Effect of decalcification on bone strength.

Introduction

Osteoporosis is a condition that weakens bones making them fragile and more likely to break. This condition affects many millions of people around the world, and specifically around 3 million people in the UK. (1)

(1) http://www.nhs.uk/conditions/osteoporosis/pages/introduction.aspx

Osteoporosis is the result of losing bone density. This process starts gradually from ages around 35 and is a normal part of the aging process, although in some people can lead to osteoporosis and an increased risk of bone fractures. Osteoporosis is more common in old people and especially in women after the menopause, as it produces hormonal changes that can result in osteoporosis. There are also other risk factors like inflammatory conditions, hormonal conditions, family history, long term medications, heavy drinking and smoking, etc. Many people like me have been affected by this condition, as elder members of my family suffer from it, so I thought it would be worth designing an experiment that would study the effect of decalcification in the structure and function of the bone.

Bones form part of our skeletal system and their main function is mainly support, protection, movement of the limbs and being a mineral reservoir, amongst many others. Most of the calcium is stored in bones as well as most of the potassium. Bones are composed of an organic matrix (mainly collagen) and a mineral component (especially hydroxyapatite, which is an insoluble salt of calcium and phosphorus), on top of small amounts of other minerals like magnesium, sodium and bicarbonate. The rest of the bone (around a 25% in adult bones) is just water.

Below, the equations for the chemical reactions mainly responsible for the decalcification of the bone using an acid.

Ca_{10}(PO_4)_6(OH)_2(s) \rightleftharpoons \ce{10Ca^{2+} + 6PO_4^{3-} + 2OH^{-}}

PO_4^{3-}(aq) + H_3O^+ (aq) \rightarrow HPO_4^{2-}(aq) + H_2O(l)

OH^{-}(aq) + H_3O^+ (aq) \rightarrow 2H_2O(l)

(2) https://www.chem.wisc.edu/deptfiles/genchem/netorial/rottosen/tutorial/modules/acid_base/04salts/salt4.htm

As portrayed in the previous chemical equations, the equilibrium for the conversion of the hydroxyapatite (insoluble solid) into soluble calcium is shifted to the right by the presence of the acid, as HCl reacts chemically with the phosphate and hydroxide ions. The result is the removal of calcium from the bone that then stays in solution. The bubbles of carbon dioxide observed during the experiment correspond with the reaction of the acid with other calcium compounds present in the bone like calcium carbonate.

Whereas collagen provides the required flexibility to the bone, the mineral salts provide the strength necessary to perform its function. In this experiment I am going to use an acid (HCl 1M) to decalcify the bone and I am going to observe changes in the strength of the bone by hanging masses from it and
measuring the flexion (how much the bond bends) obtained after different degrees of decalcification. As the bone is decalcified, the mineral salts disappear from it and only the collagen is left. Therefore, as the decalcification occurs, the bone will be less strong and more flexible, hence it will bend more when the mass is placed.

**Research question:**

What is the effect of the decalcification by HCl of lamb rib bones on their strength measured (± 1 mm) as the degree of flexion observed after hanging a 500 g mass on the tip of the bone.

**Variables:**

**Independent variable:** decalcification, measured as the amount of time that the bone spends in a given HCl 1M solution (20 minutes intervals). The more time in the acid, the more decalcification will occur.

**Dependent variable:** length of the bone flexion after hanging a 500g mass in a specific point of the bone, measured in mm (± 1 mm).

**Controlled variables:**

**Concentration** of the acid solution: must be maintained constant. Concentration refers to the amount of moles of acid dissolved in one liter of solution (water). The higher the concentration, the more acid available per volume, and the faster the decalcification process.

**Volume of the acid solution:** for a given concentration, there is a fixed amount of acid in a given volume of the solution. As the decalcification chemical reactions occur, the acid is used up. In 20 minutes the amount of acid of the solution would have decreased significantly, so using always the same volume and therefore the same amount of acid is important for it to be a fair test.

**Same (HCl) acid used in all trials:** Different acids produce different amount of hydrogen ions, which are the ones which react with the calcium in the bone. The coefficients in the chemical reactions would change with other acids, for example with sulfuric acid, meaning that the reaction would be basically different. Also, the strength of the acid affects its dissociation, and the chemical reaction. HCl is a strong monoprotic acid (2). This is why the same acid has to be used each time.

(2) Brown, Ford (2008); “Chemistry”, Person Baccalaureate.

