COMICS: developing high throughput comet assays for DNA damage and DNA repair

Amaya Azqueta, on behalf of COMICS partners
Department of Nutrition
University of Oslo
...is an EC strategic targeted project (STREP) of the 6th frame work, aimed at turning the comet assay into a high throughput assay suitable for use in screening chemicals for potential genotoxic and cytotoxic effects.

It relates to the EC policy on REACH and the philosophy of the 3R.
The comet assay

1) Cells embedded in agarose on microscope slide

2) Lysis: 2.5 M NaCl, Triton X-100

3) Alkaline incubation: 0.3 M NaOH, 10 mM EDTA

4) Electrophoresis: 0.8 V/cm, 30 min

5) Neutralisation, DAPI stain, fluorescence microscopy

Relative intensity of tail/head reflects DNA break frequency
The comet assay modified with lesion-specific enzymes

1) Cells embedded in agarose on microscope slide

2) Lysis: 2.5 M NaCl, Triton X-100

3) Alkaline incubation: 0.3 M NaOH, 10 mM EDTA

± Digestion with lesion-specific endonuclease (FPG, endoIII, T4endoV, AlkA)

4) Electrophoresis: 0.8 V/cm, 30 min

5) Neutralisation, DAPI stain, fluorescence microscopy

Enzymes recognise the lesions (oxidized and alkylated bases or dimers of thymine) and introduce breaks

Relative intensity of tail/head reflects DNA break frequency
- Basic research on mechanism of genotoxicity
- Biomonitoring
- Chemical testing (both in vitro and in vivo)

Fast and very versatile but:
• Labour-intensive
• Number of samples is limited by the size of the electrophoresis tanks
• Scoring is very laborious and very time-consuming

Medium- or high-throughput versions of the comet assay + an automated scoring system are necessary for monitoring damage in a large number of samples
Objectives of COMICS:

• To increase the throughput of the comet assay, using multi-well format and ‘cell arrays’.

• To develop further the cell array system as a parallel assay for cytotoxicity.

• To seek optimal cell types (e.g. metabolically active HepaRG).

• To develop a faster scoring method based on differential fluorescence of head and tail DNA.

• To develop and compare methods for measuring DNA repair activity.

• To use lesion-specific enzymes for different kinds of DNA damage – oxidised and alkylated bases, UV-induced damage and bulky adducts.
• To develop fluorescent in situ hybridisation on comets, to study gene-specific DNA repair.

• To validate the comet assay in its various forms, assessing reproducibility and robustness, comparing results in different laboratories.

• To develop reference and internal standards for use in the comet assay.

• To make the various innovative products available for use by companies and researchers investigating DNA damage and repair.
- University of Oslo, Oslo, Norway (CO-ORDINATOR: Andrew Collins)
- Commissariat à l'Energie Atomique (CEA), Grenoble, France
- Institut National de la Santé et de la Recherche Médicale (INSERM), Rennes, France
- Slovak Medical University, Bratislava, Slovakia
- Uppsala University, Uppsala, Sweden
- Institute of Experimental Botany, Prague, Czech Republic
- IMSTAR S.A., Paris, France
- Chiron AS, Trondheim, Norway
- TATAA Biocenter AB, Göteborg, Sweden
- Astra Zeneca, Macclesfield, United Kingdom
- Thistle Scientific Limited, Glasgow, Scotland
- Severn Biotech LTD, Kidderminster, United Kingdom
- BIOPREDIC INTERNATIONAL, Rennes, France
- Norwegian Institute of Public Health (NIPH), Oslo, Norway
- JOINT RESEARCH CENTRE (JRC-ECVAM), Ispra, Italy
University of Oslo, Norway

• To develop and calibrate a cell array for a medium-throughput assay.

• To collaborate in the development of automated analysis.

• To develop an in vitro repair assay based on the comet assay to detect different pathways, and to compare this method with DNA repair chips.

