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Underlying Mechanisms at the Bone–Biomaterial Interface

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Abstract In order to understand how biomaterials influence bone formation in vivo, it is necessary to examine cellular response to materials in the context of wound healing. Four interrelated properties of biomaterials (chemical composition, surface energy, surface roughness, and surface topography) affect mesenchymal cells in vitro. Attachment, proliferation, metabolism, matrix synthesis, and differentiation of osteoblast-like cell lines and primary chondrocytes are sensitive to one or more of these properties. The nature of the response depends on cell maturation state. Rarely do differentiated osteoblasts or chondrocytes see a material prior to its modification by biological fluids, immune cells and less differentiated mesenchymal cells in vivo. Studies using the rat marrow ablation model of endosteal wound healing indicate that ability of osteoblasts to synthesize and calcify their extracellular matrix is affected by the local presence of the material. Changes in the morphology and biochemistry of matrix vesicles, extracellular organelles associated with matrix maturation and calcification, seen in normal endosteal healing, are altered by implants. Moreover, the material exerts a systemic effect on endosteal healing as well. This may be due to local effects on growth factor production and secretion into the circulation, as well as to the fact that the implant may serve as a bioreactor.

Key words: implant, bone formation, osteoblast, matrix vesicles, bone/implant interface

Historically, studies on the effects of materials on bone apposition have focused on issues of biocompatibility. Those materials that are well tolerated, in that they do not cause an overt adverse systemic reaction and are not aggressively rejected immunologically, are deemed to be biocompatible. Even among this select group of materials, there is a broad range of tissue response. Histologic examination of the interface reveals that bone formation may occur in close apposition to the material, even appearing to form a chemical bond. In other instances, bone is separated from the material by fibrous connective tissue, suggesting that scar formation may have occurred.

To understand how a single material can elicit a disparate set of responses, one must establish an alternative to the paradigm presented above. Rather than restrict thinking to mechanisms of bone formation, one must recast the process in terms of wound healing, with bone being the primary healing tissue, but not the only tissue, in contact with the material. Certainly, variability in host physiology is important. Nutritional status, hormonal status, use of pharmaceuticals, immunology, lifestyle choices, age, presence of organic disease or infection, and other factors, all influence the healing response. Particularly important for bone healing, the mechanical and physical stresses exerted on the material or on the healing tissue can have profound effects on the healing process, as well as on the eventual remodeling of the newly formed osseous tissue.

The contribution of the material itself to the wound healing response and the production of new bone around the implant is less well understood. This paper discusses these issues.

MATERIAL PROPERTIES AFFECTING MESENCHYMAL CELL RESPONSE

It is increasingly clear that cells of mesenchymal origin are sensitive to several surface properties of biomaterials. As shown in Figure 1, those properties that influence behavior of cells,

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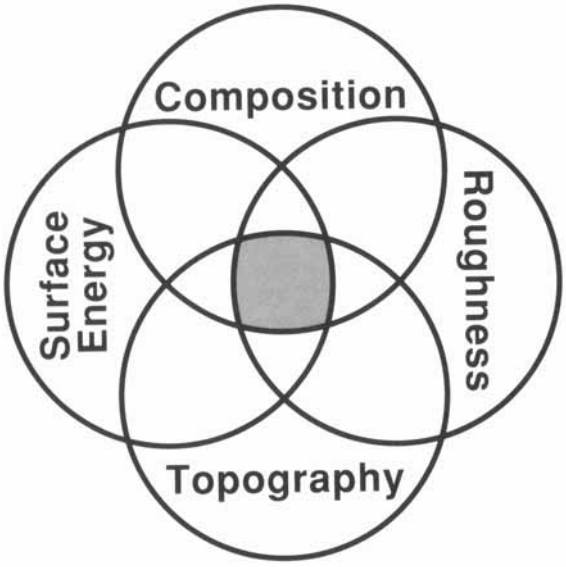

Fig. 1. Implant surface characteristics affecting bone formation and apposition.

including matrix production and calcification, can be grouped into four interrelated categories: composition, surface energy, roughness, and topography.

