Extraction of total RNA from human synovial tissue explants following tissue homogenization in the 6775 Freezer/Mill

Author: Alexander Stoegner MSc.
Reviewed and approved by: Assoc. Prof. Priv.-Doz. Mag. Dr. Stefan Toegel
Karl Chiari Lab for Orthopaedic Biology, Medical University of Vienna, Austria

Reasoning: *The grinding of whole tissues allows for the extraction of total RNA while avoiding the shifts in genetic phenotype caused by classical cell culture methods.*

Equipment:

6757 microvials, polycarbonate with metal impactor

6775 Freezer/Mill

6758 Extractor for microvials

6753C Sample extraction tool

Collection of samples: After dissection from an orthopedic joint explant, the synovial tissue is cut into small pieces in preparation for flash-freezing. Due to the limited diameter of 6757 vials, it is recommended to ensure the tissue pieces do not exceed 6 mm in width before flash-freezing.

When flash-freezing the tissue, we recommend using a Styrofoam box with a wide opening, filled about ¼ with liquid nitrogen. Prior to freezing the tissues, cryovials should be prepared and cooled to -190°C to avoid samples freezing to the tubes.

Using a pair of long tweezers, the tissue pieces are submerged in the liquid nitrogen until it stops boiling, then transferred to the cold tube. Simply submerging soft tissue samples in liquid nitrogen can sometimes cause problems, as soft tissues like synovium tend to compact to spheres when submerged in liquid nitrogen, which cannot be loaded into the 6757 vials. To avoid this, the tweezers should be dragged through the liquid nitrogen (=stirring motion) to ensure the pieces freeze in an elongated shape.

Loading of samples: *Before beginning the milling process, we recommend having a large, open Styrofoam box to work in, as well as a second container with a lid to keep samples cold. After setting a rack into the open Styrofoam box, take the milling vials, cap one end and add the impactors. The vials are then placed on the rack in the liquid nitrogen with the open end upwards for cooling. Make sure to avoid nitrogen entering the vials (both liquid and gas), as this will lead to pressure buildup in the vial during the milling process.*

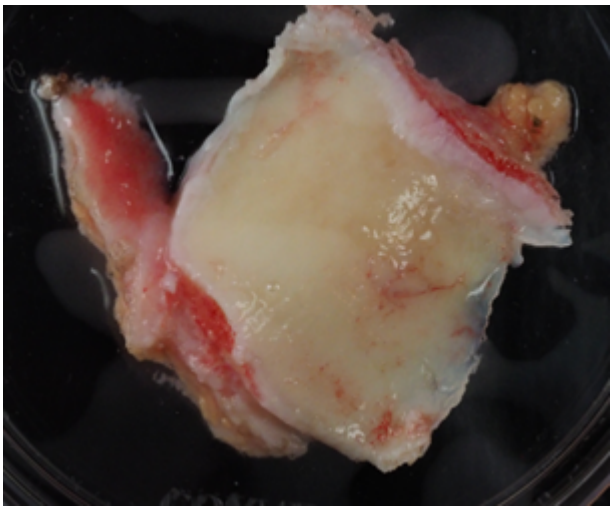
With the vials cooled down, samples can be transferred into the vials using a long pair of forceps and the remaining end capped. We suggest loading the microvials with up to 200 mg of synovial tissue for optimal results while grinding. After loading, make sure that the impactor can still freely move in the vial and remove some sample if this is not the case.

Milling*: For the milling process itself, the machine is set up to pre-cool samples for 10 minutes. Then, samples are milled at a rate of 12 contacts per second (cps) for 1:30 minutes, then cooled again for 2 minutes.*

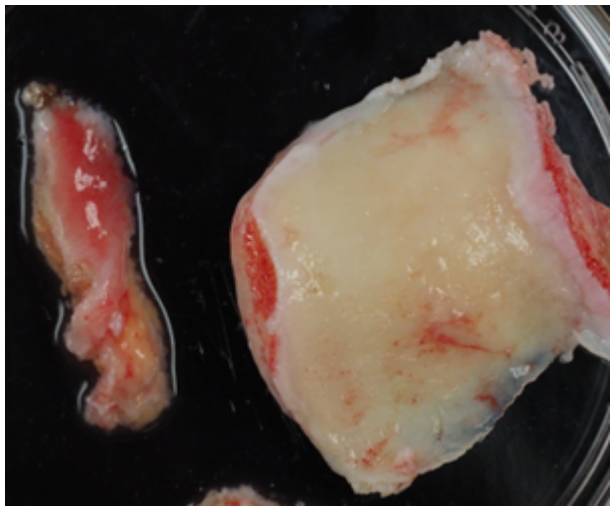
After 3 cycles the milling process is complete. During the milling process, we suggest preparing collection tubes (eg. Eppendorf tubes) and pre-chilling them in liquid nitrogen. Once the milling is complete, make sure that all of the sample in each vial was grinded into a fine powder before opening the microvials. If the sample is not milled, repeating the milling program can sometimes suffice as a solution.

