

Bone repair in the twenty-first century: biology, chemistry or engineering?

BY KARIN A. HING

*Interdisciplinary Research Centre in Biomedical Materials,
Queen Mary, University of London, London E1 4NS, UK
(k.a.hing@qmul.ac.uk)*

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Increases in reconstructive orthopaedic surgery, such as total hip replacement and spinal fusion, resulting from advances in surgical practice and the ageing population, have lead to a demand for bone graft that far exceeds supply. Consequently, a number of synthetic bone-graft substitutes (BGSs) have been developed with mixed success and surgical acceptance. Skeletal tissue regeneration requires the interaction of three basic elements: cells, growth factors (GFs) and a permissive scaffold. This can be achieved by pre-loading a synthetic scaffold with GFs or pre-expanded cells; however, a 'simpler' approach is to design intrinsic 'osteinductivity' into your BGS, i.e. the capability to recruit and stimulate the patient's own GFs and stem cells. Through investigation of the mechanisms controlling bone repair in BGSs, linking interactions between the local chemical and physical environment, scientists are currently developing osteoinductive materials that can stimulate bone regeneration through control of the scaffold chemistry and structure. Moreover, this body of research is providing the foundations for future generations of BGSs and bone-repair therapies and may ultimately contribute towards improving the quality of life through maintenance of the skeleton and reversal of disease states, as opposed to the mending of broken bones that we currently practice. Will we be able to grow our own bones in a bio-reactor for use as autologous graft materials in the future? Could surgery be limited to accidental trauma cases, with greater restoration of function through biochemical or gene therapies? The technology and research probes necessary to this task are currently being developed with the advent of nanotechnology, genomics and proteomics: are we about to embark on a chemical revolution in medicine? This paper aims to discuss some of the current thinking on the mechanisms behind bioactivity and biocompatibility in bone and how a fuller understanding of the interactions between cells and the materials used today could bring about completely new approaches for the treatment of bone fracture and disease tomorrow.

Keywords: tissue engineering; cell–material interactions;
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1. Introduction

From a biological perspective, bone is a remarkable living tissue that performs several key functions within the body. Bone not only provides structural support and

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protection to bodily organs, but it is involved in the metabolism of minerals such as calcium, and is the primary site for the synthesis of blood cells. Furthermore, it is capable of maintaining an optimal shape and structure throughout life via a continual process of renewal through which it is able to respond to changes in its mechanical environment by ‘remodelling’ to meet different loading demands, so maintaining an optimal balance between form and function (Wolff 1870). However, as a living tissue, bone requires a constant supply of oxygen and nutrients, and is limited in the size of fracture or defect it is able to restore to healthy tissue. Furthermore, bone can suffer from pathological conditions, (e.g. cancer) and is subject to degeneration as a result of age and disease (i.e. osteoporosis). In these cases, patient comfort and bone function can often only be restored by surgical reconstruction. Bone grafting, the procedure of replacing missing bone with material from either the patient’s own body (autografting) or that of a donor (allografting) was first established in the 1800s (Czitrom & Gross 1992; Meeder & Eggers 1994; Sanan & Haines 1997). Evidence for the use of artificial, synthetic or natural substitutes, however, predates this in the form of gold and silver plates and pieces of coconut shell found in cranial defects within prehistoric skulls (Sanan & Haines 1997). Furthermore, archaeological studies of the skeletons of ancient Egyptian mummies have demonstrated the successful practice of external fracture fixation using splints made of bamboo, reeds, wood or bark, padded with linen (Wangenstein & Wangenstein 1978). In modern medicine, autografting is regarded as the ‘gold standard’; however, the amount of bone that can be safely harvested is limited, while the additional surgical procedure may be complicated by donor-site pain and morbidity. Modern allografting using material stored within regulated bone banks overcomes these difficulties. However, the demand far outstrips the supply, there is no assurance of freedom from disease (Barriga *et al.* 2004; McCann *et al.* 2004) and healing can be inconsistent (Togawa *et al.* 2004). Consequently, there is an increasing demand for synthetic bone-graft products that would avoid these complications, in addition to overcoming the problem of an inadequate supply of material.

In 2001 worldwide sales for orthopaedic products approached \$15 billion and continue to expand at an annual growth rate of 13% (Clinica Reports 2002). Furthermore, the bone-grafting segment, valued at over \$1 billion globally, has been estimated to represent 408 000 procedures in Europe and 605 000 procedures in the USA alone (Clinica Reports 2002). In the period 2002–2003, over 77 000 primary hip operations were performed within a total of 617 000 bone and joint procedures recorded by the NHS in England alone (Government Statistical Service 2003). Moreover, an estimated further 19 000 (20%) hip operations are performed annually within the private sector; thus the true number of bone-repair procedures performed annually within England is likely to be well in excess of this figure. This ‘boom’ in reconstructive surgery is due in part to favourable demographics. Currently, there is a projected annual increase of 2–3% in the global 65+ population (i.e. a population increase of 100 000 000 people aged 65 and over from 2000 to 2010). However, it also results from lifestyle changes, increased expectations regarding the quality of life, developments in surgical technique (such as minimally invasive surgery) and advancements in technology (leading to new innovations in implant materials) resulting in a greater demand for and a wider application of orthopaedic devices. Ironically, the desire to lead an active healthy lifestyle results in an increase in sports-related injuries and joint damage, while those that lead an overly sedentary lifestyle can suffer from poor

posture leading to spinal problems. Moreover, joint replacements performed in the 1980s are now reaching the end of their lifetimes and will require revision. Fortunately, advancements in biomaterials technology have led to the development of new more durable or more 'biocompatible' materials that make devices such as hip prostheses more suitable for younger patients who wish to continue to lead an active lifestyle. For instance, the use of tough alumina (Al_2O_3) or yttria-stabilized zirconia ceramic heads coupled with durable high molecular weight polyethylene cups in total hip replacement has been shown to significantly reduce the generation of wear particle debris as compared with metal/polymer combinations (Hernigou & Bahrami 2003; Santavirta *et al.* 2003; Tanaka *et al.* 2003). These submicrometre or nanosized wear particles, when produced in a high enough concentrations, have in turn been found to upregulate or 'switch on' the body's natural immune response, which sets in motion a cascade of events ultimately leading to loosening and failure of the implant (Orishimo *et al.* 2003; Warashina *et al.* 2003). Moreover, the inclusion of a 'bioactive' coating on the stem of the femoral component can also increase the rate of healing and extend the lifetime of the implant by promoting direct bonding between the bone and the implant (Kroon & Freeman 1992; Reikeras & Gunderson 2003; Skinner *et al.* 2003).

However, as a result of bones' load-bearing function in the body, much of the orthopaedic surgery performed today uses engineering solutions; for instance, in a simple break the fracture site will be externally or internally fixated using splints and casts or metal plates and pins, respectively, which provides the local stability necessary to facilitate bone regeneration. Similarly, spinal injuries are treated with the use of metal cages and hips and knees are reconstructed using components selected for their mechanical properties rather than their biological functionality. Where internal fixation is required to facilitate healing the patient often has to undergo a second operation to remove the metalwork, particularly in the young and active where it is vital to retrieve devices once they become surplus to requirements lest they interfere with normal patterns of bone growth, lead to wasting of bone tissue due to stress shielding (a side effect of bone's ability to respond to functional demand, which is why astronauts lose bone mass in space and why highly trained athletes have denser bones), or have the potential to severely complicate a second fracture should the patient be unlucky enough to injure themselves again. In order to spare patients the ordeal of retrieval (and to save healthcare resources) there is a considerable body of research focused on the development of bioresorbable fixation devices (Steinmann *et al.* 1990) that use bones' natural remodelling characteristics to degrade the device and dispose of the degradation products, similar to the resorbable sutures commonly used in wound closure (Chu *et al.* 1996). Examples of the materials used in these devices include resorbable polymers such as polyurethane, and poly(L-lactic acid). However the application of bioresorbable plates, screws and cements is generally limited to the treatment of small defects, such as in maxillofacial surgery, and to the fixation of tendon grafts into bone (McGuire *et al.* 1999), as a result of their relatively low strength and concerns regarding the biocompatibility of their degradation products (Böstman 1998; Ignatius & Claes 1996). However, they do have the potential to act as drug-delivery devices, releasing growth factors (proteins that can stimulate bone repair) to promote rapid bone healing as they degrade, the main technical challenge being the control of the rate at which they degrade and thus also control of the targeted area of delivery and the drug dosage (Di Silvio *et al.* 1994).

