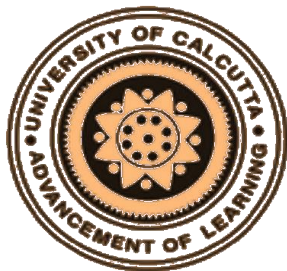


*PEGylated-thymoquinone-nanoparticle mediated retardation
of breast cancer cell migration by deregulation of cytoskeletal
actin polymerization through miR-34a*

Presented by:

Arghya Adhikary Ph.D

DST INSPIRE Faculty, Assistant Professor



**Centre for Research in Nanoscience and Nanotechnology,
University of Calcutta**

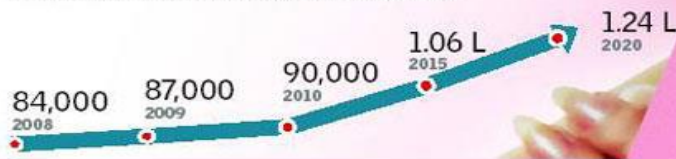
Breast cancer

Spiralling Incidence

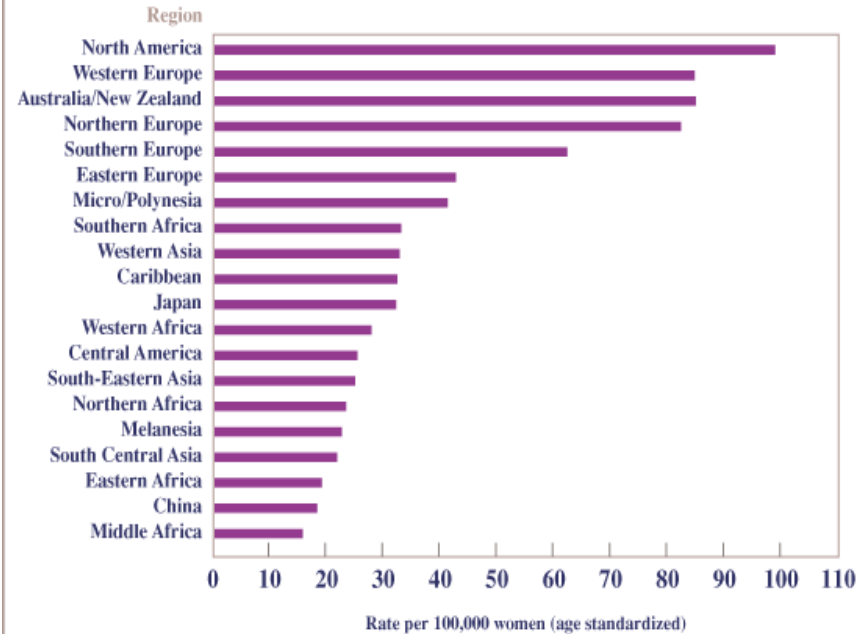
An analysis of cancer cases among women in Delhi, Mumbai, Chennai and Bangalore from 1982 to 2005 shows that the incidence of breast cancer has nearly doubled in metropolitan cities. The figures below represent incidence per one lakh women in %



PREDICTION | India's National Health Profile 2010 predicts that by 2020, breast cancer will affect 1.24 lakh women across India



Breast Cancer Incidence Worldwide

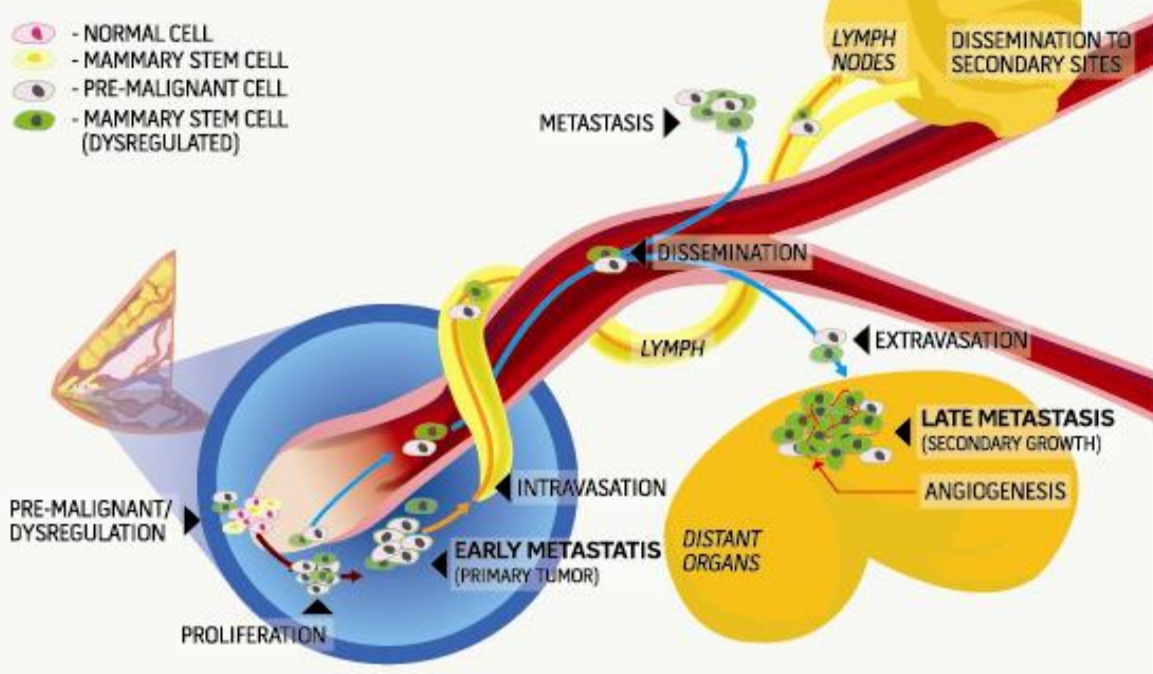


- ❖ Breast Cancer: **second** most commonly diagnosed cancers worldwide (11.9% of all cancers) **fifth** cause of death in cancer (6.3% of all cancers)
- ❖ Since the 2008 estimates, breast cancer incidence has increased by more than **20%**, while mortality has increased by **14%**.
- ❖ The most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). **It now represents one in four of all cancers in women.**
- ❖ It is the **slightly most common cancer in less developed regions** (883,000 cases) than in more developed (794,000) regions.

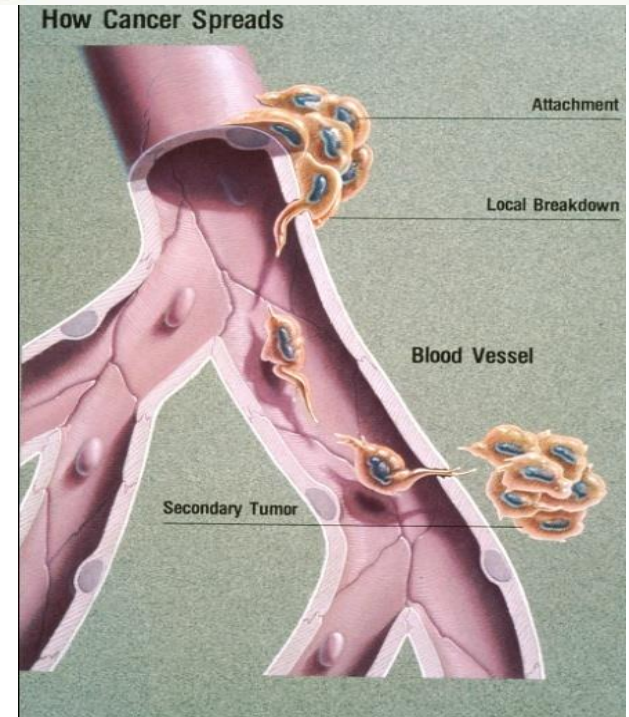
Metastasis

90% death occur in Breast cancer due to metastasis.

Metastasis refers to the capability to leave a primary tumor, travel via the circulation to a distant tissue site, and form a secondary tumor.

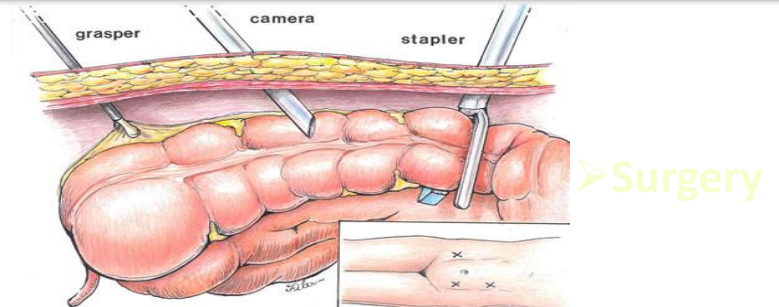


- 1 Migration
- 2 Intravasation
- 3 Circulation
- 4 Extravasation
- 5 Colonization, proliferation and angiogenesis



Present modalities of cancer therapy and reasons of failure

- **Surgery:** 100% tumor mass can not be removed
- **Chemotherapy:** Results in severe immuno-suppression and general toxicity
- **Radiotherapy:** Normal tissues are also affected
- **Hormonal therapy:** Develops resistance



Chemoradiation



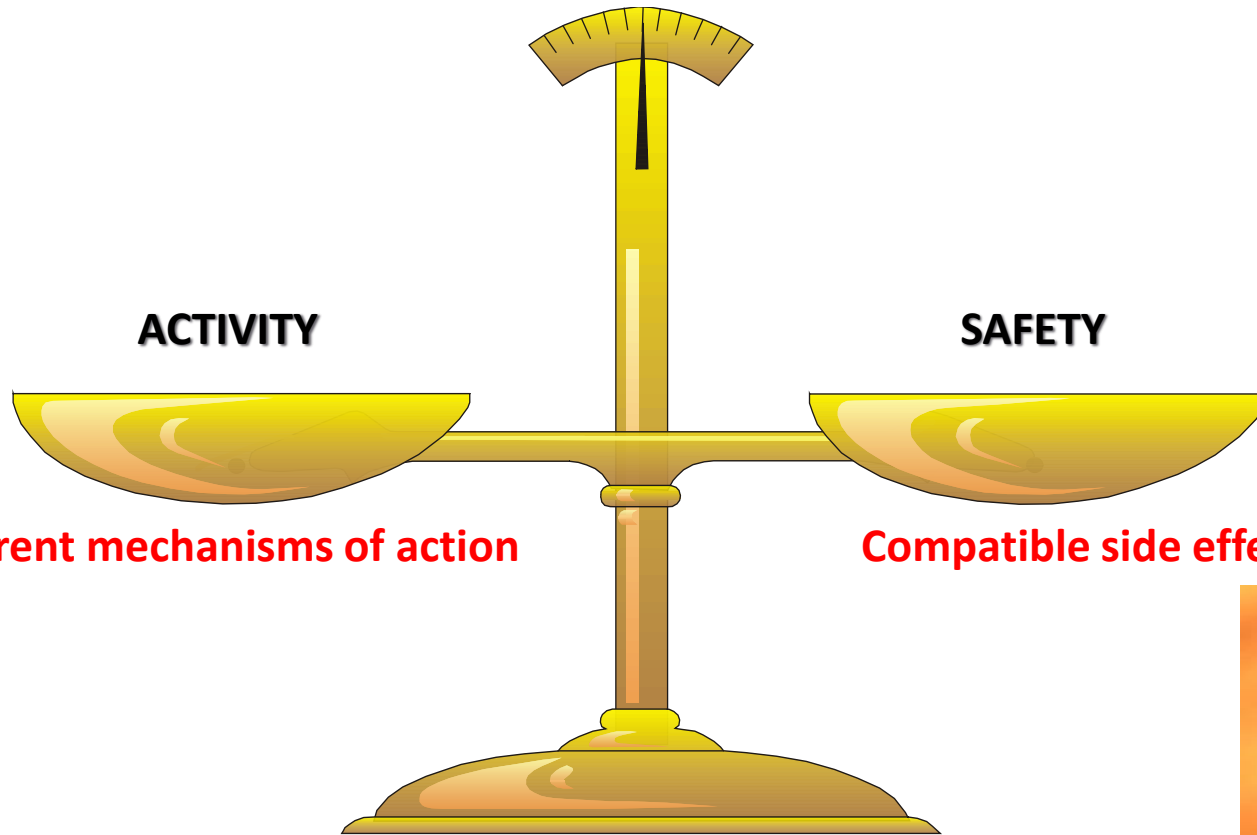
Chemotherapy of Breast Cancer

Most Commonly Used Agents

- Doxorubicin, Epirubicin, Mitoxantrone
- Paclitaxel, Docetaxel
- Cyclophosphamide
- Methotrexate
- Fluorouracil
- Vinorelbine
- Capecitabine
- Gemcitabine

Treatment and side effects : Maintaining a balance

INCREASED EFFICACY



Different mechanisms of action

Compatible side effects

Which way to look ?

