Autoradiography: Detection and Analysis of Radioactive Entities

Nida Tabassum Khan*
Department of Biotechnology, Faculty of Life Sciences and Informatics, Balochistan University of Information Technology Engineering and Management Sciences, (BUITEMS), Quetta, Pakistan

Abstract
Autoradiography is a specific biological tool used to detect radioactive materials by using X-ray photographic films. A technically simple technique to be used for characterizing receptors and localizing their positions in the tissues. Moreover its detection sensitivity could be enhanced using fluorography by transforming radioactive emissions into light.

Keywords: Fluorography; Photographic emulsion; Silver halide

Introduction
Autoradiography is employed for the detection of materials that possess radioactive properties. By using X-ray films, autoradiography determine the relative positions and intensities of radiolabeled bands in a gel or blot. In 1867 the first autoradiography was observed accidentally when an emulsion of silver chloride and iodide turns black by uranium salts [1]. With the advent of photographic emulsions and photographic films after World War II, autoradiography was used as a biological technique for the detection of radioactive substances or materials labelled with radioactive isotopes [2].

Mechanism
Penetration of negatively charged beta particles emitted by radioactive salts through silver halide film emulsion causes activation of silver present in the emulsion. Activated silver crystals are very unstable therefore quickly reduced to black silver particles which is easily detectable. Autoradiography sensitivity is improved by carrying the detection process at 70°C and preflashing the film before use. Preflashing needs only one hit per crystal deposited to increases sensitivity [3]. Autoradiography detection limits vary for different radioisotopes as given in the table below (Table 1) [4,5].

Sequential steps of autoradiography
• Brief exposure of living cells to a pulse of specific radioactive material for a variable time.
• Preparation of samples are for microscopy either light or electron.
• Dissection of samples into sections for coverage with thin film of photographic emulsion which are then incubated in the dark for few days for radioactive decay. The exposure time depends on isotope activity, temperature and the background radiation.
• Development of photographic emulsion.
• Toluidine blue is used for counter staining to reveal tissue histology. Instead Osmium or dipping emulsion can be used for pre-staining of the entire tissue before exposure to the photographic emulsion to avoid for individual post- staining each slide.

Table 1: Autoradiography detection limit.

| Isotope | Count per minute (CPM) for Detection | Energy per Emission (MEV) |
|---------|--------------------------------------|---------------------------|
| H       | >10^7                                | 0.0055                    |
| ^14C     | 2000                                 | 0.050                     |
| ^32P     | 1000                                 | 0.167                     |
| ^35S     | 100                                  | 0.70                      |
| ^125I    | 10                                    | Gamma                     |

Fluorography
Autoradiography sensitivity is greatly enhanced through fluorography which transforms radioactive emissions into light which efficiently penetrates the film to be readily detected [8]. A number of phosphor compounds absorb energy from beta particles and re-emit it as light e.g. Autofluor [9,10].

Advantages
• Technically easy not much expertise required,
• Highly specific detection tool,
• Unlike tissue bath preparations, pharmacologically characterize and localize receptors in tissues,
• Enables characterization of receptors in different tissues in different animals or brain regions [11,12].

Disadvantages
• Lack of assessment criteria to determine whether the binding site really corresponds to an actual receptor,
• Non-physiological significance of high affinity radiolabelled receptor,
• Non-specificity of ligands can easily cause misinterpretation of results [13].

Autoradiography practical applications
Autoradiography provides qualitative as well as quantitative...
information regarding a specimen. Some of the following applications of this technique are given below:

- Autoradiography is used to determine receptor distribution and localization while studying neurodegenerative disorders [14].
- Application of autoradiography in electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets during blotting [15].
- To study cyogenesis of the forebrain [16].
- Applications in radiopharmaceutical research [17].
- Applications in radioimmunoelectrophoresis to study viruses [18].
- In imaging and analyzing rock porosity [19].
- In matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI), and secondary ion mass spectrometric imaging (SIMS-MSI) for pharmaceutical discovery and development [20].
- In whole body imaging [21].
- Tool for genetic studies [22].
- For comparison of complex mixtures of proteins [23]
- Applications in microbial ecology [24].
- Determining gross absorption and utilization of foliar applied nutrients etc. [25].

Conclusion

Today, autoradiography is employed as an important detection tool for the identification of different target receptors in various tissues to provide us with a better understanding of molecular pharmacological pathways.

