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Human fibroblast-like synoviocyte isolation matter: a comparison between cell isolation from synovial tissue and synovial fluid from patients with rheumatoid arthritis

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SUMMARY

OBJECTIVE: Cell culture technology has become a popular method in the field of cell biology, pharmacology, and medical researches. Primary cells represent the normal physiological condition of human cells. Fibroblasts are the most common native cells of connective tissue that play a crucial role in the entire pathogenesis of various disorders, such as rheumatoid arthritis (RA). Fibroblast-like synoviocytes (FLSs), which overlie the loose connective tissue of the synovial sublining, are known to be the central mediators of joint damage. The most routine approach for the isolation of FLS is an enzymatic digestion of synovial tissue. This experimental study is designed to introduce an easy, fast, and high-throughput method compared with enzymatic digestion for isolation of FLS.

METHODS: The synovial tissue and synovial fluid (SF) samples were collected from eight patients with RA who underwent routine knee replacement surgery. Synovial tissue was incubated with collagenase VIII enzyme, while SF was washed with a similar volume of phosphate-buffered saline. The cells were further subcultured and stored based on the standard protocols. The purity of isolated synoviocytes was confirmed using flow cytometry analysis.

RESULTS: Isolation of FLS from SF was more successful with a faster rate, 3–5 days after culture. The morphological assessment and flow cytometry analysis confirmed the purity of SF-derived cells in passage 4.

CONCLUSIONS: SF could be a more accessible source of FLS than synovial tissue. Obtaining primary FLS from SF is a simple, fast, and cost-effective way to have a large-scale cell during a short time.

KEYWORDS: Cell culture. Cell isolation. Fibroblast-like synoviocyte. Synovium. Synovial fluid.

INTRODUCTION

Rheumatoid arthritis (RA) is a complex and multisystem disorder that primarily affects the synovial joints[1,2]. Fibroblast-like synoviocytes (FLSs) and macrophage-like synoviocytes (MLSs) are two main resident cells in the intimal layer of the synovial membrane, which play a central role in the joint pathology of RA[3]. Nevertheless, FLSs are the most common cell at the bone–pannus interface with a more abundant population than MLSs[4,5]. The migration and invasion of FLS into cartilage and bone are a key event in synovial hyperplasia, resulting in cartilage destruction in patients with RA[6,7]. Activated FLSs produce a wide range of inflammatory mediators, which promote

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the recruitment and activation of circulating and resident immune cells. Also, the migration of arthritis to unaffected joints has been attributed to the transmigration of FLSs[8,9]. Although there is still much to be learned about their resolution in RA disease, FLSs have sparked a lot of attention in recent studies. The culture and growth of FLSs is the first step for the investigation and analysis of these cells. At present, the synovium is the main and traditional source of FLS, and access to the synovium is feasible only during arthroplasty or arthroscopy surgery. In advanced RA, total joint arthroplasty (TJA) has been suggested as a successful intervention when optimal medical and supportive therapies have failed[10,11]. It has been reported that early and continuous use of biological and disease-modifying antirheumatic drugs (DMARDs) delays TJA[12,13]. Given the importance of FLS in RA pathology, there is a great need to find a reliable and repeatable source for quick access to them. As we know, the extraction of FLSs from synovium obtained during surgery is time-consuming and prone to contamination with microorganisms as well as needs a lot of materials. So, the establishment of an optimized procedure with low cost and shorter time compared with an enzymatic method is essential. In this study, for comparing the efficiencies of different isolation, we aimed to compare two currently being used FLS isolation methods to find a simple and effective procedure.

Materials and reagents

Dulbecco's modified Eagle medium: nutrient mixture F-12 (DMEM/F12)+PSF (penicillin, streptomycin, and fungizone) (Biosera) (for the digestion mixture), fetal bovine serum (FBS) (Biosera), 0.25% trypsin-EDTA (Biosera), collagenase type VIII (Sigma C-2139), fluorochrome-labeled antibodies, cell culture flasks, dishes and tubes, and surgical blade (NO.17) were used.

METHODS

Patients and tissue samples

Human synovial tissue and synovial fluid (SF) were obtained from eight female patients with RA who underwent total knee arthroplasty in the Department of Orthopedics, Shafa Hospital, Sari, Mazandaran, with an average age of 58.8±9.66 years, ranging from 48 to 77 years. All patients fulfilled the 2010 RA classification criteria[14]. This study was approved by the Institutional Medical Ethics Committee of Mazandaran University of Medical Sciences (MAZUMS; IR.MAZUMS.REC.1398.1364), and informed consent was acquired from all patients before surgery.

