

# ALUMINIUM AND BREAST CANCER

## Aluminium und Brustkrebs

Dr. Philippa Darbre

University of Reading, UK



- ❖ Human body is exposed to aluminium from diet, vaccination adjuvant, personal care products
- ❖ As underarm antiperspirant, it is applied to the breast area
- ❖ This upper outer quadrant of the breast is the site of greatest cancer incidence
- ❖ Aluminium has been measured in several human breast structures
  - ❖ Human breast tissue
  - ❖ Milk
  - ❖ Nipple aspirate fluid
  - ❖ Breast cyst fluid



# RISK FACTORS FOR BREAST CANCER

## • GENETICS

- Female
- Loss of function of the BRCA1 / BRCA2 genes (loss of DNA repair)

## • DIET / OBESITY / ALCOHOL / RADIATION

## • HORMONAL EFFECTS (lifetime exposure to oestrogen)

- PHYSIOLOGICAL (age of puberty, menopause)
- CHILDBIRTH (age of first child, breastfeeding)
- PERSONAL DECISIONS (oral contraceptive, HRT)

## • OESTROGENIC CHEMICALS FROM THE ENVIRONMENT WHICH CAN ENTER THE HUMAN BREAST?

- POLLUTION OF DIET / WATER
  - Persistent organochlorine pollutants (POPs)
- POLLUTION OF THE DOMESTIC ENVIRONMENT
  - Polybrominated diphenylethers (flame retardants)
  - Phthalates, bisphenol A (plastics)
  - Alkyl phenols (detergents)
- DERMAL ABSORPTION OF COSMETIC CHEMICALS
  - Parabens, triclosan (preservatives, deodorant)
  - Cyclosiloxanes (conditioning, spreading)
  - UV screens (absorb UV light)
  - Polycyclic & nitro musks, Lilial, benzyl salicylate/benzoate (fragrance)
  - ALUMINIUM (ANTIPERSPIRANT) (metalloestrogen)



# THE CASE FOR AN INVOLVEMENT OF COSMETIC CHEMICALS IN BREAST CANCER

- **Exposure**
  - Applied to underarm and breast area
  - Left on skin allowing for continuous exposure
  - Antiperspirants, deodorants, body lotions, body creams, body sprays, moisturising creams, breast firming/enhancing creams, tanning creams, sun-care creams.
- **Chemical overload / individual susceptibility**
  - Used with increasing frequency & quantity
  - Used by ever younger children & babies
- **Molecular basis**
  - DNA damage
  - Cell growth - oestrogenic chemicals
  - Enable "Hallmarks of Cancer"
- **Chemical toxicity**
  - Long-term low dose exposure
  - Exposure to mixtures of chemicals
  - Absorption, metabolism, clearance
  - Consideration of timing of exposure

Darbre 2001 Eur J Cancer Prev 10, 389-393  
Darbre 2003 J Appl Toxicol 23, 89-95  
Darbre 2006 Best Pract Res Clin Endo Metab 20, 121-143  
Darbre 2009 Breast Cancer Research 11 (S3)  
Darbre 2010 Anticancer Research 30, 815-828



# EVIDENCE FOR AN INVOLVEMENT OF UNDERARM COSMETICS

- Proximity of application to the breast region
- Disproportionate incidence of breast cancer in the upper outer quadrant of the breast
  - Studies in the 1920-30s reported 31%, now over 50%
  - Disproportionate incidence is increasing linearly each year
    - England & Wales 47.9% in 1979 → 53.3% in 2006
    - Scotland 38.3% in 1980 → 57.0% in 2006
  - This is inconsistent with the disproportionality relating solely to more epithelial tissue in that region

Darbre & Charles. 2010. Anticancer Research 30, 815-828

- Genomic instability in outer breast quadrants
  - Tissues from breast quadrants of 21 patients
  - Assayed 26 chromosomal regions commonly deleted in breast cancer
  - 17 regions showed more genomic instability in outer regions

Ellsworth et al. 2004. Ann Surg Oncol 11, 861-868

- Two epidemiological studies with conflicting results

Mirick et al. 2002. JNCI 94, 1578-1580; McGrath. 2003. EJCP 12, 479-485



# THE CASE FOR AN INVOLVEMENT OF ALUMINIUM ANTIPERSPIRANT SALTS

- Aluminium salts are applied at high levels
  - Aluminium chlorohydrate 20% w/v
  - Aluminium zirconium chlorohydrate glycine complexes 25% w/v
  - Antiperspirant is left on skin allowing continuous exposure
  - Shaving may enhance entry to underlying tissues
- Aluminium chlorohydrate is absorbed through the skin
  - Using  $^{26}\text{Al}$  underarm in human subjects
    - (Flarend *et al*, Food Chem Toxicol 39, 163, 2001)
  - High plasma Al (4mM) by transdermal uptake from antiperspirant (Guillard *et al*, Am J Med 117, 956, 2004)
  - Aluminium absorbed through intact skin  $1.81\mu\text{g}/\text{cm}^2$  but more through stripped (shaved) skin  $11.5\mu\text{g}/\text{cm}^2$ . (Pineau *et al*, J Inorg Biochem 110, 21, 2012)
- During my lecture I want to make three main points:
  1. Aluminium has been measured in a range of human breast tissue structures
  2. Aluminium is a metalloestrogen and oestrogen is a risk factor for breast cancer development
  3. Aluminium can influence migration of breast cancer cells and tumour spread is the main reason for mortality from breast cancer



1.  
Aluminium has been measured  
in a range of human breast tissue structures

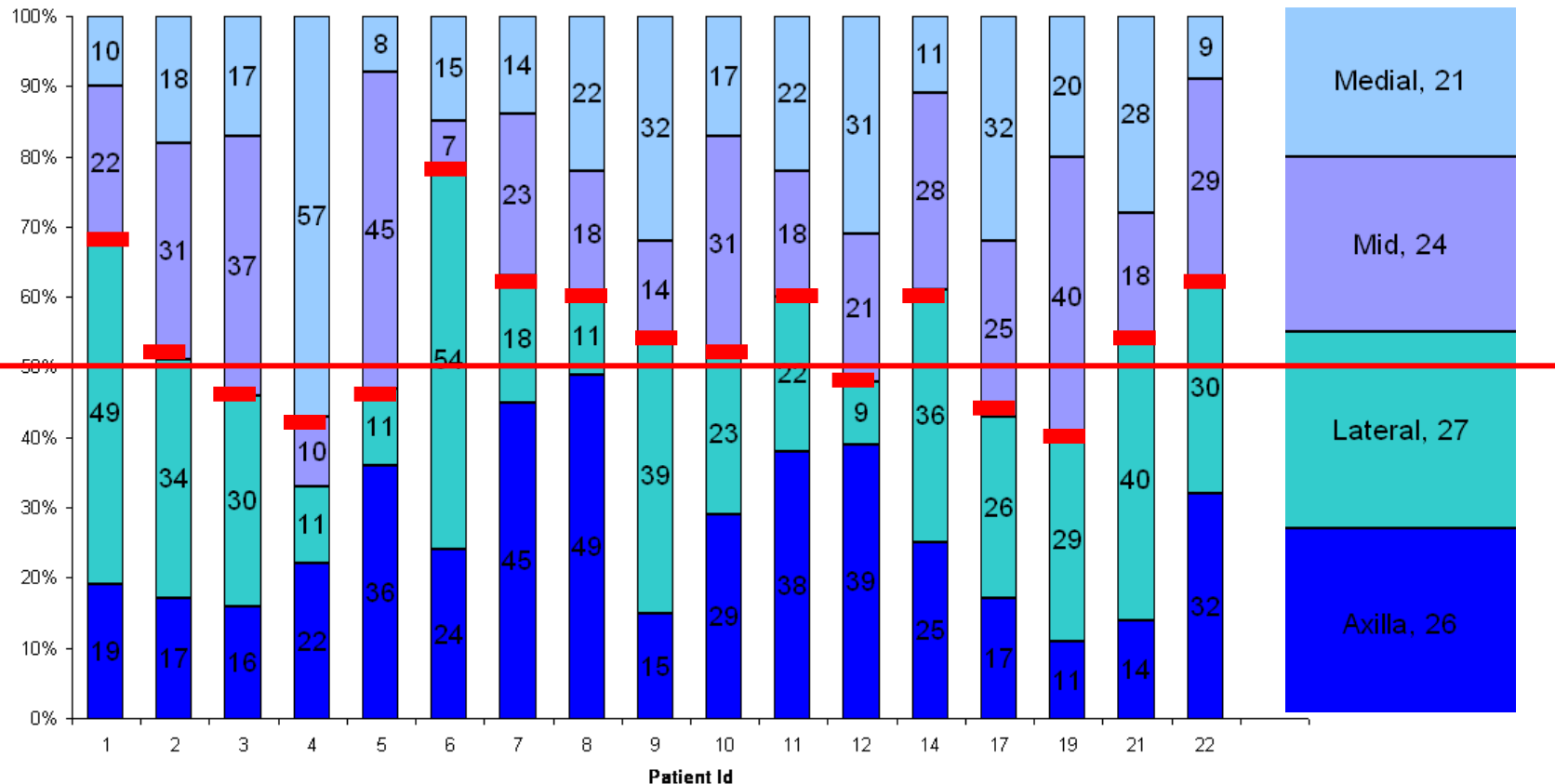




# ALUMINIUM CAN BE MEASURED IN HUMAN BREAST TISSUE

- VARIED FROM 4-437 nmol/gm DRY WT

Aluminium content of the outer regions (axilla+lateral) was higher than inner regions (mid+medial) ( $P=0.033$ )