Composition

Surface chemistry can directly affect the adsorption of serum components and factors present in the extracellular fluid onto the material. This initial interaction between the host environment and the biomaterial may have significant downstream consequences on cellular attachment. The sequential binding of serum proteins and factors has been the focus of a number of laboratories [Hench and Paschall, 1973; Jarcho et al., 1977; Ziats et al., 1988]. It has also been shown that fibronectin binding is nearly instantaneous [Pearson et al., 1988]. Fibronectin has been shown to mediate cell attachment and spreading on artificial substrates by interaction with glycosaminoglycans and the cytoskeleton [Doillon et al., 1987]. This conditioning of the surface also involves adsorption of metal ions [Dearnalley, personal communication], which in itself can alter the binding characteristics of serum proteins as well as the conformation of proteins after binding. For example, altered fluoride content of enamel can change binding of salivary proteins, their conformation, and the ability of oral bacteria to adhere to the surface. Similarly, in wound healing, the differential adsorption of attachment proteins such as fibronectin, osteopontin, and laminin, or variations in their stereochemistry, may alter the migration, attachment, and differentiation of different mesenchymal cell populations. While much attention has been paid to protein adsorption, it is likely that the chemical composition of the surface will also influence adsorption of lipids and sugars as well. The consequences of this are not known.

Chemical composition of the material at time of implantation may not reflect chemical composition following exposure to biologic fluids or following modification by the adjacent cells. Corrosion products and leached ions are frequently found in the adjacent tissue [Pappas and Cohen, 1968; Golijanin and Bernard, 1988]. Even Ti, long presumed to be nonleachable due to the formation of TiO_2 on the material surface, has been found deposited in surrounding tissue [Steinman and Mausli, 1989; Osborn et al., 1990]. The success of some materials, such as bone-bonding bioglasses, depends in part on the chemical activity of the material [Gross and Strunz, 1980, 1985; Gross et al., 1981; Blumenthal et al., 1988]. Conversely, the corrosion of materials like stainless steel contributes to their rejection by bone [Steinman, 1992; Evans and Benjamin, 1987; Keller et al., 1989; Ducheyne et al., 1990]. Moreover, implants removed from patients have a demonstrated positive correlation between metal corrosion and tissue response [French et al., 1984]. It is becoming increasingly clear that no material is truly chemically inert, nor is being inert necessarily desirable for all applications.

Cell culture studies in our lab demonstrate the sensitivity of bone and cartilage cells to surface chemistry. By sputter coating culture dishes with a variety of metals and ceramics, we were able to isolate chemical composition as a single variable. All surfaces were 800 Å thick and had a surface morphology like that of the underlying plastic. Osteoblast-like cell lines, as well as primary chondrocytes, were cultured on surfaces prepared by sputtering the following target materials: Al_2O_3, calcium phosphate with a ratio of 1.67:1, calcium phosphate with a ratio of 1.5:1, commercially pure Ti, Ti in which molecular O_2 was introduced during sputtering (TiO_2) and Zr in which molecular O_2 was introduced during sputtering (ZrO_2). One strength of this approach was that it allowed the comparison of two calcium phosphate chemistries as well as two different crystallinity states of

TiO_2:Ti that formed an oxide layer upon exposure to the ambient atmosphere versus a more crystalline TiO_2 created during the sputtering process.

The transformed osteoblast-like cell studies showed that cell proliferation alone was an inadequate measure of the effects of surface chemistry on cell response [Windeler et al., 1991]. Phenotypic expression was modulated by the surface, however, including differences in gene expression. The chondrocyte studies showed that sensitivity to surface chemistry was also a property of nontransformed cells [Hambleton et al., in press]. Moreover, the response of the chondrocytes was dependent on the state of cell maturation. In these studies, all aspects of cell function were affected by culture on the different surfaces, including cell proliferation, cell metabolism, extracellular matrix synthesis, and cell differentiation. The chondrocytes were able to discriminate between closely related surface chemistries, including the degree of TiO_2 crystallinity. These observations have important implications for cellular response to materials during wound healing as cells progress through complex differentiation cascades.

Surface Energy

Related to the chemical composition of the material is the surface energy. Wetability influences the rate at which serum components adhere (see above section on composition) as well as the extent of the surface that will be affected.

Surface energy may also be affected by the method used to finish the material. One of the best examples of this concept is the use of glow discharge to maximize the charge density of tissue culture polystyrene prior to use. The subtleties among commercially prepared tissue culture dishes are well known to cell biologists, who quickly learn to select the optimal surface for their particular application based on empirical assessments of cell attachment and spreading. The cells are able to discriminate among the charge densities resulting from the differences in proprietary glow discharge techniques.

The importance of surface energy has been demonstrated both in vivo and in vitro [Baier et al., 1984; Kasemo and Lausmaa, 1986; Chehroudi et al., 1991]. It is important to note that those surface characteristics most prized by cell biologists may have little merit in vivo. While cell spreading and proliferation are desired in vitro, cell differentiation may be more important in vivo.

