Double Lavage Technique for Determination of Synovial Fluid Volume and Constituent Concentrations

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Introduction:
Synovial fluid (SF) provides nutrients, regulatory factors, and lubricant molecules that are important to the health, biological modulation, and biomechanics of diarthrodial joints. The concentrations of such molecules are typically assessed in fluid collected by arthrocentesis. However, for healthy and small joints, obtaining fluid can be difficult. Lavage is useful to obtain diluted SF, but changes the concentration of constituents in unknown ways. Determination of two unknown, but related quantities during lavage dilution, the original solute concentration and SF volume, can be achieved theoretically from two measurements. Recent studies have advocated measures of Ca²⁺ or urea both in the joint lavage fluid and in the serum obtained from the same donor as the two measures. However, such small molecules rapidly diffuse from the synovium into the fluid, potentially confounding such measures. An alternative approach is to perform two sequential lavages with known volumes and constituents, and to measure the concentration of relatively large molecules in the sequential lavage fluids. The objective of this study was to establish a simple technique for SF collection based on this principle to allow estimation of SF volume and SF solute concentration.

Methods:

Theoretical Model. A mass balance at the different steps of the two sequential lavages (Fig. 1) yields equations for initial SF volume, and concentration of a solute of interest, \( x \):

\[
V_{SF} = V_i^1 - V_{i1} + \frac{c_{x1}^1 V_{i1}^2}{c_{x1}^0 - c_{x1}^2} \quad (1)
\]

\[
c_{x}^{SF} = c_{x1}^0 \left( 1 - \frac{V_{i1}}{V_{SF}} \right) \quad (2)
\]

Addition in quadrature was performed to estimate how parameters contribute to uncertainty in the equations. Numerical analysis was then performed by introducing error terms in the parameters in order to determine injection and withdrawal volumes that would be feasible for in vivo applications and yield relatively small errors.

Experimental Studies. In Vitro. Using known volumes of pooled adult bovine SF ranging from 0.080 ml simulating the volume in a small human joint, an in vitro study was conducted by adding a known volume of saline and mixing, removing a portion of the mixture, and adding a second known volume of saline and mixing. The fluids were analyzed for protein concentration (BCA Assay). In Vivo. Human cadaveric knee joints (n=4) were obtained from tissue banks within 48h of death. A small amount of neat SF was drawn for later comparison to concentration estimates. Then, 5ml normal saline was injected into the knee joint, which was then flexed 10 times through the full flexion/extension range. Roughly 4ml of this diluted synovial fluid was removed from the joint. 10ml normal saline was then injected, the joint flexed 10 more times, and the fluid mixture was then removed. Neat and lavage fluids were weighed to estimate the volume obtained. Then, neat fluid was centrifuged at 15,000G for 30min and lavage fluids were centrifuged at 3,000G for 30min. Supernatants were removed and stored at -80°C until further analysis. Protein (BCA Assay) and hyaluronan (HA; ELISA-like assay) were quantified in neat and lavage fluids. Statistics. Data reported as mean±SD.

Results:

Theoretical Analysis. Addition in quadrature showed that the largest uncertainties in the equation are due to errors in concentration measurements. Numeric analysis and knowledge of reasonable volumes for practical use led to the selection of the injection/withdrawal/injection ratio of 5:4:10.

Experimental Studies. The in vitro study validated the theoretical model, providing estimates that were 105.0%±9.3% of the known volume. Fluid volume estimates from in vivo lavages were 5.1±2.2ml by protein analysis (n=4) and 4.8±2.5ml by HA analysis (n=3). Fluid concentrations of protein and HA were estimated to be 11.8±6.2mg/ml and 1.6±0.8mg/ml respectively. Relative to the native fluid concentrations, these estimates correspond to 78% of the protein concentration and 81% of the HA concentration.

Discussion: Presented here is a simple method for estimating SF volume and solute concentration that is applicable to small or large joints, with only injection of saline. Volume estimates for human knee joints were within the range of data for human knee SF volumes. The low estimates for native concentrations from the in vivo fluids may be due to the need for more mixing of fluids within the joint and loss of injected fluid across the synovial lining with pressurization. In the latter case, use of more viscous fluids may be helpful. The model here could also be improved by considering transport due to intraarticular pressure gradients that are increased or by injection of a tracer that does not freely diffuse.

Fig. 1. Illustration of the double lavage technique and definition of parameters. (A) There is an initial volume of SF and concentration of a particle \( x \) inside the joint, \( V_i^x \) and \( c_{x1}^0 \), respectively. The first injection volume, \( V_{i1} \), is added and the joint is flexed and extended through its full range of motion 10x. (B) A sample of this fluid mixture is collected, with volume \( V_i^x \) and concentration \( c_{x1}^0 \). (C) The second injection volume, \( V_i^x \), is then added, the joint ranged again, and a sampling of this mixture is collected, with concentration \( c_{x2} \).

Fig. 2. Representative images of (A) neat, (B) first lavage, and (C) second lavage fluids obtained from cadaveric knee joints.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Known Value</th>
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<tbody>
<tr>
<td>protein</td>
<td>5.1 ± 2.2</td>
<td>11.8 ± 6.2</td>
</tr>
<tr>
<td>HA</td>
<td>4.8 ± 2.5</td>
<td>1.6 ± 0.8</td>
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Table 1. Estimates of fluid volume and concentration compared to the initial concentration.


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