Evaluation of a Pretreatment Method for Two-Dimensional Gel Electrophoresis of Synovial Fluid Using Cartilage Oligomeric Matrix Protein as a Marker

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Osteoarthritis (OA) is the most common rheumatic pathology. One of the major objectives of OA research is the development of early diagnostic strategies such as those using proteomic technology. Synovial fluid (SF) in OA patients is a potential source of biomarkers for OA. The efficient and reliable preparation of SF proteomes is a critical step towards biomarker discovery. In this study, we have optimized a pretreatment method for two-dimensional gel electrophoresis (2DE) separation of the SF proteome, by enriching low-abundance proteins and simultaneously removing hyaluronic acid, albumin, and IgG. SF samples pretreated using this optimized method were then evaluated by 1DE and 2DE separation followed by immunodetection of cartilage oligomeric matrix protein (COMP), a known OA biomarker, and by the identification of 3 proteins (apolipoprotein, haptoglobin precursor, and fibrinogen D fragment) that are related to joint diseases.

Keywords: Osteoarthritis, synovial fluid, two-dimensional gel electrophoresis, biomarker, cartilage oligomeric matrix protein, hyaluronic acid

Osteoarthritis (OA) is a degenerative joint disease that is characterized by progressive cartilage destruction and bone changes, accompanied by synovial inflammation [5]. A major objective for OA research is the development of early diagnostic technologies, including those based on genomics, proteomics, and metabolomics [7, 15]. Proteomic tools have been used to discover diagnostic or therapeutic biomarkers in OA [2, 9]. Successful separation of proteins is acknowledged to be a critical step in OA proteomics [11].

Synovial fluid (SF) is a potential source of OA biomarkers because it is derived directly from the joint disease site [4]. Hyaluronic acid, a high molecular weight glycosaminoglycan, is the major constituent of SF (1–3 mg/ml) and forms a complex with protein components [3]. Enzymatic digestion of hyaluronic acid facilitates the proteomic analysis of SF [17]. However, protein concentrations in SF range from ng/ml (e.g., protein biomarker) to mg/ml scales (e.g., albumin and IgG) [5], and the verification and quantification of protein OA biomarkers in SF using two-dimensional polyacrylamide gel electrophoresis (2DE) are therefore challenging [6]. Enriching low-abundance proteins and simultaneously removing highly abundant ones in SF would greatly facilitate the proteomic study of SF.

In this study, we systematically compared and optimized SF pretreatment methods, particularly the removal of hyaluronic acid, albumin, and IgG. The pretreated SF samples were then evaluated both by 1DE and 2DE immunodetection of cartilage oligomeric matrix protein (COMP) [8], a known OA biomarker, and by the identification of 3 proteins that are related to joint diseases. Because COMP is a biomarker for cartilage turnover and its level is elevated in the SF of OA patients [14], it can serve as a marker for evaluating SF preparations from OA patients.

MATERIALS AND METHODS

Clinical SF Samples
Human SF samples were obtained from 5 OA patients whose ages ranged from 39 to 64 years. Informed consent was obtained from each patient. The Institutional Review Board (IRB) of Ajou University approved all procedures. SF samples were centrifuged at 3,000 rpm for 20 min at 4°C, and supernatants were stored at −80°C in 1 ml aliquots until use.

Digestion of Hyaluronic Acid in SF Samples
SF sample contains highly viscous hyaluronic acid, which can be removed by treatment with hyaluronidase [6]. To prepare hyaluronidase stock solution, SHSE buffer (60 mM NaOAc, 1 mM EDTA; pH 6.0) was added to 1 vial of hyaluronidase at a final concentration of 1,300 units/ml. Hyaluronic acid digestion was carried out at 37°C
Depletion of Albumin and IgG in SF Samples by Affinity Chromatography

To deplete albumin and IgG in SF samples, hyaluronidase-treated or untreated SF samples were loaded onto the Aurum Serum Protein Mini Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer’s instructions. To determine the extent of nonspecific binding to the spin affinity columns, the resin used for removal of albumin and IgG was washed with elution buffer and collected for further study.

Two-Dimensional Gel Electrophoresis (2DE)

Protein concentrations in pretreated or untreated SF samples were measured using the 2D Quant kit (GE Healthcare, Waukesha, WI, USA) according to the manufacturer’s instructions. To determine the extent of nonspecific binding to the spin affinity columns, the resin used for removal of albumin and IgG was washed with elution buffer and collected for further study.

Effect of Hyaluronic Acid Removal from SF on 2DE Proteome Resolution Profiles

The high concentration of hyaluronic acid in SF makes it difficult to analyze the SF proteome by two-dimensional liquid chromatography-coupled tandem mass spectrometry because of the high viscosity of hyaluronic acid [6]. The SF of OA patients had bubbles (Fig. 1A) and was viscous, suggestive of high hyaluronic acid content. When the SF was treated with hyaluronidase to remove hyaluronic acid, the bubbles disappeared (Fig. 1B) and the viscosity of SF was significantly reduced. Next, we compared the proteome separation profiles of untreated and hyaluronidase-treated