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REGULAR ARTICLE

A new convenient technique for making cell blocks

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Abstract Cell block sections serve as an important diagnostic annex for cytological smears, liquid-based SurePath cytology and the Liquid-based Thin-prep Cytology Test (TCT). A variety of methods for the preparation of cell blocks are described in the literature and the techniques in cell blocks are in continuous improvement. A new technique for making cell blocks was introduced in the present study. We first used pregelatinized starch as the frame for the cell block, which is a really simple and economic method, because it can be carried out at room temperature without additional special instruments. We have performed hematoxylin and eosin (HE) staining, immunohistochemistry analysis and fluorescence in situ hybridization (FISH) in the cell block sections in 122 cytological specimens. The results demonstrated in this article show that pregelatinized starch is a useful frame for cell blocks. The pregelatinized starch can effectively collect even a few cells with powerful adhesiveness. Therefore, this new technique for making cell blocks is especially useful for cytologic samples with low cellularity, such as cerebrospinal fluid specimens.

Keywords Body fluid · Cell block · Pregelatinized starch · Cytology

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Introduction

Cell block sections serve as an important diagnostic annex not only for cytological smears, liquid-based SurePath cytology and the Liquid-based Thin-prep Cytology Test (TCT) but they are also used today as a reliable preparation for immunohistochemical (IHC) studies, which is especially useful in delineation of the primary origins of tumor cells in effusion fluid cytology specimens (Wagner et al. 2010). Furthermore, molecular pathological analysis can be performed in cell block sections (Collins et al. 2011; Ouansafi et al. 2010). A variety of methods for the preparation of cell blocks have been described and the techniques are in permanent improvement. Techniques for making cell blocks include direct embedding of the precipitate after centrifugation and cell block adjuvant-assisted embedding. When the quantity of the specimen is scarce, the direct embedding of the precipitate after centrifugation is difficult or impossible to perform and for that reason cell block adjuvant-assisted embedding is almost compulsory.

To the authors' knowledge, there are several cell block adjuvants that have been used to date including: agar, thrombin, egg albumen and so on. However, the above-mentioned adjuvant usually requires complex preparation. Recently, a cell block method using sodium alginate has been claimed to be simple and inexpensive (Noda et al. 2010). Nonetheless, using sodium alginate is still a time-consuming method when compared with the new technique for making cell blocks described in this study.

The pregelatinized starch was first used as the frame of the cell block, which is really simple and inexpensive, since this method can be carried out at room temperature without additional special instruments. We have performed hematoxylin and eosin (HE) staining, immunohistochemistry analysis, or fluorescence in situ hybridization

(FISH) in the cell block sections prepared with this technique in 122 cytological specimens, including pleural fluid, ascites fluid, cerebrospinal fluid and several more. The results demonstrated that pregelatinized starch is a useful frame for cell blocks, with simple manipulation and a clear background in the sections of HE staining and immunohistochemistry analysis. Especially, the recent review concluded that cell blocks can be prepared from all types of cytological specimens, except preparations with low cellularity such as cerebrospinal fluids (Skoog and Tani 2011). However, the pregelatinized starch can effectively collect even a few cells with powerful adhesiveness. Therefore, this new technique for making cell blocks is especially useful for cytologic samples with low cellularity, such as cerebrospinal fluid specimens.

Materials and methods

Case selection

In the present study, 122 cell blocks were obtained from nongynecologic cytology specimens between June, 2010 and March 2012 in the pathology department of Guangdong Provincial Hospital of TCM with the approval of the IRB of Guangzhou University of Chinese Medicine and prepared according to the undermentioned protocols. The specimens include pleural fluid, ascitic fluid, cerebrospinal fluid, hydatid fluid from mammary gland and several more. The array of non-gyn specimens evaluated are listed in Table 1. Each of the patients gave an informed consent.