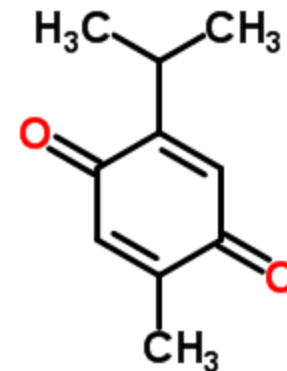
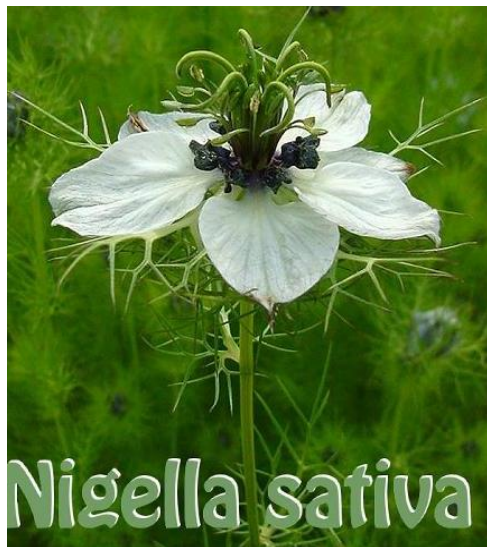


Use of Dietary Plant Products: A New Concept

Benefits of Using Phytochemicals

- **Provide opportunities to develop strategies for curing cancer by directly or indirectly altering specific cellular targets**
- **These are the products of nature**
- **Many phytochemicals are already used as dietary supplements**
- **Have antitoxic and anti-inflammatory properties**
- **Normally are devoid of immunosuppressive activity**
- **Many are rich sources of anti-oxidants**
- **Can be a cheap rescue in comparison to other traditional treatments**

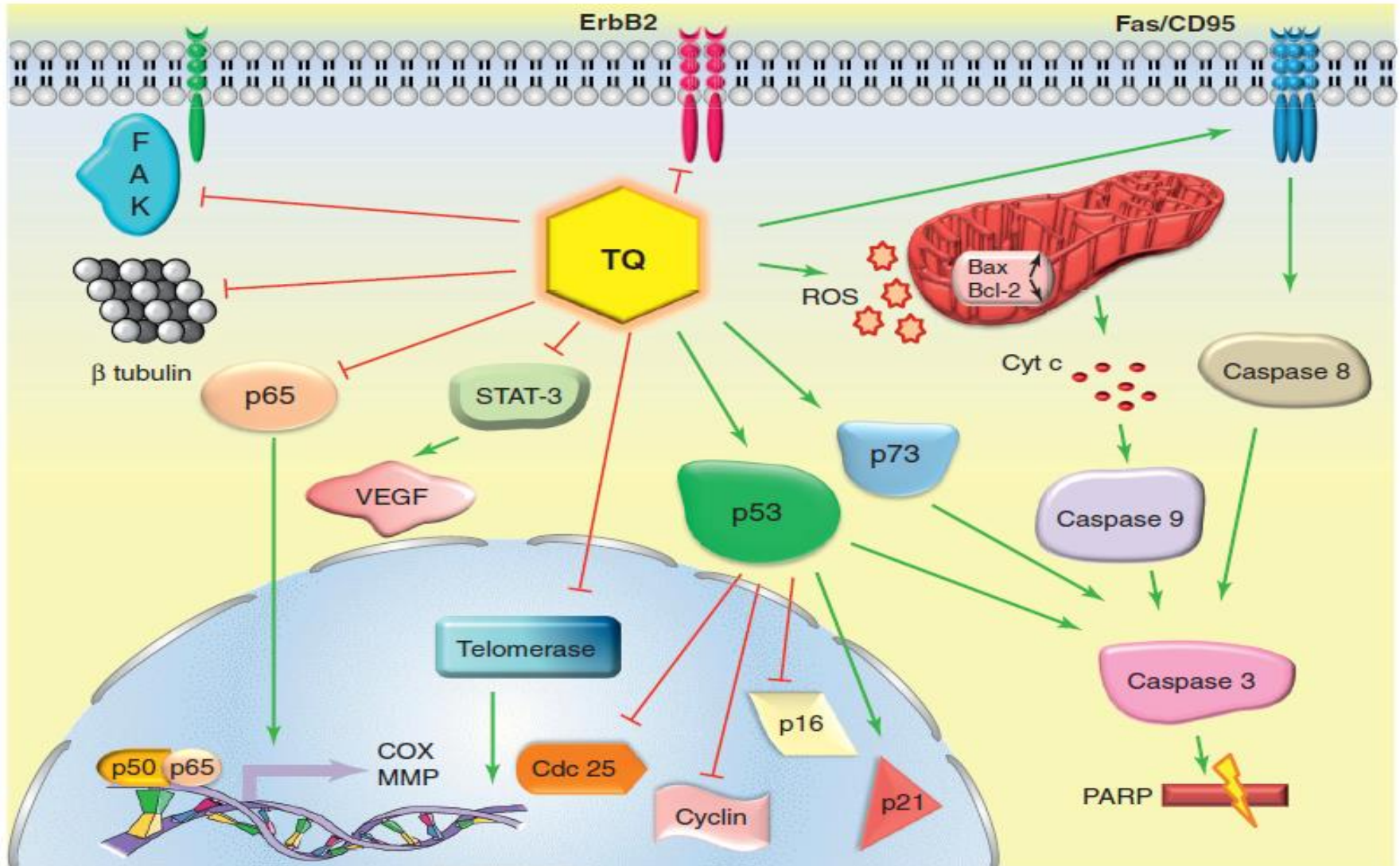
Thymoquinone



- ❖ Thymoquinone (TQ) is a major active constituent of black seeds of *Nigella sativa*.
 - ❖ frequently used in Unani, Ayurveda, Chinese and Arabic medicines.
 - ❖ Fever, common cold, headache, asthma, rheumatic diseases, microbial infections and to expel worms from the intestines as well as “Sartan” (cancer).
-
- ❖ The prophet Muhammad said “Use the black seed, which is a healing for all diseases except As-Sam” [‘As-Sam’ means ‘death’]
 - ❖ The content of TQ in seed is 2200 mg/kg on fresh weight basis.
 - ❖ The first report of TQ, for its cytotoxic activity was against Ehrlich’s ascites carcinoma, Dalton’s lymphoma ascites and sarcoma-180 cells (Salomi et al., 1992).



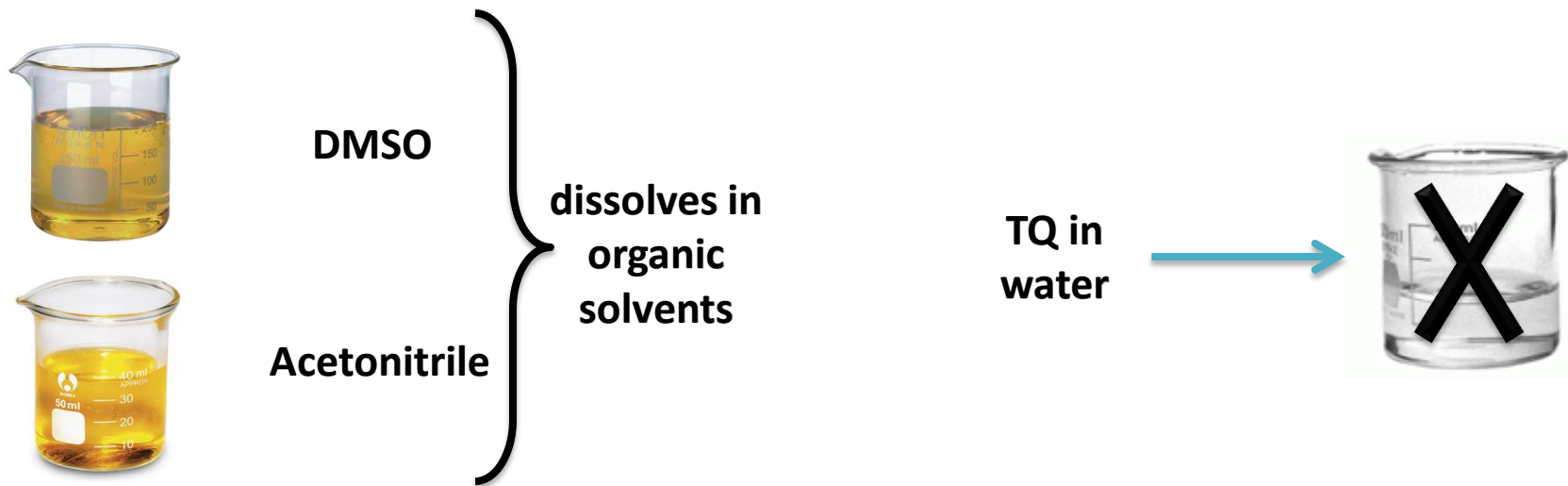
Effects of Thymoquinone



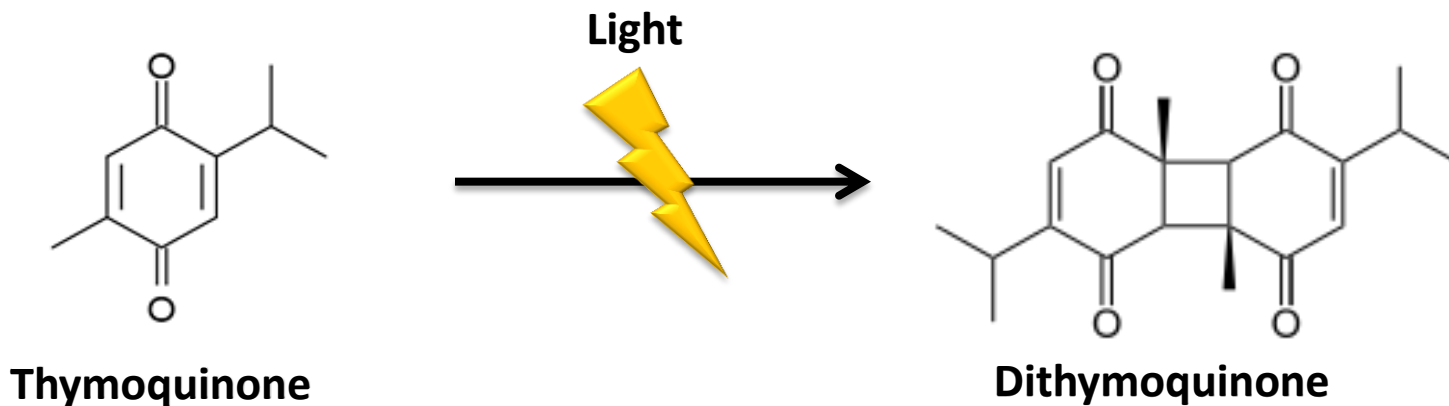
* Schneider-Stock R et al. Drug Discov Today. 2014 Jan;19(1):18-30.

Drawbacks of Thymoquinone

1. TQ is hydrophobic.

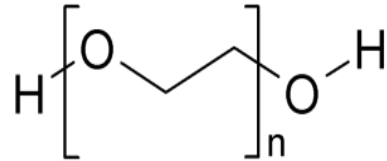


2. TQ is light sensitive.



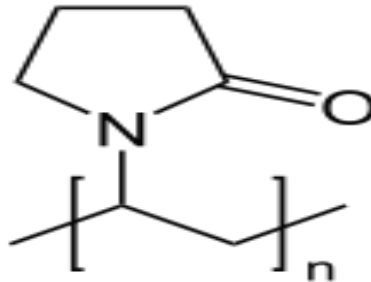
Encapsulating agents

Polyethylene glycol



1. PEG₂₀₀
2. PEG₄₀₀₀

Polyvinyl pyrrolidon



3. PVP K30

Advantages of them:

1. Hydrophilic
2. Biodegradable
3. Used in pharmaceuticals, food additives etc.

Synthesis

Synthesis of PVP-TQ-Nps

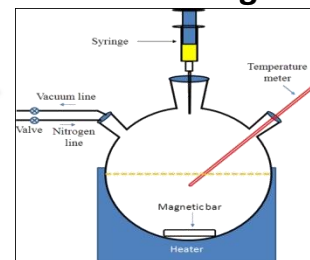
100mM TQ solution
in Acetonitrile



PVP solution, prepared
in water, boiled at 160°C



TQ solution added in PVP
solution; boiling at 160°C and
continuous stirring for O/N



Organic solvents were
veporized using Rotary
evaporator at 90°C



Synthesis of PEG-TQ-Nps

100mM TQ solution
in Acetonitrile



Added dropwise into
PEG solution under
ultrasonic condition.