Table 1. *A selection of bone-graft substitutes available for clinical use*

name	chemistry	morphology	features
ApaPore	HA	macroporous	three porosity grades 60, 70, 80%; interconnected microporosity
Bonesave	HA/TCP (60/40)	macroporous	50% porosity
Endobon	HA	macroporous	bovine cancellous structure
Grafton	demineralized bone matrix	putty, gel, granules, sheet, matrix	natural cocktail of proteins/GFs
Hedrocel trabecular metal	tantalum	macroporous	high toughness
InFuse	BMP 2	GF loaded onto collagen sponge	contains GF
Norian	CO ₃ apatite	dense dahllite cement on setting	injectable cement
OsSatura	HA/TCP (80/20)	macroporous	scaffold with biomimetic coating
OssiGraft (OP-1)	BMP 7	powder	contains GF
Osteoset	CaSO ₄	dense pellets	resorbable
Pro Osteon	HA	macroporous	coral exoskeletons; two 200 and 500 mm pore-size grades
Pro Osteon-R	CaCO ₃ HA coated	macroporous	resorbable
Skelite	Ca-PO ₄	macroporous	resorbable; interconnected microporosity
Vitoss	TCP	macroporous	resorbable; interconnected microporosity

In orthopaedics the closest we come to harnessing bone's natural regenerative powers is in bone grafting. In severe trauma cases, some hip revisions and in the correction of large 'bony defects', where a significant part of the bone is missing or damaged, bone grafts are used to replace or augment the missing or fractured bone (generally in combination with fixation devices as mechanical stabilization demands must still be met). As discussed earlier, this can be done using either the patient's own bone (autograft), removed from another site, or bone allograft (human bone obtained from a bone bank). The graft should not only replace missing bone but encourage osseointegration, i.e. act as a scaffold for guided bone growth into the graft, so helping the body to repair its own lost bone. This bone in-growth strengthens the grafted area by forming a bridge between the existing bone and the graft material. Ideally

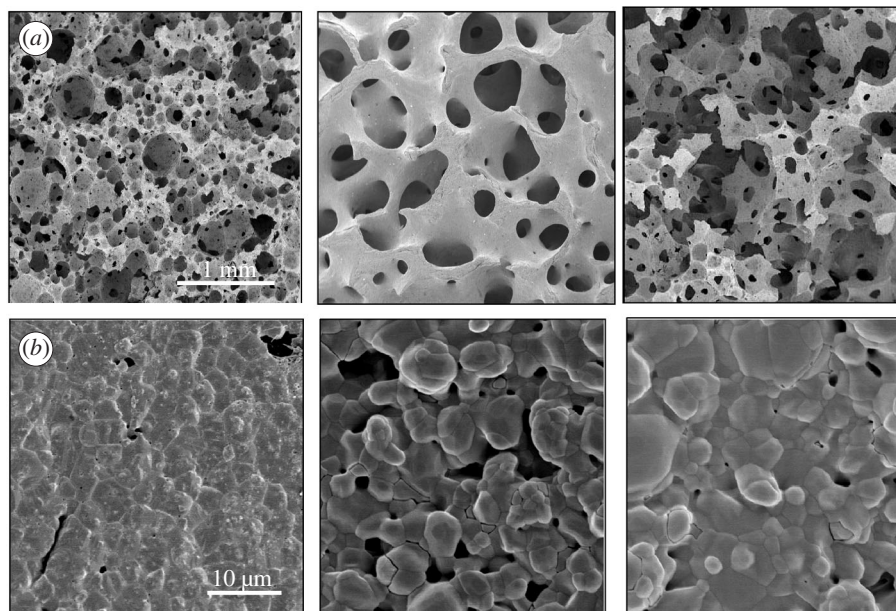


Figure 1. Scanning electron micrographs of some typical ceramic BGSs. Note the variation in (a) macrostructures and (b) microstructures and surface topographies.

with remodelling over time, the newly formed bone should replace much of the graft. However, with the rising usage of bone grafts there are now not enough to meet demand, surgeons have reported that in autografting there is generally insufficient bone 20% of the time, while a single hip-revision procedure can require 4–6 femoral heads' worth of banked allograft.

In response to this a number of synthetic bone-graft substitutes (BGSs) have been developed and are in use clinically, with mixed success and surgical acceptance (see figure 1 and table 1). As can be seen from table 1 the term 'bone-graft substitute' includes materials with a wide range of chemistries and structural morphologies; all these materials can be said to be biocompatible and most are osteoconductive (i.e. they support the formation of bone on their surfaces by mature bone-forming cells, osteoblasts). Some of these materials are intended for use with cells or growth factors to produce a biologically active graft—a practice known as tissue engineering. However, some also claim to be 'osteoinductive' (i.e. they are able to induce bone formation by influencing the differentiation or maturation of stem cells into bone-forming cells) by interaction of the material's surface with local cells and proteins, through either their chemistry or their micro-topography. The availability of a synthetic BGS that would reliably replicate the best results observed with the use of fresh healthy autograft, which contains the patient's own bone cells and growth factors, would be of great benefit to both surgeon and patient alike.

This paper aims to discuss and elaborate on some of the current thinking on the mechanisms behind bioactivity and biocompatibility in bone and how a fuller understanding of the interactions between cells and the materials used today could bring about completely new approaches for the treatment of bone fracture and disease tomorrow. However, we will first start by considering bone, its make-up and its normal healing and remodelling cycles.

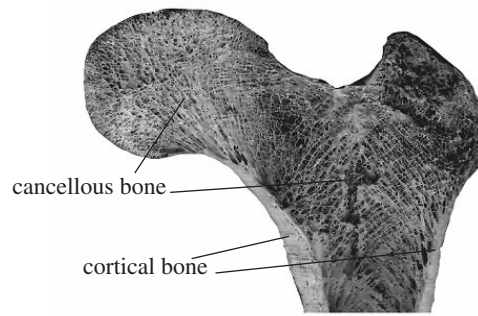


Figure 2. Sectioned human femoral head demonstrating the variation in bone structure.

(a) *Basics of bone*

Bone is astonishing. It is a living, highly vascular, dynamic, mineralized, connective tissue, which is characterized by its hardness, resilience and growth mechanisms, and its ability to remodel and repair itself. Simply, bone is a dense multi-phase material or ‘composite’ made up of cells embedded in a matrix composed of both organic (collagen fibres, lipids, peptides, proteins, glycoproteins, polysaccharides and citrates) and inorganic (calcium-phosphates, carbonates, sodium, magnesium and fluoride salts) elements (Cameron 1972). However, its structure and proportion of its components differ widely with age, site, and history, resulting in many different classifications of bone that exhibit very different mechanical and functional characteristics. Moreover, as previously mentioned, bone is not purely a structural tissue, but is also responsible for maintaining mineral homeostasis and providing a source of haematopoietic stem cells, i.e. it acts as a mineral (notably calcium) and blood cell reservoir for the rest of the body. Consequently, bone in its natural environment is engaged in a constant cycle of resorption and renewal, undergoing continual chemical exchange and structural remodelling, due to both internal hormonal regulation and external mechanical demands. Moreover, the mineral function can supersede the structural function, resulting in loss of integrity in the bone structure. Indeed, many pathological bone diseases are instances where an imbalance in the body’s normal hormonal regulatory system results in depletion of bone (osteoporosis) or overproduction of bone (Paget’s disease).

