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

Presented by:

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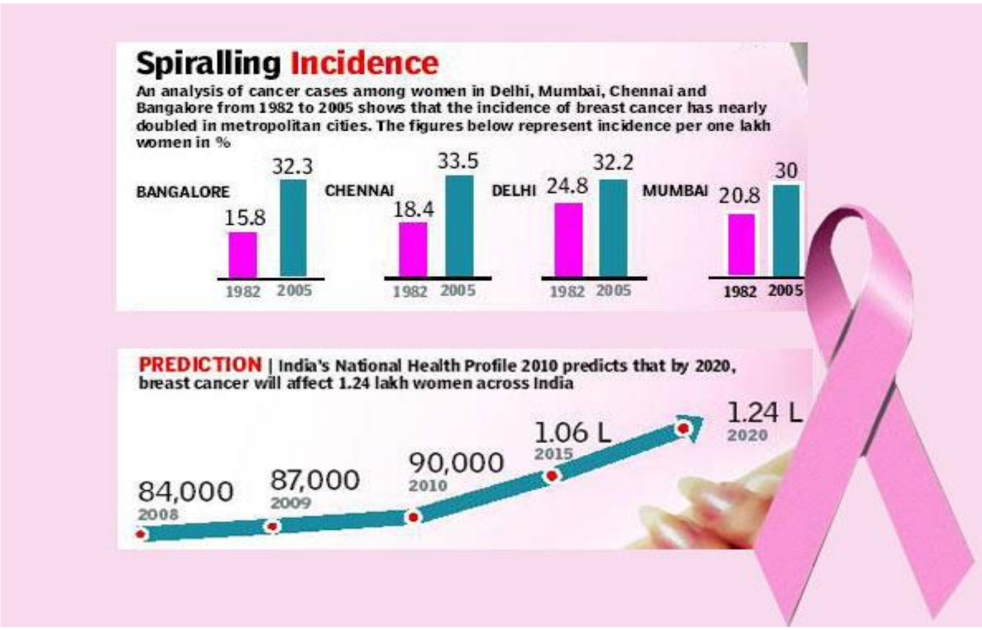
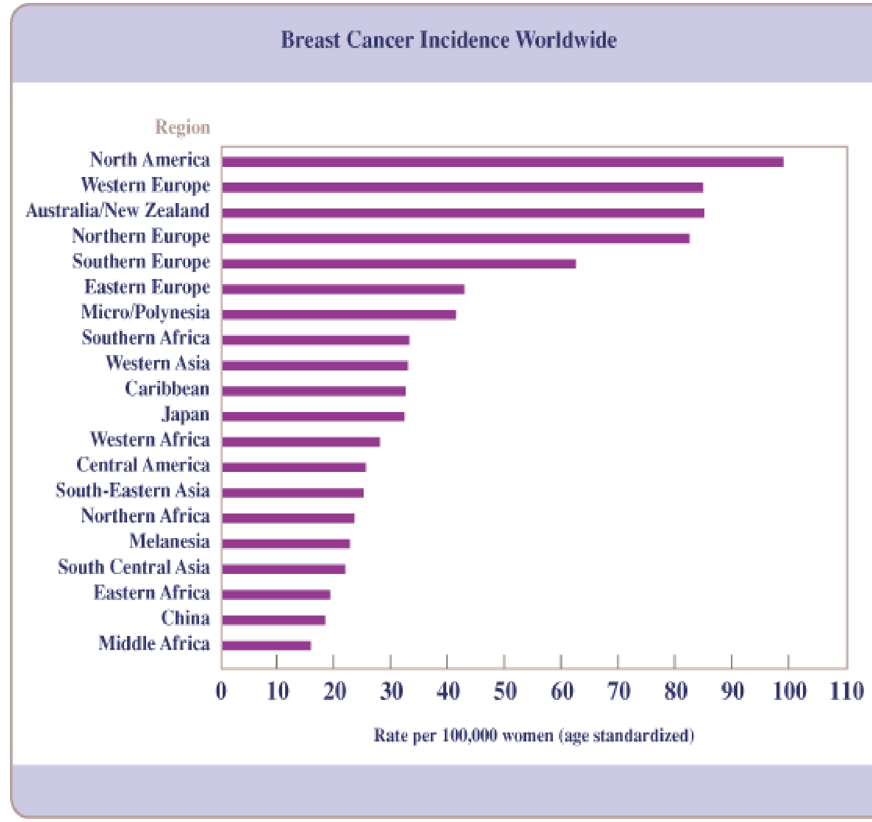
Breast cancer



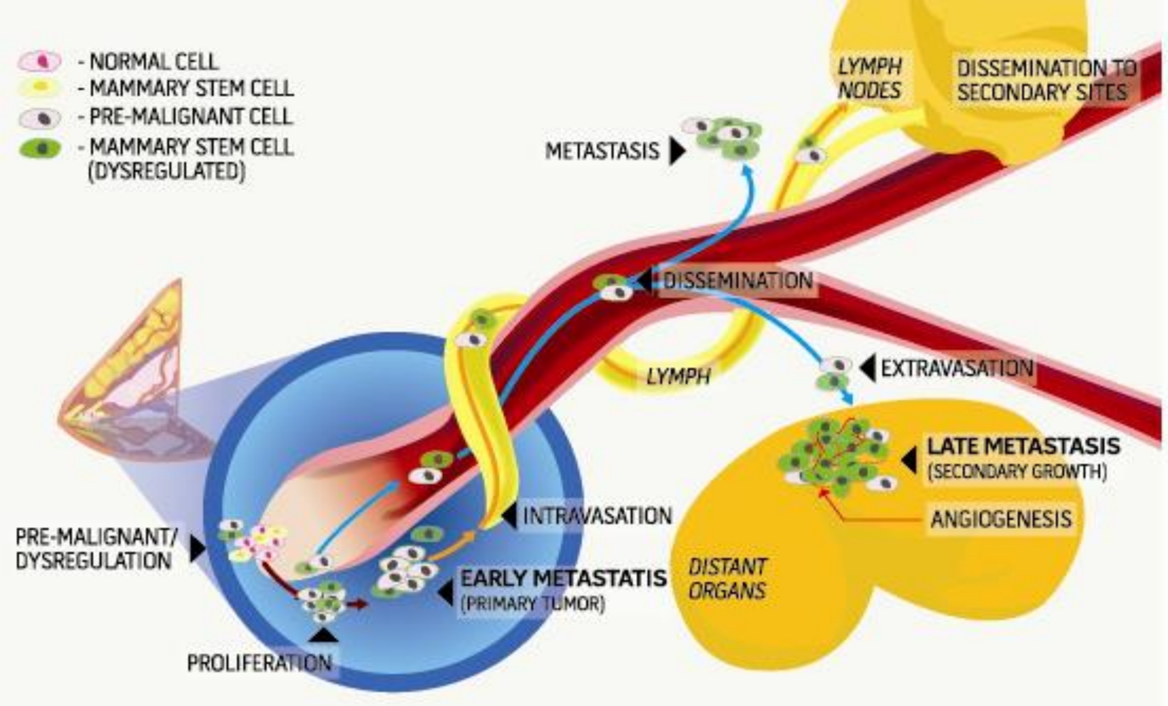


- ❖ Breast Cancer: **second** most commonly diagnosed cancers worldwide (11.9% of all cancers)
  fifth cause of death in cancer (6.3% of all cancers)
- ❖ Since the 2008 estimates, breast cancer incidence has increased by more than **20%**, while mortality has increased by **14%**.
- ❖ The most frequent 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.

*International Agency for Research on Cancer, GLOBCON 2012

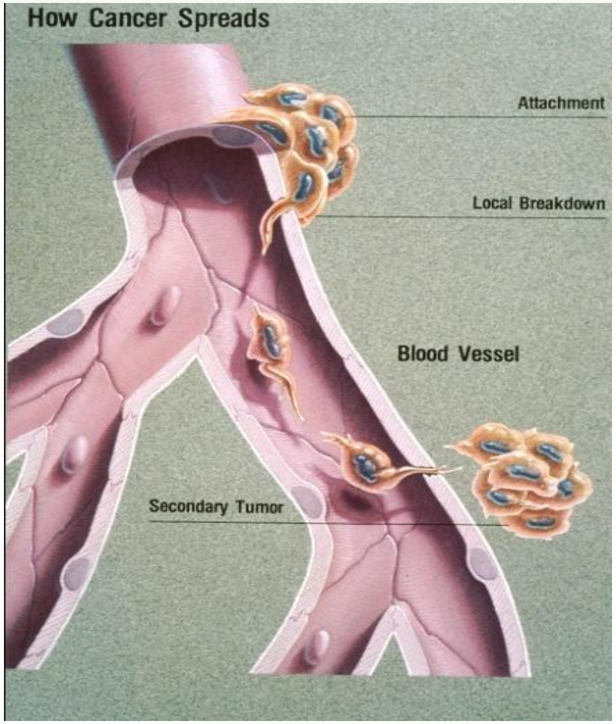
Metastasis

90% death occur in Breast cancer due to metastasis.



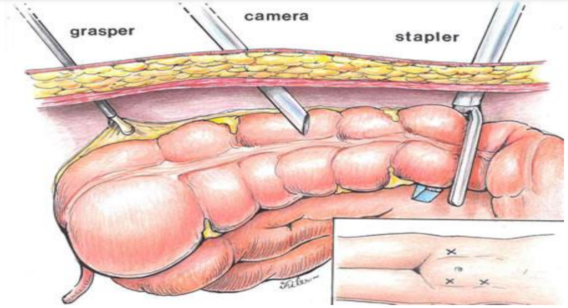
Metastasis refers to the capability to leave a primary tumor, travel via the circulation to a distant tissue site, and form a secondary tumor.

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**



➤Surgery

Chemotherapy

Chemoradiation

Chemotherapy of Breast Cancer
Most Commonly Used Agents

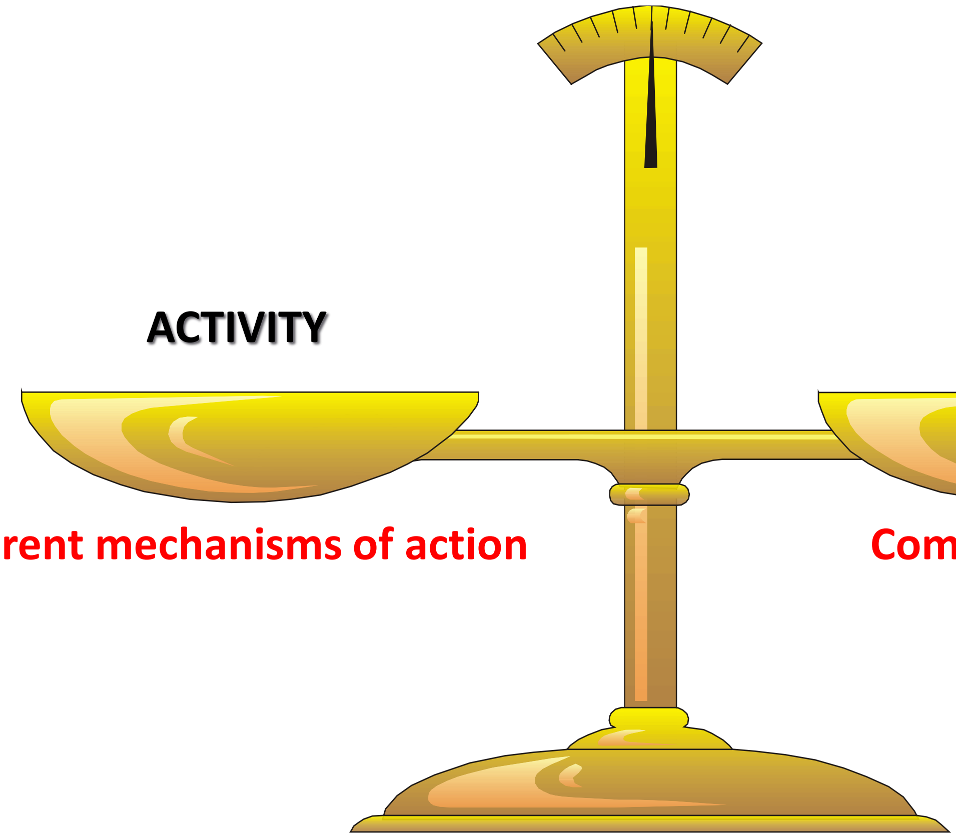
- Doxorubicin, Epirubicin, Mitoxantrone
- Paclitaxel, Docetaxel
- Cyclophosphamide
- Methotrexate
- Fluorouracil
- Vinorelbine
- Capecitabine
- Gemcitabine

Treatment and side effects : Maintaining a balance

INCREASED EFFICACY

ACTIVITY

SAFETY



Different mechanisms of action

Compatible side effects

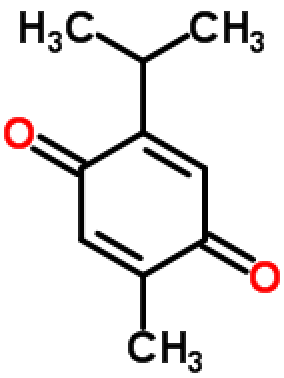
Which way to look ?