• To develop stains and staining procedures for two-colour fluorescence

• To develop FISH combined with cell array to make scoring easier and quicker

• To apply padlock probes to detect gene-specific DNA repair in comets
University of Oslo, Norway

• To develop and calibrate a cell array for a medium-throughput assay.
• To collaborate in the development of automated analysis.
• To develop an in vitro repair assay based on the comet assay to detect different pathways, and to compare this method with DNA repair chips.
• To develop stains and staining procedures for two-colour fluorescence
• To develop FISH combined with cell array to make scoring easier and quicker
• To apply padlock probes to detect gene-specific DNA repair in comets
University of Oslo, Norway

• To develop and calibrate a cell array for a medium-throughput assay.
• To collaborate in the development of automated analysis.
• To develop an in vitro repair assay based on the comet assay to detect different pathways, and to compare this method with DNA repair chips.
• To develop stains and staining procedures for two-colour fluorescence
• To develop FISH combined with cell array to make scoring easier and quicker
• To apply padlock probes to detect gene-specific DNA repair in comets
IN VITRO REPAIR ASSAY: estimates the repair activity of cell extracts by measuring their incision activity

- Alkaline treatment
- Electrophoresis
- Neutralization
- Staining
- Scoring

SUBSTRATE: Nucleoids of cells + damage

Nucleoids containing 8-oxoguanine ⇒ BER
Nucleoids with cyclobutane pyrimidine dimers ⇒ NER

* Comparison of in vitro repair assay with DNA repair chips
* Development of an assay to detect the capacity for repair of alkylation damage
University of Oslo, Norway

- To develop and calibrate a cell array for a medium-throughput assay.
- To collaborate in the development of automated analysis.
- To develop an in vitro repair assay based on the comet assay to detect different pathways, and to compare this method with DNA repair chips.
- To develop stains and staining procedures for two-colour fluorescence
- To develop FISH combined with cell array to make scoring easier and quicker
- To apply padlock probes to detect gene-specific DNA repair in comets
MEDIUM THROUGHPUT ASSAY

12 gels/slide

Perfect for testing different chemicals and for testing different cell extract in the in vitro repair assay
MEDIUM THROUGHPUT ASSAY: $\text{H}_2\text{O}_2$ dose response

1 and 12: 0 $\mu$M $\text{H}_2\text{O}_2$
2 and 11: 12.5 $\mu$M $\text{H}_2\text{O}_2$
3 and 10: 25 $\mu$M $\text{H}_2\text{O}_2$
4 and 9: 50 $\mu$M $\text{H}_2\text{O}_2$
5 and 8: 100 $\mu$M $\text{H}_2\text{O}_2$
6 and 7: 2000 $\mu$M $\text{H}_2\text{O}_2$

Cells were treated after embedding in agarose.
- No leakage between wells
- Good dose response
- Excellent reproducibility between duplicates
MEDIUM THROUGHPUT ASSAY: comparison with standard assay

1: Buffer (30 µl)
2: FPG (30 µl)

1,3 and 5: Buffer (25 µl)
2, 4 and 6: FPG (25 µl)
7, 9 and 11: FPG (50 µl)
8, 10 and 12: Buffer (50 µl)

Good agreement between the two systems
25 µl are enough to incubate the minigels in the well
MEDIUM THROUGHPUT ASSAY

Next steps:

- Decrease the amount of extract/compounds/enzymes for incubating minigels

- Validation of the new medium-throughput assay (12 gels/slide) and the high-throughput assay (96 gels/Gelbond film)
HIGH THROUGHPUT ASSAY

Gelbond film with 96 gels/film
University of Oslo, Norway

- To develop and calibrate a cell array for a medium-throughput assay.
- To collaborate in the development of automated analysis.
- To develop an in vitro repair assay based on the comet assay to detect different pathways, and to compare this method with DNA repair chips.
- To develop stains and staining procedures for two-colour fluorescence
- To develop FISH combined with cell array to make scoring easier and quicker
- To apply padlock probes to detect gene-specific DNA repair in comets
AUTOMATED SCORING (IMSTAR) vs VISUAL SCORING

Cells treated with different concentrations of H₂O₂

Lymphocytes

HeLa cells
AUTOMATED SCORING (IMSTAR)

Next steps:

- Adapt the IMSTAR system to the new prototypes: medium-throughput assay (12 gels/slide) and high-throughput assay (96 gels/Gelbond film).

  Until now - very promising!!!

  Scoring will be even faster if we manage to find a staining method to give a direct measure of total tail DNA
THANK YOU FOR YOUR ATTENTION!!!