# NIPPLE ASPIRATE FLUID

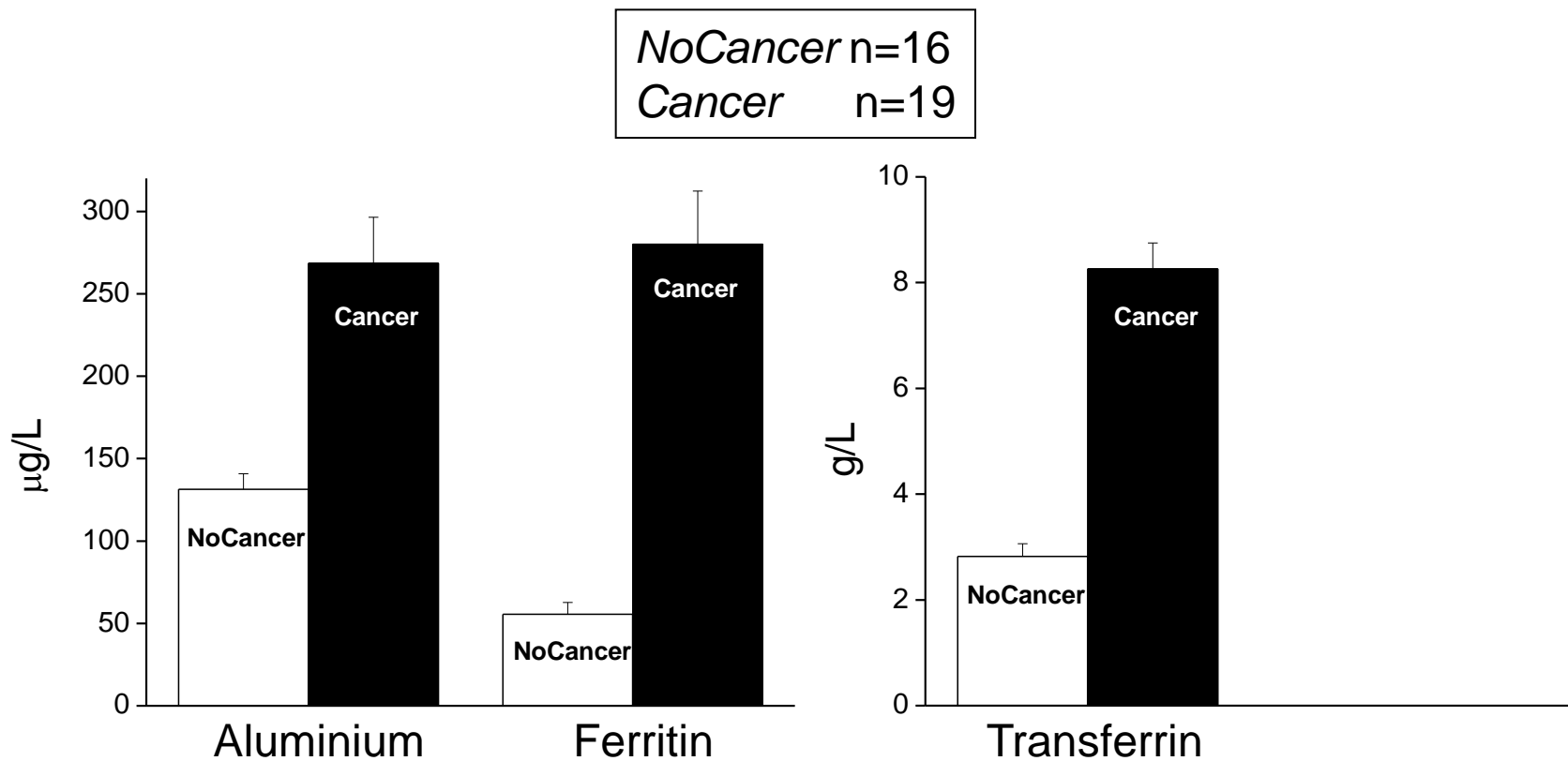
- Biological fluid secreted from ductal / lobular epithelial cells
- Reflects breast microenvironment
- Can be collected non-invasively through nipple aspiration
- Contains secreted proteins and cells shed from ductal and lobular epithelium
- Used to identify biomarkers in women at higher risk of developing breast cancer
  
- Aluminium measured in nipple aspirate fluids
  - from healthy women (*NoCancer*)
  - from women affected by breast cancer (*Cancer*)





# MEASUREMENT OF ALUMINIUM IN HUMAN NIPPLE ASPIRATE FLUID

Al 5.6	Ferritin 41.0	Transferrin 2.9	Serum(n=15)
Al 24.8	Ferritin 25.2	Transferrin 2.9	Milk (n=45)