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

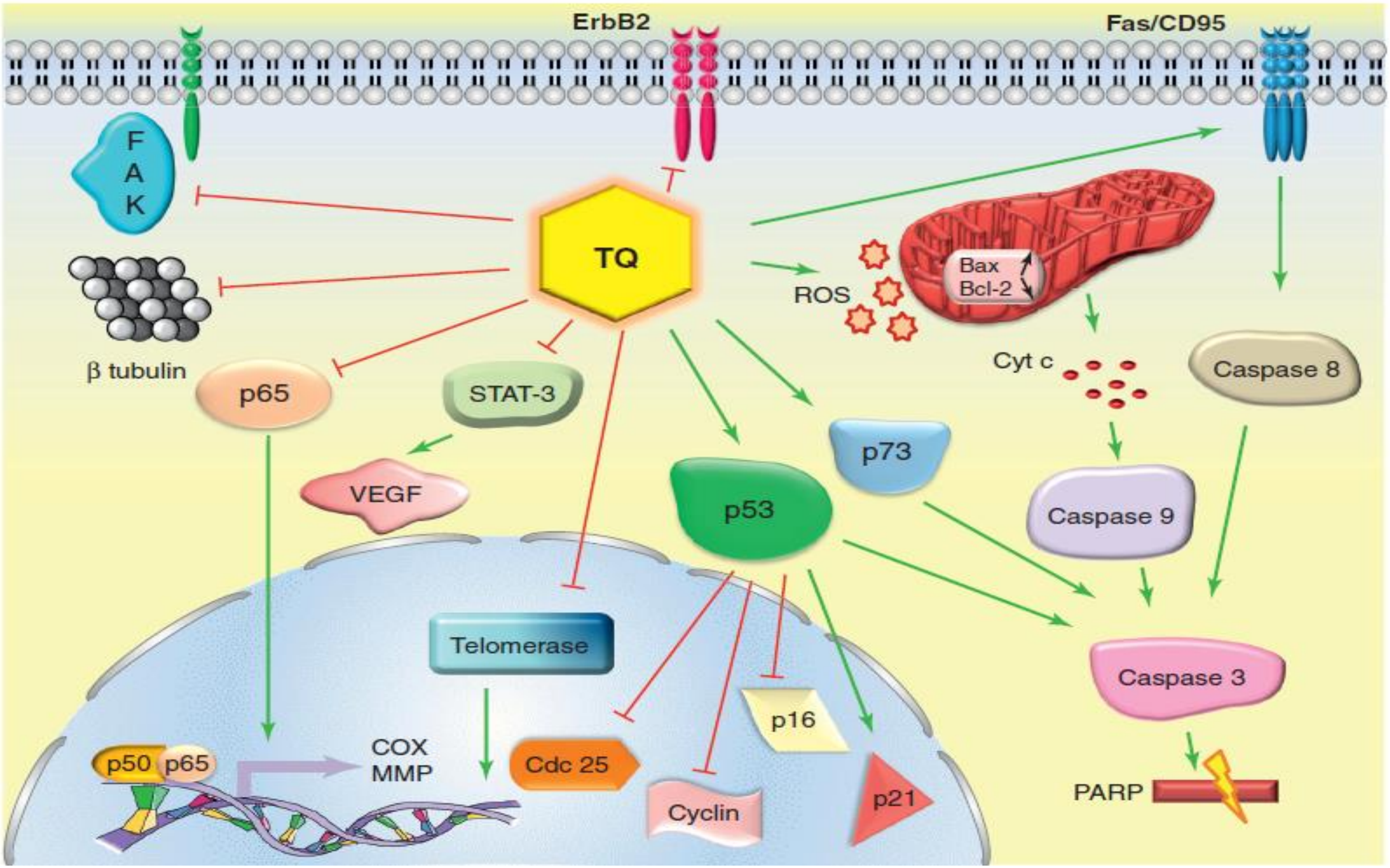
Thymoquinone



- Thymoquinone (TQ) is a major active constituent of black seeds of *Nigella sativa.*
- frequently used in Unani, Ayurveda, Chinese and Arabic medicines.
- Fever, commoncold, 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 Ehrilch’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.**

DMSO

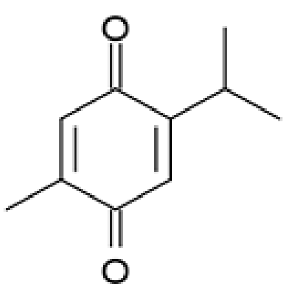
Acetonitrile

dissolves in organic solvents

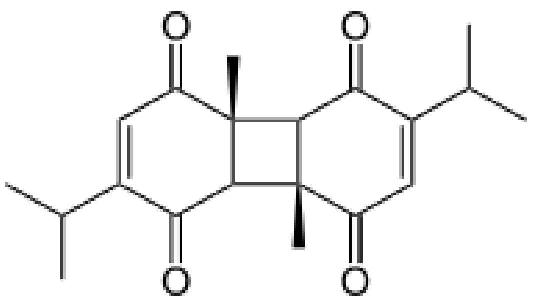
TQ in water

2. **TQ is light sensitive.**

Light



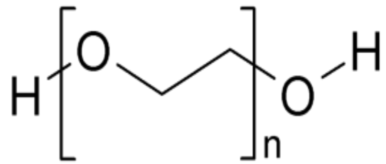
Thymoquinone



Dithymoquinone

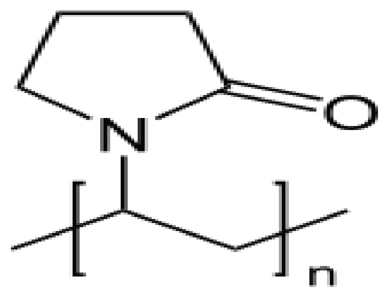
Encapsulating agents

Polyethylene glycol



1. PEG_{200}
2. PEG_{4000}

Polyvinyl pyrrolidon



3. PVP K30

<u>Advantages of them</u>:

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

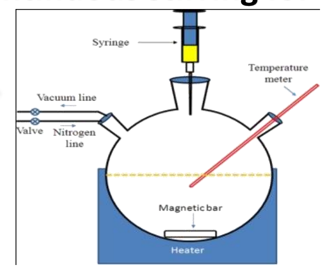
Synthesis

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 Rotery evaporator at 90°C

Synthesis of PEG-TQ-Nps

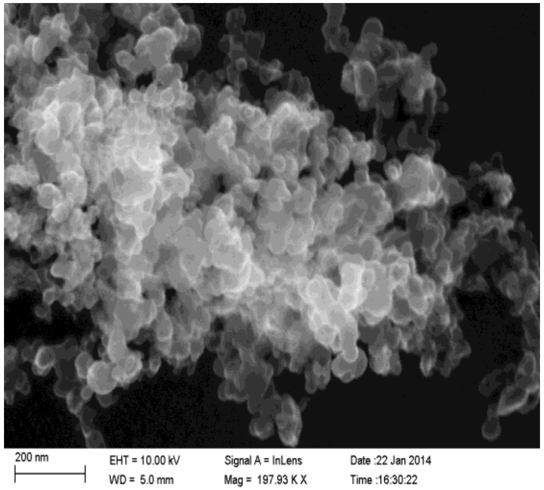
100mM TQ solution in Acetonitrile

Added dropwise into PEG solution under ultrasonic condition.

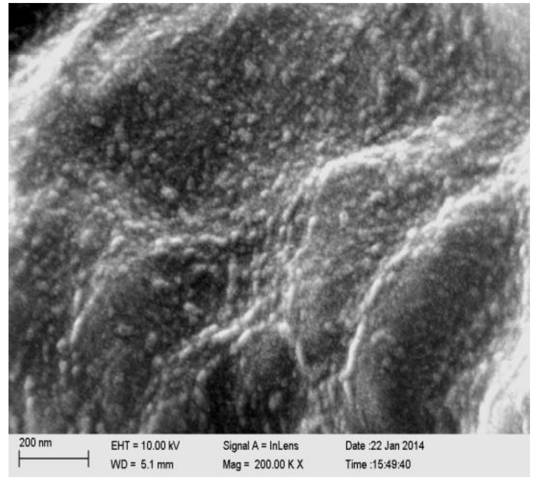
Mixture was kept on continuous stirring for O/N

Organic solvents were veporized using Rotery evaporator at 90°C

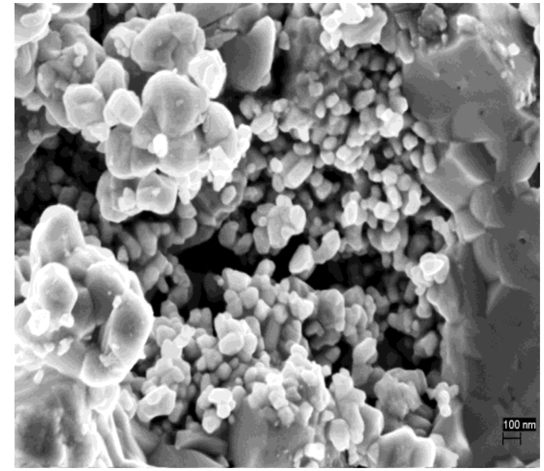
Morphology of the three NTQs evaluated by Field Emission Scanning Electron Microscope (FESEM)



