Strain imparted during impaction grafting may contribute to bony incorporation

AN IN VITRO STUDY OF THE RELEASE OF BMP-7 FROM ALLOGRAFT

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In order to investigate the osteoinductive properties of allograft used in impaction grafting and the effect of strain during impaction on these properties, we designed an in vitro experiment to measure strain-related release of bone morphogenetic protein-7 (BMP-7) from fresh-frozen femoral head allograft. A total of 40 10 mm cubes of cancellous bone were cut from ten samples of fresh-frozen femoral head. The marrow was removed from the cubes and the baseline concentrations of BMP-7 were measured. Specimens from each femoral head were allocated to four groups and subjected to different compressive strains with a material testing machine, after which BMP-7 activity was reassessed. It was present in all groups. There was a linear increase of 102.1 pg/g (95% confidence interval 68.6 to 135.6) BMP-7 for each 10% increase in strain. At 80% strain the mean concentration of BMP-7 released (830.3 pg/g bone) was approximately four times that released at 20% strain. Activity of BMP-7 in fresh-frozen allograft has not previously been demonstrated. This study shows that the freezing and storage of femoral heads allows some maintenance of biological activity, and that impaction grafting provides a source of osteoinductive bone for remodelling.

We have shown that BMP-7 is released from fresh-frozen femoral head cancellous bone in proportion to the strain applied to the bone. This suggests that the impaction process itself may contribute to the biological process of remodelling and bony incorporation.

Impaction grafting is a successful technique for restoring the lost bone stock in both the femur and the acetabulum during revision hip arthroplasty. Up to 100% survival of the implant has been reported at ten years using this technique. The procedure involves progressive compaction of morcellised cancellous bone chips into the femoral canal or acetabular cavity. The bone most commonly used to create the morcellised graft is fresh-frozen femoral head allograft. In order to achieve long-term survival of the prosthesis, two requirements must be met. First, initial stability must be achieved at the time of surgery, and second, the allograft must be incorporated into the host skeleton and undergo remodelling in response to the stress applied to it. If either of these steps fails, the implant will subside and further revision will be required. It has been shown in histological studies that impaction grafted bone does incorporate, but to a variable degree. We wished to investigate the potential contribution of the impaction process itself to the incorporation of allograft bone. It was hypothesised that, during impaction, the microfractures created in the bone chips may allow the exposure and release of factors in the non-collagenous matrix that would help to stimulate the subsequent incorporation and remodelling. We decided to investigate the release of bone morphogenetic protein-7 (BMP-7) or osteogenic protein-1 (OP-1), as this is a well-studied member of the transforming growth factor (TGF)-β superfamily, and in recombinant form has been used to stimulate healing in spinal fusions, segmental defects of long bone and nonunions of the scaphoid. The use of additional recombinant BMP-7 in revision arthroplasty of the hip has also been studied.

Materials and Methods
Preparation of the bone cubes. The bone used was taken from fresh-frozen femoral heads which had been cleared for use in research by the quality control team of Tissue Services at the National Blood Service, Liverpool, United Kingdom. The consent from donors included use for research and development. The heads were not suitable for clinical use, usually because of incomplete serological tests. Ten femoral heads were chosen at random and
Bone cubes following different levels of strain: from left to right 0%, 20%, 40%, 60% and 80%. All cubes showed a degree of recoil after strain, as demonstrated by the final resting heights.

Table I. Summary statistics of each strain group

<table>
<thead>
<tr>
<th>Strain group</th>
<th>Mean (SD) baseline (pg/g bone)</th>
<th>Mean (SD) after strain (pg/g bone)</th>
<th>Difference (pg/g bone)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>425.2 (435.7)</td>
<td>830.3 (466.2)</td>
<td>405.1 (455.7)</td>
</tr>
<tr>
<td>60%</td>
<td>373.5 (287.8)</td>
<td>570.7 (507.9)</td>
<td>197.2 (499.6)</td>
</tr>
<tr>
<td>40%</td>
<td>299.1 (214.9)</td>
<td>333.2 (261.2)</td>
<td>34.2 (265.1)</td>
</tr>
<tr>
<td>20%</td>
<td>3173 (2472)</td>
<td>216.6 (199.1)</td>
<td>-100.7 (102.8)</td>
</tr>
</tbody>
</table>

Discussion

We have shown that BMP-7 is present in fresh-frozen femoral head allograft. Although BMPs have been shown to be
Bone morphogenetic proteins are sequestered in the bone matrix during osteogenesis and are bound to various types of collagen, including type I, IV and IX. The proposed mechanism of release as observed in this study is that during strain, microfractures are created, exposing fresh areas of bone matrix. Each microfracture exposes some new collagen strands to the surrounding micro-environment. The BMPs present on the collagen are also exposed. The open-pore nature of the cancellous bone used in these experiments allows fluid to flow around these newly-exposed surfaces, washing off any cytokines that have been exposed into either solution or suspension. This mechanism is passive and is as equally applicable to fresh bone as any other form of bone in which BMPs are present.