Table 1 Clinic-pathological data of 122 cases of non-gyn specimens evaluated

Clinicopathological characteristics	
Sex (*n* %)	
Male	50 (40.98)
Female	72 (59.02)
Age (years), mean ± SD (range)	58.75±14.81 (20-87)
Specimen type (*n* %)	
Pleural fluid	66 (54.10)
Ascitic fluid	28 (22.95)
Hydatid fluid from mammary gland	6 (4.92)
Cerebrospinal fluid	6 (4.92)
urine	6 (4.92)
Other	10 (8.20)
Diagnosis (*n* %)	
Malignant	92 (75.41)
Benign	12 (9.84)
Nondiagnostic	18 (14.75)

Protocols for making cell block sections using pregelatinized starch

1. Liquid-based cytology specimen (not less than 5 ml) is placed in the tube.
2. Centrifuge the tube at 2,000 rpm for 5-10 min.
3. Pour off the supernatant and add 10 % neutral formalin to the precipitate for 5-10 min.
4. Centrifuge the tube at 2,000 rpm for 1 min and decant the supernatant.
5. Add the pregelatinized starch to the precipitate according to 1:1 volume ratio and mix them with a glass rod, whereupon the pregelatinized starch will immediately encapsulate the precipitate with a little of the residual supernatant. Roll the pellet with a glass rod until it is thoroughly wrapped by the starch powder. Alternatively, remove the precipitate with the residual supernatant using a suctionpipe and directly drip it into the pregelatinized starch powder in one pellet, when the pregelatinized starch will encapsulate the precipitate immediately, wrap the pellet in the pregelatinized starch quickly just like rolling a snowball. Note: Do not wrap too much pregelatinized starch or wrap the pellet in the pregelatinized starch powder for too long.
6. The solid pellet (pregelatinized starch encapsulated cell specimens) is then placed in a labeled cassette and submitted for tissue processing to prepare paraffin-embedded cell blocks as a standard histology specimen.

A comparison of the agar method for cell block preparation was performed in some cases to demonstrate that the pregelatinized starch technique is as good as what is already available with respect to morphology as well as phenotypic and molecular analyses. The agar method for cell block preparation was performed according to conventional standard laboratory protocols.

HE staining and Immunohistochemistry analysis on cell block sections

Additional immunohistochemical studies were performed in some malignant cases for further identification of the neoplasm, such as carcino-embryonic antigen (CEA), cytokeratin (CK), P53, CD15 and several more. A simultaneous Biopsy specimen from the same patient as well as cell block sections prepared by the agar method from the same pleural fluid specimen were performed in some cases to determine the accuracy of this method in regards to IHC. Appropriate positive and negative immunohistochemical controls were performed to ensure an accurate interpretation of the results.

HE staining and Immunohistochemistry analysis were performed with conventional laboratory methods and

equipment. The 10 % neutral formalin, xylene, ethanol and acetone were purchased from BoJing, China. The primary antibody for CEA, CK7, P53 or CD15 and EnVision testing kit were purchased from DaKo (Glostrup, Denmark). The pregelatinized starch was provided by GuoNong Starch, China.

Fluorescence in situ hybridization (FISH) analysis on cell block sections

Molecular diagnostics were used on cell block sections in 2 cases of recent, histologically or clinically confirmed malignant effusions and epidermal growth factor receptor (EGFR) molecular analysis on these 2 cases of pulmonary adenocarcinoma from pleural fluid samples were performed. To determine the accuracy of this method in regard to molecular tests, a biopsy specimen from the same patient and cell block sections prepared by the agar method from the same pleural fluid specimen were performed simultaneously as a control. The cases were assessed by the ratio of the red signal to the green signal according to the standard assessment of conventional tissue paraffin sections. FISH analysis of cell blocks was performed according to the standard protocols of conventional tissue paraffin sections in the molecular pathology department. The probes and reagent of FISH analysis were provided by JinPuJia Biotechnology, China.

Results

Sections of cell blocks prepared by the new method using pregelatinized starch as an adjuvant were assessed by morphologic features such as cellularity, architecture and nuclear morphologic features.