PVP-TQ-Np

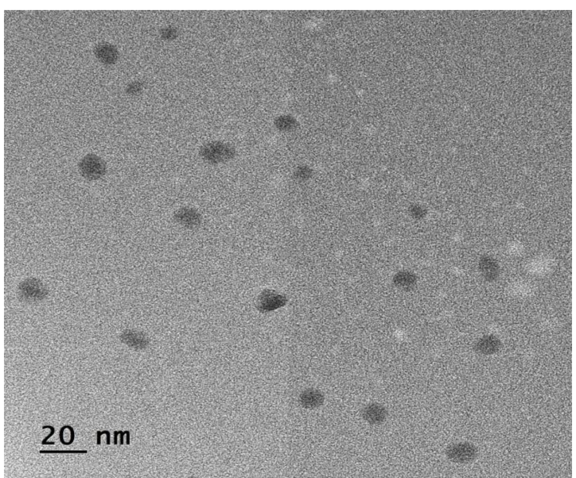


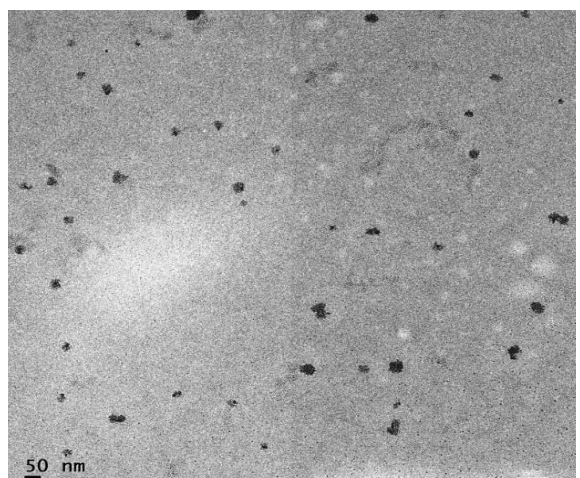
PEG_{200}TQ-Np

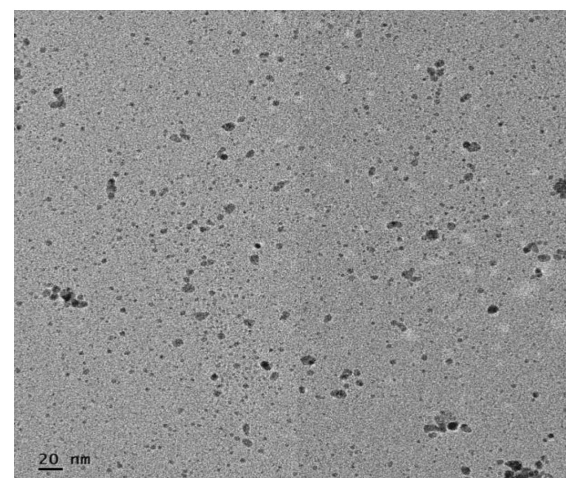


PEG_{4000}TQ-Np

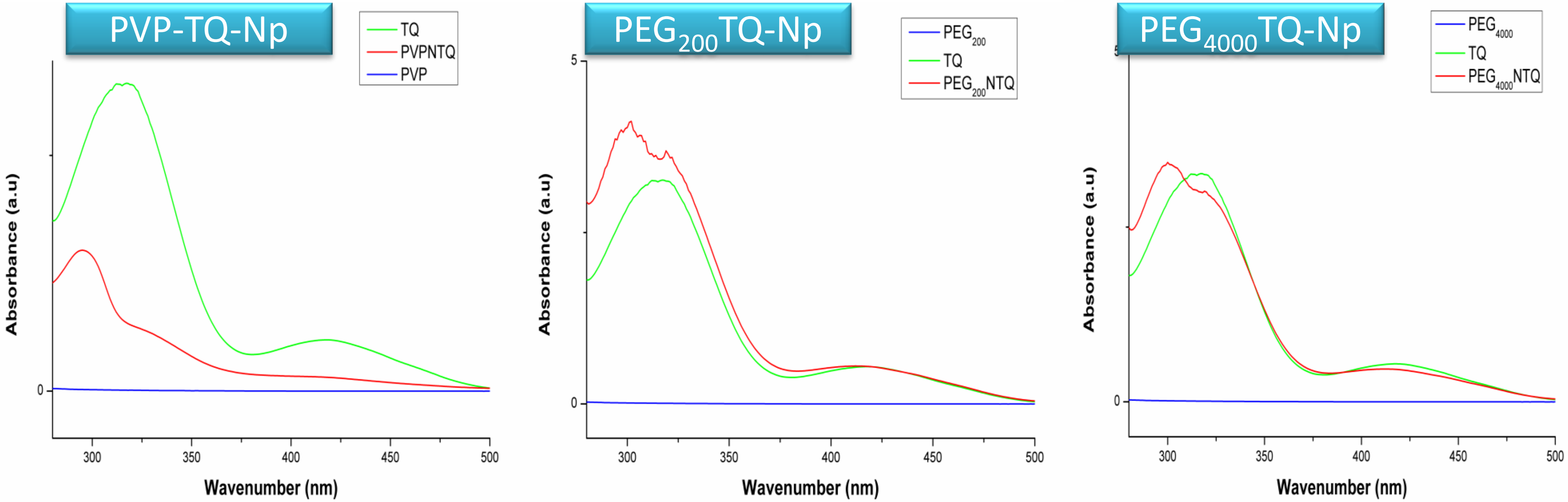
Size analysis of the three NTQs evaluated by Transmission Electron Microscope



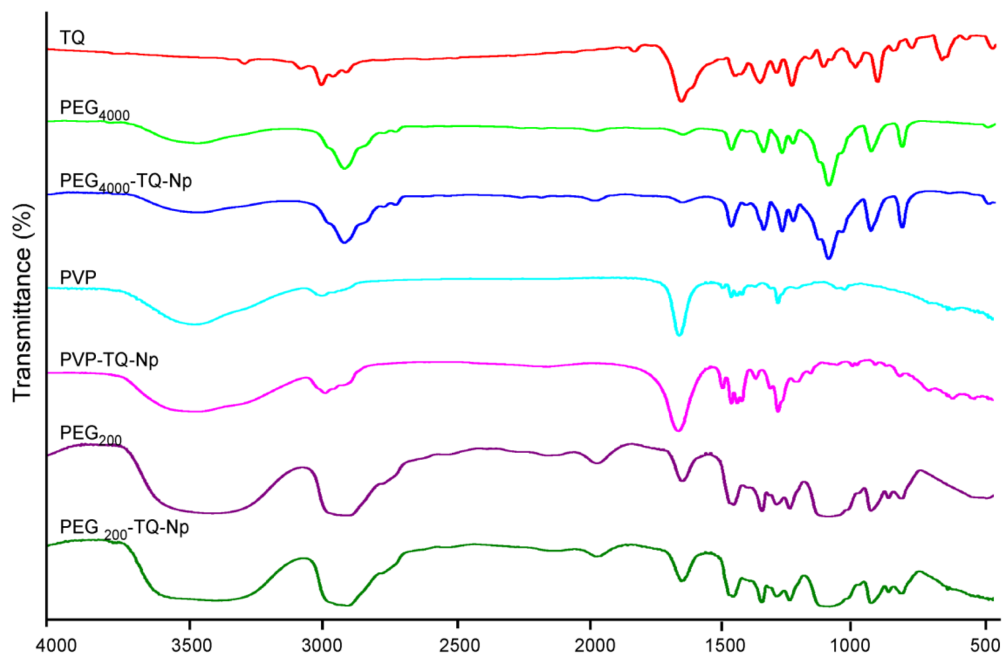




UV/Vis spectra of PVP-TQ-Nps, PEG200-TQ-Nps, PEG4000-TQ-Nps.

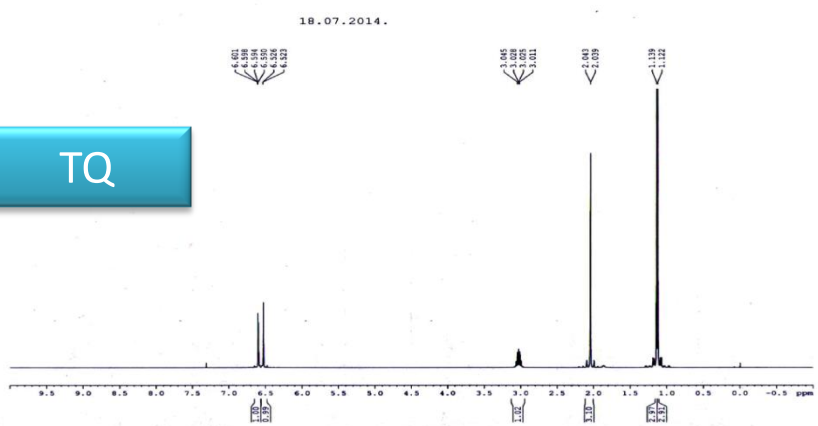


FTIR spectra of TQ, PVP, PEG200, PEG4000, PVP-TQ, PEG200-TQ and PEG4000-TQ Nanoparticles