In impaction grafting the process of impaction produces a biomechanically stable bed by creating interdigitation between bone particles. The aim of this experiment was to define whether the impaction process contributes to the biological incorporation of the graft bone. Having shown that BMP-7 is released following strain and microfracture, we can now postulate that the process itself may be beneficial to incorporation of the bone. Typical dosing regimens for in vivo use of recombinant BMPs are of the order of milligrams, rather than the picogram level found in our study. However, it has been noted that the osteoinductive properties of recombinant BMPs are reduced compared with purified BMPs, and although this study only measured BMP-7, this is a reflection of all the other BMPs that will be present in the matrix. Indeed, a study into the use of human BMPs for nonunion in long bones indicated that 100 ng of endogenous BMP effected union in 20 of 30 patients.

A femoral head weighing 80 g would yield approximately 66 ng of BMP-7 after impaction, and combined with the other BMPs present, this dose may have a significant effect. In addition to the release of growth factors after impaction, the compacted graft will also act as a slow-release depot for BMPs. As the bone is resorbed and remodelled, the matrix will be broken down, releasing growth factors.

In terms of the observed quantity of BMP-7 measured in the samples, it is currently not known how much BMP-7 is present within a given amount of bone. However, one study showed that 20 μg of highly purified BMP could be produced from 10 kg of bovine bone by heparin-sepharose chromatography and reverse-phase high-pressure liquid chromatography. The largest release of BMP-7 in our experiment was 830.3 pg/g bone after the application of 80% strain. If we take 20 ng per 10 kg bone to be indicative of BMP content, this equates to 2 ng/g bone. The 830.3 pg/g would therefore represent 42% of the total BMP present in the bone, which seems unlikely. However, the technique used to quantify the BMP-7 in this study, namely ELISA, is highly sensitive and the guanidinium chloride extraction process used by Wang et al may underestimate the total amount of BMP present. Furthermore, recent studies of BMP in demineralised bone matrix have indicated levels of BMP-7 of 227 ng/g. As the non-organic mineral content of bone is known to be 60% of the weight, extrapolation indicates that there should be 90 ng BMP-7 per gram of bone. The observation of 830.3 pg/g bone therefore only account for 0.92% of the total BMP-7, a figure that seems more realistic.

The bone used for this experiment was taken from fresh-frozen femoral heads procured from live donors at the time of primary hip replacement. Although many of these donors are elderly, whose bone is not always of good quality in terms of bone mineral content, and the articulating surface itself is, by definition, osteoarthritic, these heads are most commonly used in impaction grafting, which is the focus of this research. It is therefore logical to use this bone in these cytokine release experiments.

We have shown that the change in BMP-7 is markedly variable between femoral heads, even having accounted for the baseline value and strain effects (SD 289.7). However, the variability between individual cubes of bone is of a similar magnitude (SD 234.9). There are a number of possible explanations for this effect. There may be a relationship between age and cytokine sequestration in the matrix. However, as all of the femoral heads were procured from patients undergoing primary total hip replacement, this group is from a relatively narrow age range, precluding this analysis. Alternatively, the presence of disease such as osteoarthritis may have a significant effect on cytokines in the matrix. Although all the femoral heads showed evidence of osteoarthritis, both the extent and the cause of degeneration were variable. The variability between individuals observed in all the tests, from the haemoglobin levels to the bone mass, is unlikely to be the sole explanation for the large variation observed, as the mean concentration of BMP-7 for the femoral heads varied by a factor of 10. No information on this variation in cytokine sequestration between individuals, or in different disease states, is available in the literature, although two recent studies used similar ELISA techniques to show marked variability in different BMPs in preparations of demineralised bone matrix. Other confounding factors, such as differences in the treatments of the 40 bone cubes, were kept to a minimum by the simultaneous processing of all cubes in the marrow removal and incubation wash stages.

If our hypothesis is correct and growth factors are released following microfracture of bone particles, it could be suggested that ‘large’ bone morsels should be used when under-
taking this technique. Using larger particles will naturally create more microfracture when the bone is impacted. This assumes that it is beneficial to release growth factors in this way, an assumption that is easy to make in light of the recent clinical applications of recombinant BMPs to fracture repair. This recommendation is in line with current biomechanical knowledge of impacted bone stability.23

The final practical implication of this work is in relation to washing of the graft. Provided the graft has been washed after morcellisation, as suggested by biomechanical26-28 and bony ingrowth studies,29 washing should not be repeated following impaction, otherwise some of the released growth factors may be lost.

Supplementary Material

A table showing the full data set for each of the 40 cubes is available with the electronic version of this article on our website at www.jbjs.org.uk

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No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

References


