Mammosphere Culture Supports Short But Not Long-Term Propagation of Human Mammary Epithelial Progenitors

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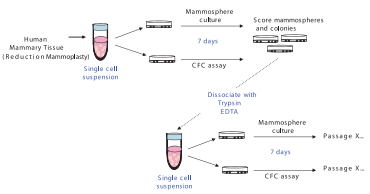
Introduction

Investigation of mammary stem cells and their regulation has been greatly limited by a lack of methods to isolate and propagate these cells in vitro. Recently, evidence has demonstrated that primitive human mammary cells may be cultured in suspension as multicellular clusters, or "mammospheres" (1,2). Mammospheres were reported to include cells whose phenotypic and functional properties indicated their primitive bi-potential origin. Presence of primitive cells has been detected over multiple generations of mammospheres, indicating the potential of this system to preserve cells capable of self-renewal. In this study we have looked at a dynamic change in the number of mammosphere-forming units, colony-forming cells (CFC) as well as relative distribution of progenitors (luminal, myoepithelial and bipotential) over multiple generations of mammospheres. The purpose of the study was to evaluate the capability of this culture system to preserve primitive mammary epithelial progenitors.

Experimental Method

FIGURE 1: Media Evaluation and Long-Term Culture Analysis

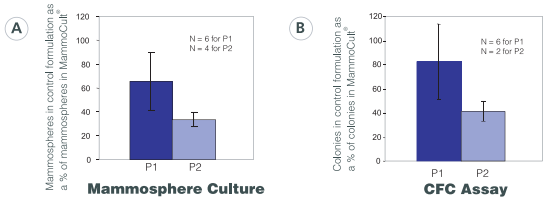


Single cells obtained from dissociated reduction mammoplasty samples were plated in mammosphere culture (containing MammoCult® or control medium*) and CFC assay in EpiCult®-B medium to measure progenitor content within the start population. After 7 days mammospheres were dissociated. A fraction of the sample was used for CFC analysis while the rest of the suspension was used to generate new mammospheres. This step was repeated every 7 days.

* Control medium was prepared according to Dontu et al. (1)

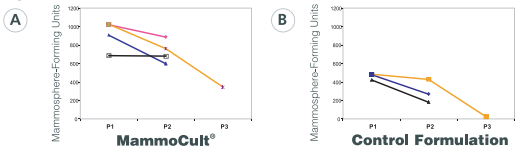
Results

FIGURE 2: Relative Numbers of Mammosphere-Forming Units and Colony-Forming Cells Detected in the Control Medium Compared to MammoCult®



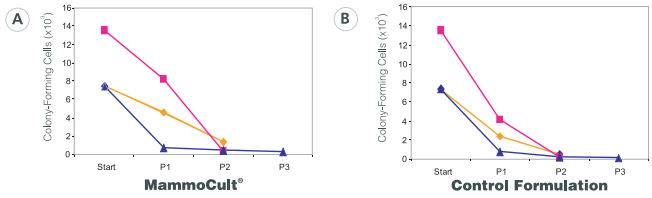
Over two passages MammoCult® medium produced higher number of mammospheres containing higher progenitor content compared to the control medium. A) Mammosphere numbers in control detected in two consecutive passages were divided by the respective numbers of mammospheres detected in MammoCult® and multiplied by 100%. B) CFCs detected in mammospheres generated in each passage (2 passages tested) in control medium were divided by the number of CFCs in mammospheres generated in each passage in MammoCult®.

FIGURE 3: Mammosphere-Forming Units Enumerated over Three Passages of Mammospheres



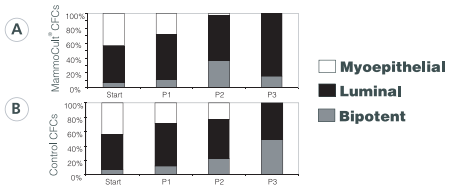
Absolute numbers of mammosphere-forming units detected over three passages in A) MammoCult® and in B) the control medium. The number of mammosphere-forming units declines over time. This decline is more dramatic in cultures maintained in the control medium. The frequency of mammosphere-forming units decreases to 33% in MammoCult® and 5.7% in the control medium.

FIGURE 4: Colony-Forming Cell Numbers Detected over Three Passages of the Mammospheres



Absolute numbers of CFCs detected in mammospheres cultured in A) MammoCult® or B) control medium at each passage for three passages. The number of colony-forming cells also declines over time suggesting that the mammosphere culture system does not allow for long-term propagation of mammary epithelial progenitors. This decline is more dramatic in cultures maintained in the control medium. Colony-forming cells detectable in mammospheres in passage 3 were reduced to 1.1% in the control medium compared to 2.6% in MammoCult®.

FIGURE 5: Distribution of Bipotent, Luminal, and Myoepithelial CFCs in Start Cell Population and Mammospheres Derived over Three Passages



A) Distribution of the three types of colony-forming cells detected in mammospheres generated in MammoCult® and B) control medium. In both media, luminal and mixed colonies can be detected in mammospheres generated in at least three passages while myoepithelial colonies seem to disappear after the second passage.

Conclusions

- MammoCult® is a specialized, defined, serum-free medium that supports formation of mammospheres from single-cell suspensions enriched in human mammary epithelial cells.
- In MammoCult®, cells from dissociated human mammary gland samples form clusters of >60µm diameter in size after 7 days in culture at a frequency of 0.51% ± 0.29 (n=15).
- Cells (clonal anaylsis not shown) from dissociated mammospheres have the ability to differentiate in standard colony-forming cell assay conditions and generate colonies containing both lineages of mammary epithelial cells.
- To date, MammoCult® is the best medium for the culture of mammospheres and maintenance of mammary epithelial progenitors.

Select Publications:

1. Dontu et al., 2003. Genes & Dev. 17: 1253-1270
2. Ginestier et al., 2007. Cell Stem Cell 1: 555-567