Effective Antibody Production by Reusing Culture Medium Previously Used in Antibody Purification

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Culture medium used in antibody purification contains growth factors, but is usually discarded after antibody purification. We examined to determine whether the growth factors can be (i) recovered and (ii) used in subsequent cell culture. Using this medium enhanced the survival of hybridoma cells and improved antibody production. Culture medium previously used in antibody purification offers an attractive resource for mammalian cell culture.

Key words: hybridoma; monoclonal antibody; spent medium; transferrin; insulin

Many monoclonal antibodies are produced by mammalian cells such as hybridomas. In the manufacturing field, these cells are cultured in serum-free medium, because using serum carries the risk of contamination by prions, viruses, and mycoplasmas.¹ But serum-free medium requires the addition of costly growth factors to maintain cellular function. In particular, transferrin and insulin are essential for the production of antibodies; transferrin functions as an iron transporter and a mitogen, while insulin improves glucose uptake. In their absence, hybridoma cells fail to proliferate or to produce antibodies.² Some of these growth factors remain after cell culture, but the spent culture medium is typically discarded. Riese et al. performed a pilot-scale culture and reported that the spent culture medium was useful in the production of mouse monoclonal antibodies when it was mixed with additional amino acids and glucose, after purification by microfiltration and ultrafiltration.³ They concluded that using a spent medium helped save pure water and supplemented proteins such as transferrin and insulin, and that it was effective at decreasing the volume of waste water. In industrial antibody production, the antibody purification process is carried out after the culture process and growth factors might lose their bioactivities during pre-treatment, due for example to condensation, change in pH, or the addition of salt. Therefore, additional treatment steps are necessary to recover the bioactivity of the residual growth factors.

Generally, mammalian cells synthesize and release various bioactive factors, including autocrine or paracrine growth factors, which maintain cellular functions and promote cell differentiation. Used culture medium is rich in such factors, and it is often used as a conditioned medium. For example, human embryonic stem cells can be cultured in mouse embryonic fibroblast-conditioned medium.⁴ However, the use of conditioned medium is limited to small-scale culture owing to difficulties in quality control.

In this study, we focused on recycling culture medium that was previously used in antibody purification as a source of supplements, and evaluated methods of recovering the bioactivity of the growth factors contained in the used medium. A mouse hybridoma cell line, 2E3-O, which produces the mouse IgG1 antibody specific for trinitrophenyl hapten,⁵ was used as a model of antibody production.

First the culture medium, previously used in antibody purification, was prepared as follows: 2E3-O cells were seeded on 90-mm culture dishes (Sumitomo Bakelite, Tokyo) at 22,000 cells/ml and cultured in a commercially available serum-free medium, ASF104 (Ajinomoto, Tokyo), at 37 °C in humidified air containing CO₂ at 5%. Two formulations of ASF104 are available: a basal medium containing glucose, amino acids, and vitamins, and a completely supplemented medium that also contains insulin and transferrin. An advantage of using ASF104 is that it is easy to change the supplement amounts. In our study, the concentrations of transferrin and insulin in the completely supplemented ASF104 were both adjusted to 5 mg/l. On day 4 of culture, with a viable cell number of 708,000 cells/ml, the culture supernatant was collected and loaded onto an affinity protein G column (GE Healthcare UK, Buckinghamshire, UK) to purify the mouse monoclonal IgG. The flow-through fraction in this process, which contains various materials that do not attach to the column, is referred to as the recycled culture medium. The recycled culture medium was concentrated 12-fold by ultrafiltration using Amicon Ultra-15 centrifugal filter units (Millipore, Billerica, MA), and then it was diluted to one-eighth concentration using fresh basal ASF104 medium. This is referred to as the recovered medium.

SDS–PAGE analysis was carried out to determine whether transferrin remained in the culture medium after antibody purification. As shown in Fig. 1, a band

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corresponding to transferrin was detected not only in the fresh completely supplemented medium but also in the culture supernatant of 2E3-O cells before antibody purification, and in the recycled culture medium obtained by purification. However, the insulin content in the recycled culture medium could not be estimated by SDS–PAGE. A band corresponding to the light chain (lower 30 kDa) of the monoclonal mouse antibody was detected in the recycled culture medium, even though no band corresponding to the heavy chain was detected. This result suggests that 2E3-O cells produced greater amount of the light chain than of the heavy chain, and that the affinity protein G column captured only the complete antibody, a complex of the heavy and the light chains, while the excess light chain remained in the recycled medium.

Next, to test whether the recovered medium was effective as a supplement for increasing cell survival and antibody productivity as compared with the fresh supplemented ASF104, the recovered medium was used in the subsequent culture of 2E3-O cells. Based on SDS–PAGE analysis, it was assumed that the amount of transferrin in the recycled medium was one-third of that in the completely supplemented ASF104 medium. At 20 h, 2E3-O cells died in the basal ASF104 medium (Fig. 2), but they survived over this culture period when they were cultured in the recovered medium. Furthermore, the cells had proliferated by 25-fold at 68 h in the presence of the mixed supplement, i.e., the recovered medium was mixed with one-third of the complete amount of the fresh supplement. The final viable cell numbers in this culture condition were similar to those of the completely supplemented ASF104 containing both transferrin and insulin at 5 mg/l. These results indicate that the recovered medium is effective for cell survival but does not promote cell proliferation.

The amount of antibodies produced was also measured by ELISA (Fig. 3). After 2E3-O cells were cultured for 68 h under various medium conditions including the recovered medium supplemented with and without the fresh supplement, the culture supernatants were collected and the antibody amounts were measured. When 2E3-O cells were cultured in recovered medium with one-third of the complete amount of the fresh supplement, the concentration of mouse IgG was significantly higher than when the cells were cultured in completely supplemented ASF104. In addition, the recovered medium appeared to contain a large amount of the IgG light chain based on the results in Fig. 1, but we have reported that the ELISA system was not affected by the light chain, and we were able to determine the concentration of intact molecule correctly.5,7) The transferrin concentration in the recovered medium appeared to be reduced to half of that in the fresh ASF104 containing 5 mg/l transferrin; therefore, the total amount of transferrin in the recovered medium supplemented with one-third of the complete amount of the fresh supplement was calculated to be 4 mg/l, less than the concentration of transferrin in the fresh ASF104 (5 mg/l), suggesting that some factors other than transferrin in the recovered medium enhanced antibody productivity. On the other hand, insulin was not involved in cell proliferation or antibody production. Insulin might not have been captured, because the cut-off molecular weight of the ultrafiltration unit we used was 5 kDa, which is slightly smaller than the insulin molecule, and insulin was not detected in the recycled medium by SDS–PAGE (data not shown). As shown in

![Fig. 1. SDS–PAGE Analysis of the Recycled Cultured Medium, Previously Used in Antibody Purification.](image1)

![Fig. 2. Effect of Recovered Medium on the Growth of Mouse Hybridoma 2E3-O Cells.](image2)
In conclusion, recovered medium prepared from the culture medium, which was previously used in antibody purification, was effective in promoting cell survival as well as in increasing antibody production, although it did not completely overcome the need for additional supplements. To improve the efficacy of recovered medium, various ultrafiltration units of appropriate pore size or materials to reduce the loss of bioactive molecules during the condensation of the recycled culture medium should be used. Nevertheless, reusing culture medium obtained after antibody purification should contribute not only to reducing the cost of preparing culture medium but also to improving antibody production.

**References**


