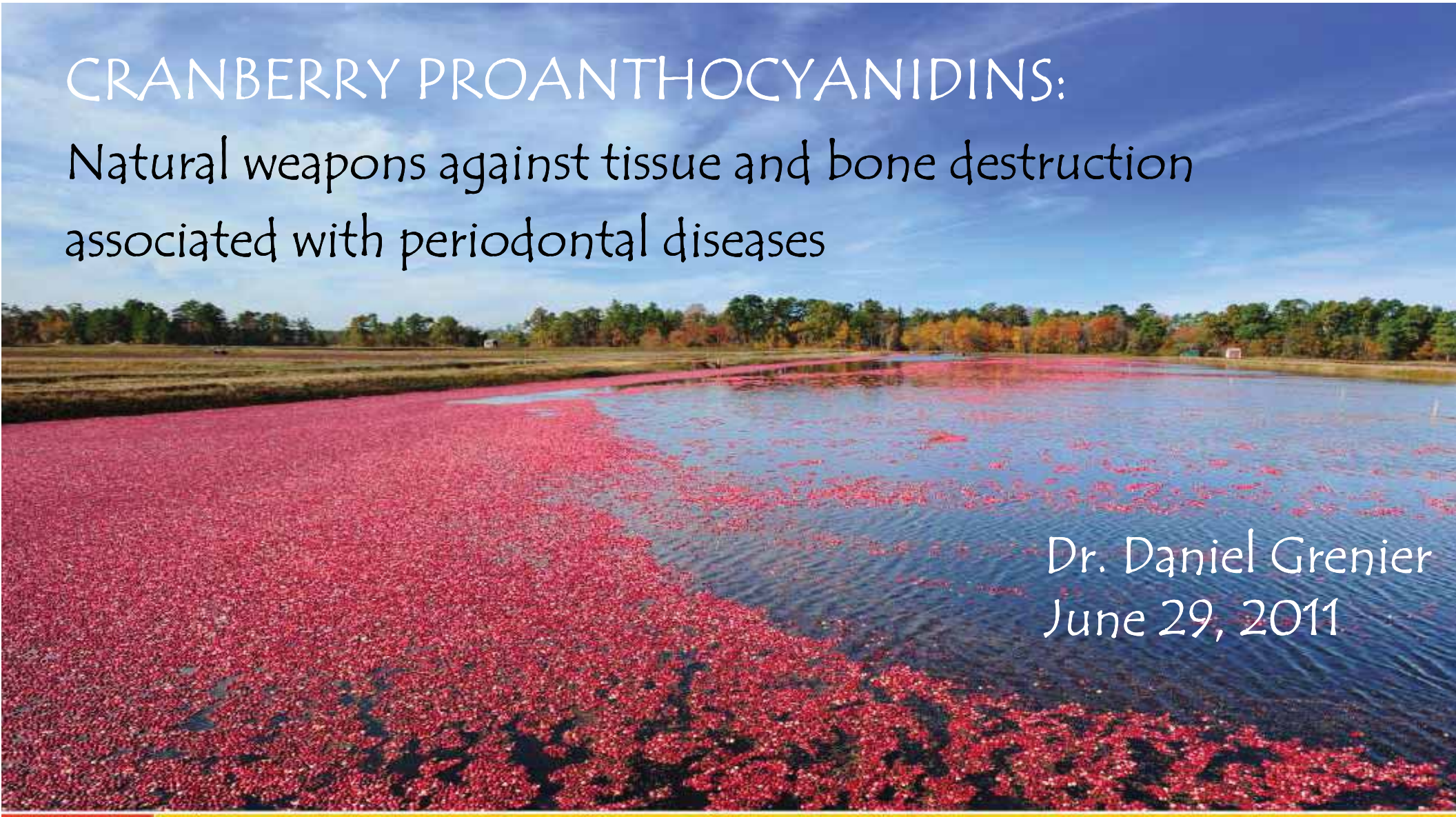
CRANBERRY PROANTHOCYANIDINS:

Natural weapons against tissue and bone destruction associated with periodontal diseases



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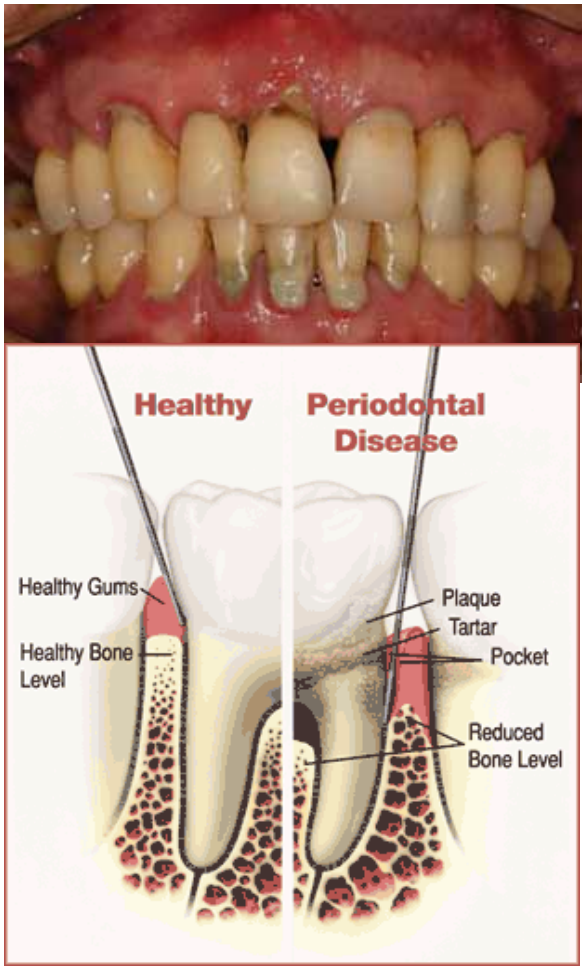
UNIVERSITÉ LAVAL