The HE staining showed excellent results for the abovementioned aspects. The cellularity was high with small clusters and single cells present in most cases. Even a few cells can be effectively captured by the pregelatinized starch with its powerful glutinosity (Fig. 1a). The cell block section of even the cerebrospinal fluid specimen showed good architecture and cellularity (Fig. 1b, c). The morphology features of cell block sections prepared by the pregelatinized starch method were similar to those displayed in the cell block sections prepared by the agar method from the same pleural fluid specimen (Fig. 3a, b) as well as the biopsy tissues. Most cases showed a clear background in HE staining sections, while in a few cases the starch crystals could be observed in the interspace between tissues or cells. This was related to a higher amount of pregelatinized starch added to the samples (Fig. 1c). However, the limit residual starch crystals are achromatic and peripheral and they do not have any effect on the morphological features of the tissues and cells.

The IHC staining pattern was assessed as cytoplasmic, cell membrane, nuclear or cytoplasmic and nuclear staining. CEA staining was positive for some of the pleural fluid or ascitic fluid cases showing a cytoplasmic staining pattern. P53 and CD15 showed a nuclear staining pattern and cell membrane pattern, respectively. Some adenocarcinomas were positive for CK7 with a cytoplasmic staining pattern (Fig. 1d). The cell block sections prepared by the pregelatinized starch method and the agar method as well as the biopsy section from the same patient showed the same IHC staining pattern. Both the cell block section from the pleural fluid and the biopsy section from the same patient with lung cancer were positive for CD56 with a cytoplasmic staining pattern (Fig. 2a, b). Both the cell block sections prepared by the pregelatinized starch method and the agar method from the same pleural fluid specimen were positive for Ki 67 with a nuclear staining pattern (Fig. 3c, d). The starch crystals were dismissed during the IHC processing and the background was clear in every cell block section.

EGFR gene amplification on cell block sections by FISH analysis showed clear signals of red and green and the ratio of the red to green signal was 1.78 in the cell block section prepared by the pregelatinized starch method from pleural fluid of a patient with pulmonary adenocarcinoma (Fig. 2c). The ratio of the red signal to green signal is 1.80 in the biopsy section from the same patient (Fig. 2d). Both the cell block sections prepared by the pregelatinized starch method and the agar method from the same pleural fluid specimen of another patient with pulmonary adenocarcinoma showed clear signals of red and green and the ratio of the red to green signals was 1.09 and 1.17, respectively (Fig. 3e and f).

Discussion

The diagnostic yield of cytological smears is often low and may be related to loss of viable diagnostic material during the smearing process. The cell block technique captures not only aspirated cells but also small tissue fragments, improving the diagnostic yield and enabling additional ancillary studies (Nguyen et al. 2011), such as immunohistochemistry analysis, which is an indispensable technique in routine cytology (Skoog and Tani 2011). It is helpful for classification or subclassification of the tumor and it is critical for guiding the targeted treatment, predicting response to systemic adyuvant chemotherapy and hinting at the prognostic of some tumor patients before surgical resection.

As the era of molecular medicine has continued to rapidly evolve, there has been an increased demand for molecular testing on cytological specimens (Collins et al. 2011). Cytology specimens with small amounts of diagnostic cellular material can still provide acceptable material for various

