



# Down Regulation of MMP-2 Expression and Invasion Potential of Human Gingival Fibroblasts

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## Abstract

### **Background:**

Gingivitis, a condition in which gums swell, turn red, and bleed easily, is the earliest stage of gum disease, affecting three out of four adults over the age of 35. Left untreated, it progresses to periodontitis, an advanced stage of gum disease characterized by additional destruction of gum tissue and progression to the bone. MMPs play a critical role in this process as, in concert, they degrade the majority of the extracellular matrix components.

### **Objective:**

We investigated the effect of a unique formulation (NM) containing ascorbic acid, lysine, proline, arginine, and epigallocatechin gallate on gingival fibroblast growth, MMP-2 secretion, and invasion potential.

### **Materials and Methods:**

Human gingival fibroblasts CRL-2014 (ATCC) were grown in DME medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 mg/ml) in 24-well tissue culture plates. At near confluence, the cells were treated with NM dissolved in media and tested at 0, 10, 100, 500, and 1000 µg/ml in triplicate at each dose. Cell proliferation/ cytotoxicity was evaluated by MTT assay, MMP expression by gelatinase zymography, and invasion through Matrigel.

### **Results:**

The nutrient mixture demonstrated slight, dose-dependent inhibition of gingival fibroblast growth with maximum inhibition of 30% at 1000 µg/ml ( $p=0.001$ ). NM inhibited gingival fibroblast MMP-2 expression in a dose-dependent fashion with total virtual inhibition at 500 µg/ml. The synergistically acting nutrient mixture significantly reduced the invasion of human gingival cells through Matrigel in a dose-dependent manner, with 76% inhibition at 50 µg/ml and 100% at 500 µg/ml NM ( $p<0.0001$ ).

### **Conclusion:**

Our results suggest that NM has potential as a therapeutic tool in treatment of periodontal inflammation, through inhibition of gingival fibroblast MMP-2 expression and matrix invasion.

### Comments

Gingivitis, the earliest stage of gum disease, affects three out of four adults over the age of 35. Left untreated, it progresses to periodontitis, an advanced stage of gum disease characterized by additional destruction of gum tissue and progression to the bone. MMPs play a critical role in this process as, in concert, they degrade the majority of the extracellular matrix components. We studied the effect of a combination (NM) of ascorbic acid, lysine, proline, and green tea extract on expression of MMPs by gingival fibroblasts in cell culture. NM significantly inhibited MMP-2 expression and invasion through Matrigel, suggesting NM has great potential as a therapeutic tool in prevention and treatment of gingivitis and its sequelae.

### NUTRIENT SYNERGY INHIBITS MMP-2 EXPRESSION AND INVASION OF GINGIVAL FIBROBLASTS

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#### BACKGROUND:

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#### OBJECTIVE:

We investigated the effect of a unique nutrient mixture (NM) containing ascorbic acid, lysine, proline, arginine, and epigallocatechin gallate on gingival fibroblast growth, MMP-2 secretion, and invasion potential.

#### MATERIALS AND METHODS:

1. Human gingival fibroblasts CRI-2014 (ATCC) were grown in DMEM medium supplemented with 10% FBS, penicillin (100 U/ml) and streptomycin (100 mg/ml) in 24-well tissue culture plates.

2. At near confluence, the cells were treated with NM dissolved in media and tested at 0, 10, 100, 500, and 1000 µg/ml in triplicate at each dose.

3. Cell proliferation was evaluated by MTT assay, MMP expression by gelatinase zymography, and invasion through Matrigel.

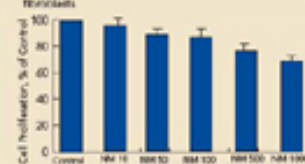
#### 4. Nutrient Mixture (NM) Composition

Nutrient	Final stock solution
Water (Deionized water in 400 µl) Control	700 mg
Ascorbic acid	1800 mg
Lysine	1800 mg
Proline	700 mg
L-Arginine	500 mg
Epigallocatechin Gallate	200 mg
Hydrolyzed Green Tea Extract 80% polyphenols	1800 mg
Selenium	30 mg
Copper	2 mg
Manganese	1 mg

#### RESULTS

1. Growth of gingival fibroblasts was slightly inhibited by the nutrient mixture with a maximum of 30% inhibition at 1000 µg/ml

Figure 1 - Effect of nutrient mixture (NM) on growth of gingival fibroblasts



2. Nutrient mixture inhibited MMP-2 expression of gingival fibroblasts in a dose-dependent fashion with complete inhibition at 500 µg/ml

Figure 2 - Effect of nutrient mixture (NM) on MMP-2 expression of gingival fibroblasts



3. The nutrient mixture (NM) inhibited gingival fibroblast Matrigel invasion in a dose-dependent fashion with complete inhibition at 500 µg/ml ( $p < 0.0001$ )

Figure 3A - Effect of nutrient mixture (NM) on Matrigel invasion by gingival fibroblasts

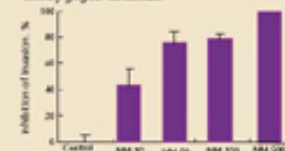
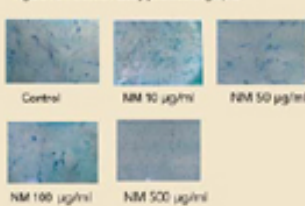
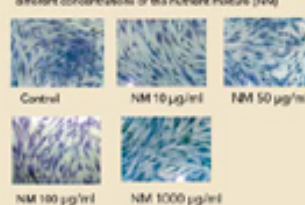


Figure 3B - Invasion study photomicrographs



4. H&E staining demonstrated morphology of gingival fibroblasts was not affected by treatment with the nutrient mixture (NM) up to 1000 µg/ml

Figure 4 - H&E stained gingival fibroblasts treated with different concentrations of the nutrient mixture (NM)



#### CONCLUSION:

Our results suggest that NM has potential as a therapeutic tool in treatment of periodontal inflammation, through inhibition of gingival fibroblast MMP-2 expression and matrix invasion.