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Semi-Annual Status Report

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Grant Period: January 1, 1993 to December 31, 1995

Principal Investigator: Andrew O. Martinez, Ph.D.
Associate Professor of Life Sciences
(210) 691-4184
FAX (210) 691-5765Institution: The University of Texas at San Antonio
7000 North Loop 1604 West
San Antonio, Texas 78249Project Title: Monoclonal Antibodies Directed Against Surface
Molecules of Multicell Spheroids

Reporting Period: January 1, 1993 to June 30, 1993

The objective of this project is to generate a library of monoclonal antibodies (MAbs) to surface molecules involved in the cell-cell interactions of mammalian cells grown as multicell spheroids (MCS). MCS are highly organized 3-dimensional multicellular structures which exhibit many characteristics of *in vivo* tissues not found in conventional monolayer or suspension culture. They also provide a functional assay for surface adhesion molecules. In brief, MCS combine the relevance of organized tissues with the accuracy of *in vitro* methodology. Further, one can manipulate these MCS experimentally to discern important information about their biology.

Studies and Results

The specific aim for the first year of the grant is to generate hybridomas producing MAbs directed against surface molecules of human and hamster MCS. To this end, during the reporting period (1) a research technician and two students were recruited to work on the project, (2) a subtractive immunization scheme to enhance the production of MAbs to MCS surface molecules was designed, and (3) mice were immunized, fusions performed and hybridomas producing antibody to MCS surface molecules generated

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and isolated.

The subtractive immunization scheme involves a two-step procedure. First, mice are given multiple injections of a suspension of single cells (tolerogen) followed by injection of the immunosuppression drug cyclophosphamide. Cyclophosphamide selectively kills off B-cells that have been stimulated to proliferate in response to surface antigens on single cells. Following cyclophosphamide treatment, subsequent exposure to these antigens results in no immunological response. Second, after the drug has been allowed to clear, the mice are given multiple injections of MCS (immunogen). Theoretically, the only antigens to which the immune system should generate antibodies after exposure to the immunogen are those molecules present in the immunogen (MCS) but not present in the tolerogen (single cells).

Two groups of five to seven week old RBF/DN mice were immunized using the subtractive immunization scheme outlined above. Monolayer cultures of WI38SV40 (human) or B14I50 (hamster) cells were trypsinized, washed twice with sterile PBS, and injected i.p. At 15 min, 24 hrs, 48 hrs, and 72 hrs the mice were given cyclophosphamide injections i.p. at a concentration of 100 mg/kg. This immunosuppression regime was repeated four times at two week intervals. Two weeks following the fourth suppression immunization, the mice were injected i.p. with seven day WI38SV40 or B14I50 MCS at a concentration of 1×10^6 MCS per ml. A boost immunization of MCS was given two weeks later. Serum was obtained from each mouse three days after the boost immunization and titered for antibodies to MCS surface antigens. Mice which exhibited high titers were killed by cervical dislocation, spleens removed, and fusions performed. Over 1000 hybridomas have been produced from the first series of fusions. We are now in process of screening these hybridomas to identify those producing MAb to MCS surface molecules.

Eighteen hybridomas producing antibody of interest (bind to points of cell-cell contact) have been identified. Representative photomicrographs showing binding patterns of these antibodies on human (WI38SV40) or hamster (B14I50) cells are presented (see Appendix). Note that the MAbs primarily bind to points of cell-cell contact. However, some photomicrographs show a binding patterns consistent with the presence of more than one type of MAb in the hybridoma supernatant. We are now in the process of cloning and subcloning these hybridomas to generate

hybridomas producing monospecific antibody.

Significance

The generation of MAbs which bind to points of cell-cell contact in MCS is a significant accomplishment because these MAbs will provide the experimental material necessary to conduct the studies proposed for the second and third year of the grant. Additionally, it is possible that these MAbs identify surface molecules involved the cell-cell interactions (aggregation, compaction, metastasis) of tumor and transformed cells.

