Homogenizers such as blenders work by shearing, which is created by a tangential force being applied to the sample. There are several tools that disrupt by shearing, including blenders, rotor-stators, and some of the glass homogenizers, all of which made their entrance into the lab in the middle of last century.

In the pre-WWII era, biological samples were homogenized by chopping and dicing, and then in the 1940s, blenders arrived. These were truly the first in a series of innovations in sample homogenization. Through the 1940s and 50s, the number of tools commercialized for sample disruption increased dramatically (including the Dounce homogenizer, Potter-Elvehjem homogenizer, and French press - named for their inventors), all of which disrupt by shearing. It was also in the 1950s that the first rotor-stator homogenizers started seeing use in the labs.

Though mistaken for bead beating, many researchers shear microorganisms and small sections of tissue using small grinding beads and vortexers. Various configurations of bench top vortex mixers can be used with micron sized grinding spheres to rupture bacteria, yeast, and molds. Larger spheres, like 7/64" stainless steel grinding balls, are usually used with tissues. Vortexers, though readily available, tend to be poor as compared to other methods discussed below.

Blender: One of the early innovations applied to sample homogenization was the Waring blender, which made its appearance early during World War II. This simple device was instrumental to early work in protein purification and analyte isolation. Samples are placed in the blender with extraction buffer and then blended. The blades shear and cut tissues, reducing tissues in size significantly. Coffee grinders have essentially the same mechanism as a blender and can be used with seeds and kernels. However, liquid samples cannot be processed with coffee grinders.

Strengths – Blenders are readily available with even household devices being suitable for many lab applications. They can process large samples quickly and are easy to use and clean. Lab blenders are available in stainless steel which allows for decontamination and sterilization.

Limitations - Blending can create vortexes that cause foaming and result in significant protein denaturation. Blenders also generate a course homogenate, which is not always suitable for efficient extractions.

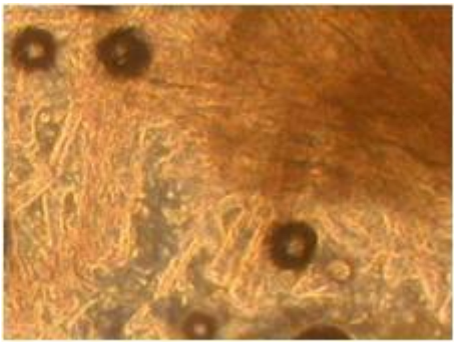

Figure . Mouse heart muscle homogenized with a blender. Blenders are an effective first step in disaggregating samples. However as depicted in this image, many microfibrils remain intact. Blenders can also introduce air bubbles (dark spots) which can be detrimental to some analytes.

Vortexer Shearing with Beads: Though not their intended use, vortexers are routinely used to disrupt samples. This method relies on adding grinding beads and sample to a tube and then repeatedly vortexing. Typically used for the lysis of microorganisms, vortexers can also disrupt tissues by using large grinding beads (>2 mm) made of zirconium or stainless steel. Homogenizing samples by vortexing can generate significant amounts of heat due to the friction created by the grinding beads. Many protocols call for bursts on the vortexer interspersed with cooling on ice. Several vortexer models are available that hold multiple microfuge tubes and that pulse (alternating on and off) in order to help dissipate heat.

Strengths – Vortexers are available to most researchers and thus can be used for homogenization at no cost. New vortex mixers are relatively inexpensive. Standard single tube vortex mixers can be used for all size tubes while pulsing vortexers can handle up to 12 microfuge tubes. Multitube vortexers can homogenize full racks of tubes.

Limitations – Vortex mixers are designed for mixing and lack the same power as true homogenizers; thus, they are usually much less effective at sample disruption. With microbes, homogenization rates are around 50% as compared to true bead beaters. Though partially effective, vortexers may be adequate for many applications.