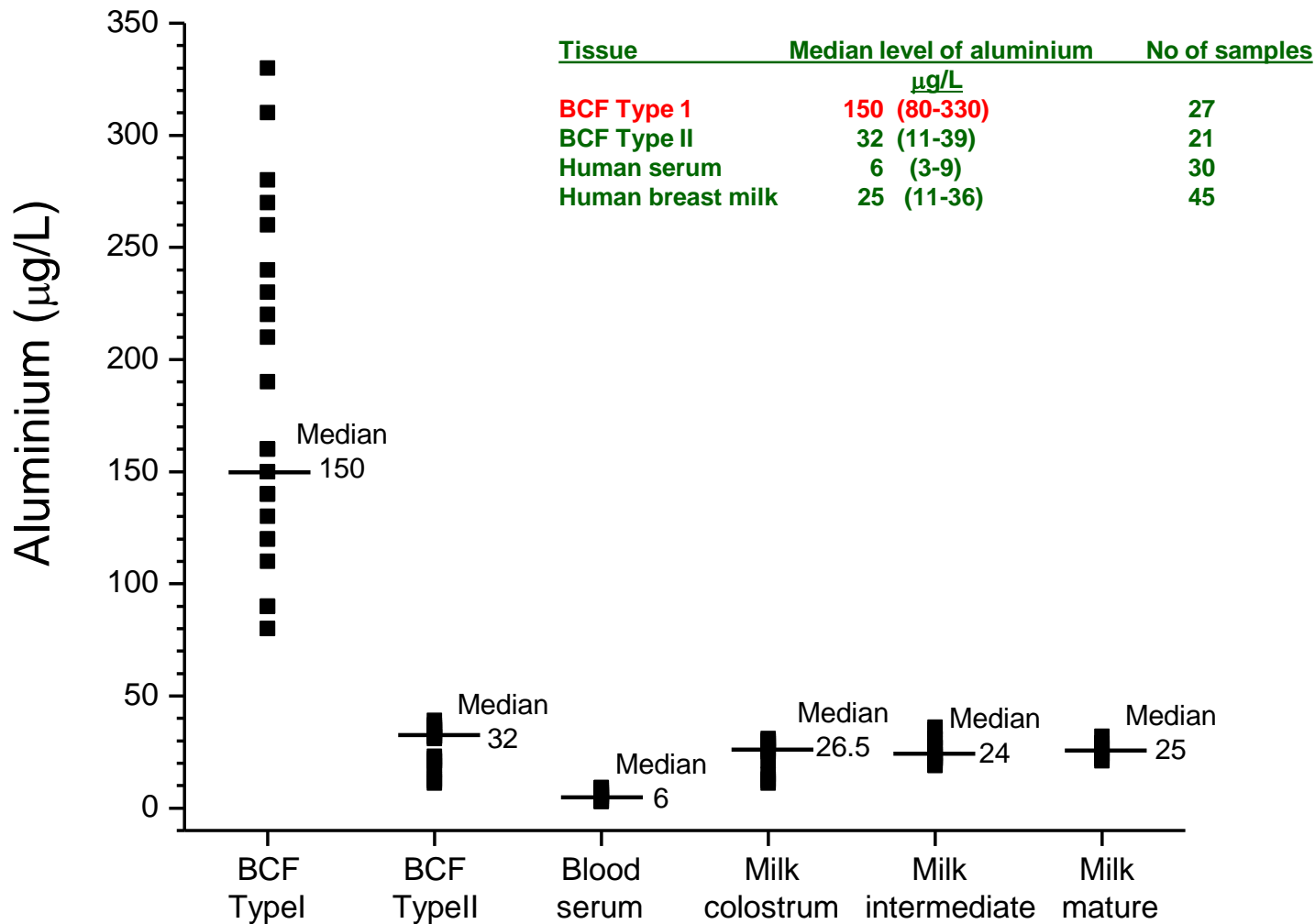
# ANTIPERSPIRANT USE AND BREAST CYSTS

## GROSS CYSTIC BREAST DISEASE

- Common benign breast disorder
- Can be associated with increased risk of breast cancer
- Greater incidence in the upper outer quadrant of the breast
- Breast cysts result from blocked breast ducts
- Antiperspirants designed to block sweat ducts of the axilla
- Aluminium measured in breast cyst fluids



# MEASUREMENT OF ALUMINIUM IN HUMAN BREAST CYST FLUID



# CONCLUSIONS 1.

- Aluminium measured in human breast tissue
  - May be higher in outer than inner regions
  - Widely distributed but patchy
- Aluminium measured in nipple aspirate fluid
  - Higher in *Cancer* NAF than *No-Cancer* NAF
  - Correlation between raised levels of aluminium and iron-binding proteins in *Cancer* NAF
- Aluminium measured in breast cyst fluid
  - Higher in Type1 BCF
- SOURCE OF ALUMINIUM CANNOT BE IDENTIFIED
- WHY IS THERE SO MUCH ALUMINIUM IN HUMAN BREAST STRUCTURES AND IS IT RELATED TO THE DISEASE STATES?



2.  
Aluminium is a metalloestrogen

and

Exposure to oestrogen is a risk factor  
for breast cancer



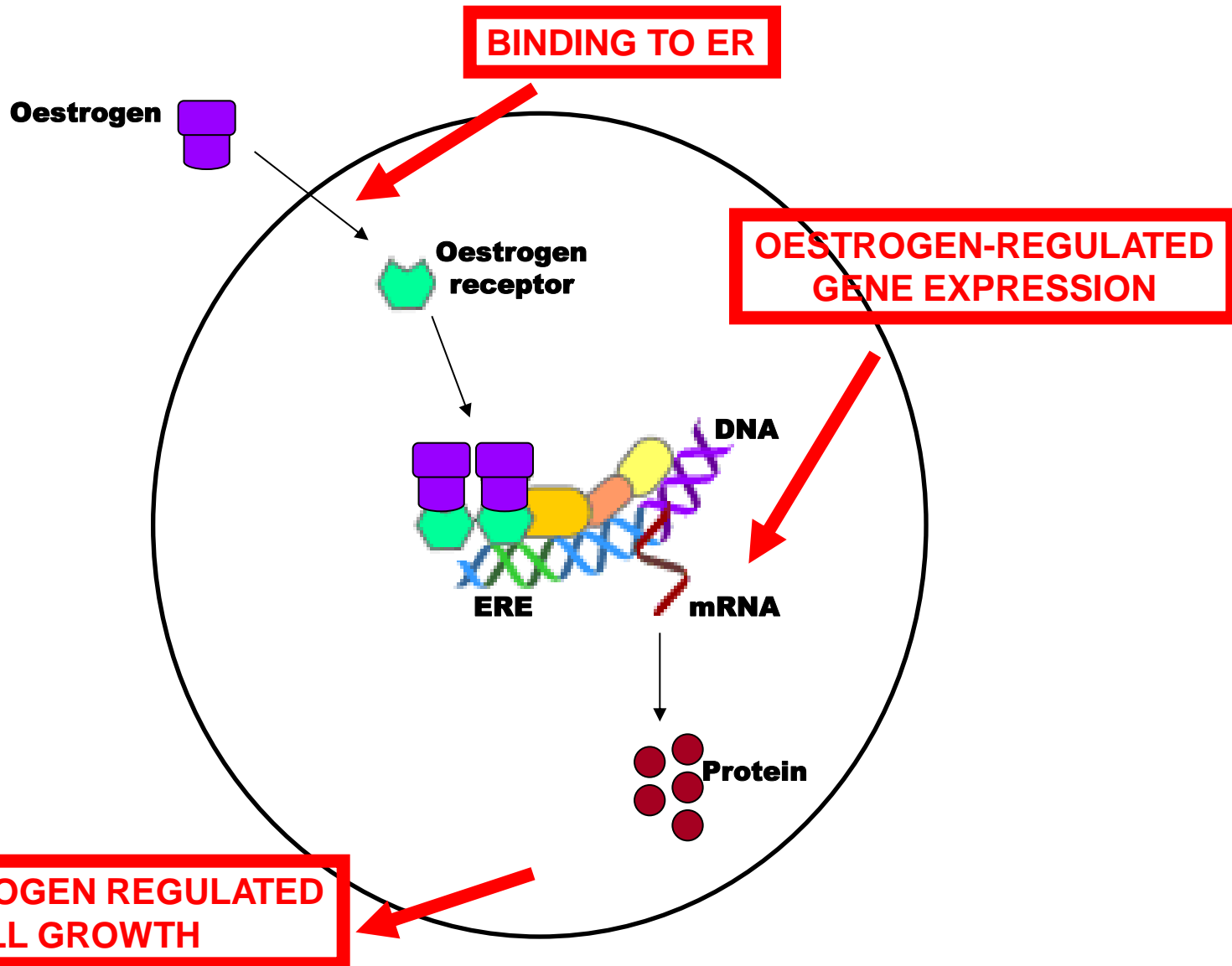
**OESTROGEN ACTS BY BINDING TO INTRACELLULAR RECEPTORS**  
- receptors function as ligand-activated transcription factors

Photograph of the structure  
of estrogen receptor  
deleted for copyright reasons.