References

1. Rogers AW (1979) Techniques of autoradiography.
2. Baserga R, Malamud D (1969) Autoradiography: techniques and applications.
3. Cross SA, Groves AD, Hesselbo T (1974) A quantitative method for measuring radioactivity in tissues sectioned for whole-body autoradiography. The International Journal of Applied Radiation and Isotopes 25: 381-382.
4. Caro LG, Van Tubergen RP (1962) High-resolution autoradiography. The Journal of Cell Biology 15: 173-188.
5. Hammarström L, Appelgren LE, Ullberg S (1965) Improved method for light microscopy autoradiography with isotopes in water-soluble form. Experimental Cell Research 37: 608-613.
6. Tribollet E, Dreifuss JJ, Charpak G, Dominik W, Zagandis N (1991) Localization and quantitation of labeled compounds in tissue sections with a gaseous detector of beta particles: comparison with film autoradiography. Proceedings of the National Academy of Sciences 88: 1466-1468.
7. Leary JJ, Brigati DJ, Ward DC (1983) Rapid and sensitive colorimetric method for visualizing biotin-labeled DNA probes hybridized to DNA or RNA immobilized on nitrocellulose: Bio-blots. Proceedings of the National Academy of Sciences 80: 4045-4049.
8. Bonner WM, Siedman JD (1978) Efficient fluorography of 3H and 14C on thin layers. Analytical Biochemistry 89: 247-256.
9. Johnston RF, Pickett SC, Barker DL (1990) Autoradiography using storage phosphor technology. Electrophoresis 11: 355-360.
10. Clark CR, Hall MD (1986) Hormone receptor autoradiography: recent developments. Trends in Biochemical Sciences 11: 195-199.
11. Hamilton JG (1941) The applications of radioactive tracers to biology and medicine. Journal of Applied Physics 12: 440-460.
12. Reubi JC (2003) Peptide receptors as molecular targets for cancer diagnosis and therapy. Endocrine Reviews 24: 389-427.
13. Palacios JM, Neihoff DL, Kuhar MJ (1981) Receptor autoradiography with tritium-sensitive film: potential for computerized densitometry. Neurosciences Letters 25: 101-105.
14. Whitehouse PJ (1985) Receptor autoradiography: applications in neuropathology. Trends in Neurosciences 8: 434-437.
15. Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Sciences 76: 4350-4354.
16. Fujita S (1966) Applications of light and electron microscopic autoradiography to the study of cyogenesis of the forebrain. In Evolution of the Forebrain.
17. Som P, Yonekura Y, Oster ZH, Meyer MA, Pelletier ML, et al. (1983) Quantitative autoradiography with radiopharmaceuticals, Part 2: Applications in radiopharmaceutical research: concise communication. Journal of nuclear medicine: official publication, Society of Nuclear Medicine 24: 235-244.
18. Tsotsos AS, Corbitt G (1973) Radioimmunoelectrophoresis a technique combining immuneolectrophoresis with autoradiography: applications to virology. Journal of Immunological Methods 3: 53-62.
19. Hellmuth K, Siltari-Kauppi M, Klöb P, Meyer K, Goebelis J (1999) Imaging and analyzing rock porosity by autoradiography and Hg-porosimetry/X-ray computerradiography-applications. Physics and Chemistry of the Earth, Part A: Solid Earth and Geodesy 24: 569-573.
20. Salom EG, Schwetzer A, Stoepcki M, Prideaux B (2010) Autoradiography, MALDI-MSI, and SIMS-MS imaging in pharmaceutical discovery and development. The AAPS Journal 12: 11-26.
21. d'Arny R, Ullberg S, Stålnacke CG, Längström B (1984) Whole-body autoradiography using 11C with double-tracer applications. The International Journal of Applied Radiation and Isotopes 35: 129-134.
22. Bertolucci E, Conti M, Mettivier G, Russo P, Amendola SR, et al. (1999) GaAs pixel radiation detector as an autoradiography tool for genetic studies. Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers. Detectors and Associated Equipment 422: 242-246.
23. McConkey EH (1979) Double-label autoradiography for comparison of complex protein mixtures after gel electrophoresis. Analytical Biochemistry 96: 39-44.
24. Karl DM (1980) Cellular nucleotide measurements and applications in microbial ecology. Microbiological Reviews 44: 739.
25. Wittwer SH, Lundahl WS (1951) Autoradiography as an aid in determining the gross absorption and utilization of foliar applied nutrients. Plant Physiology 26: 792.