(i) *Bone structure*

Mature bone is composed of two types of tissue, one of which is relatively dense, known as cortical bone, while the other consists of a network of struts or trabeculae surrounding interconnected spaces or cancelli and is known as trabecular or cancellous bone. Bone surfaces consist of cortical bone, and the thickness of this protective skin increases in mechanically demanding regions such as the shafts of long bones, while cancellous bone is found in the interior of bones, such as within the femoral head, and vertebra (figure 2).

There are two kinds of cancellous bone: coarse and fine. Coarse cancellous bone is characteristic of healthy adult mammalian skeleton, while fine cancellous bone is characteristic of the foetal skeleton or early fracture callus and comes in two forms, fine cancellous membranous bone and fine cancellous endochondral bone. There are

Table 2. Data for the mechanical properties of bone (Bonfield 1989; Cowin *et al.* 1986; Goldstein *et al.* 1983; Kuhn *et al.* 1989; Martens *et al.* 1983), HA (Akao *et al.* 1981; Best 1990; de With *et al.* 1981) and collagen (Bennet *et al.* 1986)

	testing direction	compressive strength (MPa)	tensile strength (MPa)	Young's modulus (GPa)
cortical bone	longitudinal	193	133–150	17–25
	transverse	133	50	12
cancellous bone	longitudinal	3.6–9.3	—	0.26–0.90
	transverse	0.6–4.9	—	0.01–0.40
HA	n/a	—	9–120	80–117
collagen	longitudinal	—	100	1.5

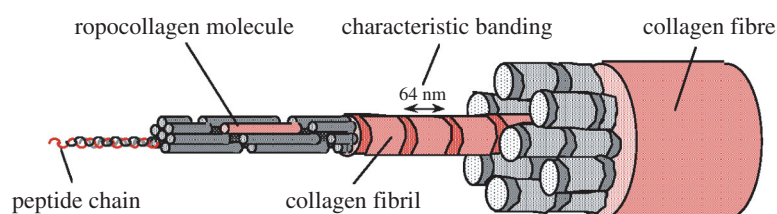


Figure 3. Hierarchical organization of collagen fibres.

also several types of cortical bone: surface, primary and secondary osteonal cortical bone, and as with the cancellous bone the distinctions are dependent on the age and origins of the bone.

This variation in structure leads to considerable variation in its stiffness, strength and toughness in both cortical and cancellous bone. The osteonal microstructure of cortical bone makes it highly anisotropic, although its density is relatively consistent ($1.85\text{--}2.05\text{ g cm}^{-3}$ in human bone). The mechanical properties of cancellous bone (which can be considered to be a foam) are highly dependent upon porosity and architecture, both of which vary widely with anatomic site (Goldstein 1987) and age (Burstein *et al.* 1976; Wong *et al.* 1985). In addition, cancellous bone is often anisotropic due to the orientation of major trabeculae along lines of principle stress (figure 2). Table 2 summarizes some of the data available from the literature.

On an elementary level the fabric of bone may be split into three main components: bone matrix, bone cells, bone marrow and its associated vascular network. The bone matrix provides mechanical strength and acts as the body's mineral store, the various bone cells are responsible for maintaining the structure of the matrix, regulating its oxygen and nutrient supply, and storing or releasing minerals as required, while the marrow and vasculature provides the source of stem cells and the main means of communication and interaction with the rest of the body.

(ii) *The matrix*

The extra-cellular matrix has two main components: the organic collagen fibres and the inorganic bone mineral crystals. Together they make up *ca.* 95% of the dry weight of bone, the remainder being composed of other organic molecules (known collectively

Table 3. *Variation in the composition of bone mineral*

(References: 1, McConnel (1973); 2, Driessens (1980); 3, Aoki (1991); 4, Le Geros & Le Geros (1993).)

reference	Ca	P	Mg	Na	K	CO ₃	F	Cl	Sr, Zn, Cu	Ca:P ratio
1	26.7	12.5	0.44	0.73	0.06	3.48	0.07	0.08	Sr = 0.04	1.66
2	36.7	16.0	0.46	0.77	—	8.00	0.04	—	—	1.77
3	34.0	15.0	0.50	0.80	0.20	1.60	0.08	0.2	—	1.75
4	24.5	11.5	0.55	0.70	0.03	5.80	0.02	0.10	traces	1.65

as the non-collagenous proteins) and ‘amorphous’ or poorly crystalline inorganic salts. It is this combination of highly ordered elastic collagen fibres reinforced by sub-microscopic inorganic crystallites together with some latitude in composition and density at any one point that enables bone to display a wide range of mechanical properties and to retain elasticity, toughness and hardness for a minimal weight.

Collagen. Collagen is the most abundant protein found in the body, and occurs in a number of different connective tissues both calcified and non-calcified. Collagen accounts for 70–90% of the non-mineralized component of the bone matrix and varies from an almost random network of coarse bundles to a highly organized system of parallel-fibred sheets or helical bundles. Collagen consists of carefully arranged arrays of tropocollagen molecules, which are long rigid molecules (300 nm long, 1.5 nm wide) composed of three left-handed helices of peptides (‘monomers’ of proteins composed of amino acid sequences) known as α -chains that are bound together in a right-handed triple helix. Although all α -chains contain the glycine–X–Y sequence, different types of collagen may be produced via the combination of different amounts and sequences of other amino acids within the tropocollagen molecule. To date, 13 different types of collagen have been identified. Bone contains mostly type-I collagen with some type-V collagen. Type-I collagen is the most abundant form, accounting for 90% of the body’s total collagen; it contains two identical and one dissimilar α -chains ($\alpha 1(I)_2\alpha 2$) within its tropocollagen molecule. Molecules of both types I and V are organized into collagen fibrils, which are formed by the assembly of tropocollagen molecules in a $\frac{3}{4}$ stagger, parallel array (figure 3). As a result of this assembly, the fibrils exhibit characteristic cross-striations or banding, which occurs in a repeating pattern every 55–75 nm, average 64 nm (Robinson & Watson 1952). The fibrils are stabilized by inter- and intra-molecular cross-links (the number and distribution of which determine whether the tissue will mineralize), and have individual diameters of 40–120 nm, average 100 nm. In type-I collagen the fibrils are wound into bundles to form collagen fibres that range in diameter from 0.2 to 12 μ m (Kielty *et al.* 1993).

Bone mineral. The main inorganic phase within bone is usually incorrectly referred to as hydroxyapatite (HA), a hydrated calcium phosphate ceramic, with a similar (but not identical) crystallographic structure to natural bone mineral (de Jong 1926), which has a chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and a Ca:P ratio of 5:3 (1.66). However, bone-apatite is characterized by calcium, phosphate and hydroxyl deficiency (reported Ca:P ratios of 1.37–1.87) (McConnel 1973; Posner 1969), internal

crystal disorder and ionic substitution within the apatite lattice resulting in the presence of significant levels of additional trace elements within bone mineral (table 3). It is not a direct analogue of HA as is commonly believed, but more closely related to an A–B type carbonate-substituted apatite (Elliot 1994; Le Geros & Le Geros 1993). These factors all contribute to an apatite that is insoluble enough for stability, yet sufficiently reactive to allow the *in vivo* sub-microscopic (5–100 nm) crystallites to be constantly resorbed and reformed as required by the body.

While many investigators of calcification agree that mineralization originates within matrix vesicles (Anderson 1980), there is some disagreement about the exact mechanisms of the process. It seems likely that both cellular and physiochemical factors are involved. Generally amorphous calcium phosphates with a Ca:P ratio varying between 1.44 and 1.55 are believed to be deposited under the control of osteoblasts. Once deposited, this amorphous tricalcium phosphate is present as a reservoir for HA crystallite nucleation and growth independently of the cells (Posner 1969). *In vitro* studies have been used to try to provide an insight into the formation of bio-apatite *in vivo*, where variation in the Ca and PO₄ saturation above and below physiological levels influenced nucleation and crystallite shape (Blumenthal & Posner 1973; Boskey & Posner 1976). Other organic substances present in the matrix have long been thought to be responsible for promoting the initial nucleation and deposition of bio-apatite, and regulating the orientation, size and growth rate of the crystals. More recent studies have focused investigation on the role of some of the non-collagenous proteins found in bone with interesting results.

