# Biophysical Characteristics Of Proteins And Living Cells Exposed To The Polyphenol

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**Introduction:** Traditional medicines and Herbs have been applied for thousands of years, but researchers started to study their role of action at the molecular and cellular levels only in last years. Here investigate the molecular scale interactions of proteins and polyphenols with special focus on its limited stability and antioxidant properties. Here observed biophysical effects of different polyphenols on various cell lines and cultures. The changes of cell adhesion, motility, migration, stiffness, apoptosis, proliferation as well as the different efficacy on normal and cancer cells are all covered here.

## Polyphenols & TNF in pro-inflammatory diseases

TNF → has been now shown to be → primary mediator of inflammation

Most members of this family exhibit pro-inflammatory activities, in part through the activation of the transcription factor, nuclear factor-kappaB (NF jB).

Thus

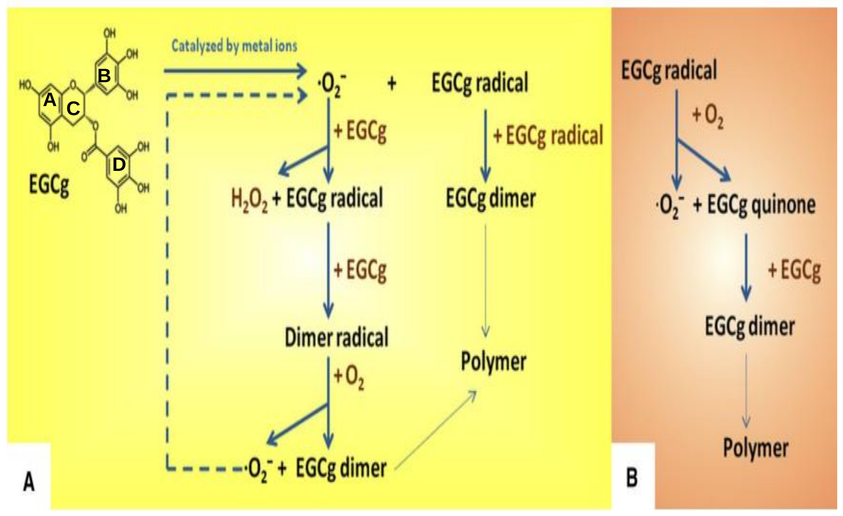
TNF and the related pro-inflammatory cytokines have been shown to play a key role in most chronic diseases such as cancer, rheumatoid arthritis, cardiovascular diseases, neurologic diseases, Crohn's disease, and metabolic diseases.

Most members of this family exhibit pro-inflammatory activities, in part through the activation of the transcription factor, nuclear factor-kappaB (NF jB).

Thus

These polyphenols are likely to have potential against various pro-inflammatory diseases.

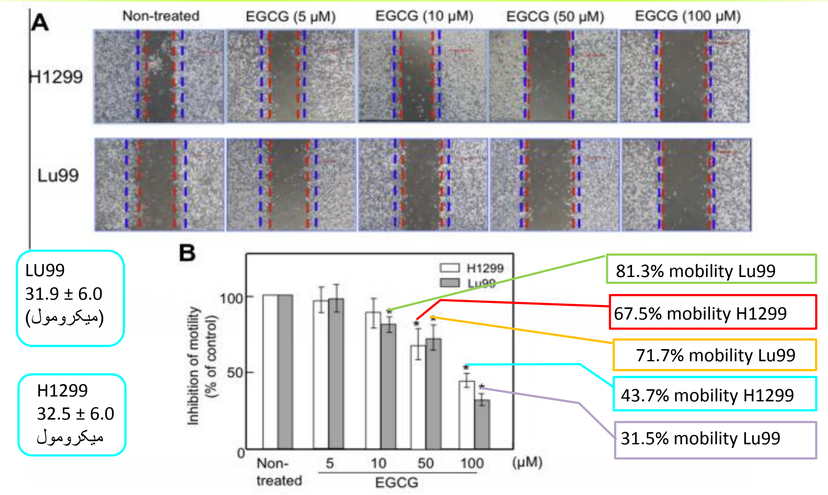
## Proposed mechanisms of EGCg auto-oxidation under cell culture conditions