Surface Roughness and Topography

In vivo studies on bone apposition to implants have demonstrated repeatedly that rougher surfaces promote bone formation whereas smoother surfaces tend to promote a fibrous interface [Schroeder et al., 1981; Buser et al., 1991; Rich and Harris, 1981; Thomas and Cook, 1985]. Smooth and rough surfaces result in different contact areas between cells and matrix. This produces different types of bonding of the biological unit. These observations have led to a variety of modifications of surface morphology to produce rougher surfaces, including plasma-sprayed materials, sintered beads, and metal fibers. The intent with these variations in surface roughness is to elicit bony ingrowth, better bone closure and fit, and better function [Carlsson et al., 1988; Wilkes et al., 1990]. Those modifications that have survived clinical testing tend to share a porous structure that is conducive to osteoblast migration and differentiation [Bowers et al., 1992; Michaels et al., 1989; Itakura et al., 1988; Groessner-Schreiber and Tuan, 1992].

Only recently have we begun to differentiate between the role of surface roughness and surface topography in cell response. To do this we established a cell culture model which maintains constant chemistry, but varies roughness and/or topography. Commercially pure Ti disks were prepared with five different surfaces. Four of the surfaces varied in roughness and the fifth had a roughness comparable to one of the test surfaces but varied in the topographical distribution of rough areas. When cells of the transformed human osteoblast cell line, MG63, were cultured on the surfaces, the cells differed in terms of cell morphology, adherence, proliferation, extracellular matrix synthesis, and differentiation [Martin et al., in press]. Moreover, MG63 cells cultured on surfaces of comparable roughness but different topography behaved in a differential manner. In general, cells cultured on the rougher surfaces exhibited more cuboidal cell morphology and a greater degree of differentiation.

At this point, we can only speculate about the mechanism by which surface roughness alters phenotypic expression in osteoblasts. Enhanced surface area may lead to greater binding of attachment proteins and regulatory factors. However, greater surface area would also in-