Non-collagenous proteins. The non-collagenous organic constituents of bone matrix include a number of sulphated and acid mucopolysaccharides. There are four proteins of particular interest, osteocalcin (OC), bone sialoprotein (BSP) osteopontin (OP) and osteonectin (ON). These are members of the group generally referred to as the non-collagenous proteins (NCPs), are produced by bone cells and are believed to regulate bone mineralization and remodelling. Only OC and BSP are bone specific, all appear to be multi-functional and their relative composition within the bone matrix appears to be self-regulating through a feedback effect on the expression of NCPs by osteoblasts (Butler 2000; Gerstenfeld *et al.* 2000).

Osteocalcin, also known as bone Gla protein, is one of the most abundant NCPs in bone, comprising up to 20% of the total NCPs in bone. OC has a strong affinity for HA but not amorphous Ca–PO₄, binding to HA through orientation of the Gla residues with the Ca ions in the mineral lattice (Butler 2000). Furthermore, OC has been shown to be a potent inhibitor of HA formation by delaying nucleation (Hunter *et al.* 1996); its expression is mediated by Ca²⁺ regulated hormones, and recent experiments have demonstrated that OC acts to regulate remodelling through suppression of bone formation by osteoblasts (Butler 2000). Several metabolic bone diseases such as Paget's and osteomalacia are associated with elevated levels of OC.

Bone sialoprotein (BSP) is an acidic sialoglycoprotein and comprises 15% of the total non-collagenous proteins in bone. BSP is thought to be involved in both bone mineralization and remodelling (Wuttke *et al.* 2001). It is a multi-functional protein promoting both osteoblast differentiation and bone resorption in a dose-dependent manner (Wuttke *et al.* 2001). BSP has an arginine–glycine–aspartate (RGD) tripeptide sequence, the minimal structure required for cell binding and has been shown to promote osteoblasts adhesion *in vitro* (Gerstenfeld *et al.* 2000). Furthermore, BSP

has a high affinity for HA and has been shown to facilitate HA nucleation (Hunter *et al.* 1996).

Osteopontin is an acidic phosphorylated glycoprotein present in many tissues. OP is a highly potent inhibitor of HA formation, acting by inhibition of crystallite growth (Hunter *et al.* 1996). Furthermore, protein conformation has been shown to affect its ability to inhibit nucleation. It appears that the high level of phosphorylation and associated negative charge density, which may be regulated, is key to its inhibition potency. This supports the theory that crystallite growth is suppressed by OP binding to the HA and sterically preventing further ionic growth through electrostatic repulsion of ions (Pampena *et al.* 2004). OP also has an RGD sequence that promotes cell attachment and is involved in the regulation of osteoclast motility during bone resorption (Butler 2000) and it has been implicated in the regulation of both osseous and ectopic calcification (Pampena *et al.* 2004).

Osteonectin is expressed by osteoblastic cells. However, its abundance in the bone matrix is highly variable and it is also present in many other tissues. As a result of this variability, its role in bone formation is unclear, ON has been reported to inhibit HA crystallite growth but only when it is present in sufficiently high concentrations (Hunter *et al.* 1996). Furthermore, studies failed to detect ON in newly formed bone, questioning previously proposed roles for this protein in tissue mineralization (Kasugai *et al.* 1991; Nagata *et al.* 1991). ON is a single-chain polypeptide containing two glutamate-rich segments that can bind eight Ca^{2+} ions. Unsurprisingly, ON binds strongly to HA (Romberg *et al.* 1986). However, it also binds to other extracellular matrix proteins including collagens I and V. ON will also bind to, and inhibit the spreading of, endothelial and smooth muscle cells (Sage *et al.* 1989). ON is therefore believed to be involved in cell–matrix interactions rather than being directly involved in mineralization, facilitating changes in cellular shape and cell disengagement from the matrix.

Growth factors. Growth factors (GFs) are peptides that regulate cell growth, function and motility, resulting in the formation of new tissue. Bone GFs influence the synthesis of new bone by acting on the local cell population present in bone marrow and on bone surfaces. They either act directly on specific osteoblasts as local regulators of cell growth and function or by inducing angiogenesis (vascularization) (basic fibroblast growth factors 1 & 2, bFGF-1/2, vascular endothelial growth factor, VEGF), or osteogenesis by promoting endothelial or osteoprogenitor cell migration and differentiation (Urist 1965). Bone matrix contains a great number of growth factors (Solheim 1998; Yoon & Boden 2002) including fibroblast growth factors (FGFs), insulin-like growth factor I and II (IGF-I, IGF-II), platelet-derived growth factors (PDGF), and the transforming growth factor beta (TGF- β) supergene family, which currently has 43 members and includes, among others, TGF- β 1–5 and the bone morphogenic proteins, BMP 2–16 (Burt & Law 1994). The proteins of the TGF- β superfamily regulate many different biological processes, including cell growth, differentiation and embryonic pattern formation (Zhu *et al.* 1999).

BMPs play a critical role in modulating mesenchymal differentiation inducing the complete sequence of endochondral bone formation where cartilage forms first and is subsequently replaced by bone (Wozney & Rosen 1998). Other GFs such as TGF- β , IGF and FGFs all affect the already differentiated bone-forming cells, causing them to divide or increase secretion of extracellular matrix and proteins. In contrast BMPs

are the only known GFs with the ability to stimulate the differentiation of mesenchymal stem cells in the chondroblastic and osteoblastic direction (Chen *et al.* 1991; Reddi *et al.* 1987; Skinner *et al.* 2003). Therefore, the proximity and quality of the local bone is less of a factor in the regeneration. These findings suggest that the use of recombinant BMP has a broad therapeutic potential for orthopaedic reconstruction. However, different BMPs are not identical in their osteoinductive potential. For example, BMP 5 is needed in larger amounts to induce the same amount of bone compared with BMP 2 or 7 (Wozney & Rosen 1998) and only BMP 7 (OP-1) has been shown to regulate *Cbfa1*, which has been identified as the only transcriptional factor responsible for osteoblastic differentiation and expression of osteocalcin and osteopontin.

Moreover, native BMP is present in cortical bone in minute amounts (*ca.* 1–2 μg BMP per kg of cortical bone) and, while recombinant human (rh) BMPs 2, 4 and 7 have been shown to induce bone in many experiments and are now also being tested in clinical studies (Boden 1999), it is interesting that the amount of rhBMP 2 necessary to produce bone induction *in vivo* is of the order of 0.7–17 μg BMP per mg of collagen carrier and that the activity of rhBMP 2 is one-tenth that of purified human BMP 2 (Bessho *et al.* 1999). This fact suggests that native BMP activity is a combination of the activities of different BMPs or the synergistic activity between them (Wozney *et al.* 1990) leading to some concerns regarding their use at such high concentrations (Poynton & Lane 2002).