Proposed mechanisms of EGCg auto-oxidation under cell culture conditions by Hou and co-workers (Hou et al. 2005). **a** The reaction in cell culture medium is probably catalyzed by metal ions such as Cu2+, and produces EGCg radicals and superoxide radicals (Hou et al. 2005). The unpaired electron may delocalize around the B ring (Hou et al. 2005). The superoxide radical can further react with another EGCg molecule to produce EGCg radical and H2O2 . Two EGCg radical molecules probably collide to form a dimer, or it can be possible that the EGCg radical may attack the B ring of another EGCg molecule, which is more abundant, to form a dimer radical (Hou et al. 2005). It can react with oxygen molecule to form the EGCg dimer and regenerate the superoxide radical (Hou et al. 2005).
**b** An alternative mechanism by the same authors is that the EGCg radical is oxidized by an oxygen molecule to form ·O2− and EGCg quinone (oxidized derivative), and the quinone mayreact with another molecule of EGCg to form the dimer (Hou et al. 2005)

1. Beatrix Peter, Szilvia Bosze and Robert Horvath, Eur Biophys J, 2016.

## Inhibition of cell motility in H1299 and Lu99 cells by treatment with EGCG in in vitro wound healing assay

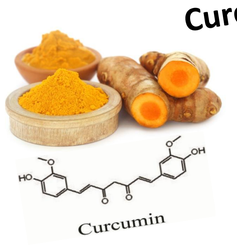



Inhibition of cell motility in H1299 and Lu99 cells by treatment with EGCG in in vitro wound healing assay. (A) Representative wound healing with H1299 cells (upper) and Lu99 cells (lower). Blue dotted line indicates the edge of scratch at 0 h, and red dotted line is the leading edge of scratch 24 h after. (B) Inhibition of cell motility by treatment with EGCG. The values are means of three independent experiments. White bar indicates the result of H1299, and gray bar is that of Lu99.

2. Atsushi Takahashi, Tatsuro Watanabe, Anupom Mondal, Kaori Suzuki, Miki Kurusu-Kanno, Zhenghao Li, Takashi Yamazaki, Hirota Fujiki, Masami Suganuma, Biochemical and Biophysical Research Communications, 2014, 1-6.

## Down modulation of TNF-a by natural products in vivo

### Curcumin




1. Attenuated tnf-a levels, exhibited an anti-hyperglycemic effect, and improved insulin sensitivity in rats on a high-fat diet.
2. Decreased expression of tnf-a and reduced mortality in a rat model of sepsis.
3. Significantly reduced lps-induced overproduction of circulating tnf-a, il-1b, and IL-6, brain glutamate, PGE2, and hydroxyl radicals in rabbits.

### Resveratro


1 .Inhibited release of tnf- a and il-6 and expression of inos in the rats brain.
2. Reduced the levels of malondialdehyde, tnf-a, IL-6, and myeloperoxidase in diabetic rats.

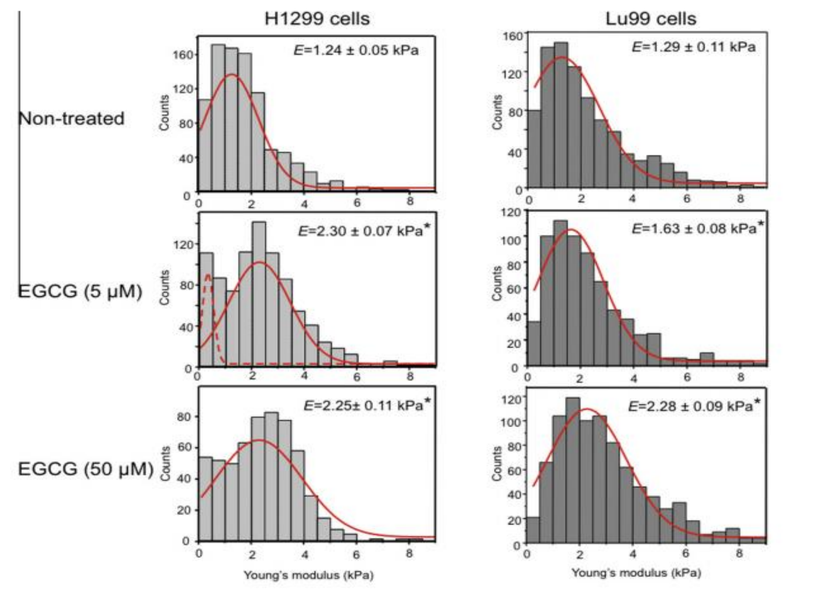
### EGCG


1. Chronic treatment with EGCG significantly reduced tnf-a level and fatigue in mice.
2. Significantly reduced cognitive deficits and levels of TNF- a, il-1b, and nf-jb in ethanol-exposed rat