This project relates to NASA's interest in Biotechnology and Cell Science Research and involves investigations of cell-cell interactions of tumor and transformed cells grown as MCS in a gravity-based environment. The results obtained will provide a base of scientific information necessary to expand the focus of this research to investigations of cell-cell interactions in microgravity- and hypergravity-based environments.

This project also has the potential to yield important materials (e.g. cell model systems, monoclonal antibodies, cellular factors) which could be of biomedical and/or research interest to NASA.

Plans

The generation of MAbs to MCS surface antigens using the subtractive immunization scheme described above has proved to be very labor intensive and time consuming. During the remainder of the first year of the grant, we will continue to generate MAbs to MCS surface molecules using the subtractive immunization scheme in combination with single cells or cell-aggregates as the tolerogen and MCS as the immunogen.

In the second year of the grant, we will initiate studies to characterize selected MAbs from the library. Using MAb-mediated immunofluorescent microscopy, ELISA, and flow cytometry, we will determine the binding pattern of MAbs and quantitate the distribution of surface molecules on panels of tumor, transformed and normal cell lines. In addition, using biological functional assays, we will determine whether the MAbs or Fab-fragments block/promote cell-cell interactions, and whether surface molecules recognized by MAbs are cell adhesion molecules (CAMs).

Student Development

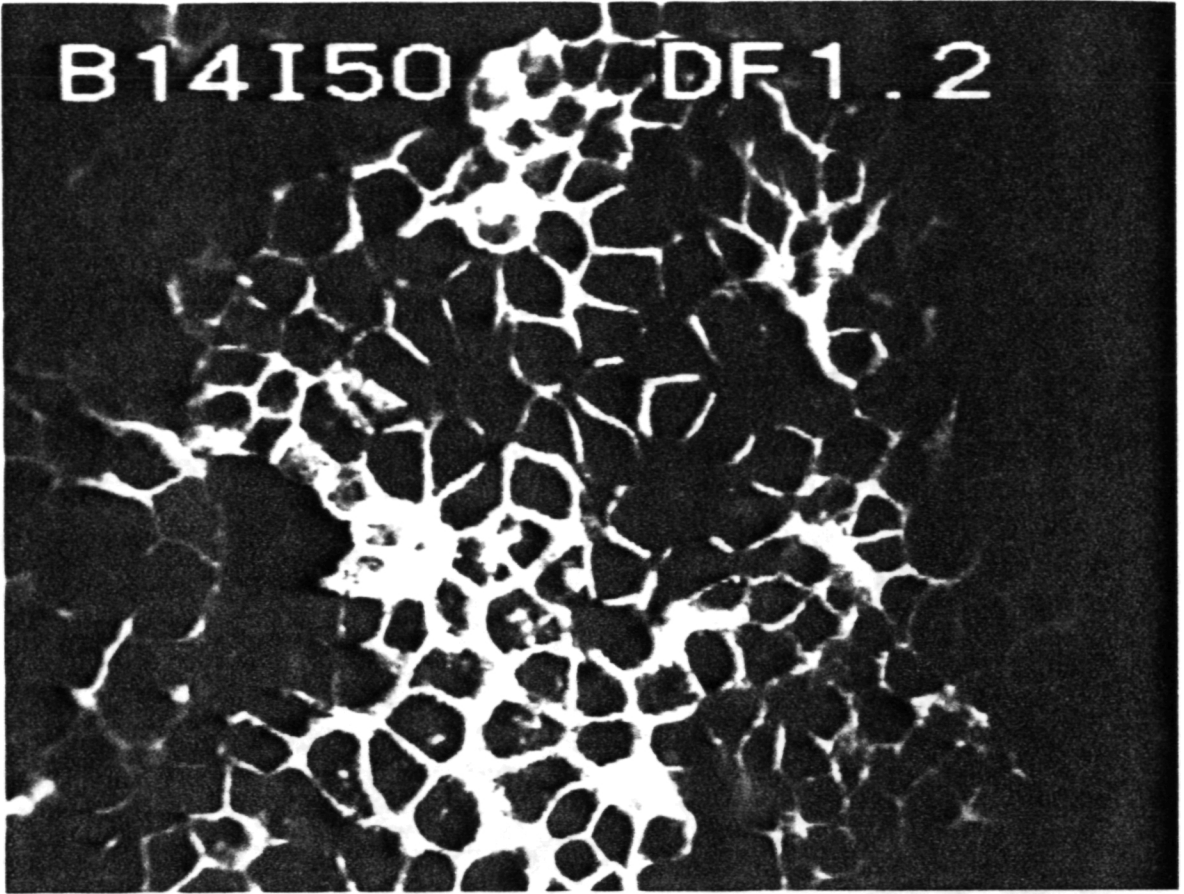
This project supports the training of two underrepresented minority students in biotechnology and cell science research. relevant to NASA's mission. Thus, this project will assist in developing a pool of minority graduates in research areas relevant to NASA's mission. Two underrepresented minority students, one undergraduate and one graduate, participated in the project during the reporting period.

