

## Cell Culture Media Chronicle

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Cell culture media has been one of the key factors in cell culture technology since media supports cell survival and proliferation, as well as cellular functions. The quality and characteristics of a medium directly impact the research experimental results, the biopharmaceutical production rate, even the treatment outcomes of patients. Here we summarized the chronological development of balanced saline solution, natural media, synthetic media, classical media, serum free media, chemically define media, industrial media, protein free media, and animal component free media. This review has provided the information needed to facilitate investigators to decide an appropriate medium according to their aims.

**Keywords:** Chronological Development; Classical Media; Natural Media;  
Synthetic Media; Serum Free Media.

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The impact of cell culture technology on human society has been immeasurable. Currently, cell culture-based technologies are essential for protein base therapeutics, gene therapy and cell therapy.<sup>1-3</sup> Cell culture media is one of the key factor in cell culture technology because media supports cell survival and proliferation, as well as cellular functions.<sup>3</sup> The quality of a medium directly impact the research experimental results, the biopharmaceutical production rate, and the treatment outcomes of patients. Therefore, it is essential for investigators to select an appropriate medium for their specific aims. In order to accelerate researchers' familiar process of the cell

culture media, this review chronologically account for experiments that are indispensable to develop animal-cell culture media. This review should be able to provide the prospective types of media regarding their characteristics and components.

### 1882: Balanced Salt Solution

In 1882, Sydney Ringer developed a balanced saline solution with its composition comparable to that of bodily fluids. This solution enable frog hearts beating after removal from the body.<sup>4</sup> Several balanced saline solutions were then developed to cultivate animal tissues.<sup>5-7</sup> Although the compositions of these balanced salt solutions are simple, their compositions are chemically



defined and their pH, osmotic pressure, and inorganic salt concentrations were calibrated to physiological conditions that enable tissues and cells survive outside the body for short periods<sup>8</sup>. These balanced saline solutions are considered the foundation of modern culture media.

#### **1907: Natural Media**

In 1907, Ross G. Harrison successfully grew nerve fibers of a frog in the fresh lymph extract.<sup>9</sup> Using similar strategy, Burrows then used plasma instead of lymph fluid for the cultivation of animal cells. Thereafter, blood plasma had become a major culture medium component for most animal cells<sup>10</sup> including mammalian cells.<sup>11</sup> Natural media, such as lymph, plasma, and embryonic extract etc., were later identified as facilitating agents for cell culture, and used as culture media.<sup>10,11</sup>

#### **1911: Synthetic Media**

Since the composition of the lymph, plasma, and embryonic extract was not defined, researchers attempted to replace the natural media with ingredients of definite composition by identifying the growth-promoting substances within these natural media. In 1911, Margaret R. Lewis and Warren H. Lewis demonstrated that addition of amino acids, bouillon, and glucose (or maltose) into their balanced saline solution can improve the chick embryo cell cultivation.<sup>12,13</sup> Following years, more researchers identified additional components that promote the performance of media. These components contain vitamin A, ascorbic acid, vitamin B1, vitamin B2, glutathione, and blood plasma.<sup>14</sup> Nevertheless, natural media are still needed to supplement these synthetic media for optimizing cell culture performance.

#### **1948: Classical Media**

In 1948, Fischer demonstrated that cells were supported by adding defined amino acids to dialyzed serum<sup>15</sup> Following these findings, Harry Eagle then developed the minimum essential medium (MEM) by studying the amino acid requirements of different cells in 1955. Eagle's findings are significant due to the versatility of MEM toward varieties of cells. Hence, MEM set up a foundation for several classical media such as Dulbecco's modified MEM (DMEM) etc.<sup>8</sup> In 1958, McCoy used the serum prepared by Fischer's method to develop Roswell Park Memorial Institute (RPMI) modified the 5A medium that for

carcinoma cells.<sup>16</sup> These studies defined that serum is used as a media supplement to improve the performance of different types of cell culture.