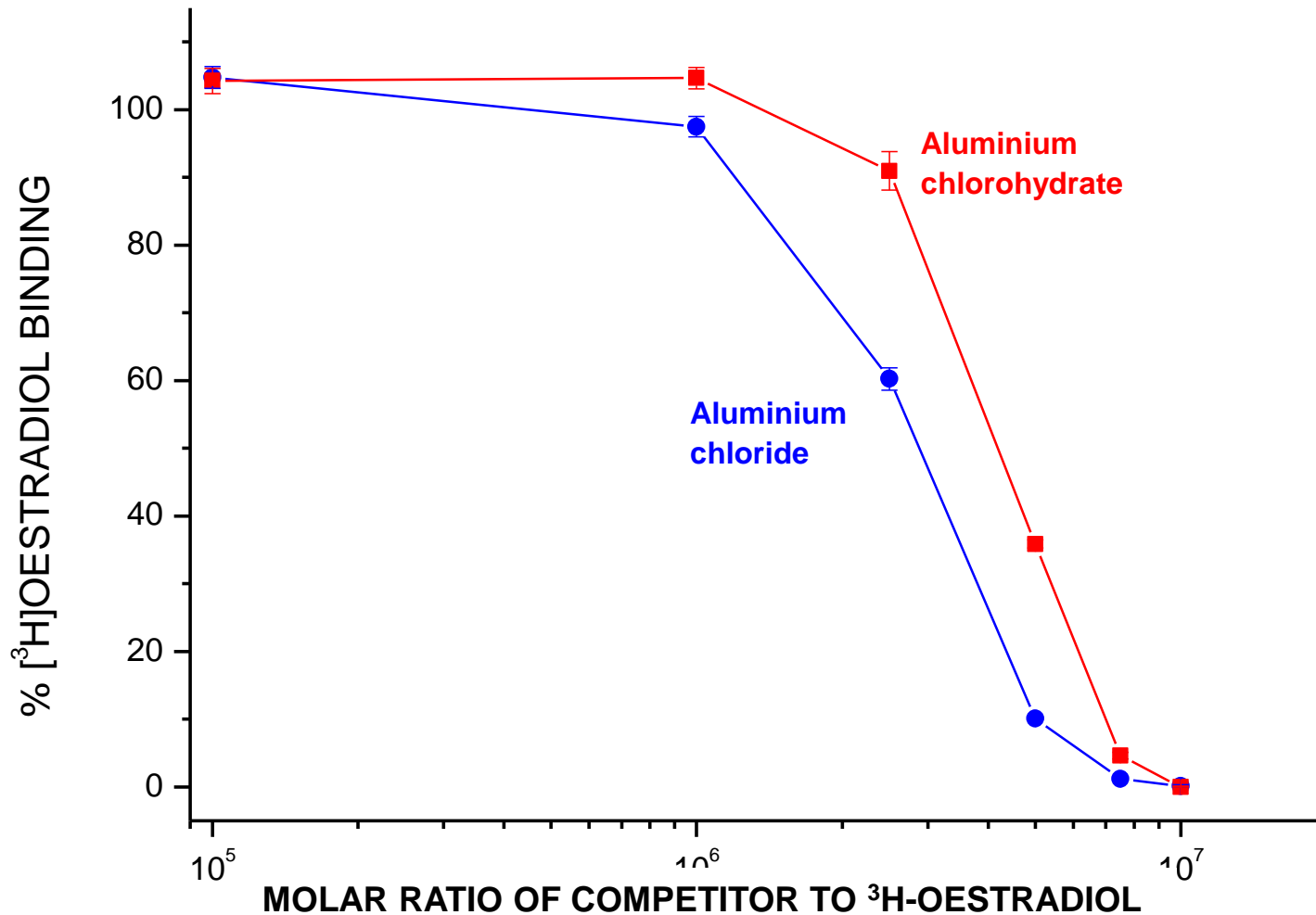


# HOW COULD ALUMINIUM INTERFERE IN THE OESTROGEN ACTION?

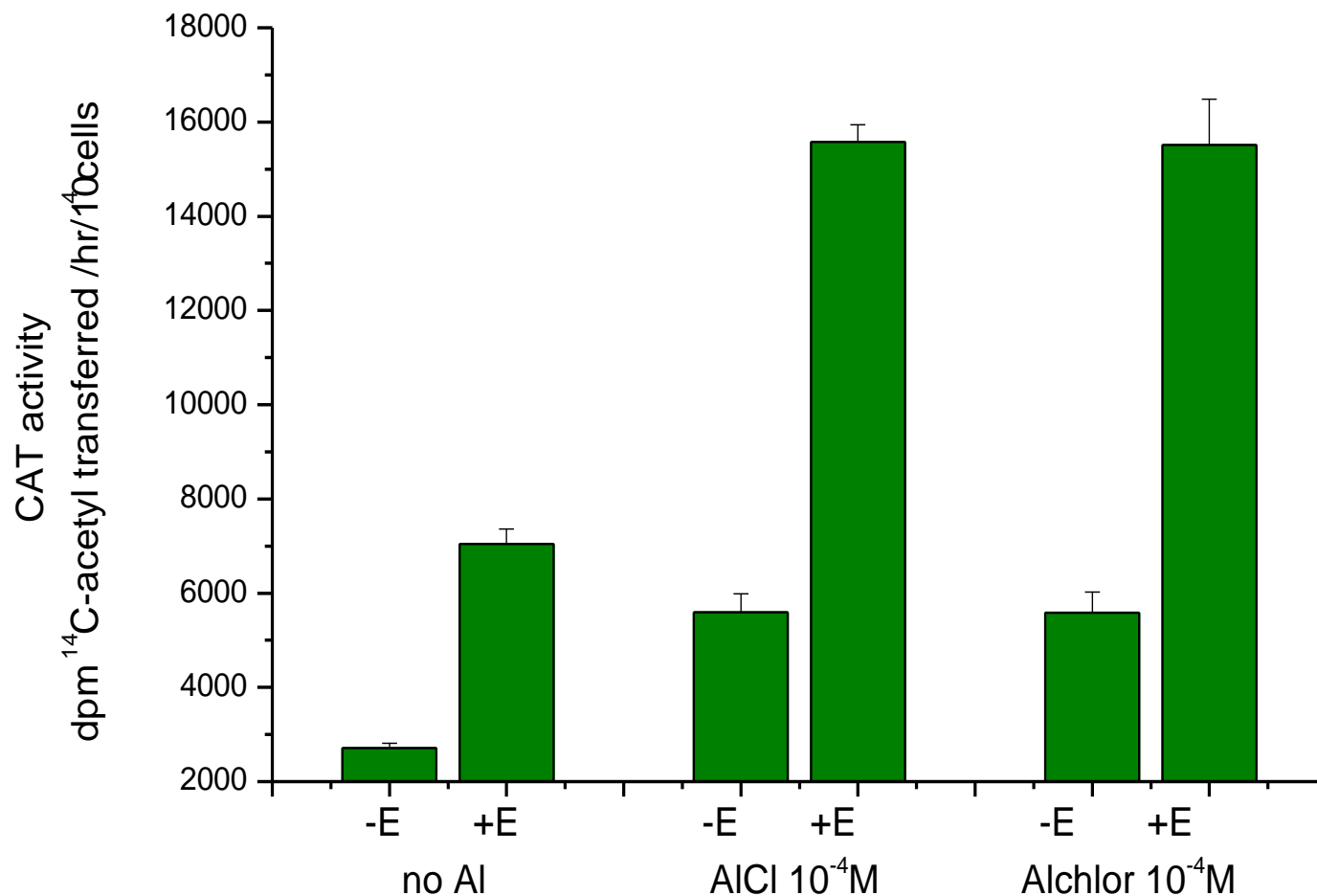


# Can aluminium chlorohydrate interfere with [<sup>3</sup>H]-oestradiol binding to oestrogen receptor of MCF7 human breast cancer cells?

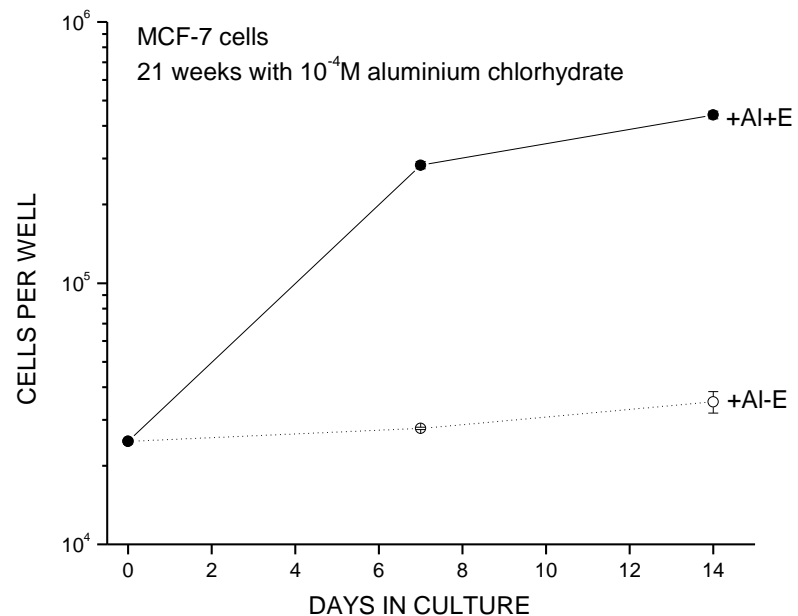
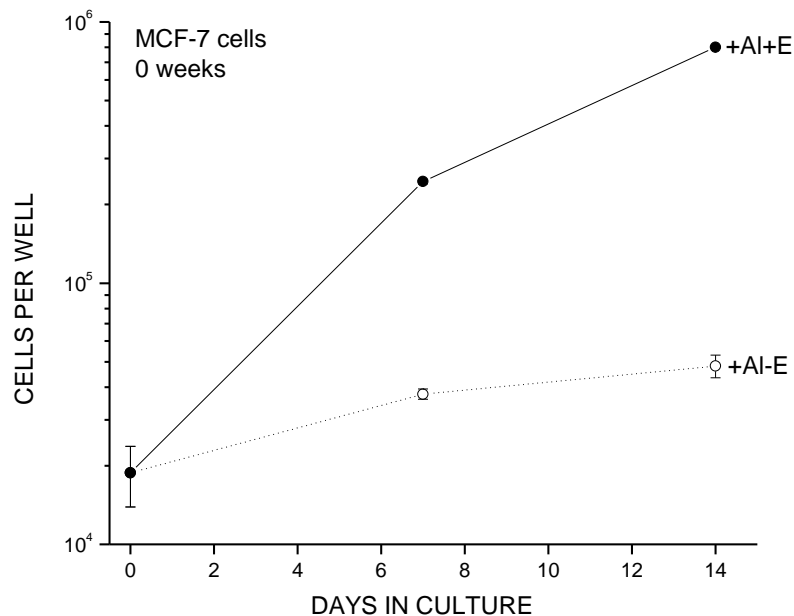
## -competitive binding assay



