Synchronized Mammalian Cells in Culture:

A Comparison of Normal and KB Cancer Cell Growth

I. C. Baianu[1] and M. M. Marinescu[+]
[1]AFC- NMR and NIR Microspectroscopy Facility, College of ACES
FSHN and NPRE Departments,
College of ACES and College of Engineering,
University of Illinois at Urbana,
305/350 Burnsides Research Laboratory,
Urbana, Illinois 61801, US
Email: ibaianu@illinois.edu

Nature Precedings : hdl:10101/npre.2012.7128.1 : Posted 3 Apr 2012

1. Introduction

Experimentation and tests with cells in culture and cell synchronization or synchrony are important in order to be able to make both reliable and high-sensitivity measurements. This is also very important for mechanistic studies of cell division control, oncogenesis and tumor growth.

Experimental Models and Samples that were employed:

1. *Synchronized cells in culture:*

i. Human and rabbit lymphocytes in culture
ii. HeLa and KB cell lines (Figures 14C and 14D)
iii. Stabilized cultures of primate kidney cells and Fibroblast Rabbit cells in culture (Figures1A and 1B).

All cell cultures were grown on Roux, Khele, and Chance sterile plates at 37 $\pm$ 0.2 °C.

2. *Synchronization Methods and Timing employed*:

i. Methroxate (MTX) blockage
ii. Amethopterin blockage of DNA synthesis
iii. Thymidine double-blockage reversal after 16 hours (Steffen et al., 1969, 1970).

3. *Observation Methods employed:*

i. Confocal Epi-Fluorescence Microscopy and cell counting of cell culture plates
ii. FT-NIR Hyperspectral Imaging
iii. FT-IR /Focal Plane Array Chemical Imaging
iv. Single- and Two- photon Fluorescence Correlation Spectroscopy (FCS).

Nature Precedings : hdl:10101/npre.2012.7128.1 : Posted 3 Apr 2012

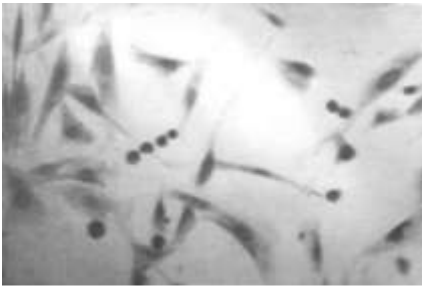
2.Experimental Results

Microphotographs obtained of cells synchronized in culture are shown in Figure1. Degrees of synchronization obtained with KB and rabbit fibroblast cells in culture by the thymidine double-blockage method were consistently high (between 78 and 85%) as determined by cell counting and biochemical analyses. Two mitotic waves were clearly distinguished up to 50 hours from the beginning of cell growth from the primary cultures. Additional experiments with cell mixtures such as KB/ Rabbit cells synchronized in culture were compared with KB and rabbit cell controls, and it was found that the different cell types exhibited a distinct pattern of interactions, as expected from current models of synchronized cells in culture.

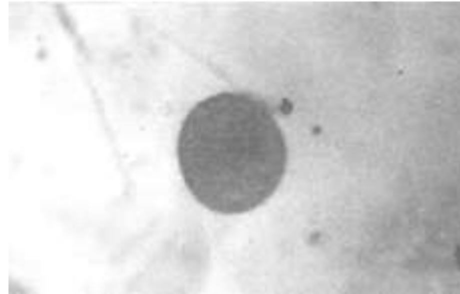
A. Rabbit Cells in Culture

B. Single Rabbit Cell in Metaphase

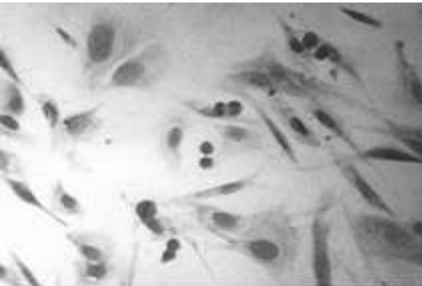
A.



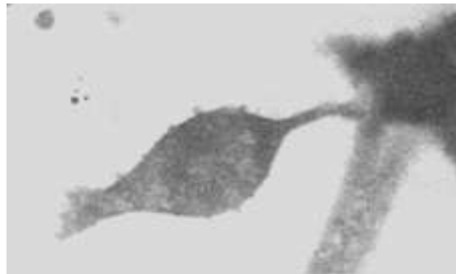
B.



C.



D.



C. KB Cells in Culture

D. Single KB Cell

Figure 1. Photomicrographs of Synchronized KB and Fibroblast Rabbit Cells in Culture.

A. Fibroblast Rabbit Cells in Culture; **B.** Single Rabbit Cell in Metaphase; (both A. and B. were viewed through air with a 25x objective);
C. KB Cells in Culture**; D**. Single KB Cell (both C. and D. were viewed with a 50x oil immersion objective).