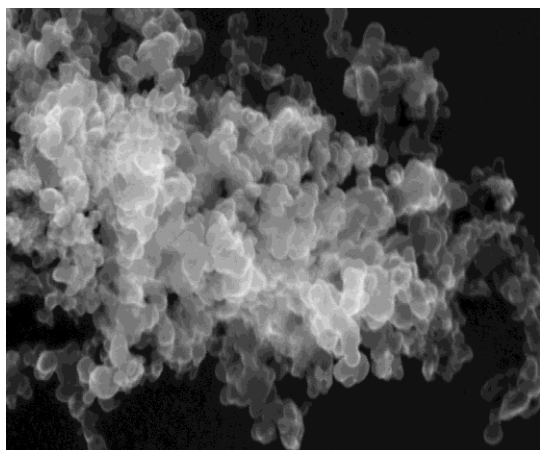
Mixture was kept
on continuous
stirring for O/N



Organic solvents were
veporized using Rotary
evaporator at 90°C

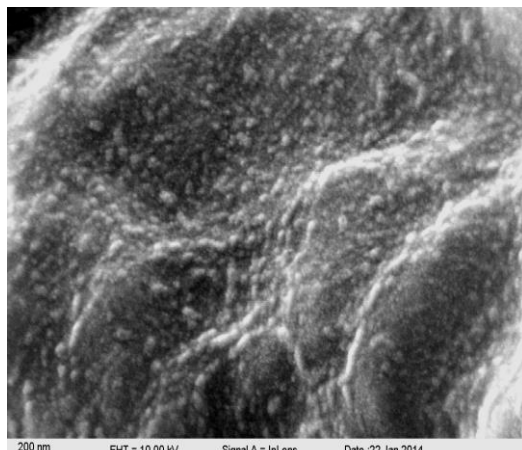


Morphology of the three NTQs evaluated by Field Emission Scanning Electron Microscope (FESEM)



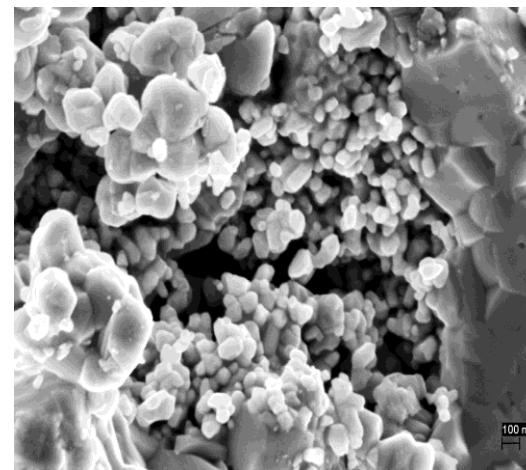
200 nm EHT = 10.00 kV Signal A = InLens Date :22 Jan 2014
WD = 5.0 mm Mag = 197.93 K X Time :16:30:22

PVP-TQ-Np



200 nm EHT = 10.00 kV Signal A = InLens Date :22 Jan 2014
WD = 5.1 mm Mag = 200.00 K X Time :15:49:40

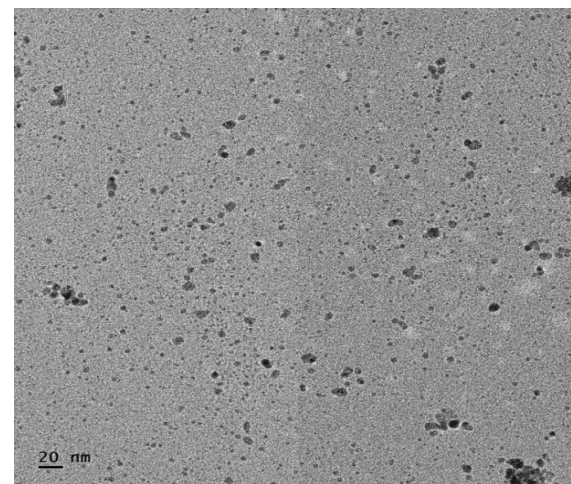
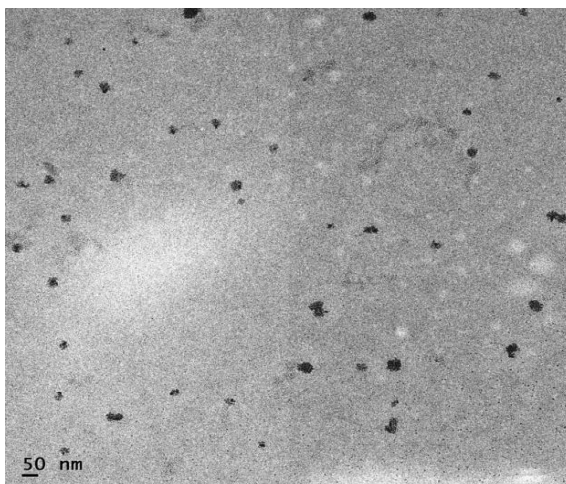
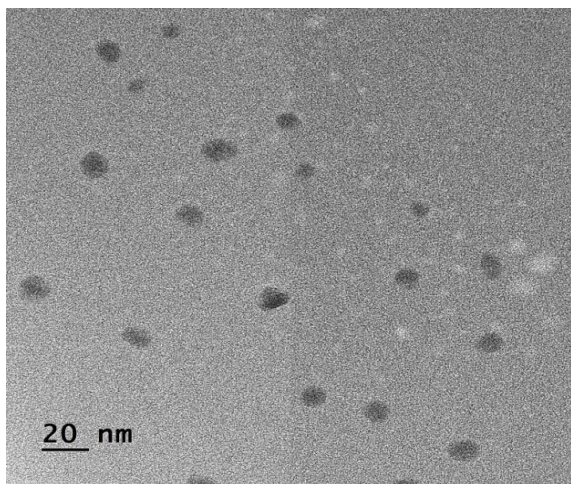
PEG₂₀₀TQ-Np



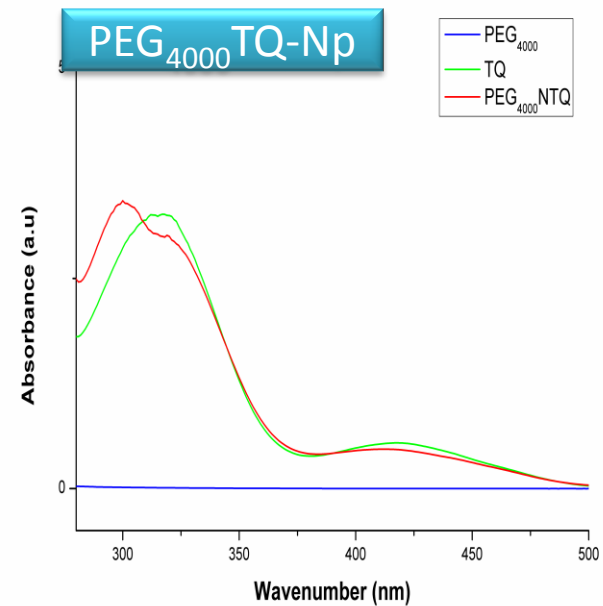
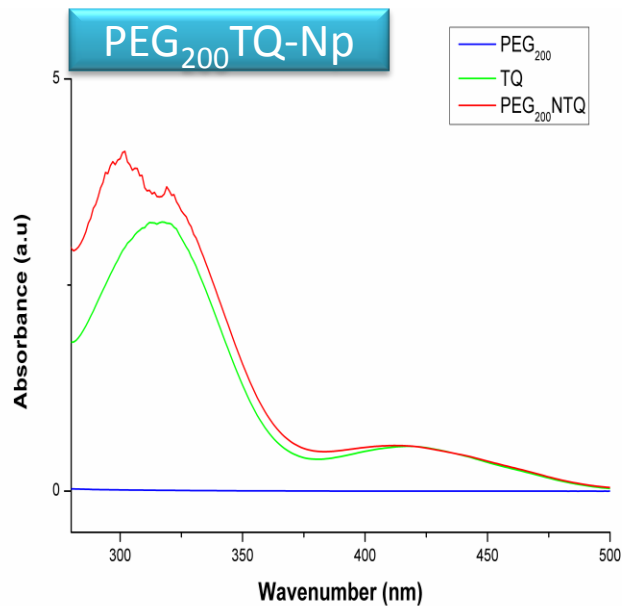
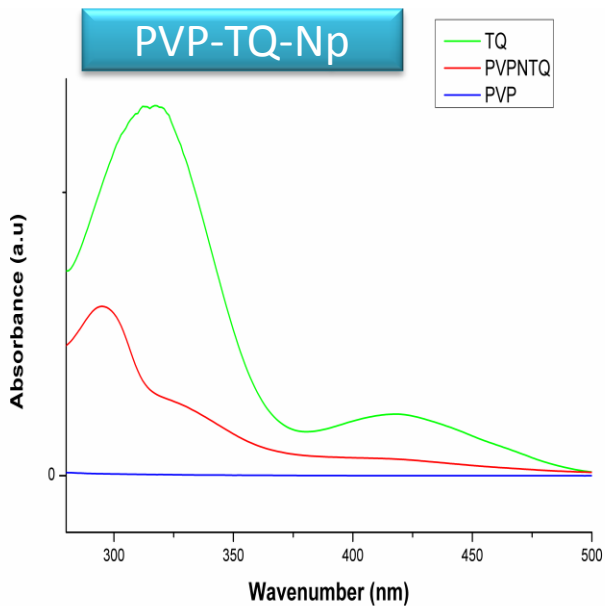
100 nm

PEG₄₀₀₀TQ-Np

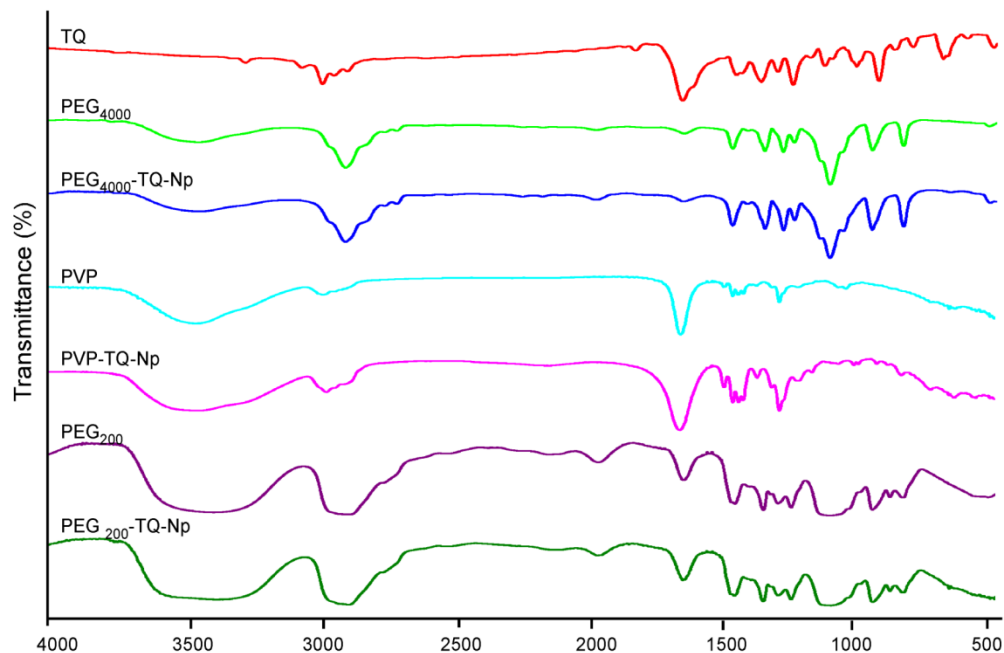
Size analysis of the three NTQs evaluated by Transmission Electron Microscope



UV/Vis spectra of PVP-TQ-Nps, PEG200-TQ-Nps, PEG4000-TQ-Nps.



FTIR spectra of TQ, PVP, PEG200, PEG4000, PVP-TQ, PEG200-TQ and PEG4000-TQ Nanoparticles



1H-NMR Spectra

18.07.2014.

6.652
6.598
6.556
6.513

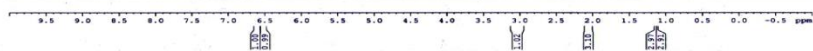
3.045
3.025
3.011

2.043

1.129

1.122

TQ



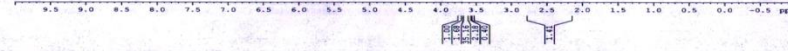
23.07.2014.

7.288

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3.654
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PEG₄₀₀₀



23.07.2014.

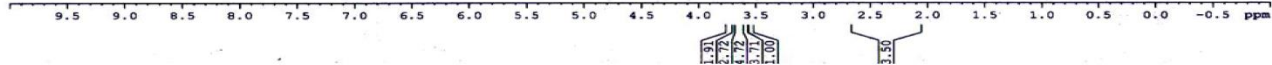
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3.583