#### **1950 Serum Free Media**

In 1950, Raymond C. Parker developed Connaught Medical Research Laboratories (CMRL) 199 by adding low-molecular-weight chemical substances instead of serum.<sup>17</sup> Using this medium (CMRL-199), chick embryo-derived cells were able to culture for 3-4 weeks without serum.<sup>17</sup> This coined the first serum free media was developed. Since most of the serum-free media were developed in mouse L929 cells, they were not necessarily suitable for the serum-free culture of other cell types.<sup>8</sup> In 1963, Richard G. Ham developed Ham's F-10 medium by replacing serum with albumin and fetuin to grow a single Chinese hamster ovary (CHO) cell to form a colony under serum-free conditions.<sup>18,19</sup> Ham's studies are significant due to the commonly used of CHO cells in current biopharmaceutical industry.

#### **1956 Chemically Define (CD) Media**

In 1956, Wilton R. Earle at the National Cancer Institute's Tissue Culture Section (NCTC), developed a chemically defined medium NCTC109.<sup>20</sup> In 1959, Charity Waymouth developed the MB 752/1 medium to simplify the number of ingredients for easier formulation.<sup>21</sup> Since NCTC109 and MB 752/1 have been designed for the mouse L929 cells, other cell types are still lack chemically define media for their culture. In 1963, Ham modified Ham's Media F10 to develop a chemically defined Ham's F12 medium by replacing the undefining ingredients with low-molecular-weight substances.<sup>18,19</sup> In 1976, Izumi Hayashi and Gordon H. Sato found that combinations of several hormones and growth factors are effective serum substitutes.<sup>22</sup> Following this study, many researchers attempt at developing CD media to substitute serum for their specific cell type of interest.

#### **1986 Industrial Cell Culture Media**

In 1986, the first therapeutic protein, recombinant tissue-type plasminogen activator (tPA), was obtained in the culture of immortalized CHO cell line.<sup>23,24,3</sup> Soon after, many other recombinant protein pharmaceuticals such as erythropoietin, interferon  $\beta$ , and monoclonal antibodies are expressed in different mammalian cell lines for therapeutic purposes.<sup>23,3</sup>

These cell lines included, CHO cells, mouse myeloma (NS0), baby hamster kidney (BHK), and human embryo kidney (HEK-293) etc.<sup>23,25,3</sup> Although the media used in these industrial production processes are proprietary, one can speculate that these therapeutics were first produced by using classical media supplemented with serum, and then went through the development from classical media to CD media.<sup>26</sup>

### 1993 Protein Free Media

The protein in the media can sometime affect downstream purification and then cause unwanted cell activation in the biotherapeutic manufacturing process.<sup>27</sup> In 1993, a protein free media was developed by replacing protein with various trace elements stabilized by metal chelators.<sup>28</sup> Various other protein free media were then developed for laboratory and industrial usages.

### 1996: Animal Component Free Media

Following the mycoplasma contamination, endotoxin concerns, and bovine spongiform encephalopathy (BSE) scares, the biopharmaceutical industry demand origin of raw materials used in the manufacture of therapeutics to be free of animal. Cell culture media suppliers are often asked for documentation of the animal-component free (ACF) status of media and reagent components.<sup>29</sup> It should be noted that the industrial media developments are not disclosed, and the development of these media are speculated by the dated publications.<sup>30,31</sup>

## CONCLUSION

Since Ringer's first successful cultivation of cells in balanced salted solution, cell culture technology has developed quickly with many breakthroughs.<sup>3</sup> It should be emphasized that the current culture media and their formulations are developed by timeless efforts of many researchers who cannot be all referenced in our current review. The continuous improvement of cell culture media performance along with the innovation of cell culture equipment make it possible for many scientists to work with cell culture conveniently and systematically. The cell culture technologies development is expected to be continued, and intensified in the future since significant progress was made in regenerative medicine, biopharmaceuticals cosmetic medical

procedure, and cell base agriculture.<sup>32,34,33,8,3</sup> While the trend of cell culture development intensified, the culture medium is still the key to any kind of cell culture experiment breakthrough. This review could provide the chronologically description for researchers to obtain insights into understanding the development of diverse types of cell culture media

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