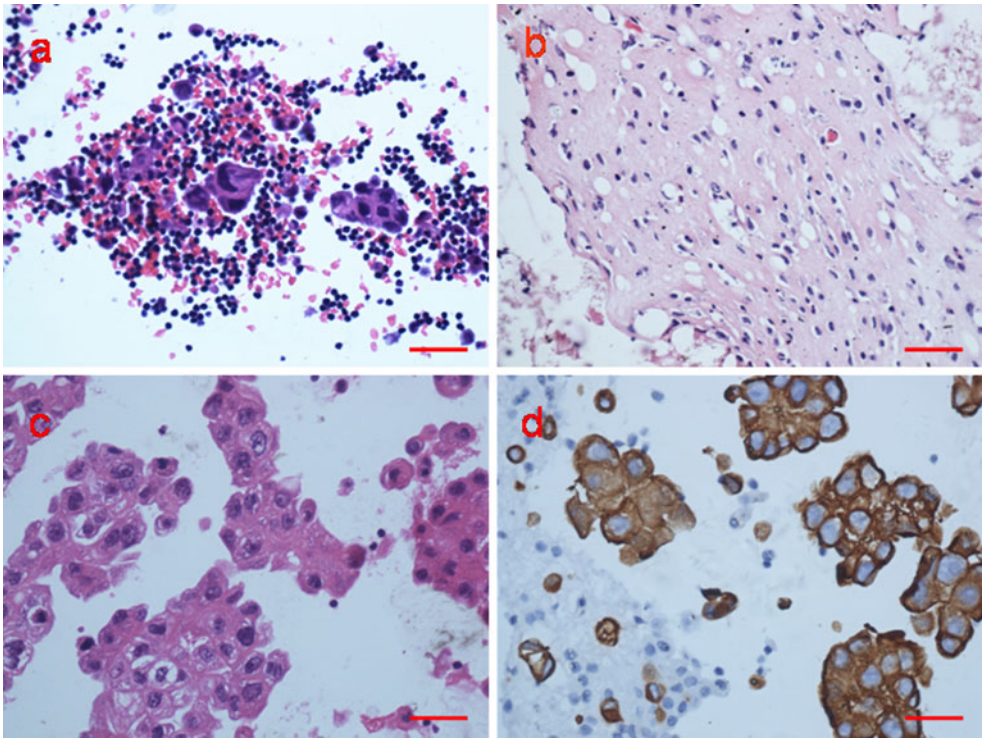

Fig. 1 Limit aspirated cells in pleural fluid were captured effectively with a clear background (**a** ×400, HE staining).The cell block section of the cerebrospinal fluid specimen showed tissue fragments with good architecture and cellularity (**b** ×400, HE staining). The cell block section of the hydropericardium specimen showed high cellularity and glandular architecture (**c** ×400, HE staining). Immunohistochemistry showed that the tumor cells of the hydropericardium specimen were cytoplasmic positive for CK7 (**d** ×400). HE, hematoxylin and eosin; CK, cytokeratin. *Bars* (**a**–**d**) 100 μm

molecular studies, including testing for markers such as EGFR and KRAS (da Cunha et al. 2010; Halling et al. 2006; Mok et al. 2006; Riely et al. 2006). Cohen and Weydert recently stated that, in the future, the challenge and opportunity exist for pathologists to enhance patient care by developing ways in which adequate cytological material can be obtained (Cohen and Weydert 2011).

Adequate cellularity within the cell block sections is difficult to achieve without an adjuvant. Among the current cell block adjuvants we can mention agar, thrombin and egg albumen. Agar is difficult to dissolve in ethanol or xylene with a higher density and lower permeability after solidification. Moreover, the agar needs to be heated first and then cooled every time, which is not so convenient. The thrombin