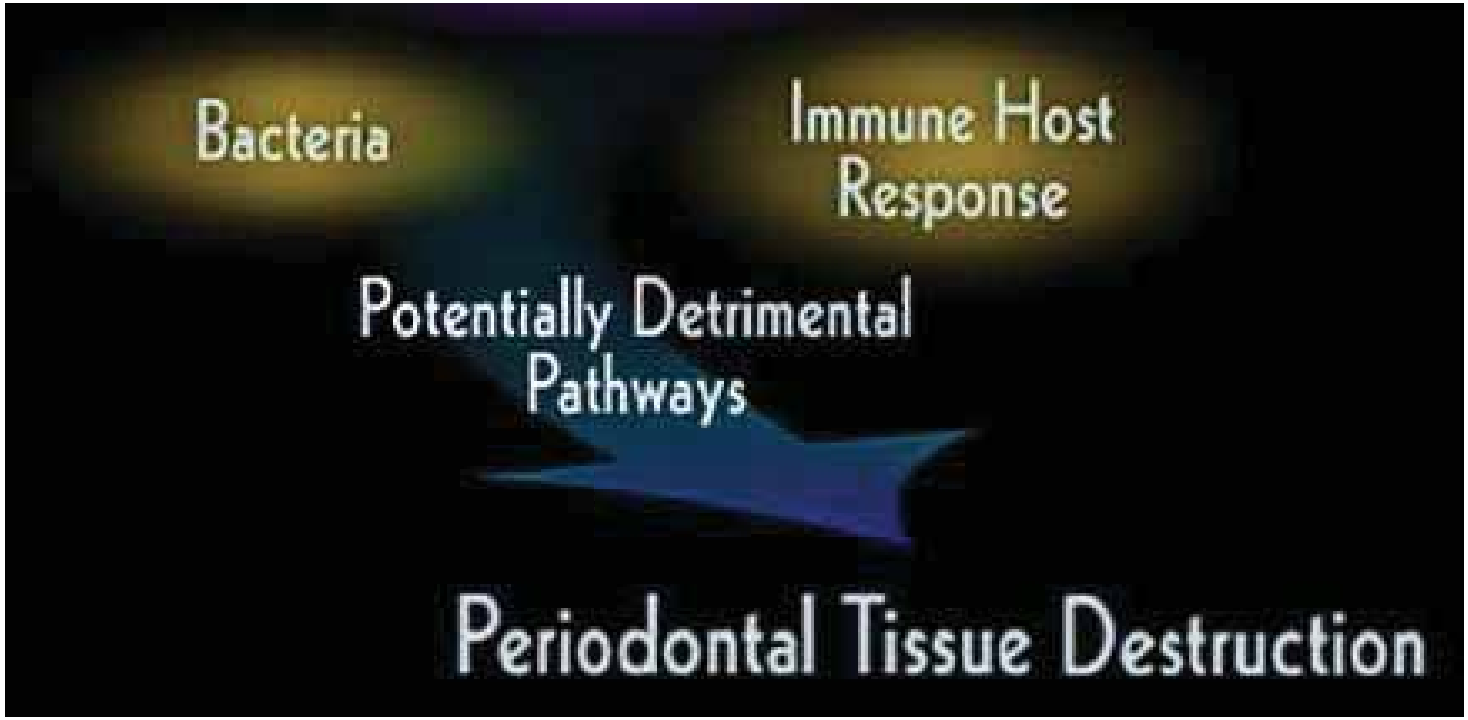
BERRY HEALTH BENEFITS SYMPOSIUM

What are periodontal diseases?

- **Inflammatory diseases of bacterial origin that affect the tooth-supporting tissues**
- **In North America, approximately 75% of individuals are affected to various degrees**
- **Two major groups**
  - Gingivitis: Reversible inflammatory condition
  - Periodontitis: Permanent damages that may lead to tooth loss. Severe forms affect about 10% of the population
- **Risk factors**
  - Poor oral hygiene
  - Age
  - Tobacco use
- **Systemic consequences**
  - Preterm baby delivery
  - Cardiovascular diseases
  - Pneumoniae



Etiology of periodontitis



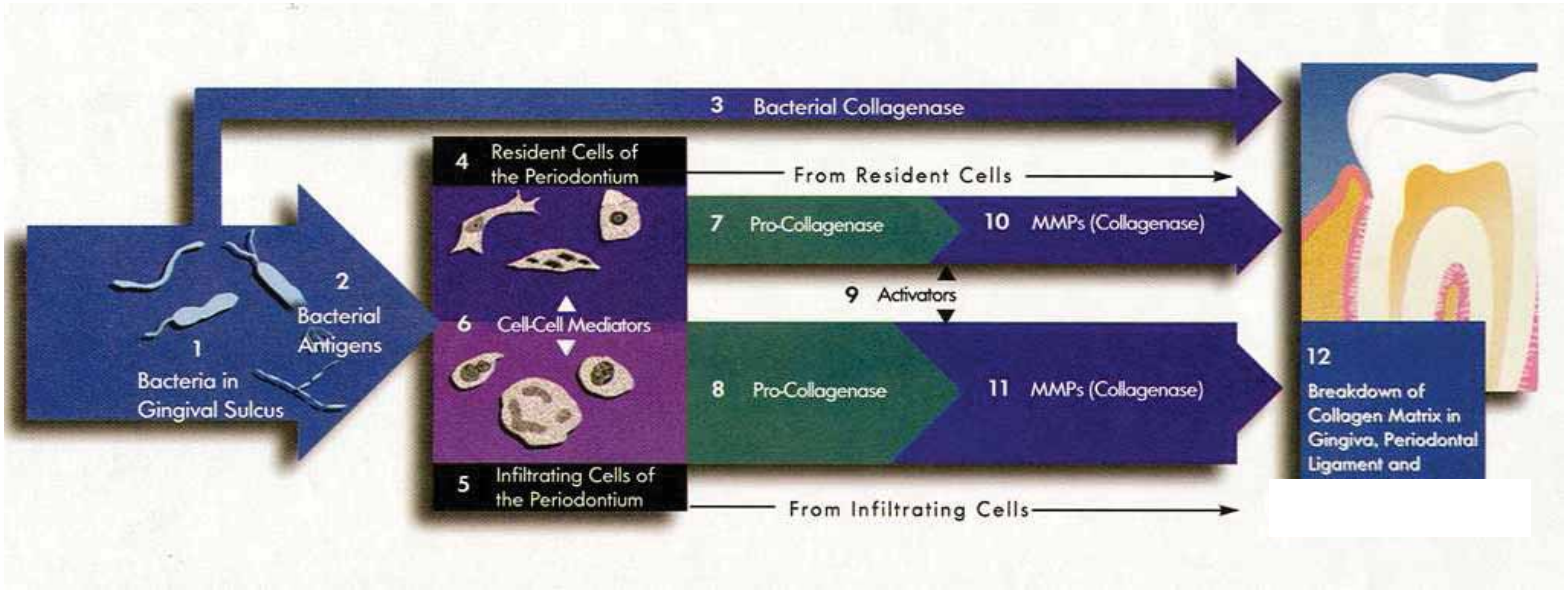
Two etiological factors are involved in the disease process leading to periodontal tissue destruction

1. Gram negative anaerobic bacteria in a structurally-organized biofilm
2. Host immune response to the bacterial challenge that results in secretion of large amounts of inflammatory mediators

How is destroyed the tooth support?