Sandra Garcia (Hispanic) is an undergraduate biology student. She started working on this project in January 1993. During the reporting period, she gained experience with a number of experimental techniques, including cell culture, subtractive immunization, cell fusion, single-cell cloning, fluorescent microscopy, and MAb isolation and purification. She also assisted the PI and research technician with immunizations, cell fusions, and hybridoma production. In addition, she independently isolated several hybridomas producing MAbs to surface molecules of B14150 MCS. She will present results of this work at a national NSF meeting to be held in Washington, D.C. in October 1993. She was also the recipient a prestigious research award presented by the Cancer Federation to a University of Texas at San Antonio undergraduate student conducting cancer-related research. Sandy will continue to work on this project during the second year of the grant. She expects to graduate in August 1994.

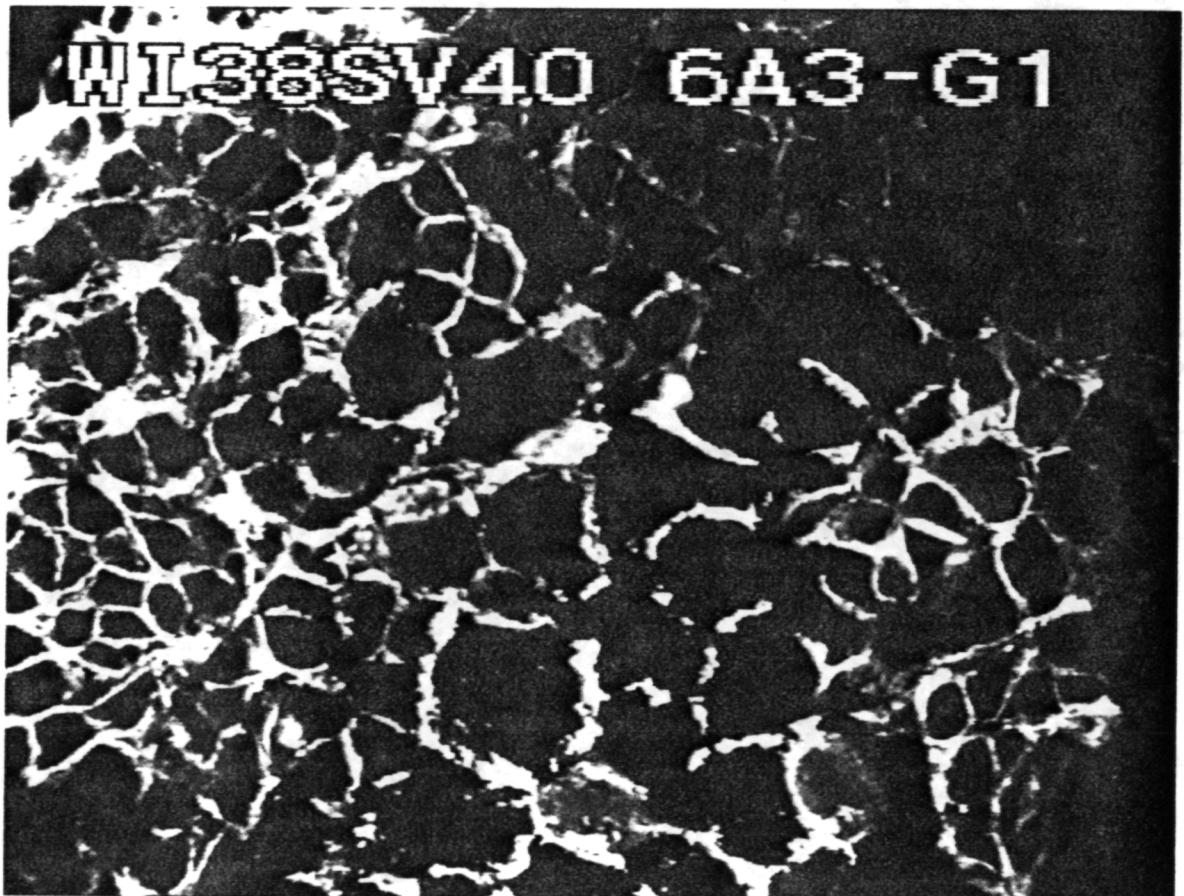
Cynthia Cantu (Hispanic) is a graduate biology student. She also started working on this project in January 1993. During the reporting period, she learned and gained experience with a number of experimental techniques used in hybridoma production and MAb research. In addition, she was involved in developing protocols for extracting membrane proteins using detergents and for fractionating membrane proteins by electrophoresis, and for determining MAb-surface protein interactions by Western blotting. This work will form the basis of her M.S. thesis research project. Cynthia has already completed all coursework required for the M.S. degree in Biology. She will concentrate on her thesis research during the second year of the grant. She expects to graduate in May 1994.