## Summary of the listed references on motility and migration experiments

| Cell line | Method | Observed effects | Concentration of EGCg |
|---|---|---|---|
| **Lu99** (Human non-small cell lung cancer) | AFM | 1. Inhibition of expression of vimentin and Slug<br>2. Inhibition of epithelial–mesenchymal transition phenotypes | |
| **HeLa** (human, cervix epithelial adenocarcinoma derived from cervix) | 1. RNeasy Mini Kit<br>2. PCR<br>3. ELISA | 1.Downregulation of genes involved in the stimulation of proliferation, adhesion, motility, invasion processes<br>2. Upregulation of several genes known to have antagonist effects | 10 μM |
| | 1. Hypoxic chamber with an auto-purge airlock<br>2. Transient transfection and luciferase3. Reporter assays Western blot | 1. Inhibition of hypoxia- and serum-induced HIF-lα protein Accumulation<br>2. EGCg abolishes both chemoattractant- and hypoxia-stimu lated HeLa cell migration | 50, 100 μmol/l |
| | Holomonitor M4 | Reduced migration, motility and motility speed | 500 μg/ml |

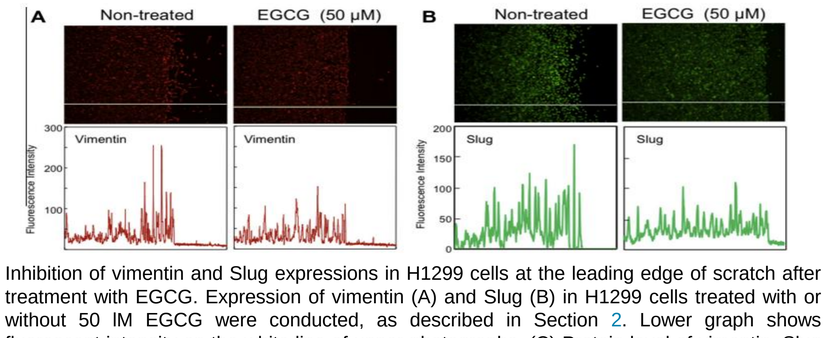
## Increase in Young's moduli of H1299 and Lu99 cells by treatment with EGCG




Increase in Young's moduli of H1299 and Lu99 cells by treatment with EGCG. Young's moduli were obtained from 861 force-curves in 70 non-treated cells, 896 forcecurves in 79 cells treated with 5 lM EGCG, and 555 force-curves in 35 cells treated with 50 lM EGCG for H1299 cells (left). Young's moduli were also obtained from 895 force-curves in 62 cells for non-treated cells, 687 force-curves in 57 cells for 5 lM EGCG, and 885 force-curves in 44 cells for 50 lM EGCG for Lu99 cells (right), after treatment for 4 h, as described in Section 2. Red curves are Gaussian fitting curves. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Atsushi Takahashi, Tatsuro Watanabe, Anupom Mondal, Kaori Suzuki, Miki Kurusu-Kanno, Zhenghao Li, Takashi Yamazaki, Hirota Fujiki, Masami Suganuma, Biochemical and Biophysical Research Communications, 2014, 1-6.

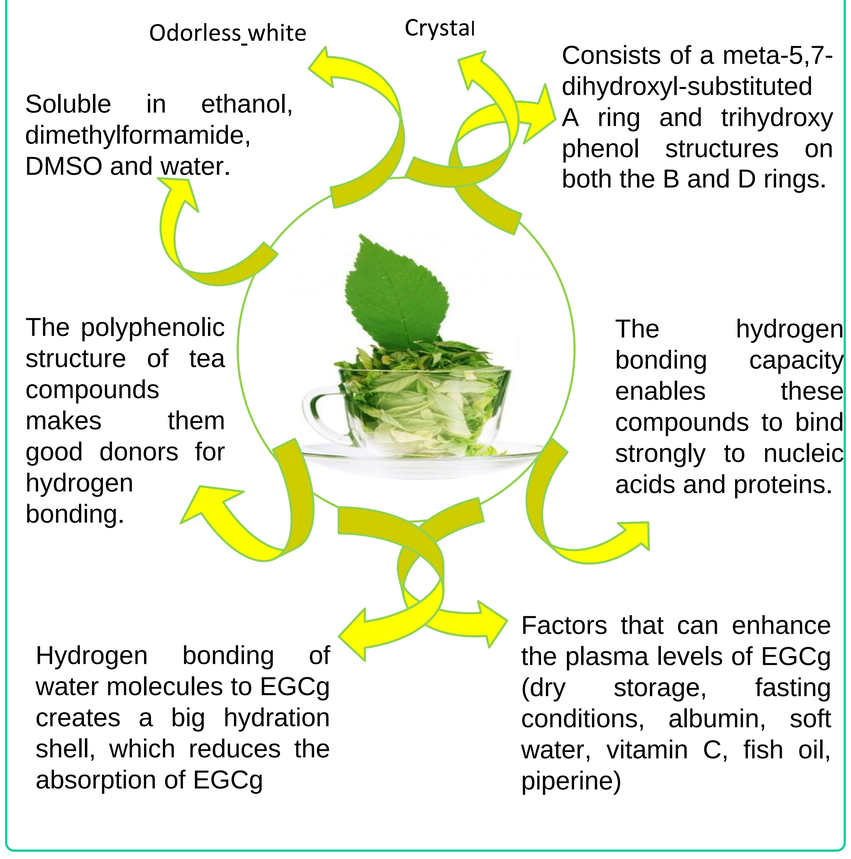
## Inhibition of vimentin and Slug expressions in H1299 cells at the leading edge of scratch after treatment with EGCG