Nature Precedings : hdl:10101/npre.2012.7128.1 : Posted 3 Apr 2012

3. Conclusions and Discussion

Very detailed, automated chemical analyses of biomolecules in cell cultures are now becoming possible by FT-NIR microspectroscopy of single cells, both *in vitro* and *in vivo*. Such rapid analyses have potentially important applications in cancer research, pharmacology and clinical diagnosis for selectively detecting the distinctive molecular signatures of cancer cells and tumors. Our recent, preliminary results presented above in Section C indicate that the sensitivity range of FT-NIR micro-spectroscopy observations can be extended to the *femtogram* level, with submicron spatial resolution. Such FT-NIR/IR microspectroscopy instrumentation developments are potentially very important for biomedical and pharmacological applications that require rapid and sensitive analyses, such as those relevant to cancer diagnosis and treatment, or cancer research involving the screening of high-content micro-arrays (HCMA) recently beginning to be utilized by Post-Genomics and Proteomics biomedical programs. Other related, possible developments in such programs are the applications of Fluorescence Cross-Correlation Spectroscopy detection to monitoring: *DNA-telomerase interactions*, DNA hybridization kinetics, cell surface oncogenic carbohydrate pattern detection, ligand-receptor interactions and HIV-HBV testing, as well as their utilization in combination with other advanced bioimaging techniques, such as: NIR/IR Microspectroscopy, SNIRF, Multiple Micro-Array Analyses and NMRSI.

Our results presented in the previous section illustrate the currently available microspectrosopy capabilities for bioimaging applications in cancer research and diagnosis; they also indicate several of their severe limitations in terms of image acquisition speed and sensitivity, as well as the urgent need for developing ultra-sensitive and rapid bioimaging technologies for reliable cancer detection, well beyond the current state-of-the-art. Supporting evidence was provided by our preliminary results described in Section **C** for the feasibility of our novel approaches --described next-- for developing ultra-sensitive and rapid microspectroscopic instruments with advanced capabilities for the *in vivo*, reliable detection of cancer in its early stages.

Bibliography

1. Baianu, I.C., D.M. Costescu, and T. You. 2002. Novel Techniques for Microspectroscopy and Chemical Imaging Analysis of Soybean Seeds and Embryos. *The 9th Biennial Conference of the Cellular and Molecular Biology of the Soybean Proceedings*, Aug 11-14, 2002, pp.395-396.

2. Baianu, I.C., T. You, and D.M. Costescu. 2003. Near Infrared Microspectroscopy / Fluorescence Correlation Spectroscopy, Infrared Chemical Imaging and High-Resolution Nuclear Magnetic Resonance Analysis of Soybean Seeds, Somatic Embryos and Single Cells. *AOCS Publs.* : Washington, DC. 95th AOCS Proceed:, pp.204-235.

3. Baianu, I.C., Costescu, D.M., Hofmann, N., and Korban, S.S. (2003). Near Infrared Microspectroscopy, Chemical Imaging and NMR Analysis of Oil in Developing and Mutagenized Soybean Embryos in Culture. *AOCS Meeting, Analytical Division.*

4. Bohmer at al. (2001). Time-resolved confocal device for ultra-sensitive fluorescence detection *Rev. Sc. Instr.*,72(11): 4145-4152.

5. Clarke, F.C., Jee, D.R., Moffat, A.C., and Hammond, S.V. (2001). Effective sample volume for measurements by Near-Infrared Microscopy. (e.g.: 40,000 mm^3, or ~30 ng); (Abstract,116), British Pharmaceutical Conference. UK.

6.Compton, J. (1991). Nucleic acid sequence-based amplification. *Nature*, **350**:91-92.

7.Costescu, D.M. (2002). Analysis of oil and protein content by Nuclear Magnetic Resonance (NMR) and Electron Microscopy (EM) of samples of somatic embryogenic suspension cultures of soybean, soybean seeds and soybean flours, MS Thesis, UIUC.

8. Cooper, S. 1998. *Cell Prolif*. **31**:9-16.

9.Diaspro, A., and Robello, M. (1999). Multi-photon Excitation Microscopy to Study Biosystems. *European Microscopy and Analysis*, **5**:5-7.

10.Eigen, M., and Rigler, R. (1994). Sorting single molecules: Applications to diagnostics and evolutionary biotechnology, *Proc. Natl. Acad. Sci. USA*, **91**:5740.

11. Elson, E.L., and Magde, D. (1974). Fluorescence correlation spectroscopy. I: Conceptual basis and theory, *Biopolymers*, **13**:1.

12. Gamborg, O.L., Miller, R.A., and Ojim, K. (1968). Nutrient requirement of suspension cultures of soybean root cells. *Exp. Cell Res*. **50**:151-158.

13.Glover, P. and P. Mansfield. (2002). Limits to Magnetic Resonance Microscopy. *J. Rep. Prog. Phys*., **65**: 1489-1511.

14.Helstetter, C.E. et al., 2003. *Cell Cycle*, 2(1): 42-45.

15.Hofmann, N.E, Raja, R., Nelson, R.L., and Korban, S.S. (2003). Mutagenesis of embryogenic cultures of soybean and detecting polymorphisms using RAPD markers. *Biol. Plant*. 21:33-48.