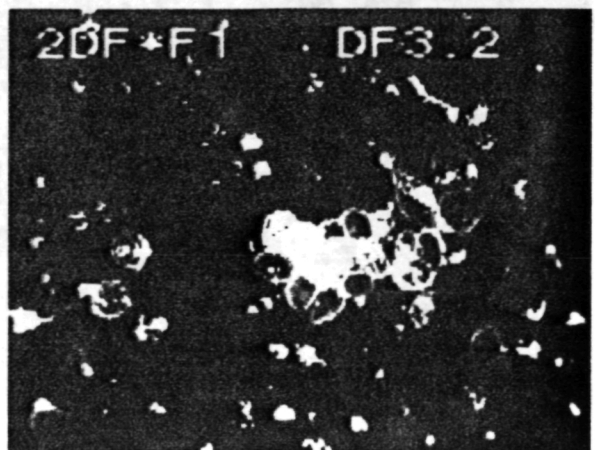
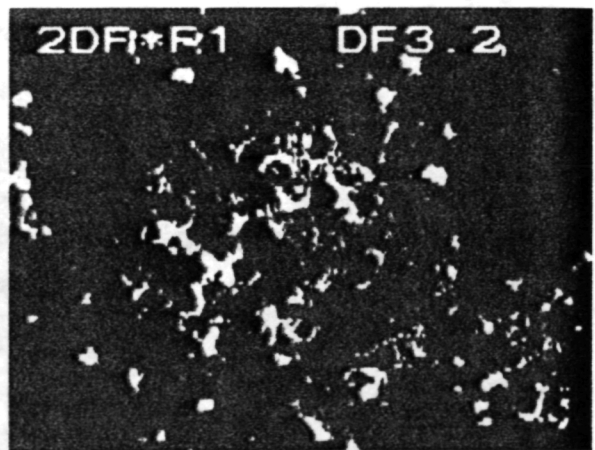
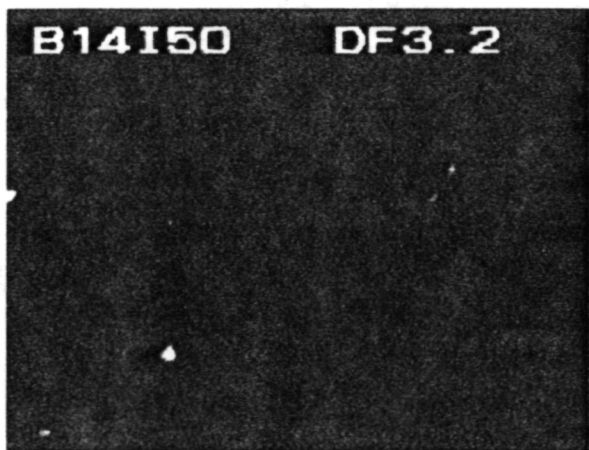
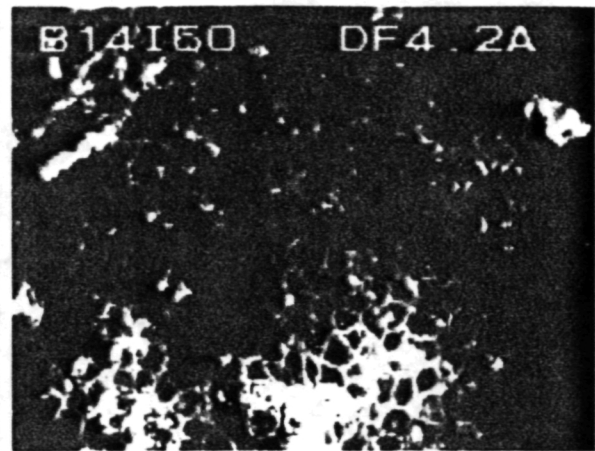