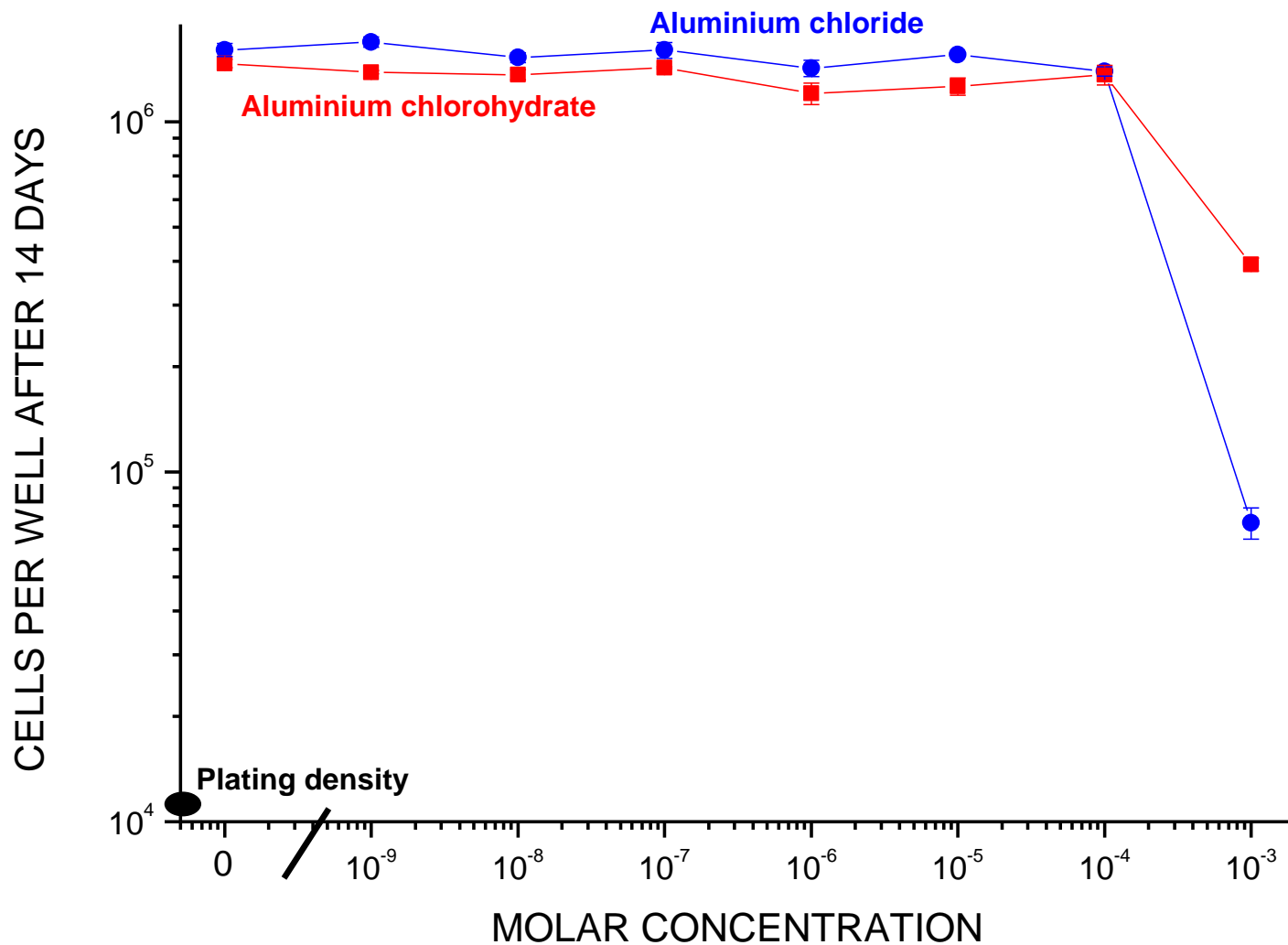
# Can aluminium chlorohydrate interfere with expression of an oestrogen-responsive reporter gene (ERE-CAT) in MCF7 human breast cancer cells?



# CAN ALUMINIUM CHLOROHYDRATE INTERFERE IN OESTROGEN REGULATION OF GROWTH OF MCF-7 HUMAN BREAST CANCER CELLS?



# Can aluminium chlorohydrate inhibit oestrogen-stimulated growth of MCF7 human breast cancer cells?



# Aluminium chloride induces anchorage-independent growth of MCF10A human non-transformed, immortalised breast epithelial cells

Photographs of results  
deleted for copyright reasons,  
please see reference

Sappino et al. 2012. J Appl. Toxicol. 32, 233.





## CONCLUSIONS 2

- Aluminium can displace  $^3\text{H}$ -oestradiol from oestrogen receptors
- Aluminium can increase expression of oestrogen regulated genes in the absence or presence of oestradiol
- Aluminium does not alter growth regulation of MCF-7 human breast cancer cells by oestradiol
- Aluminium does induce anchorage-independent growth of MCF10A human mammary epithelial cells



**3.**  
**Aluminium can influence migration and invasion  
of human breast cancer cells**



# COULD ALUMINIUM INFLUENCE THE EARLY PROCESSES OF METASTASIS WHERE CELLS BREAK AWAY FROM THE INITIAL TUMOUR MASS?

Diagrams of the processes of metastasis deleted for copyright reasons

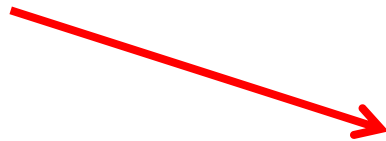
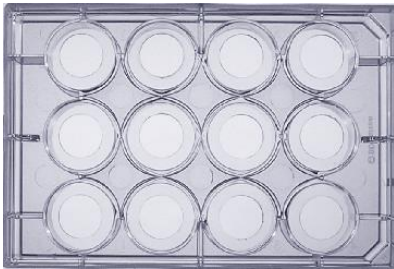
## Alterations to cell behaviour:

- Decreased adhesion
- Increased motility
- Increased migration
- Invasive properties



# MOTILITY OF CELLS CAN BE STUDIED USING TIME-LAPSE MICROSCOPY

- Cells are plated at low density in a 12-well culture dish
- Placed on an automatic stage in a 37°C/5%CO<sub>2</sub> chamber
- Photographs are taken of each well every 15 minutes for 24 hours
- Cells can be tracked using ImageJ software

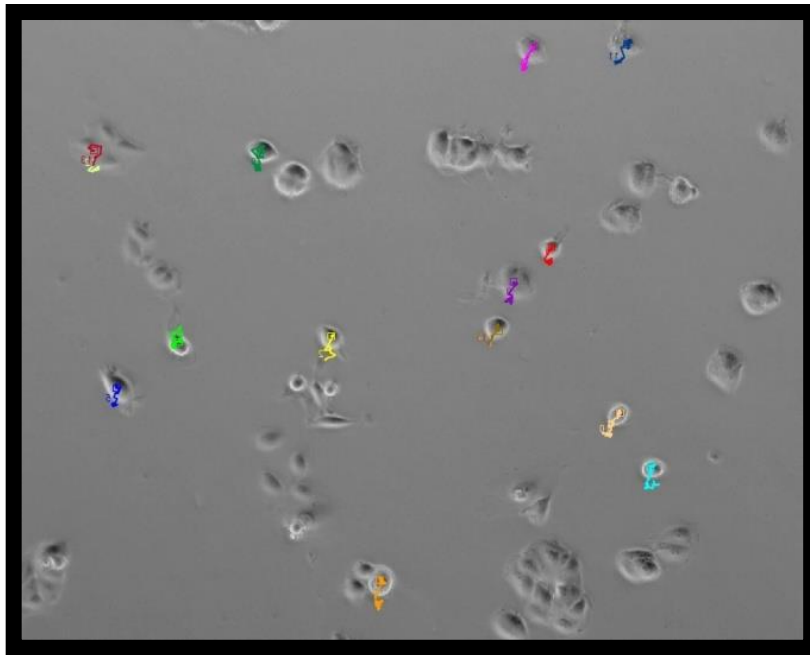


Picture of the microscope  
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copyright reasons

