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HOW REVERSIBLE ARE CONSOLIDANTS USED ON FRAGILE ARCHAEOLOGICAL BONES? A PRACTICAL EVALUATION OF B-72 IMPREGNATION

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ABSTRACT

This work presents the results of a pilot study to investigate the uptake of, and subsequent extraction of B-72 as a consolidant for fragile archaeological bones. A 10% solution of B-72 in acetone has a viscosity of approximately 5.80 ± 1.06 μcP and surface tension of around 20.5 ± 2.0 mNm-1. Despite a higher viscosity and lower surface tension, this concentration of consolidant penetrates porous archaeological bone extremely efficiently under capillary forces down to the nano-pore level. There is no requirement for vacuum impregnation to achieve full consolidation and strengthening of fragile bone. A single impregnation with a 10% solution resulted in a 7.7% increase in dry weight attributable to the solid B-72 resin within the structure. A second impregnation raised introduced up to 17.8 wt% of resin. B-72 is detectable in the consolidated bone using FTIR-ATR spectrometry but is not quantifiable. On un-aged consolidated bone specimens only approximately 54% of the resin could be removed by two immersion washes in acetone. Surprisingly, the resin proved easier to remove from both heat-aged (64-74% extractable) and uv-aged (61-62% extractable) specimens.

Keywords: Archaeological bone, Paraloid B-72, Consolidation, Extraction efficiency, FTIR-ATR.

1. INTRODUCTION

The consolidation of fragile archaeological bones has been a problem for the archaeologist, conservator and curator for many decades. Several different approaches have been made to solve the problem of reinforcing/strengthening individual bones or skeletons so that they can be first cleaned and documented (plans, photographs, etc.) in the field, and then later lifted for safe transport to a museum for further work and study. In the early days, natural resins and glues (*e.g.* shellac and animal glue) were used as adhesives and to strengthen fragile specimens. Shellac is a natural resin, often applied as flakes dissolved in hot alcohol (Koob 1979). It was commonly used as an adhesive on old museum repairs on ceramics and bone but went out of favor in the mid-1960s (Johnson 1994) due to color changes and poor aging properties (Brock *et al*. 2018). Animal glues are collagen-based glues derived from any number of sources such as rabbit skin, fish skin, cow hide or even bones themselves. In 1905 Rathgen recommended the application of natural resins such as dammer, shellac, isinglass and/or animal glues in appropriate solvents as methods of preserving fossil bones (Rathgen 1905, Cook and Ward 2008). Since the middle of the 20th century the use of synthetic resins in the adhering and consolidation of fragile or fragmentary archaeological and fossil bones became more popular. These resins include those that cured by solvent evaporation (*e.g.* Butvar B-76, Butvar B-98) by evaporation of water in various emulsions and colloidal dispersions (PVAc emulsions and Primal WS-42) or by chemical reaction (cyanoacrylates and epoxy resins) (Goldberg and Davison 2014, Brock *et al*. 2018). Later, the specific conservation resin Paraloid™ (Acryloid) B-72 became the resin of choice, both as an adhesive and, in more dilute form, as a consolidant for fragile and porous finds, including bones (Koob 1984; 1986, Cronyn 1990, Johnson 1994).

In the later part of the 20th century several authors attempted to evaluate the performance of these resins in terms of their penetration, curing and strengthening properties (Phillips 1978, Nakhla 1985, Wang and Schniewind 1985, Kres and Lovell 1994). More recently authors have considered the impact of different consolidants on later chemical, isotopic and biochemical analyses (Moore *et al*. 1989, Bruhn *et al*. 2001, D'Elia *et al*. 2007, Brock *et al*. 2018).

Our ongoing research aims to investigate the reversibility of consolidants applied during the excavation, cleaning and lifting of fragile archaeological bones in Taiwan. This is particularly relevant to the study of ancient human remains on the island. When a skeleton is discovered during an archaeological excavation it must generally be carefully cleaned *in situ* so that it may be photographed and drawn in detail. Often the bones are fragmentary and held together only by the surrounding sediment. In European excavations the soils are usually damp and resins dissolved in an organic solvent cannot be used. Instead, water-based acrylic dispersions or poly-vinyl acetate emulsions have been commonly used. In Taiwan, the soils are relatively dry, allowing resins dissolved in organic solvents to be applied. Consequently, Paraloid™ B-72 is the most commonly used acrylic resin for the temporary or permanent strengthening and gluing of fragmentary skeletal remains found in Taiwan.