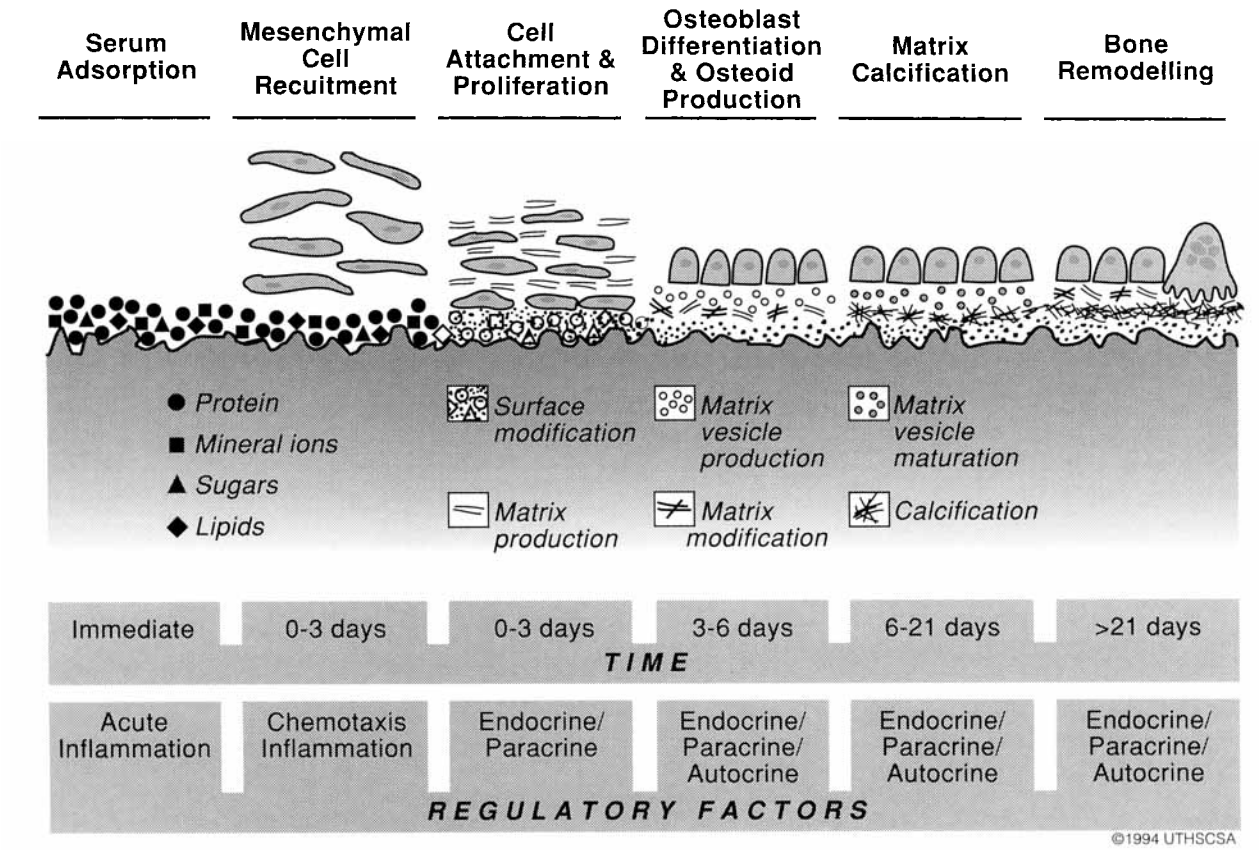

Fig. 2. Implant materials and the wound-healing paradigm. Following implantation, the surface of the material is conditioned by serum proteins, mineral ions, sugars, and lipids, as well as cytokines produced by immune cells. During the first 3 days, undifferentiated mesenchymal cells migrate to the surface of the material, attach and proliferate. During this time, they synthesize their own extracellular matrix, including growth factors and cytokines, and modify the surface of the implant. Between three and six days of healing, the mesenchymal cells undergo osteoblastic differentiation. They produce osteoid including matrix vesicles and growth factors. The osteogenic cells respond to endocrine, paracrine, and autocrine regulatory agents. At 6–14 days, the cells begin to calcify their matrix. Matrix vesicle maturation is indicated by increased alkaline phosphatase and phospholipase A_2 activities and phosphatidylserine content in tissue adjacent to materials which promote bone formation. After 21 days, the newly formed woven bone is remodeled, involving osteoclast recruitment.

crease ion leaching [Osborn et al., 1990]. Cells on the roughest surfaces, with the higher density of rough areas, cannot spread but must form focal attachments which enable them to span from one site to another. How the variation in cytomechanics modulates proliferation, matrix synthesis, and differentiation is not known, but certainly integrins and transmembrane signaling are involved.

WOUND HEALING PARADIGM

Most in vitro studies are limited by the fact that well-differentiated cells, such as osteoblasts or chondrocytes, are frequently used. In vivo, however, these more differentiated cells do not see the material until late in the wound healing cascade. Consequently, the material surface has been conditioned by serum factors, as well as by cells earlier in the process. As shown in Figure 2, the in vivo events involved in bone apposition may be conceived to occur in a series of discrete but overlapping stages. Depending on the success of the system at each stage, bone formation will progress, or fibrous connective tissue formation will ensue.

Studies describing the healing of critical-size craniotomy defects have elucidated the stages of lamellar bone formation following surgical injury [Schmitz et al., 1990]. Following an acute inflammatory response, which includes clot formation and release of wound healing factors, such as platelet-derived growth factor and transforming growth factor beta, mesenchymal stem cells are recruited to the wound site. Once at the wound site, the cells elaborate a type III collagen matrix and proliferate. As they undergo differen-

