MINI-SYMPOSIUM: FRACTURE HEALING

(v) Which scaffold for which application?

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Summary
Scaffolds are usually osteoconductive, but rarely osteoinductive and extremely rarely osteogenic. They do however have the potential advantage of providing structural support. The load bearing capability of each type of scaffold needs to be determined prior to clinical use if it is to be used in a load-bearing situation. Some scaffolds are resorbable. These are best used in clinical situations where the biology of the host will promote effective new bone formation. It is preferable to have a scaffold that osteointegrates as this will allow bonding at a molecular level between the scaffold and host bone. Synthetic scaffolds have the lowest risk of infection and antigenicity. At present, there is no scaffold available that is suitable for all bone grafting procedures.

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Introduction
Orthobiologics can be divided into scaffolds, growth factors and stem cells. This article is a brief overview of scaffolds. The epidemiology of bone grafting as well as the advantages and disadvantages of autograft and allograft are discussed. A classification system for scaffolds is proposed and then different types of scaffolds are examined with particular reference to their advantages, disadvantages and clinical uses.

Semantics
It will be useful to start this chapter with a few definitions. Osteointegration means bonding at a molecular level between a given substance and bone. Examples of substances that osteointegrate are titanium, hydroxyapatite and tricalcium phosphate. Examples of substances that do not osteointegrate are stainless steel and polymethylmethacrylate. When a substance osteointegrates, the bond between it and the bone is incredibly strong, often exceeding the tensile strength of both the substance itself and of bone. Dental studies have shown that when ceramics osteointegrate with bone, the bond between the bone and the ceramic is so strong that attempts to separate the two usually result in fracture of either the ceramic or the bone, but rarely separation at the interface.

A osteoconductive material provides a scaffold for bone to grow along. This is dependant on the material characteristics as well as the morphology of the material.

An osteogenic material forms new bone on its own. For this, it needs to contain both the cellular element as well as the morphogens required to produce bone.

An osteoinductive material induces pluripotential stem cells to become osteoblasts.

Orthobiologics
Orthobiologics can be divided into three fields: (A) scaffolds, (B) growth factors and (C) stem cells.
Scaffolds are usually osteoconductive, but rarely osteoinductive and extremely rarely osteogenic. They do however have the potential advantage of providing structural support (Figures 1 and 2).

Growth factors include the Bone Morphogenic Proteins (BMPs), which are part of the Transforming Growth Factor Beta superfamily of proteins. The term Bone Morphogenic Protein was coined by Marshall Urist in the 1960s, but is probably a misnomer. These proteins do not just promote bone morphogenesis, but are involved in organogenesis in general. It would be more accurate to refer to them as Morphogenetic proteins. Currently, BMP’s 2, 4 and 7 are commercially available. BMP 7 is extracted from Chinese hamster ovaries. Under the influence of BMP’s, bone is regenerated by pluripotential stem cells that are converted to osteoblasts.  

Bone grafting

Bone is the second most transplanted tissue after skin. In the USA in 2001, 643,000 bone grafting procedures were performed. The cost of these procedures has been estimated at approximately $600 million p.a. The current breakdown by type of procedures is as follows:

- 45% autologous bone
- 45% allogenic bone
- 10% synthetics.

In Europe, 287,300 procedures of bone grafting were carried out in 2000 and 429,660 procedures in 2003.

Autografting is still considered to be the gold standard in bone grafting, but there are problems with autografting. These include: bulk limitations, graft donor site morbidity (particularly pain and injury to the lateral cutaneous nerve of thigh) and lengthening of the procedure whilst harvesting occurs. For these reasons, allografting has become more popular.

Allografting, however, has introduced new problems, which include cost, availability, antigenicity, infectivity, reproducibility and structural stability.