Mechanism 1: Degradation of connective tissue

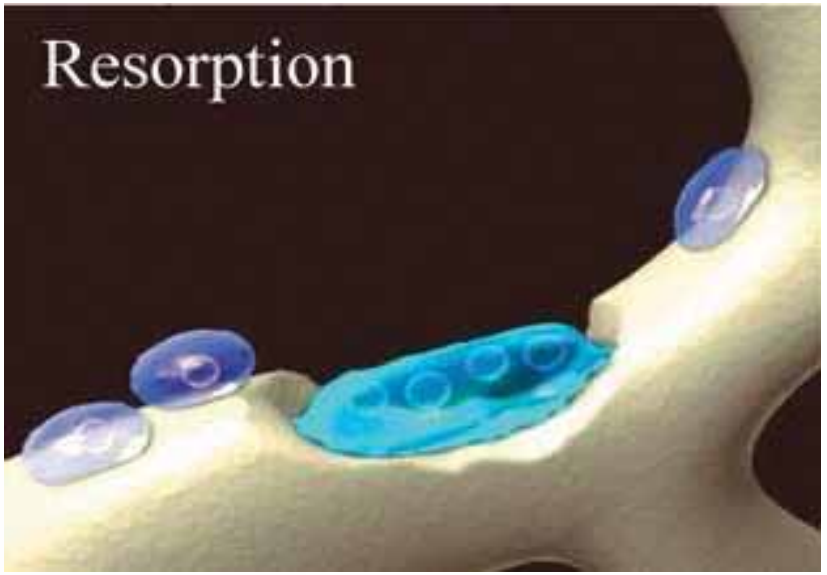
- Mainly collagens (I and IV) and fibronectin
- Bacterial collagenase (*Porphyromonas gingivalis*)
- Matrix metalloproteinases (MMPs) secreted by resident (fibroblasts) and immune cells (neutrophils, macrophages) stimulated by periodontopathogens (lipopolysaccharide)



How is destroyed the tooth support?

Mechanism 2: Alveolar bone resorption

- Increased secretion of RANKL (Receptor Activator for Nuclear Factor Kappa B Ligand) by gingival fibroblasts
- RANKL mediates the differentiation of osteoclast precursors into mature cells
- Mature osteoclasts destroy the bone structure through secretion of acids and MMPs

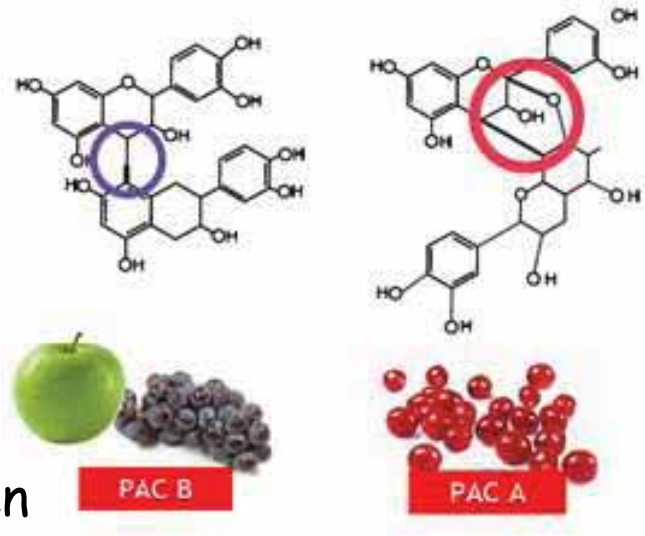


Hypothesis

1. Tissue-degrading enzymes as well as osteoclasts represent key targets for preventing periodontal disease progression
2. Proanthocyanidins from cranberry are active on these targets

Cranberry and proanthocyanidins (PACs)

- Small berry fruits from *Vaccinium macrocarpon*
- Cranberry is rich in flavonoids
  - Proanthocyanidins (condensed tannins)
  - Flavonols (quercetin and myricetin)
  - Anthocyanins (cyanidin- and peonidin-glycoside)
- Structural analysis by mass spectrometry revealed that cranberry proanthocyanidins have a unique feature: A-type linkages between epicatechin units (up to 12)



- Cranberry and dental caries
  - A crude fraction enriched in proanthocyanidins prevents biofilm formation by the cariogenic bacterium *Streptococcus mutans*
  - The fraction also reduces acid formation by *S. mutans*
  - The use of a mouthwash containing this fraction reduces salivary levels of *S. mutans*

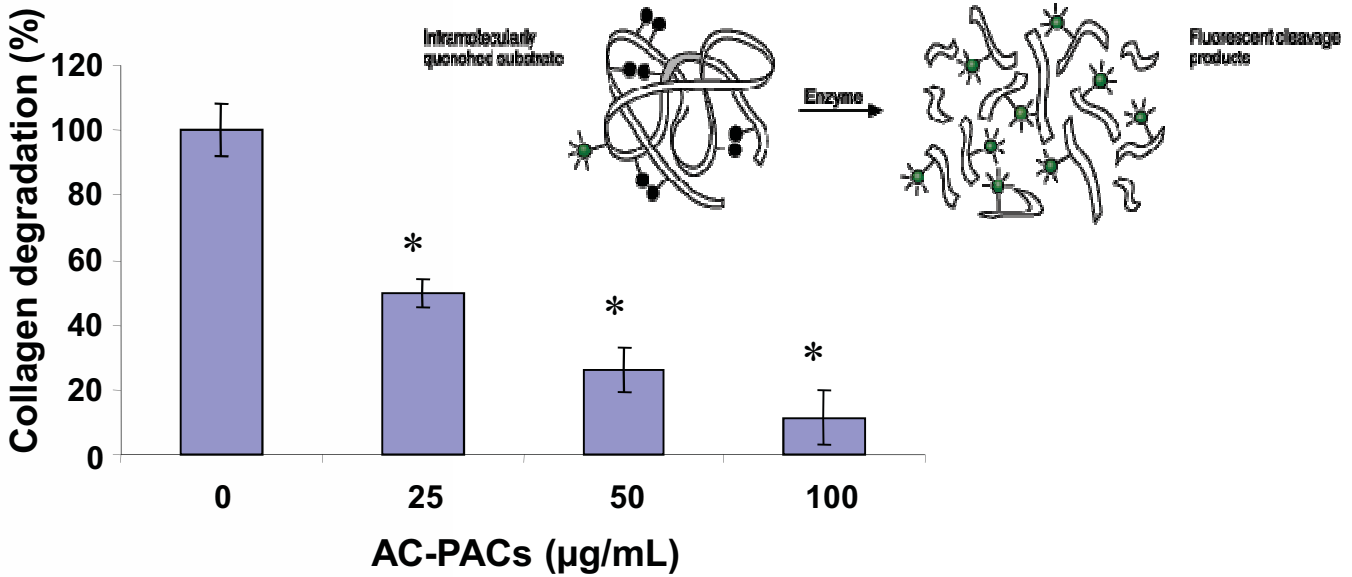
Cytotoxicity of cranberry proanthocyanidins