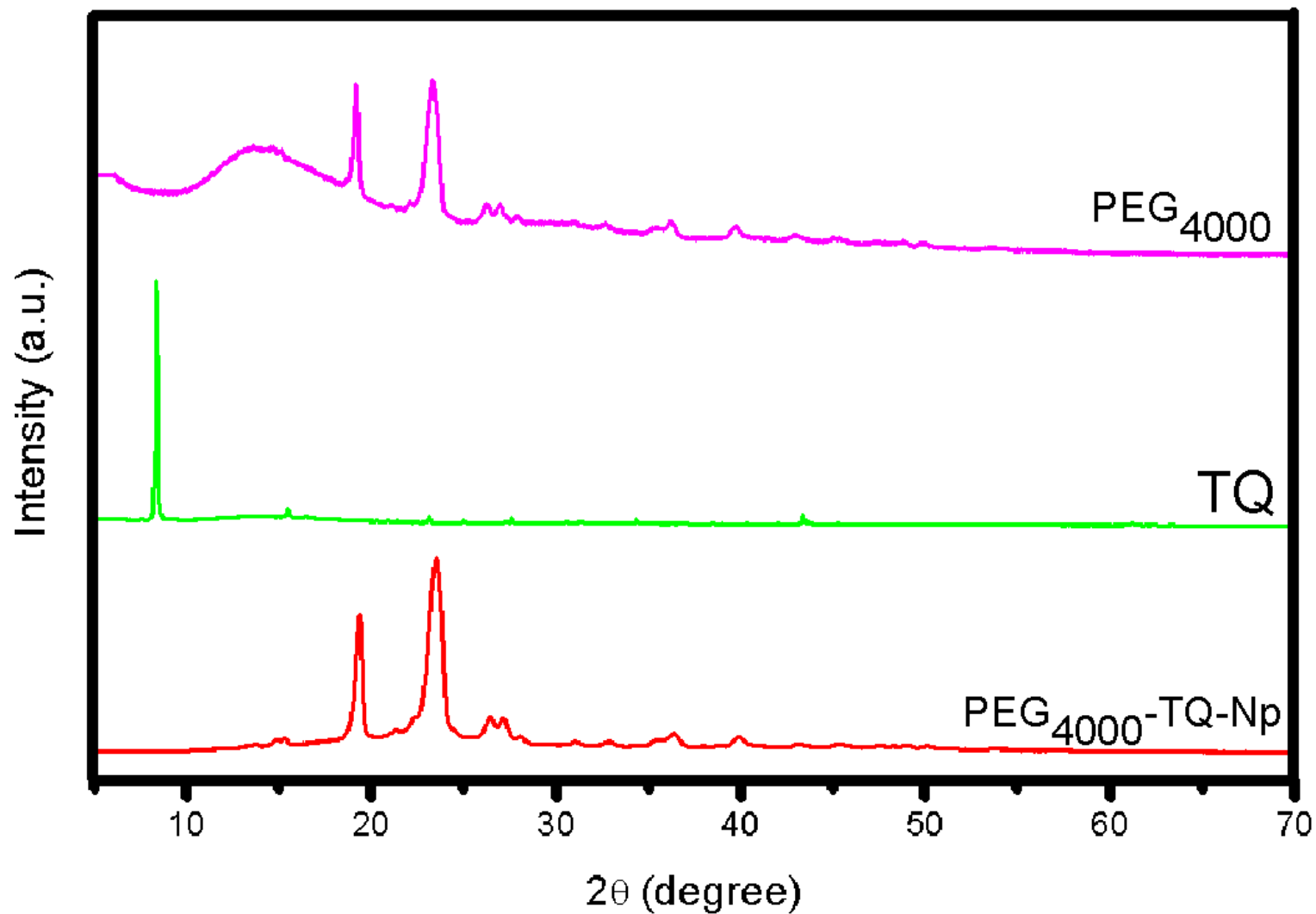
2.418

-0.001

PEG₄₀₀₀TQ-Np

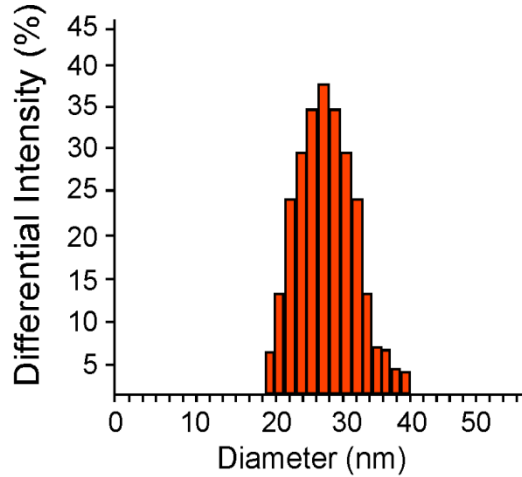


X-ray diffraction peaks

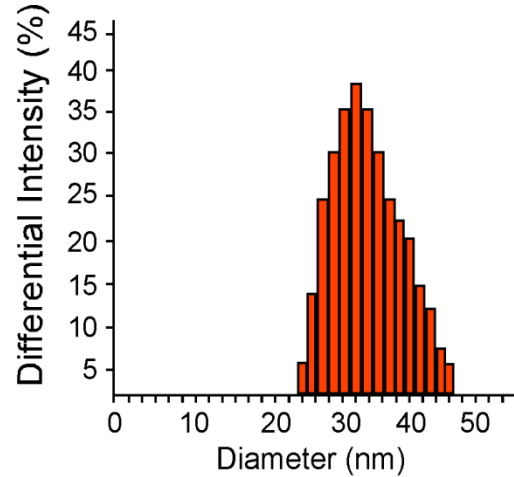


Size distribution and surface charge

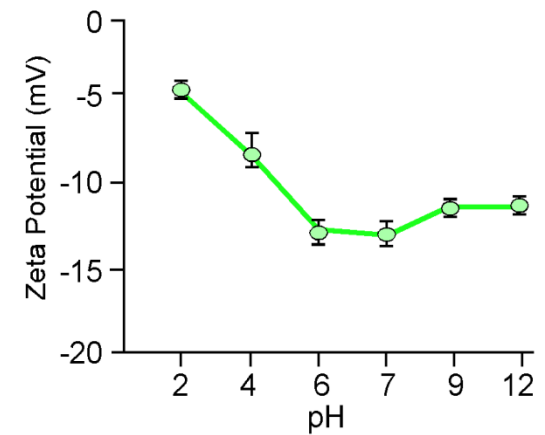
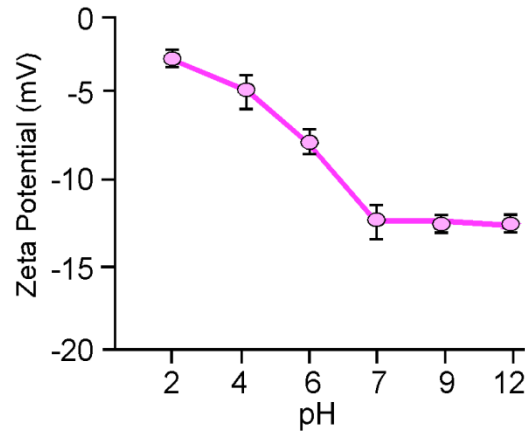
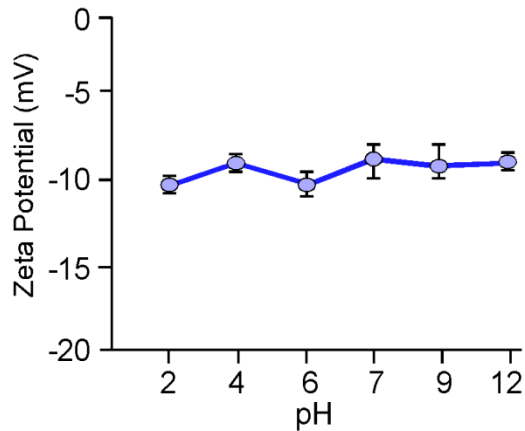
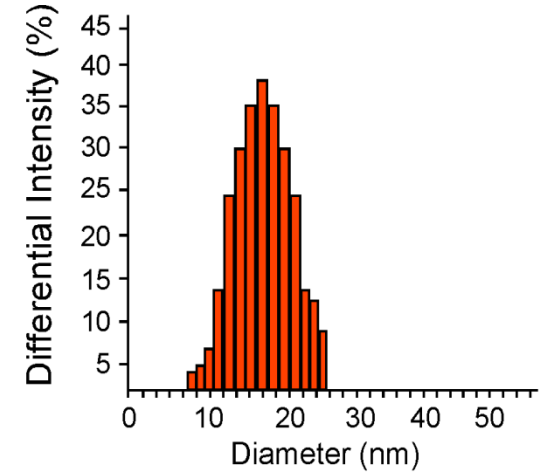
PVP-TQ-Np



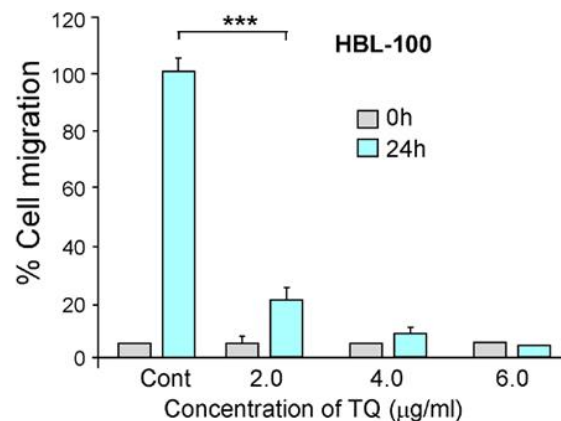
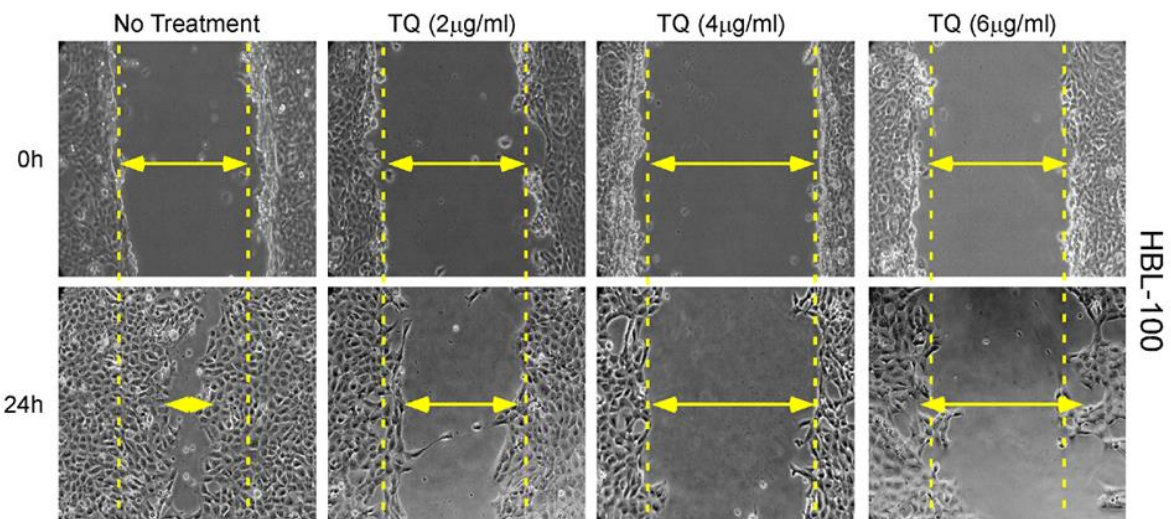
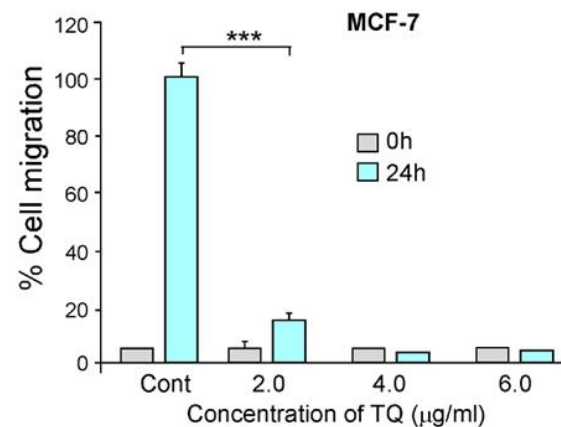
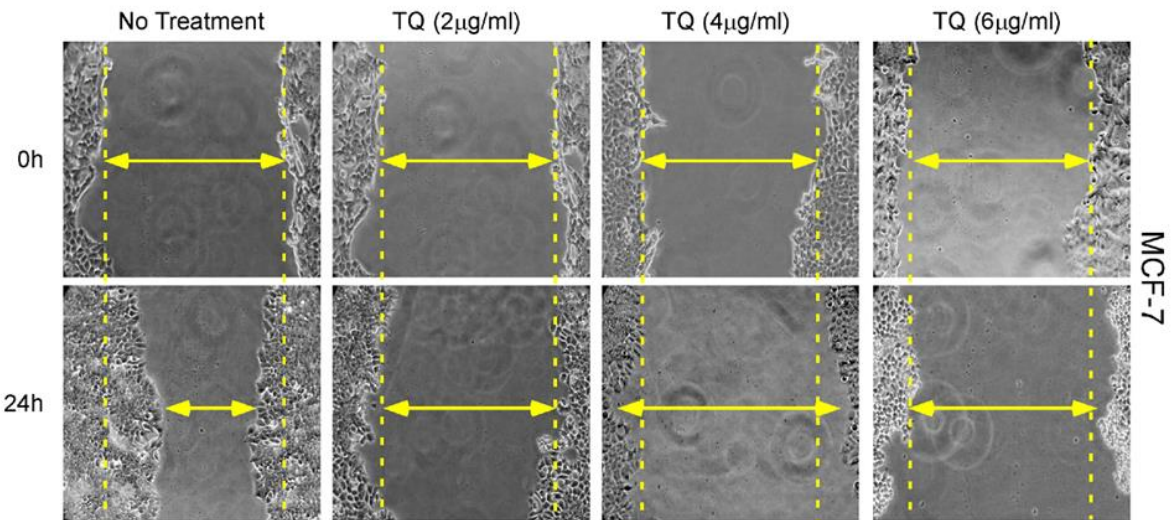
PEG₂₀₀TQ-Np