Since the initial reports of morsellised impaction grafting appeared in the literature in the early 1990s, the technique has become extremely popular. Demand for bone has risen without a corresponding increase in supply. For example, in Scotland, the Scottish National Blood Transfusion Service performs Bone Banking. They collect and distribute all the bone that is used in revision total hip arthroplasty in Scotland. In 1993, they collected 1559 femoral heads and issued 1061. In 1995, they collected 1778 heads and issued 1567. This represents a 13% increase in supply, against a 50% increase in demand over a 2-year period. Between 1993 and 1995, the number of primary total hip arthroplasties performed increased by approximately 1.8%, whilst the number of revision arthroplasties increased by approximately 20%. Galea et al., predicted that between 1995 and the year 2000, the number of primary total hip replacements would increase by 12% and the number of revisions would increase by 100%. They predicted that their supply of donor femoral heads would have been outstripped by demand before 2000 and concluded: "This source cannot..."
meet the demand for revision surgery of the hip or for other operations because of the increase in the number of revisions and the use of techniques which require more bone, such as impaction grafting, which may use up to five femoral heads. Other studies have reached similar conclusions.4–9

Allografts have the potential to invoke graft rejection by activating T cell-mediated immune responses in the host. Fresh, untreated allograft bone provokes both humoral and cell-mediated host responses. Bone that has been frozen and stored prior to implantation evokes a much diminished host response, probably due to diminished antigen presentation by the graft. Freeze-drying diminishes this response even further.10 Friedlander et al. have identified donor-specific anti-HLA antibodies in human recipients of freeze-dried allografts.11 Although sensitisation is a potential cause for concern, Musculo et al. did not show a correlation between sensitisation and poor clinical outcome12 and at present most bone banks make no effort at tissue typing between donors and recipients.

Although most bone banks screen donors meticulously for potential sources of infection, harvest and store bone under sterile conditions, and in many cases irradiate bone, there is still a problem with both viral and bacterial infection.

The literature records a number of cases of transmission of the Human Immunodeficiency Virus (HIV) through bone allograft transplantation despite rigorous precautions. It is imperative to exclude people with high risk factors from donating. According to Huo et al., the first recorded case of HIV transmission via allograft bone was in a woman who developed AIDS 3 1/2 years after receiving bone from a frozen femoral head that was used for spinal fusion.13 Simonds et al. reported a tragic case where seven recipients developed AIDS after receiving tissue from a single donor. Four had received organs and three had received bone. The donor was a victim of a homicide who had no known risk factors for HIV infection. Two serum samples taken at the time of harvest tested negative for HIV. Subsequent culture of the donor’s cryopreserved spleen cells grew HIV.14 Buck studied the risks of unknowingly implanting HIV infected bone when utilising bone-banked bone in the United States of America. If all the recommended checks are rigorously adhered to, the risk is slight. When there is failure to take adequate precautions, he estimates the risk to be 1/161.15

The problem with screening for HIV infection is that there is a highly variable window between infection and the presence of detectable antibodies. This window may range from a few months to several years. HIV is usually tested for by means of ELISA and Western blot assay. The sensitivity and specificity of these methods is between 90% and 99%.

The incidence of bacterial infection with allograft bone varies considerably, depending on a number of factors including the storage and processing of the bone, the immune status of the recipient and the host site. The post-operative incidence of bacterial infection is twice as high with allograft bone as compared with autograft bone when used in similar surgery.16 Infection rates in total hip arthroplasty with the use of allograft bone vary between authors. Gie et al.17 and Harris et al. reported no infections.18 Jofe et al. reported four infections in 28 cases19 and Mankin et al. reported 13 infections in 91 cases.20 Reconstruction following tumour resection carries a higher risk of infection.

Meding et al.21 published a study in the Journal of Bone and Joint Surgery in 1997. They outlined the cost of the disposables used during impaction grafting surgery in their institution. Femoral heads cost $950 each. Often impaction grafting requires up to five femoral heads.9 Highly polished double taper femoral stems cost approximately $2000 each and cortical strut allografts cost $500 each. If the use of allograft from femoral heads can be substituted with a cheaper, but as effective source, then the cost of some operations can be reduced by as much as 4500 US dollars.

Allograft bone is not reproducible. The quality of the bone is dependant on both donor factors (age, osteoporosis, and co-morbidities) and processing (sterilisation methods, storage). This is reflected in the variable clinical outcomes. Eldridge et al. reported a 12% early massive subsidence when using allograft bone in impaction grafting of the femur.22

### Synthetic scaffolds

Due to the problems outlined above, there has recently been a surge in interest in synthetic scaffolds.

Bone grafts and bone graft substitutes can be broadly classified as shown in Table 1.