Cell types	% viability in the presence of PACs (µg/ml)		
	25	100	250
Epithelial cells	100	97	92
Fibroblasts	99	99	97
Macrophages	100	98	97

- Cell viability evaluated with an MTT assay (mitochondrial respiration)
- AC-PACs do not have any cytotoxic effects on major cell types found in oral cavity

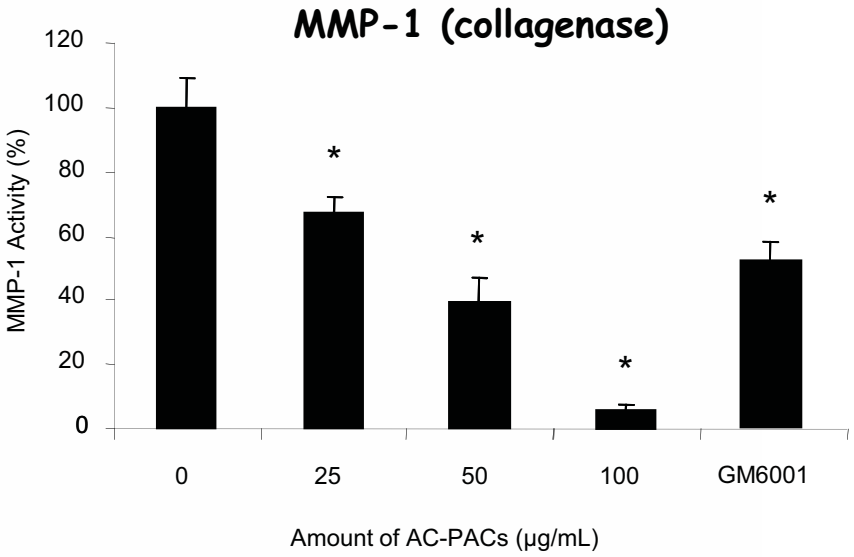
Cranberry PACs and *P. gingivalis* collagenase

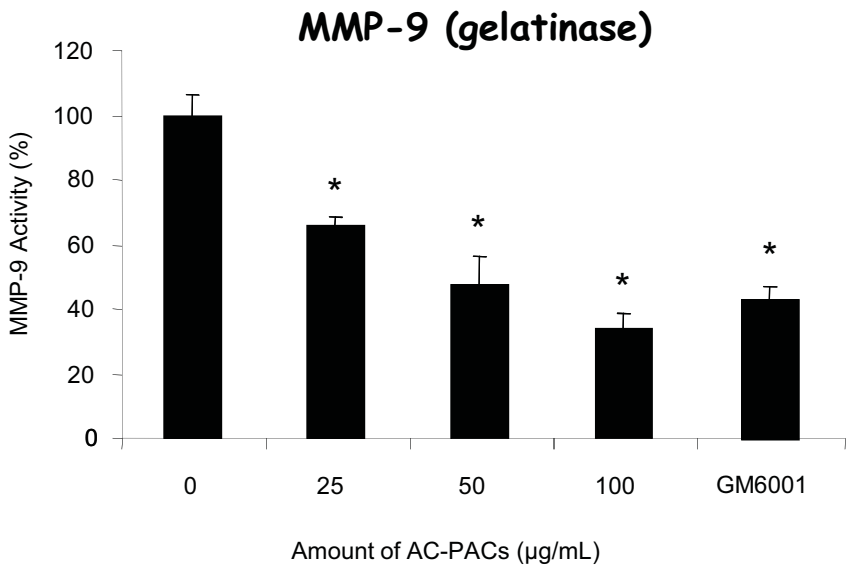
- *P. gingivalis* is the only oral bacteria known to produce a collagenase
- This bacterial species increases in numbers in diseased periodontal sites
- Cranberry PACs dose-dependently inhibit the degradation of type I collagen by *P. gingivalis*
- A 50% inhibition was obtained at 25 µg/ml while almost complete inhibition occurred at 100 µg/ml



Cranberry PACs and MMP activity

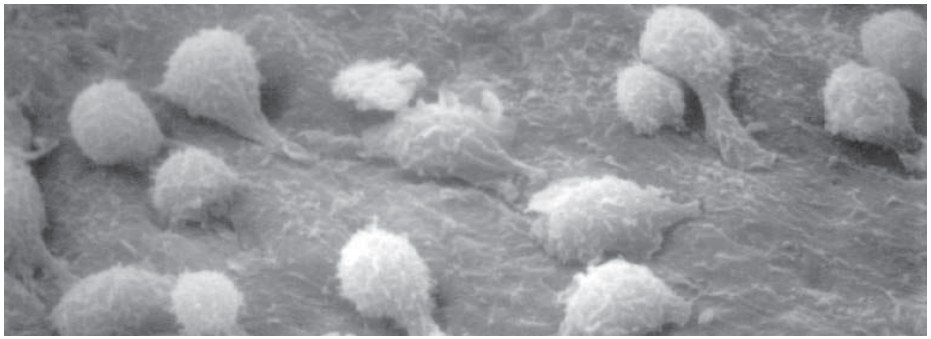
- MMPs secreted by resident (epithelial cells, fibroblasts) and immune cells (macrophages) increase in concentration in diseased periodontal sites
- MMPs degrade most tissue proteins: collagen, fibronectin, entactin, etc
- Cranberry PACs dose-dependently inhibit the activity of recombinant MMP-1 and MMP-9
- A significant inhibition was obtained at the lowest concentration tested (25 µg/ml)
- Conformational changes of the enzyme structure