PEG₄₀₀₀TQ-Np

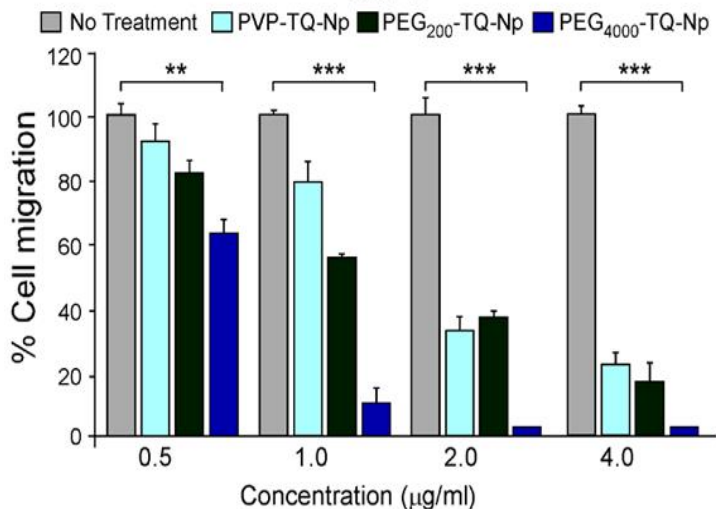


In vitro effect of TQ on retardation of breast cancer cell migration

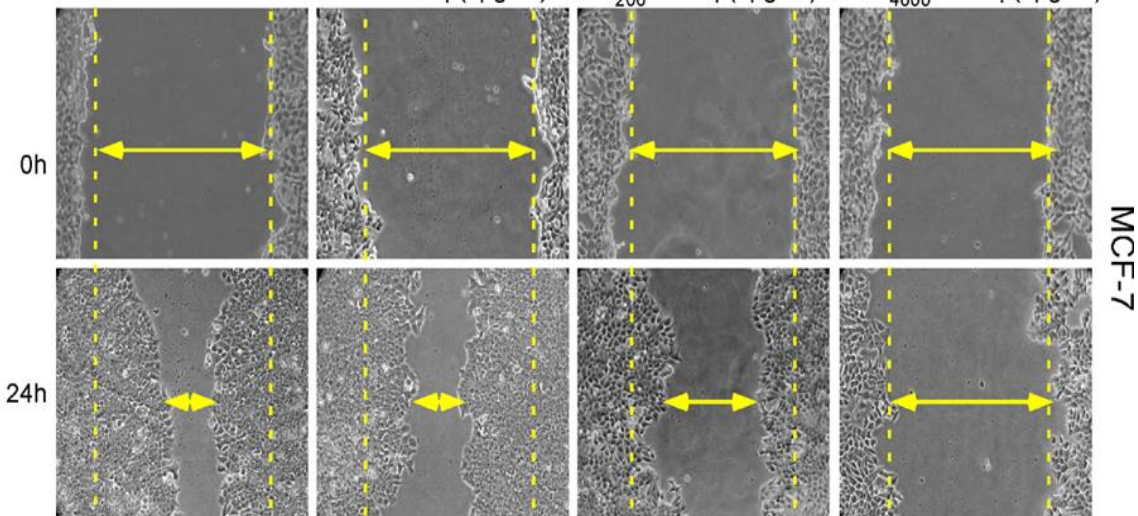


In comparison to the other two TQ-Nps, PEG4000-TQ-Np was much more effective in retarding migration of both MCF-7 and HBL-100 even at a much lower dose

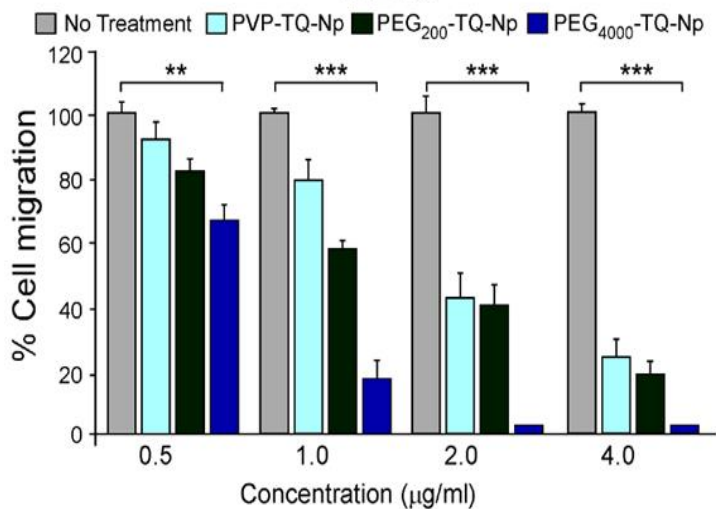
MCF-7



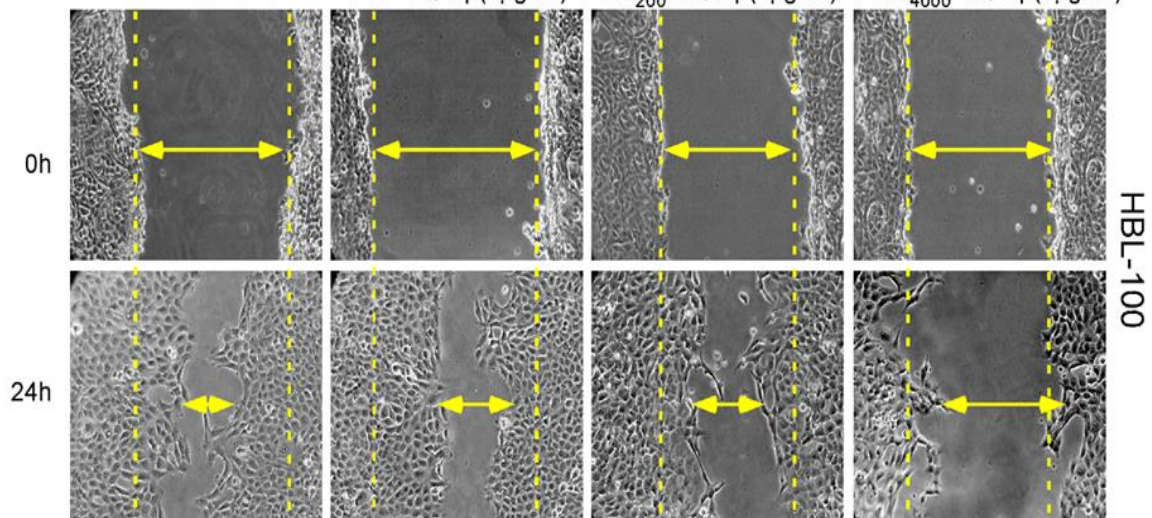
No Treatment PVP-TQ-Np(1µg/ml) PEG₂₀₀-TQ-Np(1µg/ml) PEG₄₀₀₀-TQ-Np(1µg/ml)



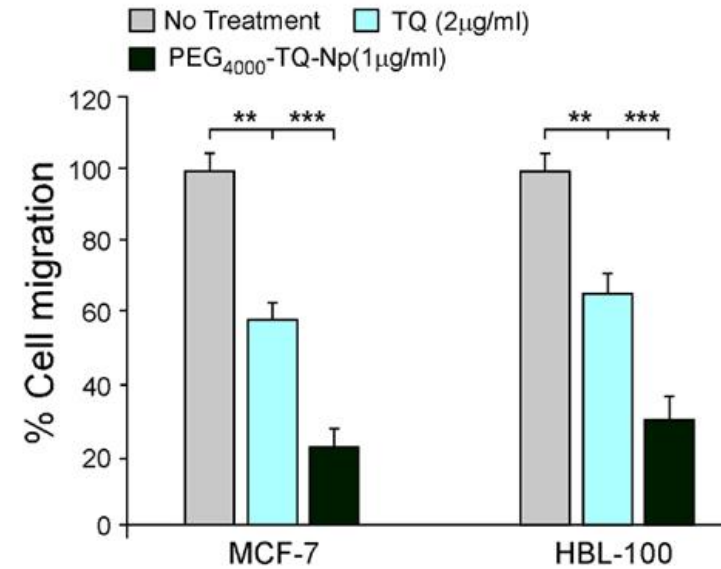
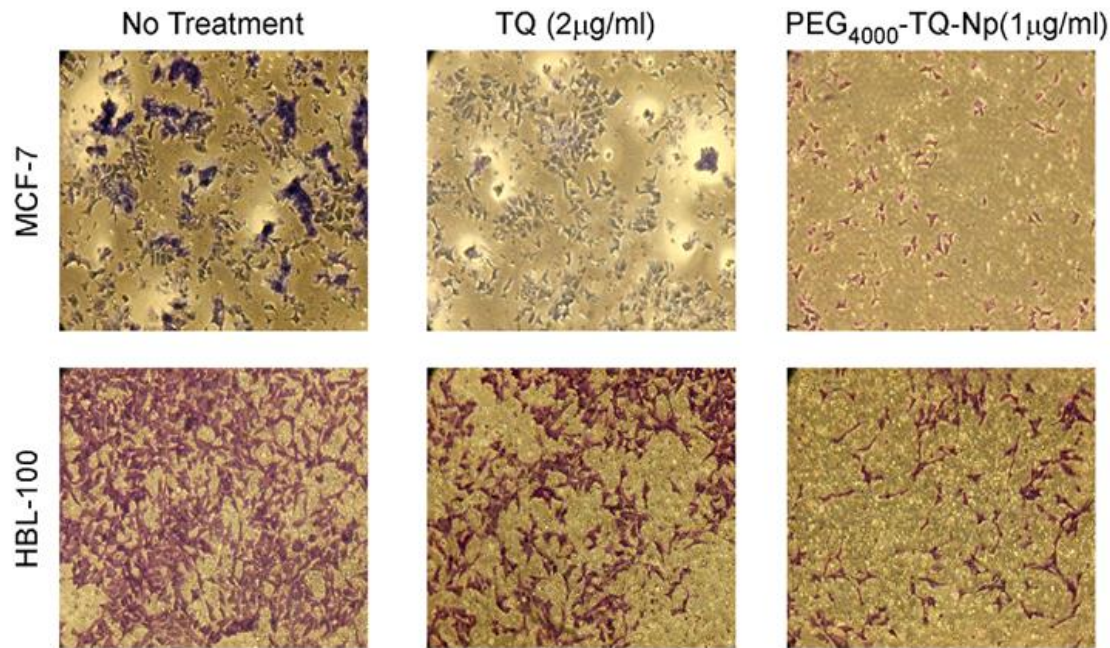
HBL-100



No Treatment PVP-TQ-Np(1µg/ml) PEG₂₀₀-TQ-Np(1µg/ml) PEG₄₀₀₀-TQ-Np(1µg/ml)



Effect of TQ and TQ-Nps on migration of breast cancer cells shown through transwell migration assay



Lamellipodium formation during Cell migration

1.

- Extension of lamellipodium

2.

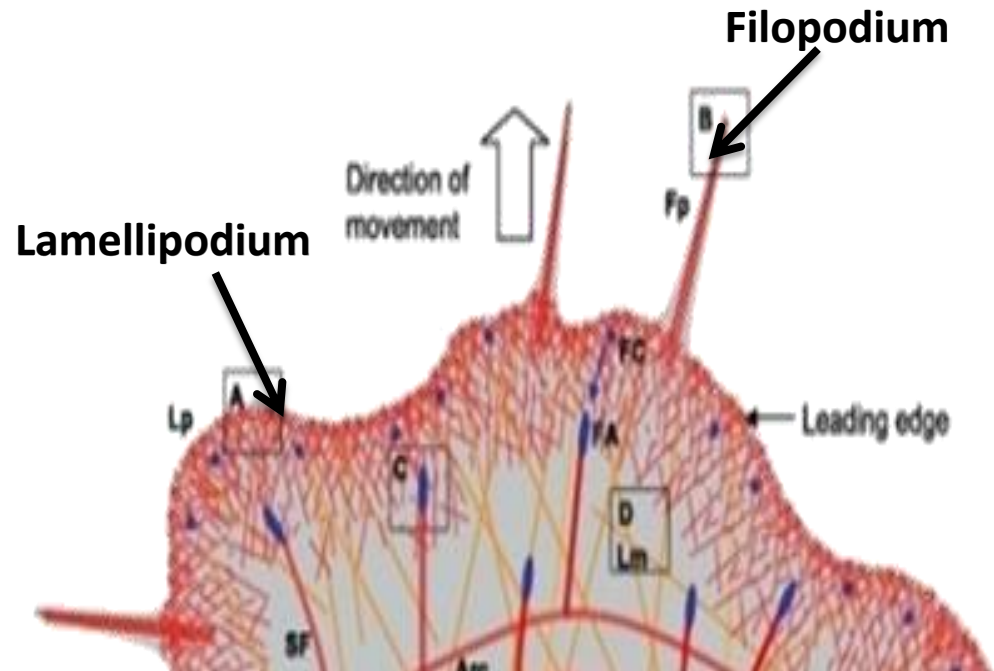
- formation of new focal adhesion at the leading edge

3.

- breaking of adhesions at the rear end

4.