Using the 6758 extractor for microvials, open one end of the vials and invert the vial into an Eppendorf tube (the microvial fits into an Eppendorf tube opening) to slide the impactor into the tube. After removing the impactor and placing the open end back in the Eppendorf tube the other end cap can be removed. A 6753C sample extraction tool can be used to transfer the powder sample into the collection tube. If the vials were filled with too much tissue, the powder will sometimes compact at the ends of the vials, which can be loosened with a pipette tip or similar long and thin object. Using the 6753c sample extraction tool in these situations can lead to the tool breaking and may result in rubber flakes in your samples. Therefore, always make sure there is no resistance when using the 6753c sample extraction tool. In order to avoid enzymatic degradation by RNAses, we suggest keeping the samples at -80°C prior to RNA isolation.

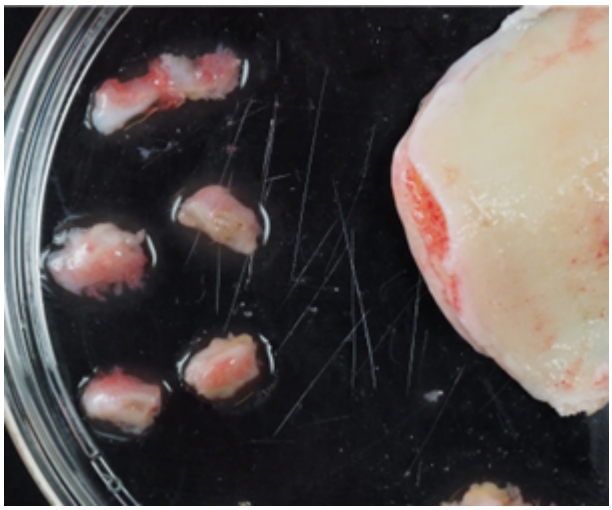
The Steps of the Sample Preparation Process



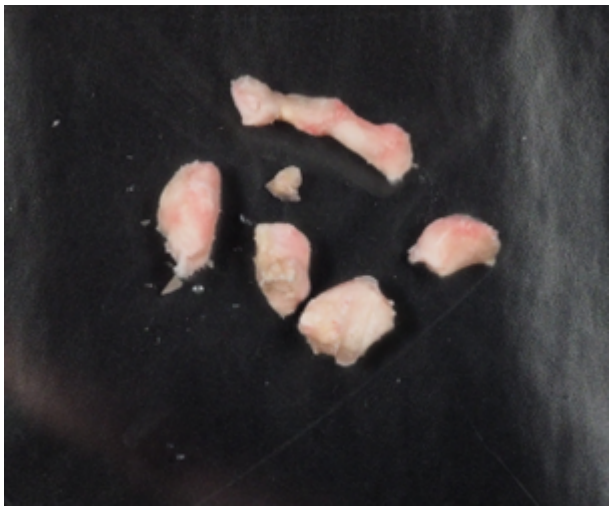
1. Clinical specimen of articular knee cartilage with attached synovial tissue



2. Synovial tissue cut off the osteochondral part



3. Synovial tissue cut into pieces



4. Synovial tissue pieces after freezing in liquid nitrogen

5. Freeze-milling of synovial tissue resulting in a fine powder

Cleaning: *The steel impactors and endcaps are washed with warm water and scrubbed to remove possible remnants of milled sample. Following this, the steel pieces can be autoclaved to ensure sterility.*

The plastic vials cannot be autoclaved and therefore need to be cleaned by hand. We suggest using high percentage EtOH to flush the tubes, then using water and hand soap to thoroughly remove any traces of EtOH. Tubes are then set to air dry.

RNA isolation: *For the isolation of RNA from ground tissue samples, we use the innuPREP RNA Mini Kit 2.0 provided by IST Innuscreen GmbH. Briefly, 350 µL of the contained lysis buffer (Guanidium Thiocyanate-buffer) are added to each sample, and samples are vortexed thoroughly for approx. 2 minutes and spun down using a table centrifuge. The resulting supernatant can then be collected and used to isolate RNA as described in the kit.*

After the isolating procedures, total RNA concentrations between 80-350 ng/µg can be expected, depending on the amount of tissue milled and the state of pathological progression of the tissue.

Karl Chiari Lab for
ORTHOPAEDIC BIOLOGY

DEPARTMENT OF ORTHOPEDICS
AND TRAUMA SURGERY
MEDICAL UNIVERSITY OF VIENNA

General notes:

- Make sure to be wearing full protective gear (face shield, apron, cryogloves) when working with liquid nitrogen, as contact with skin can cause serious burns.

- When using the microvial set, make sure to mark the vials for easier differentiation (Nail polish) and note which sample is loaded in which vial.

- If the vials contain nitrogen (liquid or gas) prior to closing, the milling process will cause a buildup of pressure in the vials. This results in some loss of the powder content, as opening the vials causes a sudden release of pressure.

- Make sure the area around the freezer mill is free of other items, as the condensation that occurs during the milling will make everything wet. We suggest placing the freezer mill on an absorbent pad which should be changed regularly.

- While the freezer/mill is running, make sure to wear ear protection and keep doors are closed, as the machine running generates substantial noise.