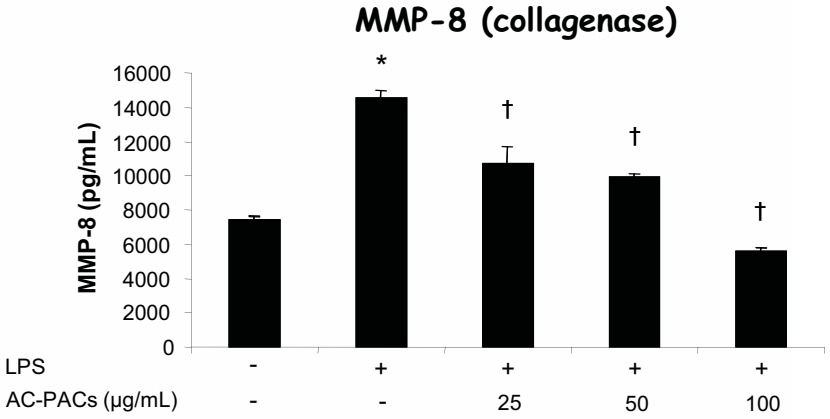


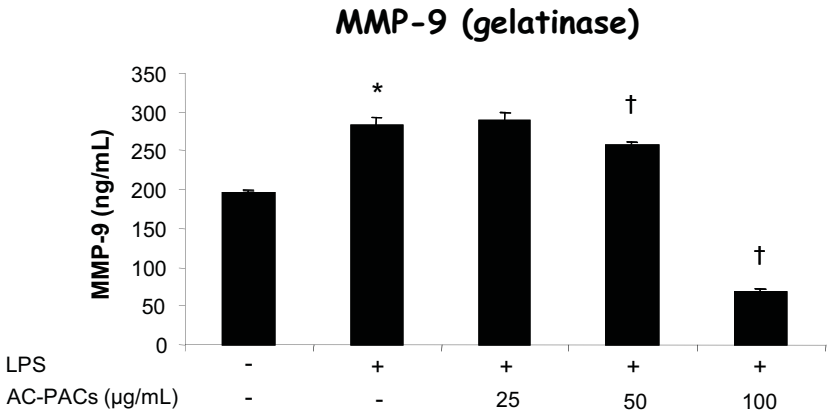
Cranberry PACs and MMP secretion by macrophages

- Macrophages migrate through periodontal tissues and accumulate in the periodontal pockets
- *P. gingivalis* LPS induces the secretion of MMP-8 and MMP-9 by human macrophages



- Cranberry PACs dose-dependently inhibit the secretion of both MMPs by LPS-stimulated macrophages
- Inhibition of MMP-3 and MMP-8 secretion by gingival fibroblasts was also observed

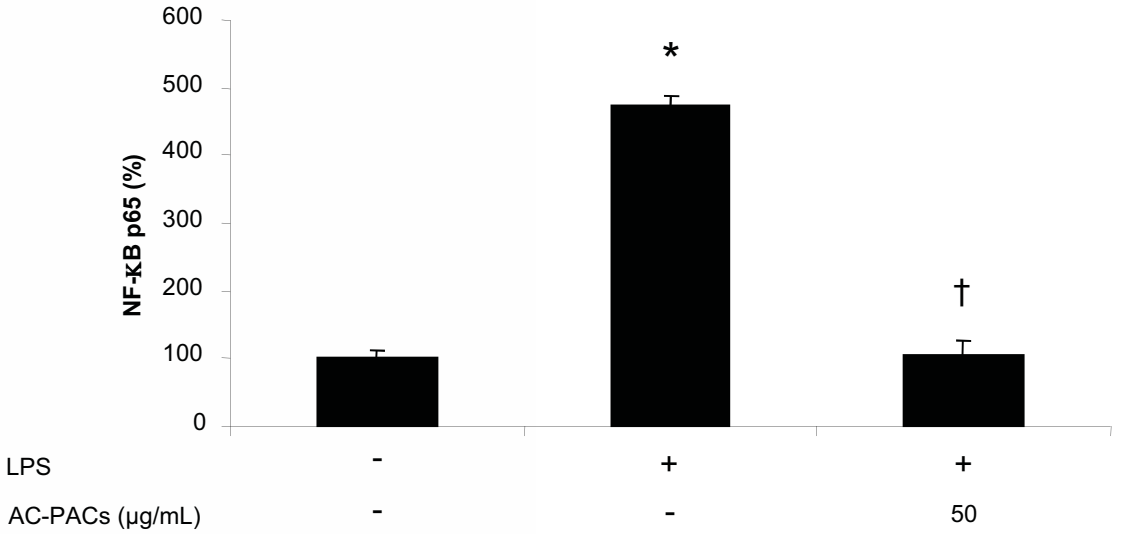




Cranberry PACs and MMP secretion by macrophages

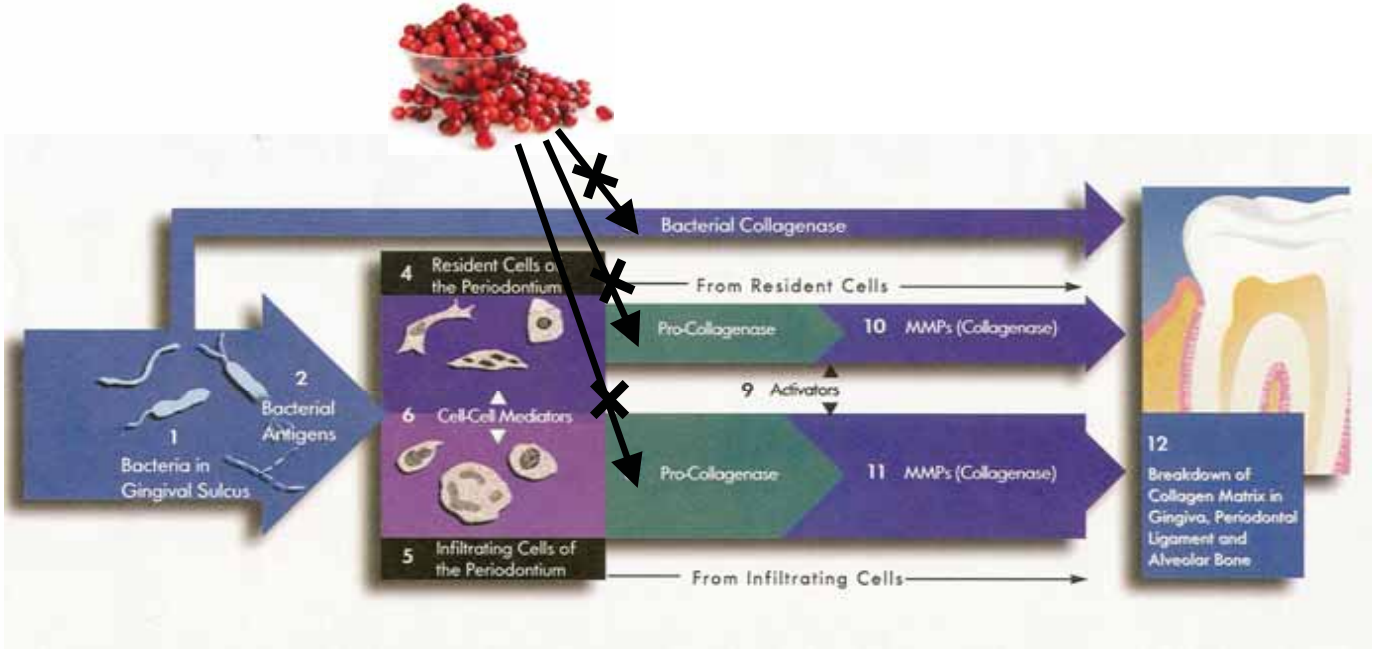
Cranberry PACs prevent LPS-induced MMP secretion in macrophages by interfering with the signaling cascade leading to gene expression