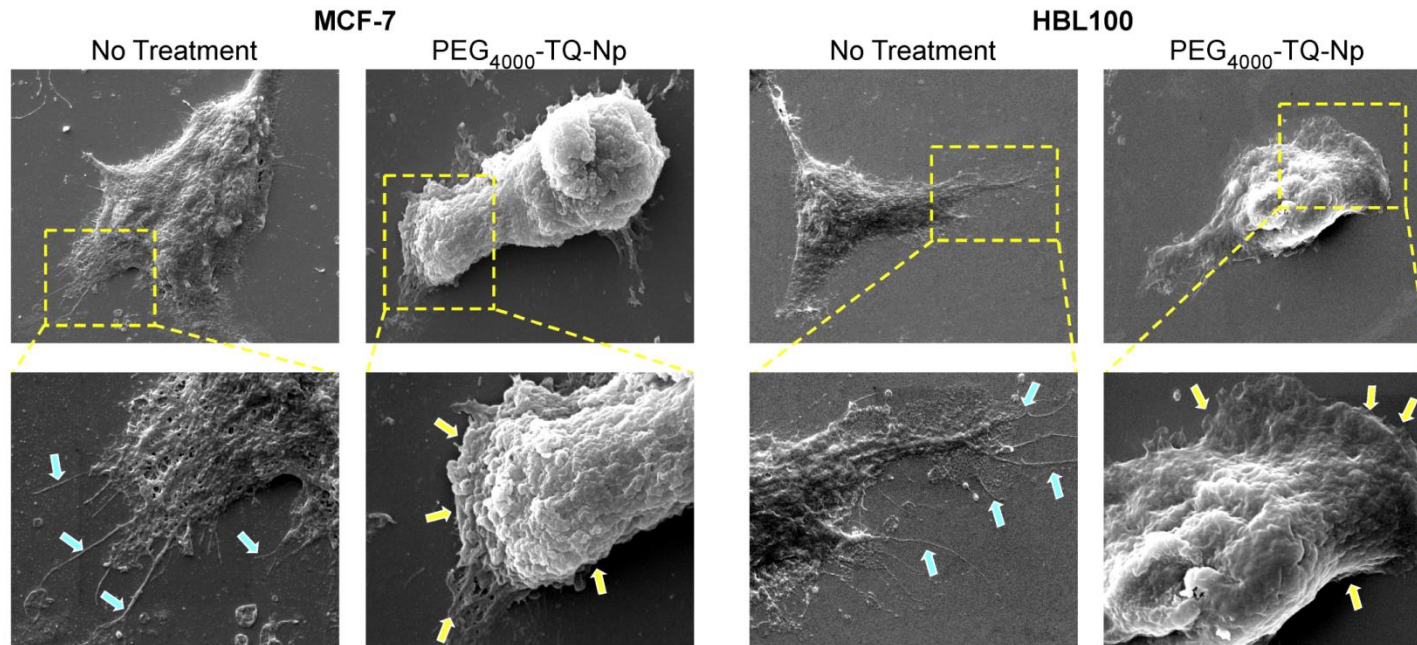
- translocation of the cell mass



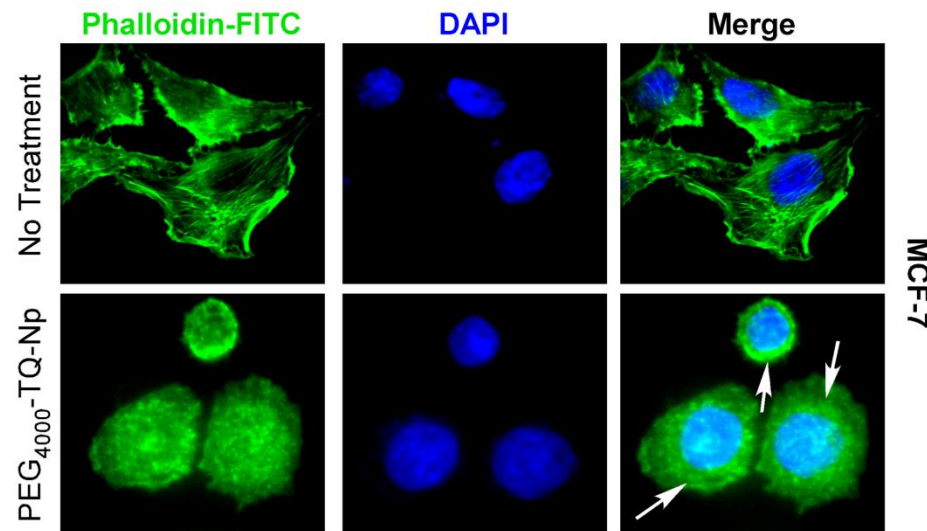
The lamellipodium is a broad, flat cell protrusion formed at the leading edge of migrating cells.

The filopodium is a thin, tubular, finger-like cell protrusion filled with straight bundled, crosslinked actin filaments.

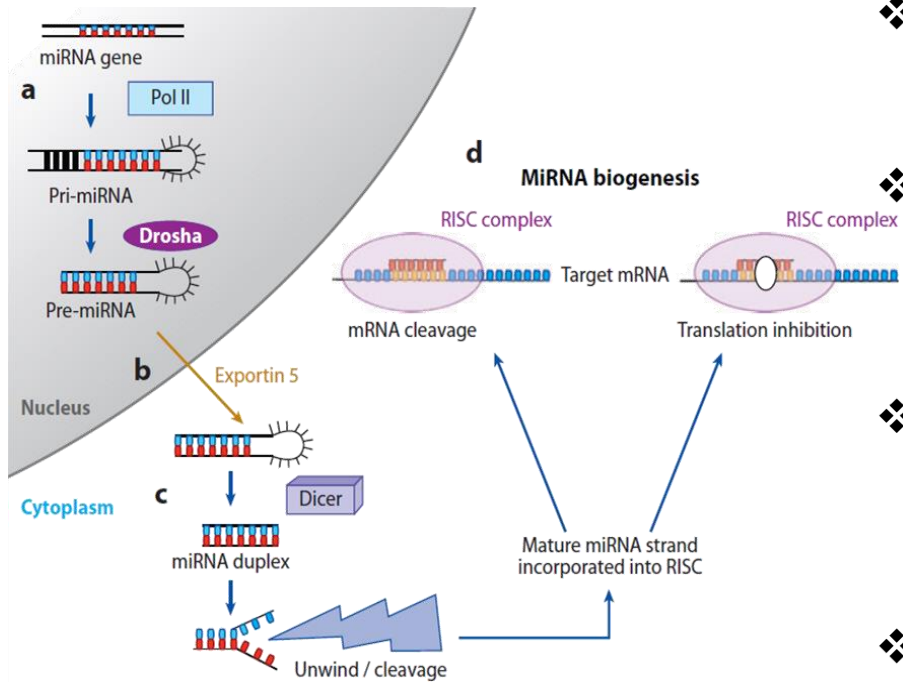
Effect of PEG4000TQ-Nps on the cellular protrusions



Modulation of rearrangement of actin cytoskeleton on treatment of Nps



microRNA



- ❖ small non-coding RNA molecule (ca. 22 nucleotides)

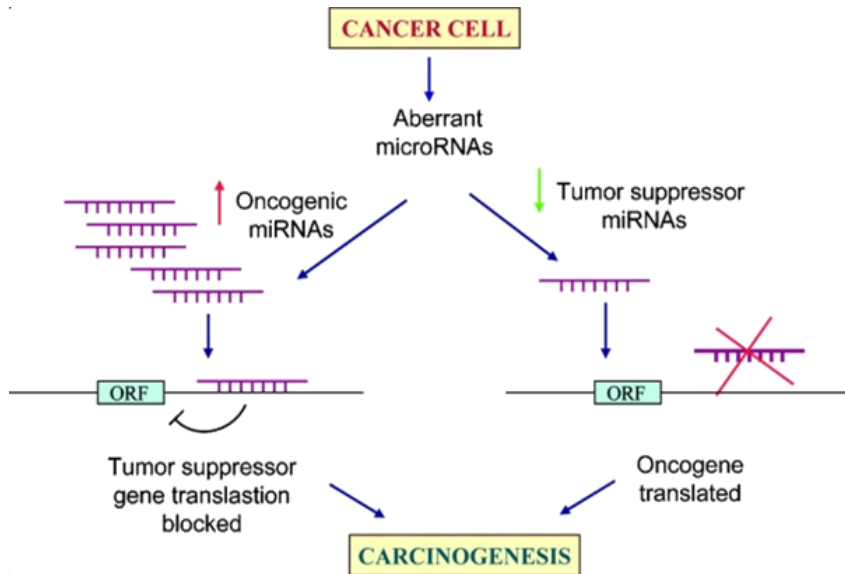
- ❖ found in plants, animals, and some viruses, which functions in transcriptional and post-transcriptional regulation of gene expression.

- ❖ miRNAs silence genes by base pairing with complementary mRNA sequence in 3'UTR of their targets.

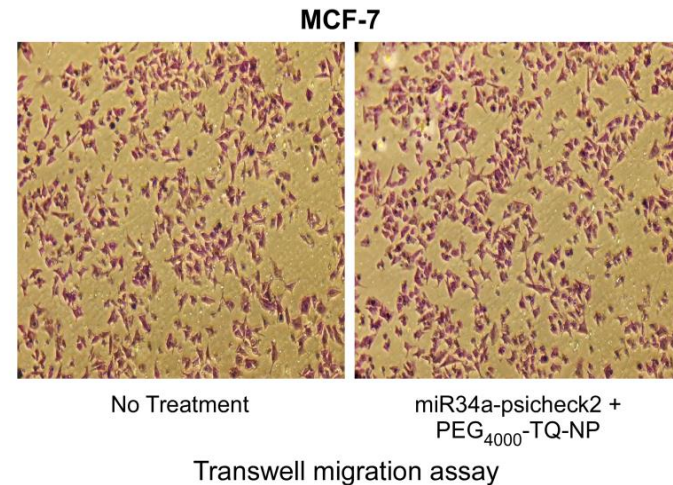
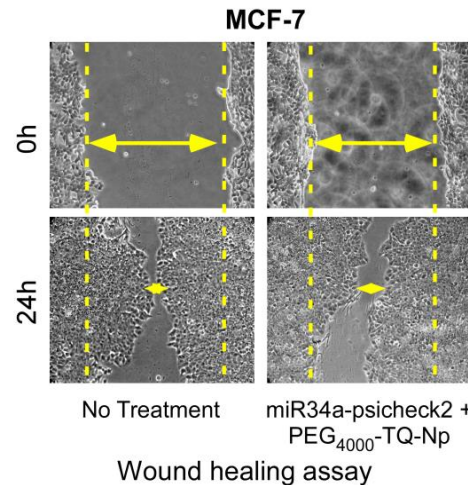
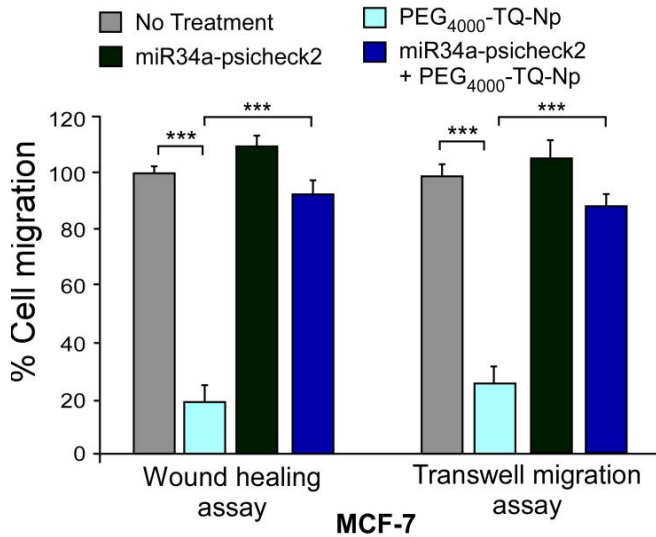
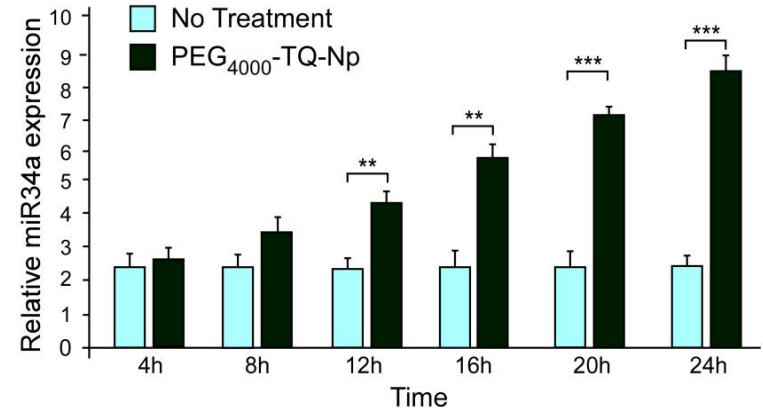
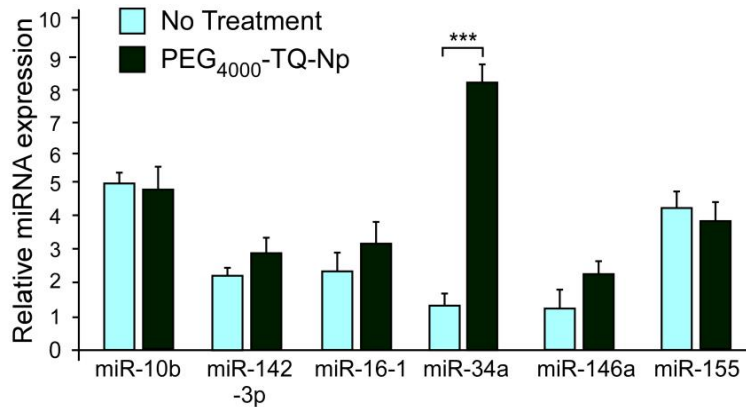
- ❖ The human genome may encode over 1000 miRNAs which may target about 60% of mammalian genes

- ❖ miRNAs function in a variety of biological processes including tissue differentiation, organ differentiation, organ development, control of cell proliferation, apoptosis.