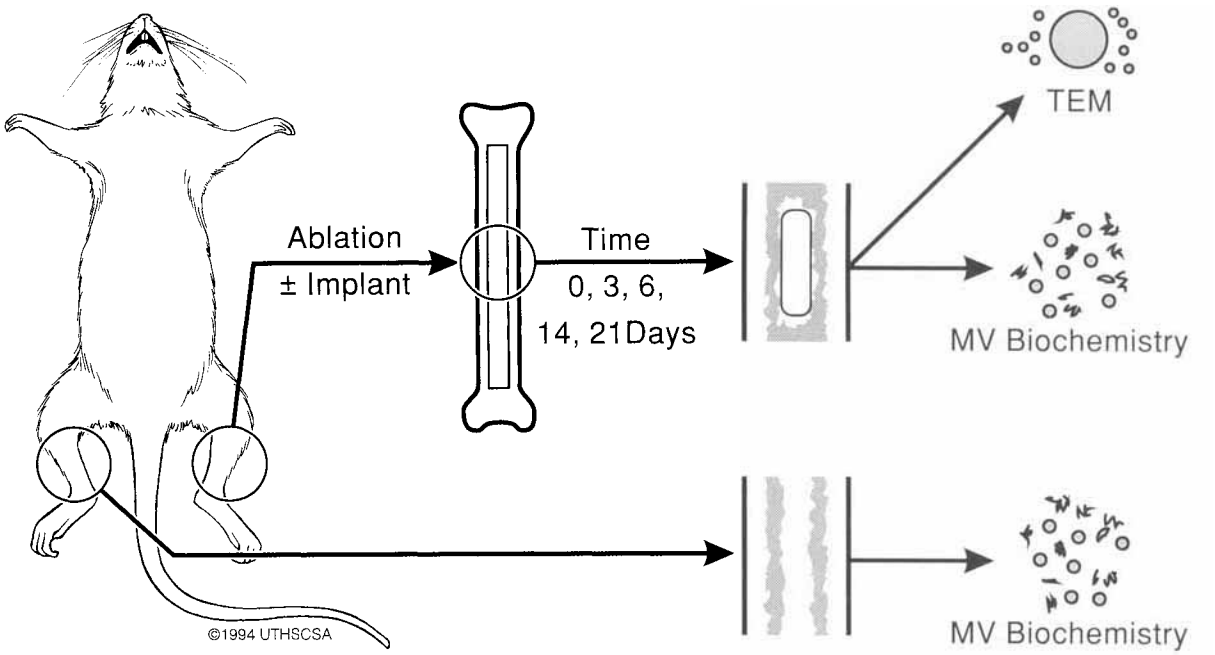

Fig. 3. Bone marrow ablation model for assessing local and systemic response to materials during endosteal bone healing. Marrow in the left tibia is ablated by repeated washing with saline under pressure. Implants are introduced into the marrow cavity and the rats are returned to normal weight bearing. At time = 0, 3, 6, 14, and 21 days post ablation, material adjacent to the implant is removed for transmission electron microscopy (TEM) (two animals per N with an N = six per time point) or for matrix vesicle (MV) isolation (six animals per matrix vesicle preparation × six preparations per time point). Matrix vesicles are also isolated from the endosteum of the contralateral tibia. TEM studies include matrix vesicle morphology, diameter, distance from the calcification front, and number per μm^2 of matrix. Biochemical analyses include alkaline phosphatase specific activity, phospholipase A_2 specific activity, and phospholipid composition, particularly phosphatidylserine content.

tiation, presumably in response to factors like bone morphogenetic protein, they exhibit a chondrogenic phenotype in sites of low O_2 tension and an osteoblastic phenotype in sites where O_2 tension is high. As long as micromotion in the defect is minimized, appropriate endocrine and paracrine factors are available, and the supply of osteoprogenitor cells is sufficient, bone will form. However, the interrelationships within the cascade are complex, and slight differences can lead to fibroblastic, rather than osteogenic, differentiation.

It is unknown how the introduction of a biomaterial affects mesenchymal stem cell recruitment and the early stages of cell differentiation. To begin to understand these phenomena, we adapted the rat marrow ablation model of endosteal bone healing (Fig. 3). In this model, the marrow of one tibia is ablated and events related to primary bone formation, specifically the calcification of osteoid, are monitored in both the ablated limb and the contralateral limb as a function of time [Schwartz et al., 1989; Sela et al., 1987]. To do this, we measured changes in matrix vesicles, extracellular organelles which play a role in initial hydroxyapatite formation. Changes in matrix vesicle morphology and number were visualized by transmission electron microscopy and monitored as a function of time. Bone-bonding materials tend to increase matrix vesicle numbers per area of matrix and to delay their time-dependent shifts in electron density, diameter, distance from the calcification front, and rupture [Schwartz et al., 1991] when compared with healing in unimplanted tibias. In contrast, implants which do not support bonding do not elicit increases in matrix vesicle number and appear to block the normal matrix vesicle maturation cascade [Schwartz et al., 1991].

These observations suggest that the presence of the material has a direct effect on cellular production of matrix vesicles as well as a direct effect on matrix vesicle maturation in the matrix. Biochemical studies of matrix vesicles isolated from the healing tissue adjacent to the implant support this interpretation. In the absence of implants, matrix vesicle alkaline phosphatase is elevated at 6 days postablation, a time when mineralization of osteoid is initiating. Similarly, matrix vesicle phospholipase A_2 activity

and phosphatidylserine content, both associated with initial calcification, are elevated at 6 days [Schwartz et al., 1992, 1993; Marshall et al., 1991]. When bone bonding implant materials are present, matrix vesicle alkaline phosphatase specific activity is elevated by three days post ablation, commensurate with an increase in matrix vesicle number. In absolute terms, however, specific activity of alkaline phosphatase as well as of phospholipase A_2 and phosphatidylserine content in tissues adjacent to the bone bonding materials is never as great as that seen in normal healing tissue. Moreover, matrix vesicles from tissue adjacent to nonbonding materials show no increase in these parameters at 3 days, and by 6 days, the wound healing effect is virtually abrogated [Schwartz et al., 1992, 1993; Marshall et al., 1991]. These observations suggest that the matrix vesicles produced by cells adjacent to implants are defective, either due to changes in composition during their biogenesis or to direct effects of the material after biosynthesis.