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Although aqueous acrylic dispersions have been employed in the past in Taiwan, this current project focuses on consolidation of fragile bones with Paraloid™ B-72 dissolved in acetone. There are many reasons for the choice of solvent-based acrylics over acrylic dispersions. Soil degraded archaeological bone has a very large pore volume and a very small pore size. The total porosity of modern cow bone is approximately 23% vol/vol and much of the porosity is made up of natural channels and pores which in life hold bone cells and blood vessels. However, archaeological cow bone can have a total porosity of up to 65% and much of this lies below 500 nanometres diameter (Turner-Walker & Parry 1995). Surprisingly, even in bones buried in soils much of this porosity is empty. In damp soils the smallest pores may be filled with water but soil generally does not fill pores smaller than a few millimetres in diameter. The droplet sizes of the dispersions are of the order of 1 μm for PVAc and 200 nanometres for the acrylics. So, in dispersions and emulsions the consolidant is unlikely to penetrate the smallest pores. In the case of damp bones the large particle sizes of dispersions also restricts diffusion rates within the complex pore networks present in degraded bones. Coalescence of emulsion droplets is likely to block pores in the degraded bone as it cures and make later removal unpredictable and difficult to assess. For these reasons, and because B-72 is now the most common consolidant used in Taiwan, our initial studies have focused entirely on B-72 since this makes our experiments both simpler and more reproducible.

Paraloid™ B-72 is a clear, colorless, thermoplastic resin – an ethyl methacrylate (70%) methyl acrylate (30%) copolymer – that is considered to be one of the most stable and reversible consolidants used in conservation (Feller *et al*. 1792). Originally created by Rohm and Haas as a surface coating and binder for printing inks it is described as being a durable and non-yellowing acrylic resin that is stronger and harder than polyvinyl acetate (PVAc) without being as brittle as some other consolidants used on porous archaeological materials (Koob 1986). It is soluble in acetone, ethanol, toluene, and xylenes, and various solvent mixtures. B-72 has a glass transition temperature (T_g) of approximately 40°C which makes its use as an adhesive in Taiwan potentially risky in uncontrolled environments where ambient temperatures may approach or exceed 40°C. Nevertheless, B-72 is in widespread use in museums in Taiwan and has been routinely used in the consolidation and adhesion of fragile bone specimens – both on site prior to lifting skeletons and during post-excavation cleaning.

2. MATERIALS AND METHODS

Two groups of bone samples were used in this study. In the initial round of experiments, a heavily degraded archaeological cow metapodial from the Romano-British site of Stanwick in the North of England from the author's (GTW) reference collection was used. This bone was excavated from a typical aerated soil and was heavily degraded by soil bacteria, with a low residual collagen content and high porosity. It was still intact and was strong enough to permit several slices to be sawn from the mid-shaft. This bone could be sacrificed for destructive analysis.

In the second round a larger group of assorted small bone specimens from an archaeological site in Taiwan (*Niu Hong Kong Site*) was borrowed from the Tree Valley Foundation and consisted of fragments of deer rib and long bone. These were used to evaluate reversibility without destroying the physical form of the samples and to better approximate conditions in the field. The samples are illustrated in Fig. 1.

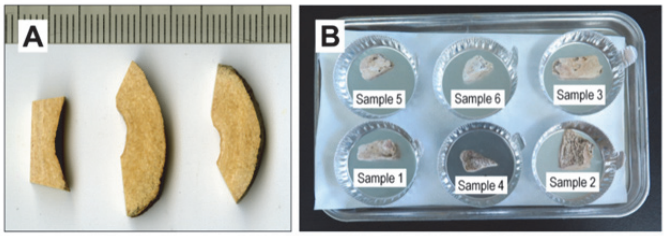

Fig. 1 Cow metapodial samples for Experiment 1 (A) and the samples for non-destructive Experiment 2 (B)

The consolidant used in the experiments was a precisely measured 10% solution of B-72 in acetone with the specifications given in Table 1, below. Knowing the exact concentration was important so that the volume of the pores filled by consolidant could be calculated. Density of the solution was measured by volumetric methods using a density bottle. This solution was used in all the impregnation experiments.

Table 1 Measured parameters of the 10% B-72 solution and the given densities of acetone and B-72

Density[1]	Viscosity[2] (av. n = 6)	Surface Tension[3](av. n = 2)
0.827 g cm^{-3}	5.80 ± 1.06 μcP	20.5 ± 2.0 mNm^{-1}

1 = Density bottle method;
2 = Falling ball method;
3 = capillary rise method

2.1 Experiment 1

Volume Determination and Consolidant Penetration

The cow metapodial was sawn into slices approximately 5 mm thick and the sawn faces ground to flat, approximately parallel sides on an electric grinder-polisher (Metkon Gripo 2v). The bulk volume (the external volume of the sample including pores) of each experimental bone sample was calculated by making a high resolution scan of each side of the parallel-sided slice, measuring the average cross-sectional areas using image processing software (Gimp 2.8.16) and multiplying this by the average slice thickness measured using a precision micrometer (Teclock Corporation). The results are expressed in cubic centimeters (cm^3).