**Temperature:** (performed inside a lab with a constant temperature of about 23°C). Temperature affects the speed of chemical reactions. If the temperature would change in this experiment, the rate of decalcification would change as well. To keep the rate constant temperature should be constant as well.

**Type of bone:** Rib bones from the same lamb will be used for the experiment. They are similar in shape (shape would be another factor that would affect the strength of the bone) and being from the same animal the level of calcification will be similar (different animals would have different amounts of calcium compounds in their bones depending on factors like diet, activity, etc).

**Mass used to test flexion:** the more mass, the more flexion would be expected, so it needs to be kept constant.

**Safety and Ethical Considerations**
Safety goggles must be worn at all times since I will be working with an acid (even when the concentration is only 1M)

Extra care must be taken when removing the meat from the bones before the experiment. A knife will be used to remove the bigger chunks, so there is a risk of getting cut.

As I will be hanging masses from the bones, extra care will be placed in case any of the bones shatter and their surfaces might get sharp edges as a result.

Latex gloves will be worn at all times since I will be dealing with raw meat and bone.

Lamb bones obtained from an ethical source through local butcher

**Apparatus:**
- Ruler
- Stand
- Clamp
- Camera
- Measuring cylinders
- Burette
- HCl solution 1M
- NaOH solution 0.5M
- Phenolphthalein
- Stop watch
- Pipette
- Distilled water
- Lamb rib bones
- 500g mass.

**Procedure**
1. Clean the lamb rib bones, remove all the meat and wash them thoroughly.
2. Prepare 300 ml of HCl 1M and place them into 3 separated measuring cylinders, 100 ml each.
3. Add 500 ml of distilled water in a beaker
4. Place the 3 bones into the measuring cylinders with the acid for 20 minutes in 3 minutes intervals to allow for enough time to make the measurements.
5. Start the stop watch and time 3 minutes.
6- Position the camera and the ruler so they do not move and so the camera is looking at the meter ruler.
7- After 20 minutes, remove the bone from the measuring cylinder, rinse it with distilled water, dry it with a paper towel and clamp it to the table.
8- Measure with a ruler the distance from the edge of the table and the place where the mass will be positioned.
9- Using the camera, which is fixed in a position during all the experiment in front of the clamp and the ruler, take a picture of the initial point to measure the length.
10- Position the 500 g mass close to the tip of the bone (see diagram below)
11- Take a second picture with the camera to record the bone flexion.
12- Repeat the same procedure for the other two bones.
13- Put the bones in the measuring cylinders again and repeat the whole process 5 times per bone.
14- At the end of the experiment get a sample of the acid in each measuring cylinder and perform a titration using phenolphthalein and sodium hydroxide 0.5M to find out the amount of acid that reacted with the bone minerals.
15- Look at the pictures and calculate the bone flexion in mm using the camera zoom for accuracy.

Data table to show measured bone flexion and calculated averages for 3 different bone samples.

<table>
<thead>
<tr>
<th>Time in HCl 1M (minutes ±10 s)</th>
<th>Bone 1 flexion (mm ± 1.0 mm)</th>
<th>Bone 2 flexion (mm ± 1.0 mm)</th>
<th>Bone 3 flexion (mm ± 1.0 mm)</th>
<th>Average (mm ± 1.0 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.0</td>
<td>2.0</td>
<td>4.0</td>
<td>3.3</td>
</tr>
<tr>
<td>20</td>
<td>8.0</td>
<td>3.0</td>
<td>7.0</td>
<td>6.0</td>
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<tr>
<td>40</td>
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<td>8.0</td>
<td>7.0</td>
</tr>
<tr>
<td>60</td>
<td>6.0</td>
<td>6.0</td>
<td>8.0</td>
<td>6.7</td>
</tr>
<tr>
<td>80</td>
<td>9.0</td>
<td>6.0</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>100</td>
<td>9.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.7</td>
</tr>
<tr>
<td>120</td>
<td>10.0</td>
<td>7.0</td>
<td>8.0</td>
<td>8.3</td>
</tr>
<tr>
<td>140</td>
<td>11.0</td>
<td>9.0</td>
<td>12.0</td>
<td>10.7</td>
</tr>
</tbody>
</table>
Analysis, conclusion and evaluation

In the graph we can observe a positive correlation between the two sets of data, meaning that as the time the bone is immersed in HCl and therefore the higher the decalcification of the bone, the more it will bend under stress. These results were expected, as the collagen remains initially unaffected by the action of the acid, so the bone remains flexible and can bend, although the calcium salts that give the strength to the bone dissolve due to the action of the acid (see chemical equations in the introduction) and have a clear impact in the ability of the bone to resist flexion. When the bone is within normal levels of calcium, it hardly bends when the force (in this case the 500 g mass) is exerted, whereas as it loses calcium the bone bends more easily. The effects of the decalcification were noticeable also just by touch, as the bone after being for some time in the acid did feel smoother and bend easier in my hands.

