.



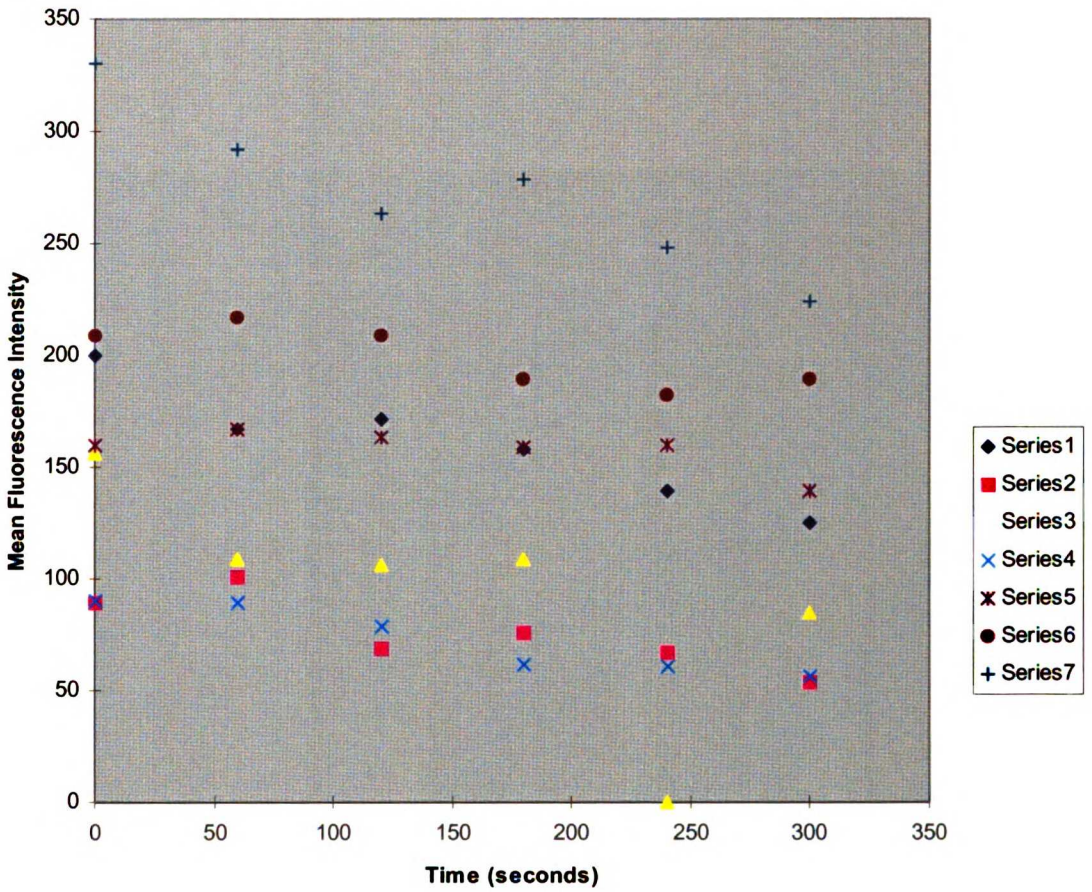
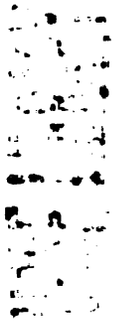


Figure 11. Distribution of F-actin stain of PMNs in individual smokers at baseline and at 1-5 minutes following smoke exposure.

Compared to nonsmokers, smokers had a more scattered distribution of F-actin values at baseline and at different time intervals following smoke exposure.



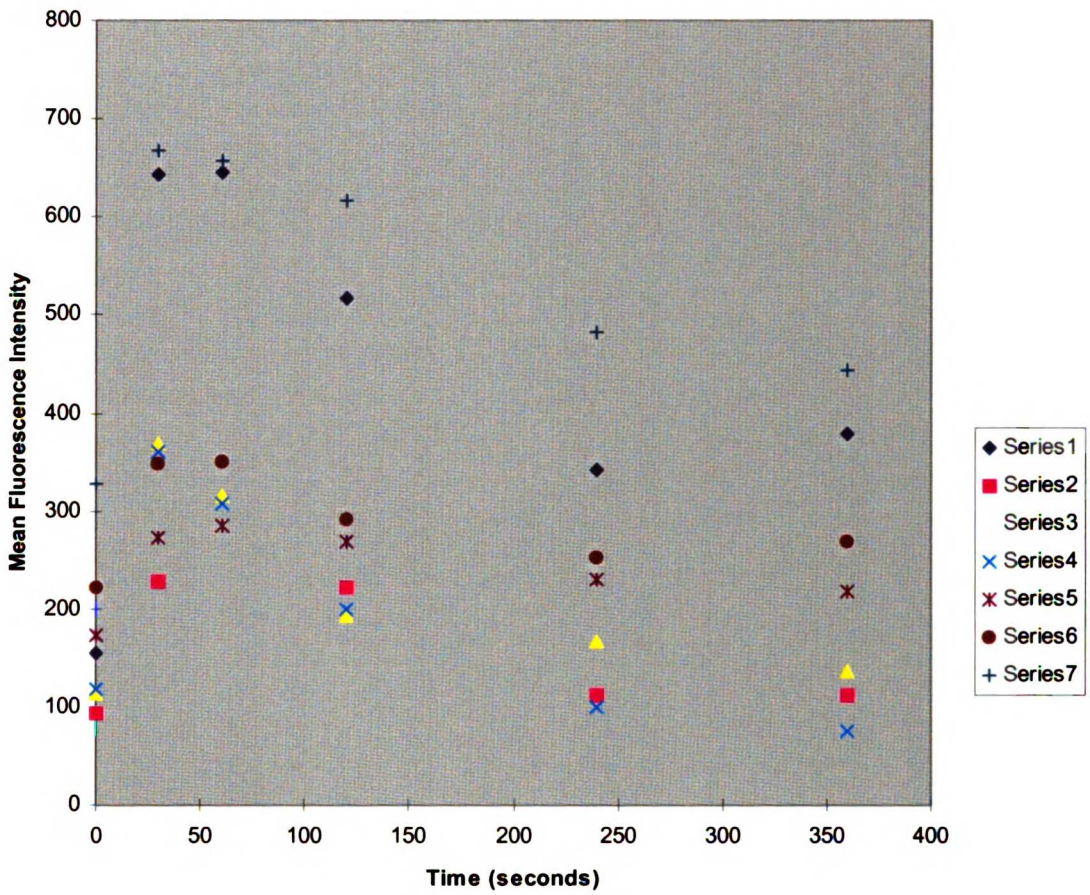
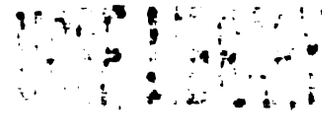