(iii) Bone cells

Of the many cells associated with bone, three are of special interest: the osteoblast, osteocyte and osteoclast, which are responsible for the production, maintenance, and resorption of bone, respectively. However, they are highly specialized differentiated cells and do not generally proliferate (replicate). Less differentiated (immature) cells of the same lineage are required for the control of the cell population, and, as demands are made on or by the bone, these cells proliferate and differentiate as required. Such cells are generally known as stem cells (which have engendered considerable media attention over recent months) and in the case of bone formation are often referred to as osteogenic cells. The osteogenic bone-forming cells originate from the mesenchymal bone marrow stromal cell line and exist in the endosteum and periosteum (Owen 1978). Biochemical signalling molecules stimulated during remodelling and fracture healing result in local population increases in these cells. However, the local environment also determines the route of differentiation undertaken by an osteogenic cell, resulting in the evolution of either osteoblasts or chondroblasts (Bourne 1972). High vascularity, *i.e.* the availability of nutrients and oxygen in the surrounding environment, is necessary for the growth and nourishment of healthy bone. Bone metabolizes significant amounts of oxygen, but, being densely mineralized, is unable to sustain itself via osmosis or similar methods of diffusion over long distances. It therefore relies on an internal vascular network to circulate oxygen and essential nutrients, which then diffuse over shorter distances. Conversely, cartilage requires little or no oxygen for metabolism and, being composed mainly of unmineralized, although densely packed, collagen fibres, is able to sustain itself via the diffusion of nutrients through the cartilaginous matrix. Thus, if the environment surrounding a differentiating osteogenic cell has a high vascular content, as in healthy bone, the cell

will differentiate into an osteoblast which will produce bone. Once the osteoblast has been surrounded by bone it differentiates into an osteocyte and becomes involved in the nutrition and maintenance of the local bone. However, if the environment surrounding a differentiating osteogenic cell has little or no vascular content, as in a recent fracture site, the cell will differentiate into a chondroblast and cartilage will be produced. Once the chondroblast is surrounded by cartilage it then differentiates into a chondrocyte, which maintains the surrounding collagenous matrix until it is replaced by bone during endochondral ossification (Jee & Kimmel 1976; Owen 1978). In contrast osteoclasts are derived from monocytes that originate from the haematopoietic stem cells lineage. Under the influence of specific signalling proteins or cytokines, mononuclear monocytes migrate to the resorption site and fuse with either other monocytes or an established multi-nucleated macrophage before differentiating into the specialized osteoclast (Ross 2003). Osteoclasts are aggressive cells that are responsible for the majority of bone resorption, although the monocytes and the multi-nuclear macrophages are also involved in bone resorption during remodelling and fracture repair (Baron *et al.* 1980; Parfitt 1993). They are easy to distinguish, being the multi-nucleated cells (the largest having 100 nuclei, the smallest two nuclei, with an average of 10–20) lying in resorption pits on the bone and having a ruffled border adjacent to the surface undergoing resorption.

(iv) *Bone repair*

Bone is unique among all vertebrate tissue in its ability to heal via the formation of new bone; all other tissue, such as heart, muscle and brain heal by replacement with connective tissue rather than original tissue. Furthermore, in a mature animal, the molecular and cellular patterns of bone repair after injury are similar to bone formation in an embryo, suggesting analogous mechanisms for the control of bone formation in adult and embryonic skeletons (Rosen & Thies 1992). In an embryo a condensation (concentration) of primitive mesenchymal cells can transform into bone via either intramembranous ossification or endochondral ossification. Intramembranous ossification occurs when the mesenchymal cells are transformed into osteoprogenitor cells and then directly into osteoblasts, resulting in the direct formation of bone. While in endochondral ossification bone formation occurs via a two-step process where the mesenchymal cells transform into chondroblasts that lay down a collagenous template which is subsequently ossified by invading osteoblasts. The final mature bone formed by both processes is virtually indistinguishable and the mechanisms that dictate which route is taken are poorly understood. In the embryo, skull and jaw bones form through intramembranous ossification whereas the shoulder, pelvic and limb bones form through endochondral ossification.

Fractured bone invariably heals through endochondral ossification in a five-step process.

- (i) Firstly, a haematoma (blood clot) is formed, resulting from injury to the periosteum (the fibrous membrane that covers most bone surfaces containing blood vessels which nourish the bone while also acting as an attachment point for tendons and muscles) and local soft tissue.
- (ii) As a consequence of this disruption in the blood supply, osteocytes nearest to the fracture die, resulting in local necrosis of the bone around the fracture (under normal conditions these osteocytes are dependent on nourishment

derived from the fluids circulating within the canaliculi, which in turn make contact with the blood supply). Simultaneously, there is a demand for the repair of the bone, the stabilization of the damaged area and the removal of the dead tissue.

- (iii) In response to this, macrophages and fibroblasts are recruited to the site to remove tissue debris and to express extracellular matrix, respectively. In response to GFs and cytokines released by these inflammatory cells, mesenchymal stem cells are recruited from the bone marrow and periosteum, which then proliferate and differentiate into osteoprogenitor cells.
- (iv) This leads to an apparent thickening of the periosteum and the production of collars of external fracture callus around the fracture site. Those osteoprogenitor cells that lie close to undamaged bone (and are thus within reach of a ready supply of oxygen) differentiate into osteoblasts and form osteoid which is rapidly calcified into bone, while those farther away become chondroblasts and form cartilage. Angiogenesis is induced concurrently and, almost as soon as the cartilage has been formed and the fracture site stabilized, it is replaced by woven cancellous bone, via endochondral ossification, in which osteoclasts and osteoprogenitor cells invade the cartilaginous callus preceded by capillary formation.
- (v) The uncalcified material is resorbed and new bone is deposited on remaining spicules of calcified cartilage. The woven bone is then remodelled into lamellar bone and the process is completed by the return of normal bone marrow within cancellous regions, while in repairing cortical bone the spaces between trabeculae are gradually filled in with successive layers of bone so forming new Haversian canals. Thus load-bearing capabilities and a new vascular network are restored (Bourne 1972).

(b) *Bone grafting*

So what should an ideal BGS do? A bone graft should not only temporarily replace missing bone, but also provide a framework into which the host bone and vascular network can regenerate and heal. It should act as a scaffold to support the new bone, blood vessels and soft tissue as they grow to connect fractured bone segments, so strengthening the grafted area and fixating it by forming a bridge between existing bone and the graft material. However, ideally it should also interact with the host tissue, recruiting and even promoting differentiation of osteogenic stem cells, rather than acting as a passive stage for the performance of any itinerant cells. There are clearly two important (but not unrelated) factors—selection of the correct chemistry to support or stimulate an appropriate host response and the engineering of an appropriate scaffold structure. Here we see that biology, chemistry and engineering have begun to overlap.

(i) *Chemical mediation of bioactivity*

Since the early 1970s researchers have been investigating the use of HA in the treatment of bone fractures or defects. Over the years there has been much disagreement over the characterization of its biological response. Generally researchers agree

that the material is osteoconductive, i.e. that it supports the formation of bone on its surface by osteoblasts. However, there is some dispute as to its osteoinductivity, i.e. its ability to induce bone formation by influencing the differentiation of stem cells into osteoblasts. Fundamental to this dispute is the fact that the nature of bone response to HA (indeed any replacement material) is far from fully understood—why should either bone or stem cells find the surface chemistry of HA conducive to the formation of bone whereas (fortunately for those with hip replacements) a polyethylene (PE) surface is not, and thus suitable as a bearing surface? Moreover, why should HA appear osteoconductive in some circumstances and osteoinductive in others?

It is accepted that the difference in response between HA and PE results from the difference between their chemistries, although the precise mechanisms through which the chemistry of an implant and biology interact are still unknown. This may in part result from the complexity of the likely interaction. Cells interact with their environment through a number of sensors known as receptors. These receptors may be transmembrane or intracellular and are coded to bind to specific molecules or ligands which mediate a specific intracellular response, such as cell division (proliferation), cell maturation (differentiation), gene expression, collagen synthesis or ion channel regulation. Transmembrane cell surface receptors interact with ligands to directly or indirectly mediate a specific intracellular response. Intracellular receptors are triggered by interaction with molecules that either diffuse across the cell membrane or have been activated intracellularly following ligand binding at a cell surface receptor. This phenomenon is known as cell signalling, where signalling molecules include ions, peptides and proteins generally released by signalling cells, but can also include segments of proteins attached to an adjacent cell or a tissue or implant surface. The sequential process initiated by binding of a signalling molecule is known as signal transduction (Lodish *et al.* 1999). It is through this mechanism that GFs act on cells—most work through activation of factor specific receptors to stimulate cell division (i.e. they are mitogenic)—whereas the BMPs are unique in their ability to act both mitogenically and morphogenically, i.e. to stimulate osteoprogenitor stem cell differentiation. Another form of cell interaction occurs through cell adhesion molecules. Again, these are transmembrane proteins that include integrin and cadherin receptors that bind specific segments on a number of extracellular matrix proteins, and other cells, respectively, while their intracellular component interacts with the cell cytoskeleton, so enabling cell–matrix or cell–cell adhesion in addition to signal transduction. Furthermore, cells are able to communicate directly via direct exchange of ions through gap junctions (Anselme 2000).