16.Laferte S, Prokopishyn NL, Moyana T, Bird RP.(1995). Monoclonal antibody recognizing a determinant on type 2 chain blood group A and B oligosaccharides detects oncodevelopmental changes in azomethane-induced rat colon tumors and human cancer cell lines. *J. Cell Biochem*. **157**:101-19.

17.Lakowicz, J. R. 2001. Radiative Decay Engineering: biophysical and biomedical applications. *Anal. Biochem*. **298**:1-24

18.Malicka, J., et al. 2003a. Fluorescence spectral properties of cyanine dye-labeled DNA oligomers on surfaces coated with silver particles. *Analytical Biochem*., **317**:136-146.

19. Malicka, J., et al. 2003b. DNA hybridization assays using metal-enhanced fluorescence. *Biochem. Biohys. Res. Comm*. **306**: 213-218.

Nature Precedings : hdl:10101/npre.2012.7128.1 : Posted 3 Apr 2012

20. Lee, S. C. et al., (2001). One Micrometer Resolution NMR Microscopy. *J. Magn. Res.*, **150**: 207-213.

21. Magde D., Elson E.L. and Webb W.W. (1972). Thermodynamic fluctuations in a reacting system - measurement by fluorescence correlation spectroscopy, *Phys. Rev. Lett.* **29**:705.

22. Magde D., Elson E.L. and Webb W.W. (1974). Fluorescence correlation spectroscopy II: An experimental realization, *Biopolymers,* **13**:29.

23. Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **15**:473-497.

24.Oehlenschläger F., Schwille P. and Eigen M. (1996). Detection of HIV-1 RNA by nucleic acid sequence-based amplification combined with fluorescence correlation spectroscopy, *Proc. Natl. Acad. Sci. USA*, **93**:1281.

25. Otting, G. and K. Wutrich. (1989). Studies of Protein Hydration in Aqueous Solutions by Direct NMR Observation of Individual Protein-Bound Water Molecules. *J. Am. Chem. Soc.*, **111**:1871-1875.

26. Palli D, Caporaso NE, Shiao YH, et al. Diet, *Helicobacter pylori*, and p53 mutations in gastric cancer: a molecular epidemiology study in Italy. *Cancer Epidemiol Biomarkers Prev* 1997 Dec; **6**(12):1065-1069.

27. Rigler R. and Widengren J. (1990). Ultrasensitive detection of single molecules by fluorescence correlation spectroscopy, *BioScience* (Ed. Klinge & Owman), p.180.

28. Rigler R. and Mets Ü. (1992). Diffusion of single molecules through a Gaussian laser beam, *SPIE* 1921:239.

29. Rigler R., Mets Ü., Widengren J. and Kask P. (1993). Fluorescence correlation spectroscopy with high count rate and low background: Analysis of translational diffusion, *Eur. Biophys. J.* **22**:169.

30. P. Schwille.(2001) *in* "Fluorescence Correlation Spectroscopy. Theory and applications" (R. Rigler & E.S. Elson, eds.), p. 360. Springer Verlag: Berlin.

31. Schwille P., Oehlenschläger F. and Walter, N. (1996). Analysis of RNA-DNA hybridization kinetics by fluorescence correlation spectroscopy, *Biochemistry*, **35**:10182.

32. Schwille P., Meyer-Almes F.-J. and Rigler R. (1997). Dual-color fluorescence cross-correlation spectroscopy for multicomponent diffusional analysis in solution*, Biophys. J.*, **72**:1878.

33. Schwille P., Bieschke J. and Oehlenschläger F. (1997). Kinetic investigations by fluorescence correlation spectroscopy: The analytical and diagnostic potential of diffusion studies, Biophys. Chem. 66:211-228.

34. Schwille, P., Haupts, U., Maiti, S., and Webb. W.(1999). Molecular dynamics in living cells observed by fluorescence correlation spectroscopy with one- and two-photon excitation. *Biophysical Journal*, **77**(10):2251-2265.

Nature Precedings : hdl:10101/npre.2012.7128.1 : Posted 3 Apr 2012

35. Schwille P., Bieschke J. and Oehlenschläger F. (1997). In: Kinetic investigations by fluorescence correlation spectroscopy: The analytical and diagnostic potential of diffusion studies. *Biophys. Chem*. **66**:211-228.

36. Schuman S. (1994). Rapid TOPO cloning. *J. Biol. Chem*, **269**: 32678-84.

37. Shay, J.W. (1995). Aging and cancer: are telomeres and telomerase the connection? *Mol. Med. Today*. **1**: 378-384.

38. Shay, J.W. and Gazdar, A.F. (1997). Telomerase in the early detection of cancer. *J. Clin. Pathol.* **50**: 106-109.

39. Tamura, Y. et al. (1998). Highly selective and orally active inhibitors of type IV collagenase (MMP-9 and MMP-2): *N*-sulfonylamino acid derivatives. *J. Med. Chem*., **41**: 640–649.

+ Deceased.

Nature Precedings : hdl:10101/npre.2012.7128.1 : Posted 3 Apr 2012