Cell culture

Synovium

The synovial tissues were carried from the surgery department to the cell culture laboratory in a 50-ml falcon tube containing 15-ml DMEM culture medium with 100 U/ml penicillin, 100 U/ml streptomycin, and 1% amphotericin B as transport media and stored at 4°C before processing. Then, the collected tissues were, respectively, washed with phosphate-buffered saline (PBS) (pH 7.3–7.4), alcohol (75%), and finally with PBS+100 U/ml penicillin, 100 U/ml streptomycin, and 1% amphotericin B, each for a period of 2 min. Tissues were minced into 1 mm×1 mm pieces with the help of sterile BP blade No. 17 in a sterilized Petri dish containing DMEM media and then incubated with collagenase VIII for 2 h at 37°C in a shaker incubator to promote isolation of FLS. After incubation time, cell pellets were harvested by centrifugation at 1000 g for 10 min. Then, pellets were suspended in DMEM supplemented with 10% (vol/vol) heat-inactivated FBS, 100 U/ml penicillin, and 100 U/ml streptomycin and placed in a T-25 flask at 37°C with 5% CO_2 in a humidified atmosphere. After 48 h, the medium was changed, and the isolation of cells from synovial tissues was checked every day. The medium was changed twice a week.

Synovial fluid

After collection of SF in a 15-ml falcon tube containing anticoagulant, it was diluted twice with sterile PBS and centrifuged at 400*g* for 10 min. Harvested pellets were suspended in DMEM with 10% FBS and antibiotics containing 100 U/ml penicillin and 100 mg/ml streptomycin. The cells were placed in T-25 flasks and incubated under the standard condition at 37°C with 5% CO_2 with saturated humidity. Following the 48 h incubation, fresh DMEM was added to the cells. The culture medium was refreshed every 3–4 days.

SF and synovial tissue culture processing steps are shown in Figure 1.

Identification of FLS by flow cytometry

FLSs at passage 4 were identified by flow cytometry. The purity of FLS was checked for the presence of MLS. The cells were stained with the following antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-CD68, phycoerythrin (PE)-conjugated anti-CD14 antibody, and allophycocyanin (APC)-conjugated anti-CD90.

RESULTS

The results showed that three of eight primary cultures of FLS by enzymatic digestion method were successful. The median

time to the presence of the first cells was 15 days, and they reached 70–80% of confluency around 30–35 days of culture. The remaining tissue samples were discarded if any cells were observed after 30 days. In contrast, cell isolation of all SF samples was successful, and the first cells were seen after about 3–4 days of culture. Getting 70–80% of confluency was dependent on the initial volume of SF.

Morphology of FLS cells

The cells were monitored for morphological assessment after the first day of their presence in the T-25 flask. Most of the cell population were spindle-shaped fibroblast, even in passage 0; however, few number of round to spherical shape, stellate-shaped, and epithelioid-shaped cells were also observed, especially in initial passages (Figure 2). Overall, isolated FLSs from synovium showed more similarity in shape in passages 1–3 compared with those from SF. It should be mentioned that the homogeneity of cells was different from one sample to another but all get to purity from passages 3 and 4 onward.

Percentage of FLS

Isolated synovial cells from synovium and SF were evaluated by flow cytometry at passage 4. We found a similar percentage of positive cells in specific markers in both procedures used after passage 4.

Overall comparison between the two methods is summarized in Table 1.

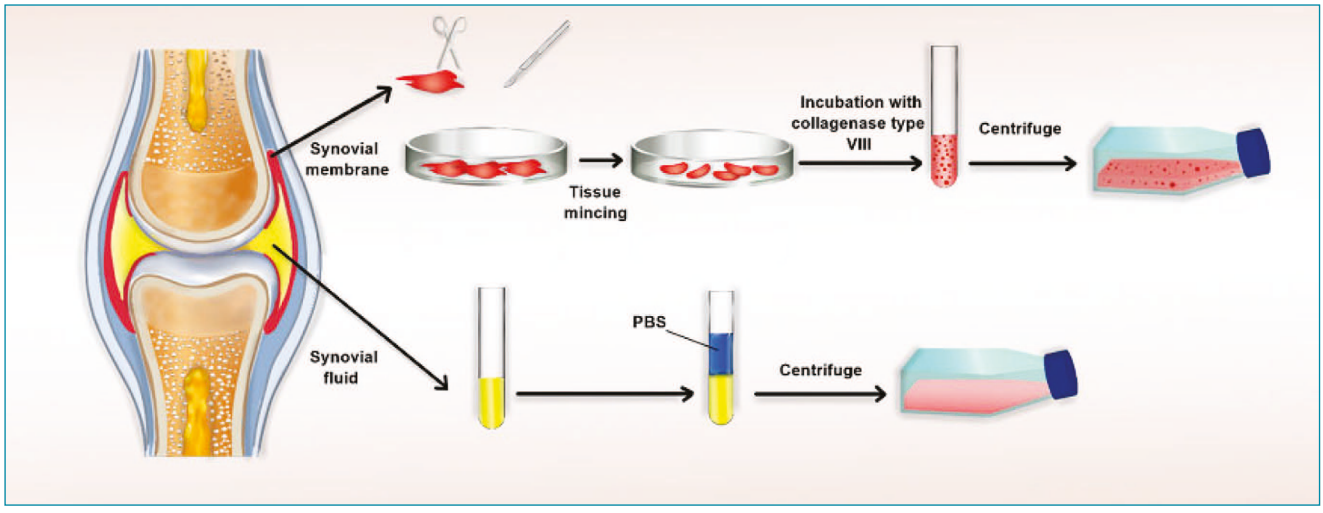

Figure 1. Schematic illustration of synovial fluid and synovial tissue culture processing steps.