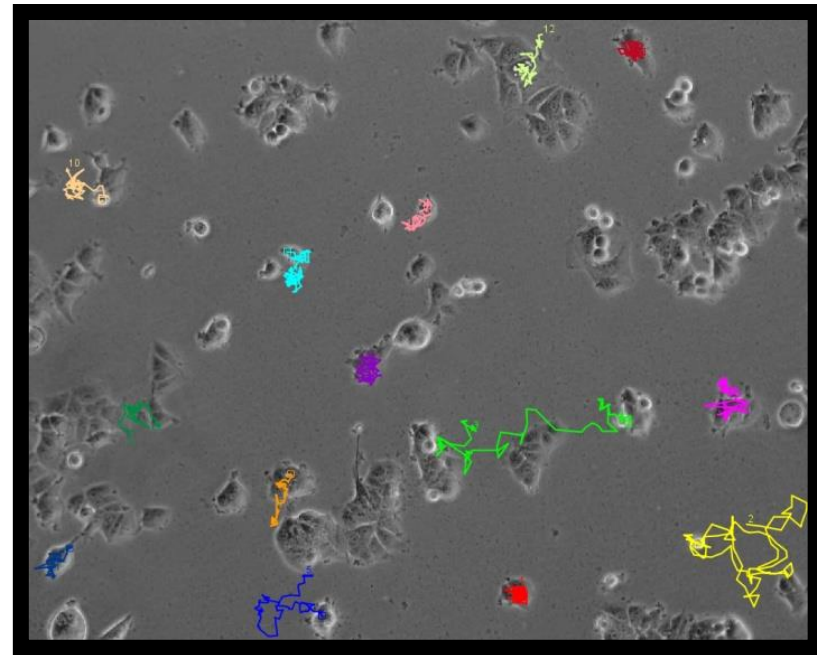


# USE OF TIME-LAPSE MICROSCOPY TO DETERMINE THE EFFECT OF ALUMINIUM ON MOTILITY OF MCF-7 HUMAN BREAST CANCER CELLS

Pictures show the final of 96 time points.  
Coloured lines show the movement from the first point.



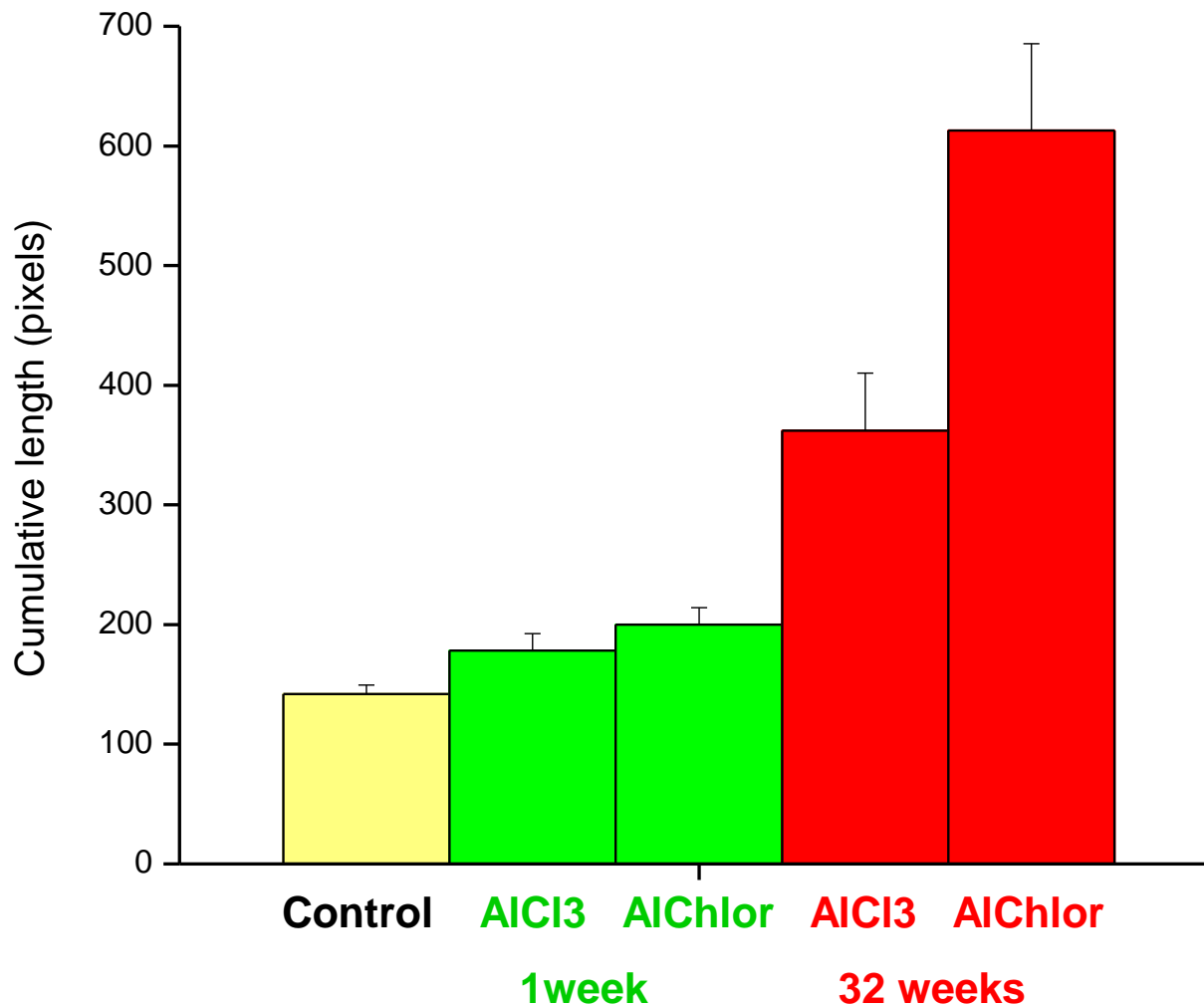
**Control**



**Al Chlorohydrate 32 weeks**

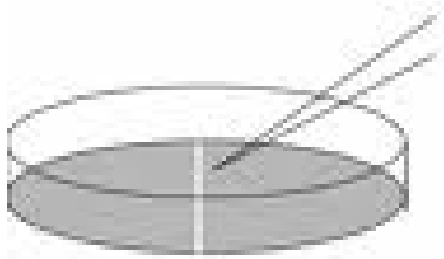


# EFFECT OF ALUMINIUM ON MOTILITY OF MCF-7 HUMAN BREAST CANCER CELLS





# MOTILITY OF CELLS CAN BE STUDIED USING A WOUND-HEALING OR SCRATCH ASSAY



**Picture of the microscope  
deleted for  
copyright reasons**



0hr



24hr

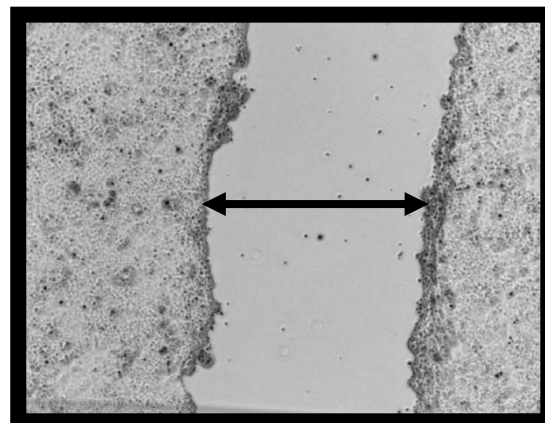
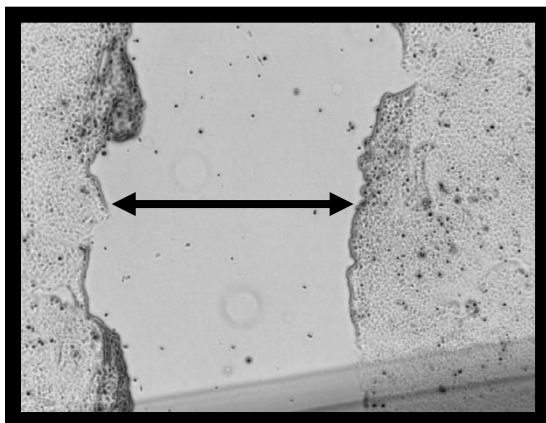