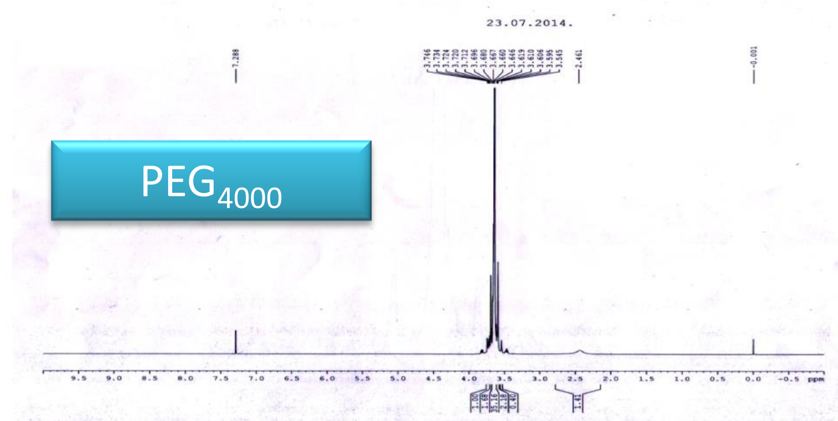


1H-NMR Spectra

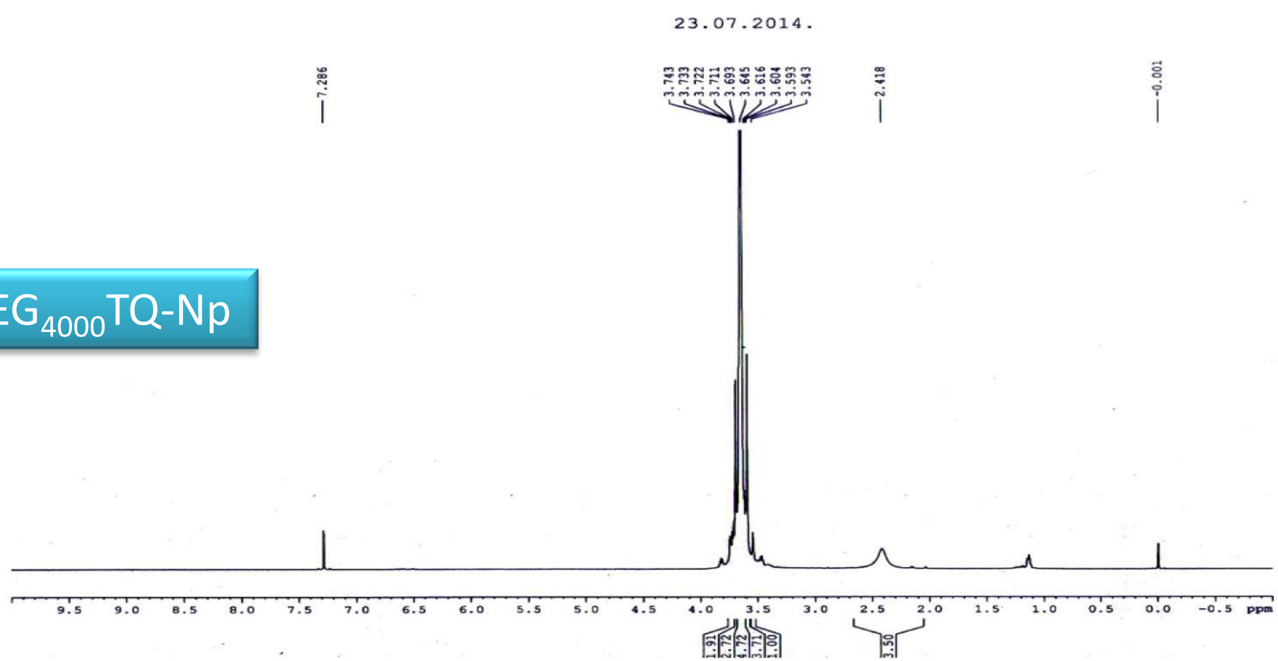
TQ



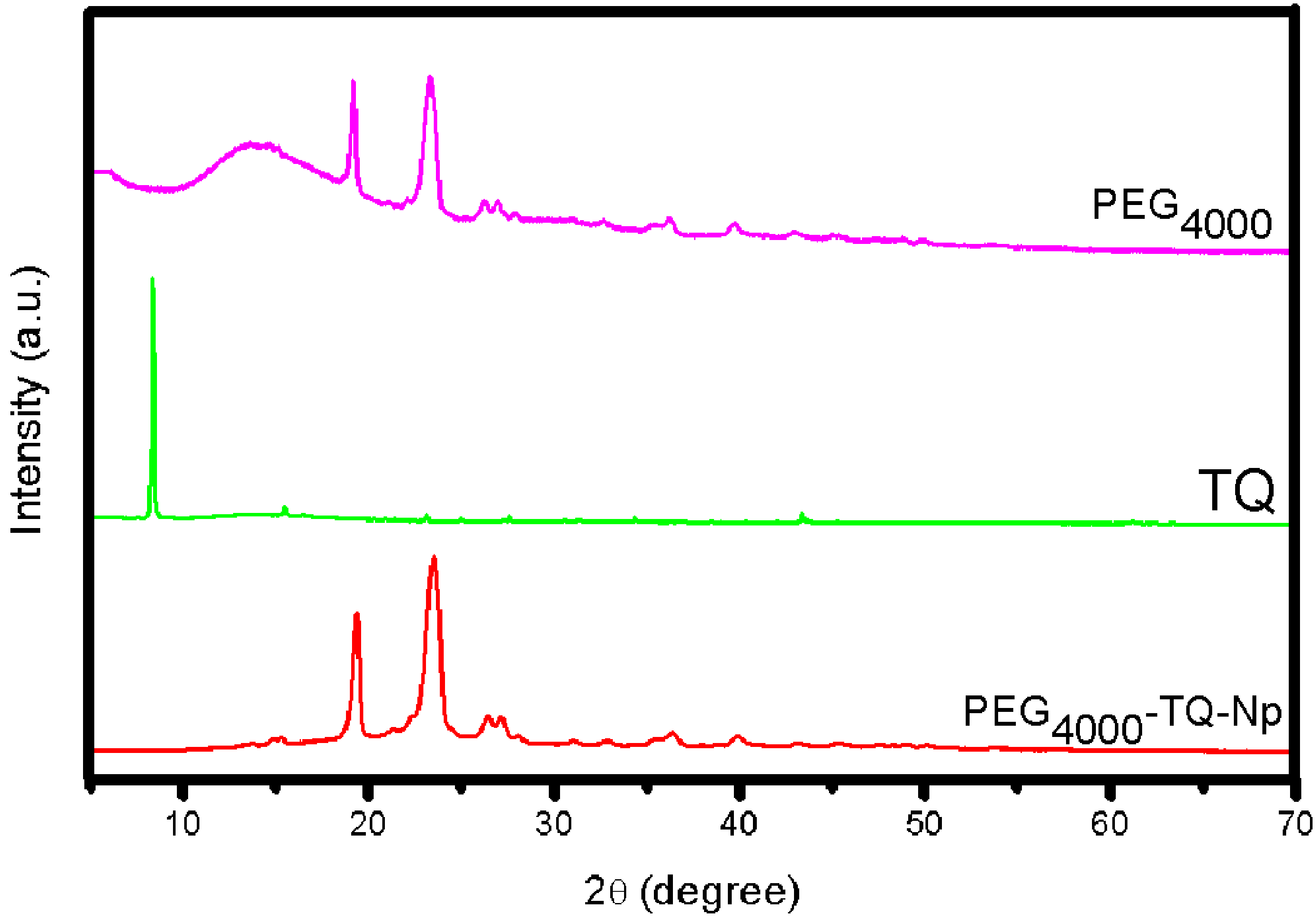
PEG_{4000}



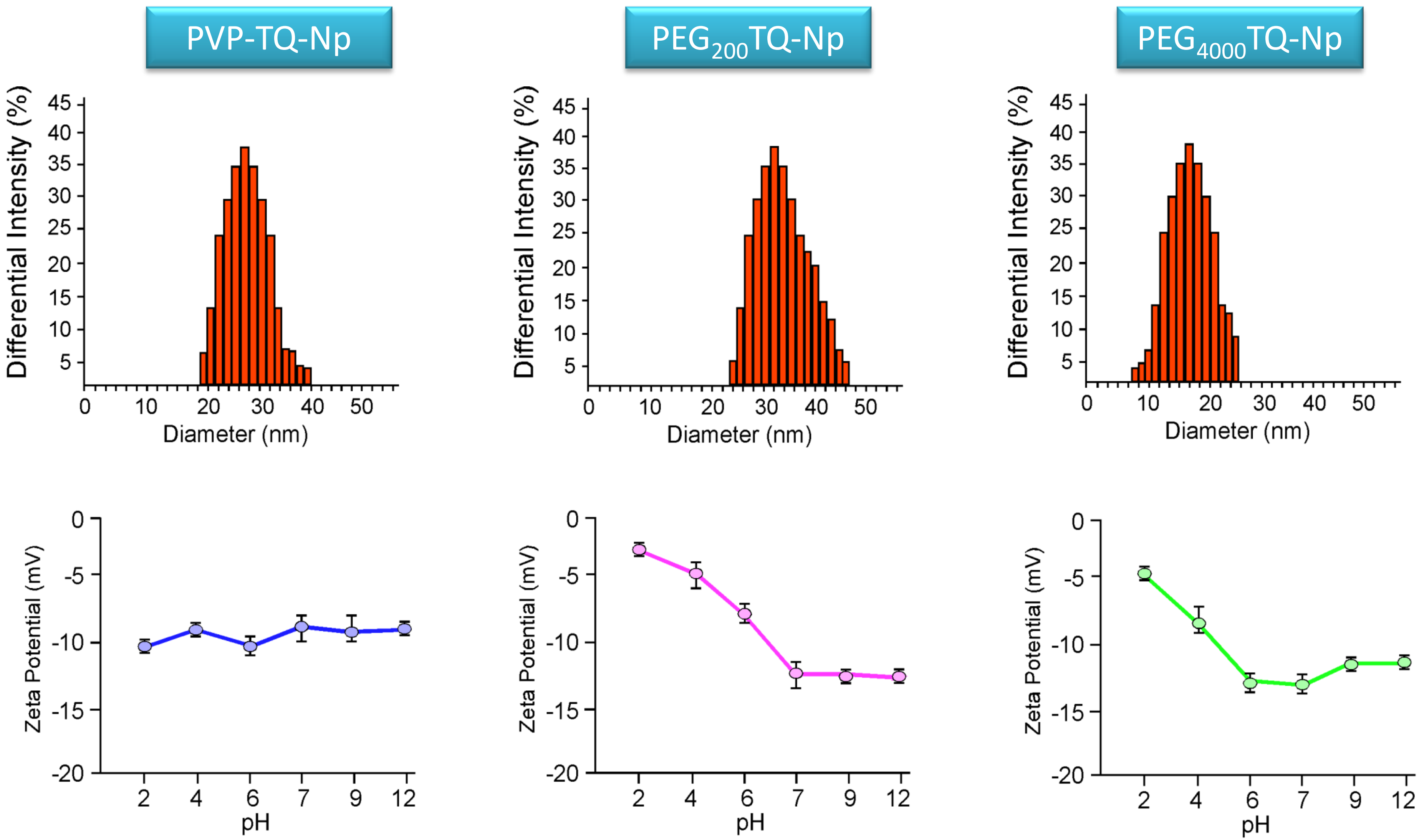
PEG_{4000}TQ-Np



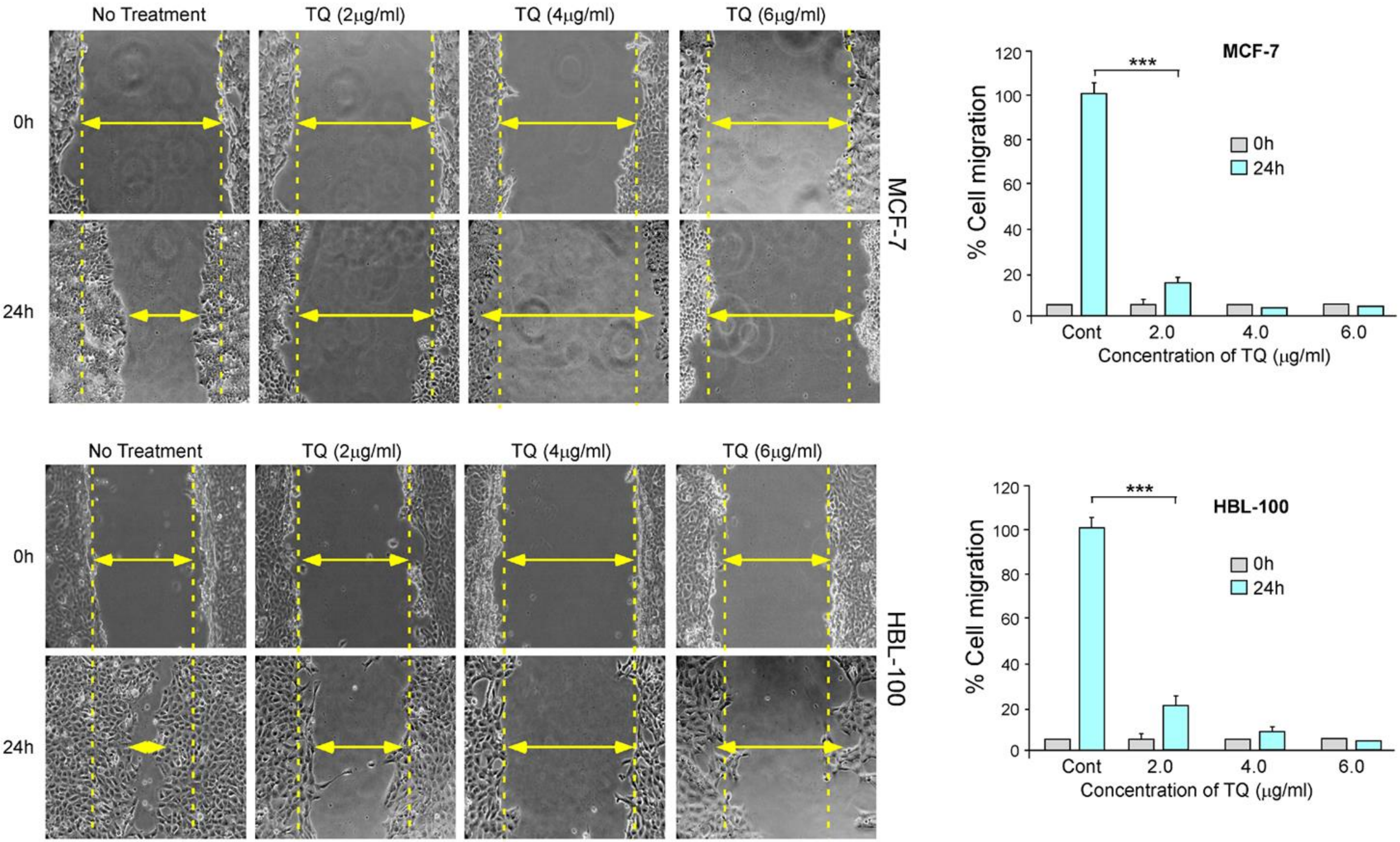
X-ray diffraction peaks



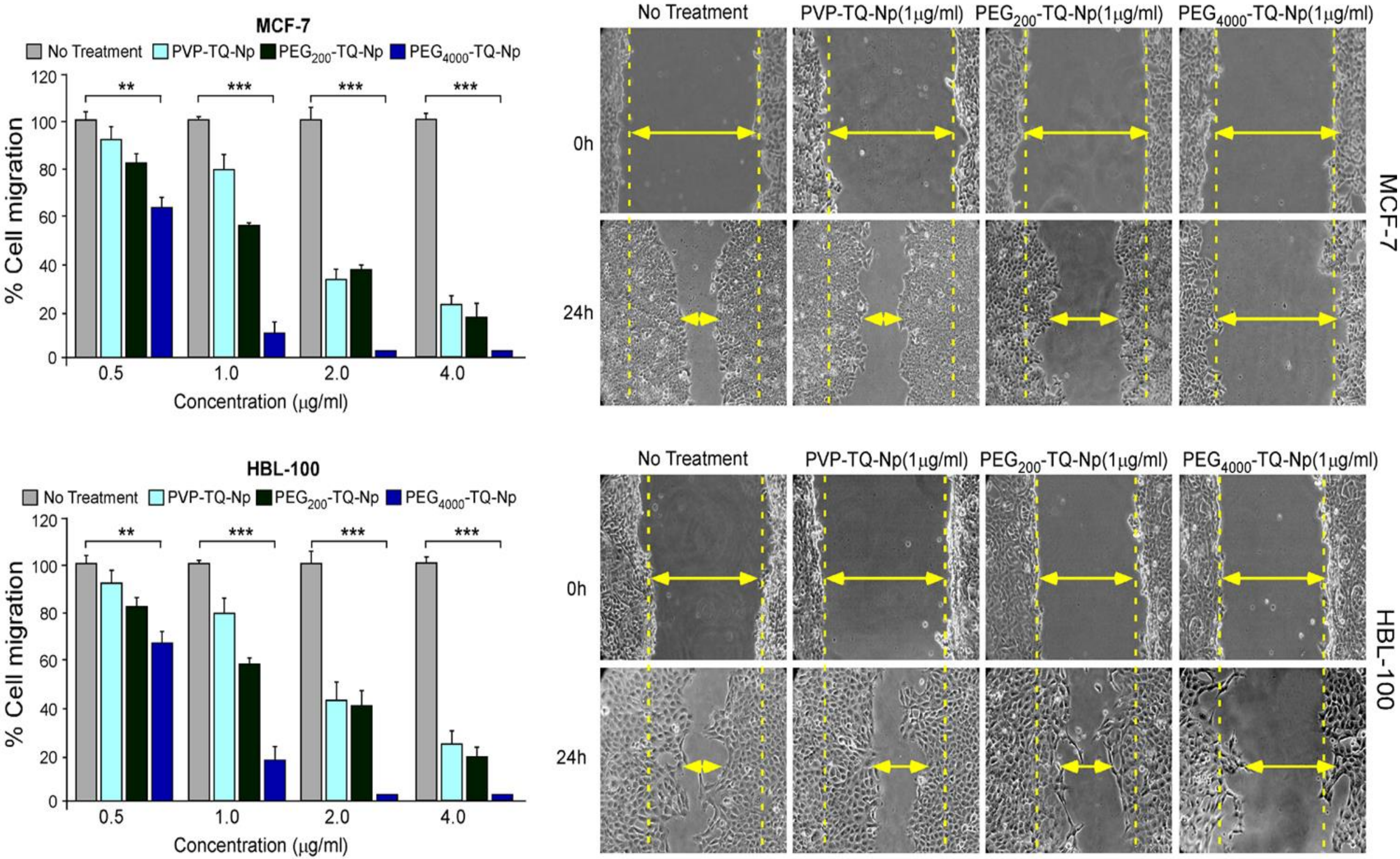
Size distribution and surface charge



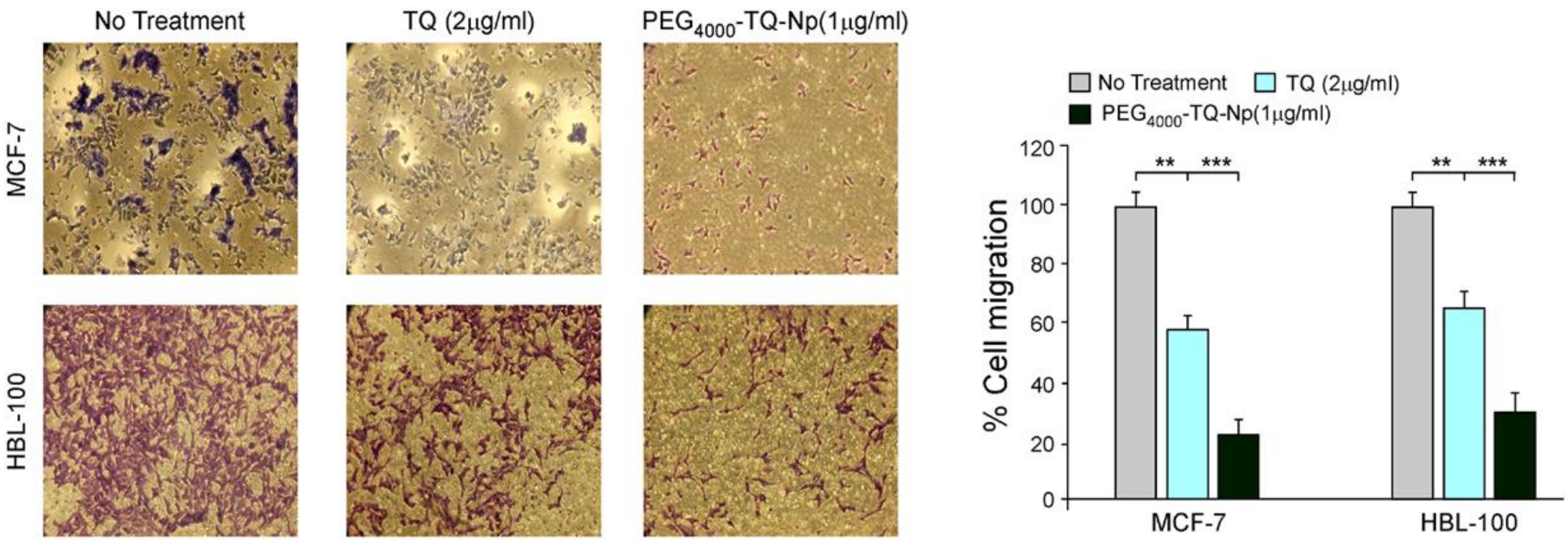
In vitro effect of TQ on retardation of breast cancer cell migration



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

