A stainless-steel mortar, pestle and sleeve design for the efficient fragmentation of ancient bone

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Different types of milling equipment – such as oscillating ball mills, freezer mills, mortar and pestle – can be used to fragment ancient bone prior to DNA extraction. However, each of these tools is associated with practical drawbacks. Here, we present the design for a stainless-steel mortar and pestle, with a removable sleeve to contain bone material. The tool is easy to clean, practical and its simplicity allows university workshops equipped with a lathe, boring tools and a milling machine to make these components at local expense. This design allows for the efficient fragmentation of ancient bone and improves sample throughput. This design is recommended as a useful, economical addition to existing laboratory equipment for the handling of ancient bone.

To improve the economy and throughput of research projects, laboratory protocols require periodic evaluation with an aim to reduce human handling time and/or increase accuracy or efficiency. Such evaluation is of particular importance in ancient DNA (aDNA) laboratories where laboratory time associated with minimizing contamination forms a significant cost. Here, we present a design for an easily cleaned, stainless steel mortar and pestle with a removable sleeve for fragmenting and pulverizing ancient bone, which is a fundamental procedure prior to DNA extraction [1]. Even though a broad range of laboratory mills, grinders and crushers such as rotor-, knife-, disc- or ball-based mills, mortar grinders, jaw crushers or drills are available, not all tools can be easily adapted to cleanroom standards. The most commonly used equipment and approaches in aDNA studies are grinding balls with shaking or freezer mills [2–8], but also mortar and pestle is used [9–11], or drills are used at low rotational velocity [12,13]. While these approaches are suitable, each method has practical disadvantages. For instance, bone fragmentation in oscillating ball or freezer mills occurs in closed beakers during a pre-set milling time. After this period, the containers are removed from the machine for visual inspection of the extent of fragmentation. This process leads to iterative tool (re)assembling to ensure complete milling and may result in over-processing of easily fragmented samples. Moreover, these mills require time-consuming cleaning routines and represent a significant capital investment. While mortar and pestle are significantly cheaper and more straightforward to clean, it can be difficult to control impact and to keep fragments contained within the mortar, leading to the scattering of bone material throughout the working area. In addition, some samples may simply be too hard to be processed this way. Finally, the low rotational velocity required for drills to avoid the burning of bone results in substantial handling time in order to obtain sufficient powder. The design presented here addresses several of the drawbacks mentioned above.

The design consists of three parts – mortar, pestle and sleeve – that adhere closely together (Figure 1). The design is adapted from commercially available mortars that are used for the fragmentation of ores and minerals, for instance Impact Mortar and Pestle (cat. no 845/850 Chemplex®, FL, USA) or Plattner's mortar and pestle (cat. no. 6883L10, Thomas Scientific, NJ, USA) with several modifications for the specific purpose of fragmenting ancient bone. First, we elongated the pestle to provide easy grip and prevent fine bone powder from reaching hand level while exiting the sleeve during repetitive grinding. Second, we elongated the sleeve to allow the pestle to remain in the sleeve to help contain the material. Third, we reduced the depth of the mortar chamber for easy access during cleaning. Fourth, we removed a 1-mm thick section over the entire length of the pestle. This allows the pestle to move more freely inside the mortar chamber and prevents the build-up of air-pressure. Initial tests of an earlier design without such section removed revealed that a tight fit of the pestle within the sleeve can result in the build-up of air-pressure with each downward move of the pestle. This pressure can then push fine bone powder through small seams between the mortar chamber and sleeve. Finally, each separate item (mortar, sleeve and pestle) is identified by an engraved number so that all pieces of a set can be kept together during handling.

All items have been constructed of stainless steel (AISI 316L) in the Instrument Workshop at the University of Oslo (Norway) using a lathe and boring tools. The mortar

METHOD SUMMARY

We present an economical design of a stainless-steel mortar, pestle and sleeve for the efficient and controlled fragmentation of ancient bone.