So how do cells sense the difference in HA or PE and why should it matter? Several theories have been proposed. Some believe that it is the surface chemistry of the material which dominates behaviour, through either the influence of its charge density and atomic array on adherent or passing cell populations, or the influence that surface charge has on the population and conformation (and thus potency) of proteins, such as matrix proteins or growth factors, absorbed on its surface and their subsequent influence on local cell behaviour (figure 4a). Work by Dalby & co-workers (Dalby *et al.* 2002b; Di Silvio *et al.* 2002) demonstrated that in either HA–PE or HA–PMMA composites osteoblast proliferation and differentiation increased with HA addition and that this may be related to increased focal contact (with HA acting as stepping stones for cell adhesion) and rates of cytoskeletal organization. Further-

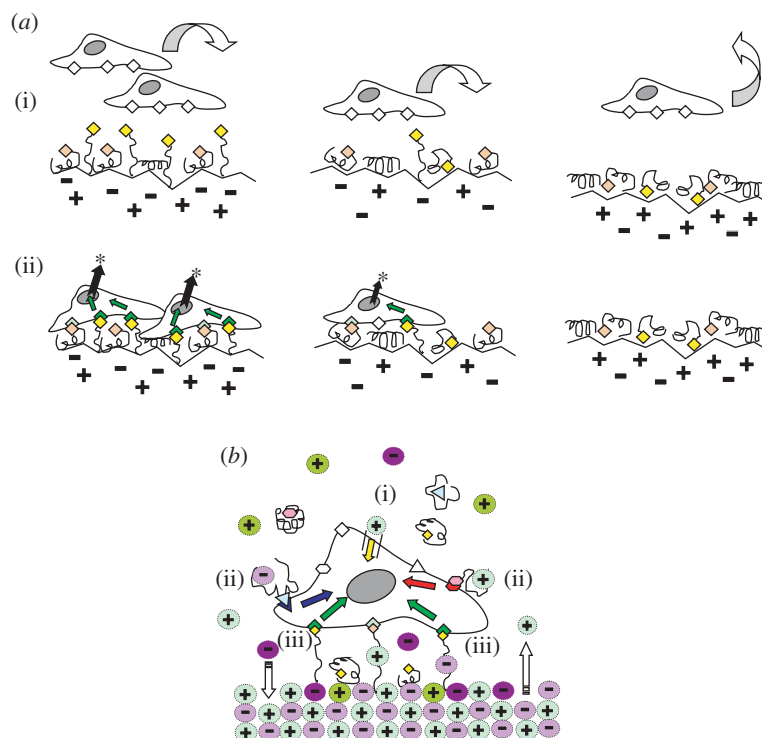


Figure 4. Schematic representing some of the theories behind cell sensitivity to BGS chemistry. (a) Surface charge/hydrophobicity affects speciation and conformation of adsorbed proteins so influencing exposure of the protein segments specific to cell surface receptors and thus mediating (i) cell recruitment and (ii) subsequent regulation of cell response (*). (b) Dissolution and reprecipitation (exchange) of ionic species may initiate: (i) direct intracellular chemical signalling via ion channels, (ii) modification of signalling molecules to facilitate/amplify interaction with surface receptors, (iii) surface modification and protein entrapment influencing speciation and conformation of adsorbed proteins.

more, HA coatings have been demonstrated to reduce fibrous tissue encapsulation of implants exhibiting micromotion and to entice bone formation into blind chambers (Søballe *et al.* 1992; Stephenson *et al.* 1991). It is clear that optimizing the chemistry of a BGS will greatly enhance the chances of a successful clinical outcome. In order to understand these phenomena, other studies have demonstrated the importance of surface charge and wettability in controlling these responses (Redey *et al.* 1999, 2000) and this has been linked to their influence on gene expression and protein attachment (Webb *et al.* 1998) in terms of both the quantity (Kilpadi *et al.* 2001) and the 'quality' (Ducheyne & Qiu 1999) of protein attachment. Given the sensitivity to charge density observed in the ability of osteopontin to bind and thus inhibit HA crystallite growth (Pampena *et al.* 2004), it is not surprising that the surface charge of any implant surface in turn influences the type and confirmation of proteins absorbed on the surface and thus mediates the subsequent cell response. This may also explain why deviation in the phase purity of HA can reduce bone apposition (Hing *et al.* 2001), accounting for some of the variable clinical outcomes observed with differing calcium-phosphate BGSs. In contrast, recognition of the chemical sensitivity of bone

repair has led to considerable activity in the development of substituted apatites (Gibson & Bonfield 2002*a,b*; Gibson *et al.* 1999; Ikeuchi *et al.* 2003; Layani *et al.* 2000; Merry *et al.* 1998). As mentioned earlier, in addition to calcium, phosphate and hydroxyl ions, bone mineral contains significant concentrations of other ions such as carbonate, sodium, magnesium and trace levels of silicon and zinc and aluminium. The inclusion of these ions within the apatite lattice has thus been postulated to improve the bioactivity of the material. This has been most effectively demonstrated with Si substitution which has been demonstrated to have a significant influence on the rate and pattern of bone formation *in vivo* (Gibson *et al.* 2002; Hing *et al.* 2004*a*; Patel *et al.* 2002). Carbonate has been recognized to increase the solubility of HA and to facilitate resorption by osteoclasts (Hasegawa *et al.* 2003), while Mg plays an important role in the mineralization of bone (Marie *et al.* 1983) and in the promotion of bone formation (Toba *et al.* 2000). Furthermore, recent studies of Mg ion incorporation by ion-beam implantation in a range of biomaterials, including HA (Zreiqat *et al.* 1999, 2002), have established its stimulatory effect on bone formation.

Can these responses be solely a result of changes in cell-protein-material interface communications? Some believe that direct interaction with the intrinsic chemistry of the material is responsible for osteoinduction through the dissolution and release of specific key ions that act directly on local cells to up regulate gene expression or influence cell differentiation (figure 4*b*). This would extend the range of the materials influence from a few micrometres (and thus only cells already at the material surface) up to several metres, assuming release products migrate and diffuse into the blood stream, significantly extending the range of cells that may be involved in the response. This is part of the rationale behind the use of β -tricalcium phosphate (β -TCP, $\text{Ca}_3(\text{PO}_4)_2$) or calcium sulphate (CS, CaSO_4) BGS. These materials can be resorbed within six months and three weeks of implantation, respectively, through combined chemical and cellular action, resulting in the release of high concentrations of Ca and PO_4 ions. Ca is an important signalling molecule implicated in cell adhesion, movement, enzyme regulation, carbohydrate release, nerve and muscle cell function. Within the context of bone repair an increase in the local Ca and P concentration has been shown to be important in mineralization *in vitro* (Chang *et al.* 2000). However, it appears that too rapid a dissolution rate may be detrimental to bone formation (Glazer *et al.* 2001; Jamali *et al.* 2002; Liljensten *et al.* 2003; Wang *et al.* 2004). This is unsurprising given the importance of substrate stability to osteoblast adhesion and function. It may also be a consequence of an inflammatory response induced by either a high concentration of ceramic particles and/or degradation products generated by highly porous resorbable materials (Hing *et al.* 2004*b*; Saeed *et al.* 2004). This has resulted in the development of a number of bi-phasic materials, where it has been demonstrated that a composition of 70/30 HA/TCP has similar osteoconductivity to HA (Wang *et al.* 2004). However, the degree of resorbability is disputable even for pure TCP under non-loaded conditions (Handschel *et al.* 2002).