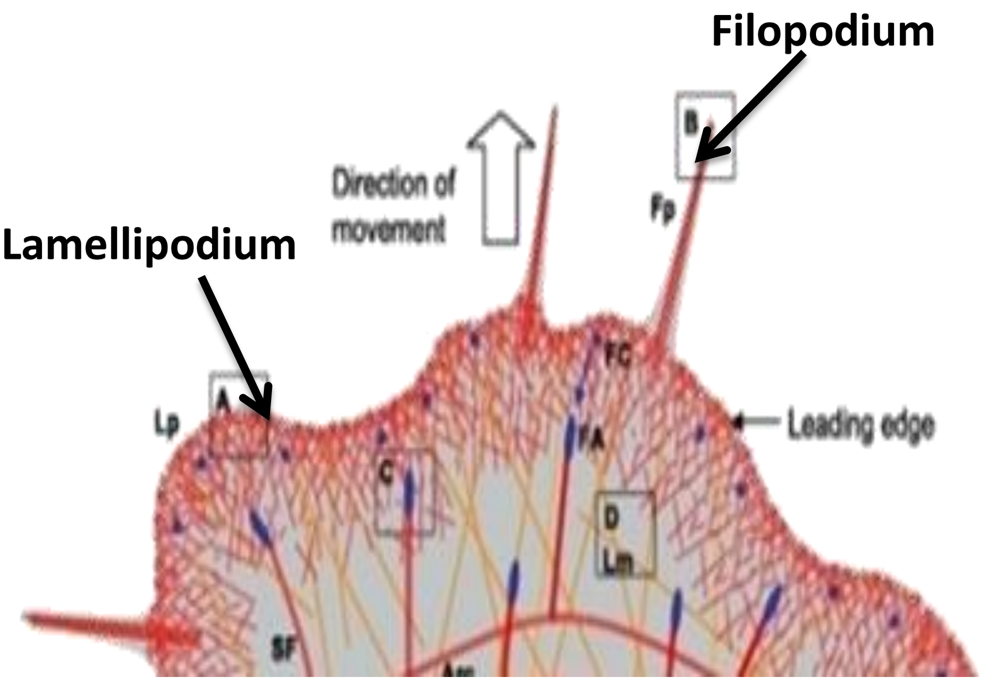


Effect of TQ and TQ-Nps on migration of breast cancer cells shown through transwell migartion assay



Lamellipodium formation during Cell migration

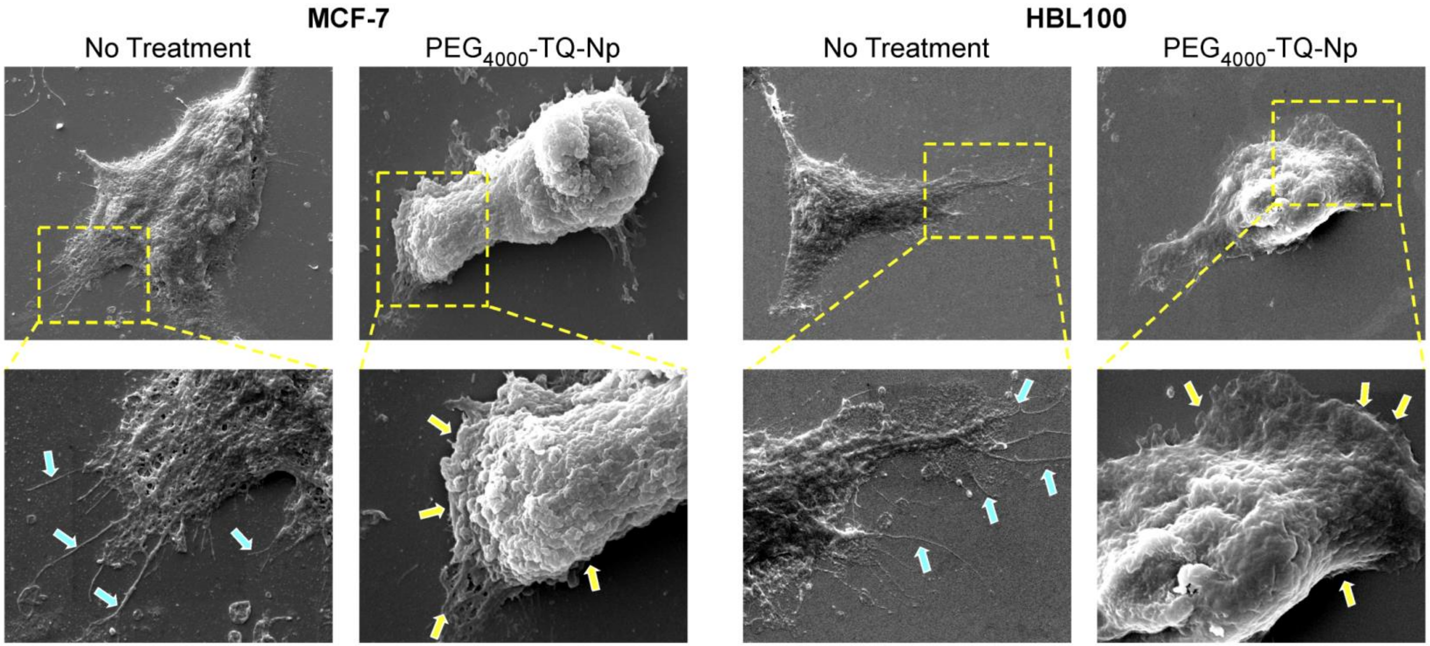
1. Extension of lamelipodium
2. formation of new focal adhesion at the leading edge
3. breaking of adhesions at the rear end
4. translocation of the cell mass



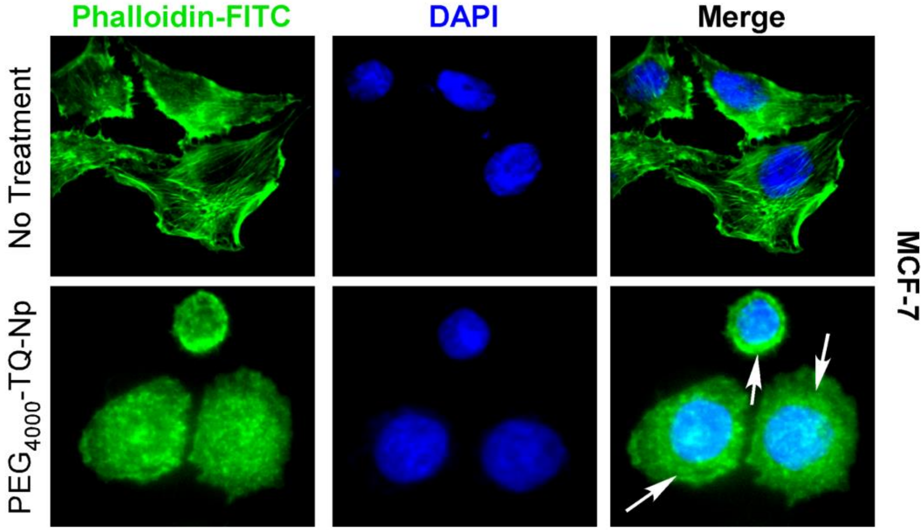
The lamellipodium is a broad, flat cell protrusion formed at the leading edge of migrating cells.