The total porosity of each sample was calculated by the water infiltration method as described elsewhere (Turner-Walker & Parry 1995; Turner-Walker 2011). This involved drying the samples to constant weight in an oven at 105°C, then immersing in pure water under vacuum overnight. Samples were quickly blotted of excess water and reweighed. The gain is weight was assumed to be water filling all of the pore spaces below 1 mm and this was converted into a volume by assuming the absorbed water had a density of 1 g cm^{-3}. Samples were then allowed to dry in air for 3 days and transferred to a vacuum desiccator containing dry silica gel before reweighing.

It is considered that water impregnation for approximately 12 hours will flood pores down to a pore diameter of approximately 40 nanometres (Turner-Walker 2011). This is the size of the "gap zone" in the collagen molecules. Water will also penetrate the smaller pores between tropocollagen molecules but this will be much slower, because this water becomes part of the mo-

lecular structure of the collagen.

The metapodial bones slices were impregnated by placing the sample in a glass petri dish and adding 10% B-72 solution to the dish so that the consolidant was wicked up into the pore spaces by capillary forces. This impregnation took from 3-6 minutes depending upon the thickness of the sample. The lid was placed on the petri dish during impregnation to reduce evaporation of the solvent.

With the capillary impregnation experiments the bone slice was assumed to be fully consolidated when liquid had reached the top of the sample and the upper surface looked "wet". However, the hydroxyapatite found in bone is also used in thin layer chromatography and it was possible that the B-72 acrylic resin was separating out and only the acetone was reaching the upper surface. To check that it was actually the B-72 reaching the top of the sample and not just the acetone we labelled the 10% B-72 solution with a fluorescent dye. A pink or red highlighter pen contains "Solvent Red 49" a rhodamine dye with a large molecular weight (479) compared to that of acetone (58) and might be expected to separate out in the same way as B-72.

Fluorescent labelling has been used in the past by other researchers to investigate the penetration of consolidants used to stabilize powdery paint layers on paper. Hummert et al. (2013) used this method to investigate the penetration of gelatin and methylcellulose into handmade rag papers. Tests with the fluorescently labelled B-72 solution suggested that acetone and resin did not fractionate appreciably during the impregnation process. Figure 2 suggests that the solvent reaches the top of the slice slightly before the fluorescent dye (note the difference in fluorescence between the high points – blue – and the rest of the slice – red. For this reason, in the test samples the bone slice was impregnated for slightly longer than it took to wet the specimen.

Fig. 2 Impregnation of a bone slice with fluorescently labelled B-72 (A-B); and viewed under uv light (C).

After impregnation the samples were allowed to dry by placing them on a stainless steel mesh in the open air for several days, then transferring to a vacuum desiccator filled with silica gel before reweighing. Solvents can remain trapped in the resin and take several days to escape if the polymer film is below its glass transition temperature (Cairncross 2002). The mass of the B-72 resin absorbed could be calculated from the weight gain compared to the silica gel dry bone and this was converted to volume using the density of B-72, and the equivalent volume of B-72/acetone solution absorbed.

2.2 Experiment 2

For the irregular fragments of bone from the Tree Valley Foundation the 10% B-72 solution was dripped onto the fragment using a polyethylene Pasteur pipette. This more closely simulated the conditions under which a bone or skeleton may be consolidated in the field. In many cases the pores sizes were much larger than in the cow metapodial due to the larger proportion of spongy (cancellous) bone in the specimens. This method was harder to control than the capillary impregnation technique used for the bone slices and the irregular porosity meant that it was hard to estimate how much of the pore volume was filled. Nevertheless, total weight and volume of B-72 resin absorbed in the consolidated samples were estimated by the methods described above.

These six samples were divided into four groups. Two that were to be that were to be impregnated only one time and then exposed to 60°C for 100 hours; two that were impregnated one time and exposed to intense uv light for 36 hours, one sample that was impregnated but left unaged and one that was not impregnated at all.

2.3 B-72 Extraction

Resin extraction was achieved by suspending the samples (one by one) in a stainless steel basket in an excess of clean acetone for 24 hours while continuously stirring (Fig. 3). Each sample (1.5 ~ 2.5 g) was immersed in 200 ml (ratio of ~ 1:100 wt:wt) of acetone. This was repeated to make a total of 48 hours and 400 ml of acetone. Samples were removed from the acetone bath, blotted quickly and allowed to dry on a stainless steel mesh. They were then allowed to acclimatize to room temperature and humidity for three days before being transferred to a vacuum desiccator containing dry silica gel and reweighed.