The influence of Si on bone formation and in bone repair has been of interest for a number of years. Carlisle (1970) first reported that silicon deficiency resulted in abnormal bone formation, while the role of silicon in promoting bone induction in resorbable 'bioactive' glasses (which usually contain $\text{CaO-P}_2\text{O}_5\text{-SiO}_2\text{-Na}_2\text{O}$) has been studied intensively with demonstration of up regulation of a number of genes expressed by osteoblasts including BMP 2 when exposed to the ionic products of these

bioactive glasses (Gao *et al.* 2001; Xynos *et al.* 2001). In addition Reffitt *et al.* (2003) have recently demonstrated that orthosilicic acid present in physiological concentrations (5–20 μM) stimulated collagen type-I synthesis and enhanced osteoblast differentiation, while treatment at a higher concentration resulted in a smaller increase in collagen synthesis (Reffitt *et al.* 2003). This agrees well with work performed on silicate substituted apatites, where Porter *et al.* (2003) observed increased dissolution (and thus release of Si) with Si content *in vivo* and recent studies have demonstrated an optimal level of *ca.* 0.8 wt % Si when implanted in a highly porous form which would presumably be able to under go a greater degree of chemical surface interaction (Hing *et al.* 2004a). Furthermore, there is increasing evidence to suggest that either form of chemical interaction may not only act directly on the bone or stem cells but may act further downstream, influencing events such as angiogenesis prior to ossification or the production of specific cytokines or signal molecules by ‘controller’ T cells that then direct the differentiation of stem cells and the function of bone cells. For example, in a study of angiogenesis following HA implantation, a close relationship was demonstrated between the HA surface and newly formed capillaries where immunohistochemical localization demonstrated association of VEGF with the HA surface (Ohtsubo *et al.* 2003). Similarly, vascularization was found to progress more rapidly within a macroporous HA orbital implant than within a macroporous PE implant (Rubin *et al.* 1994), while investigation of T lymphocyte activity (controller cells that express inflammatory cytokines) on exposure to HA particles combined with histological evaluation of distant organs suggests that implantation of HA with or without GFs may have a short-term systemic effect (Damien & Revell 2004; Saeed *et al.* 2004).

(ii) *Engineering osteoinductive bone scaffolds*

The sensitivity of bone cells and matrix proteins is not limited to chemical phenomena. Bulk geometry, surface geometry and topography also influence the process of osteogenesis apparently through mechanical and physical considerations that promote or screen the osteoconductive or osteoinductive potential of a material. Geometrical structural features such as the volume fraction, size, shape and degree of interconnectivity of the pores that characterize the scaffold, and even the density or rigidity of the scaffold struts, have been found to influence the biological response.

Many authors have reported a greater degree and faster rate of bone penetration with increasing macroporosity (i.e. pores greater than 50 μm in diameter) in a wide variety of BGSs (Eggl *et al.* 1988; Hing *et al.* 2004c; Holmes *et al.* 1984; Klawitter *et al.* 1976; Kühne *et al.* 1994). Again there are a number of possible explanations: is it a result of the greater volume available for in-growth or is it related to the openness or interconnectivity of the structure? Bone is a mineralized tissue, i.e. nutrients and oxygen do not readily diffuse through it so it relies heavily on the presence of an internal blood supply. Any new bone formation or repair must always be preceded by the formation of a vascular network, the rapidity and extent of which is strongly influenced by the degree of structural interconnectivity between pores (Rubin *et al.* 1994). Unsurprisingly, a greater penetration of bone was observed in porous PE implants with increasing pore interconnection size up to a 100–135 μm limit (Kilpadi *et al.* 2004) and in-growth was improved within a coralline apatite with a greater pore size as a result of 260 μm interconnections which were not present in the smaller-

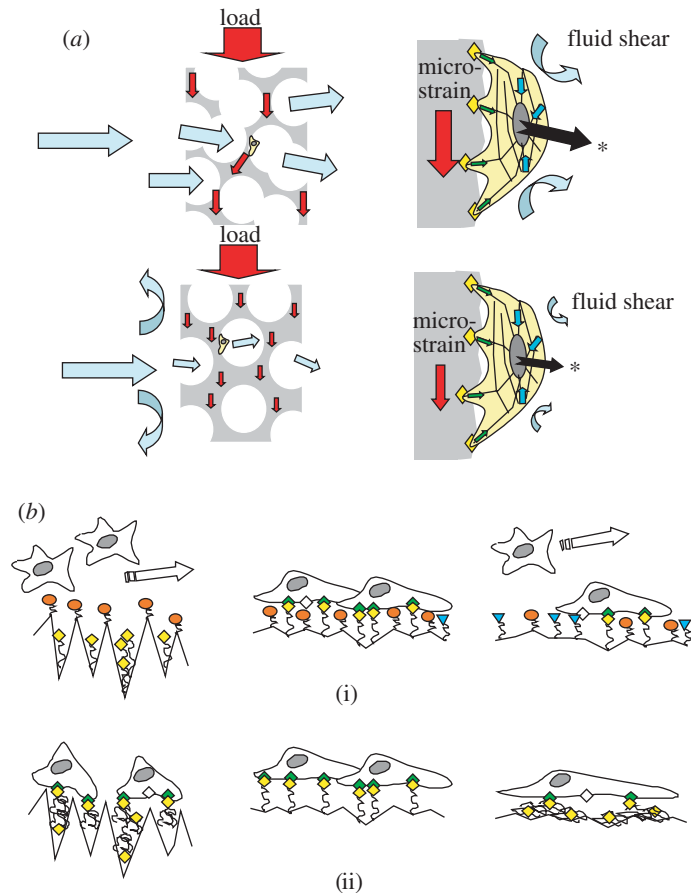


Figure 5. Schematic representing some of the theories behind cell sensitivity to BGS Macrostructure and microstructure/topography. (a) As a result of differences in strut volume and pore interconnectivity, cells on internal pore surfaces of higher percentage porosity BGS may experience elevated levels of strain and shear leading to regulation of cell response via mechano-transduction (*). (b) Microstructure/surface topography is thought to regulate both cell attachment and behaviour through its influence on surface energy and hence (i) speciation and (ii) conformation of adsorbed proteins.

pored ceramic (Kühne *et al.* 1994). Thus the degree of scaffold porosity could be responsible for altering the perceived bioactivity of a BGS as a function of increasing the structural permeability.

Alternatively, or probably concurrently, it is well known that bone is functionally adaptive, i.e. that it responds to external mechanical stimuli to either reduce or increase its mass as required (Wolff 1870)—one of the reasons astronauts have difficulty maintaining their bone mass in space despite rigorous exercise regimes. Unsurprisingly, the mechanical behaviour of various BGSs vary widely with pore structure and to a lesser extent with fabric (Hing *et al.* 1999; Holmes *et al.* 1984; Le Huec *et al.* 1995; Peelen *et al.* 1978). Mechanical forces regulate many cell types, including both osteoblasts and osteocytes (Frost 1987), through a process known as mechano-transduction. Mechano-transduction loosely describes the chain of molec-

ular events that enable mechanical stresses to be converted into biological signals and physiological responses. Intergrin-mediated adhesion sites are believed to be mechano-sensitive and to be involved in mediating cytoskeletal organization, gene expression cell mobility and proliferation (Katsumi *et al.* 2004). Furthermore, certain extra-cellular proteins (such as fibronectin) have been found to alter conformation under strain to expose new binding sites suggesting possible mechanisms for strain-mediated assembly of the extra-cellular matrix (Zhong *et al.* 1998). While mechanical stimulation has recently been shown to stimulate osteoblastic differentiation of osteoprogenitor cells (Mauney *et al.* 2004). Therefore, it is possible to see how variation of local strain in scaffold struts as a result of porosity variation may induce or inhibit bone formation (figure 5a). Moreover, there is some evidence to suggest that the final level of bone in-growth within a BGS may be dependent on this phenomenon (Hing *et al.* 2004c) provided that the level of pore interconnection is sufficient to support adequate vascularization (Eggl *et al.* 1988).