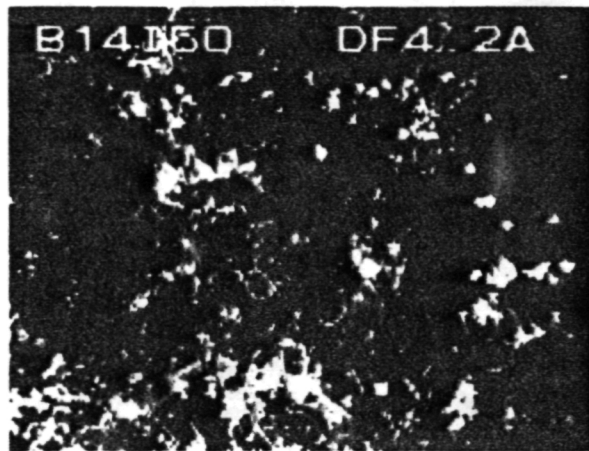
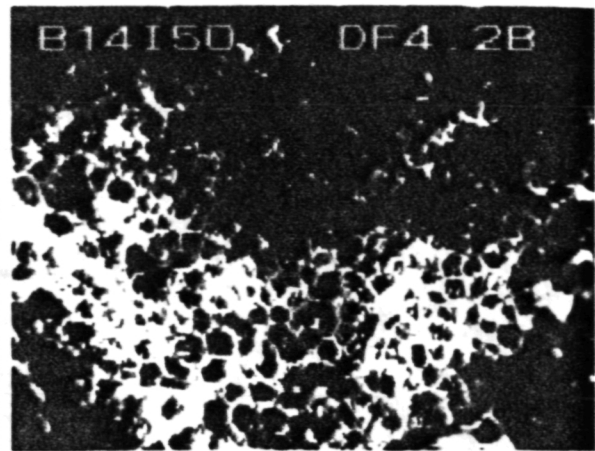
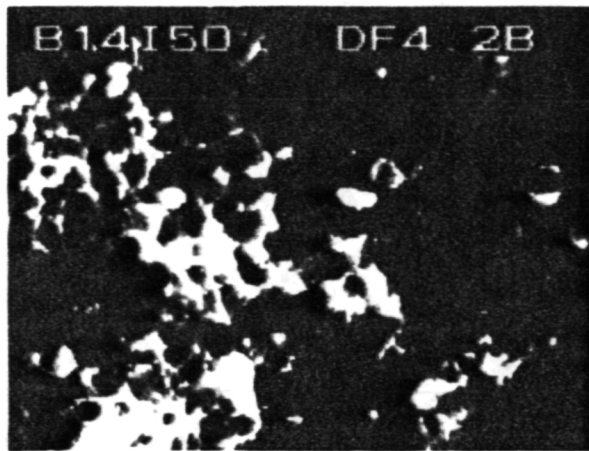
Appendix

