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Chapter 1

History of Cell Culture

Magdalena Jedrzejczak-Silicka

Abstract

From the ancient Romans, through the Middle Ages, to the late of the nineteenth century, the Aristotelian doctrine of spontaneous generation was one of the most basic laws. Even the invention of the microscope and investigations of Leeuwenhoek and Hook did not disprove the Aristotelian doctrine. Finally, in the eighteenth century, the spontaneous generation doctrine was laid by Louis Pasteur. Moreover, in the first decade of the eighteenth century, nucleus was observed in plant and animal tissues, and Virchow and other scientists presented the view that cells are formed via scission of preexisting cells. In the first decade of the twentieth century, Ross Harrison developed the first techniques of cell culture in vitro, and Burrows and Carrel improved Harrison’s cell cultures. In mid-twentieth century, the basic principles for plant and animal cell cultures in vitro were developed, and human diploid cell lines were established. On the basis of knowledge about the cell cycle and gene expression regulation, the first therapeutic proteins were produced using mammalian cell cultures. The end of twentieth century and early twenty-first century brought the progress in 3-D cell culture technology and created the possibility of the tissue engineering and the regenerative medicine development.

Keywords: spontaneous generation, Harrison’s hanging drop culture method, HeLa cell line, Hayflick limit, cell culture history

1. Introduction

At the present time, animal and human cell cultures are significant tools widely used in many branches of life science. Different variants of cell culture found application in modeling diseases, IVF technology, stem cell and cancer research, monoclonal antibody production, regenerative medicine and therapeutic protein production. All those different scientific approaches would not be possible without some crucial discoveries that had been made over the centuries from Aristotelian spontaneous generation doctrine through Pasteur’s experiments and Carrel’s cell culture to large-scale cultures for therapeutic proteins production and vision of
the future of regenerative medicine and in situ bioprinting of wounds. The main milestones in cell cultures are presented in proposed chapter (see Figure 1).

Figure 1. Timeline: key milestone in cell cultures.
2. Live under the microscope

The development of biological sciences would not have been possible without one of the greatest inventions—microscopes. In the sixteenth and seventeenth centuries—two countries—the Netherlands and Italy played a crucial role in constructing and using microscopes and telescopes. In the Netherlands, around 1590, Hans Janssen and his son invented a compound microscope—constructed of two convex lenses. In the early 1600s (about 1610), the great Galileo Galilei (1564–1642) constructed several simple microscopes and telescopes, which he called as “occhialino.” The term “microscope” was used for the first time in 1625 by the Italian physician Giovanni Faber [1].

The first publication, in which Petrus Borellus described the use of microscope in medicine, was written in 1653. He presented 100 microscopic observations and applications (e.g., removing ingrowing eyelashes invisible with the naked eye). In 1646, Athanasius Kircher (1601–1680), a Jesuit priest, described that “in the blood of fever patients a number of things might be discovered.” Kircher showed later (in 1658) that maggots and other living creatures (some of them he called microscopic “worms”) occurred and developed in decaying tissues [1–3]. Two other microscopists—Swammerdam (1637–1680) in 1667 and Malpighi (1628–1694)—characterized red blood cells [1,2]. In Bologna, another scientist, Joseph Campini, illustrated the first use of the microscope in the clinical examination of a wound on the leg of a patient [1, 4].

At the beginning of the seventeenth century, two inventors—Robert Hook (1635–1702) and Antonie van Leeuwenhoek (1632–1732)—made an unusual discovery. Both of them made their first observations of life under the microscope and made the previously invisible microscopic world real [3].

The English physicist, Hooke, published in 1665 the first important work on microscope construction, its components and microscopic observations. In his Micrographia, he illustrated microscopic structures of many biological samples (e.g., insects, plants, sponges, bryozoans, fossils), as observed through microscopes and described the microscopic units. The “cells” or “pores”, as he called small compartments of a slice of cork (thickened walls of dead cells) were chosen to refer to these microscopic units. Although Robert Hooke used the term “cell” differently compared to the later biologists, the today’s term “cell” comes directly from Hooke’s Micrographia [3, 5, 6].