Rotor-Stator: The rotor-stator, or what is commonly known as the handheld homogenizer, was first commercialized for the laboratory in the 1950s. This homogenizer is one of the most widely used tools for homogenizing plant and animal tissues. Rotor-stators are designed with an outer stationary tube (stator) and an inner turning shaft (rotor) which is connected to a motor. At the bottom of the rotor-stator are slots on both the stator and rotor. This design is essentially the same as an electric razor. When running at 10,000-20,000 rpm, samples pressed into the slots of the rotor-stator are efficiently sheared.

Rotor-stators come in many different widths and bottom slot configurations. It would be speculative to identify the intended application for each rotor-stator type, but in practice these larger shaft assemblies are used to macerate animal and plant tissues of increasing mass (P). The shearing action of the homogenizers produces a very uniform homogenate in relatively little time. Like other homogenization techniques, the rotor-stators can generate heat; thus, some of the more advanced models come with temperature probes that shut down the units if the temperature rise is extreme.

Figure. Top (upper) and side (bottom) views of a rotor/stator. The outer shaft (stator) remains stationary while the inner shaft (rotor) turns at speeds up to 20,000 rpm. Rotor/stators can be very effective on homogenizing powders and pills, but can generate only course homogenates when used on tougher tissues.

Rotor-stators are available as handheld units and larger stand supported models. Some are modified workshop routers, but they can also be very complex programmable models with numerous features. Most are the size of handheld kitchen mixers, though with much greater power. Sample sizes which can be processed on handheld homogenizers range from less than 1 ml up to 40 L or more.

Strengths – Rotor-stators can be very effective at homogenizing a wide array of samples. The shafts are designed for different sample types and sizes. Indeed, process scale rotor-stators are commonly used in various industries. Samples disrupted with rotor-stators can be very homogeneous.
Limitations – The initial cost for rotor-stators varies, but at minimum it will be an investment of several thousand dollars. Furthermore, different motor units will be required for very small and large samples. Each rotor-stator is $1000 or more. Shafts are difficult to clean, which requires the unscrewing of the rotor shaft from the stationary outer stator housing. When homogenizing fibrous samples such as muscle, threads of connective tissue can become caught within the shaft assembly, making rapid cleaning and decontamination a problem. High speed homogenization also generates heat and possible vortexes that can denature proteins.

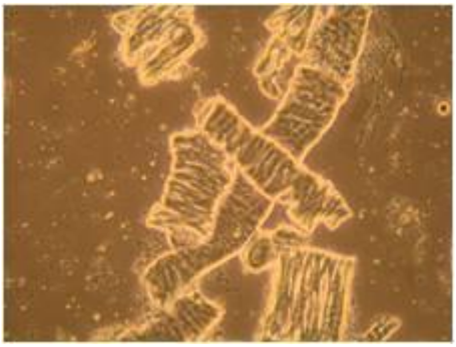

Figure . Mouse muscle disrupted using a rotor-stator homogenizer. Rotor-stator homogenizer had a relative efficiency of 27.6% as compared to other methods (see Fig. 20).

Dounce Homogenizer: Not long after the arrival of the Waring blender, the Dounce homogenizer was introduced. Though this device looks like a ground glass homogenizer, it relies on pushing a sample between the sides of the tube and the pestle. Shearing forces are created as the sample and liquid squeeze up and past the pestle. The Dounce homogenizer is most effective at lysing tissue culture cells and finely diced tissue in order to generate lysates where there are still intact subcellular particles. If there is a need for membrane fragments and organelles, then the Dounce homogenizer is a good tool to use. Once the sample is placed in the tube, the pestle is inserted, pressed down, and then lifted. This up and down motion is repeated, causing the sample to be sheared repeatedly. The shearing force can be controlled to an extent by using different pestles with different diameters. The larger diameter pestle is tighter fitting and creates a greater shear, while the opposite is true for the smaller pestle.
Strengths – The Dounce homogenizer is an inexpensive device that is very effective for mildly lysing cells. They typically are purchased with two different diameter pestle heads (type A and B) which provides some control over the extent of shearing. Dounce homogenizers are easy to use, clean, and decontaminate.
Limitations – Solid tissue is not effectively homogenized using a Dounce homogenizer. If individual cells are processed, they must first be disaggregated from solid tissue or dissociated from tissue culture plates, which is time consuming. Throughput is low. The devices are fragile and can break easily.