48hr

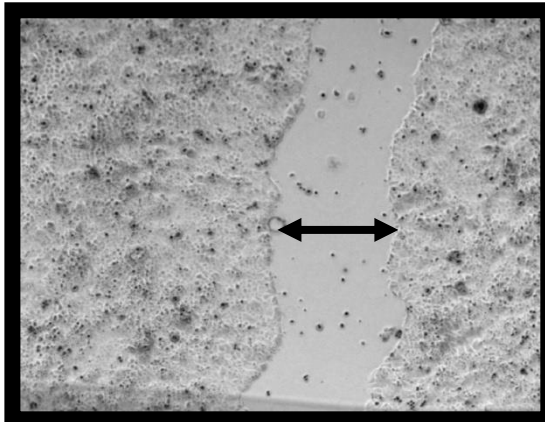
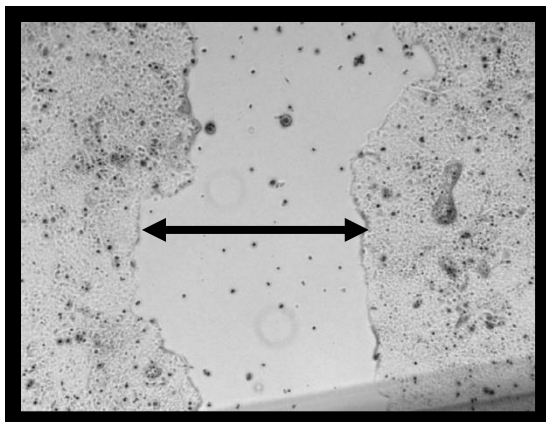


# USE OF A WOUND HEALING ASSAY TO DETERMINE THE EFFECT OF ALUMINIUM ON MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS

T=0



T=21hr

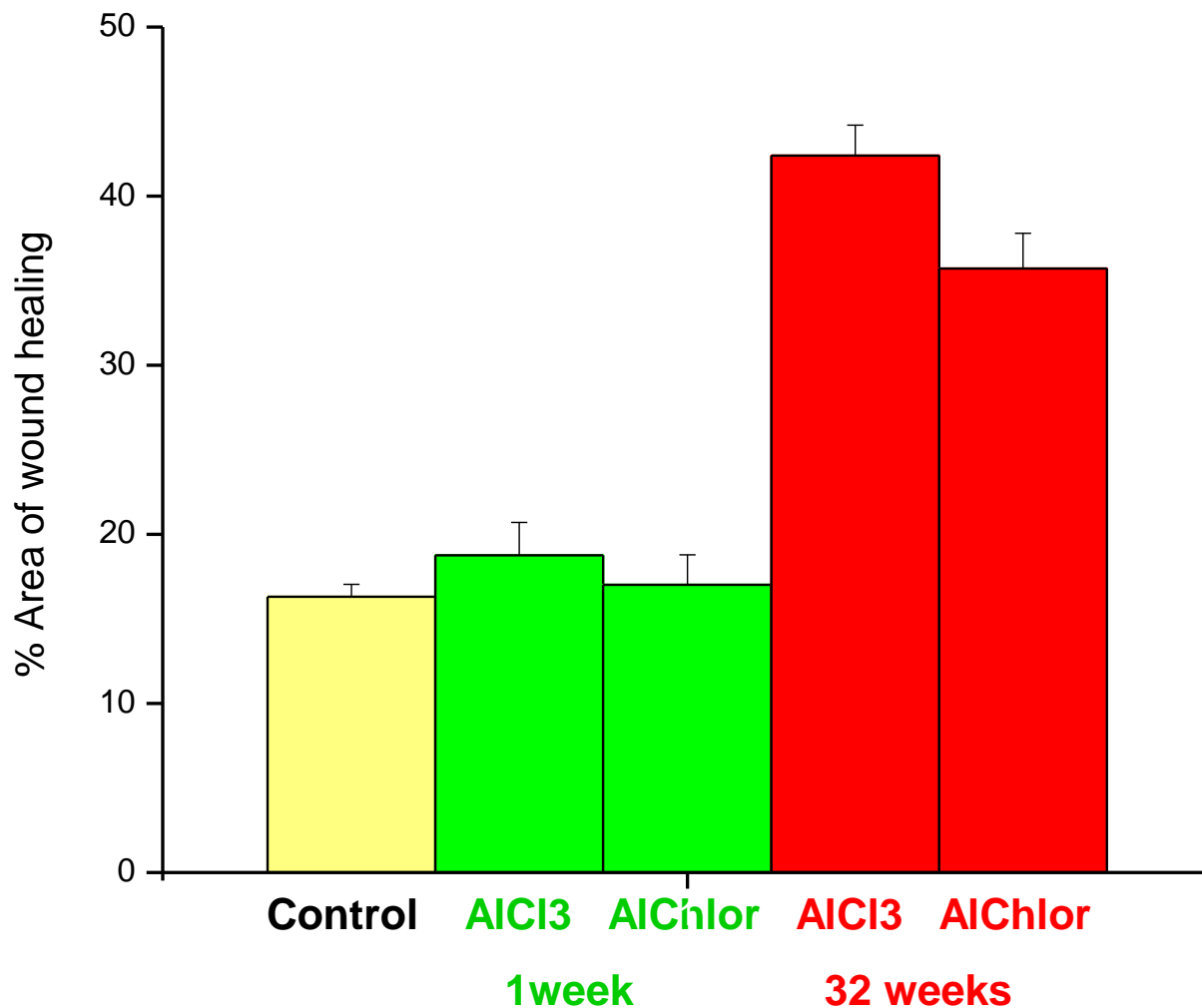


Control

AlCl<sub>3</sub> 32 weeks

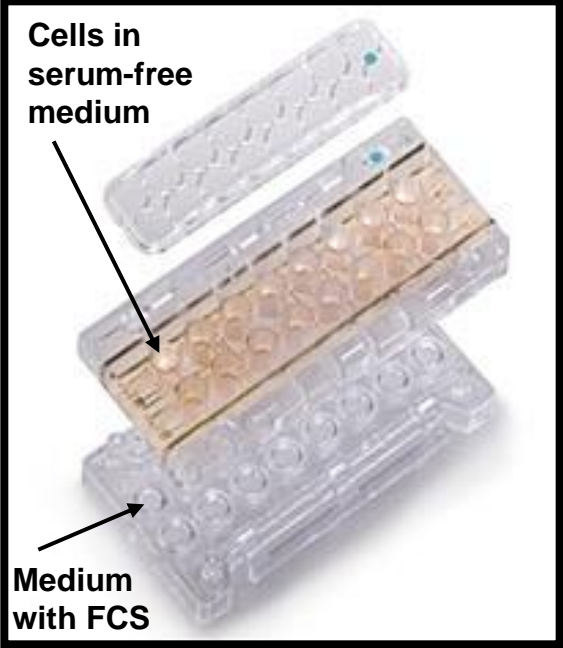


# EFFECT OF ALUMINIUM ON MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS



# USE OF XCELLIGENCE TECHNOLOGY TO DETERMINE THE EFFECT OF ALUMINIUM ON MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS