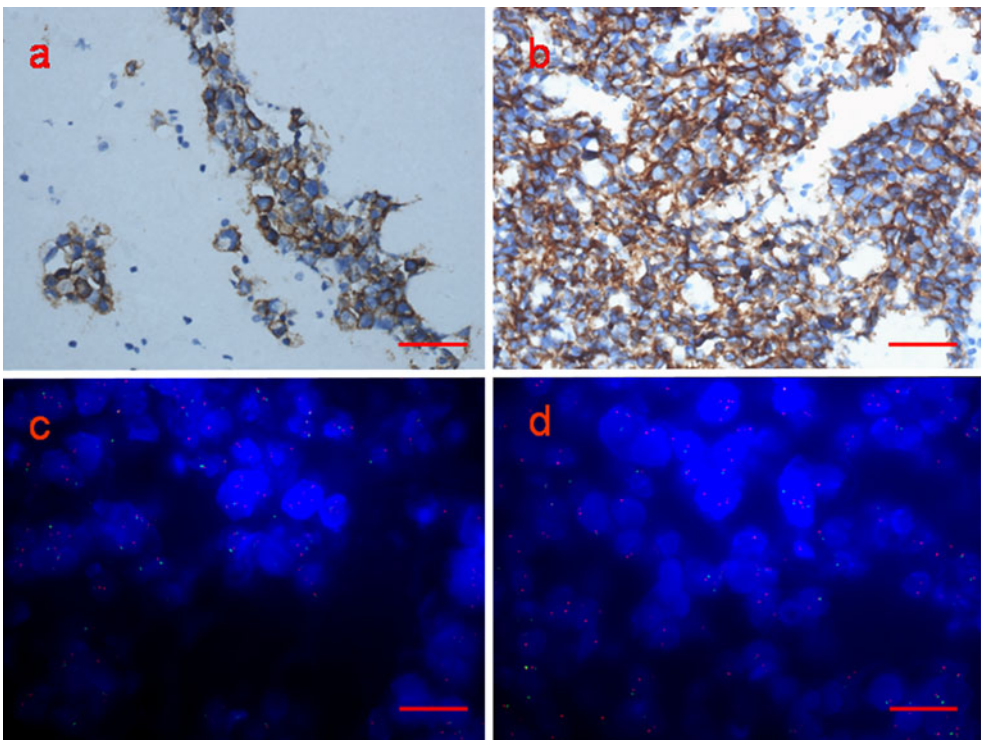

Fig. 2 Immunohistochemistry showed that both the cell block section from pleural fluid (**a** ×400) and the biopsy section (**b** ×400) from the same patient with lung cancer were positive for CD56 with a cytoplasmic staining pattern. FISH analysis showed clear signals of EGFR gene amplification in the cell block section from the pleural fluid specimen(**c** ×oil) and the biopsy section (**d** ×oil) from the same patient . FISH, Fluorescence in situ hybridization; EGFR, epidermal growth factor receptor. *Bars* (**a**, **b**) 100 μm, (**c**, **d**) 20 μm