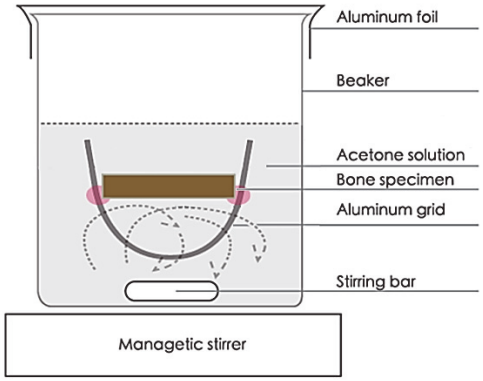

Fig. 3 Extraction method for bone samples

2.4 SEM Examination

A polished section of the impregnated metapodial bone slice was viewed under backscatter SEM (BSEM) to evaluate the deterioration of the bone. Embedding, polishing, and preparation for electron microscopy followed protocols described in Turner-Walker and Jans (2008). An EDX analysis was conducted along a line scan close to one of the larger pores to evaluate resin penetration into the bone's interior. In addition, one small sample each of unconsolidated bone and one sample of 10% B-72 consolidated bone were palladium coated and examined in the SEM using secondary electron (SE) imaging. The two samples came from the same slice of the metapodial bone shaft and were separated by approximately 10 mm so it is safe to assume that their structures should be nearly identical.

2.5 FTIR Spectrometry

Infrared spectrometry was undertaken on un-impregnated and impregnated (Romano-British metapodial) bone and on a thin film of pure B-72 in acetone cast onto a glass slide and removed with a scalpel. Attenuated total reflectance (ATR) analyses were undertaken on the thin film and KBr disks were prepared for the powdered bone samples. Infrared spectra were recorded for each disc using a Tensor 27 FTIR interferometer (Bruker Optics) in the 4000–400 cm^{-1} region, averaging 64 scans at a resolution of 4 cm^{-1}.

3. RESULTS AND DISCUSSION

3.1 Experiment 1

The Fig. 4A shows plots of oven dried weights against total volume (bulk volume) and pore volume for the three samples analyzed in Experiment 1. The results are very consistent (R^2 values 0.97 ~ 0.99) and the pore volumes typical for heavily degraded archaeological bones (~ 53%). This suggested that several slices from the same bone could be used to evaluate resin penetration, extraction efficiency and achieve comparable results (Table 2).

Turning to the B-72 impregnation tests on Experiment 1 we find that the volume of B-72/acetone solution entering the pore structure was very nearly the same as the water penetration. This suggests that the viscosity and surface tension forces for the 10% B-72 in acetone were not appreciably different from water. In fact, the viscosities are quite different (B-72 solution = 5.80 ± 1.06 μcP; water = 0.89 μcP) and the surface tension of water is considerably higher (B-72 solution = 20.5 ± 2.0 mNm^{-1}; water = 72.0 mNm^{-1}). A single impregnation with 10% B-72 solution reduced the total porosity of the bones by 8.2%. A second impregnation of the dried specimen reduced the remaining porosity by a further 6.6%. This probably means that some of the smaller pores becoming completely blocked with solid resin from the first impregnation that does not re-dissolve when the second impregnation is applied.

Comparing the weight gain in the fully dried, B-72 impregnated bone samples it was possible to calculate the amount of 10% B-72 in acetone absorbed into the bone using the density of B-72 and vol/vol concentration of the solution. Surprisingly, the consolidant solution filled approximately the same volume of pores as did the water. This suggests that a 10% solution of B-72 penetrates the degraded bone tissues down to a sub-micron level. This implies that the bone will be fully consolidated (strengthened) by a 10% solution of B-72 in acetone, but how easy is it to remove a resin that has penetrated so thoroughly? For only a single impregnation the extraction efficiency was surprisingly good at 88 ~ 90% (Table 3).