### Xenografts

Bovine bone has been investigated as a potential substitute for human allograft bone since the 1960s.26 More recently,

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**Table 1** Classification of bone graft substitutes.

<table>
<thead>
<tr>
<th>Biological human</th>
<th>Biological non-human</th>
<th>Synthetic non-absorbable</th>
<th>Synthetic absorbable</th>
<th>Biological/synthetic combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autograft</td>
<td>Xenograft</td>
<td>Methylmethacrylate</td>
<td>Calcium sulphate</td>
<td>Collagen matrix (ceramic/fibrillar collagen)</td>
</tr>
<tr>
<td>Allograft</td>
<td>Coralline</td>
<td>Glass-ionomers</td>
<td>Ceramic: hydroxyapatite tricalcium phosphate Polyhydroxyacids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other (such as bamboo, eggshell)</td>
<td></td>
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Levai et al. reported good results in 27 out of 30 cases using bovine bone in acetabular reconstruction in total hip revision. Bovine bone is biocompatible for human osteoblasts. Hubbell et al. showed that when cyclically loaded, bovine bone exhibited stability similar to human bone when used as a morcellised graft in impaction grafting of the femur. Their pilot studies in sheep showed graft incorporation with new bone formation comparable with allograft. Other studies have highlighted problems with bovine xenograft. Begley demonstrated that bovine xenograft causes intense inflammatory reactions that are not provoked by coral when used in identical circumstances. Charalambides et al. reported very poor results in revision hip surgery using bovine bone, with more than 25% of cases needing re-revision at an average follow-up of 36 months. There are also fears with regard to infection and patient acceptability in view of recent scares caused by bovine spongiform encephalopathy.

**Coralline-derived hydroxyapatite**

This biomaterial is derived from reef building coral of the genus *Porites*. The calcium carbonate exoskeleton is converted to hydroxyapatite by means of a hydrothermal chemical exchange, whilst still maintaining the original microstructure. The microstructure of the coral is similar to bone with a porous structure and pore size that facilitates bony ingrowth. A pore size of around 500 μm has been demonstrated to be optimal for bony ingrowth. Coral has a low potential for infectivity and anti-gencicity.

Coralline hydroxyapatite has been shown to osseointegrate well in rabbits, rats, dogs, baboons and sheep. It has successfully been used in humans as a space filling material in maxillofacial surgery. However, it is fragile and does not appear to possess the mechanical strength to be used in load-bearing bone such as the proximal femur.

**Polymethylmethacrylate (bone cement)**

This is a non-absorbable material widely used to fix prostheses in joint replacement surgery. It is a filling material and not glue. Although it has been used as a bone graft substitute in tumour surgery, it has many limitations including low tensile strength, brittleness and a tendency to provoke an aggressive osteolytic response. When used to replace lost bone stock in revision total hip arthroplasty the results have invariably been poor. Its application as a bone graft substitute is therefore limited.

**Calcium sulphate (plaster of paris)**

Calcium sulphate was used as long ago as 1892 by Dreesman to fill bony defects caused by tuberculous osteomyelitis. Since then it has fallen into disfavour as it is quickly absorbed (within 4–8 weeks) and thus provides poor structural stability. In addition, it is prone to fracture when shear-loaded. Nevertheless, Coetzee reported excellent results in 110 patients when using calcium sulphate to repair cranial defects. He reported complete substitution of calcium sulphate with bone within 8 weeks. There have been no other reports in the literature to support these dramatic results.

**Glass-ionomer ceramics**

These are formed by sintering glass in different proportions of SiO2, Al2O3, CaF2 and AlPO4 with or without hydroxyapatite. Glass-ionomers are not resorbable, as bone cannot eliminate the silicate and aluminium from which they are constructed.

They have been demonstrated to have good osseoconductive potential between particles, but not within them, as well as being biocompatible without causing foreign body reactions. After bony ingrowth has occurred, the glass-ionomer remains permanently within the new fibro-osseous matrix. It remains unclear whether this enhances structural stability or whether the persistence of unresorbed foreign particles prevents restitution of normal morphology with permanent weakening of the bone. These issues are still to be resolved in long-term studies. An ovine study using a glass-ionomer as a bone graft expander in impaction grafting of the femur performed at the University of Bristol showed good clinical, but poor histological results (with multiple voids within the graft and little graft incorporation) at 6 months.