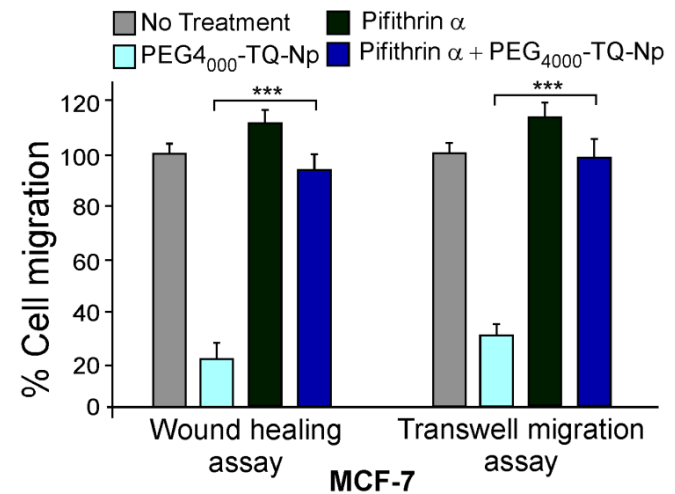
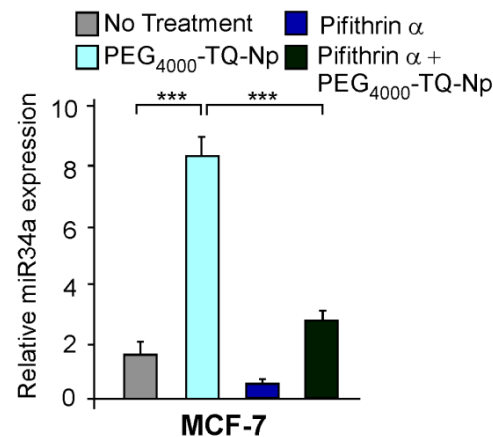
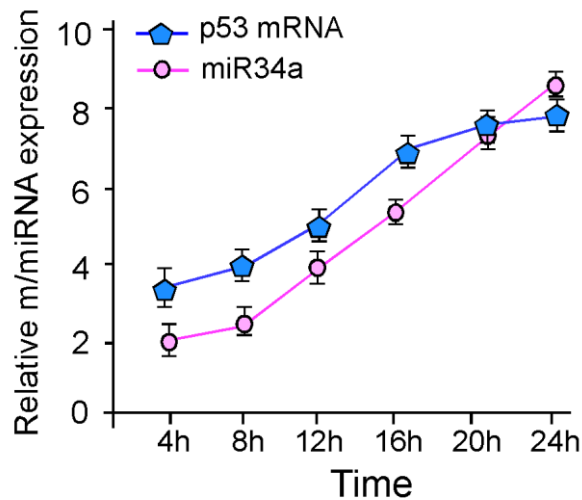
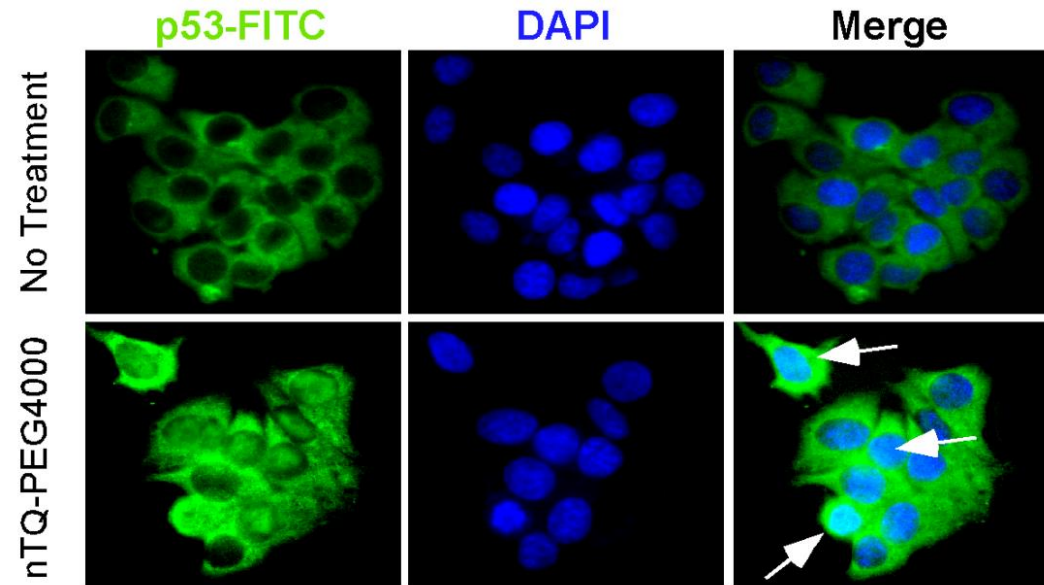
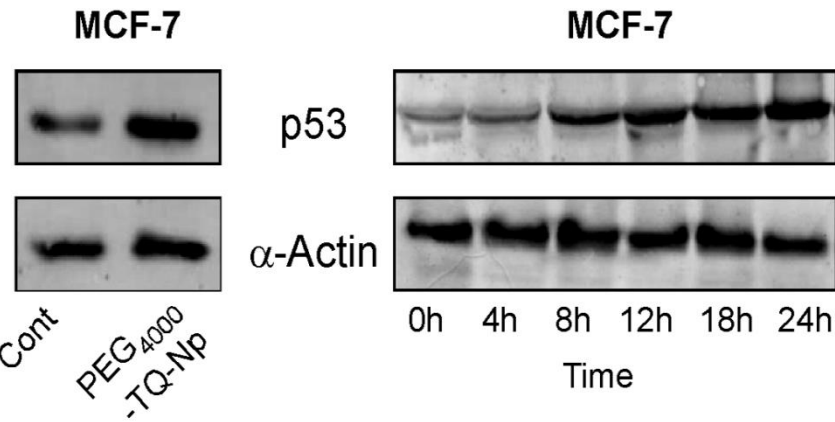
- ❖ Deregulation of these miRNAs may lead to different diseases like cancer.



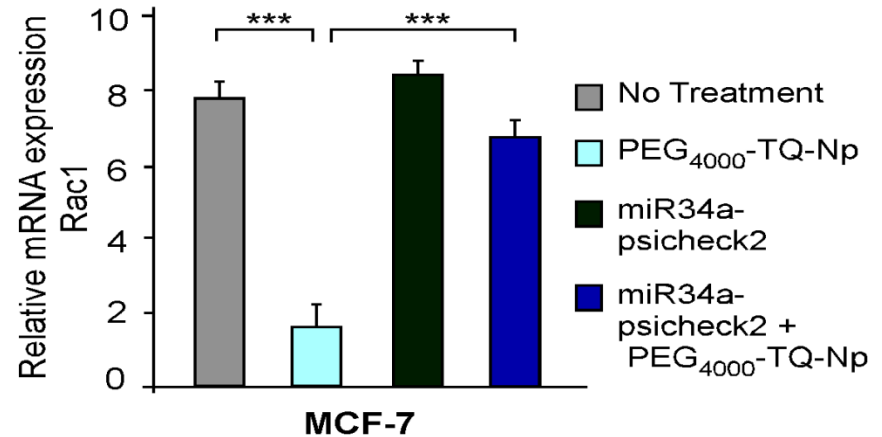
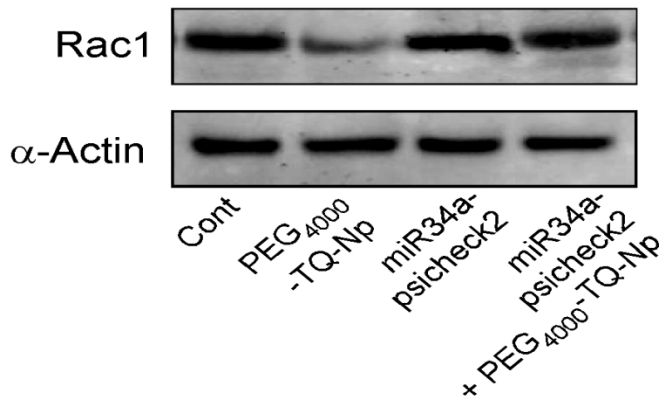
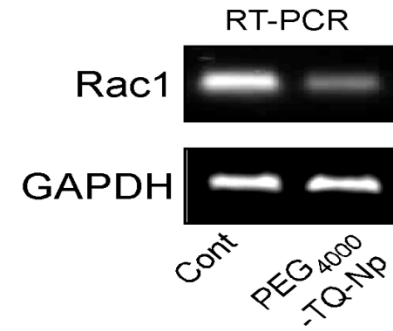
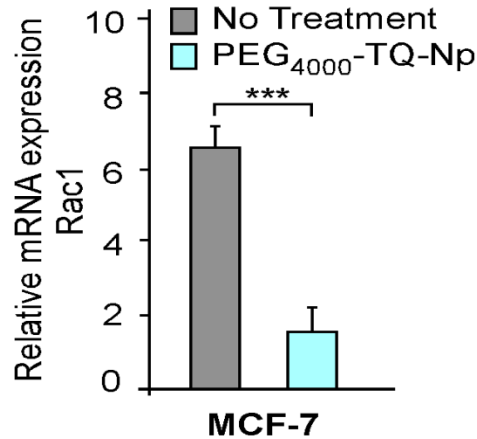
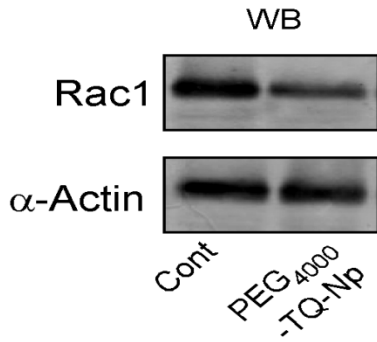
Effect of PEG4000TQ-Nps on miRNA expression profile



Effect of PEG4000-TQ-Np in regulation of miR-34a expression by p53

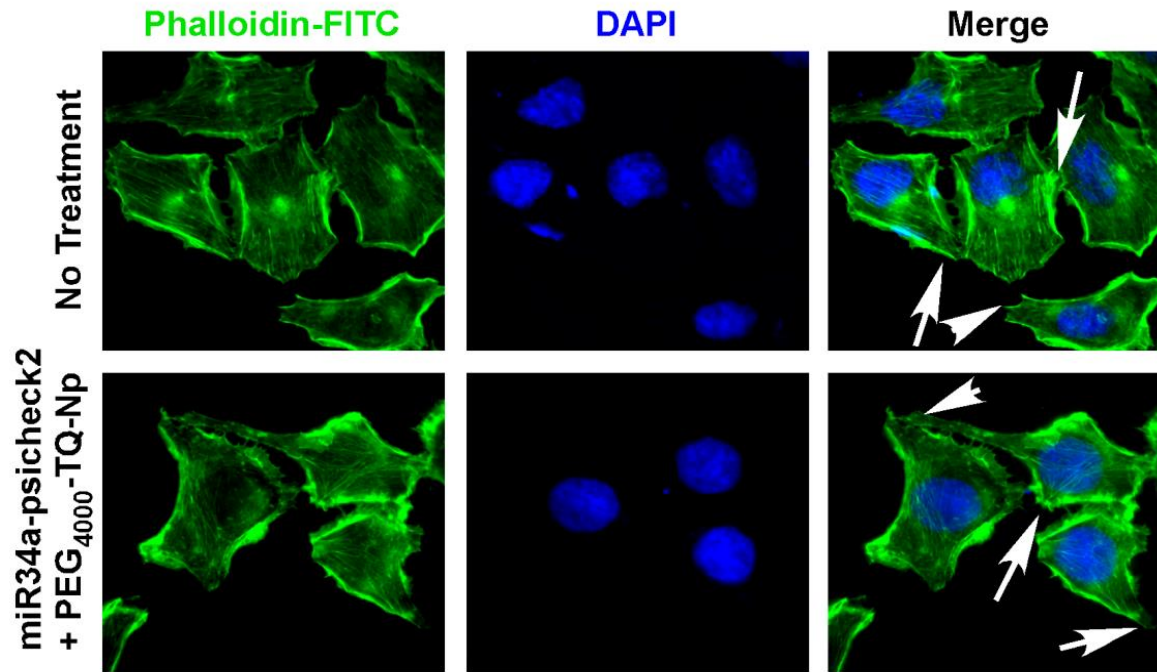


Effect of PEG4000TQ-Nps on Rac1

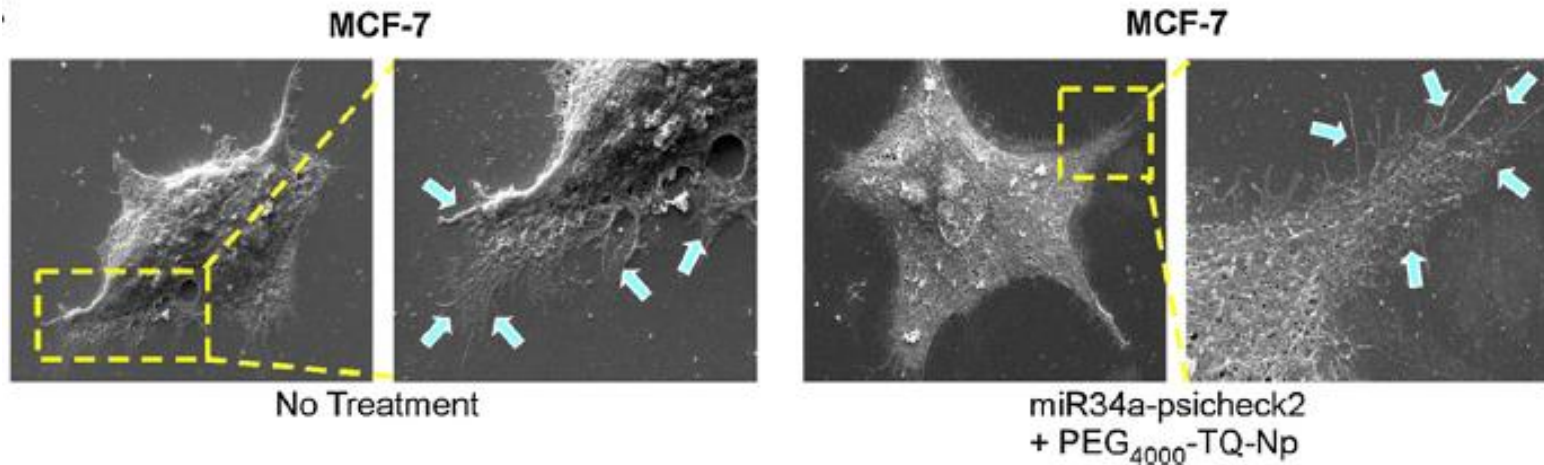


The results obtained significantly displayed that only TQ-Nps treated cells showed prominent down-regulation of Rac1 protein as well as mRNA whereas TQ-Np treatment in psi-CHECK2- AS34a transfected cells failed to down-regulate the expression of Rac1 protein as well as mRNA confirming the direct regulation of Rac1 by miR-34a