Figure 12. Distribution of F-actin stain of PMNs in smoking individuals from control group at baseline and at 30-360 seconds following fMLP stimulation.
Two individuals in the control group showed a dramatic rise in F-actin stains after fMLP stimulation and maintained at a higher level for 6 minutes compared to values of PMNs in other smokers.



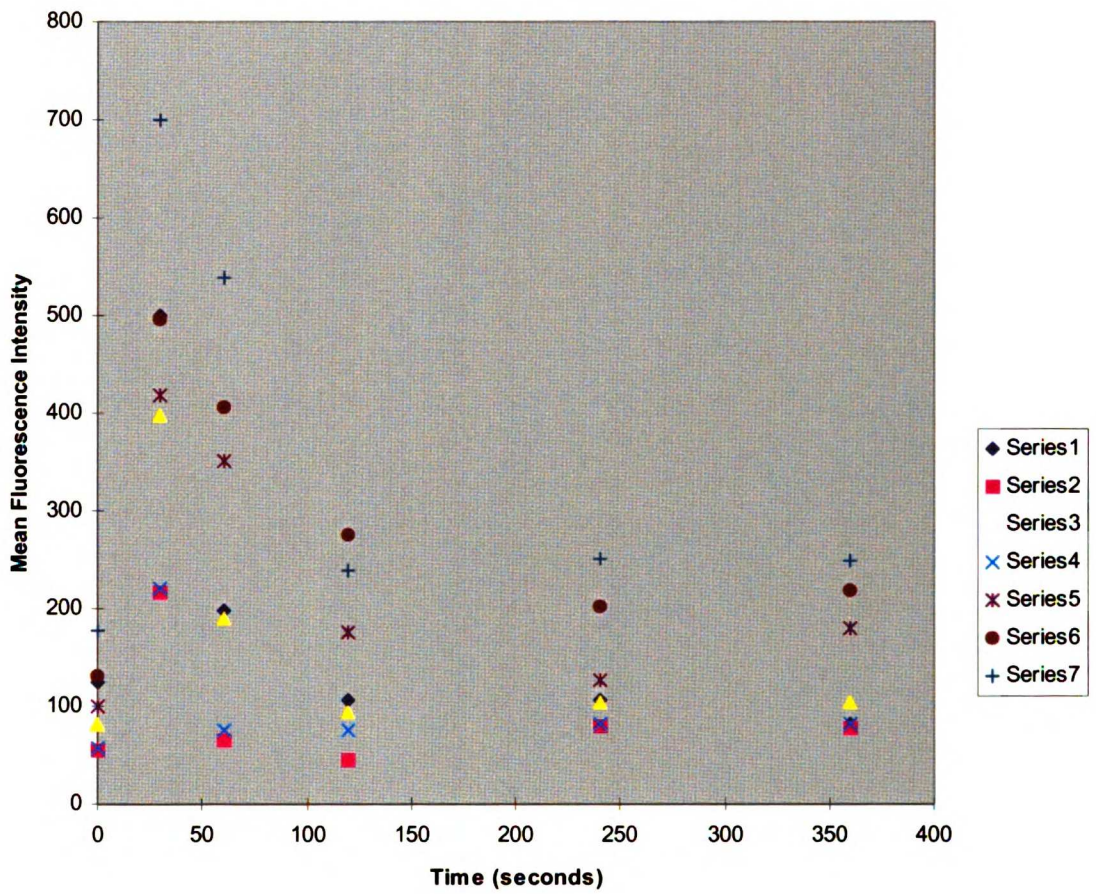


Figure 13. Distribution of F-actin stain of PMNs in smoking individuals from test group at baseline and at 30-360 seconds following fMLP stimulation.

Individual F-actin values were more clustered and in the lower fluorescence range in 5-minute smoke exposure group at baseline and at different time intervals compared to the values in control group. However, one individual continued to show a higher F-actin value at 30 and 60 seconds following fMLP stimulation.