1. Reduction of phosphorylation of intracellular kinases: JNK2 (c-Jun N-terminal kinase 2) and MEK1 (mitogen-activated protein kinase kinase 1)
2. Inhibition of NF-kB p65 activation



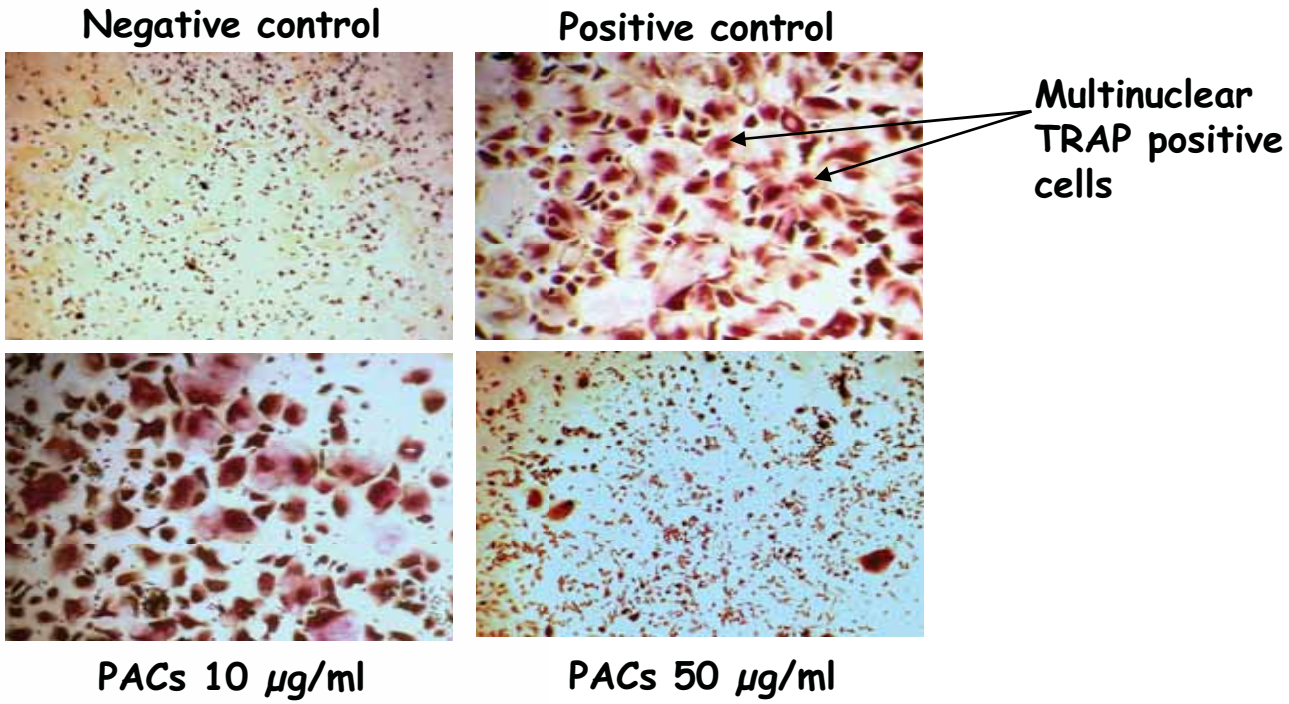
Conclusions on cranberry PACs and connective tissue destructive enzymes

1. Inhibition of *P. gingivalis* collagenase activity by cranberry PACs may prevent tissue degradation and invasion mediated by this enzyme
2. Cranberry PACs may contribute to limit extracellular matrix degradation and tissue destruction during periodontitis by inhibiting both the catalytic activity and the production of MMPs by resident and immune cells



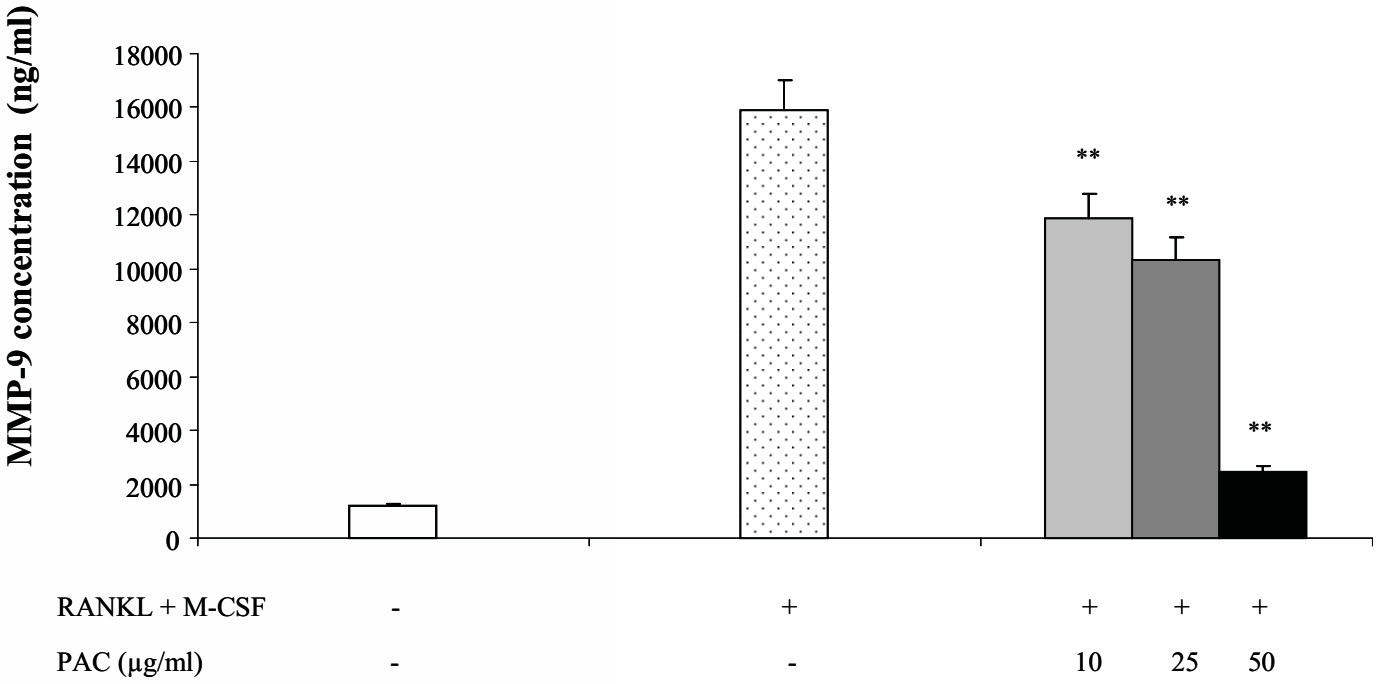
Cranberry PACs and osteoclast differentiation