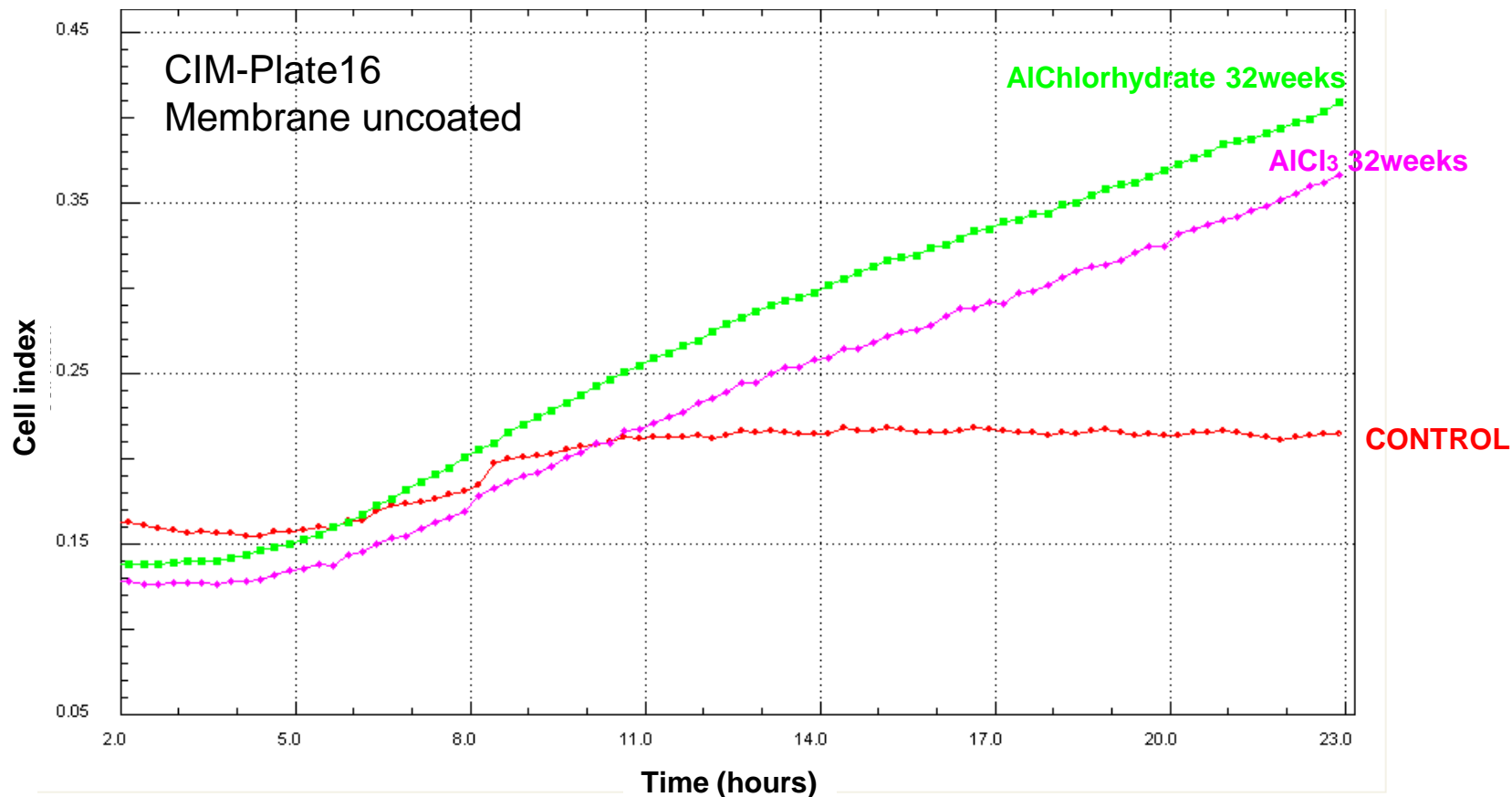
Picture of the machine deleted for copyright reasons



Cells pass through 8 $\mu$ m pores by chemotaxis and are detected on gold electrodes on the underside of the membrane



# EFFECT OF ALUMINIUM ON MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS





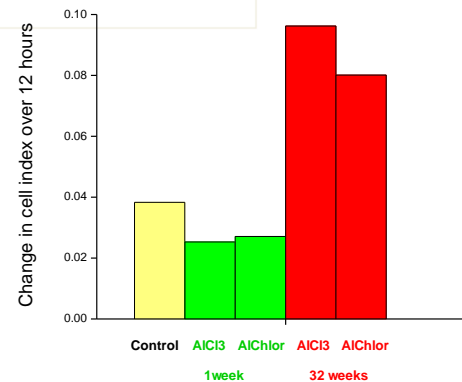
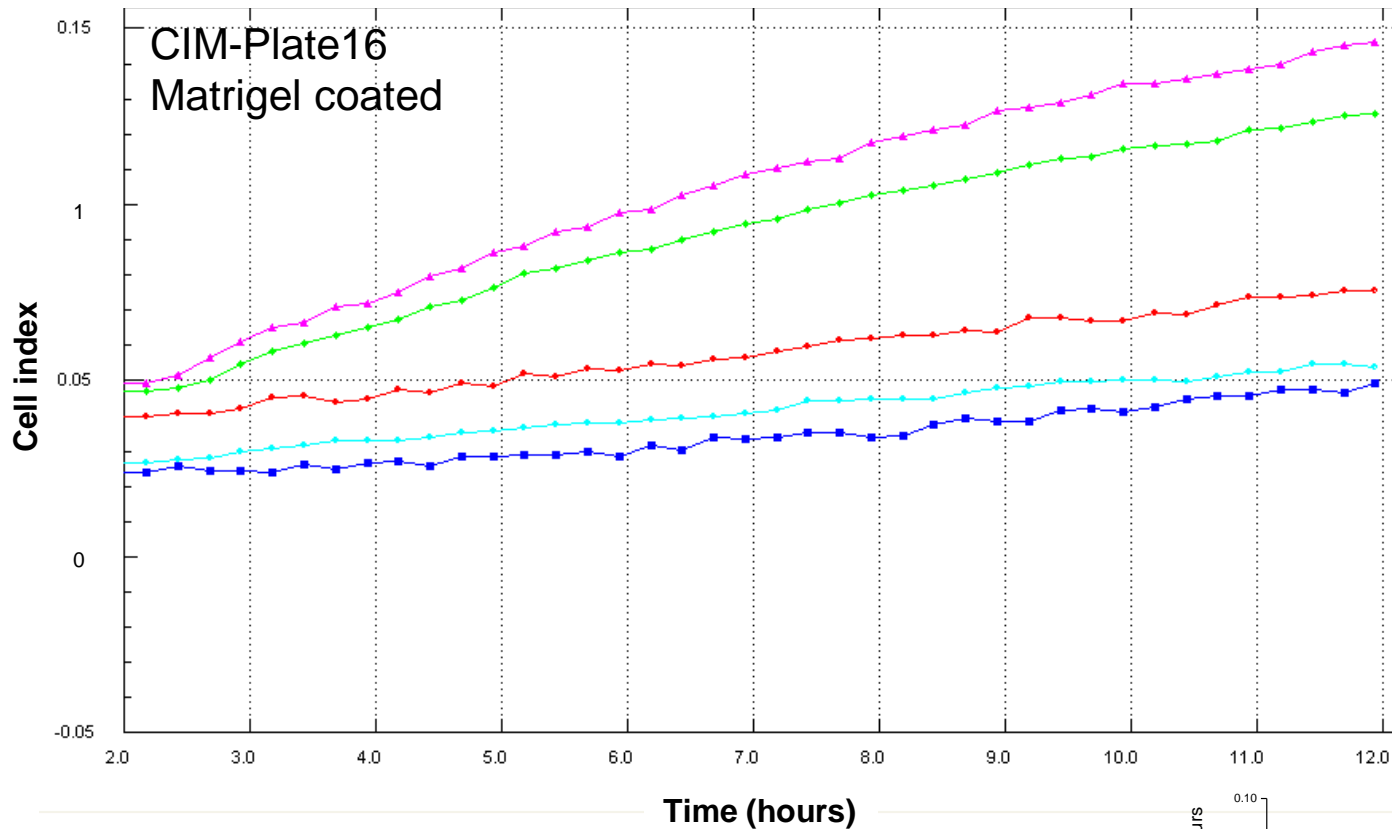
# USE OF XCELLIGENCE TECHNOLOGY TO DETERMINE THE EFFECT OF ALUMINIUM ON INVASION OF MCF-7 HUMAN BREAST CANCER CELLS

Upper membrane surface coated with matrigel

Picture of the machine deleted for copyright reasons



# EFFECT OF ALUMINIUM ON INVASION OF MCF-7 HUMAN BREAST CANCER CELLS





# CONCLUSIONS 3.

- Aluminium can increase motility/migration of MCF-7 cells
  - as measured using time-lapse microscopy
  - as measured using the wound healing assay
  - as measured using xCELLigence technology
- Aluminium can increase invasive activity of MCF-7 cells
  - as measured through matrigel using xCELLigence technology
- Molecular mechanisms remain to be determined



# CONCLUSIONS

- The human breast is exposed to aluminium
- Aluminium has been measured in human breast tissue structures
- Aluminium is a metalloestrogen
- Aluminium can turn human breast epithelial cells into a transformed phenotype in culture
- Aluminium can influence migratory and invasive properties of human breast cancer cells in culture

**Vielen Dank für Ihre Einladung  
Danke für Ihre Aufmerksamkeit**