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

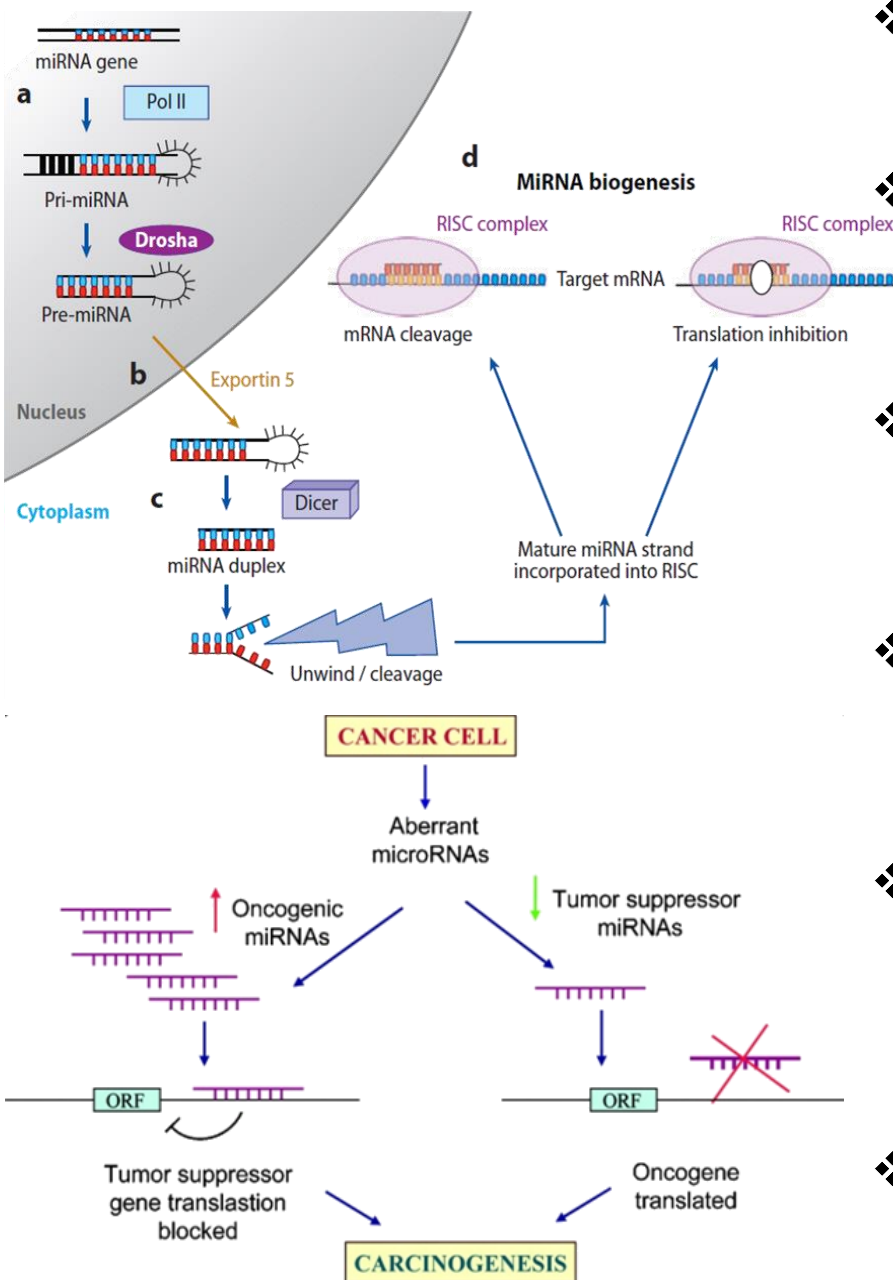
Effect of PEG4000TQ-Nps on the cellular protrusions



Modulation of rearrangement of actin cytoskeleton on treatment of Nps

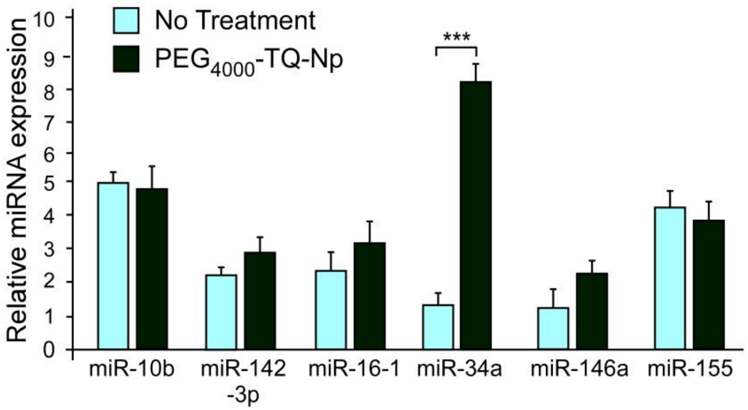


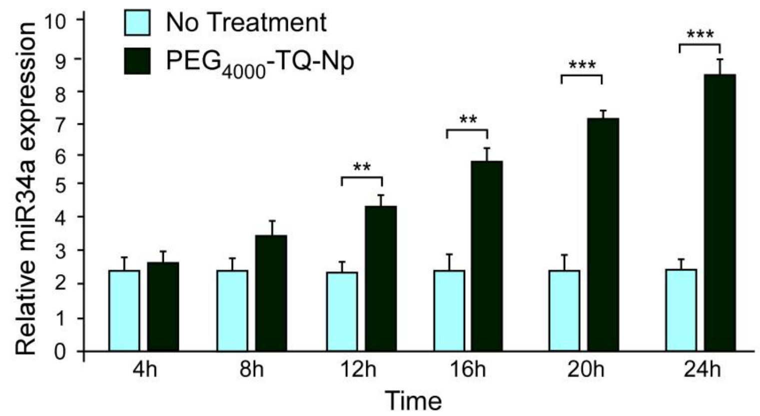
microRNA

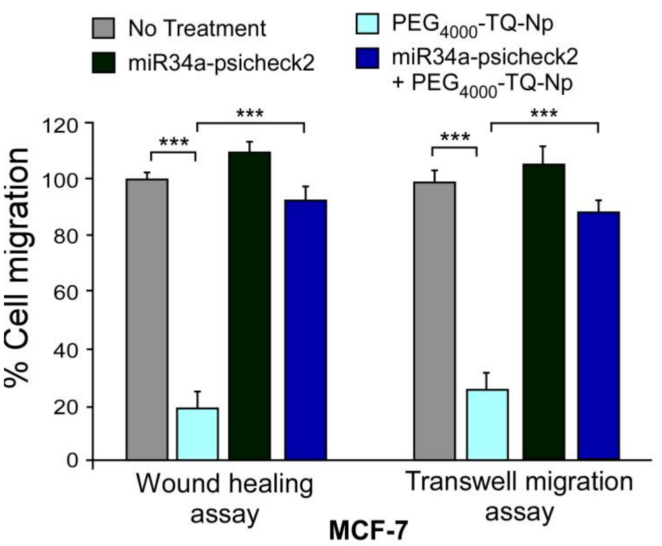


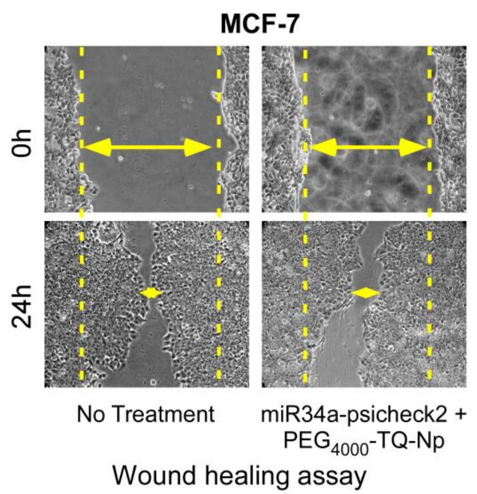
- small non-coding RNA molecule (ca. 22 nucleotides)
- found in plants, animals, and some viruses, which functions in transcriptional and post-transcriptional regulation of gene expression.
- miRNAs silence genes by base pairing with complementary mRNA sequence in 3'UTR of their targets.
- The human 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.

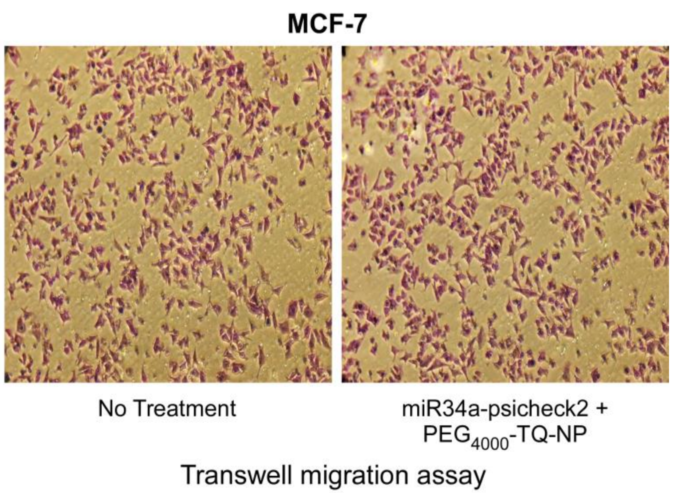
Effect of PEG4000TQ-Nps on miRNA expression profile