- Osteoclasts are monocyte/macrophage precursors possessing a bone-resorbing activity
- Osteoclast precursors can be differentiated into mature multinucleated cells by treatment with RANKL (Receptor Activator for Nuclear Factor Kappa B Ligand) and M-CSF (Macrophage Colony-Stimulating Factor)
- PACs at 50 μg/ml completely block the differentiation



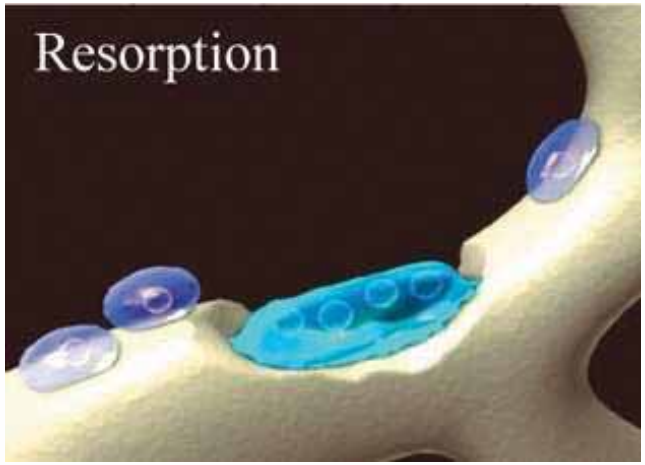
Cranberry PACs and osteoclast formation

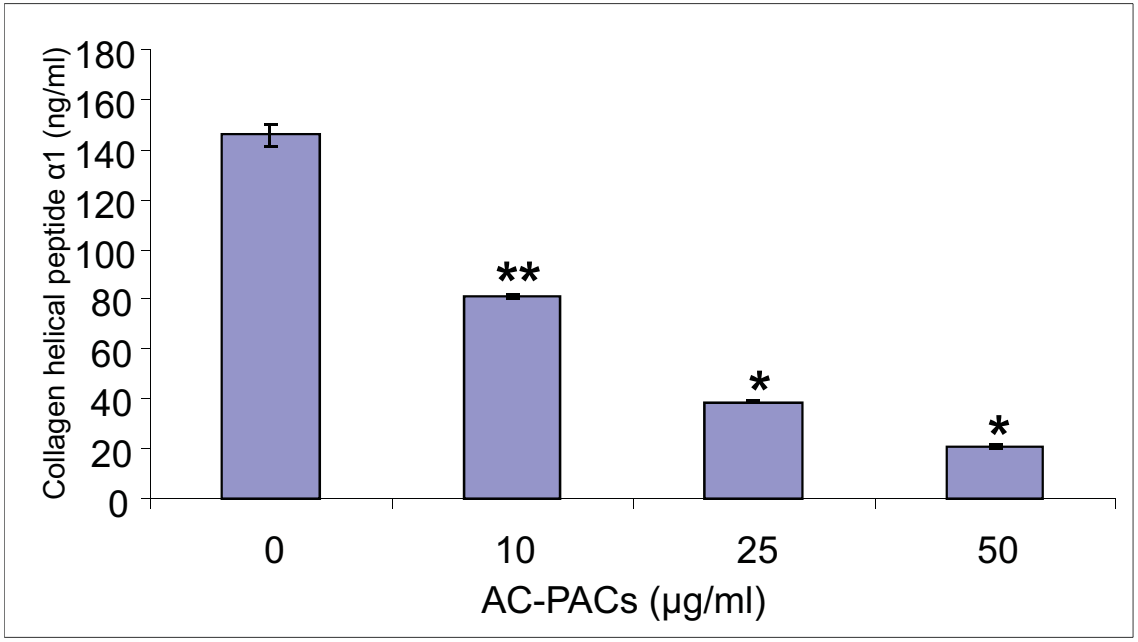
- Blocking osteoclast differentiation was associated to a reduced secretion of MMP-9 by cells
- Cranberry PACs at 50 µg/ml completely inhibited MMP-9 secretion



Cranberry PACs and osteoclast activity

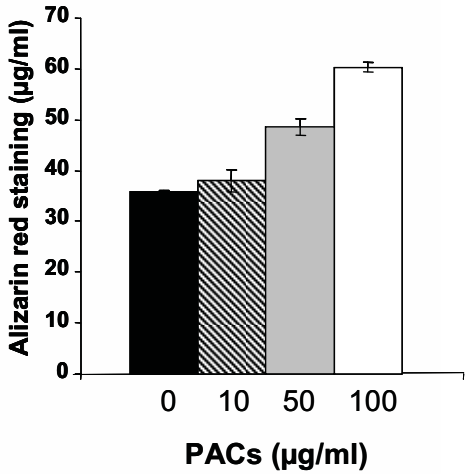
- Mature osteoclasts produce MMPs that mediate the bone destruction
- Cultivation of mature osteoclasts onto a bone plate was associated with the release of collagen peptides
- Bone degradation by osteoclasts was inhibited by cranberry PACs even at the lowest concentration tested (10 µg/ml)