A strength of this model is the opportunity to assess systemic effects of implants. Ablation itself elicits a systemic response. Changes in matrix vesicles in the endosteum of the contralateral limb mimic those of the treated limb, but at lower amplitude. However, introduction of implants alters the contralateral effect in an implant-specific manner. If one accepts the hypothesis that wounding results in systemic as well as local release of wound healing factors, one would expect the contralateral limb to behave as if the implants were not present. But the implant alters this normal healing effect. One possibility is that the cells adjacent to the material are producing and secreting an altered mix of factors. Another is that the material acts as a mini "bioreactor" which binds, modifies, and releases factors in dynamic equilibrium with surrounding tissue [Lee et al., 1992].

EFFECTS OF MATERIALS ON OSTEOBLAST DIFFERENTIATION

The rat marrow ablation studies described above focus on markers of osteoblastic differentiation, in particular markers of matrix calcification. In terms of bone apposition, the ability of cells to synthesize a calcifiable matrix and to deposit hydroxyapatite is the litmus test. The results of these studies show that materials that promote bone apposition in vivo tend to have a net local effect over and above the systemic response observed in the contralateral limb, which enhances matrix vesicle enzyme activity. In contrast, materials which do not support bone apposition in vivo tend not to enhance matrix vesicle function locally. In fact, the local response is identical to that observed systemically. For some materials, this effect is neutral, while for others there is a suppression of matrix vesicle enzyme activity as a function of time, not only with respect to the normal wound healing response, but below that observed even in the absence of surgery.

Hydroxyapatite remains an enigmatic material. In vivo many investigators have found an enhancement of bone formation with this material [Schwartz et al., 1993; Sartoris et al., 1992; Cook et al., 1988]. When implants coated with hydroxyapatite by a variety of techniques are examined, bone appears to form on the surface, as well as on the endosteal surface of the healing bone. One interpretation is that hydroxyapatite is bioactive due to its binding of serum proteins [Hjorting-Hansen et al., 1990; Bagambisa et al., 1990] and growth factors [Luyten et al., 1992]. It has also been hypothesized that hydroxyapatite is the same mineral as found in bone so it "jumpstarts" the osteoprogenitor cells to continue matrix synthesis and calcification.

The hydroxyapatites used in implant materials are not comparable to those found in bone, however. They differ in carbonate content, other trace mineral ions, crystal size, and perfection, and other features. Perhaps even more importantly, they lack the normal biologic matrix associated with hydroxyapatite crystals in bone. Thus, it is possible that the rapid bone formation seen on these surfaces may be due to an adaptive response of the osteoblast to synthesize osteoid, and distance itself from the defective mineral.

Rapid bone formation is not synonymous with good bone formation. Subtle differences in matrix composition may have consequences downstream for bone remodeling. For example, changes in crystal size and perfection may affect the nature of the associated matrix proteins [Boskey et al., 1992]. The rat marrow ablation studies clearly demonstrate that there is a time dependent production and maturation of matrix vesicles associated with bone wound healing, which includes a systemic response. This series of events is modified by the presence of hydroxyapatite granules in the marrow cavity [Schwartz et al., 1993]. The data at this point are phenom-

enological, but they do indicate that the nature of bone formation in the presence of these materials is not identical to that seen under normal conditions. That hydroxyapatite does affect the mineralization process has been further shown using ^{99m}Tc-MD^{32}P as an indicator. These studies indicate that the normal distribution of the ^{99m}Tc and MD^{32}P moieties of this imaging agent is altered by the presence of hydroxyapatite granules (data not shown).

SUMMARY

This paper stresses the importance of understanding how materials alter the physiology of cells that interact with them locally, as well as of those cells that may experience systemic consequences of their presence in the organism. Our knowledge of these processes is at best rudimentary. To best use materials in the body, we must learn to manage these effects optimally.

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