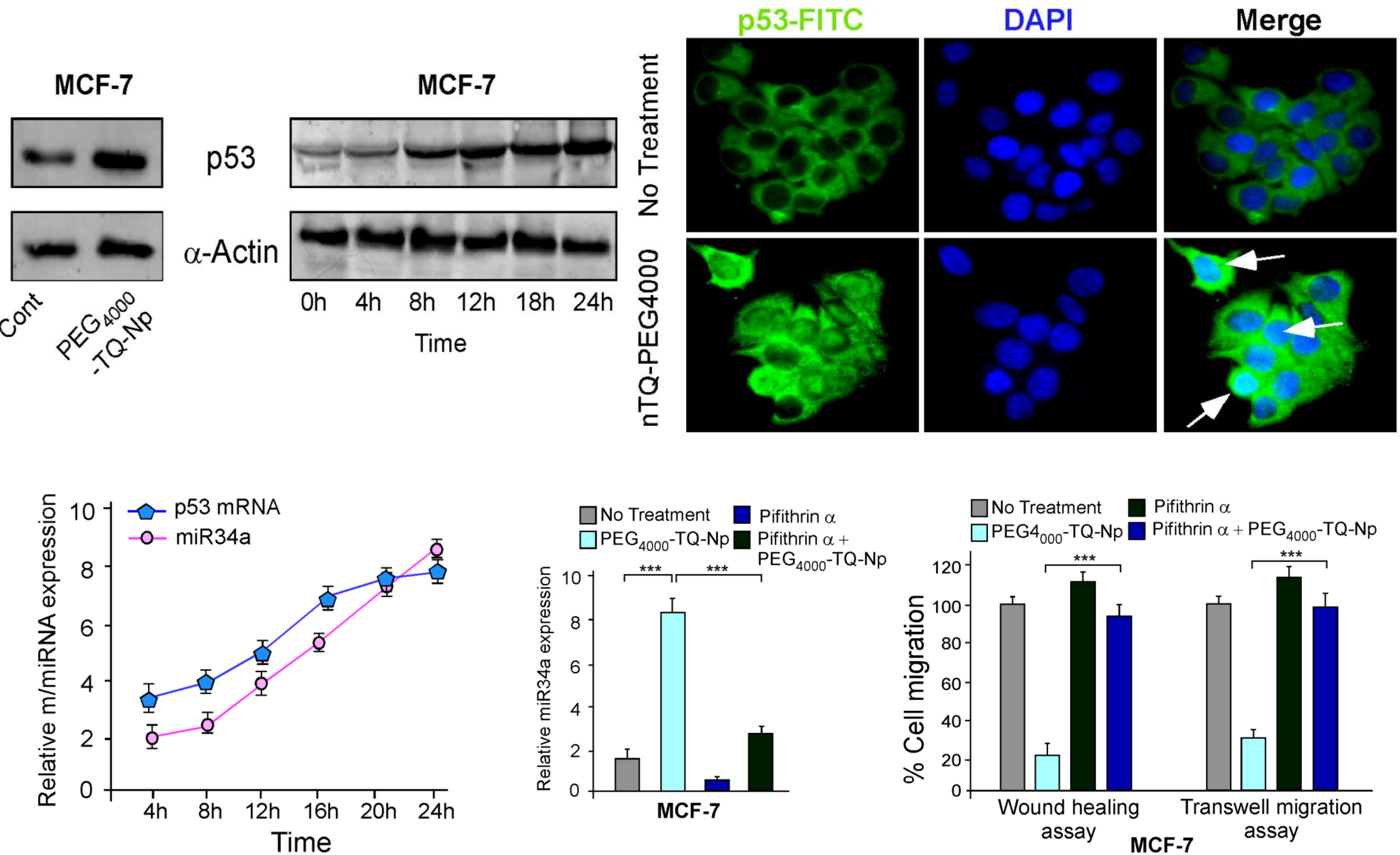




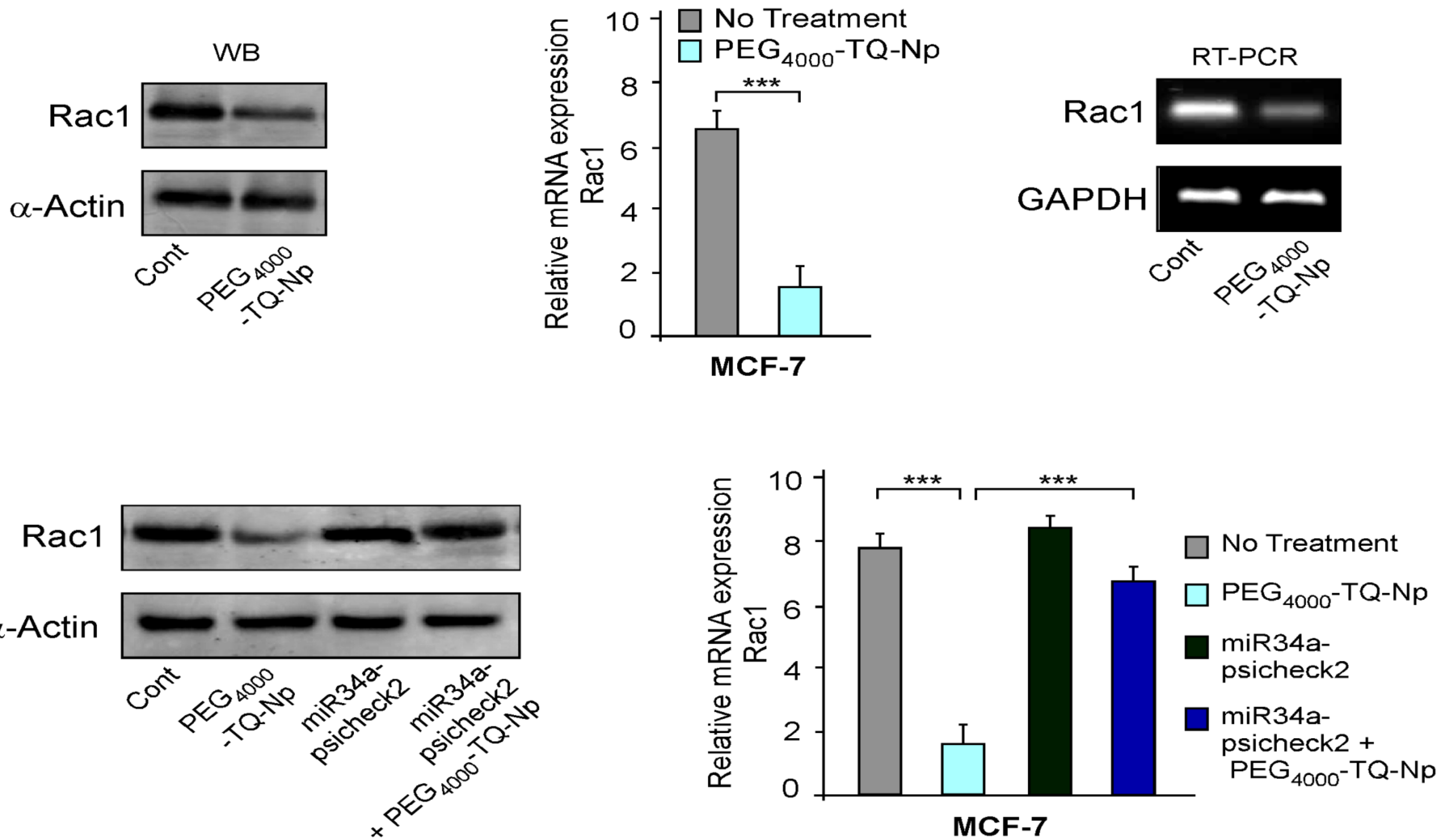




Effect of PEG4000-TQ-Np in regulation of miR-34a expression by p53

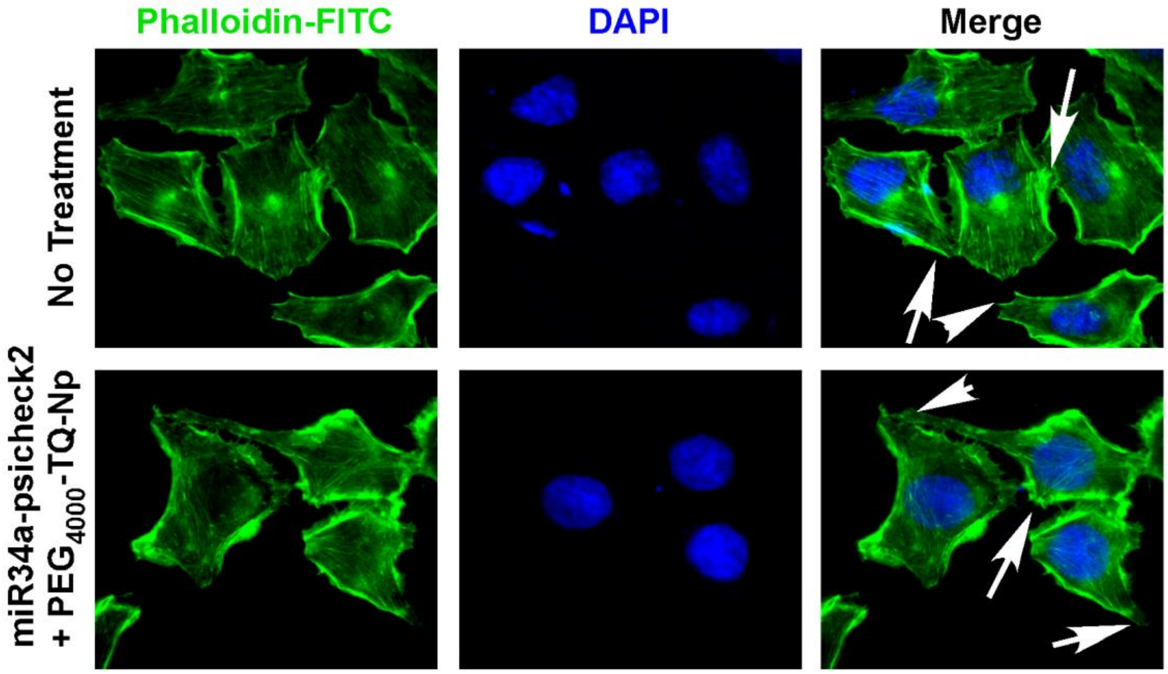


Effect of PEG4000TQ-Nps on Rac1

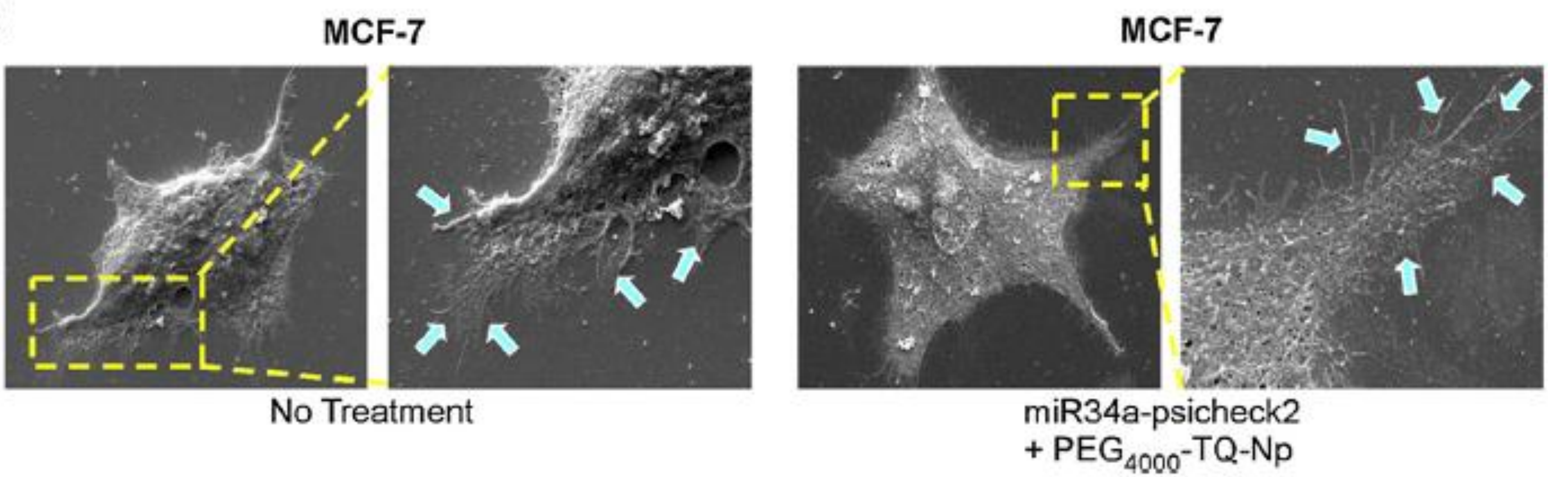


The results obtained significantly displayed that only TQ-Nps treated cells showed prominent down-regulation of Rac1 protein as well as mRNA whereas TQ-Np treatment in psi-CHECK2- AS34a transfected cells failed to down-regulate the expression of Rac1 protein as well as mRNA confirming the direct regulation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