**Absorbable ceramics**

Ceramics are manufactured by baking or firing minerals. The ceramics most commonly used as bone graft substitutes are made of tricalcium phosphate (Ca3(PO4)2) (TCP) and hydroxyapatite (Ca10(PO4)6(OH)2) (HA). Ceramic powder is obtained by precipitation from an aqueous solution, for example by adding ammonium phosphate ((NH4)HPO4) to a calcium nitrate solution (Ca(NO3)2) at pH of 11–12. This is then cold-pressed to form tablets. These are baked or fired at a high temperature (>800°C) causing their crystals to fuse. This process is called sintering. This produces a dense material with a porosity by volume of between 1% and 5% (depending on parameters such as sintering temperature, sintering time and grain distribution). High porosity is achieved in the manufacturing process by one of a number of methods including the addition of glucose (which expands when heated and is then combusted), or the addition of hydrogen peroxide (H2O2) or naphthalene. Porosity is important as it allows effective ingrowth of bone (osseoconduction within the ceramic particles).

Hanft states: "The principle limitation of calcium phosphate materials as hard-tissue implants has apparently been their mechanical properties." He goes on to say "Unfortunately, these mechanical weaknesses have prevented this material from being used in cases where they must bear the initial structural load alone."

In rebuttal Jarcho cites compressive strength of porous calcium phosphate as similar to that of cancellous bone while the tensile strength is 72% of the tensile strength of cancellous bone. Non-porous calcium phosphate has a tensile and compressive strength far in excess of both cancellous and cortical bone.
Bouler et al. studied the influence of five synthesis parameters on compressive strength of porous biphasic calcium phosphate ceramics. These parameters were as follows:

1. Chemical composition
2. Percentage of macropores
3. Mean size of macropores
4. Isostatic compaction pressure
5. Sintering temperature.

Macroporosity and final sintering temperature exerted the major influences on compressive strength.

Two ratios of HA:TCP were studied. These were 45% HA, 55% TCP and 75% HA, 25% TCP. The lower ratio of HA provided slightly better compressive strengths.

Isostatic compaction had comparatively little influence on compressive strength.

The more porous the ceramic, the less resistant it was to compressive forces. For a given volume percentage porosity, a few 500 μm pores were better than many 100 μm pores. When the thickness of the bridges between the pores fell below a critical size, the ceramic structure disintegrated when subjected to even low compressive forces.

Bouler’s study also showed that compressive strength increased significantly with a rise in sintering temperature. At 900 °C, boundaries between grains of ceramic were formed. Mechanical properties of biphasic ceramics are dependent on the number and size of these grain boundaries. At temperatures between 900 and 1100 °C densification (the elimination of connected and non-connected micropores) occurred. This densification corresponded with further increase in compressive strength.

Blom et al. have demonstrated that porous biphasic absorbable ceramics can be manufactured that are both more stable and give more reproducible stability than allograft.

Tsugura et al. have demonstrated the importance of porosity in allowing osteoconduction. They compared the same ceramic with different pore sizes, thus isolating pore size as the only variable. Their studies showed that a porosity of around 500 µm allowed greater bony ingrowth than smaller pores. Guillemin et al. compared the bony ingrowth into two species of coral implanted into both ovine and porcine long bones. Porites coral resorbed twice as fast as Acropora coral and had twice the bony ingrowth. Interestingly, Porites has a mean pore diameter of 250 µm (range 150–400) and Acropora coral has a mean pore diameter of 500 µm (range 200–800). It would therefore appear that both pore size and rate of resorption of the ceramic influence osteoconduction.

Porosity also allows a greater degree of resorption to occur (by both dissolution and phagocytosis) as the surface area of the ceramic is increased.

The body resorbs ceramics at different rates, depending on their chemical composition and structure. Two different biological processes govern resorption. These are dissolution (in physiological solutions) and phagocytosis. Frayssinet et al. observed both these processes causing resorption of calcium phosphate ceramics implanted into sheep bone. Multinucleated giant cells caused localised areas of resorption. In addition, a uniform dissolution was observed around the implant surfaces. Guillemin et al. have observed osteoclast resorption of implanted coral-derived ceramic.