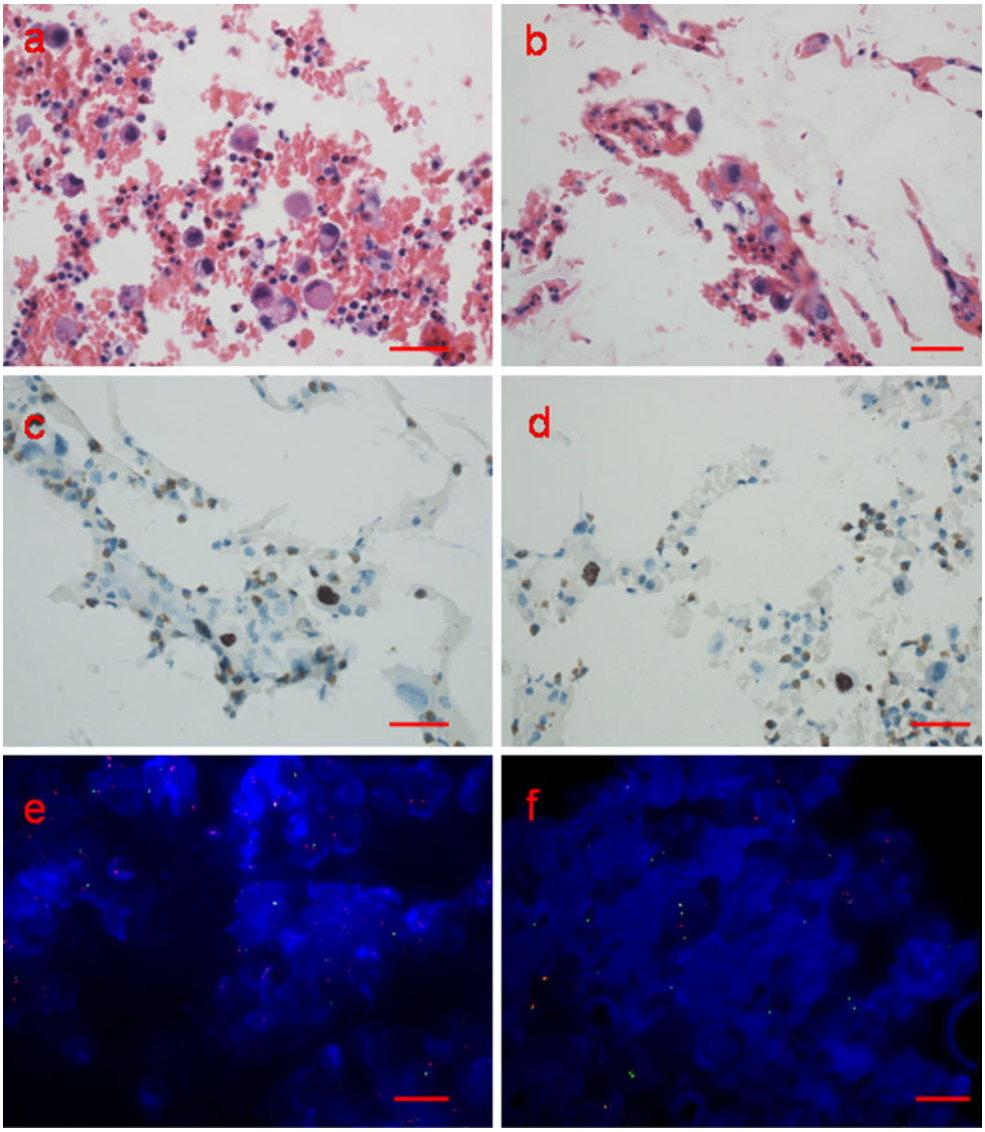

Fig. 3 The morphology features of the cell block section prepared by the pregelatinized starch method (**a** ×400; HE staining) were similar to those displayed in the cell block section prepared by the agar method from the same pleural fluid specimen (**b** ×400; HE staining). Immunohistochemistry showed that both the cell block sections prepared by the pregelatinized starch method (**c** ×400) and the cell block section prepared by the agar method (**d** ×400) from the same pleural fluid specimen were positive for Ki 67 with a nuclear staining pattern. FISH analysis showed clear signals of EGFR gene amplification in the cell block section prepared by the pregelatinized starch method (**e** ×oil) and the cell block section prepared by the agar method (**f** ×oil) from the same pleural fluid specimen. *HE* hematoxylin and eosin, *FISH* fluorescence in situ hybridization, *EGFR* epidermal growth factor receptor. *Bars* (**a**–**d**) 100 μm, (**c**, **d**) 40 μm

must be stored at low temperature to keep its activity and blood serum must be added according to certain proportions. Egg albumen needs repeated precipitation and corrosion protection, which make the procedure more complicated. The sodium alginate method has been claimed to be simple and this method needs the addition of 0.5 ml of 1 % sodium alginate to the sediment, then to be agitated and centrifuged for 10 min, followed by the addition of 1 or 2 drops of 1 M calcium chloride solution, leaving for 5-10 min, removing concretion and embedding in paraffin (Noda et al. 2010). However, the pregelatinized starch method is more convenient compared to the above-mentioned methods. A little pregelatinized starch powder was added to the sediment and the pregelatinized starch will encapsulate the sediment immediately with a little residual supernatant and then the dough was submitted to tissue processing. Moreover, pregelatinized starch is stable in room temperature and it is cheap.

Pregelatinized starch was reported to serve as the encapsulation formulation for some drugs to increase their bioaccessibility (Yu and Huang 2010) and it is useful as a binder in tableting of pharmaceutical industries due to its physiochemical properties and relative inertness (Jubril et al. 2012). Pregelatinized starch is hydrophobic but can be plasticized when it meets a little water at room temperature and, moreover, the dough of pregelatinized starch is indissoluble in tissue processing, which can therefore be used as a powerful cell block adjuvant thanks to this property. The encapsulation formulation of pregelatinized starch during capturing aspirated cells or small tissue fragments in fluid cytological specimens is useful for the cell block technique, being especially applicable for cytological samples with low cellularity.

The pregelatinized starch method for making cell blocks was validated by using biopsy tissue from the same patient as a control to determine the accuracy of this method in regard to the IHC or molecular tests. In conclusion, the present study

introduces a novel, convenient, economical and pragmatic technique for making cell blocks, showing that the pregelatinized starch technique is a promising alternative to smear cytology, liquid-based SurePath cytology and TCT.

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Conflict of interest statement The authors declare that Qing-Lian He is in the process of filing a patent application for findings described in this article.

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