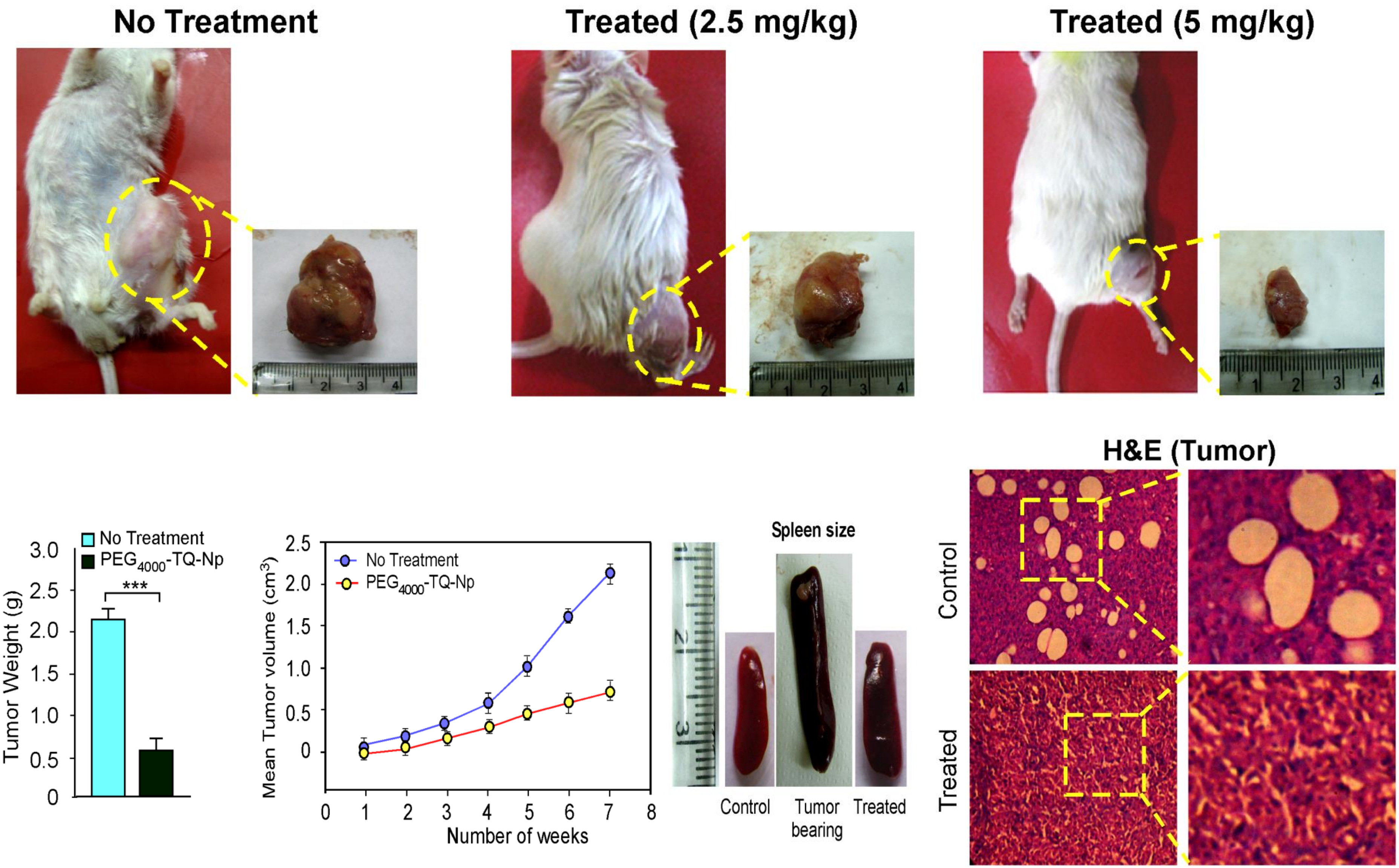
Overexpression of miR-34a disrupts actin filaments



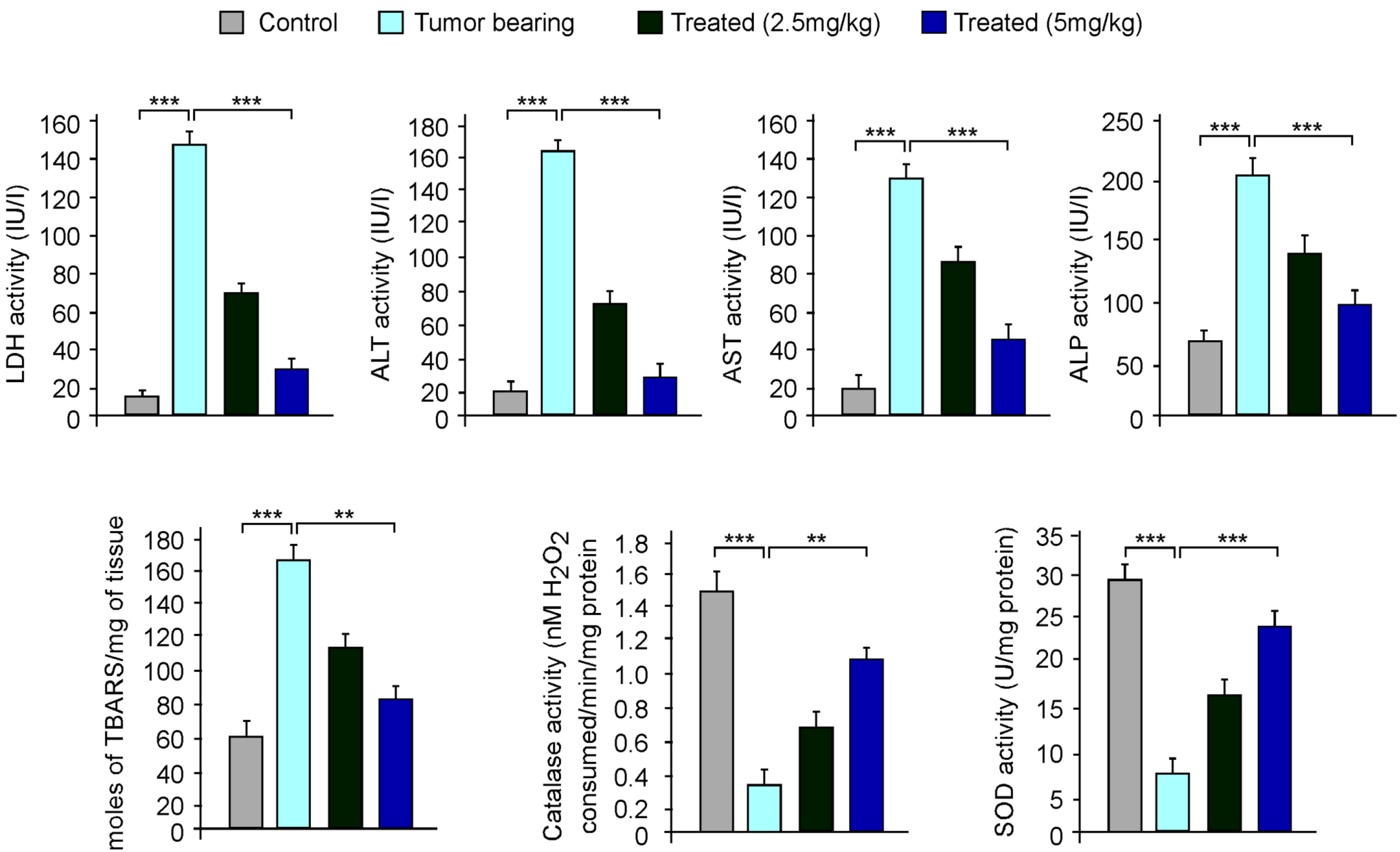
Lamelliopodia formation studied by SEM was found to be unaffected in, psi-CHECK2-AS34a transfected, treated with PEG4000- TQ-Nps cells in respect to the control



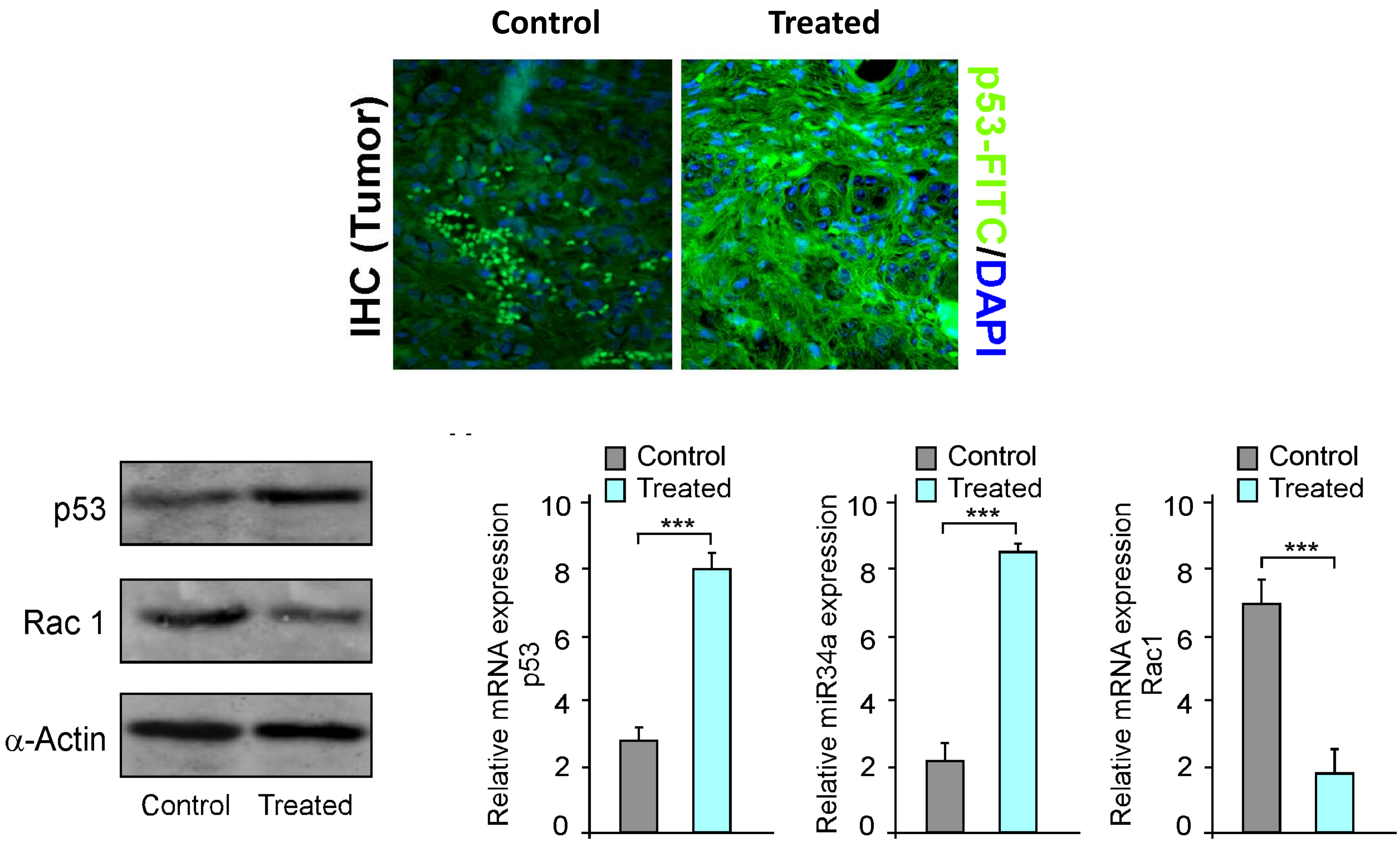
In vivo validation of the effect of PEG4000-TQ-Nps



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



Verification of molecular mechanism *in vivo*



Conclusion

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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

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Priyanka (JRF)

Mousumi (JRF)

Ayan (M.Tech trannie)

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