Overexpression of miR-34a disrupts actin filaments



Lamelliopodia formation studied by SEM was found to be unaffected in, psi-CHECK2-AS34a transfected, treated with PEG4000- TQ-Nps cells in respect to the control



In vivo validation of the effect of PEG4000-TQ-Nps

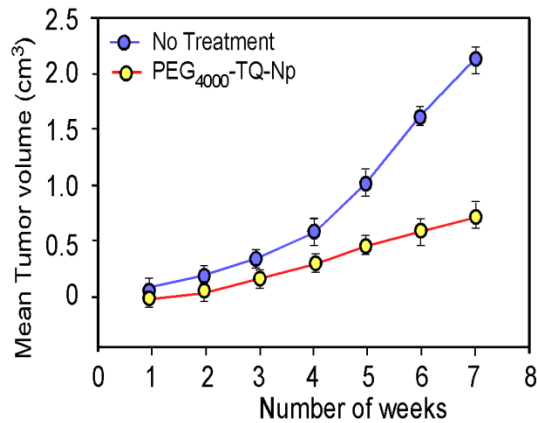
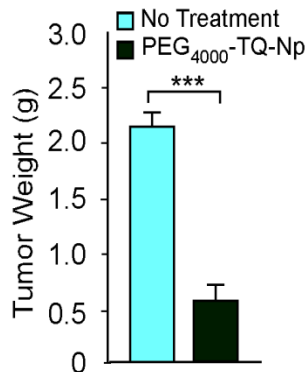
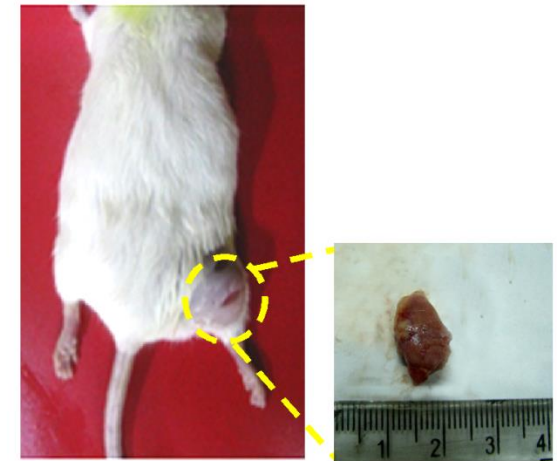
No Treatment



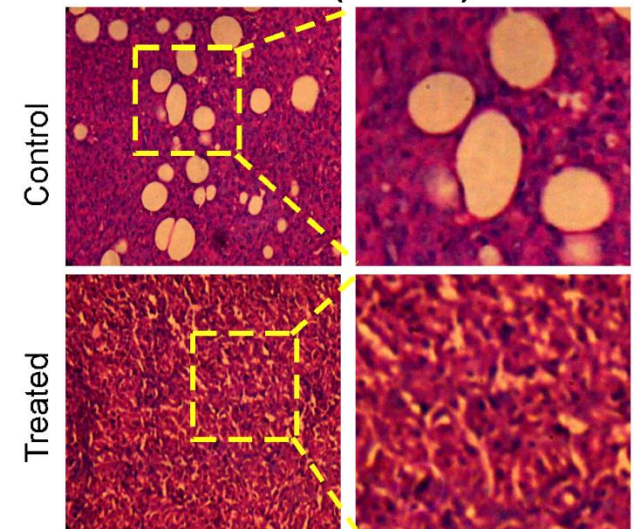
Treated (2.5 mg/kg)



Treated (5 mg/kg)

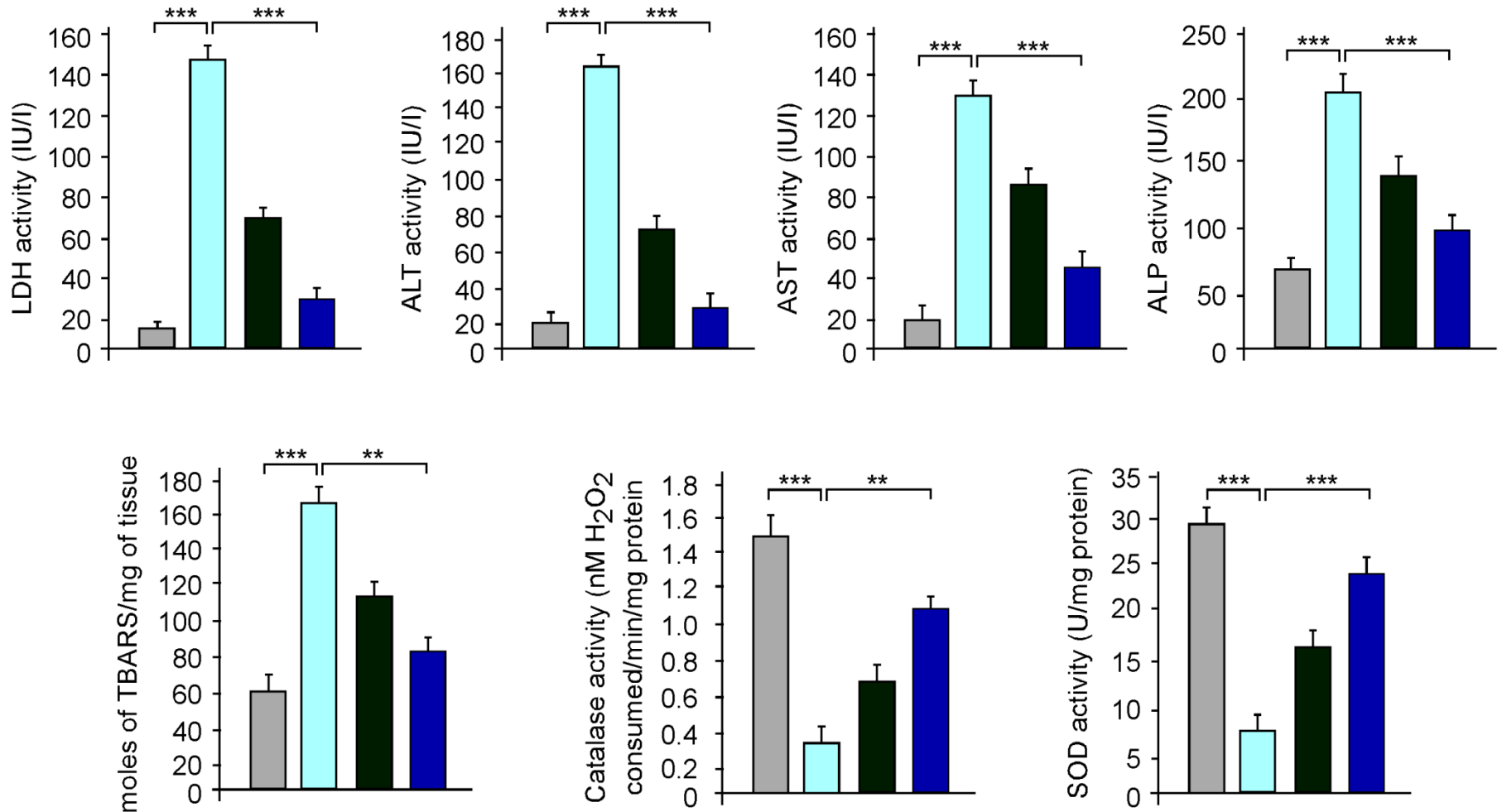


H&E (Tumor)

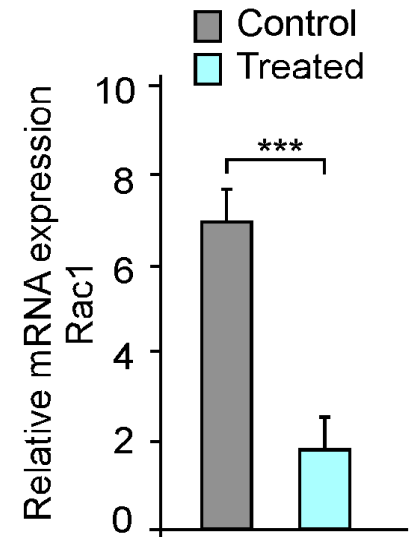
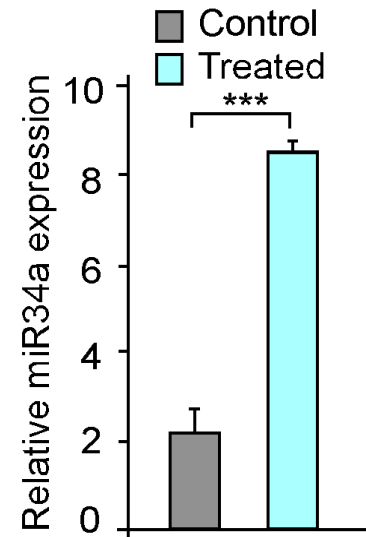
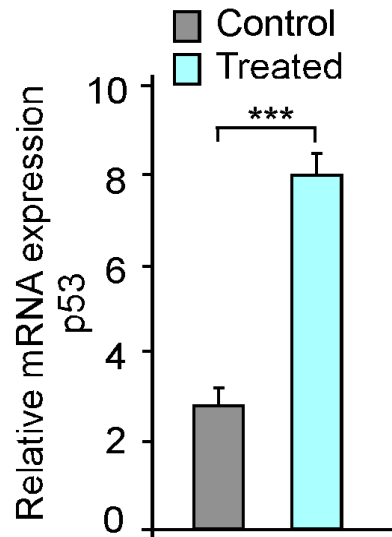
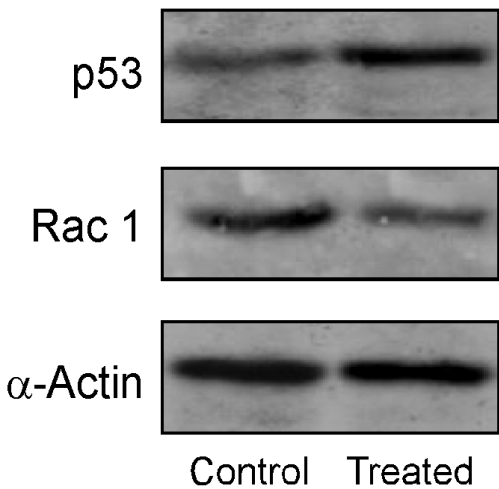
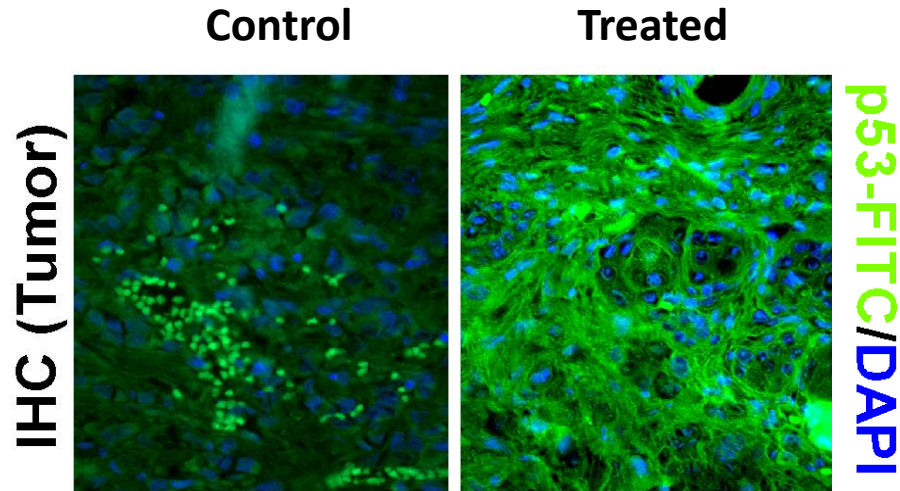


Protection of tumor-bearing mice from cancer induced systemic toxicity by PEG4000-TQ-Nps

Control Tumor bearing Treated (2.5mg/kg) Treated (5mg/kg)



Verification of molecular mechanism *in vivo*



Conclusion

Biomaterials 51 (2015) 91–107



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PEGylated-thymoquinone-nanoparticle mediated retardation of breast cancer cell migration by deregulation of cytoskeletal actin polymerization through miR-34a



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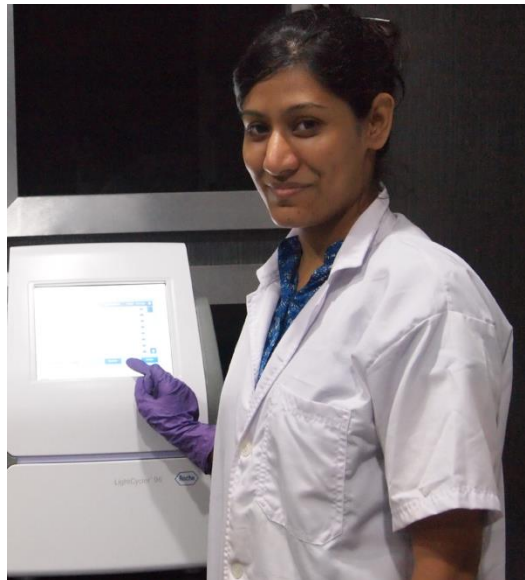
^c Division of Molecular Medicine, Bose Institute, P-1/12, Calcutta Improvement Trust Scheme VII M, Kolkata 700054, West Bengal, India



We sincerely hope that PEGylated thymoquinine nanoparticle may emerge as a potential and effective anti-cancer agent for battling breast cancer in the not so distant future



Saurav (SRF)



Manisha (SRF)



Priyanka (JRF)



Mousumi (JRF)



Ayan (M.Tech trannie)

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