The experiment was performed at the same time for 3 different rib bones and averages were calculated. Logically, different bones had different characteristics in terms of shape and dimensions, but they were all coming from the same lamb from the local butcher, so we can assume that the levels of calcification would be very similar in the three of them.

As for the decalcification process, slight differences were observed at the end of the experiment when the titrations were performed to find out the amount of acid used in each of the solutions and therefore the final amount of calcium removed. These fluctuations, especially comparing the first bone with the other two, for which we got the same value (see table below *), could be due to the different surface
area in each of the bones in contact with the acid solution, or simply to experimental error from the titration process. On average, the 1M HCl initial solution concentration went down to 0.7 M, which indicates that part of the acid reacted with chemicals in the bone (this was easily observable as during the time the bone was immersed in the solution, tiny bubbles of carbon dioxide gas were constantly coming of the bone). Therefore it can be concluded that the bone did effectively lose calcium and, as seen in the graph, this had an impact in the strength of the bone.

<table>
<thead>
<tr>
<th></th>
<th>Acid final solution 1</th>
<th>Acid final solution 2</th>
<th>Acid final solution 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml) NaOH 0.5M solution used in the titration</td>
<td>2.6</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Molarity HCl</td>
<td>0.65M</td>
<td>0.725M</td>
<td>0.725M</td>
</tr>
</tbody>
</table>

The error bars in the graph represent the uncertainty in the measurement (± 1 mm). To try to decrease this uncertainty, as it was difficult to read the results from exactly the same point of view, I used an camera fixed in one position in front of the experiment and would take two pictures per measurement (example pictures provided next to the procedure), one before the mass was hanged and the next one with it. This way I did not have to worry about the measurements at the time and I could focus on finishing the experimental procedure quick and proved to be strength of the investigation. After finishing with the measurements and the titration of the final acid solution, I did have a look and the before and after pictures in the camera and using the digital zoom feature of the camera it was very easy to read the change in distance, being this measurement much more objective as the camera would always keep the same perspective.

Other than that, and looking at the points in the graph, there are some obvious fluctuations of the averages, but the trend is easily identified as a positive correlation and the coefficient of correlation calculated (r= 0.92) suggests a very strong positive correlation between the two sets of data. I calculated as well the coefficient of determination (R² = 0.8483), which suggests that the line of best fit produced by Excel fits pretty well the data points in the graph.
Weaknesses

One of the main difficulties I faced during the experiment was the correct positioning of the clamped bone in the table, as for it to be a fair test the distance between the point where the mass was hanged and the contact point between the bone and the table border had to be the same. For future investigations I think I could design an apparatus where bones would be placed resting in two different points in both sides and then hang the mass from the center, measuring the flexion of the bone at this point.

The selection of the bone proved to be challenging as well because first I tried with chicken leg bones, but as the size was small and in the femur there can be found two different types of bone in the center and in the sides, the bone would bend unequally as the decalcification would affect the two parts differently. Also, the shape of the bone made it difficult to clamp it always in the same position to measure the flexion. After several trials I decided to use lamb rib bones, as they are flatter and easier to clamp on the table, they are longer and I could measure the flexion specifically in the middle part, where the bone is the same type. This type of bone gave me better and more reliable results than the chicken femurs and was a strength of the investigation.

For future investigations I would try with different concentration of acids to see which one is more effective to decalcify the bone without degrading it. I used 1M because is safe to work with (I was wearing safety glasses at all times anyway) and provides acceptable results. Higher concentrations would require more careful safety procedures to handle the acid. I could also have left the bones for longer time in every trial (20 minutes trials in my experiment), but logically I had access to the lab and the materials for a limited amount of time and it wasn’t practical to do it longer. Finally, the temperature could be more effectively controlled with a water bath, but for this experiment I did not have one sufficiently large to fit the three measuring cylinders at the same time and I assumed that the room temperature would be maintained constant all through the experiment.
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