In 1676, the Royal Society (RS) received a letter from Antonie van Leeuwenhoek, in which the microscopist had described his exciting discoveries—observations and records of small living particles. These microorganisms, which Leeuwenhoek called “animalcules,” were mainly protozoa and bacteria [3, 4]. Implementation of his scientific project was inspired by the Hooke’s bestseller, Micrographia. He started by handcrafting lenses and constructing microscopes (he was known to make over 500 microscopes). The Leeuwenhoek’s single lens microscopes were smaller than magnifying glasses (3–4 inches long) but were capable of 270× magnifications or even more (while the Hooke’s microscopes could only achieve magnifications of about 50×), with clear and bright images. The observed specimen was mounted in
Leeuwenhoek began his microscopic observations with insect samples (e.g., parts of bees) and continued with observations of spirogyra, vorticella, protozoa and motile bacteria (e.g., from the human mouth). He also examined many human and animal tissue samples, and he described blood cells (for the first time illustrated in his Arcana in 1695), sperm cells (he called semen “sperm animals”), skeletal muscle fibres, epithelial cells, teeth and circulatory system structures. He was the first to use histological staining (he stained muscle tissue with saffron) and described most of his observations (most of them involved microorganisms) in 560 letters to the Royal Society (RS) during his lifetime, and thus, he became the “Father of Microbiology” [1, 3, 4, 7].

Since Leeuwenhoek’s invention microscopes have been one of the most fundamental tools, particularly, in the biological sciences, but also in clinical pathology and medical diagnosis [8]. In the twentieth century, many discoveries have been made in the field of life sciences, due to modern microscopy techniques. In 1941, Fritz Zernike constructed first phase contrast microscope. Another invention was a microscopic differential interference contrast technique (phase contrast) evolved by Georges Nomarski. Invention of fluorescence and confocal microscopy revolutionized life sciences. Confocal scanning microscopy gives possibility to examine fixed or alive biological specimens. This technique allows the selective and specific detection and visualization of molecules at small concentrations with good signal-to-background ratio [8]. Technique of confocal microscopy was evolved by Marvin Minsky in 1957 [9]. Confocal scanning microscopy technique is based on the restriction of photodetection to light originating from the focal point, whereas in fluorescence microscopy, the entire sample is excited indiscriminately, where the fluorescent photons arise from out-of-focus fluorofores. The optical sectioning gives three-dimensional microscopic reconstruction of biological samples. For photodamage and photobleaching reduction, the confocal microscopy was improved by the use of spinning disk scanners that were based on the disk invented by Nipkow (in 1884). Use of many pinholes enhances detection of the fluorescence and reduces excitation [8].

The fluorescent microscopy was also revolutionized by the two-photon microscopy invention. In this technique, two-photon excitation is applied, that means that using ultrafast laser (infrared) is possible to obtain locally very high photon concentration that occurs only at the focal point of the microscope. The two low-energy photons excite together a chromophore (only at the scan plane) and generate fluorescence. Use of infrared results in lowering the light scattering cross section of living tissues, which gives possibility to examine fluorophores deep in living samples. In contrast to confocal microscopy, the two-photon microscopy ensures that the problem of photodamage and photobleaching is reduced, but disadvantage of that method is worse spatial resolution in comparison with confocal microscopes [8]. In the 1990s, Stefan Hell developed super-resolution fluorescence microscopy technique and gave the scientists possibility to examine structures of the size of a few nanometers.

Immunofluorescence techniques with the new fluorescence molecules (immunofluorescence reagents, organic dyes, quantum dots) and discovery of fluorescent proteins (e.g., GFP) and use of confocal microscopy made new possibilities to examine biological specimens [10, 11]. For example, confocal microscopy allows the live-cell imaging (time-laps microscopy) to monitor cell movements, cell and tissue structures in one (1-D), two (2-D), three (3-D) spatial
dimensions or 4D—(3D × time) [12]. The variant of live-cell imaging techniques—fluorescence loss in photobleaching (FLIP)—utilizes repeated photobleaching that can be used to assess the continuity of membrane of endoplasmic reticulum or Golgi apparatus. Fluorescence resonance energy transfer (FRET) technique gives opportunity to display interactions between two molecular species. The energy transfer from fluorescent “donor” to fluorescent “acceptor” is possible when fluorophores are in nanometer proximity [12].

Using fluorescent dyes, it is possible to label live or death cell nuclei, for example, SYTO59 or SYTO61 for live cells, DAPI, podipidum iodide (Figure 2), Sytox Green or T0-Pro-3 for the nuclei of death cells [12], and fluorescently labeled antibodies used for, for example, HeLa cell mitoses with anti-tubulin staining [13], anti-cytokeratin staining (Figure 2).