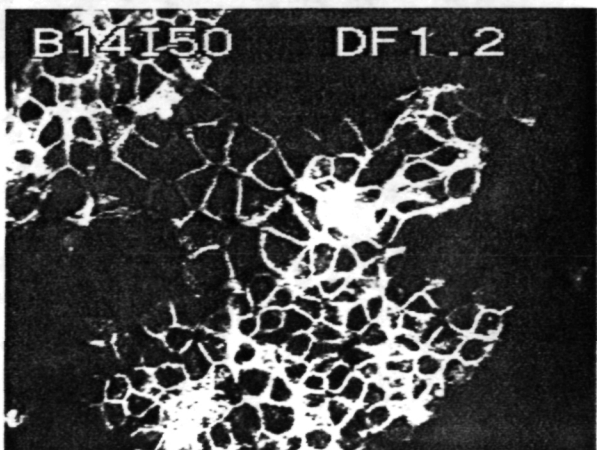
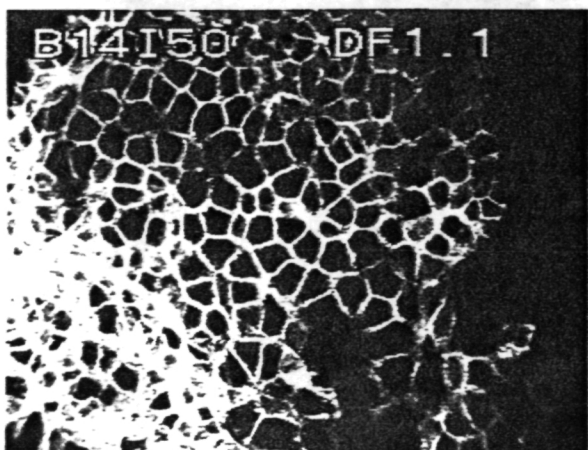
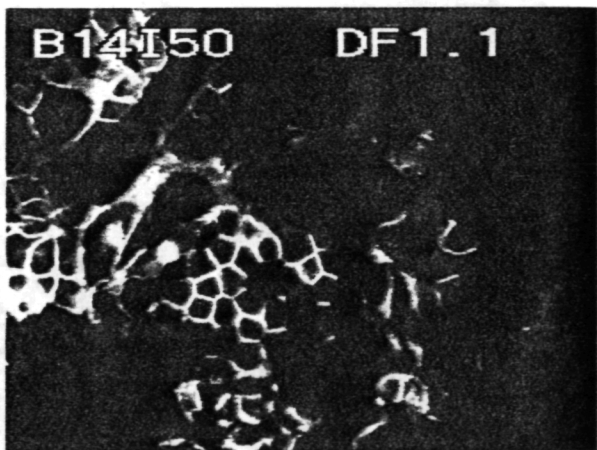
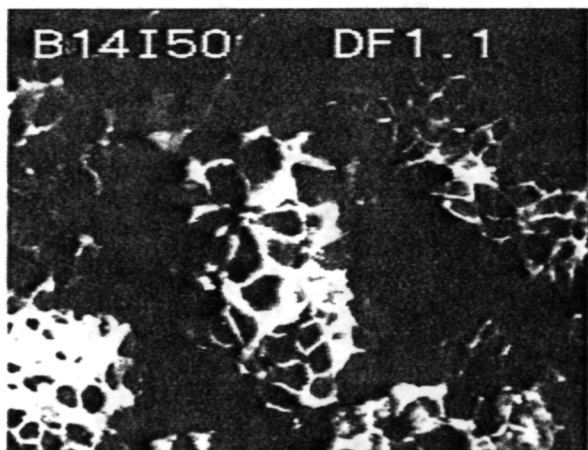
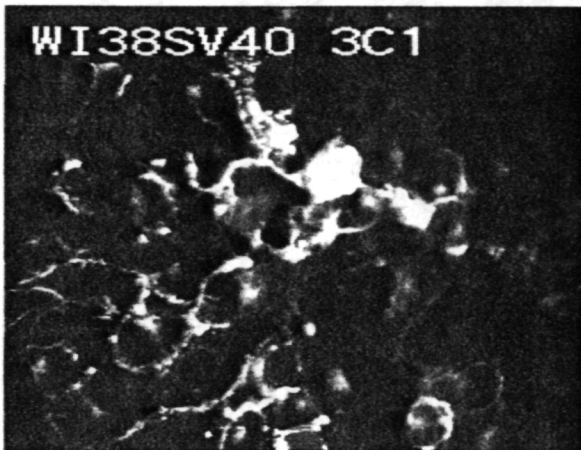
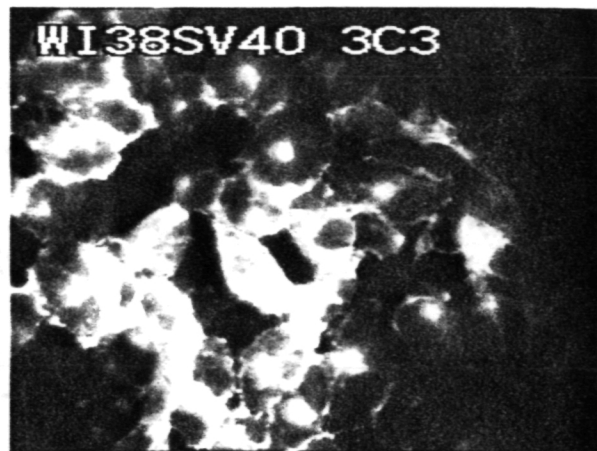
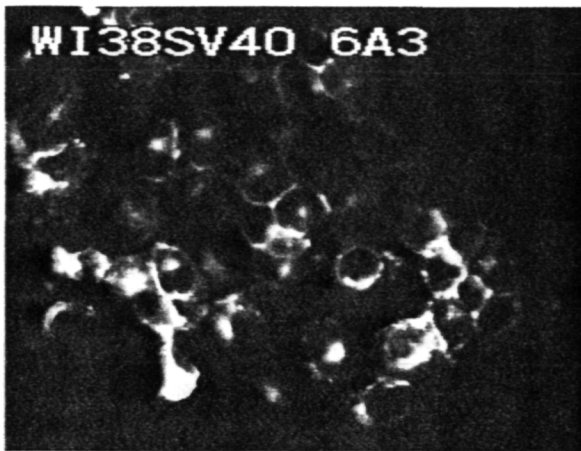
B14I50 DF1.2