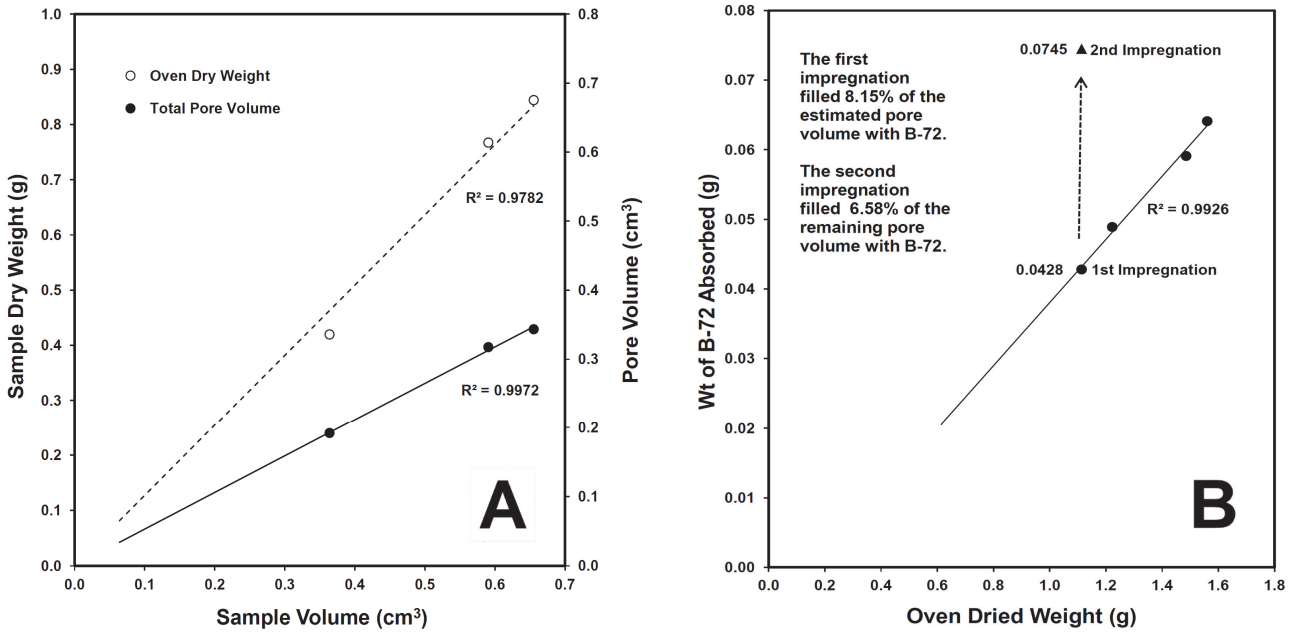

Fig. 4 Results for porosity measures (A) and B-72 impregnations (B) for Experiment 1

Table 2 Measured parameters of vol/vol and vol/wt porosities for samples in Experiment 1

Sample	Area (cm^2)	Thickness (cm)	Volume (cm^3)	Dry wt. (g)	Wet wt. (g)	Total pore vol.	vol/vol porosity	vol/wt porosity
A	0.6645	0.5476	0.3638	0.4198	0.6116	0.1918	52.7	45.7
B	1.3656	0.4798	0.6552	0.8442	1.1876	0.3434	52.4	40.7
C	1.2319	0.4793	0.5904	0.7673	1.0848	0.3175	53.8	41.4
			Average (SD)				53.0 (0.7)	42.6 (2.7)

Porosity of Experiment 1 samples in terms of volume/volume and volume weight.

Table 3 Measured parameters of vol/vol and vol/wt porosities for samples in Experiment 1

Sample	Wt after B-72 (g)	B-72 content (g)	B-72 (%)	Wt after Extraction (g)	B-72 removed (g)	Extraction efficiency (%)
A*	1.1973	0.0745	17.8	1.1637	0.0336	45.10
B	1.6356	0.0641	7.6	1.5778	0.0578	90.17
C	1.5568	0.0591	7.7	1.5043	0.0525	88.83

Sample A was consolidated twice (Figure 4B) and had a considerably higher final loading of B-72 resin (17.8% of the dry weight of bone) compared to the samples that received only one impregnation (~ 7.7% of the dry weight). This had a serious effect on the subsequent extraction efficiency (~ 45% compared to ~ 90%). This may be because acetone in the second impregnation swelled the original film, effectively increasing its volume sufficiently to block pores and create bottlenecks. For heavily impregnated bones a double extraction regimen may be required – the second one being a variation of "Sohxlet extraction" where clean acetone is recirculated to run through the sample continuously. The success of any solvent extraction, however, depends on the interconnectedness of the pore network and the orientation of the sample in the solvent flow. The extraction may have worked much better with powdered or granulated samples rather than with whole pieces of bone but this would have made measuring the weights of the various samples almost impossible.

3.2 SEM Results

The results of SEM investigations proved interesting. As expected from the appearance and the weight of the cow metapodial, as well as the porosity measurements, the bone tissues had been severely degraded by soil bacteria resulting in a large increase in the microporosity (Fig. 5). It is not possible to visualize the B-72 directly in SEM backscatter mode because it is indistinguishable from the embedding resin. However, it was possible to detect the depth of penetration of the B-72 into the bone tissue by performing a line scan EDX to measure relative Ca, P, C and O concentrations across one of the larger resin filled pores and the into the bone tissues (Fig. 6).