Some of the most striking evidence for the importance of the geometrical configuration of a BGS is in the work of Ripamonti and co-workers, who demonstrated that osteoinductivity in HA was linked to the precise shape of surface concavities in implants (Kuboki *et al.* 1998; Magan & Ripamonti 1996; Ripamonti *et al.* 1993). By using immunolocalization, they demonstrated that this osteoinductivity occurred as a result of a concentration of BMP 3 and BMP 7 within the surface concavities. A similar mechanism was proposed to explain the bioactivity seen in bioactive glass microspheres, in which *de novo* bone formation is believed to initiate from the centre of the hollowed out beads following dissolution of the Si-rich glass and reprecipitation of a CaP-rich shell loaded with adsorbed proteins (Ducheyne & Qiu 1999).

Furthermore, on a smaller scale, surface texture has also been demonstrated to influence cell response to a material. This is proposed to be through mediation of cell attachment (Dalby *et al.* 2002a; Lampin *et al.* 1997), possibly as a result of the influence of roughness on surface energy (Lampin *et al.* 1997) and thus on protein adsorption (figure 5b), where protein patterning on surfaces has been shown to influence cell attachment (McFarland *et al.* 2000; Webster *et al.* 2000). Conversely, studies on the effect of roughness of HA have demonstrated an insensitivity to attachment but found that events such as proliferation and differentiation were affected (Rosa *et al.* 2003), highlighting the complicated nature of response to bioactive materials that are predisposed to bind serum proteins (Kilpadi *et al.* 2001). On a similar scale, microporosity in BGSs has clearly been shown to have a significant impact on bioactivity (Bignon *et al.* 2003; Hing *et al.* 2004d). With evidence to suggest that this is through a combination of enhanced angiogenesis (Hing *et al.* 2004d) and cell adhesion (Annaz *et al.* 2004; Bignon *et al.* 2003), presumably through entrapment and adsorption of adhesion proteins or GFs within the micropores. Furthermore, it has recently been shown that primary human stromal cells and committed osteoblasts adhere to HA via different mechanisms (Kilpadi *et al.* 2004). All the evidence suggests that the geometric dependence of HAs osteoinductivity reflects changes in GF/protein adhesion and subsequent selective cell recruitment, which may have a temporal component reflecting the dynamic nature of the protein population at the BGS interface. These geometrical mediators of bioactivity in combination with other factors would help to explain the differences often observed in response to the 'same' biomaterial.

(c) Bone-graft substitutes—the next generation

It is clear that skeletal tissue regeneration requires the interaction of three basic elements: cells, growth factors and a permissive matrix scaffold. All these factors are necessary for successful bone regeneration, and several studies have demonstrated the effectiveness of combining some (Arnaud *et al.* 1999; Boden *et al.* 2000; Ripamonti *et al.* 2001; Solheim 1998; Steffen *et al.* 2001) or all (Niederwanger & Urist 1996; Reddi 2000; Takagi & Urist 1982) of these elements. Another ‘simpler’ approach is to design a reliably osteoinductive BGS capable of recruiting and stimulating the patient’s own cells where bone induction may be manipulated by a combination of the surface chemistry, the physical macrostructure and the microstructure or surface topography of the BGS (Ducheyne & Qiu 1999; Gibson *et al.* 1999; Hench & Paschall 1973; Hing *et al.* 2004c; Lampin *et al.* 1997; Ripamonti *et al.* 1993). Either way, the resultant body of research concerned with understanding the mechanisms of osteoinductivity and linking interactions between the local chemical and physical environment is paving the way for future generations of BGSs and bone-repair therapies.

One such future direction is gene therapy (Boden 2000; Yoon & Boden 2004). Currently the clinical use of BMPs is restricted to a limited number of applications (or indications) and has a high price tag (€4500 per unit (Giltaij 2002)). Moreover, BMPs are degraded rapidly once released and there are concerns regarding the high doses used (Posner 1969). Gene therapy refers to the practice of delivering a viral vector to transfect local cells to manufacture BMPs (Riew *et al.* 1998; Wang *et al.* 2003) or to express genes that subsequently stimulate BMP expression in local cells (Minamide *et al.* 2003). This treatment strategy was originally conceived as a way to correct single gene mutations associated with genetic diseases. However, sustained expression has proven difficult to achieve. This is not a limitation in bone repair, as only a relatively short term of gene expression is required. However, there are several factors which need to be considered in the development of a successful approach; these include choice of an appropriate osteoinductive gene and selection of a delivery vector that will not invite an inflammatory response but will result in timely gene transfer such that delivery of a temporally and spatially potent dose of GF is achieved.

A further or concurrent step into the future would ironically be a return to auto-grafting, although this time one could imagine future bone engineers taking bone marrow aspirate samples, isolating and expanding the angiogenic and osteogenic cell fractions and introducing them into a ‘bioreactor’, where they are stimulated to produce functional live bone, i.e. with all the macro and microscopic features of bone such as a trabecular network of struts containing osteocyte lacunae communicating with their surroundings and local blood supply via canaliculi. This approach would provide a patient with as much of their own good quality bone as they required without any fear of donor-site pain or morbidity. Furthermore, although we are some way off knowing exactly what environment and stimuli will be required inside a bone bioreactor to produce functional bone, it is not quite as futuristic as it may sound, as similar technologies are already in clinical practice for treatment of damaged cartilage (Carticel, Genzyme, MACI Verigen) and in skin grafting (Myskin CellTran, Dermagraft, Smith & Nephew) where autologous cells are biopsied from the patient, expanded and reintroduced to the wound/defect site usually supported within a matrix. Currently, the main limitations of these technologies (besides cost

and regulatory approval) are the requirement of a specific period of time in which to expand and grow your tissue (approximately three weeks) and long-term stabilization/integration of the 'graft' tissue (especially in the treatment of cartilage). The current success achieved with bone autografting suggests that integration of 'live bioengineered' bone tissues will not be a problem. However, while a three-week delay will not be an issue when dealing with elective surgery such as hip revision, in the treatment of trauma it could be critical. Moreover, current surgical trends towards minimally invasive surgery would favour development of a more therapeutic injectable 'magic bullet' treatment modality. This approach would favour either a gene-therapy-based methodology or direct delivery of the cues required to invoke osteogenesis or bone augmentation *in vivo*, where the patient acts as the bioreactor.

Nevertheless, in the short to medium term, optimization of a BGS to act as a delivery vehicle for viral vectors or as a scaffold for bioengineered bone formation may still be required. At the very least the use and understanding of the way in which current and next generation osteoinductive BGS scaffolds stimulate bone formation and interact with their surroundings to control bone morphology will be an essential prerequisite to designing future 'magic bullet' or 'regeneration tank' therapies. Thus these future options will be guided by an understanding of the exact mechanism by which bone cells probe the chemistry and physics of their environment and how this influences the biological pathways involved in osteogenic function. Increasingly this appears to be based in the realms of physical chemistry, with cells acting as micro-computers transmitting and receiving physical and chemical signals that orchestrate the basic functions that underpin our lives. It would appear that biology and chemistry might be more intimately linked than one merely being the building blocks of the other; biology appears to be about harnessing chemical and physical phenomena to sustain life.

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AUTHOR PROFILE

Karin A. Hing

Karin Hing is 35. Born in Watford, Hertfordshire, she studied Materials Science at Brunel University, where she graduated with First Class honours in 1991. After a year spent backpacking throughout Asia and Australasia, she continued her studies at Interdisciplinary Research Centre in Biomedical Materials, Queen Mary University of London, obtaining a PhD in 1996. Since 1999 she has held an EPSRC Advanced Research Fellowship at Queen Mary. Her research interests have focused on the mechanisms underpinning successful osseointegration of macroporous ceramics and the development of optimized synthetic BGSs for guided bone repair. More recently she has been concentrating on the relative importance of the physical and chemical characteristics of these biomaterials. She has over 30 publications, and is one of the founding scientists of ApaTech Ltd, a start-up orthopaedic company specializing in bone-substitute materials. Married with one son, she is enjoying the time-honoured challenges and rewards associated with the definitive tissue-engineering project.