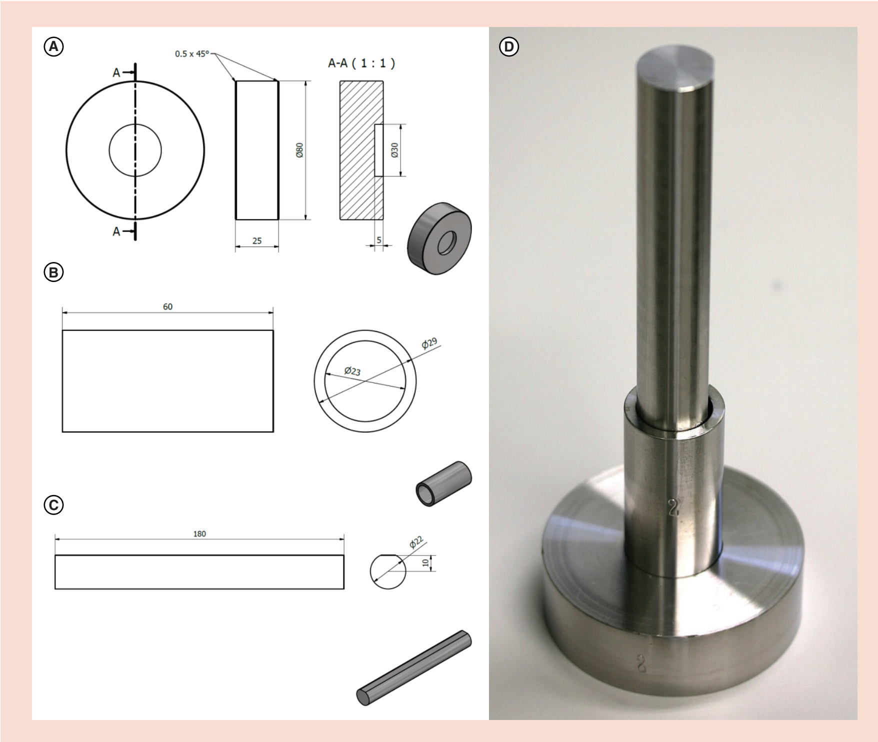

Figure 1. Design specifications. (A) Mortar, **(B)** sleeve and **(C)** pestle for the fragmentation of ancient bone. All measurements are in millimetres. Each item is made of stainless steel (AISI 316L). **(D)** Photo of the assembled tool set.

base is 25-mm high with a diameter of 80 mm, and the chamber has a depth of 5 mm with a diameter of 30 mm (Figure 1A). The sleeve has an outer diameter of 29 mm, inner diameter of 23 mm and a height of 60 mm (Figure 1B). The pestle has a diameter of 22 mm, a length of 180 mm and a 1-mm thick segment is removed from the transverse section over the entire length of the pestle (Figure 1C). All pieces are engraved with a respective set number (Figure 1D).

In our laboratory, the stainless-steel mortar, pestle and sleeve design compares favorably to an oscillating mixer mill (Table 1). The design is quicker to assemble and clean, the material withstands a harsh cleaning routine and is corrosion resistant. Moreover, during milling with the mortar, pestle and sleeve, it is easy to directly observe the progress of fragmentation. These features result in an improved sample throughput for a given number of working hours (Table 1). The design is capable of handling hard bones, including Atlantic walrus [14], and is sufficiently robust to be used in combination with a rubber hammer, with no risk of overheating. We find that the tool proved to be especially useful while handling petrous bone samples, whereby the lower density parts of the bone break off preferentially after initial impacts of the pestle, making it easy to isolate the dense section of the petrous bone, which contains the most endogenous DNA [15,16], for further fragmentation. Finally, this design can be acquired at a fraction of the cost of an oscillating mill and beaker (Table 1).

In our experience, the three-piece stainless-steel mortar, pestle and sleeve is a versatile, economical and effective tool for fragmenting ancient bone. We have introduced specific modifications that are particularly useful for aDNA applications and find that the possibility to monitor the degree of bone pulverization while containing bone fragments within the sleeve provides a distinct advantage. The tool's simple design allows university workshops equipped with a lathe, boring tools and a milling machine to make these sets at local expense, which – in Norway – is more economical compared with commercially available varieties designed for ores and minerals. Moreover, the capital investment is significantly less than oscillating mixer mills. We recommend this design as a useful, economical addition to existing laboratory equipment for the efficient handling of ancient bone.

Author contributions

BS & ATG conceptualized the design. ATG designed the mortar, pestle and sleeve. ATG tested all designs. BS and SB provided

Table 1. Comparison of milling tools for the fragmentation of ancient bone.

		Zirconium beakers	Steel beakers	Mortar, pestle and sleeve
Time (min)	Tool assembly	2	2	<0.5
	Cleaning; one set (batch of six sets)	35 (75)	30 (70)	20 (55)
Cost (US$)	Single tool set†	$2,683	$1,636	$186‡
	Oscillating mixer mill§	$11,604	$11,604	N/A
Throughput (no. of samples per 6 h)		12	12	18
Observation during milling		Requires (re)assembly	Requires (re)assembly	No (re)assembly
Maximum bone hardness		Hard	Medium	Hard
UV resistance		No	Yes	Yes

Estimates for time, cost and workflow are based on laboratory routines and samples handled at the aDNA laboratory of the University of Oslo. These values are therefore approximate and affected by differences in laboratory routines, type of bone, exchange rates, shipping and local labor costs.
†A minimum of two matching beakers is required for operating the oscillating mill. A single tool set consists of a beaker and ball (Zirconium; Retsch™ 014620201, 053680093. Steel; Retsch™ 024620213, 053680105).
‡The cost for mortar, pestle and sleeve includes material and labor.
§Retsch™ 207450001 Mixer Mill.

funding and consumables. BS & ATG wrote the manuscript in collaboration with SB.

Competing interests

The authors declare no competing interests.

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