Inhibition of vimentin and Slug expressions in H1299 cells at the leading edge of scratch after treatment with EGCG. Expression of vimentin (A) and Slug (B) in H1299 cells treated with or without 50 lM EGCG were conducted, as described in Section 2. Lower graph shows fluorescent intensity on the white line of upper photographs. (C) Protein level of vimentin, Slug and N-cadherin in H1299 cells treated with EGCG.

2. Atsushi Takahashi, Tatsuro Watanabe, Anupom Mondal, Kaori Suzuki, Miki Kurusu-Kanno, Zhenghao Li, Takashi Yamazaki, Hirota Fujiki, Masami Suganuma, Biochemical and Biophysical Research Communications, 2014, 1-6.

## Physical and biochemical properties and bioavailability of EGCg

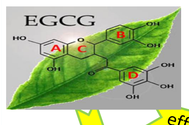



## EGCG causes a significant increase in the membrane elasticity of cancer cells

The effects of EGCG on the metastatic potential of highly metastatic human lung cancer cell lines H1299 and Lu99 in vitro wound healing assay, and on cell stiffness. → measured by → Atomic Force Microscope (AFM)

Highly metastatic cancer cells → show →
1. Low Young's modulus
2. Indicating lower cell stiffness (softer elasticity)
3. Higher cell motility

treatment with




1 . EGCG dose-dependently inhibited cell motility
2. Increased of H1299 and Lu99 cells, resulting in higher cell stiffness and rigid elasticity.

effects of on → Cell motility, cell stiffness, and expression of vimentin and Slug, which are molecular phenotypes of epithelial–mesenchymal transition (EMT).

Inhibits both mechanical and biochemical phenotypes of EMT, including cell motility, cell stiffness, and expression of vimentin and Slug in the cells by in vitro wound healing assay.

Cell motility and cell stiffness are now recognized as mechanical phenotypes of epithelial–mesenchymal transition (EMT): EMT is theoretically understood as acquisition of the phenotypes of mesenchymal cells, such as fibroblasts, by epithelial cells, and cancer cells undergo a partial or complete EMT, resulting in increase in cell motility and invasiveness.

Demonstrate that → Inhibition of EMT is associated with alteration of cell membrane organization induced by methyl-b-cyclodextrin (MbCD), which depletes cholesterol in cell membranes

## Results and discussion

Results strongly indicate that membrane organization directly reflects cell motility and cell stiffness. Also polyphenols treatment resulted in a dose dependent (i) inhibition of cell growth, (ii) G0/G1-phase arrest of the cell cycle, and (iii) induction of apoptosis.

## Conclusion

Polyphenols has been shown to inhibit spontaneous metastasis of cancer cell. Polyphenols can suppress TNF- activated inflammatory pathways both in vitro and in vivo.

## References


- 1. Beatrix Peter, Szilvia Bosze and Robert Horvath, Eur Biophys J, 2016.
- 2. Atsushi Takahashi, Tatsuro Watanabe, Anupom Mondal, Kaori Suzuki, Miki Kurusu-Kanno, Zhenghao Li, Takashi Yamazaki, Hirota Fujiki, Masami Suganuma, Biochemical and Biophysical Research Communications, 2014, 1-6.
- 3. Thejass Punathil, Trygve O. Tollefsbol, Santosh K. Katiyar, Biochemical and Biophysical Research Communications, 2008,375, 162–167.
- 4.Subash C. Gupta, Amit K. Tyagi, Priya Deshmukh-Taskar, Myriam Hinojosa, Sahdeo Prasad, Bharat B. Aggarwal, Archives of Biochemistry and Biophysics, 2014
- 5. Nihal Ahmad, Sanjay Gupta, and Hasan Mukhtar, Archives of Biochemistry and Biophysics, 2000, 376, 338–346