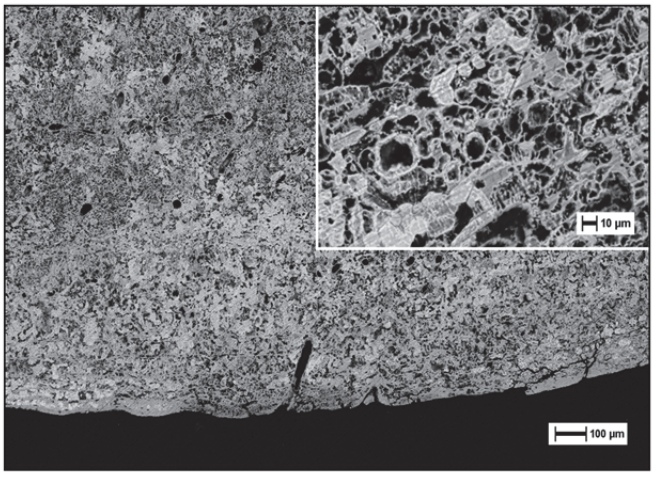

Fig. 5 BSEM image of impregnated bone section at 55X and 500X (inset). Note the high porosity caused by bacterial degradation.

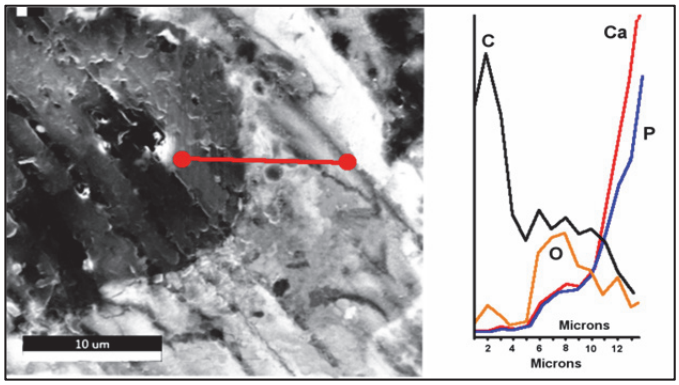

Fig. 6 BSEM image and EDX line scan across a large pore and the surrounding bone tissue

1-5µm epoxy resin; 5 ~ 10µm B-72 & porous bone; 10 ~ 13µm compact bone.

Looking at the line scan we see from the carbon line that the high peak represents the epoxy resin in which the bone sample was embedded (0-5 microns). The calcium and phosphorus content is effectively zero in the pore as one would expect. As the line passes from the epoxy-filled pore the carbon value drops to less than half and the oxygen content increases dramatically (5-11 microns). Acrylic resin has an oxygen content approximately 2.5 times that of epoxy resin and therefore a carbon content of less than half. This shows that the B-72 is present in the degraded bone tissues. Deeper into the sample the calcium and phosphorus contents increase sharply as an area of densely redeposited bone mineral lies on the line. The B-72 penetration falls of steeply because little resin enters this very dense material.

Results for the secondary electron investigation of the unconsolidated and consolidated bone are shown in Fig. 7. Figures. 7A and 7B show the very porous nature of bacterially degraded bone. At higher magnifications that it is possible to see the tunnels made by soil bacteria Fig. 7C and 7D. The bacterial tunnels are very small, with diameters typically in the range 300 ~ 500nm. There is little obvious difference between the consolidated and un-consolidated bone. At 20,000X the bacterial tunnels in the consolidated sample appear to be lined with a layer of thin, needle-like crystals (Fig. 7D), whereas these are not evident in the unconsolidated bone (Fig. 7C). These are not visible in the un-consolidated sample. However, there is no evidence for a continuous film or layer of smooth resin on the surfaces of the tunnels. Drying polymer films can sometimes exhibit a wrinkled or puckered surface as a result of surface tension and other forces experience during drying (Cairncross 2002, Pauchard, and Allain 2003, Poulard and Damman 2007). However, none of these authors describes anything like the needle structures seen in the SEM images. Thus, it seems likely that the 10% B-72 solution is able to penetrate into the very smallest pores in the degraded bone structure and does not simply act as a pore filler. The nature of the needle-like structures requires further investigation with SEM-EDX.

