Collagen Isolation from Bioapatite

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Please contact the Stable Isotope Ecology Laboratory Technical Director with any questions or clarifications. **StableIsotopes@ucmerced.edu**

Supplies

- ☐ Gloves
- ☐ Eye protection
- ☐ Lab coat
- ☐ 0.1 M hydrochloric acid
- ☐ 5.75" glass pipettes and rubber bulbs
- ☐ 1.5 mL Microcentrifuge tubes
- ☐ microcentrifuge tube rack
- ☐ 1000 µL pipette with tips
- ☐ DI water
- ☐ One glass beaker for chemical waste

Introduction

This guide covers the extraction of collagen from well preserved bioapatite (bone, dentine) samples[1]. This guide assumes that the samples were collected using a dental drill or have otherwise been ground into a fine (flour-like) powder. If you are working with chunks of bone the timings will need to be adjusted. This guide is intended for well preserved modern samples and does not cover gelatinization, filtration, lipid extraction, or contaminant removal from collagen. If these steps are necessary for your samples please contact Dr. Robin Trayler (rtrayler@ucmerced.edu), or Dr. Sora Kim (skim380@ucmerced.edu).

Safety

This procedure uses 0.1 M hydrochloric acid (HCl). Hydrochloric acid is corrosive and toxic, therefore familiarize yourself with the chemical MSDS for HCl found here. When handling concentrated HCl, always work in the fume hood with proper PPE (minimum of nitrile gloves, lab coat, and eye protection). When mixing 0.1M HCl be sure to always add acid to water. Be sure to store HCl in the proper labeled cabinets.

[1] Trayler, Landa, P. V., & Kim, S. L. (2023). Evaluating the efficacy of collagen isolation using stable isotope analysis and infrared spectroscopy. Journal of Archaeological Science, 151, 105727–. https://doi.org/10.1016/j.jas.2023.105727

Reagents

Hydrochloric Acid (0.1 M)

Working in the fume hood, use a 10 mL pipet add 4.13 mL of stock (12.1 M) HCl to 495.87 mL of deionized water. This will make 500 mL of 0.1 M HCl. Store in a labeled glass bottle in the laboratory refrigerator.

Steps

Preparation

1. Weigh about 3.0 mg of powdered bioapatite into 1.5 mL micro centrifuge tubes. **Label the tubes with the sample name**.
    - Record the sample weights in your lab notebook.
    - If working with larger samples (> 4 mg) use a larger glass or plastic tube.
2. Store with the tubes closed until ready to demineralize.

Demineralization

1. Add 1 mL of 0.1 HCl to each micro centrifuge tube using a 1000 µL pipet.
    - If working with larger samples, scale the amount of acid by 0.33 mL/mg.
2. Note the time acid was added to the samples.
3. Vortex the samples for about 5 seconds each.
4. Transfer the samples to the laboratory refrigerator.
    - The ~4°C temperature of the refrigerator slows the demineralization reaction time.
5. After **1 hour** take the samples out of the refrigerator.
6. Visually inspect the samples for any chunks of un-demineralized bioapatite. Fully demineralized samples should be pale white and translucent.
    - If there are opaque white spots, these may be partially demineralized chunks of bioapatite. Return the samples to the refrigerator and check again after 15 minutes. Repeat as necessary.
7. Once the demineralization reaction is complete, centrifuge at 10,000 RPM for 5 minutes. **Make sure the microcentrifuge is balanced**.
8. Using clean glass pipette for each sample, discard the supernatant into a waste beaker. Be careful not to pipette the collagen. It is better to leave more liquid in than taking too much out.
9. Add ~ 1mL of DI water to each sample and centrifuge again at 10,000 RPM for 5 minutes.

10. Repeat steps 7 - 8 for a total of 5 rinses.

    ☐ Rinse 1
    ☐ Rinse 2
    ☐ Rinse 3
    ☐ Rinse 4
    ☐ Rinse 5

11. After the water from fifth rinse is removed, cap the vials and place them in the lab freezer until frozen.
12. Once the samples are frozen, lyophilize (freeze dry) them overnight.
13. The final product should resemble fluffy/cotton-candy like texture.