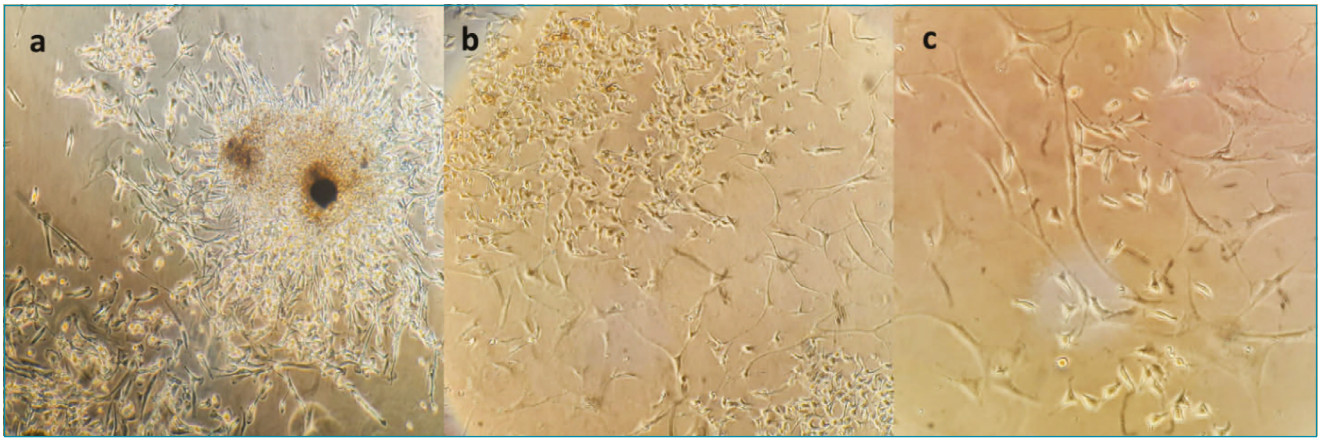

Figure 2. Light microscopic features of synovial fluid and synovial tissue culture. Cell outgrowth from synovial tissue on days 15–25 (a) (100×), a mixture of spindle-shaped and spherical shape fibroblasts with different size in passage 0 from synovial tissue (b) (100×) and synovial fluid (b) (400×).

Table 1. Comparison of important characteristics between two isolation methods.

FLS source	Volume	Sample preparation time	Primary incubation	Required materials	Number of days to see the first cell	Getting to 95% purity
Synovial tissue	1 cm^2 (1 mg)	3.5–4 h	+	Digestion enzyme, PBS, alcohol	15–25 days	Passage 3
Synovial fluid	500 µl (minimum)	20 min	–	Anticoagulant, PBS	3–5 days	Passages 4 and 5

PBS: phosphate-buffered saline.

DISCUSSION

Isolation of primary cell lines is required for researchers to investigate the morphological, functional, and cellular characteristics of a special tissue[15,16]. Nevertheless, a single-standard protocol has not yet been optimized for the isolation of many cells, especially recently discovered ones. FLSs were recently proven to be key players of observed inflammation in RA context[17,18]. These cells locate inside joints in the synovium and are involved in pannus formation, a hallmark pathological change in patients with RA[19-21]. Recent advances in the treatment of rheumatologic disorders have resulted in a reduction of access to synovial tissues by investigators[22]. Accordingly, it is essential to find a more proximal source of FLSs than the replaced joints during arthroplasty. In this study, we readily extracted adherent fibroblast cells from SF compared with synovial tissues. Also, flow cytometry analysis of the SF-derived cells confirmed the phenotype of FLS cells. We found that FLS does not migrate from all synovial tissues, but FLS extraction from all SF samples was successful. However, extracted cells from SF were more heterogeneous in initial passages than those from synovial tissue, but in the following, they reach to a similar phenotype like synovial tissue-derived cells. These results are in the same direction as those obtained by Stebulis et al. who indicated that FLS isolation from SF can facilitate the study of synovial cells when synovial tissues are not available. They found that both SF- and tissue-derived FLS are the same in functional and cellular measures such as the presence of some specific enzymes and production of inflammatory mediators[23]. Moreover, Ahn et al. reported that there is no significant difference between the invasive capacity of the two FLS types and that both of them are identical in surface markers[24]. Neidhart et al. demonstrated that SF-derived FLSs mediate cartilage destruction similar to tissue-derived ones[25]. These reports are in agreement with our results regarding the identical characteristics of extracted FLSs from both methods.

CONCLUSIONS

We found that the extraction of FLS from synovial tissues is not 100%, and FLS isolation from SF is the most convenient and effective method. The use of 500-µl SF is sufficient to get a higher number of FLS with a shorter time and lower cost of cultivation than synovial tissues. These data may support the selection of FLS isolation from SF for downstream analysis.

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AUTHORS' CONTRIBUTIONS

PZ: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **AR:** Investigation, Writing – review & editing. **FF:** Formal analysis. **SG:** Investigation, Methodology. **AH:** Investigation, Methodology. **MT:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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