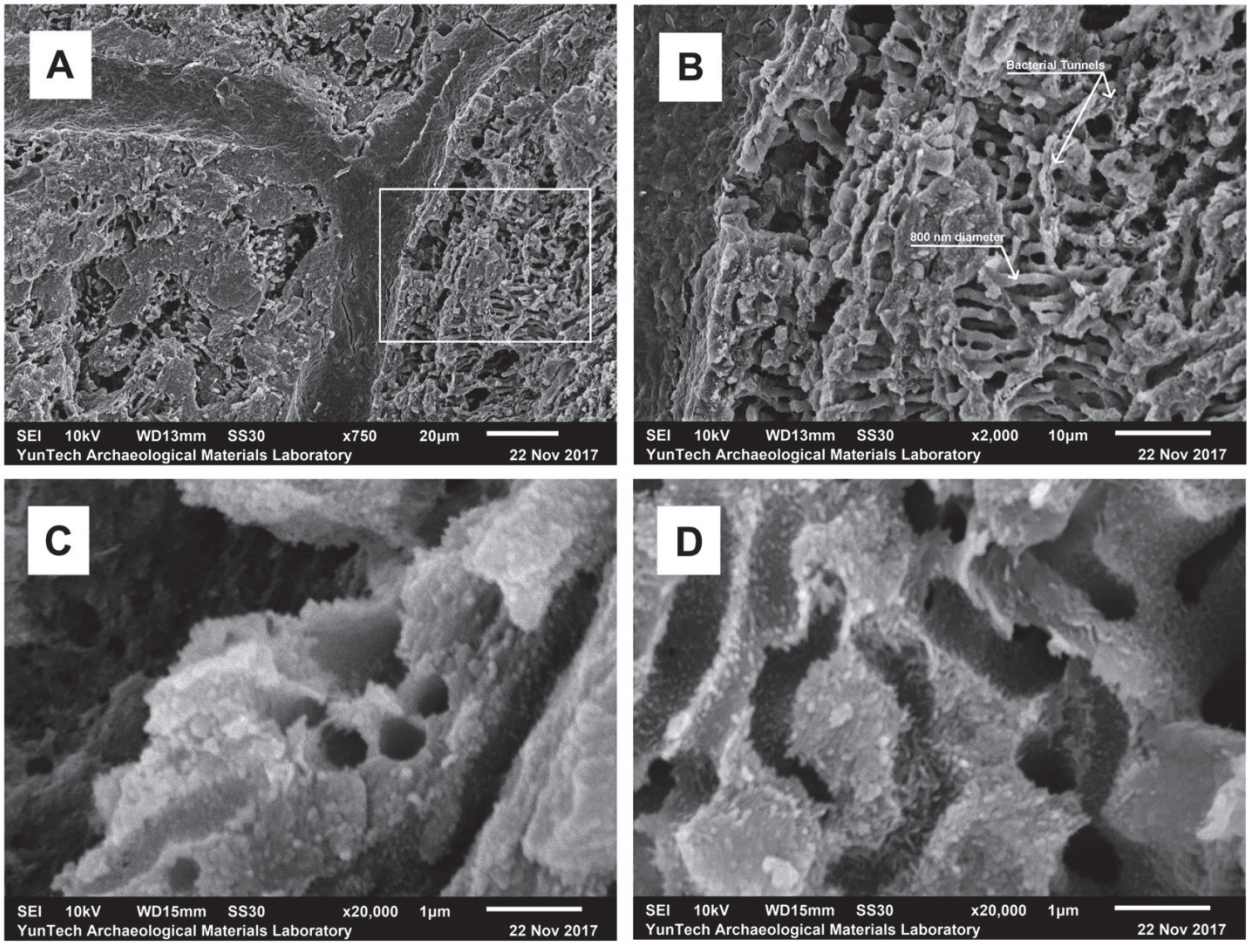

Fig. 7 SE images of unconsolidated archaeological bone (A-C) and bone consolidated with 10% B-72 (D). Even at high magnification there is little evidence for a resin film covering the surfaces.

3.3 FTIR Spectrometry

The spectra for the bone powders are typical for archaeological bone. The principal absorption bands are shown for the collagen and mineral components. The full spectrum for a typical archaeological bone illustrating the principal absorption bands is shown as the inset in Fig. 8. Despite the severe bacterial degradation of the specimen there is still some residual collagen in the compact tissues – shown by the Amide I and II absorption bands. B-72 has a major absorption band at 1725 cm^{-1} which also corresponds to the Amide I band in collagen. There is a visible deepening of the Amide I absorption in the bone sample that was consolidated with B-72. A slightly enhanced absorption is seen at the $\delta(CH_2, CH_3)$ position. Unfortunately, the major absorption for B-72 at 1165 cm^{-1} is lost in the major phosphate ν_1, ν_3 absorptions bands. This demonstrates that FTIR can be a very sensitive method for detecting consolidants in bone, even when these consolidants make up only a small percentage of the total weight. It may be more difficult to detect in bones with larger amounts of residual collagen but these are unlikely to require consolidation.