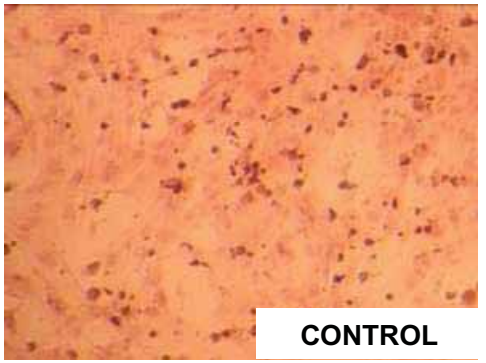


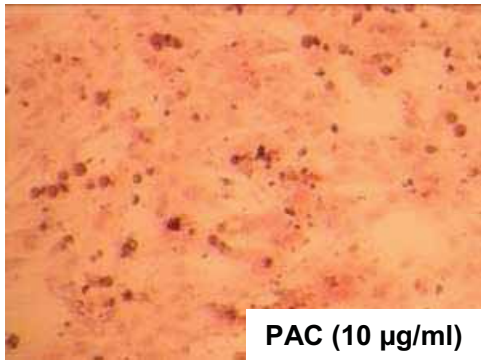


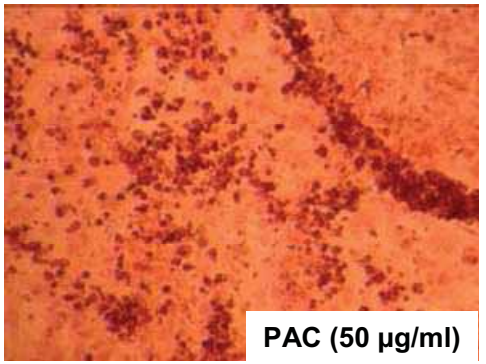
Cranberry PACs and bone formation

- Osteoblasts are mononucleate cells responsible for bone formation
- They build up the matrix of the bone structure and are involved in the mineralization of the bone matrix
- Preliminary data showed that the mineralizing activity of human osteoblasts (hFOB) is dose-dependently increased when cells were grown in the presence of cranberry PACs



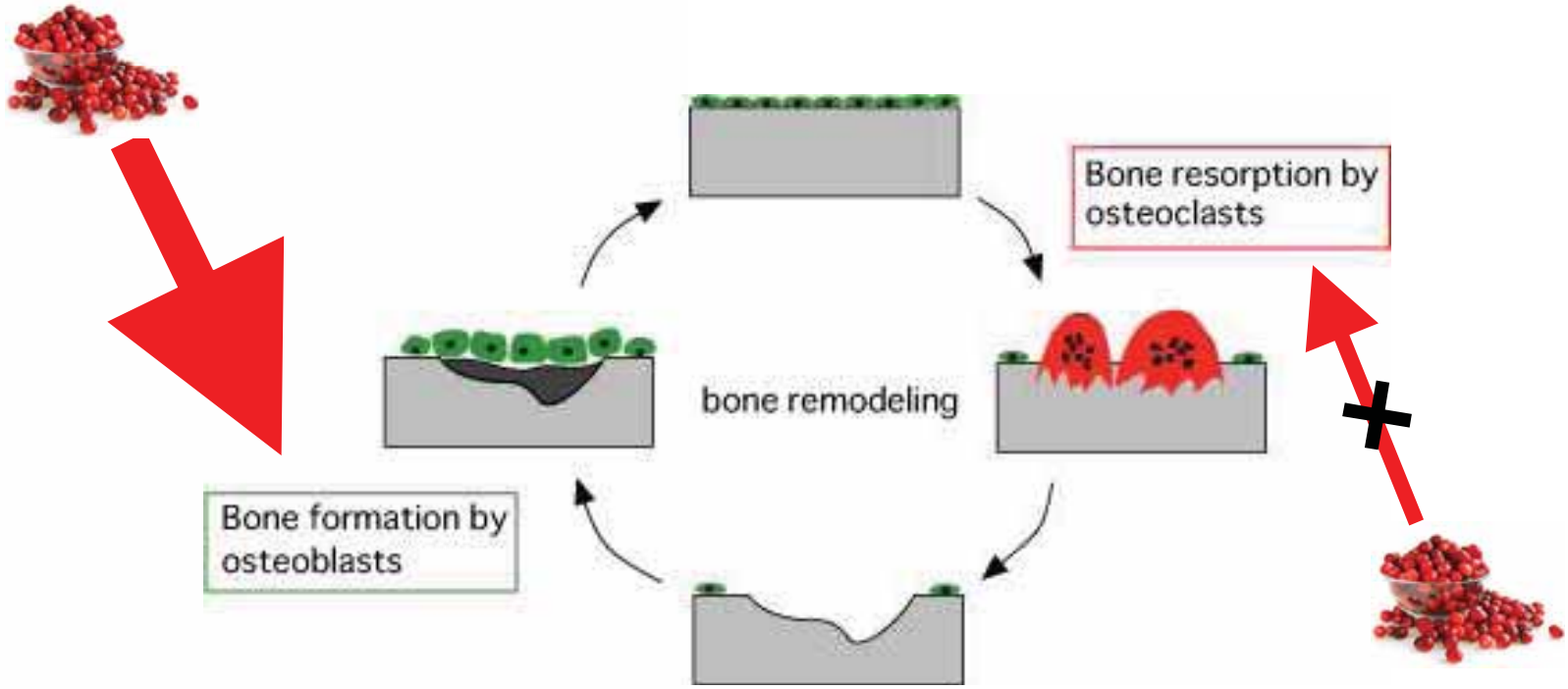






Conclusions on cranberry PACs and bone resorption/formation

Cranberry PACs may contribute to reduce bone resorption by inhibiting the formation and bone-degrading activity of osteoclasts while promoting bone formation by increasing the minerilizing activity of osteoblasts



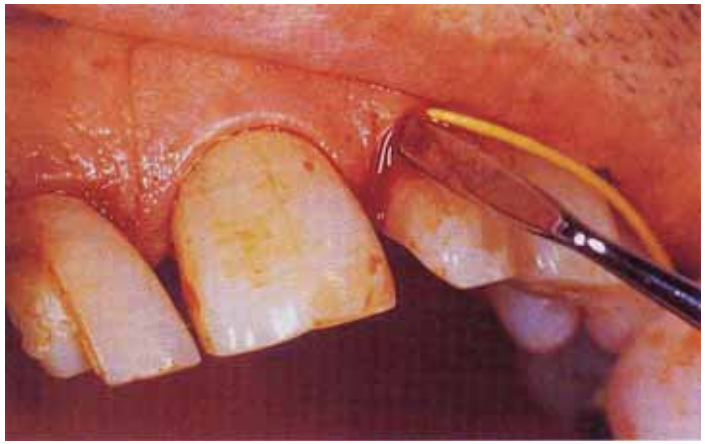
Summary of the potential benefits of cranberry PACs for periodontal health

Periodontopathogen colonization

- Inhibition of biofilm formation by *P. gingivalis*
- Inhibition of adherence properties of *P. gingivalis*

Virulence factors

Cranberry PACs



Host immuno-inflammatory response

- Inhibition of cytokine production

Connective tissue and bone destruction

- Inhibition of *P. gingivalis* collagenase activity
- Inhibition of MMP production and activity
- Inhibition of osteoclast formation and activity

Signs of Periodontitis

- Potential periodontal health benefits

Acknowledgements

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- **Contributors**
  - Dr. Amy Howell for providing our laboratory with purified A-type cranberry proanthocyanidins
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Thank you