The dissolution of HA and TCP in both buffered acid and buffered basic solutions has been compared. The HA and TCP ceramics studied were prepared with similar structural characteristics, so that any difference in resorption would be due to their chemical compositions. The TCP ceramic dissolved 12.3 times faster in the acid and 22.3 times faster in the basic solution than the HA ceramic.

Kay compared the dissolution rate of various calcium phosphates in an aqueous solution at 37 °C and pH of 7.3. TCP was 25 times more soluble than HA. Calcium phosphate was 667 times more soluble than HA.

Shimazaki and Mooney compared both implant resorption and new bone formation between HA and TCP ceramics implanted into rabbit tibiae. At 24 weeks post-implantation, 46.4% of the TCP had resorbed compared with 27.5% of the HA. The HA however allowed 8% more new bone formation than the TCP.

Ceramics have been used extensively as bone graft substitutes in humans. In a randomised study of spinal fusion in 341 patients, Ransford et al. obtained similar results with autograft and Triosite (a ceramic consisting of 60% hydroxyapatite and 40% tricalcium phosphate). As these materials contain no proteins, they do not provoke an antigenic response from host tissue. Porous hydroxyapatite has been used to repair tibial plateau fractures in a series of 17 patients with fracture union occurring in all cases.

Oonishi reports excellent results using hydroxyapatite to fill massive acetalubar defects at the time of revision hip replacement, despite the loads of up to 240% of body weight achieved whilst mobilising with crutches. Hydroxyapatite and tricalcium phosphate ceramics have demonstrated marked osteointegration and osteoconduction both radiologically and histologically in a number of studies in humans and animals. One of these ceramics has been successfully used in an impaction grafting model in sheep with clinical, radiological and histological changes comparable to allograft.

**Polyhydroxyacids**

Polyhydroxyacids have been used for the past 30 years to manufacture absorbable sutures such as Dexon, which is made from polyglycolic acid (PGA). Glycolic acid is a naturally occurring substance produced during normal human metabolism. It belongs to the same family of acids as lactic acid.

PGA is most commonly used to manufacture multifilament yarns, but a variety of substances can be manufactured including screws, pins and mesh. These have a wide range of clinical applications ranging from the internal fixation of wrist and elbow fractures, to the fixation of osteotomies. These products have the advantage of obtaining good fracture fixation and then gradually resorbing.

PGA and polylactic acid (PLA) multifilament yarns have been synthesised as delivery agents for BMPs. These yarns
have very consistent and predictable rates of bioabsorption and thus produce a controlled delivery of BMPs. In 1995, Robinson et al. described the use of blocks of porous PGA, which structurally mimic cancellous bone, to repair calvarial bone defects. Polyhydroxyacids have not been demonstrated to provide the structural support needed in high load bearing bone such as the acetabulum, proximal femur and proximal tibia.

Collagen matrix

This are formed by a combination of purified fibrillar collagen (usually bovine) and ceramic composed of hydroxyapatite and tricalcium phosphate. The collagen provides a structure similar to extracellular matrix; however, it potentially has the same problems of bovine bone xenograft with regard to infection and antigenicity. Chapman et al. reported raised antibody titres in 12 patients treated with a bovine collagen—calcium phosphate graft material for long bone fractures. Despite this, the patients showed no ill effects. With regard to fracture healing, those treated with the collagen matrix material did as well as those treated with autograft.

Collagen matrix substances have also been used experimentally in animals as cranial onlay grafts and to heal tibial defects in sheep with regard to infection and antigenicity. Chapman et al. reported raised antibody titres in 12 patients treated with a bovine collagen—calcium phosphate graft material for long bone fractures. Despite this, the patients showed no ill effects. With regard to fracture healing, those treated with the collagen matrix material did as well as those treated with autograft. Collagen matrix substances have also been used experimentally in animals as cranial onlay grafts and to heal tibial defects in sheep. Not all reports have been favourable. Muschler compared a collagen matrix substance to autograft when attempting spinal fusion in dogs. The collagen matrix substance performed markedly worse than the autograft. At present, these materials are available in a paste form and have FDA approval in the United States of America for fixation of long bone fracture defects, provided they are used in conjunction with internal or external fixation.

References