Figure . Two glass homogenizers used for shearing cells. The Dounce homogenizer (left) is manually operated and disrupts cells by forcing them between the pestle and tube wall. The Potter-Elvehjem homogenizer can be hand operated or attached to a motor. The pestle can be PTFE or glass.

Figure . Though the homogenate appears very fine, significant tissue was left intact. The Dounce homogenizer produces 32% relative homogenization of solid muscle as compared to other processes (see Fig. 20).

Potter-Elvehjem with PTFE Pestle: Potter-Elvehjem homogenizers can be used for both grinding tissues and shearing cells. By changing from a ground glass pestle to a PTFE one, it effectively changes from a grinder to a shearing homogenizer. For shearing, the pestle can be connected to a variable speed lab motor. For sample processing, tissue is placed in the sample and the pestle is rotated at 600-750 rpm. The tube is repeatedly pressed up on the pestle where shearing forces disrupt the sample.

Strengths – The Potter-Elvehjem homogenizer is relatively inexpensive, though the motor can cost several hundred dollars. They are easy to use and clean, and samples can be kept cold on ice during processing. The homogenizer is effective for disrupting animal cells for the generation of subcellular components.
Limitations – Using a Potter-Elvehjem homogenizer with a motorized PTFE pestle is good for disrupting cells, but not very efficient at homogenizing solid tissue. Muscle homogenized by this method was incomplete. For solid tissue, homogenizing with a Potter-Elvehjem is not overly effective. Overall the process was poor in a one-step method. When used in conjunction with other homogenizers, it was effective.

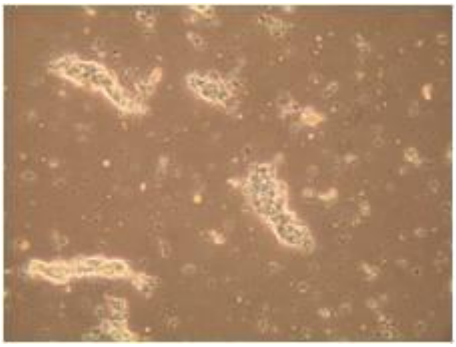

Figure . Potter-Elvehjem homogenizer with motorized PTFE pestle yields 36% relative disruption of muscle as compared to other systems. Significant solid tissue remained intact (see Fig. 20).

French Press or French Pressure Cell: The French press came into use in the early 1960s. It was developed in the late 1940s for the disruption of microorganisms. It works by forcing cells through a tiny orifice under extremely high pressure, e.g., 20,000 psi. As the cells move from high pressure to low pressure, they expand and rupture.

The French press is very useful for periodic cracking of microorganisms, but it is much less useful for routine cell disruption. The samples that are used in French pressure cells must be fluid, such as previously homogenized tissue, blood, microbes, or other fine particulate fluids. When disrupting microbes, cells are often harvested by centrifugation and the cell paste is processed.

French pressure cells cost upward of $3500, with the smaller cells being used for samples of 1-2 ml. Larger presses process upwards of 30-40 ml.

Strengths – The French press is a very effective and efficient tool. Homogenates generated by French press rival ultrasonication in degree of thoroughness of disruption. Sample homogenates are very uniform.
Limitations – Sample sizes are relatively small and throughput is very low. For any samples other than single cells or microbial cultures, a pre-homogenization step is first necessary. French pressure cells can be expensive relative to the number of samples that can be processed. Due to the small orifice, the French press can clog.