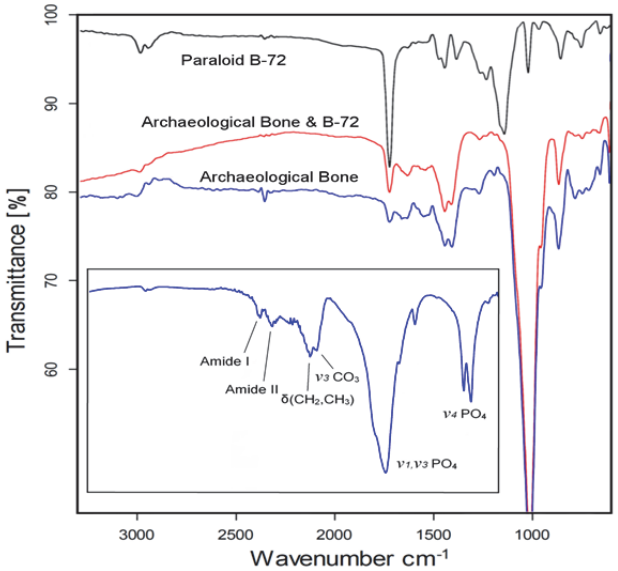

Fig. 8 FTIR-ATR spectra for the consolidated and unconsolidated bone, together with that for the B-72 film. Note the deepening of the absorption bands at the positions of the Amide I and δ (CH_2,CH_3) for the sample consolidated with B-72

Experiment 2: Extraction Efficiency

The weight changes accompanying B-72 impregnation and subsequent acetone extractions for the six archaeological bone samples are shown in Fig. 9. The control bone shown no change in weight in terms of the silica gel dried weight. The extraction efficiency was slightly higher for the heat aged bones compared to the uv aged specimens, but it is hard to state this with any confidence because of the differing porosities and pore size distributions of the different specimens. One surprise finding is that it was harder to extract B-72 from the un-aged sample. Only a little more that 50% was extracted compared to the aged samples where more than 60% was extracted. This is even more surprising because the un-aged sample (Sample 5) was the smallest and lightest specimen. One possible explanation is that the aging mechanism actually causes a shortening of the polymer lengths, thus making it easier for the polymer strands to dissolve and diffuse out of the pore spaces.

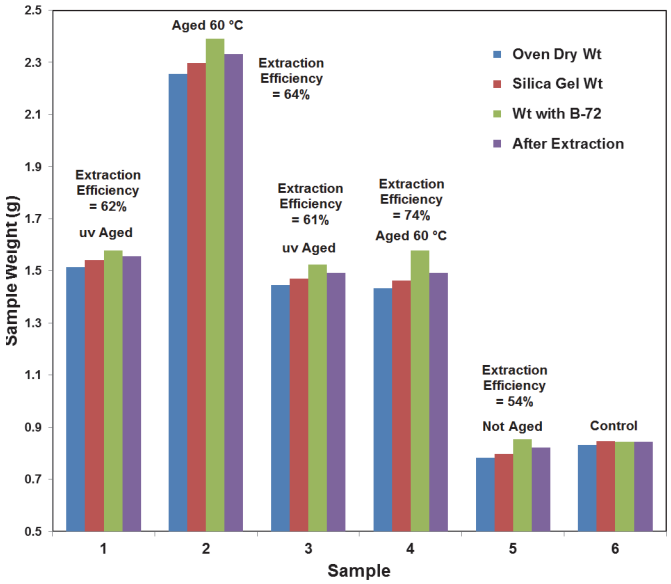

Fig. 9 Results of extraction efficiency after heat and uv ageing of B-72

The control sample did not get impregnated.

Extraction efficiencies in all of the second experiments were much poorer than the efficiency seen in Experiment 1. Sample 4 had the greatest extraction efficiency at 74% but this also had the most open pore structure. Samples 1 and 3 where the spongy bone was enclosed in a thin shell of compact bone both had extraction efficiencies of around 61-62%. Sample 2 had the spongy bone more exposed and had a slightly higher extraction efficiency at 64%.

4. CONCLUSIONS

Our pilot study demonstrates that relatively consistent results for porosity and uptake of consolidant can be achieved using comparable bone samples. Penetration of a 10% solution of B-72 in acetone is extremely efficient and the solution will infiltrate the bone under capillary forces down to the nano-porosity. There is no requirement for vacuum impregnation to achieve full consolidation and strengthening of fragile bone. A single impregnation with a 10% solution resulted in a 7.7% increase in dry weight due to the consolidating resin. A second impregnation introduced up to 17.8% resin. In bones with low collagen contents the presence of even ~7% of the resin is detectable by FTIR spectrometry.

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