WI38SV40 6A3-G1







WI38SV40 8E10



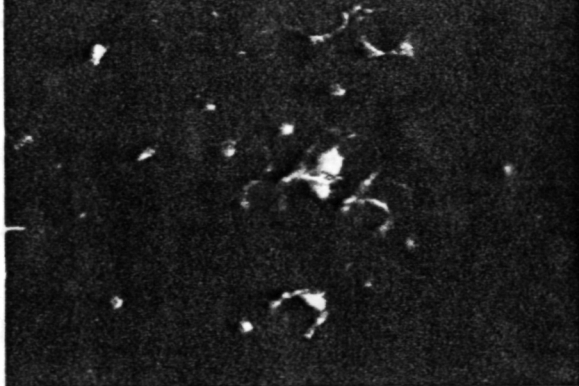
WI38SV40 9H12



WI38SV40 10D8



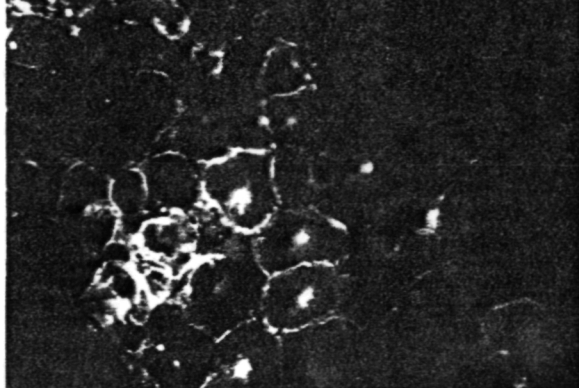
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WI38SV40 TM2C5



WI38SV40 TM8E3



WI38SV40 TM7H2