Cellular junction identification is based on detection of structural components and proteins that are associated with those components. For studying cell adhesion and cellular junctions monoclonal, polyclonal antibodies labeled with conjugates for visualization of the target cellular structures are used for gap junction—Connexin-40, CX40; Connexin-43, CX43; pannexin (1, 2) for synapses; for tight junctions (TJ)—claudins, occludins, JAMs (junctional adhesion molecules) and CRB1 (human Crumbs homolog 1); for adherents junctions—cadherin-catenin-actin modules; for desmosomes and hemidesmosomes—cadherins (desmogleins and desmocollins) and intergins [15–17].

Mentioned techniques can be used for determination of ion concentration, for example, pH, Ca\(^{2+}\), K\(^+\), Na\(^+\), O\(_2\) in biological systems (for example within cells) [18]. Many of fluorescence probes are ion indicators with a different fluorescence lifetime (\(\tau_f\)) of the free form of probes and the form bound to ions. This property allows to selective and quantitative imaging of several different ions (pH, Ca\(^{2+}\), K\(^+\), Na\(^+\)) in the same time. The intracellular pH determination is commonly analyzed using c-SNAFL-1 fluorescence probe. Different values of \(\tau_f\) for bounded and unbounded form of fluorophore and different emission spectra are measured. The Ca\(^{2+}\) determination can be performed with the [Ca\(^{2+}\)]-sensitive probe Fluo-3 or indo-1. The Fluo-3

![Figure 2](http://dx.doi.org/10.5772/66905)
reacts on the presence of Ca\textsuperscript{2+} ions, the higher intensity of fluorescence, the higher Ca\textsuperscript{2+} concentration [18], whereas the O\textsubscript{2} concentration analysis is based on reduction of $\tau_f$ that can be also used for imaging purposes [18].

Using modern microscopy technique gives the possibility to study cell structures, motility of cells and organelles, cell-cell communication and membrane potential in single cells. Microscopic techniques found important application in biomedical field (e.g., confocal endomicroscopy, oftalmology) [19, 20].

The live cells in vitro and in vivo imaging techniques accelerate drug discovery. Real-time imagine provides analysis of drug response upon target activity and pathophysiology and results in higher clinical predictivity [21]. Based on in vitro model, the monitoring of cellular phenotypes within complex samples such as co-cultures, 3-D culture models, is now possible. Cell attachment, migration (velocity, direction), vesicle formation, angiogenesis, stem cell differentiation can be recorded using automated imaging platforms [21, 22]. Some of them are based on the label-free phase holographic microscopy. In this technique, the low-power (635 nm) red diode laser divided into two beams—reference and an object beam—that passes through the unlabeled cell cultures on T-flask surface merged together can be recorded as the hologram imagines (Figure 3) [23–25].

Another microscope—the atomic force microscopy (AFM)—gives unique possibility to visualize structure, topography (Figure 4) and examine mechanical properties of cells (e.g., adhesion force distribution, cells stiffness—Young’s modulus as a biomarker of the relative metastatic potential). This method is a variant of scanning probe microscopy that demonstrated better resolution, than the optical diffraction limit [26].
From the ancient Romans, through the Middle Ages, to the late of the nineteenth century, the Aristotelian doctrine of spontaneous generation was one of the most basic laws in biological sciences [3]. This idea was presented for the first time by Aristotle in his History of Animals, where he described the generation of insects from animal flesh, mud, and other organic and inorganic matter [27]. According to this thesis, non-living matter (water, land or hay) bears the potential to generate spontaneously different and complex organisms. For example, in the seventeenth century literature, recipes for mice were known—the mixture of old shirts and wheat placed in a jar for 21 days produced mice [28, 29]. Even the invention of the microscope and investigations of Leeuwenhoek and Hook did not refute the Aritostelian doctrine. The existence of micro-organism—protozoa, and unicellular living organisms was a specific link between the inanimate substance and living organisms, and perversely, supported the spontaneous generation doctrine [3].

The first attempt to verify the idea of spontaneous generation was made by an Italian physician Francesco Redi (1626–1697). In 1668, Redi tested his hypothesis (described in the “Experiments on the Generation of Insects”) that maggots did not arise spontaneously in decaying tissues.


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New Insights into Cell Culture Technology
History of Cell Culture

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